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(54) Titre : PROCEDES D'EDITION D'UN GENE ASSOCIE A UNE MALADIE A L'AIDE D'EDITEURS DE BASES D'ADENOSINE DESAMINASE, Y COMPRIS POUR LE TRAITEMENT D'UNE MALADIE GENETIQUE

(54) Title: METHODS OF EDITING A DISEASE-ASSOCIATED GENE USING ADENOSINE DEAMINASE BASE EDITORS, INCLUDING FOR THE TREATMENT OF GENETIC DISEASE

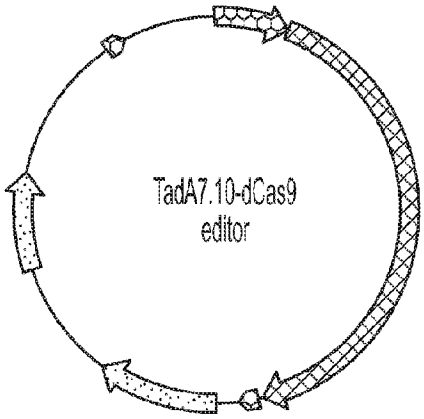

FIG. 1A

(57) Abrégé/Abstract:
The invention provides compositions comprising novel programmable adenosine base editor systems (e.g., ABE8) that provide methods of treating a disease or disorder, (e.g., Parkinson's disease, Hurler syndrome, Rett syndrome, or Stargardt disease) in a subject by administering to the subject a programmable adenosine base editor system (e.g., ABE8) that have increased efficiency and methods of using these adenosine deaminase variants for editing a disease-associated gene.

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(54) Title: METHODS OF EDITING A DISEASE-ASSOCIATED GENE USING ADENOSINE DEAMINASE BASE EDITORS, INCLUDING FOR THE TREATMENT OF GENETIC DISEASE

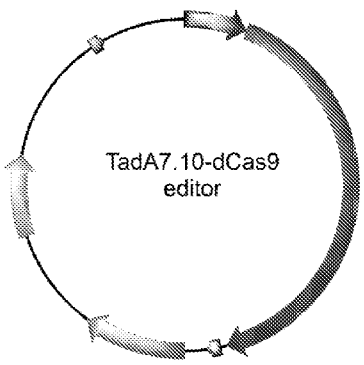

FIG. 1A

(57) Abstract: The invention provides compositions comprising novel programmable adenosine base editor systems (*e.g.*, ABE8) that provide methods of treating a disease or disorder, (*e.g.*, Parkinson's disease, Hurler syndrome, Rett syndrome, or Stargardt disease) in a subject by administering to the subject a programmable adenosine base editor system (*e.g.*, ABE8) that have increased efficiency and methods of using these adenosine deaminase variants for editing a disease-associated gene.

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CONTENANT LES PAGES 1 À 259

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 3
CONTAINING PAGES 1 TO 259

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NOTE POUR LE TOME / VOLUME NOTE:

METHODS OF EDITING A DISEASE-ASSOCIATED GENE USING ADENOSINE DEAMINASE BASE EDITORS, INCLUDING FOR THE TREATMENT OF GENETIC DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/805,271 filed on February 13, 2019; U.S. Provisional Application No. 62/852,228 filed on May 23, 2019; U.S. Provisional Application No. 62/852,224 filed on May 23, 2019; U.S. Provisional Application No. 62/873,138 filed on July 11, 2019; U.S. Provisional Application No. 62/888,867 filed on August 19, 2019; U.S. Provisional Application No. 62/931,722 filed on November 6, 2019; U.S. Provisional Application No. 62/941,569 filed on November 27, 2019; U.S. Provisional Application No. 62/966,526 filed on January 27, 2020, the disclosures of which are hereby incorporated by reference in their entirety.

INCORPORATION BY REFERENCE

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. Absent any indication otherwise, publications, patents, and patent applications mentioned in this specification are incorporated herein by reference in their entireties.

BACKGROUND OF THE DISCLOSURE

Targeted editing of nucleic acid sequences, for example, the targeted cleavage or the targeted modification of genomic DNA is a highly promising approach for the study of gene function and also has the potential to provide new therapies for human genetic diseases. Currently available base editors include cytidine base editors (*e.g.*, BE4) that convert target C•G base pairs to T•A and adenine base editors (*e.g.*, ABE7.10) that convert A•T to G•C. There is a need in the art for improved base editors capable of inducing modifications within a target sequence with greater specificity and efficiency.

SUMMARY OF THE DISCLOSURE

The invention provides compositions comprising novel adenine base editors (*e.g.*, ABE8) that have increased efficiency and methods of using base editors comprising adenosine deaminase variants for editing a target sequence.

In some aspects, provided herein, is a method of treating a neurological disorder in a subject, the method comprising: administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a target gene or a regulatory element thereof associated with the neurological disorder in the subject, thereby treating the neurological disorder in the subject. In another embodiment of this aspect, the target gene is an alpha-L-iduronidase (IDUA) gene and the neurological disease is Hurler syndrome. In one embodiment of this aspect, the target gene is a leucine-rich repeat kinase-2 (LRRK2) gene and the neurological disease is Parkinson's disease. In one embodiment of this aspect, the target gene is a methyl CpG binding protein 2 (MECP2) gene and the neurological disease is Rett syndrome. In another embodiment of this aspect, the target gene is an ATP-binding cassette subfamily member 4 (ABCA4) gene and the neurological disease is Stargardt disease.

In some aspects, provided herein, is a method of treating Hurler syndrome in a subject, the method comprising administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in an alpha-L-iduronidase (IDUA) gene or a regulatory element thereof in the subject, thereby treating Hurler syndrome in the subject.

In some embodiments, the administration ameliorates at least one symptom related to Hurler syndrome. In some embodiments, the administration results in faster amelioration of at least one symptom related to Hurler syndrome as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

In some embodiments, the IDUA gene or regulatory element thereof comprises a SNP associated with Hurler syndrome. In some embodiments, the A-to-G nucleobase

alteration is at the SNP associated with Hurler syndrome. In some embodiments, the SNP associated with Hurler syndrome results in a W402X or a W401X amino acid mutation in an IDUA polypeptide as numbered in SEQ ID NO: 4, or a variant thereof, encoded by the IDUA gene, wherein X is a stop codon. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Hurler syndrome. In some embodiments, the A-to-G alteration at the SNP associated with Hurler Syndrome changes a stop codon to a tryptophan in an IDUA polypeptide encoded by the IDUA gene.

In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *IDUA* gene or regulatory element thereof comprising the SNP associated with Hurler syndrome. In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the IDUA gene or regulatory element thereof comprising the SNP associated with Hurler syndrome. In some embodiments, the sgRNA comprises a nucleic acid sequence selected from the group consisting of: 5′- GACUCUAGGCAGAGGUCUCAA -3′, 5′- ACUCUAGGCAGAGGUCUCAA-3′, 5′- CUCUAGGCCGAAGUGUCGC -3′, and 5′- GCUCUAGGCCGAAGUGUCGC-3′.

In some aspects, provided herein, is a method of treating Parkinson's disease in a subject, the method comprising: administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration a leucine-rich repeat kinase-2 (LRRK2) gene or a regulatory element thereof in the subject, thereby treating Parkinson's disease in the subject.

In some embodiments, the administration ameliorates at least one symptom related to Parkinson's disease. In some embodiments, the administration results in faster amelioration of at least one symptom related to Parkinson's disease as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

In some embodiments, the LRRK2 gene or regulatory element thereof comprises a SNP associated with Parkinson's disease. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Parkinson's disease. In some embodiments, the SNP associated with Parkinson Disease results in a A419V, a R1441C, a R1441H, or a G2019S amino acid mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a non-wild type nucleobase that results in one or more ameliorated symptoms of Parkinson's disease. In some embodiments, the A-to-G nucleobase alteration changes a Cysteine or Histidine to an Arginine in a LRRK2 polypeptide encoded by the LRRK2 gene. In some embodiments, the A-to-G alteration changes a Serine to a Glycine in a LRRK2 polypeptide encoded by the LRRK2 gene. In some embodiments, the A-to-G alteration replaces the Cysteine (C) or Histidine (H) with an Arginine (R) at position 144 or replaces the Serine with a Glycine (G) at position 2019 of a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

In some aspects, provided herein, is a method of treating Parkinson's disease in a subject, the method comprising: administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration at a SNP in a LRRK2 gene associated with Parkinson's disease, wherein the SNP does not encode a G2019S mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof.

In some embodiments, the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof. In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson's Disease. In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising

the SNP associated with Parkinson Disease. In some embodiments, the sgRNA comprises a nucleic acid sequence: 5′-AAGCGCAAGCCUGGAGGGAA -3′; or 5′-ACUACAGCAUUGCUCAGUAC-3′.

In some aspects, provided herein, is a method of treating Rett syndrome in a subject, the method comprising administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a methyl CpG binding protein 2 (MECP2) gene or a regulatory element thereof in the subject, thereby treating Rett syndrome in the subject.

In some embodiments, the administration ameliorates at least one symptom related to Rett syndrome. In some embodiments, the administration results in faster amelioration of at least one symptom related to Rett syndrome as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase. In some embodiments, the MECP2 gene or regulatory element thereof comprises a SNP associated with Rett syndrome. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Rett Syndrome. In some embodiments, the SNP associated with Rett syndrome results in a R106W or a T158M amino acid mutation in a MECP2 polypeptide as numbered in SEQ ID NO: 5, or a variant thereof, encoded by the MECP2 gene. In some embodiments, the SNP associated with Rett syndrome results in a R255X or a R270X amino acid mutation in a MECP2 polypeptide encoded by the MECP2 gene, wherein X is a stop codon.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a non-wild type nucleobase that results in ameliorated Rett syndrome symptoms. In some embodiments, the A-to-G nucleobase alteration at the SNP associated with Rett Syndrome changes a stop codon to tryptophan in MECP2 polypeptide.

In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP

associated with Rett syndrome. In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome. In some embodiments, the guide polynucleotide comprises a nucleic acid sequence selected from the group consisting of: 5′-CUUUUCACUUCCUGCCGGGG-3′, 5′-AGCUUCCAUGUCCAGCCUUC-3′, 5′-ACCAUGAAGUCAAAAUCAUU-3′, and 5′- GCUUUCAGCCCCGUUUCUUG-3′.

In some aspects, provided herein, is a method of treating Stargardt disease in a subject, the method comprising administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in an ATP-binding cassette subfamily member 4 (ABCA4) gene or a regulatory element thereof in the subject, thereby treating Stargardt disease in the subject.

In some embodiments, the administration ameliorates at least one symptom related to Stargardt disease. In some embodiments, the administration results in faster amelioration of at least one symptom related to Stargardt disease as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

In some embodiments, the ABCA4 gene comprises a SNP associated with Stargardt disease. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Stargardt disease. In some embodiments, the SNP associated with Stargardt disease results in a A1038V or a G1961E amino acid mutation in an ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof, encoded by the ABCA4 gene. In some embodiments, the SNP associated with Stargardt disease results in a G1961E amino acid mutation in the ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a non-wild type nucleobase that results in one or more ameliorated symptoms of Stargardt disease. In some

embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt disease.

In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease. In some embodiments, the sgRNA comprises the sequence 5′-CUCCAGGGCGAACUUCGACACACAGC-3′.

In various aspects, the treatment described herein results in ameliorated symptoms of the neurological disorder compared to treatment with a base editor comprising an adenosine deaminase domain without the amino acid substitutions.

In some aspects, provided herein, is a method of editing a target gene or regulatory element thereof associated with a neurological disorder, the method comprising contacting the target gene or regulatory element thereof with (i) an adenosine base editor and (ii) a guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a target gene or a regulatory element thereof associated with the neurological disorder. In one embodiment of this aspect, the target gene is a leucine-rich repeat kinase-2 (LRRK2) gene and the neurological disease is Parkinson's disease. In another embodiment of this aspect, the target gene is an alpha-L-iduronidase (IDUA) gene and the neurological disease is Hurler syndrome. In one embodiment of this aspect, the target gene is a methyl CpG binding protein 2 (MECP2) gene and the neurological disease is Rett syndrome. In another embodiment of this aspect, the target gene is an ATP-binding cassette subfamily member 4 (ABCA4) gene and the neurological disease is Stargardt disease.

In some aspects, provided herein, is a method of editing a leucine-rich repeat kinase-2 (LRRK2) gene or a regulatory element thereof, the method comprising contacting the LRRK2 gene or regulatory element thereof with (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a

programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the LRRK2 gene a regulatory element thereof.

In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Parkinson's disease. In some embodiments, the SNP associated with Parkinson Disease results in a A419V, a R1441C, a R1441H, or a G2019S amino acid mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a non-wild type nucleobase that results in one or more ameliorated symptoms of Parkinson's disease.

In some embodiments, the A-to-G nucleobase alteration changes a Cysteine or Histidine to an Arginine in a LRRK2 polypeptide encoded by the LRRK2 gene. In some embodiments, the A-to-G alteration changes a Serine to a Glycine in a LRRK2 polypeptide encoded by the LRRK2 gene. In some embodiments, the A-to-G alteration replaces the Cysteine (C) or Histidine (H) with an Arginine (R) at position 144 or replaces the Serine with a Glycine (G) at position 2019 of a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

In some aspects, provided herein, is a method of editing a leucine-rich repeat kinase-2 (LRRK2) gene or a regulatory element thereof, the method comprising contacting the LRRK2 gene or regulatory element thereof with (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration at a SNP in a LRRK2 gene, wherein the SNP does not encode a G2019S mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof.

In some embodiments, the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof. In some embodiments, the guide polynucleotide comprises a

nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson's Disease.

In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson Disease. In some embodiments, the sgRNA comprises a nucleic acid sequence: 5′-AAGCGCAAGCCUGGAGGGAA -3′; or 5′-ACUACAGCAUUGCUCAGUAC-3′.

In some aspects, provided herein, is a method of editing an alpha-L-iduronidase (IDUA) gene or a regulatory element thereof, the method comprising contacting the IDUA gene or regulatory element thereof with (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the IDUA gene or a regulatory element thereof.

In some embodiments, the IDUA gene or regulatory element thereof comprises a SNP associated with Hurler syndrome. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Hurler syndrome. In some embodiments, the SNP associated with Hurler syndrome results in a W402X or a W401X amino acid mutation in an IDUA polypeptide as numbered in SEQ ID NO: 4, or a variant thereof, encoded by the IDUA gene, wherein X is a stop codon.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Hurler syndrome. In some embodiments, the A-to-G alteration at the SNP associated with Hurler Syndrome changes a stop codon to a tryptophan in an IDUA polypeptide encoded by the IDUA gene.

In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *IDUA* gene or regulatory element thereof comprising the SNP associated with Hurler syndrome. In some embodiments, the adenosine base editor is in

complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the IDUA gene or regulatory element thereof comprising the SNP associated with Hurler syndrome. In some embodiments, the sgRNA comprises a nucleic acid sequence selected from the group consisting of: 5′- GACUCUAGGCAGAGGUCUCAA -3′,5′- ACUCUAGGCAGAGGUCUCAA-3′, 5′- CUCUAGGCCGAAGUGUCGC -3′, and 5′-GCUCUAGGCCGAAGUGUCGC-3′.

In some aspects, provided herein, is a method of editing a methyl CpG binding protein 2 (MECP2) gene or regulatory element thereof, the method comprising administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the MECP2 gene or a regulatory element thereof.

In some embodiments, the MECP2 gene or regulatory element thereof comprises a SNP associated with Rett syndrome. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Rett Syndrome. In some embodiments, the SNP associated with Rett syndrome results in a R106W or a T158M amino acid mutation in a MECP2 polypeptide as numbered in SEQ ID NO: 5, or a variant thereof, encoded by the MECP2 gene. In some embodiments, the SNP associated with Rett syndrome results in a R255X or a R270X amino acid mutation in a MECP2 polypeptide encoded by the MECP2 gene, wherein X is a stop codon.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Rett syndrome. In some embodiments, the A-to-G nucleobase alteration at the SNP associated with Rett Syndrome changes a stop codon to tryptophan in MECP2 polypeptide.

In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP

associated with Rett syndrome. In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome. In some embodiments, the guide polynucleotide comprises a nucleic acid sequence selected from the group consisting of: 5'-CUUUUCACUUCCUGCCGGGG-3', 5'-AGCUUCCAUGUCCAGCCUUC-3', 5'-ACCAUGAAGUCAAAAUCAUU-3', and 5'- GCUUUCAGCCCCGUUUCUUG-3'.

In some aspects, provided herein, is a method of editing an ATP binding cassette subfamily member 4 (ABCA4) gene or regulatory element thereof, the method comprising contacting the ABCA4 gene or regulatory element thereof with (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the ABCA4 gene or a regulatory element thereof.

In some embodiments, the administration ameliorates at least one symptom related to Stargardt disease. In some embodiments, the administration results in faster amelioration of at least one symptom related to Stargardt disease as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

In some embodiments, the ABCA4 gene comprises a SNP associated with Stargardt disease. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Stargardt disease. In some embodiments, the SNP associated with Stargardt disease results in a A1038V, or a G1961E amino acid mutation in an ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof, encoded by the ABCA4 gene. In some embodiments, the SNP associated with Stargardt disease results in a G1961E amino acid mutation in the ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a non-wild type nucleobase that results in one or more ameliorated symptoms of Stargardt disease. In some

embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease.

In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease. In some embodiments, the sgRNA comprises the sequence 5′-CUCCAGGGCGAACUUCGACACACAGC-3′.

In various embodiments of the above aspects, the contacting is in a cell. In some embodiments, the contacting results in less than 10% indels in a genome in the cell, wherein indel rate is measured by mismatch frequency between sequences flanking the single nucleotide modification and an unmodified sequence. In some embodiments, the contacting results in less than 5% indels in a genome in the cell, wherein indel rate is measured by mismatch frequency between sequences flanking the single nucleotide modification and an unmodified sequence. In some embodiments, the contacting results in less than 1% indels in a genome in the cell, wherein indel rate is measured by mismatch frequency between sequences flanking the single nucleotide modification and an unmodified sequence.

In various embodiments of the above aspects, the cell is a neuron. In some embodiments, the contacting is in a population of cells. In some embodiments, the contacting results in the A-to-G nucleobase alteration in at least 40% of the population of cells after the contacting step. In some embodiments, the contacting results in the A-to-G nucleobase alteration in at least 50% of the population of cells after the contacting step. In some embodiments, the contacting results in the A-to-G nucleobase alteration in at least 70% of the population of cells after the contacting step. In some embodiments, at least 90% of the cells are viable after the contacting step. In some embodiments, the population of cells was not enriched after the contacting step. In some embodiments, the population of cells are neurons. In some embodiments, the contacting is in vivo or ex vivo.

In various aspects and embodiments above, the polynucleotide programmable DNA binding domain is a Cas9. In some embodiments, the Cas9 is a SpCas9, a SaCas9, or a variant thereof. In some embodiments, the polynucleotide programmable DNA binding domain comprises a modified SpCas9 having an altered protospacer-adjacent motif (PAM) specificity. In some embodiments, the Cas9 has specificity for a PAM sequence selected from the group

consisting of NGG, NGA, NGCG, NGN, NNGRRT, NNNRRT, NGCG, NGCN, NGTN, and NGC; wherein N is A, G, C, or T; and wherein R is A or G. In some embodiments, the polynucleotide programmable DNA binding domain is a nuclease inactive variant. In some embodiments, the polynucleotide programmable DNA binding domain is a nickase variant. In some embodiments, the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof. In various aspects and embodiments provided herein, the adenosine deaminase domain comprises a TadA domain. In some embodiments, the adenosine deaminase comprises a TadA deaminase comprising a V82S alteration and/or a T166R alteration.

In various aspects and embodiments above, the adenosine deaminase further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, Q154R, or a combination thereof. In various aspects and embodiments provided herein, the adenosine deaminase comprises a combination of alterations selected from the group consisting of: Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y147T + Q154R; Y147T + Q154S; and Y123H + Y147R + Q154R + I76Y. In various aspects and embodiments provided herein, the adenosine base editor domain comprises an adenosine deaminase monomer. In various aspects and embodiments provided herein, the adenosine base editor comprises an adenosine deaminase dimer. In some embodiments, the TadA deaminase is a TadA*8 variant. In some embodiments, the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, and TadA*8.13. In some embodiments, the adenosine base editor is an ABE8 base editor selected from the group consisting of: ABE8.1, ABE8.2, ABE8.3, ABE8.4, ABE8.5, ABE8.6, ABE8.7, ABE8.8, ABE8.9, ABE8.10, ABE8.11, ABE8.12, and ABE8.13.

In some aspects, provided herein, is a cell produced by the method described in various aspects and embodiments disclosed herein. In some aspects, provided herein, is a population of cells produced by the method described in various aspects and embodiments disclosed herein.

In some aspects, provided herein, is a base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid

substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a target gene or a regulatory element thereof associated with the neurological disorder. In one embodiment of the above aspect, the target gene is a leucine-rich repeat kinase-2 (LRRK2) gene and the neurological disease is Parkinson's disease. In another embodiment of this aspect, the target gene is an alpha-L-iduronidase (IDUA) gene and the neurological disease is Hurler syndrome. In one embodiment of this aspect, the target gene is a methyl CpG binding protein 2 (MECP2) gene and the neurological disease is Rett syndrome. In another embodiment of this aspect, the target gene is an ATP-binding cassette subfamily member 4 (ABCA4) gene and the neurological disease is Stargardt disease.

In some aspects, provided herein, is a base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a LRRK2 gene a regulatory element thereof.

In some embodiments, the A-to-G nucleobase alteration is at a SNP associated with Parkinson's disease in the LRRK2 gene or regulatory element thereof. In some embodiments, the SNP associated with Parkinson Disease results in a A419V, a R1441C, a R1441H, or a G2019S amino acid mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a non-wild type nucleobase that results in ameliorated Parkinson's symptoms. In some embodiments, the A-to-G nucleobase alteration changes a Cysteine or Histidine to an Arginine in a LRRK2 polypeptide encoded by the LRRK2 gene. In some embodiments, the A-to-G alteration changes a Serine to a Glycine in a LRRK2 polypeptide encoded by the LRRK2 gene. In some embodiments, the A-to-G alteration replaces the Cysteine (C) or Histidine (H) with an

Arginine (R) at position 144 or replaces the Serine with a Glycine (G) at position 2019 of a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene. In some embodiments, the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof.

In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson's Disease. In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson Disease. In some embodiments, the sgRNA comprises a nucleic acid sequence: 5′-AAGCGCAAGCCUGGAGGGAA -3′; or 5′-ACUACAGCAUUGCUCAGUAC-3′.

In some aspects, provided herein, is a base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in an alpha-L-iduronidase (IDUA) gene or a regulatory element thereof.

In some embodiments, the IDUA gene or regulatory element thereof comprises a SNP associated with Hurler syndrome. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Hurler syndrome. In some embodiments, the SNP associated with Hurler syndrome results in a W402X or a W401X amino acid mutation in an IDUA polypeptide as numbered in SEQ ID NO: 4, or a variant thereof, encoded by the IDUA gene, wherein X is a stop codon.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Hurler syndrome. In some

embodiments, the A-to-G alteration at the SNP associated with Hurler Syndrome changes a stop codon to a tryptophan in an IDUA polypeptide encoded by the IDUA gene.

In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *IDUA* gene or regulatory element thereof comprising the SNP associated with Hurler syndrome. In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the IDUA gene or regulatory element thereof comprising the SNP associated with Hurler syndrome. In some embodiments, the sgRNA comprises a nucleic acid sequence selected from the group consisting of: 5′- GACUCUAGGCAGAGGUCUCAA -3′, 5′- ACUCUAGGCAGAGGUCUCAA-3′, 5′- CUCUAGGCCGAAGUGUCGC -3′, and 5′-GCUCUAGGCCGAAGUGUCGC-3′.

In some aspects, provided herein, is a base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a methyl CpG binding protein 2 (MECP2) gene or regulatory element thereof.

In some embodiments, the MECP2 gene or regulatory element thereof comprises a SNP associated with Rett syndrome. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Rett Syndrome. In some embodiments, the SNP associated with Rett syndrome results in a R106W or a T158M amino acid mutation in a MECP2 polypeptide as numbered in SEQ ID NO: 5, or a variant thereof, encoded by the MECP2 gene. In some embodiments, the SNP associated with Rett syndrome results in a R255X or a R270X amino acid mutation in a MECP2 polypeptide encoded by the MECP2 gene, wherein X is a stop codon.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Rett syndrome. In some embodiments, the A-

to-G nucleobase alteration at the SNP associated with Rett Syndrome changes a stop codon to tryptophan in MECP2 polypeptide.

In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome. In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome. In some embodiments, the guide polynucleotide comprises a nucleic acid sequence selected from the group consisting of: 5′-CUUUUCACUUCCUGCCGGGG-3′, 5′-AGCUUCCAUGUCCAGCCUUC-3′, 5′-ACCAUGAAGUCAAAAUCAUU-3′, and 5′- GCUUUCAGCCCCGUUUCUUG-3′.

In some aspects, provided herein, is a base editor system comprising contacting (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in an ATP binding cassette subfamily member 4 (ABCA4) gene or regulatory element thereof.

In some embodiments, the administration ameliorates at least one symptom related to Stargardt disease. In some embodiments, the administration results in faster amelioration of at least one symptom related to Stargardt disease as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase. In some embodiments, the ABCA4 gene comprises a SNP associated with Stargardt disease. In some embodiments, the A-to-G nucleobase alteration is at the SNP associated with Stargardt disease. In some embodiments, the SNP associated with Stargardt disease results in a A1038V, or a G1961E amino acid mutation in an ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof, encoded by the ABCA4 gene. In some embodiments, the SNP associated with Stargardt disease results in a G1961E amino acid mutation in the ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof.

In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a wild type nucleobase. In some embodiments, the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a non-wild type nucleobase that results in ameliorated Stargardt Disease symptoms. In some embodiments, the guide polynucleotide comprises a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease.

In some embodiments, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease. In some embodiments, the sgRNA comprises the sequence 5′-CUCCAGGGCGAACUUCGACACACAGC-3′.

In various aspects and embodiments provided herein, the polynucleotide programmable DNA binding domain is a Cas9. In some embodiments, the Cas9 is a SpCas9, a SaCas9, or a variant thereof. In some embodiments, the polynucleotide programmable DNA binding domain comprises a modified SpCas9 having an altered protospacer-adjacent motif (PAM) specificity. In some embodiments, the Cas9 has specificity for a PAM sequence selected from the group consisting of NGG, NGA, NGCG, NGN, NNGRRT, NNNRRT, NGCG, NGCN, NGTN, and NGC, wherein N is A, G, C, or T and wherein R is A or G. In some embodiments, the polynucleotide programmable DNA binding domain is a nuclease inactive variant. In some embodiments, the polynucleotide programmable DNA binding domain is a nickase variant. In some embodiments, the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

In various aspects and embodiments provided herein, the adenosine deaminase domain comprises a TadA domain. In some embodiments, the adenosine deaminase comprises a TadA deaminase comprising a V82S alteration and/or a T166R alteration.

In various aspects and embodiments provided herein, the adenosine deaminase further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, Q154R, or a combination thereof. In various aspects and embodiments provided herein, the adenosine deaminase comprises a combination of alterations selected from the group consisting of: Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y147T + Q154R; Y147T + Q154S; and Y123H + Y147R + Q154R + I76Y. In some

embodiments, the adenosine base editor domain comprises an adenosine deaminase monomer. In some embodiments, the adenosine base editor comprises an adenosine deaminase dimer.

In various aspects and embodiments provided herein, the TadA deaminase is a TadA*8 variant. In some embodiments, the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, and TadA*8.13. In some embodiments, the adenosine base editor is an ABE8 base editor selected from the group consisting of: ABE8.1, ABE8.2, ABE8.3, ABE8.4, ABE8.5, ABE8.6, ABE8.7, ABE8.8, ABE8.9, ABE8.10, ABE8.11, ABE8.12, and ABE8.13.

In some aspects, provided herein, is a vector comprising the nucleic acid sequence encoding the adenosine base editor described herein. In some aspects, provided herein, is a vector comprising the nucleic acid sequence encoding the adenosine base editor and the guide polynucleotide described herein. In some embodiments, the vector is a viral vector, a lentiviral vector, or an AAV vector.

In some aspects, provided herein, is a cell comprising the base editor system or the vector described herein. In some embodiments, the cell is a central nervous system cell. In some embodiments, the cell is a neuron. In some embodiments, the cell is a photoreceptor. In some embodiments, the cell is *in vitro*, *in vivo*, or *ex vivo*.

In some aspects, provided herein, is a pharmaceutical composition comprising the base editor, the vector, or the cell described herein and a pharmaceutically acceptable carrier. In one embodiment, the pharmaceutical composition described herein further comprises a lipid. In another embodiment, the pharmaceutical composition described herein further comprises a virus.

In some aspects, provided herein, is a kit comprising the base editor or the vector described herein.

In various embodiments of the methods described herein, at least one nucleotide of the guide polynucleotide comprises a non-naturally occurring modification. In various embodiments of the methods described herein, at least one nucleotide of the nucleic acid sequence comprises a non-naturally occurring modification. In various embodiments, at least one nucleotide of the nucleic acid sequence of the base editor system comprises a non-naturally occurring modification. In some embodiments, the non-naturally occurring modification is a chemical modification. In some embodiments, the chemical modification is

a 2'-O-methylation. In some embodiments, the nucleic acid sequence comprises a phosphorothioate.

The description and examples herein illustrate embodiments of the present disclosure in detail. It is to be understood that this disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this disclosure, which are encompassed within its scope.

The practice of some embodiments disclosed herein employ, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See for example Sambrook and Green, Molecular Cloning: A Laboratory Manual, 4th Edition (2012); the series Current Protocols in Molecular Biology (F. M. Ausubel, *et al.* eds.); the series Methods In Enzymology (Academic Press, Inc.), PCR 2: A Practical Approach (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications, 6th Edition (R.I. Freshney, ed. (2010)).

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Although various features of the present disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and in view of the accompanying drawings as described hereinbelow.

Definitions

The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.*, to any commonly

owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton *et al.*, Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991).

In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or," unless stated otherwise, and is understood to be inclusive. Furthermore, use of the term "including" as well as other forms, such as "include," "includes," and "included," is not limiting.

As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, such as within 5-fold or within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise

stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

Reference in the specification to "some embodiments," "an embodiment," "one embodiment" or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

By "abasic base editor" is meant an agent capable of excising a nucleobase and inserting a DNA nucleobase (A, T, C, or G). Abasic base editors comprise a nucleic acid glycosylase polypeptide or fragment thereof. In one embodiment, the nucleic acid glycosylase is a mutant human uracil DNA glycosylase comprising an Asp at amino acid 204 (*e.g.*, replacing an Asn at amino acid 204) in the following sequence, or corresponding position in a uracil DNA glycosylase, and having cytosine-DNA glycosylase activity, or active fragment thereof. In one embodiment, the nucleic acid glycosylase is a mutant human uracil DNA glycosylase comprising an Ala, Gly, Cys, or Ser at amino acid 147 (*e.g.*, replacing a Tyr at amino acid 147) in the following sequence, or corresponding position in a uracil DNA glycosylase, and having thymine-DNA glycosylase activity, or an active fragment thereof. The sequence of exemplary human uracil-DNA glycosylase, isoform 1, follows:

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  1 mgvfclgpwg lgrklrtpgk gplqllsrlc gdhlqaipak kapagqeepg tppssplsae
 61 qldriqrnka aallrlaarn vpvgfgeswk khlsgefgkp yfiklmgfva eerkhytvyp
121 pphqvftwtq mcdikdvkvv ilgqdpyhgp nqahglcfsv qrpvpppps eniykelstd
181 iedfvhpghg dlsgwakqgv lllnavltvr ahqanshker gweqftdavv swlnqnsngl
241 vfllwgsyaq kkgsaidrkr hhvlqtahps plsvyrgffg crhfsktnel lqksgkkpid
301 wkel
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The sequence of human uracil-DNA glycosylase, isoform 2, follows:

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  1 migqktlysf fspsparkrh apspepavqg tgvagvpees gdaaaipakk apagqeepgt
 61 ppssplsaeq ldriqrnkaa allrlaarnv pvgfgeswkk hlsgefgkpy fiklmgfvae
121 erkhytvypp phqvftwtqm cdikdvkvvi lgqdpyhgpn qahglcfsvq rpvppppsle
181 niykelstdi edfvhpghgd lsgwakqgvl llnavltvra hqanshkerg weqftdavvs
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241 wlnqnsnglv fllwgsyaqk kgsaidrkrh hvlqtahpsp **l**svy**r**gffgc rhfsktnell
301 qksgkkpidw kel

In other embodiments, the abasic editor is any one of the abasic editors described in PCT/JP2015/080958 and US20170321210, which are incorporated herein by reference. In particular embodiments, the abasic editor comprises a mutation at a position shown in the sequence above in bold with underlining or at a corresponding amino acid in any other abasic editor or uracil deglycosylase known in the art. In one embodiment, the abasic editor comprises a mutation at Y147, N204, L272, and/or R276, or corresponding position. In another embodiment, the abasic editor comprises a Y147A or Y147G mutation, or corresponding mutation. In another embodiment, the abasic editor comprises a N204D mutation, or corresponding mutation. In another embodiment, the abasic editor comprises a L272A mutation, or corresponding mutation. In another embodiment, the abasic editor comprises a R276E or R276C mutation, or corresponding mutation.

By "adenosine deaminase" is meant a polypeptide or fragment thereof capable of catalyzing the hydrolytic deamination of adenine or adenosine. In some embodiments, the deaminase or deaminase domain is an adenosine deaminase catalyzing the hydrolytic deamination of adenosine to inosine or deoxy adenosine to deoxyinosine. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenine or adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (*e.g.*, engineered adenosine deaminases, evolved adenosine deaminases) provided herein may be from any organism, such as a bacterium.

In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is TadA variant. In some embodiments, the TadA variant is a TadA*8. In some embodiments, the deaminase or deaminase domain is a variant of a naturally occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identical to a naturally occurring deaminase. For example, deaminase domains are described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078)

and PCT/US2016/058344 (WO 2017/070632), each of which is incorporated herein by reference for its entirety. Also, see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017)), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1, the entire contents of which are hereby incorporated by reference.

A wild type TadA(wt) adenosine deaminase has the following sequence (also termed TadA reference sequence):

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHP
GMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD (SEQ ID NO: 2).

In some embodiments, the adenosine deaminase comprises an alteration in the following sequence:

MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG
LHDPTAHAEI MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG
RVVFGVRNAK TGAAGSLMDV LHYPGMNHRV EITEGILADE CAALLCYFFR
MPRQVFNAQK KAQSSTD

(also termed TadA*7.10).

In some embodiments, TadA*7.10 comprises at least one alteration. In some embodiments, TadA*7.10 comprises an alteration at amino acid 82 and/or 166. In particular embodiments, a variant of the above-referenced sequence comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. The alteration Y123H is also referred to herein as H123H (the alteration H123Y in TadA*7.10 reverted back to Y123H (wt)). In other embodiments, a variant of the TadA*7.10 sequence comprises a combination of alterations selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

In other embodiments, the invention provides adenosine deaminase variants that include deletions, *e.g.*, TadA*8, comprising a deletion of the C terminus beginning at residue 149, 150, 151, 152, 153, 154, 155, 156, or 157. In other embodiments, the adenosine deaminase variant is a TadA (*e.g.*, TadA*8) monomer comprising one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In other embodiments, the adenosine deaminase variant is TadA (*e.g.*, TadA*8) a monomer comprising a combination of alterations selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

In still other embodiments, the adenosine deaminase variant is a homodimer comprising two adenosine deaminase domains (*e.g.*, TadA*8) each having one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In other embodiments, the adenosine deaminase variant is a homodimer comprising two adenosine deaminase domains (*e.g.*, TadA*8) each having a combination of alterations selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

In other embodiments, the adenosine deaminase variant is a heterodimer comprising a wild-type TadA adenosine deaminase domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In other embodiments, the adenosine deaminase variant is a heterodimer comprising a wild-type TadA adenosine deaminase domain and an adenosine deaminase variant domain (*e.g.* TadA*8) comprising a combination of alterations selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

In other embodiments, the adenosine deaminase variant is a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In other embodiments, the adenosine deaminase variant is a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant domain (*e.g.* TadA*8) comprising a combination of the following alterations: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; or I76Y + V82S + Y123H + Y147R + Q154R.

In one embodiment, the adenosine deaminase is a TadA*8 that comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMD VLHYPGMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

In some embodiments, the TadA*8 is truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8.

In particular embodiments, an adenosine deaminase heterodimer comprises a TadA*8 domain and an adenosine deaminase domain selected from one of the following:

In particular embodiments, an adenosine deaminase heterodimer comprises a TadA*8 domain and an adenosine deaminase domain selected from one of the following:

Escherichia coli TadA:

MRRAFITGVFFLSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGR HDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGA AGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD

E.coli TadA (N-terminal truncated):

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHP
GMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD

Staphylococcus aureus (*S. aureus*) TadA:

MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAHAEHIA
IERAAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCSGSLMNLLQQS
NFNHRAIVDKGVLKEACSTLLTTFFKNLRANKKSTN

Bacillus subtilis (*B. subtilis*) TadA:

MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEMLVIDEA
CKALGTWRLEGATLYVTLEPCPMCAGAVVLSRVEKVVFGAFDPKGGCSGTLMNLLQEERFNH
QAEVVSGVLEEECGGMLSAFFRELRKKKKAARKNLSE

Salmonella typhimurium (*S. typhimurium*) TadA:

MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNHRVIGEGWNRPIGR
HDPTAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAMVHSRIGRVVFGARDAKTGA
AGSLIDVLHHPGMNHRVEIIEGVLRDECATLLSDFFRMRRQEIKALKKADRAEGAGPAV

Shewanella putrefaciens (*S. putrefaciens*) TadA:

MDEYWMQVAMQMAEKAEAAGEVPVGAVLVKDGQQIATGYNLSISQHDPTAHAEILCLRSAGK
KLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGTVVNLLQHPAFNHQV
EVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE

Haemophilus influenzae F3031 (*H. influenzae*) TadA:

MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQSDPTAHA
EIIALRNGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVFGASDYKTGAIGSRFHF
FDDYKMNHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKALLKSLSDK

Caulobacter crescentus (*C. crescentus*) TadA:

MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAHDPTAHA
EIAAMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGRVVFGADDPKGGAVVHGPKF
FAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI

Geobacter sulfurreducens (*G. sulfurreducens*) TadA:

MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSNDPSAHA
EMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVVFGCYDPKGGAAGSLYDL
SADPRLNHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAKATPALFIDERKVPPEP

TadA*7.10

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

Additional TadA7.10 or TadA7.10 variants contemplated as a component of a heterodimer with a TadA*8 include:

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGE
GWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG
VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

TadA7.10 CP65

TAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGS
LMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTS
ESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDP

TadA7.10 CP83

YRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEY
WMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQN

TadA7.10 CP136

MNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTSESATPESSG
SEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMAL
RQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG

TadA7.10 C-truncate

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGE
GWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG
VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFN

TadA7.10 C-truncate 2

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGE
GWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG
VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQ

TadA7.10 delta59-66+C-truncate

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGE
GWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGA
AGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFN

TadA7.10 delta 59-66

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGE
GWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGA
AGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD.

In some embodiments, the adenosine deaminase variant comprises an alteration in TadA7.10. In some embodiments, TadA7.10 comprises an alteration at amino acid 82 or 166. In particular embodiments, a variant in the above-referenced sequence comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In other embodiments, the adenosine deaminase variant comprises a combination of alterations selected from the group consisting of Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y147T + Q154R; Y147T + Q154S; and Y123H + Y147R + Q154R + I76Y.

In other embodiments, the invention provides adenosine deaminase variants that include deletions, e.g., TadA7.10 comprising a deletion of the C terminus beginning at residue 149, 150, 151, 152, 153, 154, 155, 156, or 157. In other embodiments, the adenosine deaminase variant is a TadA monomer comprising one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, Q154R. In other embodiments, the adenosine deaminase variant is a monomer comprising the following alterations: Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y147T + Q154R; Y147T + Q154S; and Y123H + Y147 R + Q154R + I76Y. In still other embodiments, the adenosine deaminase variant is a homodimer comprising two adenosine deaminase domains each having one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, Q154R. In other embodiments, the adenosine deaminase variant is a heterodimer comprising a wild-type adenosine deaminase domain or a TadA7.10 domain and an adenosine deaminase variant domain comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, Q154R. In other embodiments, the adenosine deaminase variant is a heterodimer comprising a TadA7.10 domain and an adenosine deaminase variant of TadA7.10 comprising the following alterations: Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y147T + Q154R; Y147T + Q154S; and Y123H + Y147R + Q154R + I76Y.

"Administering" is referred to herein as providing one or more compositions described herein to a patient or a subject. By way of example and without limitation, composition administration, *e.g.*, injection, can be performed by intravenous (i.v.) injection, sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, or intramuscular

(i.m.) injection. One or more such routes can be employed. Parenteral administration can be, for example, by bolus injection or by gradual perfusion over time. In some embodiments, parenteral administration includes infusing or injecting intravascularly, intravenously, intramuscularly, intraarterially, intrathecally, intratumorally, intradermally, intraperitoneally, transtracheally, subcutaneously, subcuticularly, intraarticularly, subcapsularly, subarachnoidly and intrasternally. Alternatively, or concurrently, administration can be by the oral route.

By “agent” is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By “alteration” is meant a change (*e.g.* increase or decrease) in the structure, expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a change in a polynucleotide or polypeptide sequence or a change in expression levels, such as a 10% change, a 25% change, a 40% change, a 50% change, or greater.

By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By “analog” is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polynucleotide or polypeptide analog retains the biological activity of a corresponding naturally-occurring polynucleotide or polypeptide, while having certain modifications that enhance the analog's function relative to a naturally occurring polynucleotide or polypeptide. Such modifications could increase the analog's affinity for DNA, efficiency, specificity, protease or nuclease resistance, membrane permeability, and/or half-life, without altering, for example, ligand binding. An analog may include an unnatural nucleotide or amino acid.

By "base editor (BE)" or "nucleobase editor (NBE)" is meant an agent that binds a polynucleotide and has nucleobase modifying activity. In various embodiment, the base editor comprises a nucleobase modifying polypeptide (*e.g.*, a deaminase) and a nucleic acid programmable nucleotide binding domain in conjunction with a guide polynucleotide (*e.g.*, guide RNA). In various embodiments, the agent is a biomolecular complex comprising a protein domain having base editing activity, *i.e.*, a domain capable of modifying a base (*e.g.*, A, T, C, G, or U) within a nucleic acid molecule (*e.g.*, DNA). In some embodiments, the polynucleotide programmable DNA binding domain is fused or linked to a deaminase domain. In one embodiment, the agent is a fusion protein comprising a domain having base editing activity. In another embodiment, the protein domain having base editing activity is linked to

the guide RNA (*e.g.*, via an RNA binding motif on the guide RNA and an RNA binding domain fused to the deaminase). In some embodiments, the domain having base editing activity is capable of deaminating a base within a nucleic acid molecule. In some embodiments, the base editor is capable of deaminating one or more bases within a DNA molecule. In some embodiments, the base editor is capable of deaminating an adenosine (A) within DNA. In some embodiments, the base editor is an adenosine base editor (ABE).

By "cytidine deaminase" is meant a polypeptide or fragment thereof capable of catalyzing a deamination reaction that converts an amino group to a carbonyl group. In some embodiments the cytidine deaminase has at least about 85% identity to APOBEC or AID. In one embodiment, the cytidine deaminase converts cytosine to uracil or 5-methylcytosine to thymine. PmCDA1, which is derived from Petromyzon marinus (Petromyzon marinus cytosine deaminase 1, "PmCDA1"), AID (Activation-induced cytidine deaminase; AICDA), which is derived from a mammal (e.g., human, swine, bovine, horse, monkey etc.), and APOBEC are exemplary cytidine deaminases.

In some embodiments, the base editor is a reprogrammable base editor fused to a deaminase (*e.g.*, an adenosine deaminase or cytidine deaminase). In some embodiments, the base editor is a Cas9 fused to a deaminase (*e.g.*, an adenosine deaminase or cytidine deaminase). In some embodiments, the base editor is a nuclease-inactive Cas9 (dCas9) fused to a deaminase (*e.g.*, an adenosine deaminase or cytidine deaminase). In some embodiments, the Cas9 is a circular permutant Cas9 (*e.g.*, spCas9 or saCas9). Circular permutant Cas9s are known in the art and described, for example, in Oakes *et al.*, Cell 176, 254–267, 2019. In some embodiments, the base editor is fused to an inhibitor of base excision repair, for example, a UGI domain, or a dISN domain. In some embodiments, the fusion protein comprises a Cas9 nickase fused to a deaminase and an inhibitor of base excision repair, such as a UGI or dISN domain. In other embodiments, the base editor is an abasic base editor.

In some embodiments, the base editor is an adenosine base editor (ABE). In some embodiments, an adenosine deaminase is evolved from TadA. In some embodiments, the base editors of the present invention comprise a napDNAbp domain with an internally fused catalytic (e.g., deaminase) domain. In some embodiments, the napDNAbp is a Cas12a (Cpf1) with an internally fused deaminase domain. In some embodiments, the napDNAbp is a Cas12b (c2c1) with an internally fused deaminase domain. In some embodiments, the napDNAbp is a Cas12c (c2c3) with an internally fused deaminase domain. In some embodiments, the napDNAbp is a Cas12d (CasX) with an internally fused deaminase domain. In some embodiments, the napDNAbp is a Cas12e (CasY) with an internally fused deaminase domain.

In some embodiments, the napDNAbp is a Cas12g with an internally fused deaminase domain. In some embodiments, the napDNAbp is a Cas12h with an internally fused deaminase domain. In some embodiments, napDNAbp is a Cas12i with an internally fused deaminase domain. In some embodiments, the base editor is a catalytically dead Cas12 (dCas12) fused to a deaminase domain. In some embodiments, the base editor is a Cas12 nickase (nCas12) fused to a deaminase domain.

In some embodiments, base editors are generated (*e.g.*, ABE8) by cloning an adenosine deaminase variant (*e.g.*, TadA*8) into a scaffold that includes a circular permutant Cas9 (*e.g.*, spCAS9 or saCAS9) and a bipartite nuclear localization sequence. Circular permutant Cas9s are known in the art and described, for example, in Oakes *et al.*, Cell 176, 254–267, 2019. Exemplary circular permutants follow where the bold sequence indicates sequence derived from Cas9, the italics sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence.

CP5 (with MSP "NGC=Pam Variant with mutations Regular Cas9 likes NGG" PID=Protein Interacting Domain and "D10A" nickase):

EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRM
LASAKFLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYR
STKEVLDATLIHQSITGLYETRIDLSQLGGD*GGSGGSGGSGGSGGSGGSGGM***DKKYSIGLAI**
GTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYT
RRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTI
YHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE
ENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLA
EDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASM
IKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM
DGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILT
FRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYF
KKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM
IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF
MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK
PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQ

NGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKM
KNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNT
KYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPK
LESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

In some embodiments, the ABE8 is selected from a base editor from **Table 6-9, 13,** or **14** *infra*. In some embodiments, ABE8 contains an adenosine deaminase variant evolved from TadA. In some embodiments, the adenosine deaminase variant of ABE8 is a TadA*8 variant as described in **Table 7, 9, 13** or **14** *infra*. In some embodiments, the adenosine deaminase variant is TadA*7.10 variant (*e.g.* TadA*8) comprising one or more of an alteration selected from the group of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In various embodiments, ABE8 comprises TadA*7.10 variant (*e.g.* TadA*8) with a combination of alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In some embodiments ABE8 is a monomeric construct. In some embodiments, ABE8 is a heterodimeric construct. In some embodiments, the ABE8 base editor comprises the sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

In some embodiments, the polynucleotide programmable DNA binding domain is a CRISPR associated (*e.g.*, Cas or Cpf1) enzyme. In some embodiments, the base editor is a catalytically dead Cas9 (dCas9) fused to a deaminase domain. In some embodiments, the base editor is a Cas9 nickase (nCas9) fused to a deaminase domain. In some embodiments, the base editor is fused to an inhibitor of base excision repair (BER). In some embodiments, the inhibitor of base excision repair is a uracil DNA glycosylase inhibitor (UGI). In some embodiments, the inhibitor of base excision repair is an inosine base excision repair inhibitor.

Details of base editors are described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078) and PCT/US2016/058344 (WO 2017/070632), each of which is incorporated herein by reference for its entirety. Also see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of

A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1, the entire contents of which are hereby incorporated by reference.

By way of example, a cytidine base editor as used in the base editing compositions, systems and methods described herein has the following nucleic acid sequence (8877 base pairs), (Addgene, Watertown, MA.; Komor AC, et al., 2017, Sci Adv., 30;3(8):eaao4774. doi: 10.1126/sciadv.aao4774) as provided below. Polynucleotide sequences having at least 95% or greater identity to the BE4 nucleic acid sequence are also encompassed.

```
   1 atatgccaag tacgcccccc attgacgtca atgacggtaa atggcccgcc
tggcattatg
  61 cccagtacat gaccttatgg gactttccta cttggcagta catctacgta
ttagtcatcg
 121 ctattaccat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag
cggtttgact
 181 cacggggatt tccaagtctc caccccattg acgtcaatgg gagtttgttt
tggcaccaaa
 241 atcaacggga ctttccaaaa tgtcgtaaca actccgcccc attgacgcaa
atgggcggta
 301 ggcgtgtacg gtgggaggtc tatataagca gagctggttt agtgaaccgt
cagatccgct
 361 agagatccgc ggccgctaat acgactcact atagggagag ccgccaccat
gagctcagag
 421 actggcccag tggctgtgga ccccacattg agacggcgga tcgagcccca
tgagtttgag
 481 gtattcttcg atccgagaga gctccgcaag gagacctgcc tgctttacga
aattaattgg
 541 gggggccggc actccatttg gcgacataca tcacagaaca ctaacaagca
cgtcgaagtc
 601 aacttcatcg agaagttcac gacagaaaga tatttctgtc cgaacacaag
gtgcagcatt
 661 acctggtttc tcagctggag cccatgcggc gaatgtagta gggccatcac
tgaattcctg
```

721 tcaaggtatc cccacgtcac tctgtttatt tacatcgcaa ggctgtacca ccacgctgac
781 ccccgcaatc gacaaggcct gcgggatttg atctcttcag gtgtgactat ccaaattatg
841 actgagcagg agtcaggata ctgctggaga aactttgtga attatagccc gagtaatgaa
901 gcccactggc ctaggtatcc ccatctgtgg gtacgactgt acgttcttga actgtactgc
961 atcatactgg gcctgcctcc ttgtctcaac attctgagaa ggaagcagcc acagctgaca
1021 ttctttacca tcgctcttca gtcttgtcat taccagcgac tgcccccaca cattctctgg
1081 gccaccgggt tgaaatctgg tggttcttct ggtggttcta gcggcagcga gactcccggg
1141 acctcagagt ccgccacacc cgaaagttct ggtggttctt ctggtggttc tgataaaaag
1201 tattctattg gtttagccat cggcactaat tccgttggat gggctgtcat aaccgatgaa
1261 tacaaagtac cttcaaagaa atttaaggtg ttggggaaca cagaccgtca ttcgattaaa
1321 aagaatctta tcggtgccct cctattcgat agtggcgaaa cggcagaggc gactcgcctg
1381 aaacgaaccg ctcggagaag gtatacacgt cgcaagaacc gaatatgtta cttacaagaa
1441 atttttagca atgagatggc caaagttgac gattctttct ttcaccgttt ggaagagtcc
1501 ttccttgtcg aagaggacaa gaaacatgaa cggcacccca tctttggaaa catagtagat
1561 gaggtggcat atcatgaaaa gtacccaacg atttatcacc tcagaaaaaa gctagttgac
1621 tcaactgata aagcggacct gaggttaatc tacttggctc ttgcccatat gataaagttc
1681 cgtgggcact ttctcattga gggtgatcta aatccggaca actcggatgt cgacaaactg
1741 ttcatccagt tagtacaaac ctataatcag ttgtttgaag agaaccctat aaatgcaagt

1801 ggcgtggatg cgaaggctat tcttagcgcc cgcctctcta aatcccgacg gctagaaaac
1861 ctgatcgcac aattacccgg agagaagaaa aatgggttgt tcggtaacct tatagcgctc
1921 tcactaggcc tgacaccaaa ttttaagtcg aacttcgact tagctgaaga tgccaaattg
1981 cagcttagta aggacacgta cgatgacgat ctcgacaatc tactggcaca aattggagat
2041 cagtatgcgg acttattttt ggctgccaaa aaccttagcg atgcaatcct cctatctgac
2101 atactgagag ttaatactga gattaccaag gcgccgttat ccgcttcaat gatcaaaagg
2161 tacgatgaac atcaccaaga cttgacactt ctcaaggccc tagtccgtca gcaactgcct
2221 gagaaatata aggaaatatt ctttgatcag tcgaaaaacg ggtacgcagg ttatattgac
2281 ggcggagcga gtcaagagga attctacaag tttatcaaac ccatattaga gaagatggat
2341 gggacggaag agttgcttgt aaaactcaat cgcgaagatc tactgcgaaa gcagcggact
2401 ttcgacaacg gtagcattcc acatcaaatc cacttaggcg aattgcatgc tatacttaga
2461 aggcaggagg atttttatcc gttcctcaaa gacaatcgtg aaaagattga gaaaatccta
2521 acctttcgca taccttacta tgtgggaccc ctggcccgag ggaactctcg gttcgcatgg
2581 atgacaagaa agtccgaaga aacgattact ccatggaatt ttgaggaagt tgtcgataaa
2641 ggtgcgtcag ctcaatcgtt catcgagagg atgaccaact ttgacaagaa tttaccgaac
2701 gaaaaagtat tgcctaagca cagtttactt tacgagtatt tcacagtgta caatgaactc
2761 acgaaagtta agtatgtcac tgagggcatg cgtaaacccg cctttctaag cggagaacag
2821 aagaaagcaa tagtagatct gttattcaag accaaccgca aagtgacagt taagcaattg

2881 aaagaggact actttaagaa aattgaatgc ttcgattctg tcgagatctc cggggtagaa
2941 gatcgattta atgcgtcact tggtacgtat catgacctcc taaagataat taaagataag
3001 gacttcctgg ataacgaaga gaatgaagat atcttagaag atatagtgtt gactcttacc
3061 ctctttgaag atcgggaaat gattgaggaa agactaaaaa catacgctca cctgttcgac
3121 gataaggtta tgaaacagtt aaagaggcgt cgctatacgg gctggggacg attgtcgcgg
3181 aaacttatca acgggataag agacaagcaa agtggtaaaa ctattctcga ttttctaaag
3241 agcgacggct tcgccaatag gaactttatg cagctgatcc atgatgactc tttaaccttc
3301 aaagaggata tacaaaaggc acaggtttcc ggacaagggg actcattgca cgaacatatt
3361 gcgaatcttg ctggttcgcc agccatcaaa aagggcatac tccagacagt caaagtagtg
3421 gatgagctag ttaaggtcat gggacgtcac aaaccggaaa acattgtaat cgagatggca
3481 cgcgaaaatc aaacgactca gaaggggcaa aaaaacagtc gagagcggat gaagagaata
3541 gaagagggta ttaaagaact gggcagccag atcttaaagg agcatcctgt ggaaaatacc
3601 caattgcaga acgagaaact ttacctctat tacctacaaa atggaaggga catgtatgtt
3661 gatcaggaac tggacataaa ccgtttatct gattacgacg tcgatcacat tgtaccccaa
3721 tccttttga aggacgattc aatcgacaat aaagtgctta cacgctcgga taagaaccga
3781 gggaaaagtg acaatgttcc aagcgaggaa gtcgtaaaga aaatgaagaa ctattggcgg
3841 cagctcctaa atgcgaaact gataacgcaa agaaagttcg ataacttaac taaagctgag
3901 aggggtggct tgtctgaact tgacaaggcc ggatttatta aacgtcagct cgtggaaacc

3961 cgccaaatca caaagcatgt tgcacagata ctagattccc gaatgaatac gaaatacgac

4021 gagaacgata agctgattcg ggaagtcaaa gtaatcactt taaagtcaaa attggtgtcg

4081 gacttcagaa aggattttca attctataaa gttagggaga taaataacta ccaccatgcg

4141 cacgacgctt atcttaatgc cgtcgtaggg accgcactca ttaagaaata cccgaagcta

4201 gaaagtgagt ttgtgtatgg tgattacaaa gtttatgacg tccgtaagat gatcgcgaaa

4261 agcgaacagg agataggcaa ggctacagcc aaatacttct tttattctaa cattatgaat

4321 ttctttaaga cggaaatcac tctggcaaac ggagagatac gcaaacgacc tttaattgaa

4381 accaatgggg agacaggtga aatcgtatgg gataagggcc gggacttcgc gacggtgaga

4441 aaagttttgt ccatgcccca agtcaacata gtaaagaaaa ctgaggtgca gaccggaggg

4501 ttttcaaagg aatcgattct tccaaaaagg aatagtgata agctcatcgc tcgtaaaaag

4561 gactgggacc cgaaaaagta cggtggcttc gatagcccta cagttgccta ttctgtccta

4621 gtagtggcaa aagttgagaa gggaaaatcc aagaaactga agtcagtcaa agaattattg

4681 gggataacga ttatggagcg ctcgtctttt gaaaagaacc ccatcgactt ccttgaggcg

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4981 tatctcgacg aaatcataga gcaaatttcg gaattcagta agagagtcat cctagctgat

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5101 caggcggaaa atattatcca tttgtttact cttaccaacc tcggcgctcc agccgcattc

5161 aagtattttg acacaacgat agatcgcaaa cgatacactt ctaccaagga ggtgctagac

5221 gcgacactga ttcaccaatc catcacggga ttatatgaaa ctcggataga tttgtcacag

5281 cttgggggtg actctggtgg ttctggagga tctggtggtt ctactaatct gtcagatatt

5341 attgaaaagg agaccggtaa gcaactggtt atccaggaat ccatcctcat gctcccagag

5401 gaggtggaag aagtcattgg gaacaagccg gaaagcgata tactcgtgca caccgcctac

5461 gacgagagca ccgacgagaa tgtcatgctt ctgactagcg acgcccctga atacaagcct

5521 tgggctctgg tcatacagga tagcaacggt gagaacaaga ttaagatgct ctctggtggt

5581 tctggaggat ctggtggttc tactaatctg tcagatatta ttgaaaagga gaccggtaag

5641 caactggtta tccaggaatc catcctcatg ctcccagagg aggtggaaga agtcattggg

5701 aacaagccgg aaagcgatat actcgtgcac accgcctacg acgagagcac cgacgagaat

5761 gtcatgcttc tgactagcga cgcccctgaa tacaagcctt gggctctggt catacaggat

5821 agcaacggtg agaacaagat taagatgctc tctggtggtt ctcccaagaa gaagaggaaa

5881 gtctaaccgg tcatcatcac catcaccatt gagtttaaac ccgctgatca gcctcgactg

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6001 aaggtgccac tcccactgtc ctttcctaat aaaatgagga aattgcatcg cattgtctga

6061 gtaggtgtca ttctattctg gggggtgggg tggggcagga cagcaagggg gaggattggg

6121 aagacaatag caggcatgct ggggatgcgg tgggctctat ggcttctgag gcggaaagaa
6181 ccagctgggg ctcgataccg tcgacctcta gctagagctt ggcgtaatca tggtcatagc
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6361 cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga atcggccaac
6421 gcgcggggag aggcggtttg cgtattgggc gctcttccgc ttcctcgctc actgactcgc
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6541 tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg
6601 ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca taggctccgc ccccctgacg
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6721 accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc ctgccgctta
6781 ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat agctcacgct
6841 gtaggtatct cagttcggtg taggtcgttc gctccaagct gggctgtgtg cacgaacccc
6901 ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc aacccggtaa
6961 gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga gcgaggtatg
7021 taggcggtgc tacagagttc ttgaagtggt ggcctaacta cggctacact agaagaacag
7081 tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt
7141 gatccggcaa acaaaccacc gctggtagcg gtggtttttt tgtttgcaag cagcagatta

7201 cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg tctgacgctc
7261 agtggaacga aaactcacgt taagggattt tggtcatgag attatcaaaa aggatcttca
7321 cctagatcct tttaaattaa aaatgaagtt ttaaatcaat ctaaagtata tatgagtaaa
7381 cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg atctgtctat
7441 ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata cgggagggct
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7561 tatcagcaat aaaccagcca gccggaaggg ccgagcgcag aagtggtcct gcaactttat
7621 ccgcctccat ccagtctatt aattgttgcc gggaagctag agtaagtagt tcgccagtta
7681 atagtttgcg caacgttgtt gccattgcta caggcatcgt ggtgtcacgc tcgtcgtttg
7741 gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga tcccccatgt
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7861 cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc atgccatccg
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7981 ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca catagcagaa
8041 ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca aggatcttac
8101 cgctgttgag atccagttcg atgtaaccca ctcgtgcacc caactgatct tcagcatctt
8161 ttactttcac agcgtttct gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg
8221 gaataagggc gacacggaaa tgttgaatac tcatactctt cctttttcaa tattattgaa

8281 gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt
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8341 aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc acctgacgtc
gacggatcgg
8401 gagatcgatc tcccgatccc ctagggtcga ctctcagtac aatctgctct
gatgccgcat
8461 agttaagcca gtatctgctc cctgcttgtg tgttggaggt cgctgagtag
tgcgcgagca
8521 aaatttaagc tacaacaagg caaggcttga ccgacaattg catgaagaat
ctgcttaggg
8581 ttaggcgttt tgcgctgctt cgcgatgtac gggccagata tacgcgttga
cattgattat
8641 tgactagtta ttaatagtaa tcaattacgg ggtcattagt tcatagccca
tatatggagt
8701 tccgcgttac ataacttacg gtaaatggcc cgcctggctg accgcccaac
gacccccgcc
8761 cattgacgtc aataatgacg tatgttccca tagtaacgcc aatagggact
ttccattgac
8821 gtcaatgggt ggagtattta cggtaaactg cccacttggc agtacatcaa gtgtatc

BE4 amino acid sequence:

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHV
EVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCII
LGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI

RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDSGGSGGSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKP
ESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSGGSGGSTNLSD
IIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPW
ALVIQDSNGENKIKMLSGGSPKKKRK

By way of example, the adenine base editor (ABE) as used in the base editing compositions, systems and methods described herein has the nucleic acid sequence (8877 base pairs), (Addgene, Watertown, MA.; Gaudelli NM, *et al.*, Nature. 2017 Nov 23;551(7681):464-471. doi: 10.1038/nature24644; Koblan LW, *et al.*, Nat Biotechnol. 2018 Oct;36(9):843-846. doi: 10.1038/nbt.4172.) as provided below. Polynucleotide sequences having at least 95% or greater identity to the ABE nucleic acid sequence are also encompassed.

ATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTAC
AT
GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGC
GG
TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCAT
TG
ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCC
CC
ATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACC
GT
CAGATCCGCTAGAGATCCGCGGCCGCTAATACGACTCACTATAGGGAGAGCCGCCACCATGAAACGGA
CA
GCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCGGAAAGTCTCTGAAGTCGAGTTTAGCCACGA
GT

ATTGGATGAGGCACGCACTGACCCTGGCAAAGCGAGCATGGGATGAAAGAGAAGTCCCCGTGGGCGCC
GT
GCTGGTGCACAACAATAGAGTGATCGGAGAGGGATGGAACAGGCCAATCGGCCGCCACGACCCTACCG
CA
CACGCAGAGATCATGGCACTGAGGCAGGGAGGCCTGGTCATGCAGAATTACCGCCTGATCGATGCCAC
CC
TGTATGTGACACTGGAGCCATGCGTGATGTGCGCAGGAGCAATGATCCACAGCAGGATCGGAAGAGTG
GT
GTTCGGAGCACGGGACGCCAAGACCGGCGCAGCAGGCTCCCTGATGGATGTGCTGCACCACCCCGGCA
TG
AACCACCGGGTGGAGATCACAGAGGGAATCCTGGCAGACGAGTGCGCCGCCCTGCTGAGCGATTTCTT
TA
GAATGCGGAGACAGGAGATCAAGGCCCAGAAGAAGGCACAGAGCTCCACCGACTCTGGAGGATCTAGC
GG
AGGATCCTCTGGAAGCGAGACACCAGGCACAAGCGAGTCCGCCACACCAGAGAGCTCCGGCGGCTCCT
CC
GGAGGATCCTCTGAGGTGGAGTTTTCCCACGAGTACTGGATGAGACATGCCCTGACCCTGGCCAAGAG
GG
CACGCGATGAGAGGGAGGTGCCTGTGGGAGCCGTGCTGGTGCTGAACAATAGAGTGATCGGCGAGGGC
TG
GAACAGAGCCATCGGCCTGCACGACCCAACAGCCCATGCCGAAATTATGGCCCTGAGACAGGGCGGCC
TG
GTCATGCAGAACTACAGACTGATTGACGCCACCCTGTACGTGACATTCGAGCCTTGCGTGATGTGCGC
CG
GCGCCATGATCCACTCTAGGATCGGCCGCGTGGTGTTTGGCGTGAGGAACGCAAAAACCGGCGCCGCA
GG
CTCCCTGATGGACGTGCTGCACTACCCCGGCATGAATCACCGCGTCGAAATTACCGAGGGAATCCTGG
CA
GATGAATGTGCCGCCCTGCTGTGCTATTTCTTTCGGATGCCTAGACAGGTGTTCAATGCTCAGAAGAA
GG
CCCAGAGCTCCACCGACTCCGGAGGATCTAGCGGAGGCTCCTCTGGCTCTGAGACACCTGGCACAAGC
GA
GAGCGCAACACCTGAAAGCAGCGGGGGCAGCAGCGGGGGGTCAGACAAGAAGTACAGCATCGGCCTGG
CC
ATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAA
GG

TGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGC
GA
AACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCT
GC
TATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGA
GT
CCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTG
GC
CTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCG
AC
CTGCGGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGA
CC
TGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTC
GA
GGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCA
GA
CGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGC
CC
TGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTG
AG
CAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGT
TT
CTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCAC
CA
AGGCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAA
GC
TCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACG
CC
GGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGAT
GG
ACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGAC
AA
CGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTT
AC
CCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGG
CC

CTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGG
AA
CTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATA
AG
AACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGA
GC
TGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAG
GC
CATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCA
AG
AAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCAC
AT
ACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTG
GA
AGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATG
CC
CACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAG
CC
GGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGAC
GG
CTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGA
AA
GCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCAT
TA
AGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCC
GA
GAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGA
GA
ATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAA
CA
CCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAG
GA
ACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACG
AC
TCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGA
AG

AGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAG
TT
CGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGAC
AG
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CG
ACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTC
CG
GAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGA
AC
GCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTA
CA
AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTAC
TT
CTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGC
GG
CCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGT
GC
GGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGC
AA
AGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGA
AG
TACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAA
GT
CCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAG
AA
TCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTA
AG
TACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGG
AA
ACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAG
GG
CTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCA
TC
GAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGC
CT

ACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACC
AA
TCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCA
AA
GAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTC
TC
AGCTGGGAGGTGACTCTGGCGGCTCAAAAAGAACCGCCGACGGCAGCGAATTCGAGCCCAAGAAGAAG
AG
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TT
CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCC
AC
TGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGG
GT
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CT
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TA
ATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCG
GA
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GC
CCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGC
GG
TTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGC
GA
GCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAA
CA
TGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGG
CT
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AA
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AT
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TC

GGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCT
TA
TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGG
TA
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CACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTA
GC
TCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCG
CA
GAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACACTCAGTGGAACGAAAAC
TC
ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAAT
GA
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GG
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CT
CCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAAC
GT
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CC
CAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCC
GA
TCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTT
AC
TGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGT
GT
ATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTT
AA
AAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCC
AG

TTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGT
GA
GCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCAT
AC
TCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAA
TG
TATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCGACG
GA
TCGGGAGATCGATCTCCCGATCCCCTAGGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTT
AA
GCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACA
AC
AAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCG
AT
GTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTC
AT
TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCG
CC
CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
AT
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATC

By "base editing activity" is meant acting to chemically alter a base within a polynucleotide. In one embodiment, a first base is converted to a second base. In one embodiment, the base editing activity is cytidine deaminase activity, *e.g.*, converting target C•G to T•A. In another embodiment, the base editing activity is adenosine or adenine deaminase activity, *e.g.*, converting A•T to G•C. In another embodiment, the base editing activity is cytidine deaminase activity, *e.g.*, converting target C•G to T•A and adenosine or adenine deaminase activity, *e.g.*, converting A•T to G•C. In some embodiments, base editing activity is assessed by efficiency of editing. Base editing efficiency may be measured by any suitable means, for example, by sanger sequencing or next generation sequencing. In some embodiments, base editing efficiency is measured by percentage of total sequencing reads with nucleobase conversion effected by the base editor, for example, percentage of total sequencing reads with target A.T base pair converted to a G.C base pair. In some embodiments, base editing efficiency is measured by percentage of total cells with nucleobase conversion effected by the abse editor, when base editing is performed in a population of cells.

The term "base editor system" refers to a system for editing a nucleobase of a target nucleotide sequence. In various embodiments, the base editor system comprises (1) a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9); (2) a deaminase domain (*e.g.*, an adenosine deaminase or a cytidine deaminase) for deaminating said nucleobase; and (3) one or more guide polynucleotide (*e.g.*, guide RNA). In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the base editor is an adenine or adenosine base editor (ABE). In some embodiments, the base editor system is ABE8.

In some embodiments, a base editor system may comprise more than one base editing component. For example, a base editor system may include more than one deaminase. In some embodiments, a base editor system may include one or more adenosine deaminases. In some embodiments, a single guide polynucleotide may be utilized to target different deaminases to a target nucleic acid sequence. In some embodiments, a single pair of guide polynucleotides may be utilized to target different deaminases to a target nucleic acid sequence.

The deaminase domain and the polynucleotide programmable nucleotide binding component of a base editor system may be associated with each other covalently or non-covalently, or any combination of associations and interactions thereof. For example, in some embodiments, a deaminase domain can be targeted to a target nucleotide sequence by a polynucleotide programmable nucleotide binding domain. In some embodiments, a polynucleotide programmable nucleotide binding domain can be fused or linked to a deaminase domain. In some embodiments, a polynucleotide programmable nucleotide binding domain can target a deaminase domain to a target nucleotide sequence by non-covalently interacting with or associating with the deaminase domain. For example, in some embodiments, the deaminase domain can comprise an additional heterologous portion or domain that is capable of interacting with, associating with, or capable of forming a complex with an additional heterologous portion or domain that is part of a polynucleotide programmable nucleotide binding domain. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polypeptide. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a guide polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a polypeptide linker. In some embodiments, the additional heterologous portion

may be capable of binding to a polynucleotide linker. The additional heterologous portion may be a protein domain. In some embodiments, the additional heterologous portion may be a K Homology (KH) domain, a MS2 coat protein domain, a PP7 coat protein domain, a SfMu Com coat protein domain, a steril alpha motif, a telomerase Ku binding motif and Ku protein, a telomerase Sm7 binding motif and Sm7 protein, or an RNA recognition motif.

A base editor system may further comprise a guide polynucleotide component. It should be appreciated that components of the base editor system may be associated with each other via covalent bonds, noncovalent interactions, or any combination of associations and interactions thereof. In some embodiments, a deaminase domain can be targeted to a target nucleotide sequence by a guide polynucleotide. For example, in some embodiments, the deaminase domain can comprise an additional heterologous portion or domain (*e.g.*, polynucleotide binding domain such as an RNA or DNA binding protein) that is capable of interacting with, associating with, or capable of forming a complex with a portion or segment (*e.g.*, a polynucleotide motif) of a guide polynucleotide. In some embodiments, the additional heterologous portion or domain (*e.g.*, polynucleotide binding domain such as an RNA or DNA binding protein) can be fused or linked to the deaminase domain. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polypeptide. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a guide polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a polypeptide linker. In some embodiments, the additional heterologous portion may be capable of binding to a polynucleotide linker. The additional heterologous portion may be a protein domain. In some embodiments, the additional heterologous portion may be a K Homology (KH) domain, a MS2 coat protein domain, a PP7 coat protein domain, a SfMu Com coat protein domain, a sterile alpha motif, a telomerase Ku binding motif and Ku protein, a telomerase Sm7 binding motif and Sm7 protein, or an RNA recognition motif.

In some embodiments, a base editor system can further comprise an inhibitor of base excision repair (BER) component. It should be appreciated that components of the base editor system may be associated with each other via covalent bonds, noncovalent interactions, or any combination of associations and interactions thereof. The inhibitor of BER component may comprise a BER inhibitor. In some embodiments, the inhibitor of BER can be a uracil DNA glycosylase inhibitor (UGI). In some embodiments, the inhibitor of BER can be an inosine

BER inhibitor. In some embodiments, the inhibitor of BER can be targeted to the target nucleotide sequence by the polynucleotide programmable nucleotide binding domain. In some embodiments, a polynucleotide programmable nucleotide binding domain can be fused or linked to an inhibitor of BER. In some embodiments, a polynucleotide programmable nucleotide binding domain can be fused or linked to a deaminase domain and an inhibitor of BER. In some embodiments, a polynucleotide programmable nucleotide binding domain can target an inhibitor of BER to a target nucleotide sequence by non-covalently interacting with or associating with the inhibitor of BER. For example, in some embodiments, the inhibitor of BER component can comprise an additional heterologous portion or domain that is capable of interacting with, associating with, or capable of forming a complex with an additional heterologous portion or domain that is part of a polynucleotide programmable nucleotide binding domain.

In some embodiments, the inhibitor of BER can be targeted to the target nucleotide sequence by the guide polynucleotide. For example, in some embodiments, the inhibitor of BER can comprise an additional heterologous portion or domain (*e.g.*, polynucleotide binding domain such as an RNA or DNA binding protein) that is capable of interacting with, associating with, or capable of forming a complex with a portion or segment (*e.g.*, a polynucleotide motif) of a guide polynucleotide. In some embodiments, the additional heterologous portion or domain of the guide polynucleotide (*e.g.*, polynucleotide binding domain such as an RNA or DNA binding protein) can be fused or linked to the inhibitor of BER. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a guide polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a polypeptide linker. In some embodiments, the additional heterologous portion may be capable of binding to a polynucleotide linker. The additional heterologous portion may be a protein domain. In some embodiments, the additional heterologous portion may be a K Homology (KH) domain, a MS2 coat protein domain, a PP7 coat protein domain, a SfMu Com coat protein domain, a sterile alpha motif, a telomerase Ku binding motif and Ku protein, a telomerase Sm7 binding motif and Sm7 protein, or an RNA recognition motif.

The term "Cas9" or "Cas9 domain" refers to an RNA guided nuclease comprising a Cas9 protein, or a fragment thereof (*e.g.*, a protein comprising an active, inactive, or partially active DNA cleavage domain of Cas9, and/or the gRNA binding domain of Cas9). A Cas9 nuclease is also referred to sometimes as a Casnl nuclease or a CRISPR (clustered regularly

interspaced short palindromic repeat) associated nuclease. CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. See, *e.g.*, Jinek M., *et al.*, *Science* 337:816-821(2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. Cas9 nuclease sequences and structures are well known to those of skill in the art (see, *e.g.*, "Complete genome sequence of an M1 strain of Streptococcus pyogenes." Ferretti *et al.*, Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., *et al.*, Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., *et al.*, *Science* 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference.

An exemplary Cas9, is *Streptococcus pyogenes* Cas9 (spCas9), the amino acid sequence of which is provided below:

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI

QLVQIYNQLFEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNS
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDRGMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGHSLHEQIANLAGSPAIKKGILQTVKIV
DELVKVMGHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFIKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

(single underline: HNH domain; double underline: RuvC domain)

A nuclease-inactivated Cas9 protein may interchangeably be referred to as a "dCas9" protein (for nuclease-"dead" Cas9) or catalytically inactive Cas9. Methods for generating a Cas9 protein (or a fragment thereof) having an inactive DNA cleavage domain are known (See, *e.g.*, Jinek *et al., Science.* 337:816-821(2012); Qi *et al.,* "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression" (2013) *Cell.* 28;152(5):1173-83, the entire contents of each of which are incorporated herein by reference). For example, the DNA cleavage domain of Cas9 is known to include two subdomains, the HNH nuclease subdomain and the RuvC1 subdomain. The HNH subdomain cleaves the strand complementary to the gRNA, whereas the RuvC1 subdomain cleaves the non-complementary strand. Mutations within these subdomains can silence the nuclease activity of Cas9. For example, the mutations D10A and H840A completely inactivate the nuclease activity of *S. pyogenes* Cas9 (Jinek *et al., Science.* 337:816-821(2012); Qi *et al., Cell.* 28;152(5):1173-83 (2013)). In some embodiments, a Cas9 nuclease has an inactive

(*e.g.*, an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase, referred to as an "nCas9" protein (for "nickase" Cas9). In some embodiments, proteins comprising fragments of Cas9 are provided. For example, in some embodiments, a protein comprises one of two Cas9 domains: (1) the gRNA binding domain of Cas9; or (2) the DNA cleavage domain of Cas9. In some embodiments, proteins comprising Cas9 or fragments thereof are referred to as "Cas9 variants." A Cas9 variant shares homology to Cas9, or a fragment thereof. For example, a Cas9 variant is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to wild-type Cas9. In some embodiments, the Cas9 variant may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more amino acid changes compared to wild-type Cas9. In some embodiments, the Cas9 variant comprises a fragment of Cas9 (*e.g.*, a gRNA binding domain or a DNA-cleavage domain), such that the fragment is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of wild-type Cas9. In some embodiments, the fragment is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild-type Cas9.

In some embodiments, the fragment is at least 100 amino acids in length. In some embodiments, the fragment is at least 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, or at least 1300 amino acids in length.

In some embodiments, wild-type Cas9 corresponds to Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_017053.1, nucleotide and amino acid sequences as follows).

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGAT
CACTGATGATTATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACA
GTATCAAAAAAAATCTTATAGGGGCTCTTTTATTTGGCAGTGGAGAGACAGCGGAAGCGACT
CGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACA

GGAGATTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGT
CTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGAT
GAAGTTGCTTATCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGCAGATTC
TACTGATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTG
GTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAAACTATTTATC
CAGTTGGTACAAATCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTAGAGTAGA
TGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTC
AGCTCCCCGGTGAGAAGAGAAATGGCTTGTTTGGGAATCTCATTGCTTTGTCATTGGGATTG
ACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGA
TACTTACGATGATGATTTAGATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGT
TTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATAGT
GAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAGCGCTACGATGAACATCATCAAGA
CTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTATAAAGAAATCTTTT
TTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCCAAGAAGAATTT
TATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACT
AAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCATCAAA
TTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAA
GACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATT
GGCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCAT
GGAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACA
AACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTA
TTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTACTGAGGGAATGCGAAAACCAG
CATTTCTTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAA
GTAACCGTTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGA
AATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAGGCGCCTACCATGATTTGCTAAAAA
TTATTAAAGATAAAGATTTTTTGGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTT
TTAACATTGACCTTATTTGAAGATAGGGGGATGATTGAGGAAAGACTTAAAACATATGCTCA
CCTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTGGTTGGGGACGTT
TGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAATATTAGATTTT
TTGAAATCAGATGGTTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATAGTTTGAC
ATTTAAAGAAGATATTCAAAAAGCACAGGTGTCTGGACAAGGCCATAGTTTACATGAACAGA
TTGCTAACTTAGCTGGCAGTCCTGCTATTAAAAAAGGTATTTTACAGACTGTAAAAATTGTT
GATGAACTGGTCAAAGTAATGGGGCATAAGCCAGAAAATATCGTTATTGAAATGGCACGTGA
AAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAGAAG

GTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTCAATTGCAA
AATGAAAAGCTCTATCTCTATTATCTACAAAATGGAAGAGACATGTATGTGGACCAAGAATT
AGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTTCATTAAAG
ACGATTCAATAGACAATAAGGTACTAACGCGTTCTGATAAAAATCGTGGTAAATCGGATAAC
GTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGCCAA
GTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTGAAC
TTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTG
GCACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCGAGA
GGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAATTCT
ATAAAGTACGTGAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTCGTT
GGAACTGCTTTGATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAA
AGTTTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAA
AATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAATGGA
GAGATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGATAA
AGGGCGAGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGA
AAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCGGAC
AAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATATGGTGGTTTTGATAGTCCAAC
GGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAAAAT
CCGTTAAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAAATCCGATT
GACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGACTTAATCATTAAACTACCTAA
ATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAATTAC
AAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGTCAT
TATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTTGTGGAGCAGCA
TAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGTGTTATTTTAG
CAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAACCAATACGT
GAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGCTTT
TAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAGATG
CCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTA
GGAGGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQIYNQLFEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGL

TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNS
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDRGMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGHSLHEQIANLAGSPAIKKGILQTVKIV
DELVKVMGHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFIKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

(single underline: HNH domain; double underline: RuvC domain)

In some embodiments, wild-type Cas9 corresponds to, or comprises the following nucleotide and/or amino acid sequences:

ATGGATAAAAAGTATTCTATTGGTTTAGACATCGGCACTAATTCCGTTGGATGGGCTGTCAT
AACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTCATT
CGATTAAAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGGCGACT
CGCCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTTACTTACA
AGAAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTTGGAAGAGT
CCTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATCTTTGGAAACATAGTAGAT
GAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTCAGAAAAAAGCTAGTTGACTC
AACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTGCCCATATGATAAAGTTCCGTG
GGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTCGGATGTCGACAAACTGTTCATC
CAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCTATAAATGCAAGTGGCGTGGA
TGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAATCCCGACGGCTAGAAAACCTGATCGCAC
AATTACCCGGAGAGAAGAAAAATGGGTTGTTCGGTAACCTTATAGCGCTCTCACTAGGCCTG

ACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAAGATGCCAAATTGCAGCTTAGTAAGGA
CACGTACGATGACGATCTCGACAATCTACTGGCACAAATTGGAGATCAGTATGCGGACTTAT
TTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCCTATCTGACATACTGAGAGTTAATACT
GAGATTACCAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGTACGATGAACATCACCAAGA
CTTGACACTTCTCAAGGCCCTAGTCCGTCAGCAACTGCCTGAGAAATATAAGGAAATATTCT
TTGATCAGTCGAAAAACGGGTACGCAGGTTATATTGACGGCGGAGCGAGTCAAGAGGAATTC
TACAAGTTTATCAAACCCATATTAGAGAAGATGGATGGGACGGAAGAGTTGCTTGTAAAACT
CAATCGCGAAGATCTACTGCGAAAGCAGCGGACTTTCGACAACGGTAGCATTCCACATCAAA
TCCACTTAGGCGAATTGCATGCTATACTTAGAAGGCAGGAGGATTTTTATCCGTTCCTCAAA
GACAATCGTGAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTACTATGTGGGACCCCT
GGCCCGAGGGAACTCTCGGTTCGCATGGATGACAAGAAAGTCCGAAGAAACGATTACTCCAT
GGAATTTTGAGGAAGTTGTCGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACC
AACTTTGACAAGAATTTACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTA
TTTCACAGTGTACAATGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCG
CCTTTCTAAGCGGAGAACAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAA
GTGACAGTTAAGCAATTGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGA
GATCTCCGGGGTAGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGA
TAATTAAAGATAAGGACTTCCTGGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTG
TTGACTCTTACCCTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCA
CCTGTTCGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGAT
TGTCGCGGAAACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATTTT
CTAAAGAGCGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTTAAC
CTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACGAACATA
TTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCAAAGTAGTG
GATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACATTGTAATCGAGATGGCACG
CGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGGATGAAGAGAATAGAAG
AGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGGAAAATACCCAATTG
CAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACATGTATGTTGATCAGGA
ACTGGACATAAACCGTTTATCTGATTACGACGTCGATCACATTGTACCCCAATCCTTTTTGA
AGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAAGAACCGAGGGAAAAGTGAC
AATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACTATTGGCGGCAGCTCCTAAATGC
GAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCTG
AACTTGACAAGGCCGGATTTATTAAACGTCAGCTCGTGGAAACCCGCCAAATCACAAAGCAT
GTTGCACAGATACTAGATTCCCGAATGAATACGAAATACGACGAGAACGATAAGCTGATTCG

GGAAGTCAAAGTAATCACTTTAAAGTCAAAATTGGTGTCGGACTTCAGAAAGGATTTTCAAT
TCTATAAAGTTAGGGAGATAAATAACTACCACCATGCGCACGACGCTTATCTTAATGCCGTC
GTAGGGACCGCACTCATTAAGAAATACCCGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTA
CAAAGTTTATGACGTCCGTAAGATGATCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAG
CCAAATACTTCTTTTATTCTAACATTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAAC
GGAGAGATACGCAAACGACCTTTAATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGA
TAAGGGCCGGGACTTCGCGACGGTGAGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAA
AGAAAACTGAGGTGCAGACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGT
GATAAGCTCATCGCTCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCC
TACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGA
AGTCAGTCAAAGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCC
ATCGACTTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACC
AAAGTATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGC
TTCAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCC
CATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAGCA
GCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTCATCC
TAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAACCCATA
CGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGCCGC
ATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAG
ACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGATTTGTCACAG
CTTGGGGGTGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAAGACCATGA
CGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGGCTGCAGGA

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF

LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

(single underline: HNH domain; double underline: RuvC domain)

In some embodiments, wild-type Cas9 corresponds to Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_002737.2 (nucleotide sequence as follows); and Uniprot Reference Sequence: Q99ZW2 (amino acid sequence as follows).

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGAT
CACTGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACA
GTATCAAAAAAAATCTTATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGACT
CGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACA
GGAGATTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGT
CTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGAT
GAAGTTGCTTATCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGTAGATTC
TACTGATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTG
GTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAAACTATTTATC
CAGTTGGTACAAACCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGA
TGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTC
AGCTCCCCGGTGAGAAGAAAAATGGCTTATTTGGGAATCTCATTGCTTTGTCATTGGGTTTG
ACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGA
TACTTACGATGATGATTTAGATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGT
TTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATACT
GAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCATCAAGA
CTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTATAAAGAAATCTTTT
TTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCCAAGAAGAATTT

TATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACT
AAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCATCAAA
TTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAA
GACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATT
GGCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCAT
GGAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACA
AACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTA
TTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTACTGAAGGAATGCGAAAACCAG
CATTTCTTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAA
GTAACCGTTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGA
AATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAGGTACCTACCATGATTTGCTAAAAA
TTATTAAAGATAAAGATTTTTTGGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTT
TTAACATTGACCTTATTTGAAGATAGGGAGATGATTGAGGAAAGACTTAAAACATATGCTCA
CCTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTGGTTGGGGACGTT
TGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAATATTAGATTTT
TTGAAATCAGATGGTTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATAGTTTGAC
ATTTAAAGAAGACATTCAAAAAGCACAAGTGTCTGGACAAGGCGATAGTTTACATGAACATA
TTGCAAATTTAGCTGGTAGCCCTGCTATTAAAAAAGGTATTTTACAGACTGTAAAAGTTGTT
GATGAATTGGTCAAAGTAATGGGGCGGCATAAGCCAGAAAATATCGTTATTGAAATGGCACG
TGAAAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAG
AAGGTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTCAATTG
CAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAAGAGACATGTATGTGGACCAAGA
ATTAGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTTCCTTA
AAGACGATTCAATAGACAATAAGGTCTTAACGCGTTCTGATAAAAATCGTGGTAAATCGGAT
AACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGC
CAAGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTG
AACTTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCAT
GTGGCACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCG
AGAGGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAAT
TCTATAAAGTACGTGAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTC
GTTGGAACTGCTTTGATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTA
TAAAGTTTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCG
CAAAATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAAT
GGAGAGATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGA

TAAAGGGCGAGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCA
AGAAAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCG
GACAAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATATGGTGGTTTTGATAGTCC
AACGGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAA
AATCCGTTAAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAAATCCG
ATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGACTTAATCATTAAACTACC
TAAATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAAT
TACAAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGT
CATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTTGTGGAGCA
GCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGTGTTATTT
TAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAACCAATA
CGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGC
TTTTAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAG
ATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAG
CTAGGAGGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS

DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD (SEQ ID NO: 1)

(single underline: HNH domain; double underline: RuvC domain)

In some embodiments, Cas9 refers to Cas9 from: *Corynebacterium ulcerans* (NCBI Refs: NC_015683.1, NC_017317.1); *Corynebacterium diphtheria* (NCBI Refs: NC_016782.1, NC_016786.1); *Spiroplasma syrphidicola* (NCBI Ref: NC_021284.1); *Prevotella intermedia* (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref: NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus torquisI* (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1), *Listeria innocua* (NCBI Ref: NP_472073.1), *Campylobacter jejuni* (NCBI Ref: YP_002344900.1) or *Neisseria meningitidis* (NCBI Ref: YP_002342100.1) or to a Cas9 from any other organism.

In some embodiments, dCas9 corresponds to, or comprises in part or in whole, a Cas9 amino acid sequence having one or more mutations that inactivate the Cas9 nuclease activity. For example, in some embodiments, a dCas9 domain comprises D10A and an H840A mutation as numbered in SEQ ID NO: 1 or corresponding mutations in another Cas9. In some embodiments, the dCas9 comprises the amino acid sequence of dCas9 (D10A and H840A):

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV

DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

(single underline: HNH domain; double underline: RuvC domain).

In some embodiments, the Cas9 domain comprises a D10A mutation, while the residue at position 840 remains a histidine in the amino acid sequence provided above, or at corresponding positions in any of the amino acid sequences provided herein.

In other embodiments, dCas9 variants having mutations other than D10A and H840A are provided, which, *e.g.*, result in nuclease inactivated Cas9 (dCas9). Such mutations, by way of example, include other amino acid substitutions at D10 and H840, or other substitutions within the nuclease domains of Cas9 (*e.g.*, substitutions in the HNH nuclease subdomain and/or the RuvC1 subdomain). In some embodiments, variants or homologues of dCas9 are provided which are at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical. In some embodiments, variants of dCas9 are provided having amino acid sequences which are shorter, or longer, by about 5 amino acids, by about 10 amino acids, by about 15 amino acids, by about 20 amino acids, by about 25 amino acids, by about 30 amino acids, by about 40 amino acids, by about 50 amino acids, by about 75 amino acids, by about 100 amino acids or more.

In some embodiments, Cas9 fusion proteins as provided herein comprise the full-length amino acid sequence of a Cas9 protein, *e.g.*, one of the Cas9 sequences provided herein. In other embodiments, however, fusion proteins as provided herein do not comprise a full-length Cas9 sequence, but only one or more fragments thereof. Exemplary amino acid sequences of suitable Cas9 domains and Cas9 fragments are provided herein, and additional suitable sequences of Cas9 domains and fragments will be apparent to those of skill in the art.

It should be appreciated that additional Cas9 proteins (*e.g.*, a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9), including variants and homologs thereof, are within the scope of this disclosure. Exemplary Cas9 proteins include, without limitation, those provided below. In some embodiments, the Cas9 protein is a nuclease dead Cas9 (dCas9). In some embodiments, the Cas9 protein is a Cas9 nickase (nCas9). In some embodiments, the Cas9 protein is a nuclease active Cas9.

Exemplary catalytically inactive Cas9 (dCas9):

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR
LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDE
VAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQ
LVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLT
PNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTE
ITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFY
KFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD
NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTN
FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKV
TVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVL
TLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFL
KSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVD
ELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

Exemplary catalytically Cas9 nickase (nCas9):

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR
LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDE

VAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQ
LVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLT
PNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTE
ITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFY
KFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD
NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTN
FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKV
TVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVL
TLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFL
KSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVD
ELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

Exemplary catalytically active Cas9:

DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR
LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDE
VAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQ
LVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLT
PNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTE
ITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFY
KFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD
NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTN
FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKV
TVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVL
TLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFL

KSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVD
ELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD.

In some embodiments, Cas9 refers to a Cas9 from archaea (*e.g.*, nanoarchaea), which constitute a domain and kingdom of single-celled prokaryotic microbes. In some embodiments, Cas9 refers to CasX or CasY, which have been described in, for example, Burstein *et al.*, "New CRISPR-Cas systems from uncultivated microbes." Cell Res. 2017 Feb 21. doi: 10.1038/cr.2017.21, the entire contents of which is hereby incorporated by reference. Using genome-resolved metagenomics, a number of CRISPR-Cas systems were identified, including the first reported Cas9 in the archaeal domain of life. This divergent Cas9 protein was found in little- studied nanoarchaea as part of an active CRISPR-Cas system. In bacteria, two previously unknown systems were discovered, CRISPR-CasX and CRISPR-CasY, which are among the most compact systems yet discovered. In some embodiments, Cas9 refers to CasX, or a variant of CasX. In some embodiments, Cas9 refers to a CasY, or a variant of CasY. It should be appreciated that other RNA-guided DNA binding proteins may be used as a nucleic acid programmable DNA binding protein (napDNAbp), and are within the scope of this disclosure.

In particular embodiments, napDNAbps useful in the methods of the invention include circular permutants, which are known in the art and described, for example, by Oakes *et al.*, Cell 176, 254–267, 2019. An exemplary circular permutant follows where the bold sequence indicates sequence derived from Cas9, the italics sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence, **CP5** (with MSP "NGC=Pam Variant with mutations Regular Cas9 likes NGG" PID=Protein Interacting Domain and "D10A" nickase):

EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRM
LASAKFLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYR
STKEVLDATLIHQSITGLYETRIDLSQLGGD*GGSGGSGGSGGSGGSGGSGGM*DKKYSIGLAI
GTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYT
RRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTI
YHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE
ENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLA
EDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASM
IKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM
DGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILT
FRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYF
KKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM
IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF
MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK
PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQ
NGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKM
KNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNT
KYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPK
LESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

Non-limiting examples of a polynucleotide programmable nucleotide binding domain which can be incorporated into a base editor include a CRISPR protein-derived domain, a restriction nuclease, a meganuclease, TAL nuclease (TALEN), and a zinc finger nuclease (ZFN).

In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) of any of the fusion proteins provided herein may be a CasX or CasY protein. In some embodiments, the napDNAbp is a CasX protein. In some embodiments, the napDNAbp is a CasY protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a naturally-occurring CasX or CasY protein. In some embodiments, the

napDNAbp is a naturally-occurring CasX or CasY protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to any CasX or CasY protein described herein. It should be appreciated that Cas12b/C2c1, CasX and CasY from other bacterial species may also be used in accordance with the present disclosure.

Cas12b/C2c1 (uniprot.org/uniprot/T0D7A2#2)
sp|T0D7A2|C2C1_ALIAG CRISPR-associated endo- nuclease C2c1 OS = *Alicyclobacillus acido- terrestris* (strain ATCC 49025 / DSM 3922/ CIP 106132 / NCIMB 13137/GD3B) GN=c2c1 PE=1 SV=1

MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYRRSPNGDGEQECD
KTAEECKAELLERLRARQVENGHRGPAGSDDELLQLARQLYELLVPQAIGAKGDAQQIARKF
LSPLADKDAVGGLGIAKAGNKPRWVRMREAGEPGWEEEKEKAETRKSADRTADVLRALADFG
LKPLMRVYTDSEMSSVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQEYAKLVEQ
KNRFEQKNFVGQEHLVHLVNQLQQDMKEASPGLESKEQTAHYVTGRALRGSDKVFEKWGKLA
PDAPFDLYDAEIKNVQRRNTRRFGSHDLFAKLAEPEYQALWREDASFLTRYAVYNSILRKLN
HAKMFATFTLPDATAHPIWTRFDKLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREV
DDVTVPISMSEQLDNLLPRDPNEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRRG
ARDVYLNVSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSDYLAEHPDDGKLGSE
GLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNSKGRVPFFFPIKGNDNLVAVHERSQLL
KLPGETESKDLRAIREERQRTLRQLRTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPVDAAN
HMTPDWREAFENELQKLKSLHGICSDKEWMDAVYESVRRVWRHMGKQVRDWRKDVRSGERPK
IRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRFAITLREHIDHAKE
DRLKKLADRIIMEALGYVYALDERGKGKWVAKYPPCQLILLEELSEYQFNNDRPPSENNQLM
QWSHRGVFQELINQAQVHDLLVGTMYAAFSSRFDARTGAPGIRCRRVPARCTQEHNPEPFPW
WLNKFVVEHTLDACPLRADDLIPTGEGEIFVSPFSAEEGDFHQIHADLNAAQNLQQRLWSDF
DISQIRLRCDWGEVDGELVLIPRLTGKRTADSYSNKVFYTNTGVTYYERERGKKRRKVFAQE
KLSEEEAELLVEADEAREKSVVLMRDPSGIINRGNWTRQKEFWSMV NQRIEGYLVKQIRSR
VPLQDSACENTGDI

CasX (uniprot.org/uniprot/F0NN87; uniprot.org/uniprot/F0NH53)
>tr|F0NN87|F0NN87_SULIH CRISPR-associated Casx protein OS = *Sulfolobus islandicus* (strain HVE10/4) GN = SiH_0402 PE=4 SV=1

MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGK
AKKKKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAP
SFVKPEFYEFGRSPGMVERTRRVKLEVEPHYLIIAAAGWVLTRLGKAKVSEGDYVGVNVFTP
TRGILYSLIQNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVRIYTISDAVGQNPTTIN
GGFSIDLTKLLEKRYLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTG SKRLEDLLY
FANRDLIMNLNSDDGKVRDLKLISAYVNGELIRGEG

>tr|F0NH53|F0NH53_SULIR CRISPR associated protein, Casx OS = *Sulfolobus islandicus* (strain REY15A) GN=SiRe_0771 PE=4 SV=1

MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGK
AKKKKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAP
SFVKPEFYKFGRSPGMVERTRRVKLEVEPHYLIMAAAGWVLTRLGKAKVSEGDYVGVNVFTP
TRGILYSLIQNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVSIYTISDAVGQNPTTIN
GGFSIDLTKLLEKRDLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTGSKRLEDLLYF
ANRDLIMNLNSDDGKVRDLKLISAYVNGELIRGEG

Deltaproteobacteria CasX

MEKRINKIRKKLSADNATKPVSRSGPMKTLLVRVMTDDLKKRLEKRRKKPEVMPQVISNNAA
NNLRMLLDDYTKMKEAILQVYWQEFKDDHVGLMCKFAQPASKKIDQNKLKPEMDEKGNLTTA
GFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPVKDSDEAVTYSLG
KFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGTIASFLSKYQDIII
EHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDAYNEVIARVRMWVNLNLWQ
KLKLSRDDAKPLLRLKGFPSFPVVERRENEVDWWNTINEVKKLIDAKRDMGRVFWSGVTAEK
RNTILEGYNYLPNENDHKKREGSLENPKKPAKRQFGDLLLYLEKKYAGDWGKVFDEAWERID
KKIAGLTSHIEREEARNAEDAQSKAVLTDWLRAKASFVLERLKEMDEKEFYACEIQLQKWYG
DLRGNPFAVEAENRVVDISGFSIGSDGHSIQYRNLLAWKYLENGKREFYLLMNYGKKGRIRF
TDGTDIKKSGKWQGLLYGGGKAKVIDLTFDPDDEQLIILPLAFGTRQGREFIWNDLLSLETG
LIKLANGRVIEKTIYNKKIGRDEPALFVALTFERREVVDPSNIKPVNLIGVARGENIPAVIA
LTDPEGCPLPEFKDSSGGPTDILRIGEGYKEKQRAIQAAKEVEQRRAGGYSRKFASKSRNLA
DDMVRNSARDLFYHAVTHDAVLVFANLSRGFGRQGKRTFMTERQYTKMEDWLTAKLAYEGLT
SKTYLSKTLAQYTSKTCSNCGFTITYADMDVMLVRLKKTSDGWATTLNNKELKAEYQITYYN
RYKRQTVEKELSAELDRLSEESGNNDISKWTKGRRDEALFLLKKRFSHRPVQEQFVCLDCGH
EVHAAEQAALNIARSWLFLNSNSTEFKSYKSGKQPFVGAWQAFYKRRLKEVWKPNA

CasY (ncbi.nlm.nih.gov/protein/APG80656.1)

>APG80656.1 CRISPR-associated protein CasY [uncultured Parcubacteria group bacterium]

MSKRHPRISGVKGYRLHAQRLEYTGKSGAMRTIKYPLYSSPSGGRTVPREIVSAINDDYVGL
YGLSNFDDLYNAEKRNEEKVYSVLDFWYDCVQYGAVFSYTAPGLLKNVAEVRGGSYELTKTL
KGSHLYDELQIDKVIKFLNKKEISRANGSLDKLKKDIIDCFKAEYRERHKDQCNKLADDIKN
AKKDAGASLGERQKKLFRDFFGISEQSENDKPSFTNPLNLTCCLLPFDTVNNNRNRGEVLFN
KLKEYAQKLDKNEGSLEMWEYIGIGNSGTAFSNFLGEGFLGRLRENKITELKKAMMDITDAW
RGQEQEEELEKRLRILAALTIKLREPKFDNHWGGYRSDINGKLSSWLQNYINQTVKIKEDLK
GHKKDLKKAKEMINRFGESDTKEEAVVSSLLESIEKIVPDDSADDEKPDIPAIAIYRRFLSD
GRLTLNRFVQREDVQEALIKERLEAEKKKKPKKRKKKSDAEDEKETIDFKELFPHLAKPLKL
VPNFYGDSKRELYKKYKNAAIYTDALWKAVEKIYKSAFSSSLKNSFFDTDFDKDFFIKRLQK
IFSVYRRFNTDKWKPIVKNSFAPYCDIVSLAENEVLYKPKQSRSRKSAAIDKNRVRLPSTEN
IAKAGIALARELSVAGFDWKDLLKKEEHEEYIDLIELHKTALALLLAVTETQLDISALDFVE
NGTVKDFMKTRDGNLVLEGRFLEMFSQSIVFSELRGLAGLMSRKEFITRSAIQTMNGKQAEL
LYIPHEFQSAKITTPKEMSRAFLDLAPAEFATSLEPESLSEKSLLKLKQMRYYPHYFGYELT
RTGQGIDGGVAENALRLEKSPVKKREIKCKQYKTLGRGQNKIVLYVRSSYYQTQFLEWFLHR
PKNVQTDVAVSGSFLIDEKKVKTRWNYDALTVALEPVSGSERVFVSQPFTIFPEKSAEEEGQ
RYLGIDIGEYGIAYTALEITGDSAKILDQNFISDPQLKTLREEVKGLKLDQRRGTFAMPSTK
IARIRESLVHSLRNRIHHLALKHKAKIVYELEVSRFEEGKQKIKKVYATLKKADVYSEIDAD
KNLQTTVWGKLAVASEISASYTSQFCGACKKLWRAEMQVDETITTQELIGTVRVIKGGTLID
AIKDFMRPPIFDENDTPFPKYRDFCDKHHISKKMRGNSCLFICPFCRANADADIQASQTIAL
LRYVKEEKKVEDYFERFRKLKNIKVLGQMKKI

The term “Cas12” or “Cas12 domain” refers to an RNA guided nuclease comprising a Cas12 protein or a fragment thereof (e.g., a protein comprising an active, inactive, or partially active DNA cleavage domain of Cas12, and/or the gRNA binding domain of Cas12). Cas12 belongs to the class 2, Type V CRISPR/Cas system. A Cas12 nuclease is also referred to sometimes as a CRISPR (clustered regularly interspaced short palindromic repeat) associated nuclease. The sequence of an exemplary Bacillus hisashii Cas 12b (BhCas12b) Cas 12 domain is provided below:

MAPKKKRKVGIHGVPAAATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAI
YEHHEQDPKNPKKVSKAEIQAELWDFVLKMQKCNSFTHEVDKDEVFNILRELYEELVPSSVE
KKGEANQLSNKFLYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEEEKKKWEEDKKKDP
LAKILGKLAEYGLIPLFIPYTDSNEPIVKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWES

WNLKVKEEYEKVEKEYKTLEERIKEDIQALKALEQYEKERQEQLLRDTLNTNEYRLSKRGLR
GWREIIQKWLKMDENEPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNHPEYPY
LYATFCEIDKKKKDAKQQATFTLADPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKL
TVQLDRLIYPTESGGWEEKGKVDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGT
LGGARVQFDRDHLRRYPHKVESGNVGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKVVNFKP
KELTEWIKDSKGKKLKSGIESLEIGLRVMSIDLGQRQAAAASIFEVVDQKPDIEGKLFFPIK
GTELYAVHRASFNIKLPGETLVKSREVLRKAREDNLKLMNQKLNFLRNVLHFQQFEDITERE
KRVTKWISRQENSDVPLVYQDELIQIRELMYKPYKDWVAFLKQLHKRLEVEIGKEVKHWRKS
LSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEPGEVRRLEPGQRFAIDQLNHLNALKED
RLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFEDLSNYNPYEERSRFENSKLMKWSR
REIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSVVTKEKLQDNRFFKNLQREGR
LTLDKIAVLKEGDLYPDKGGEKFISLSKDRKCVTTHADINAAQNLQKRFWTRTHGFYKVYCK
AYQVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVNAGKLKIKKGSSKQSSSELVDS
DILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFGKLERILISKLTNQYSISTIE
DDSSKQSMKRPAATKKAGQAKKKK.

Amino acid sequences having at least 85% or greater identity to the BhCas12b amino acid sequence are also useful in the methods of the invention.

By "cytidine deaminase" is meant a polypeptide or fragment thereof capable of catalyzing a deamination reaction that converts an amino group to a carbonyl group. In one embodiment, the cytidine deaminase converts cytosine to uracil or 5-methylcytosine to thymine. PmCDA1, which is derived from Petromyzon marinus (Petromyzon marinus cytosine deaminase 1, "PmCDA1"), AID (Activation-induced cytidine deaminase; AICDA), which is derived from a mammal (*e.g.*, human, swine, bovine, horse, monkey etc.), and APOBEC are exemplary cytidine deaminases.

The term "conservative amino acid substitution" or "conservative mutation" refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., Principles of Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids can be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., supra). Non-limiting examples of conservative mutations include amino acid substitutions of amino acids, for example, lysine for arginine and vice versa such

that a positive charge can be maintained; glutamic acid for aspartic acid and vice versa such that a negative charge can be maintained; serine for threonine such that a free –OH can be maintained; and glutamine for asparagine such that a free $–NH_2$ can be maintained.

The term "coding sequence" or "protein coding sequence" as used interchangeably herein refers to a segment of a polynucleotide that codes for a protein. The region or sequence is bounded nearer the 5' end by a start codon and nearer the 3' end with a stop codon. Coding sequences can also be referred to as open reading frames.

The term "deaminase" or "deaminase domain," as used herein, refers to a protein or enzyme that catalyzes a deamination reaction. In some embodiments, the deaminase is an adenosine deaminase, which catalyzes the hydrolytic deamination of adenine to hypoxanthine. In some embodiments, the deaminase is an adenosine deaminase, which catalyzes the hydrolytic deamination of adenosine or adenine (A) to inosine (I). In some embodiments, the deaminase or deaminase domain is an adenosine deaminase catalyzing the hydrolytic deamination of adenosine or deoxyadenosine to inosine or deoxyinosine, respectively. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (*e.g.*, engineered adenosine deaminases, evolved adenosine deaminases) provided herein can be from any organism, such as a bacterium. In some embodiments, the adenosine deaminase is from a bacterium, such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Shewanella putrefaciens*, *Haemophilus influenzae*, or *Caulobacter crescentus*.

In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is TadA variant. In some embodiments, the TadA variant is a TadA*8. In some embodiments, the deaminase or deaminase domain is a variant of a naturally occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identical to a naturally occurring deaminase. For example, deaminase domains are described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078) and PCT/US2016/058344 (WO 2017/070632), each of which is incorporated herein by reference for its entirety. Also, see Komor, A.C., *et al.*,

"Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017)), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1, the entire contents of which are hereby incorporated by reference.

"Detect" refers to identifying the presence, absence or amount of the analyte to be detected. In one embodiment, a sequence alteration in a polynucleotide or polypeptide is detected. In another embodiment, the presence of indels is detected.

By "detectable label" is meant a composition that when linked to a molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an enzyme linked immunosorbent assay (ELISA)), biotin, digoxigenin, or haptens.

By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ.

The term "effective amount," as used herein, refers to an amount of a biologically active agent that is sufficient to elicit a desired biological response. The effective amount of an active agent(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount. In one embodiment, an effective amount is the amount of a base editor of the invention (*e.g.*, a fusion protein comprising a programable DNA binding protein, a nucleobase editor and gRNA) sufficient to introduce an alteration in a gene of interest in a cell (*e.g.*, a cell *in vitro* or *in vivo*). In some embodiments, an effective amount of a fusion protein provided herein, *e.g.*, of a nucleobase editor comprising a nCas9 domain and a deaminase domain (*e.g.*, adenosine deaminase or cytidine deaminase) may refer to the amount of the fusion protein that is sufficient to induce editing of a target site specifically bound and edited by the nucleobase editor. In one embodiment, an effective amount is the amount of a base editor required to achieve a

therapeutic effect (*e.g.*, to reduce or control a disease or a symptom or condition thereof). Such therapeutic effect need not be sufficient to alter a gene of interest in all cells of a subject, tissue or organ, but only to alter a gene of interest in about 1%, 5%, 10%, 25%, 50%, 75% or more of the cells present in a subject, tissue or organ.

In some embodiments, an effective amount of a fusion protein provided herein, *e.g.*, of a nucleobase editor comprising a nCas9 domain and a deaminase domain (*e.g.*, adenosine deaminase or cytidine deaminase) refers to the amount of the fusion protein that is sufficient to induce editing of a target site specifically bound and edited by the nucleobase editors described herein. As will be appreciated by the skilled artisan, the effective amount of an agent, *e.g.*, a fusion protein, a nuclease, a hybrid protein, a protein dimer, a complex of a protein (or protein dimer) and a polynucleotide, or a polynucleotide, may vary depending on various factors as, for example, on the desired biological response, *e.g.*, on the specific allele, genome, or target site to be edited, on the cell or tissue being targeted, and/or on the agent being used.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

By “guide RNA” or “gRNA” is meant a polynucleotide which can be specific for a target sequence and can form a complex with a polynucleotide programmable nucleotide binding domain protein (*e.g.*, Cas9 or Cpf1). In an embodiment, the guide polynucleotide is a guide RNA (gRNA). gRNAs can exist as a complex of two or more RNAs, or as a single RNA molecule. gRNAs that exist as a single RNA molecule may be referred to as single-guide RNAs (sgRNAs), though “gRNA” is used interchangeably to refer to guide RNAs that exist as either single molecules or as a complex of two or more molecules. Typically, gRNAs that exist as single RNA species comprise two domains: (1) a domain that shares homology to a target nucleic acid (*e.g.*, and directs binding of a Cas9 complex to the target); and (2) a domain that binds a Cas9 protein. In some embodiments, domain (2) corresponds to a sequence known as a tracrRNA, and comprises a stem-loop structure. For example, in some embodiments, domain (2) is identical or homologous to a tracrRNA as provided in Jinek *et al.*, Science 337:816-821(2012), the entire contents of which is incorporated herein by reference. Other examples of gRNAs (*e.g.*, those including domain 2) can be found in U.S. Provisional Patent Application, U.S.S.N. 61/874,682, filed September 6, 2013, entitled "Switchable Cas9 Nucleases and Uses Thereof," and U.S. Provisional Patent Application, U.S.S.N. 61/874,746, filed September 6,

2013, entitled "Delivery System For Functional Nucleases," the entire contents of each are hereby incorporated by reference in their entirety. In some embodiments, a gRNA comprises two or more of domains (1) and (2), and may be referred to as an "extended gRNA." An extended gRNA will bind two or more Cas9 proteins and bind a target nucleic acid at two or more distinct regions, as described herein. The gRNA comprises a nucleotide sequence that complements a target site, which mediates binding of the nuclease/RNA complex to said target site, providing the sequence specificity of the nuclease:RNA complex.

"Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

The term "inhibitor of base repair" or "IBR" refers to a protein that is capable in inhibiting the activity of a nucleic acid repair enzyme, for example a base excision repair (BER) enzyme. In some embodiments, the IBR is an inhibitor of inosine base excision repair. Exemplary inhibitors of base repair include inhibitors of APE1, Endo III, Endo IV, Endo V, Endo VIII, Fpg, hOGGl, hNEILl, T7 Endol, T4PDG, UDG, hSMUGl, and hAAG. In some embodiments, the IBR is an inhibitor of Endo V or hAAG. In some embodiments, the IBR is a catalytically inactive EndoV or a catalytically inactive hAAG. In some embodiments, the base repair inhibitor is an inhibitor of Endo V or hAAG. In some embodiments, the base repair inhibitor is a catalytically inactive EndoV or a catalytically inactive hAAG.

In some embodiments, the base repair inhibitor is uracil glycosylase inhibitor (UGI). UGI refers to a protein that is capable of inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a fragment of a wild-type UGI. In some embodiments, the UGI proteins provided herein include fragments of UGI and proteins homologous to a UGI or a UGI fragment. In some embodiments, the base repair inhibitor is an inhibitor of inosine base excision repair. In some embodiments, the base repair inhibitor is a "catalytically inactive inosine specific nuclease" or "dead inosine specific nuclease. Without wishing to be bound by any particular theory, catalytically inactive inosine glycosylases (*e.g.*, alkyl adenine glycosylase (AAG)) can bind inosine, but cannot create an abasic site or remove the inosine, thereby sterically blocking the newly formed inosine moiety from DNA damage/repair mechanisms. In some embodiments, the catalytically inactive inosine specific nuclease can be capable of binding an inosine in a nucleic acid but does not cleave the nucleic acid. Non-limiting exemplary catalytically inactive inosine specific nucleases include catalytically inactive alkyl adenosine glycosylase (AAG nuclease), for

example, from a human, and catalytically inactive endonuclease V (EndoV nuclease), for example, from *E. coli*. In some embodiments, the catalytically inactive AAG nuclease comprises an E125Q mutation or a corresponding mutation in another AAG nuclease.

By “increases” is meant a positive alteration of at least 10%, 25%, 50%, 75%, or 100%.

An "intein" is a fragment of a protein that is able to excise itself and join the remaining fragments (the exteins) with a peptide bond in a process known as protein splicing. Inteins are also referred to as "protein introns." The process of an intein excising itself and joining the remaining portions of the protein is herein termed "protein splicing" or "intein-mediated protein splicing." In some embodiments, an intein of a precursor protein (an intein containing protein prior to intein-mediated protein splicing) comes from two genes. Such intein is referred to herein as a split intein (*e.g.*, split intein-N and split intein-C). For example, in cyanobacteria, DnaE, the catalytic subunit a of DNA polymerase III, is encoded by two separate genes, dnaE-n and dnaE-c. The intein encoded by the dnaE-n gene may be herein referred as "intein-N." The intein encoded by the dnaE-c gene may be herein referred as "intein-C."

Other intein systems may also be used. For example, a synthetic intein based on the dnaE intein, the Cfa-N (*e.g.*, split intein-N) and Cfa-C (*e.g.*, split intein-C) intein pair, has been described (*e.g.*, in Stevens et al., J Am Chem Soc. 2016 Feb. 24; 138(7):2162-5, incorporated herein by reference). Non-limiting examples of intein pairs that may be used in accordance with the present disclosure include: Cfa DnaE intein, Ssp GyrB intein, Ssp DnaX intein, Ter DnaE3 intein, Ter ThyX intein, Rma DnaB intein and Cne Prp8 intein (*e.g.*, as described in U.S. Patent No. 8,394,604, incorporated herein by reference.
Exemplary nucleotide and amino acid sequences of inteins are provided.

DnaE Intein-N DNA:

TGCCTGTCATACGAAACCGAGATACTGACAGTAGAATATGGCCTTCTGCCAATCGGGAAGAT
TGTGGAGAAACGGATAGAATGCACAGTTTACTCTGTCGATAACAATGGTAACATTTATACTC
AGCCAGTTGCCCAGTGGCACGACCGGGGAGAGCAGGAAGTATTCGAATACTGTCTGGAGGAT
GGAAGTCTCATTAGGGCCACTAAGGACCACAAATTTATGACAGTCGATGGCCAGATGCTGCC
TATAGACGAAATCTTTGAGCGAGAGTTGGACCTCATGCGAGTTGACAACCTTCCTAAT

DnaE Intein-N Protein:

CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDR
GEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN

DnaE Intein-C DNA:

ATGATCAAGATAGCTACAAGGAAGTATCTTGGCAAACAAAACGTTTATGA

TATTGGAGTCGAAAGAGATCACAACTTTGCTCTGAAGAACGGATTCATAGCTTCTAAT

Intein-C: MIKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN

Cfa-N DNA:

TGCCTGTCTTATGATACCGAGATACTTACCGTTGAATATGGCTTCTTGCCTATTGGAAAGAT

TGTCGAAGAGAGAATTGAATGCACAGTATATACTGTAGACAAGAATGGTTTCGTTTACACAC

AGCCCATTGCTCAATGGCACAATCGCGGCGAACAAGAAGTATTTGAGTACTGTCTCGAGGAT

GGAAGCATCATACGAGCAACTAAAGATCATAAATTCATGACCACTGACGGGCAGATGTTGCC

AATAGATGAGATATTCGAGCGGGGCTTGGATCTCAAACAAGTGGATGGATTGCCA

Cfa-N Protein:

CLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEYCLED

GSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

Cfa-C DNA:

ATGAAGAGGACTGCCGATGGATCAGAGTTTGAATCTCCCAAGAAGAAGAGGAAAGTAAAGAT

AATATCTCGAAAAAGTCTTGGTACCCAAAATGTCTATGATATTGGAGTGGAGAAAGATCACA

ACTTCCTTCTCAAGAACGGTCTCGTAGCCAGCAAC

Cfa-C Protein:

MKRTADGSEFESPKKKRKVKIISRKSLGTQNVYDIGVEKDHNFLLKNGLVASN

Intein-N and intein-C may be fused to the N-terminal portion of the split Cas9 and the C-terminal portion of the split Cas9, respectively, for the joining of the N-terminal portion of the split Cas9 and the C-terminal portion of the split Cas9. For example, in some embodiments, an intein-N is fused to the C-terminus of the N-terminal portion of the split Cas9, *i.e.*, to form a structure of N--[N-terminal portion of the split Cas9]-[intein-N]--C. In some embodiments, an intein-C is fused to the N-terminus of the C-terminal portion of the split Cas9, *i.e.*, to form a structure of N-[intein-C]--[C-terminal portion of the split Cas9]-C. The mechanism of intein-mediated protein splicing for joining the proteins the inteins are fused to (*e.g.*, split Cas9) is known in the art, *e.g.*, as described in Shah et al., Chem Sci. 2014; 5(1):446-461, incorporated herein by reference. Methods for designing and using inteins are known in the art and described, for example by WO2014004336, WO2017132580, US20150344549, and US20180127780, each of which is incorporated herein by reference in their entirety.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state.

"Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of this invention is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high-performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated polynucleotide" is meant a nucleic acid (*e.g.*, a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

The term "linker", as used herein, can refer to a covalent linker (*e.g.*, covalent bond), a non-covalent linker, a chemical group, or a molecule linking two molecules or moieties, *e.g.*, two components of a protein complex or a ribonucleocomplex, or two domains of a fusion protein, such as, for example, a polynucleotide programmable DNA binding domain (*e.g.*, dCas9) and a deaminase domain (*e.g.*, an adenosine deaminase, a cytidine deaminase, or an adenosine deaminase and a cytidine deaminase) or a napDNAbp domain (e.g., Cas12b) and a deaminase domain (e.g., an adenosine deaminase or a cytidine deaminase). In particular embodiments, linkers flank a deaminase domain that is inserted within a Cas protein or fragment thereof. A linker can join different components of, or different portions of components of, a base editor system. For example, in some embodiments, a linker can join a guide polynucleotide binding domain of a polynucleotide programmable nucleotide binding domain and a catalytic domain of a deaminase. In some embodiments, a linker can join a CRISPR polypeptide and a deaminase. In some embodiments, a linker can join a Cas9 and a deaminase. In some embodiments, a linker can join a dCas9 and a deaminase. In some embodiments, a linker can join a nCas9 and a deaminase. For example, in some embodiments, a linker can join a Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, or Cas12i and a deaminase. In some embodiments, a linker can join a guide polynucleotide and a deaminase. In some embodiments, a linker can join a deaminating component and a polynucleotide programmable nucleotide binding component of a base editor system. In some embodiments, a linker can join an RNA-binding portion of a deaminating component and a napDNAbp component of a base editor system. In some embodiments, a linker can join an RNA-binding portion of a deaminating component and a polynucleotide programmable nucleotide binding component of a base editor system. In some embodiments, a linker can join an RNA-binding portion of a deaminating component and an RNA-binding portion of a polynucleotide programmable nucleotide binding component of a base editor system. A linker can be positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond or non-covalent interaction, thus connecting the two. In some embodiments, the linker can be an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker can be a polynucleotide. In some embodiments, the linker can be a DNA linker. In some embodiments, the linker can be an RNA linker. In some embodiments, a linker can comprise an aptamer capable of binding to a ligand. In some embodiments, the ligand may be carbohydrate, a peptide, a protein, or a nucleic acid. In some embodiments, the linker may comprise an aptamer may be derived from a riboswitch. The riboswitch from which the

aptamer is derived may be selected from a theophylline riboswitch, a thiamine pyrophosphate (TPP) riboswitch, an adenosine cobalamin (AdoCbl) riboswitch, an S-adenosyl methionine (SAM) riboswitch, an SAH riboswitch, a flavin mononucleotide (FMN) riboswitch, a tetrahydrofolate riboswitch, a lysine riboswitch, a glycine riboswitch, a purine riboswitch, a GlmS riboswitch, or a pre-queosine1 (PreQ1) riboswitch. In some embodiments, a linker may comprise an aptamer bound to a polypeptide or a protein domain, such as a polypeptide ligand. In some embodiments, the polypeptide ligand may be a K Homology (KH) domain, a MS2 coat protein domain, a PP7 coat protein domain, a SfMu Com coat protein domain, a sterile alpha motif, a telomerase Ku binding motif and Ku protein, a telomerase Sm7 binding motif and Sm7 protein, or an RNA recognition motif. In some embodiments, the polypeptide ligand may be a portion of a base editor system component. For example, a nucleobase editing component may comprise a deaminase domain and an RNA recognition motif.

In some embodiments, the linker can be an amino acid or a plurality of amino acids (*e.g.*, a peptide or protein). In some embodiments, the linker can be about 5-100 amino acids in length, for example, about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 amino acids in length. In some embodiments, the linker can be about 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, or 450-500 amino acids in length. Longer or shorter linkers can be also contemplated.

In some embodiments, a linker joins a gRNA binding domain of an RNA-programmable nuclease, including a Cas9 nuclease domain, and the catalytic domain of a nucleic-acid editing protein (*e.g.*, cytidine or adenosine deaminase). In some embodiments, a linker joins a dCas9 and a nucleic-acid editing protein. For example, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond, thus connecting the two. In some embodiments, the linker is an amino acid or a plurality of amino acids (*e.g.*, a peptide or protein). In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is 5-200 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 35, 45, 50, 55, 60, 60, 65, 70, 70, 75, 80, 85, 90, 90, 95, 100, 101, 102, 103, 104, 105, 110, 120, 130, 140, 150, 160, 175, 180, 190, or 200 amino acids in length. Longer or shorter linkers are also contemplated.

In some embodiments, the domains of the nucleobase editor are fused via a linker that comprises the amino acid sequence of SGGSSGSETPGTSESATPESSGGS,

SGGSSGGSSGSETPGTSESATPESSGGSSGGS, or GGSGGSPGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATSGGSGGS. In some embodiments, domains of the nucleobase editor are fused via a linker comprising the amino acid sequence SGSETPGTSESATPES, which may also be referred to as the XTEN linker. In some embodiments, a linker comprises the amino acid sequence SGGS. In some embodiments, a linker comprises $(SGGS)_n$, $(GGGS)_n$, $(GGGGS)_n$, $(G)_n$, $(EAAAK)_n$, $(GGS)_n$, SGSETPGTSESATPES, or $(XP)_n$ motif, or a combination of any of these, wherein n is independently an integer between 1 and 30, and wherein X is any amino acid. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

In some embodiments, the linker is 24 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPES. In some embodiments, the linker is 40 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGS. In some embodiments, the linker is 64 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGSSGSETPGTSESATPESSGGSSGGS. In some embodiments, the linker is 92 amino acids in length. In some embodiments, the linker comprises the amino acid sequence PGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATS.

By "marker" is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

The term "mutation," as used herein, refers to a substitution of a residue within a sequence, *e.g.*, a nucleic acid or amino acid sequence, with another residue, or a deletion or insertion of one or more residues within a sequence. Mutations are typically described herein by identifying the original residue followed by the position of the residue within the sequence and by the identity of the newly substituted residue. Various methods for making the amino acid substitutions (mutations) provided herein are well known in the art, and are provided by, for example, Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)). In some embodiments, the presently disclosed base editors can efficiently generate an "intended mutation," such as a

point mutation, in a nucleic acid (*e.g.*, a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations, such as unintended point mutations. In some embodiments, an intended mutation is a mutation that is generated by a specific base editor (*e.g.*, cytidine base editor or adenosine base editor) bound to a guide polynucleotide (*e.g.*, gRNA), specifically designed to generate the intended mutation.

In general, mutations made or identified in a sequence (*e.g.*, an amino acid sequence as described herein) are numbered in relation to a reference (or wild-type) sequence, *i.e.*, a sequence that does not contain the mutations. The skilled practitioner in the art would readily understand how to determine the position of mutations in amino acid and nucleic acid sequences relative to a reference sequence.

The term "non-conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with, or inhibit the biological activity of, the functional variant. The non-conservative amino acid substitution can enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the wild-type protein.

The term "nuclear localization sequence," "nuclear localization signal," or "NLS" refers to an amino acid sequence that promotes import of a protein into the cell nucleus. Nuclear localization sequences are known in the art and described, for example, in Plank *et al.*, International PCT application, PCT/EP2000/011690, filed November 23, 2000, published as WO/2001/038547 on May 31, 2001, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences. In other embodiments, the NLS is an optimized NLS described, for example, by Koblan *et al.*, Nature Biotech. 2018 doi:10.1038/nbt.4172. In some embodiments, an NLS comprises the amino acid sequence KRTADGSEFESPKKKRKV, KRPAATKKAGQAKKKK, KKTELQTTNAENKTKKL, KRGINDRNFWRGENGRKTR, RKSGKIAAIVVKRPRK, PKKKRKV, or MDSLLMNRRKFLYQFKNVRWAKGRRETYLC.

The terms "nucleic acid" and "nucleic acid molecule," as used herein, refer to a compound comprising a nucleobase and an acidic moiety, *e.g.*, a nucleoside, a nucleotide, or a polymer of nucleotides. Typically, polymeric nucleic acids, *e.g.*, nucleic acid molecules comprising three or more nucleotides are linear molecules, in which adjacent nucleotides are linked to each other via a phosphodiester linkage. In some embodiments, "nucleic acid" refers to individual nucleic acid residues (*e.g.*, nucleotides and/or nucleosides). In some

embodiments, "nucleic acid" refers to an oligonucleotide chain comprising three or more individual nucleotide residues. As used herein, the terms "oligonucleotide" and "polynucleotide" can be used interchangeably to refer to a polymer of nucleotides (*e.g.*, a string of at least three nucleotides). In some embodiments, "nucleic acid" encompasses RNA as well as single and/or double-stranded DNA. Nucleic acids may be naturally occurring, for example, in the context of a genome, a transcript, an mRNA, tRNA, rRNA, siRNA, snRNA, a plasmid, cosmid, chromosome, chromatid, or other naturally occurring nucleic acid molecule. On the other hand, a nucleic acid molecule may be a non-naturally occurring molecule, *e.g.*, a recombinant DNA or RNA, an artificial chromosome, an engineered genome, or fragment thereof, or a synthetic DNA, RNA, DNA/RNA hybrid, or including non-naturally occurring nucleotides or nucleosides. Furthermore, the terms "nucleic acid," "DNA," "RNA," and/or similar terms include nucleic acid analogs, *e.g.,* analogs having other than a phosphodiester backbone. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, *etc.* Where appropriate, *e.g.*, in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, and backbone modifications. A nucleic acid sequence is presented in the 5′ to 3′ direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (*e.g.*, adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (*e.g.*, methylated bases); intercalated bases; modified sugars (2′-*e.g.*,fluororibose, ribose, 2′-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (*e.g.*, phosphorothioates and 5′-*N*-phosphoramidite linkages).

The term "nucleic acid programmable DNA binding protein" or "napDNAbp" may be used interchangeably with "polynucleotide programmable nucleotide binding domain" to refer to a protein that associates with a nucleic acid (*e.g.*, DNA or RNA), such as a guide nucleic acid or guide polynucleotide (*e.g.,* gRNA), that guides the napDNAbp to a specific nucleic acid sequence. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some

embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable RNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a Cas9 protein. A Cas9 protein can associate with a guide RNA that guides the Cas9 protein to a specific DNA sequence that is complementary to the guide RNA. In some embodiments, the napDNAbp is a Cas9 domain, for example a nuclease active Cas9, a Cas9 nickase (nCas9), or a nuclease inactive Cas9 (dCas9). Non-limiting examples of nucleic acid programmable DNA binding proteins include, Cas9 (*e.g.*, dCas9 and nCas9), Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i. Non-limiting examples of Cas enzymes include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas8a, Cas8b, Cas8c, Cas9 (also known as Csn1 or Csx12), Cas10, Cas10d, Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csx11, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, homologues thereof, or modified or engineered versions thereof. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, although they may not be specifically listed in this disclosure. See, *e.g.*, Makarova *et al.* "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" *CRISPR J.* 2018 Oct;1:325-336. doi: 10.1089/crispr.2018.0033; Yan *et al.*, "Functionally diverse type V CRISPR-Cas systems" *Science*. 2019 Jan 4;363(6422):88-91. doi: 10.1126/science.aav7271, the entire contents of each are hereby incorporated by reference.

The term "nucleobase," "nitrogenous base," or "base," used interchangeably herein, refers to a nitrogen-containing biological compound that forms a nucleoside, which in turn is a component of a nucleotide. The ability of nucleobases to form base pairs and to stack one upon another leads directly to long-chain helical structures such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Five nucleobases – adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U) – are called primary or canonical. Adenine and guanine are derived from purine, and cytosine, uracil, and thymine are derived from pyrimidine. DNA and RNA can also contain other (non-primary) bases that are modified. Non-limiting exemplary modified nucleobases can include hypoxanthine, xanthine, 7-methylguanine, 5,6-dihydrouracil, 5-methylcytosine (m5C), and 5-hydromethylcytosine. Hypoxanthine and

xanthine can be created through mutagen presence, both of them through deamination (replacement of the amine group with a carbonyl group). Hypoxanthine can be modified from adenine. Xanthine can be modified from guanine. Uracil can result from deamination of cytosine. A "nucleoside" consists of a nucleobase and a five carbon sugar (either ribose or deoxyribose). Examples of a nucleoside include adenosine, guanosine, uridine, cytidine, 5-methyluridine (m5U), deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine, and deoxycytidine. Examples of a nucleoside with a modified nucleobase includes inosine (I), xanthosine (X), 7-methylguanosine (m7G), dihydrouridine (D), 5-methylcytidine (m5C), and pseudouridine (Ψ). A "nucleotide" consists of a nucleobase, a five carbon sugar (either ribose or deoxyribose), and at least one phosphate group.

The term "nucleic acid programmable DNA binding protein" or "napDNAbp" refers to a protein that associates with a nucleic acid (e.g., DNA or RNA), such as a guide nucleic acid, that guides the napDNAbp to a specific nucleic acid sequence. For example, a Cas12 protein can associate with a guide RNA that guides the Cas12 protein to a specific DNA sequence that is complementary to the guide RNA. In some embodiments, the napDNAbp is a Cas12 domain, for example a nuclease active Cas12 domain. Examples of napDNAbps include, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i. Other napDNAbps are also within the scope of this disclosure, although they may not be specifically listed in this disclosure. See, e.g., Makarova et al. "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" CRISPR J. 2018 Oct;1:325-336. doi: 10.1089/crispr.2018.0033; Yan et al., "Functionally diverse type V CRISPR-Cas systems" Science. 2019 Jan 4;363(6422):88-91. doi: 10.1126/science.aav7271, the entire contents of each are hereby incorporated by reference.

The terms "nucleobase editing domain" or "nucleobase editing protein," as used herein, refers to a protein or enzyme that can catalyze a nucleobase modification in RNA or DNA, such as cytosine (or cytidine) to uracil (or uridine) or thymine (or thymidine), and adenine (or adenosine) to hypoxanthine (or inosine) deaminations, as well as non-templated nucleotide additions and insertions. In some embodiments, the nucleobase editing domain is a deaminase domain (*e.g.*, an adenine deaminase or an adenosine deaminase; or a cytidine deaminase or a cytosine deaminase). In some embodiments, the nucleobase editing domain is more than one deaminase domain (*e.g.*, an adenine deaminase or an adenosine deaminase and a cytidine or a cytosine deaminase). In some embodiments, the nucleobase editing domain can be a naturally occurring nucleobase editing domain. In some embodiments, the nucleobase editing domain can be an engineered or evolved nucleobase editing domain from

the naturally occurring nucleobase editing domain. The nucleobase editing domain can be from any organism, such as a bacterium, human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. For example, nucleobase editing proteins are described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078) and PCT/US2016/058344 (WO 2017/070632), each of which is incorporated herein by reference for its entirety. Also see, Komor, A.C., et al., "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., et al., "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., et al., "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

As used herein, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

A "patient" or "subject" as used herein refers to a mammalian subject or individual diagnosed with, at risk of having or developing, or suspected of having or developing a disease or a disorder. In some embodiments, the term "patient" refers to a mammalian subject with a higher than average likelihood of developing a disease or a disorder. Exemplary patients can be humans, non-human primates, cats, dogs, pigs, cattle, cats, horses, camels, llamas, goats, sheep, rodents (*e.g.*, mice, rabbits, rats, or guinea pigs) and other mammalians that can benefit from the therapies disclosed herein. Exemplary human patients can be male and/or female.

"Patient in need thereof" or "subject in need thereof" is referred to herein as a patient diagnosed with, at risk or having, predetermined to have, or suspected of having a disease or disorder.

The terms "pathogenic mutation," "pathogenic variant," "disease casing mutation," "disease causing variant," "deleterious mutation," or "predisposing mutation" refers to a genetic alteration or mutation that increases an individual's susceptibility or predisposition to a certain disease or disorder. In some embodiments, the pathogenic mutation comprises at least one wild-type amino acid substituted by at least one pathogenic amino acid in a protein encoded by a gene.

The term "pharmaceutically-acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or

solvent encapsulating material, involved in carrying or transporting the compound from one site (*e.g.*, the delivery site) of the body, to another site (*e.g.*, organ, tissue or portion of the body). A pharmaceutically acceptable carrier is "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the tissue of the subject (*e.g.*, physiologically compatible, sterile, physiologic pH, etc.). The terms such as "excipient," "carrier," "pharmaceutically acceptable carrier," "vehicle," or the like are used interchangeably herein.

The term "pharmaceutical composition" can refer to a composition formulated for pharmaceutical use.

The terms "protein," "peptide," "polypeptide," and their grammatical equivalents are used interchangeably herein, and refer to a polymer of amino acid residues linked together by peptide (amide) bonds. The terms refer to a protein, peptide, or polypeptide of any size, structure, or function. Typically, a protein, peptide, or polypeptide will be at least three amino acids long. A protein, peptide, or polypeptide can refer to an individual protein or a collection of proteins. One or more of the amino acids in a protein, peptide, or polypeptide can be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a hydroxyl group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modifications, etc. A protein, peptide, or polypeptide can also be a single molecule or can be a multi-molecular complex. A protein, peptide, or polypeptide can be just a fragment of a naturally occurring protein or peptide. A protein, peptide, or polypeptide can be naturally occurring, recombinant, or synthetic, or any combination thereof. The term "fusion protein" as used herein refers to a hybrid polypeptide which comprises protein domains from at least two different proteins. One protein can be located at the amino-terminal (N-terminal) portion of the fusion protein or at the carboxy-terminal (C-terminal) protein thus forming an amino-terminal fusion protein or a carboxy-terminal fusion protein, respectively. A protein can comprise different domains, for example, a nucleic acid binding domain (*e.g.*, the gRNA binding domain of Cas9 that directs the binding of the protein to a target site) and a nucleic acid cleavage domain, or a catalytic domain of a nucleic acid editing protein. In some embodiments, a protein comprises a proteinaceous part, *e.g.*, an amino acid sequence constituting a nucleic acid binding domain, and an organic compound, *e.g.*, a compound that can act as a nucleic acid cleavage agent. In some embodiments, a protein is in a complex with, or is in association with, a nucleic acid, *e.g.*, RNA or DNA. Any of the proteins provided herein can be produced by any method known in the art. For example, the proteins provided herein can be produced via recombinant

protein expression and purification, which is especially suited for fusion proteins comprising a peptide linker. Methods for recombinant protein expression and purification are well known, and include those described by Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), the entire contents of which are incorporated herein by reference.

Polypeptides and proteins disclosed herein (including functional portions and functional variants thereof) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine. The polypeptides and proteins can be associated with post-translational modifications of one or more amino acids of the polypeptide constructs. Non-limiting examples of post-translational modifications include phosphorylation, acylation including acetylation and formylation, glycosylation (including N-linked and O-linked), amidation, hydroxylation, alkylation including methylation and ethylation, ubiquitylation, addition of pyrrolidone carboxylic acid, formation of disulfide bridges, sulfation, myristoylation, palmitoylation, isoprenylation, farnesylation, geranylation, glypiation, lipoylation and iodination.

The term "polynucleotide programmable nucleotide binding domain" or "nucleic acid programmable DNA binding protein (napDNAbp)" refers to a protein that associates with a nucleic acid (*e.g.*, DNA or RNA), such as a guide polynucleotide (*e.g.*, guide RNA), that guides the polynucleotide programmable nucleotide binding domain to a specific nucleic acid sequence. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable RNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a Cas12 protein.

The term "recombinant" as used herein in the context of proteins or nucleic acids refers to proteins or nucleic acids that do not occur in nature, but are the product of human engineering. For example, in some embodiments, a recombinant protein or nucleic acid molecule comprises an amino acid or nucleotide sequence that comprises at least one, at least two, at least three, at least four, at least five, at least six, or at least seven mutations as compared to any naturally occurring sequence.

By “reduces” is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By “reference” is meant a standard or control condition. In one embodiment, the reference is a wild-type or healthy cell. In other embodiments and without limitation, a reference is an untreated cell that is not subjected to a test condition, or is subjected to placebo or normal saline, medium, buffer, and/or a control vector that does not harbor a polynucleotide of interest.

A “reference sequence” is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, at least about 20 amino acids, at least about 25 amino acids, about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, at least about 60 nucleotides, at least about 75 nucleotides, about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween. In some embodiments, a reference sequence is a wild-type sequence of a protein of interest. In other embodiments, a reference sequence is a polynucleotide sequence encoding a wild-type protein.

The term "RNA-programmable nuclease," and "RNA-guided nuclease" are used with (*e.g.*, binds or associates with) one or more RNA(s) that is not a target for cleavage. In some embodiments, an RNA-programmable nuclease, when in a complex with an RNA, may be referred to as a nuclease:RNA complex. Typically, the bound RNA(s) is referred to as a guide RNA (gRNA). gRNAs can exist as a complex of two or more RNAs, or as a single RNA molecule. gRNAs that exist as a single RNA molecule may be referred to as single-guide RNAs (sgRNAs), though "gRNA" is used interchangeably to refer to guide RNAs that exist as either single molecules or as a complex of two or more molecules. Typically, gRNAs that exist as single RNA species comprise two domains: (1) a domain that shares homology to a target nucleic acid (*e.g.*, and directs binding of a Cas9 complex to the target); and (2) a

domain that binds a Cas9 protein. In some embodiments, domain (2) corresponds to a sequence known as a tracrRNA, and comprises a stem-loop structure. For example, in some embodiments, domain (2) is identical or homologous to a tracrRNA as provided in Jinek et ah, Science 337:816-821(2012), the entire contents of which is incorporated herein by reference. Other examples of gRNAs (*e.g.*, those including domain 2) can be found in U.S. Provisional Patent Application, U.S.S.N. 61/874,682, filed September 6, 2013, entitled "Switchable Cas9 Nucleases and Uses Thereof," and U.S. Provisional Patent Application, U.S.S.N. 61/874,746, filed September 6, 2013, entitled "Delivery System For Functional Nucleases," the entire contents of each are hereby incorporated by reference in their entirety. In some embodiments, a gRNA comprises two or more of domains (1) and (2), and may be referred to as an "extended gRNA." For example, an extended gRNA will, *e.g.*, bind two or more Cas9 proteins and bind a target nucleic acid at two or more distinct regions, as described herein. The gRNA comprises a nucleotide sequence that complements a target site, which mediates binding of the nuclease/RNA complex to said target site, providing the sequence specificity of the nuclease:RNA complex.

In some embodiments, the RNA-programmable nuclease is the (CRISPR-associated system) Cas9 endonuclease, for example, Cas9 (Casnl) from *Streptococcus pyogenes* (see, *e.g.*, "Complete genome sequence of an Ml strain of *Streptococcus pyogenes*." Ferretti J.J., *et al.*, Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., *et al.*, Nature 471:602-607(2011).

Because RNA-programmable nucleases (*e.g.*, Cas9) use RNA:DNA hybridization to target DNA cleavage sites, these proteins are able to be targeted, in principle, to any sequence specified by the guide RNA. Methods of using RNA-programmable nucleases, such as Cas9, for site-specific cleavage (*e.g.*, to modify a genome) are known in the art (see *e.g.*, Cong, L. *et al.*, Multiplex genome engineering using CRISPR/Cas systems. Science 339, 819-823 (2013); Mali, P. *et al.*, RNA-guided human genome engineering via Cas9. Science 339, 823-826 (2013); Hwang, W.Y. *et al.*, Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature biotechnology 31, 227-229 (2013); Jinek, M. *et al.*, RNA-programmed genome editing in human cells. eLife 2, e00471 (2013); Dicarlo, J.E. *et al.*, Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic acids research (2013); Jiang, W. *et al.*, RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nature biotechnology 31, 233-239 (2013); the entire contents of each of which are incorporated herein by reference).

The term "single nucleotide polymorphism (SNP)" is a variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population (*e.g.*, > 1%). For example, at a specific base position in the human genome, the C nucleotide can appear in most individuals, but in a minority of individuals, the position is occupied by an A. This means that there is a SNP at this specific position, and the two possible nucleotide variations, C or A, are said to be alleles for this position. SNPs underlie differences in susceptibility to disease. The severity of illness and the way our body responds to treatments are also manifestations of genetic variations. SNPs can fall within coding regions of genes, non-coding regions of genes, or in the intergenic regions (regions between genes). In some embodiments, SNPs within a coding sequence do not necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code. SNPs in the coding region are of two types: synonymous and nonsynonymous SNPs. Synonymous SNPs do not affect the protein sequence, while nonsynonymous SNPs change the amino acid sequence of protein. The nonsynonymous SNPs are of two types: missense and nonsense. SNPs that are not in protein-coding regions can still affect gene splicing, transcription factor binding, messenger RNA degradation, or the sequence of noncoding RNA. Gene expression affected by this type of SNP is referred to as an eSNP (expression SNP) and can be upstream or downstream from the gene. A single nucleotide variant (SNV) is a variation in a single nucleotide without any limitations of frequency and can arise in somatic cells. A somatic single nucleotide variation can also be called a single-nucleotide alteration.

By "specifically binds" is meant a nucleic acid molecule, polypeptide, or complex thereof (*e.g.*, a nucleic acid programmable DNA binding domain and guide nucleic acid), compound, or molecule that recognizes and binds a polypeptide and/or nucleic acid molecule of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical

with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (*e.g.*, a gene described herein), or portions thereof, under various conditions of stringency. (See, *e.g.*, Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, *e.g.*, formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, *e.g.*, sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a one: embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In another embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In another embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In an embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and

0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel *et al.* (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By “split” is meant divided into two or more fragments.

A "split Cas9 protein" or "split Cas9" refers to a Cas9 protein that is provided as an N-terminal fragment and a C-terminal fragment encoded by two separate nucleotide sequences. The polypeptides corresponding to the N-terminal portion and the C-terminal portion of the Cas9 protein may be spliced to form a “reconstituted” Cas9 protein. In particular embodiments, the Cas9 protein is divided into two fragments within a disordered region of the protein, *e.g.*, as described in Nishimasu et al., Cell, Volume 156, Issue 5, pp. 935-949, 2014, or as described in Jiang et al. (2016) Science 351: 867-871. PDB file: 5F9R, each of which is incorporated herein by reference. In some embodiments, the protein is divided into two fragments at any C, T, A, or S within a region of SpCas9 between about amino acids A292-G364, F445-K483, or E565-T637, or at corresponding positions in any other Cas9, Cas9 variant (*e.g.*, nCas9, dCas9), or other napDNAbp. In some embodiments, protein is divided into two fragments at SpCas9 T310, T313, A456, S469, or C574. In some embodiments, the process of dividing the protein into two fragments is referred to as “splitting” the protein.

In other embodiments, the N-terminal portion of the Cas9 protein comprises amino acids 1-573 or 1-637 S. pyogenes Cas9 wild-type (SpCas9) (NCBI Reference Sequence: NC_002737.2, Uniprot Reference Sequence: Q99ZW2) and the C-terminal portion of the Cas9 protein comprises a portion of amino acids 574-1368 or 638-1368 of SpCas9 wild-type.

The C-terminal portion of the split Cas9 can be joined with the N-terminal portion of the split Cas9 to form a complete Cas9 protein. In some embodiments, the C-terminal portion of the Cas9 protein starts from where the N-terminal portion of the Cas9 protein ends. As such, in some embodiments, the C-terminal portion of the split Cas9 comprises a portion of amino acids (551-651)-1368 of spCas9. "(551-651)-1368" means starting at an amino acid

between amino acids 551-651 (inclusive) and ending at amino acid 1368. For example, the C-terminal portion of the split Cas9 may comprise a portion of any one of amino acid 551-1368, 552-1368, 553-1368, 554-1368, 555-1368, 556-1368, 557-1368, 558-1368, 559-1368, 560-1368, 561-1368, 562-1368, 563-1368, 564-1368, 565-1368, 566-1368, 567-1368, 568-1368, 569-1368, 570-1368, 571-1368, 572-1368, 573-1368, 574-1368, 575-1368, 576-1368, 577-1368, 578-1368, 579-1368, 580-1368, 581-1368, 582-1368, 583-1368, 584-1368, 585-1368, 586-1368, 587-1368, 588-1368, 589-1368, 590-1368, 591-1368, 592-1368, 593-1368, 594-1368, 595-1368, 596-1368, 597-1368, 598-1368, 599-1368, 600-1368, 601-1368, 602-1368, 603-1368, 604-1368, 605-1368, 606-1368, 607-1368, 608-1368, 609-1368, 610-1368, 611-1368, 612-1368, 613-1368, 614-1368, 615-1368, 616-1368, 617-1368, 618-1368, 619-1368, 620-1368, 621-1368, 622-1368, 623-1368, 624-1368, 625-1368, 626-1368, 627-1368, 628-1368, 629-1368, 630-1368, 631-1368, 632-1368, 633-1368, 634-1368, 635-1368, 636-1368, 637-1368, 638-1368, 639-1368, 640-1368, 641-1368, 642-1368, 643-1368, 644-1368, 645-1368, 646-1368, 647-1368, 648-1368, 649-1368, 650-1368, or 651-1368 of spCas9. In some embodiments, the C-terminal portion of the split Cas9 protein comprises a portion of amino acids 574-1368 or 638-1368 of SpCas9.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Subjects include livestock, domesticated animals raised to produce labor and to provide commodities, such as food, including without limitation, cattle, goats, chickens, horses, pigs, rabbits, and sheep.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). In one embodiment, such a sequence is at least 60%, 80% or 85%, 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and

phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

COBALT is used, for example, with the following parameters:

a) alignment parameters: Gap penalties-11,-1 and End-Gap penalties-5,-1,

b) CDD Parameters: Use RPS BLAST on; Blast E-value 0.003; Find Conserved columns and Recompute on, and

c) Query Clustering Parameters: Use query clusters on; Word Size 4; Max cluster distance 0.8; Alphabet Regular.

EMBOSS Needle is used, for example, with the following parameters:

a) Matrix: BLOSUM62;

b) GAP OPEN: 10;

c) GAP EXTEND: 0.5;

d) OUTPUT FORMAT: pair;

e) END GAP PENALTY: false;

f) END GAP OPEN: 10; and

g) END GAP EXTEND: 0.5.

The term "target site" refers to a sequence within a nucleic acid molecule that is modified by a nucleobase editor. In one embodiment, the target site is deaminated by a deaminase or a fusion protein comprising a deaminase (*e.g.*, cytidine or adenine deaminase).

As used herein, the terms “treat,” treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith or obtaining a desired pharmacologic and/or physiologic effect. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated. In some embodiments, the effect is therapeutic, *i.e.*, without limitation, the effect partially or completely reduces, diminishes, abrogates, abates, alleviates, decreases the intensity of, or cures a disease and/or adverse symptom attributable to the disease. In some embodiments, the effect is preventative, *i.e.*, the effect protects or prevents an occurrence or reoccurrence of a disease or condition. To this end, the presently disclosed methods comprise administering a therapeutically effective amount of a compositions as described herein.

By “uracil glycosylase inhibitor” or “UGI” is meant an agent that inhibits the uracil-excision repair system. In one embodiment, the agent is a protein or fragment thereof that binds a host uracil-DNA glycosylase and prevents removal of uracil residues from DNA. In

an embodiment, a UGI is a protein, a fragment thereof, or a domain that is capable of inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a modified version thereof. In some embodiments, a UGI domain comprises a fragment of the exemplary amino acid sequence set forth below. In some embodiments, a UGI fragment comprises an amino acid sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the exemplary UGI sequence provided below. In some embodiments, a UGI comprises an amino acid sequence that is homologous to the exemplary UGI amino acid sequence or fragment thereof, as set forth below. In some embodiments, the UGI, or a portion thereof, is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% identical to a wild-type UGI or a UGI sequence, or portion thereof, as set forth below. An exemplary UGI comprises an amino acid sequence as follows:

>splP14739IUNGI_BPPB2 Uracil-DNA glycosylase inhibitor
MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSD
APEYKPWALVIQDSNGENKIKML.

The term "vector" refers to a means of introducing a nucleic acid sequence into a cell, resulting in a transformed cell. Vectors include plasmids, transposons, phages, viruses, liposomes, and episome. "Expression vectors" are nucleic acid sequences comprising the nucleotide sequence to be expressed in the recipient cell. Expression vectors may include additional nucleic acid sequences to promote and/or facilitate the expression of the of the introduced sequence such as start, stop, enhancer, promoter, and secretion sequences.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

DNA editing has emerged as a viable means to modify disease states by correcting pathogenic mutations at the genetic level. Until recently, all DNA editing platforms have functioned by inducing a DNA double strand break (DSB) at a specified genomic site and relying on endogenous DNA repair pathways to determine the product outcome in a semi-stochastic manner, resulting in complex populations of genetic products. Though precise, user-defined repair outcomes can be achieved through the homology directed repair (HDR) pathway, a number of challenges have prevented high efficiency repair using HDR in therapeutically-relevant cell types. In practice, this pathway is inefficient relative to the competing, error-prone non-homologous end joining pathway. Further, HDR is tightly

restricted to the G1 and S phases of the cell cycle, preventing precise repair of DSBs in post-mitotic cells. As a result, it has proven difficult or impossible to alter genomic sequences in a user-defined, programmable manner with high efficiencies in these populations.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A – 1C depict plasmids. **FIG. 1A** is an expression vector encoding a TadA7.10-dCas9 base editor. **FIG. 1B** is a plasmid comprising nucleic acid molecules encoding proteins that confer chloramphenicol resistance (CamR) and spectinomycin resistance (SpectR). The plasmid also comprises a kanamycin resistance gene disabled by two point mutations. **FIG. 1C** is a plasmid comprising nucleic acid molecules encoding proteins that confer chloramphenicol resistance (CamR) and spectinomycin resistance (SpectR). The plasmid also comprises a kanamycin resistance gene disabled by three point mutations.

FIG. 2 is an image of bacterial colonies transduced with the expression vectors depicted in **FIG. 1**, which included a defective kanamycin resistance gene. The vectors contained ABE7.10 variants that were generated using error prone PCR. Bacterial cells expressing these "evolved" ABE7.10 variants were selected for kanamycin resistance using increasing concentrations of kanamycin. Bacteria expressing ABE7.10 variants having adenosine deaminase activity were capable of correcting the mutations introduced into the kanamycin resistance gene, thereby restoring kanamycin resistance. The kanamycin resistant cells were selected for further analysis.

FIGs. 3A and **3B** illustrate editing of a regulatory region of the hemoglobin subunit gamma (HGB1) locus, which is a therapeutically relevant site for upregulation of fetal hemoglobin. **FIG. 3A** is a drawing of a portion of the regulatory region for the HGB1 gene. **FIG. 3B** quantifies the efficiency and specificity of adenosine deaminase variants. Editing is assayed at the hemoglobin subunit gamma 1 (HGB1) locus in HEK293T cells, which is therapeutically relevant site for upregulation of fetal hemoglobin. The top panel depicts nucleotide residues in the target region of the regulatory sequence of the HGB1 gene. A5, A8, A9, and A11 denote the edited adenosine residues in HGB1.

FIG. 4 illustrates the relative effectiveness of adenosine base editors comprising a dCas9 that recognizes a noncanonical PAM sequence. The top panel depicts the coding sequence of the hemoglobin subunit. The bottom panel is a graph demonstrating the efficiency of adenosine deaminase variant base editors with guide RNAs of varying lengths.

FIG. 5 is a graph illustrating the efficiency and specificity of ABE8 base editors. The percent editing at intended target nucleotides and unintended target nucleotides (bystanders) is quantified.

FIG. 6 is a graph illustrating the efficiency and specificity of ABE8 base editors. The percent editing at intended target nucleotides and unintended target nucleotides (bystanders) is quantified.

FIGs. 7A – 7D depict eighth generation adenine base editors mediate superior A•T to G•C conversion in human cells. **FIG. 7A** illustrates an overview of adenine base editing: i) ABE8 creates an R-loop at a sgRNA-targeted site in the genome; ii) TadA* deaminase chemically converts adenine to inosine via hydrolytic deamination on the ss-DNA portion of the R-loop; iii) D10A nickase of Cas9 nicks the strand opposite of the inosine containing strand; iv) the inosine containing strand can be used as a template during DNA replication; v) inosine preferentially base pairs with cytosine in the context of DNA polymerases; and vi) following replication, inosine may be replaced by guanosine. **FIG. 7B** illustrates the architecture of ABE8.x-m and ABE8.x-d. **FIG. 7C** illustrates three perspectives of the *E. coli* TadA deaminase (PDB 1Z3A) aligned with the *S. aureus* TadA (not shown) complexed with tRNAArg2 (PDB 2B3J). Mutations identified in eighth round of evolution are highlighted. **FIG. 7D** are graphs depicting A•T to G•C base editing efficiencies of core ABE8 constructs relative to ABE7.10 constructs in Hek293T cells across eight genomic sites. Values and error bars reflect the mean and s.d. of three independent biological replicates performed on different days.

FIGS 8A-8C depict Cas9 PAM-variant ABE8s and catalytically dead Cas9 ABE8 variants mediate higher A•T to G•C conversion than corresponding ABE7.10 variants in human cells. Values and error bars reflect the mean and s.d. of three independent biological replicates performed on different days. **FIG. 8A** is a graph depicting A•T to G•C conversion in Hek293T cells with NG-Cas9 ABE8s (-NG PAM). **FIG. 8B** is a graph depiecting A•T to G•C conversion in Hek293T cells with Sa-Cas9 ABE8s (-NNGRRT PAM). **FIG. 8C** is a graph depiecting A•T to G•C conversion in Hek293T cells with catalytically inactivated, dCas9-ABE8s (D10A, H840A in *S. pyogenes* Cas9).

FIGs. 9A-9E depict the comparison between the on- and off-target editing frequencies between ABE7.10, ABEmax and ABEmax with one BPNLS in Hek293T cells. Individual data points are shown and error bars represent s.d. for n=3 independent biological replicates, performed on different days. **FIGs 9A** and **9B** are graphs that depict on-target DNA editing frequencies. **FIGs 9B** and **9C** are graphs that depict sgRNA-guided DNA-off-target editing frequencies. **FIG 9E** is a graph depicting RNA off-target editing frequencies.

FIGs. 10A-10B depict the median A•T to G•C conversion and corresponding INDEL formation of TadA, C-terminal alpha-helix truncation ABE constructs in HEK293T cells. **FIG 10A** is a heat map depicting A•T to G•C median editing conversion across 8 genomic sites. **FIG 10B** is a heat map depicting INDEL formation. Delta residue value corresponds to deletion position in TadA. Median value generated from n=3 biological replicate.

FIG 11 are heat maps depicting the median A•T to G•C conversion of 40 ABE8 constructs in HEK293T cells across 8 genomic sites. Median values were determined from two or greater biological replicates.

FIG. 12 is a heat map depicting median INDEL % of 40 ABE8 constructs in HEK293T cells across 8 genomic sites. Median values were determined from two or greater biological replicates.

FIG. 13 is a graph depicting fold change in editing, ABE8:ABE7. Representation of average ABE8:ABE7 A•T to G•C editing in Hek293T cells across all A positions within the target of eight different genomic sites. Positions 2-12 denote location of a target adenine within the 20-nt protospacer with position 20 directly 5' of the -NGG PAM.

FIG. 14 depicts a dendrogram of ABE8s. Core ABE8 constructs selected for further studies highlighted in in black.

FIG. 15 are heat maps depicting median A•T to G•C conversion of core eight ABE8 constructs in HEK293T cells across 8 genomic sites. Median values were determined from three or greater biological replicates.

FIG. 16 is a heat map depicting median INDEL frequency of core 8 ABE8s tested at 8 genomic sites in HEK293T cells.

FIG. 17 are heat maps depicting median A•T to G•C conversion of core NG-ABE8 constructs 9 (-NG PAM) at six genomic sites in HEK293T cells. Median value generated from n=3 biological replicate.

FIG. 18 is a heat map depicting median INDEL frequency of core NG-ABE8s tested at six genomic sites in HEK293T cells. Median value generated from n=3 biological replicate.

FIG. 19 are heat maps depicting median A•T to G•C conversion of core Sa-ABE8 constructs (-NNGRRT PAM) at six genomic sites in HEK293T cells. Site positions are numbered -2 to 20 (5' to 3') within the 22-nt protospacer. Position 20 is 5' to the NNGRRT PAM. Median value generated from n=3 biological replicate.

FIG. 20 is a heat map depicting **m**edian INDEL frequency of core Sa-ABE8s tested at 8 genomic sites in HEK293T cells. Median value generated from n=3 biological replicate.

FIG. 21 are heat maps depicting median A•T to G•C conversion of core dC9-ABE8-m constructs at eight genomic sites in HEK293T cells. Dead Cas9 (dC9) is defined as D10A and H840A mutations within S. pyogenes Cas9. Median value generated from n≥3 biological replicate.

FIG. 22 are heat maps depicting median A•T to G•C conversion of core dC9-ABE8-d constructs at eight genomic sites in HEK293T cells. Dead Cas9 (dC9) is defined as D10A and H840A mutations within S. pyogenes Cas9. Median value generated from n≥3 biological replicate.

FIGs. 23A and **23B** depict Median INDEL frequency of core dC9-ABE8s tested at 8 genomic sites in HEK293T cells. Median value generated from n≥3 biological replicate.

FIG. 23A is a heat map depicting indel frequency shown for dC9-ABE8-m variants relative to ABE7.10. **FIG. 23B** is a heat map depicting indel frequency shown for dC9-ABE8-d variants relative to ABE7.10.

FIG. 24 is a graph depicting C•G to T•A editing with Hek293T cells treated with ABE8s and ABE7.10. Editing frequencies for each site averaged across all C positions within the target. Cytosines within the protospacer are indicted with shading.

FIGs. 25A-25H depict DNA on-target and sgRNA-dependent DNA off-target editing by ABE8 constructs and ABE8 constructs with TadA mutations to improve specificity for DNA. Individual data points are shown and error bars represent s.d. for n=3 independent biological replicates, performed on different days. **FIGs. 25A** and **25B** are graph depicting

on-target DNA editing frequencies for core ABE8 constructs as compared to ABE7. **FIGs. 25C** and **25D** are graphs depicting on-target DNA editing frequencies for ABE8 with mutations that improve RNA off-target editing. **FIGs. 25E** and **25F** are graphs depicting sgRNA-guided DNA-off-target editing frequencies for core ABE 8 constructs as compared to ABE7. **FIGs. 25G** and **25H** are graphs depicting sgRNA-guided DNA-off-target editing frequencies for ABE 8 constructs with mutations that improve RNA off-target editing.

FIG. 26 is a graph depicting indel frequencies at 12 previously identified sgRNA-dependent Cas9 off-target loci in human cells Individual data points are shown and error bars represent s.d. for n=3 independent biological replicates, performed on different days.

FIGs. 27A and 27**B** depict A•T to G•C conversion and phenotypic outcomes in primary cells. **FIG. 27A** is a graph depicting A•T to G•C conversion at -198 HBG1/2 site in CD34+ cells treated with ABE from two separate donors. NGS analysis conducted at 48 and 144h post treatment. -198 HBG1/2 target sequence shown with A7 highlighted. Percent A•T to G•C plotted for A7. **FIG. 27B** is a graph depicting percentage of γ-globin formed as a fraction of alpha-globin. Values shown from two different donors, post ABE treatment and erythroid differentiation.

FIGs. 28A and 2**8B** depict A•T to G•C conversion of CD34+ cells treated with ABE8 at the -198 promoter site upstream of HBG1/2. **FIG. 28A** is a heat map depicting A to G editing frequency of ABE8s in CD34+ cells from two donors, where Donor 2 is heterozygous for sickle cell disease, at 48 and 144h post editor treatment. **FIG. 28B** is a graphical representation of distribution of total sequencing reads which contain either A7 only edits or combined (A7 + A8) edits.

FIG. 29 is a heat map depicting INDEL frequency of CD34+ cells treated with ABE8 at the -198 site of the gamma-globin promoter. Frequencies shown from two donors at 48h and 144h time points.

FIG. 30 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of untreated differentiated CD34+ cells (donor 1).

FIG. 31 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE7.10-m (donor1)

FIG. 32 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE7.10-d (donor1).

FIG. 33 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.8-m (donor1)

FIG. 34 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.8-d (donor1).

FIG. 35 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.13-m (donor1).

FIG. 36 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.13-d (donor1).

FIG. 37 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.17-m (donor1).

FIG. 38 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.17-d (donor1).

FIG. 39 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.20-m (donor1).

FIG. 40 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.20-d (donor 1).

FIG. 41 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells untreated (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 42 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE7.10-m (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 43 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE7.10-d (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 44 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.8-m (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 45 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.8-d (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 46 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.13-m (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 47 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.13-d (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 48 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.17-m (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 49 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.17-d (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 50 depicts an UHPLC UV-Vis trace (220 nm) and integration of globin chain levels of differentiated CD34+ cells treated with ABE8.20-m (donor 2). Note: donor 2 is heterozygous for sickle cell disease.

FIG. 51A-51E depict editing with ABE8.8 at two independent sites reached over 90% editing on day 11 post erythroid differentiation before enucleation and about 60% of gamma globin over alpha globin or total beta family globin on day 18 post erythroid differentiation. **FIG. 51A** is a graph depicting an average of ABE8.8 editing in 2 healthy donors in 2 independent experiments. Editing efficiency was measured with primers that distinguish HBG1 and HBG2. **FIG. 51B** is a graph depicting an average of 1 healthy donor in 2 independent experiments. Editing efficiency was measured with primers that recognize both HBG1 and HBG2. **FIG. 51C** is a graph depicting editing of ABE8.8 in a donor with heterozygous E6V mutation. **FIGs. 51D** and **51E** are graphs depicting gamma globin increase in the ABE8.8 edited cells.

FIGs. 52A and **52B** depict percent editing using ABE variants to correct sickle cell mutations. **FIG. 52A** is a graph depicting a screen of different editor variants with about 70% editing in SCD patient fibroblasts. **FIG. 52B** is a graph depicting CD34 cells from healthy donors edited with a lead ABE variant, targeting a synonymous mutation A13 in an adjacent proline that resides within the editing window and serves as a proxy for editing the SCD mutation. ABE8 variants showed an average editing frequency around 40% at the proxy A13.

FIGs. 53A and **53B** depict RNA amplicon sequencing to detect cellular A-to-I editing in RNA associated with ABE treatment. Individual data points are shown and error bars represent s.d. for n=3 independent biological replicates, performed on different days.**FIG. 53A** is a graph depicting A-to-I editing frequencies in targeted RNA amplicons for core ABE 8 constructs as compared to ABE7 and Cas9(D10A) nickase control. **FIG. 53B** is a graph

depicting A-to-I editing frequencies in targeted RNA amplicons for ABE8 with mutations that have been reported to improve RNA off-target editing.

FIG. 54 is a schematic diagram illustrating the loss of dopamine that results from the loss of dopaminergic neurons in Parkinson Disease.

FIG. 55 is a schematic diagram showing a guide RNA and target sequences for the correction of R1441C and R1441H mutations in LRRK2 associated with Parkinson's Disease.

FIG. 56 is a schematic diagram showing target sequences for correction of the Y1699C, G2019S, and I2020 mutations in LRRK2 associated with Parkinson's Disease.

FIG. 57A-57C provides a graph, a schematic diagram, and a table. **FIG. 57A** quantifies the percent conversion of A to G at nucleic acid position 7 of the LRRK2 target sequence. The editors used are designated PV1-PV14, a description of this which is provided below. pCMV designates the CMV promoter; bpNLS designates a bipartite Nuclear Localization Signal; monoABE8.1 designates a monomeric form of the ABE8.1 base editor. **FIG. 57B** depicts target sequences and guide RNA for correction of the R1441C mutation in LRRK2 associated with Parkinson's Disease. **FIG. 57C** shows the percent conversion of A to G at nucleic acid position 7 of the LRRK2 target sequence. Editors PV1-14 were used to edit LRRK2 R1441C. Editors (15-28) were used to edit G2109. The editors (PV1-28) used for correction of the LRRK2 mutations follows:

PV1 (also termed PV15). pCMV_monoABE8.1_bpNLS + Y147T
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD

PV2 (also termed PV16). pCMV_monoABE8.1_bpNLS + Y147R
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCRFFRMPRQVFNAQKKAQSSTD

PV3 (also termed PV17). pCMV_monoABE8.1_bpNLS + Q154S
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRSVFNAQKKAQSSTD

PV4 (also termed PV18). pCMV_monoABE8.1_bpNLS + Y123H
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHHPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

PV5 (also termed PV19). pCMV_monoABE8.1_bpNLS + V82S
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYSTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

PV6 (also termed PV20). pCMV_monoABE8.1_bpNLS + T166R
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSRD

PV7 (also termed PV21). pCMV_monoABE8.1_bpNLS + Q154R
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRRVFNAQKKAQSSTD

PV8 (also termed PV22). pCMV_monoABE8.1_bpNLS + Y147R_Q154R_Y123H
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTD

PV9 (also termed PV23). pCMV_monoABE8.1_bpNLS + Y147R_Q154R_I76Y
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLYDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAK
TGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSST
D

PV10 (also termed PV24). pCMV_monoABE8.1_bpNLS + Y147R_Q154R_T166R
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSRD

PV11 (also termed PV25). pCMV_monoABE8.1_bpNLS + Y147T_Q154R
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCTFFRMPRRVFNAQKKAQSSTD

PV12 (also termed PV26). pCMV_monoABE8.1_bpNLS + Y147T_Q154S
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCTFFRMPRSVFNAQKKAQSSTD

PV13 (also termed PV27). pCMV_monoABE8.1_bpNLS +
H123Y123H_Y147R_Q154R_I76Y
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLYDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAK
TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSST
D

PV14 (also termed PV28). pCMV_monoABE8.1_bpNLS + V82S + Q154R
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYSTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRRVFNAQKKAQSSTD

FIG. 58A-58C provides a graph, a schematic diagram, and a table. **FIG. 58A** quantifies the percent conversion of A to G at nucleic acid position 6 of the LRRK2 target sequence. The editors used are designated PV15-PV28, a description of this which is provided above. pCMV designates the CMV promoter; bpNLS designates a bipartite Nuclear Localization Signal; monoABE8.1 designates a monomeric form of the ABE8.1 base editor. **FIG. 58B** depicts target sequences and guide RNAs for correction of the G2019S mutation in LRRK2 associated with Parkinson's Disease. **FIG. 58C** shows the percent conversion of A to G at nucleic acid positions 4 and 6 of the LRRK2 target sequence. The A to G transition at position 4 is a bystander effect.

FIGS. 59A-59L depicts the sequence reads for the A to G transition at position 7 of the LRRK2 target sequence, which encodes R1441C (See **FIG. 57A-57C**). The editor is indicated (PV1-14). A description of PV1-28 is provided **at FIG. 56**.

FIGS. 60A-60W depicts the sequence reads for the A to G transition at positions 4 and 6 of the LRRK2 target sequence, which encodes G2019S (See **FIG. 58A-58C**).

FIG. 61A provides a schematic diagram depicting the target sequence for correction of a pathogenic mutation A419V in LRRK2, which is encoded by an antisense strand G>A mutation. The mutation is corrected using an ABE targeting the A at position 12 using an SpCas9 variant that has specificity for a TGG PAM.

FIG. 61B provides a schematic diagram depicting the target sequence for correction of a pathogenic mutation L1114L in LRRK2, which is associated with Parkinson Disease. The mutation is an antisense strand T>C, which is corrected using a base editor having cytidine deaminase activity (CBE).

FIG. 61C provides a schematic diagram depicting the target sequence for correction of a pathogenic mutation I1122V in LRRK2, which is associated with Parkinson Disease. The mutation is an antisense strand T>C, which is corrected using a base editor having cytidine deaminase activity (CBE).

FIG.61D provides a schematic diagram depicting the target sequence for correction of a pathogenic mutation M1869V in LRRK2, which is associated with Parkinson Disease. The mutation is an antisense strand T>C, which is corrected using a base editor having cytidine deaminase activity (CBE).

FIGS. 62A and **62B** depict the precise base editing correction of the *Mus musculus* IDUA W401X mutation in HEK293T cells. **FIG. 62A** is a graph depicting the percentage of base editing of the *Mus musculus* IDUA W401X mutation using ABE8 base editor variants using a 21-nucleotide guide RNA. **FIG. 62B** is a graph depicting the percent indels for the ABE8 base editor variants using a 21-nucleotide guide RNA.

FIG. 63 is a graph depicting the percentage of base editing of the *Mus musculus* IDUA W401X mutation using ABE8 base editor variants using either a 20-nucleotide guide RNA or a 21-nucleotide guide RNA.

FIG. 64 depicts a diagramic illustration of the *Homo sapiens* IDUA genomic nucleic acid and amino acid sequence as a target for A-to-G nucleotide base editing to correct the W402X mutation. Also shown in the figure is the nucleic acid sequence of a corresponding guide RNA (gRNA). Noted in the figure is the target adenosine (A) nucleobase (boxed) in the IDUA nucleic acid sequence.

FIGS. 65A and **65B** depict the precise base editing correction of the *Homo sapiens* IDUA W402X mutation in HEK293T cells. **FIG. 65A** is a graph depicting the percentage of

base editing of the *Homo sapiens* IDUA W402X mutation using ABE8 base editor variants using a 20-nucleotide guide RNA. **FIG. 65B** is a graph depicting the percent indels for the ABE8 base editor variants using a 20-nucleotide guide RNA.

FIGS. 66A through **66O** are tables depicting the efficiency of percentage of A-to-G nucleotide change in the IDUA nucleic acid sequence using ABE8 base editor variants, as detected by deep sequencing (MySeq) following PCR of the genomic DNA in cells in which base editing had occurred. **FIGS. 66A** through **66M** depict the percent of A to G base editing at position 6 in the IDUA nucleic acid target site using three samples of each ABE8 base editor variants ABE8.1 through ABE8.13, respectively. **FIG. 66N** depicts the percent of A to G base editing at position 6 in the IDUA nucleic acid target site using three samples of positive control base editor ABE7.10. **FIG. 66O** depicts the percent of A to G base editing at position 6 in the IDUA nucleic acid target site using two samples of negative control.

FIG. 67 illustrates Rett/MECP2: Mutation correction. MECP2 loss of function – can result from many different *de novo* mutations. X-linked: XX patients are mosaic for MECP2 loss; XY usually results in infant mortality.

FIG. 68 illustrates Rett Syndrome R106W mutation correction for top 3 guide sequences.

FIG. 69 illustrates Rett Syndrome R255X mutation correction with editors having NGTT PAM optimization.

FIGS. 70A-C: Hurler/IDUA mutation correction. **FIG. 70A** illustrates experiment design of IDUA W402X mutation correction. **FIG. 70B** illustrates the percent editing for each editor construct. **FIG. 70C** illustrates specific activity (nmol/mg/h) for edited and unedited constructs.

FIG. 71 depicts *In vivo* base editing with ABE 8.8. From left to right for each of each sample: Guide 11 (AAV9), Guide 12 (AAV9), Guide 11 (PHP.eB), Guide 12 (PHP.eB), and Control.

FIGS. 72A-72B. **A•T to G•C conversion by ABE7.10 and ABE8 variants at the *ABCA4* G1961E allele in a model cell line. FIG. 72A:** A•T to G•C conversion in HEK293T cells at an integrated disease allele and wobble base of the *ABCA4* G1961E codon after plasmid lipofection of the 21-nt spacer sgRNA and base editor variant. Cells incubated for 5 days after lipofection and were then assessed for editing. **FIG. 72B**: The DNA sequence at the site of interest including the *ABCA4* G1961E disease allele, the wobble base of the codon, and the -NGG PAM used by the 21-nt spacer sgRNA. Error bars represent the s.d. of

three replicates. In each data set, the disease allele is on the left and the wobble base is on the right.

FIG. 73. A•T to G•C conversion by sgRNA spacer-length variants at the *ABCA4* G1961E allele in a model cell line. A•T to G•C conversion in HEK293T cells at an integrated disease allele and wobble base of the *ABCA4* G1961E codon after plasmid lipofection of the sgRNA of varied spacer lengths and ABE7.10. Cells incubated for 5 days after lipofection and were then assessed for editing. hRz = inclusion of a self-cleaving hammer head ribozyme at the 5'-end of the sgRNA. Error bars represent the s.d. of three replicates. In each data set, the disease allele is on the left and the wobble base is on the right.

FIG. 74. Schematic of the dual AAV delivery of a split base editor using split intein reconstitution. Two AAV particles are packaged separately with the components required for base editing. One virus encodes the C-terminal region of the base editor with an N-terminal split intein fusion, and a complementary virus encodes the N-terminal region of the base editor with a C-terminal split intein fusion as well as the sgRNA. Upon co-transduction of the complementary viruses, the sgRNA is transcribed and each half of the base editor is expressed and recombined through protein trans-splicing via the split intein.

FIGS. 75A-75B. A•T to G•C conversion by dual AAV delivery of split ABE variants at the *ABCA4* G1961 in wild type cells. FIG. 75A: A•T to G•C and C•G to T•A conversion in wild type ARPE-19 cells at the wild type *ABCA4* G1961 target site, in which editing at position 8A serves as a surrogate target for editing in these cells. Cells infected at a MOI of 5E+4 viral genomes per virus per cell. Cells were incubated for 2 weeks post infection and were then assessed for editing. Error bars represent the s.d. of six replicates. For each data point, samples treated with Pos. 8 (A>G)- surrogate site are shown on the left and Pos. 5 (C>T) are shown on the right.

FIG. 75B: The DNA sequence at the wild type target site including the *ABCA4* G1961 allele and the -NGG PAM used by the 21-nt spacer sgRNA targeting the wild type sequence.

FIGS. 76A-76B. Off target base editing in wild type ARPE-19 cells dual infected with AAV2 expressing split ABE7.10 and sgRNA targeting the disease allele of *ABCA4* G1961E. **FIG. 76A**: Maximum A•T to G•C conversion across the target or off-target protospacers 2 weeks after co-infection with the dual AAV (teal) compared to untreated controls (gray). **FIG. 76B**: Maximum non-A•T to G•C conversion across the target

or off-target protospacers 2 weeks after co-infection with the dual AAV (teal) compared to untreated controls (gray). For each data point, samples treated with wild type (wt) ARPE-19 cells are shown on the left and untreated wt ARPE-19 cells are shown on the right.

FIG. 77. Indel formation due to base editing in wild type ARPE-19 cells dual infected with AAV2 expressing split ABE7.10 and sgRNA targeting the disease allele of *ABCA4* G1961E. Percentage of indels formed within or proximal to the target or off-target protospacers 2 weeks after co-infection with the dual AAV (teal) compared to untreated controls (gray). For each data point, samples treated with wild type (wt) ARPE-19 cells are shown on the left and untreated wt ARPE-19 cells are shown on the right.

FIG. 78: Primate Retina Integrity and GFP expression at Day 22 post-culture. Sections were immunolabeled with anti-Rhodopsin, anti-GFP, and biotinylated peanut agglutinin antibodies overnight at 4°C. Anc80L65.hGRK.eGFP showed GFP to be observed exclusively in the photoreceptor-containing outer nuclear layer (ONL) confirming photoreceptor-specific activity of the GRK promoter. Top row is Day 0, untransduced. The second row is Day 22, untransduced. Third row is Day 22, GRK. Fourth row is Day 22, CMB. Columns are unstained (1st column), DAPI (2nd column), GFP (3rd column), PNA (4th column), and rhodopsin (5th column).

FIG. 79: Cas9 Expression in NHP. Cas9 expression is detected in primate retina as early as day 6 post-culture. Results are shown for ABE7.10 (columns 1 and 2), ABE8.5 (columns 2 and 3), and ABE8.9 (columns 3 and 4). Top row: day 6 post-culture. Bottom row: day 17 post-culture. The results demonstrate that the AAV system delivers split-inteins that express Cas9. Scale Bar: 100 μm

DETAILED DESCRIPTION OF THE INVENTION

The invention provides compositions comprising novel adenine base editors (*e.g.*, ABE8) that have increased efficiency and methods of using them to generate modifications in target nucleobase sequences.

NUCLEOBASE EDITOR

Disclosed herein is a base editor or a nucleobase editor for editing, modifying or altering a target nucleotide sequence of a polynucleotide. Described herein is a nucleobase editor or a base editor comprising a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9) and a nucleobase editing domain (*e.g.*, adenosine deaminase). A polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9), when in conjunction with a bound guide polynucleotide (*e.g.*, gRNA), can specifically bind to a target polynucleotide sequence (*i.e.*, via complementary base pairing between bases of the bound guide nucleic acid and

bases of the target polynucleotide sequence) and thereby localize the base editor to the target nucleic acid sequence desired to be edited. In some embodiments, the target polynucleotide sequence comprises single-stranded DNA or double-stranded DNA. In some embodiments, the target polynucleotide sequence comprises RNA. In some embodiments, the target polynucleotide sequence comprises a DNA-RNA hybrid.

Polynucleotide Programmable Nucleotide Binding Domain

It should be appreciated that polynucleotide programmable nucleotide binding domains can also include nucleic acid programmable proteins that bind RNA. For example, the polynucleotide programmable nucleotide binding domain can be associated with a nucleic acid that guides the polynucleotide programmable nucleotide binding domain to an RNA. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, though they are not specifically listed in this disclosure.

A polynucleotide programmable nucleotide binding domain of a base editor can itself comprise one or more domains. For example, a polynucleotide programmable nucleotide binding domain can comprise one or more nuclease domains. In some embodiments, the nuclease domain of a polynucleotide programmable nucleotide binding domain can comprise an endonuclease or an exonuclease. Herein the term "exonuclease" refers to a protein or polypeptide capable of digesting a nucleic acid (*e.g.*, RNA or DNA) from free ends, and the term "endonuclease" refers to a protein or polypeptide capable of catalyzing (*e.g.*, cleaving) internal regions in a nucleic acid (*e.g.*, DNA or RNA). In some embodiments, an endonuclease can cleave a single strand of a double-stranded nucleic acid. In some embodiments, an endonuclease can cleave both strands of a double-stranded nucleic acid molecule. In some embodiments a polynucleotide programmable nucleotide binding domain can be a deoxyribonuclease. In some embodiments a polynucleotide programmable nucleotide binding domain can be a ribonuclease.

In some embodiments, a nuclease domain of a polynucleotide programmable nucleotide binding domain can cut zero, one, or two strands of a target polynucleotide. In some embodiments, the polynucleotide programmable nucleotide binding domain can comprise a nickase domain. Herein the term "nickase" refers to a polynucleotide programmable nucleotide binding domain comprising a nuclease domain that is capable of cleaving only one strand of the two strands in a duplexed nucleic acid molecule (*e.g.*, DNA). In some embodiments, a nickase can be derived from a fully catalytically active (*e.g.*, natural) form of a polynucleotide programmable nucleotide binding domain by introducing one or more mutations into the active polynucleotide programmable nucleotide binding domain. For

example, where a polynucleotide programmable nucleotide binding domain comprises a nickase domain derived from Cas9, the Cas9-derived nickase domain can include a D10A mutation and a histidine at position 840. In such embodiments, the residue H840 retains catalytic activity and can thereby cleave a single strand of the nucleic acid duplex. In another example, a Cas9-derived nickase domain can comprise an H840A mutation, while the amino acid residue at position 10 remains a D. In some embodiments, a nickase can be derived from a fully catalytically active (*e.g.*, natural) form of a polynucleotide programmable nucleotide binding domain by removing all or a portion of a nuclease domain that is not required for the nickase activity. For example, where a polynucleotide programmable nucleotide binding domain comprises a nickase domain derived from Cas9, the Cas9-derived nickase domain can comprise a deletion of all or a portion of the RuvC domain or the HNH domain.

The amino acid sequence of an exemplary catalytically active Cas9 is as follows:

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI

REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD.

A base editor comprising a polynucleotide programmable nucleotide binding domain comprising a nickase domain is thus able to generate a single-strand DNA break (nick) at a specific polynucleotide target sequence (*e.g.*, determined by the complementary sequence of a bound guide nucleic acid). In some embodiments, the strand of a nucleic acid duplex target polynucleotide sequence that is cleaved by a base editor comprising a nickase domain (*e.g.*, Cas9-derived nickase domain) is the strand that is not edited by the base editor (*i.e.*, the strand that is cleaved by the base editor is opposite to a strand comprising a base to be edited). In other embodiments, a base editor comprising a nickase domain (*e.g.*, Cas9-derived nickase domain) can cleave the strand of a DNA molecule which is being targeted for editing. In such embodiments, the non-targeted strand is not cleaved.

Also provided herein are base editors comprising a polynucleotide programmable nucleotide binding domain which is catalytically dead (*i.e.*, incapable of cleaving a target polynucleotide sequence). Herein the terms "catalytically dead" and "nuclease dead" are used interchangeably to refer to a polynucleotide programmable nucleotide binding domain which has one or more mutations and/or deletions resulting in its inability to cleave a strand of a nucleic acid. In some embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain base editor can lack nuclease activity as a result of specific point mutations in one or more nuclease domains. For example, in the case of a base editor comprising a Cas9 domain, the Cas9 can comprise both a D10A mutation and an H840A mutation. Such mutations inactivate both nuclease domains, thereby resulting in the loss of nuclease activity. In other embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain can comprise one or more deletions of all or a portion of a catalytic domain (*e.g.*, RuvC1 and/or HNH domains). In further embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain comprises a point mutation (*e.g.*, D10A or H840A) as well as a deletion of all or a portion of a nuclease domain.

Also contemplated herein are mutations capable of generating a catalytically dead polynucleotide programmable nucleotide binding domain from a previously functional version of the polynucleotide programmable nucleotide binding domain. For example, in the case of catalytically dead Cas9 ("dCas9"), variants having mutations other than D10A and H840A are provided, which result in nuclease inactivated Cas9. Such mutations, by way of example, include other amino acid substitutions at D10 and H840, or other substitutions within the nuclease domains of Cas9 (*e.g.*, substitutions in the HNH nuclease subdomain

and/or the RuvC1 subdomain). Additional suitable nuclease-inactive dCas9 domains can be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure. Such additional exemplary suitable nuclease-inactive Cas9 domains include, but are not limited to, D10A/H840A, D10A/D839A/H840A, and D10A/D839A/H840A/N863A mutant domains (*See*, *e.g.*, Prashant *et al.*, CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nature Biotechnology*. 2013; 31(9): 833-838, the entire contents of which are incorporated herein by reference).

Non-limiting examples of a polynucleotide programmable nucleotide binding domain which can be incorporated into a base editor include a CRISPR protein-derived domain, a restriction nuclease, a meganuclease, TAL nuclease (TALEN), and a zinc finger nuclease (ZFN). In some embodiments, a base editor comprises a polynucleotide programmable nucleotide binding domain comprising a natural or modified protein or portion thereof which via a bound guide nucleic acid is capable of binding to a nucleic acid sequence during CRISPR (*i.e.*, Clustered Regularly Interspaced Short Palindromic Repeats)-mediated modification of a nucleic acid. Such a protein is referred to herein as a "CRISPR protein." Accordingly, disclosed herein is a base editor comprising a polynucleotide programmable nucleotide binding domain comprising all or a portion of a CRISPR protein (*i.e.* a base editor comprising as a domain all or a portion of a CRISPR protein, also referred to as a "CRISPR protein-derived domain" of the base editor). A CRISPR protein-derived domain incorporated into a base editor can be modified compared to a wild-type or natural version of the CRISPR protein. For example, as described below a CRISPR protein-derived domain can comprise one or more mutations, insertions, deletions, rearrangements and/or recombinations relative to a wild-type or natural version of the CRISPR protein.

CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems, correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, and then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding

and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA," or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. *See, e.g.*, Jinek M., *et al.*, Science 337:816-821(2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self.

In some embodiments, the methods described herein can utilize an engineered Cas protein. A guide RNA (gRNA) is a short synthetic RNA composed of a scaffold sequence necessary for Cas-binding and a user-defined ~20 nucleotide spacer that defines the genomic target to be modified. Thus, a skilled artisan can change the genomic target of the Cas protein specificity is partially determined by how specific the gRNA targeting sequence is for the genomic target compared to the rest of the genome.

In some embodiments, the gRNA scaffold sequence is as follows: GUUUUAGAGC UAGAAAUAGC AAGUUAAAAU AAGGCUAGUC CGUUAUCAAC UUGAAAAAGU GGCACCGAGU CGGUGCUUUU.

In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is an endonuclease (*e.g.*, deoxyribonuclease or ribonuclease) capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is a nickase capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is a catalytically dead domain capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a target polynucleotide bound by a CRISPR protein derived domain of a base editor is DNA. In some embodiments, a target polynucleotide bound by a CRISPR protein-derived domain of a base editor is RNA.

Cas proteins that can be used herein include class 1 and class 2. Non-limiting examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 or Csx12), Cas10, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i, CARF, DinG, homologues thereof, or modified versions thereof. An unmodified CRISPR enzyme can have DNA

cleavage activity, such as Cas9, which has two functional endonuclease domains: RuvC and HNH. A CRISPR enzyme can direct cleavage of one or both strands at a target sequence, such as within a target sequence and/or within a complement of a target sequence. For example, a CRISPR enzyme can direct cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence.

A vector that encodes a CRISPR enzyme that is mutated to with respect, to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence can be used. Cas9 can refer to a polypeptide with at least or at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild-type exemplary Cas9 polypeptide (*e.g.*, Cas9 from *S. pyogenes*). Cas9 can refer to a polypeptide with at most or at most about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild-type exemplary Cas9 polypeptide (*e.g.*, from *S. pyogenes*). Cas9 can refer to the wild-type or a modified form of the Cas9 protein that can comprise an amino acid change such as a deletion, insertion, substitution, variant, mutation, fusion, chimera, or any combination thereof.

In some embodiments, a CRISPR protein-derived domain of a base editor can include all or a portion of Cas9 from *Corynebacterium ulcerans* (NCBI Refs: NC_015683.1, NC_017317.1); *Corynebacterium diphtheria* (NCBI Refs: NC_016782.1, NC_016786.1); *Spiroplasma syrphidicola* (NCBI Ref: NC_021284.1); *Prevotella intermedia* (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref: NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus torquis* (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1); *Listeria innocua* (NCBI Ref: NP_472073.1); *Campylobacter jejuni* (NCBI Ref: YP_002344900.1); *Neisseria meningitidis* (NCBI Ref: YP_002342100.1), *Streptococcus pyogenes*, or *Staphylococcus aureus*.

Cas9 domains of Nucleobase Editors

Cas9 nuclease sequences and structures are well known to those of skill in the art (*See, e.g.*, "Complete genome sequence of an Ml strain of *Streptococcus pyogenes*." Ferretti *et al.*, Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., *et al.*, Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial

immunity." Jinek M., *et al.*, Science 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference.

In some embodiments, a nucleic acid programmable DNA binding protein (napDNAbp) is a Cas9 domain. Non-limiting, exemplary Cas9 domains are provided herein. The Cas9 domain may be a nuclease active Cas9 domain, a nuclease inactive Cas9 domain (dCas9), or a Cas9 nickase (nCas9). In some embodiments, the Cas9 domain is a nuclease active domain. For example, the Cas9 domain may be a Cas9 domain that cuts both strands of a duplexed nucleic acid (*e.g.*, both strands of a duplexed DNA molecule). In some embodiments, the Cas9 domain comprises any one of the amino acid sequences as set forth herein. In some embodiments the Cas9 domain comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth herein. In some embodiments, the Cas9 domain comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more or more mutations compared to any one of the amino acid sequences set forth herein. In some embodiments, the Cas9 domain comprises an amino acid sequence that has at least 10, at least 15, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, or at least 1200 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth herein.

In some embodiments, proteins comprising fragments of Cas9 are provided. For example, in some embodiments, a protein comprises one of two Cas9 domains: (1) the gRNA binding domain of Cas9; or (2) the DNA cleavage domain of Cas9. In some embodiments, proteins comprising Cas9 or fragments thereof are referred to as "Cas9 variants." A Cas9 variant shares homology to Cas9, or a fragment thereof. For example, a Cas9 variant is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least

about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to wild-type Cas9. In some embodiments, the Cas9 variant may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more amino acid changes compared to wild-type Cas9. In some embodiments, the Cas9 variant comprises a fragment of Cas9 (*e.g.*, a gRNA binding domain or a DNA-cleavage domain), such that the fragment is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of wild-type Cas9. In some embodiments, the fragment is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild-type Cas9. In some embodiments, the fragment is at least 100 amino acids in length. In some embodiments, the fragment is at least 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, or at least 1300 amino acids in length.

In some embodiments, Cas9 fusion proteins as provided herein comprise the full-length amino acid sequence of a Cas9 protein, *e.g.*, one of the Cas9 sequences provided herein. In other embodiments, however, fusion proteins as provided herein do not comprise a full-length Cas9 sequence, but only one or more fragments thereof. Exemplary amino acid sequences of suitable Cas9 domains and Cas9 fragments are provided herein, and additional suitable sequences of Cas9 domains and fragments will be apparent to those of skill in the art.

A Cas9 protein can associate with a guide RNA that guides the Cas9 protein to a specific DNA sequence that has complementary to the guide RNA. In some embodiments, the polynucleotide programmable nucleotide binding domain is a Cas9 domain, for example a nuclease active Cas9, a Cas9 nickase (nCas9), or a nuclease inactive Cas9 (dCas9).

Examples of nucleic acid programmable DNA binding proteins include, without limitation, Cas9 (*e.g.*, dCas9 and nCas9), CasX, CasY, Cpf1, Cas12b/C2C1, and Cas12c/C2C3.

In some embodiments, wild-type Cas9 corresponds to Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_017053.1, nucleotide and amino acid sequences as follows).

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGAT
CACTGATGATTATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACA
GTATCAAAAAAAATCTTATAGGGGCTCTTTTATTTGGCAGTGGAGAGACAGCGGAAGCGACT
CGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACA
GGAGATTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGT
CTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGAT
GAAGTTGCTTATCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGCAGATTC
TACTGATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTG
GTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAAACTATTTATC
CAGTTGGTACAAATCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTAGAGTAGA
TGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTC
AGCTCCCCGGTGAGAAGAGAAATGGCTTGTTTGGGAATCTCATTGCTTTGTCATTGGGATTG
ACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGA
TACTTACGATGATGATTTAGATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGT
TTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATAGT
GAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAGCGCTACGATGAACATCATCAAGA
CTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTATAAAGAAATCTTTT
TTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCCAAGAAGAATTT
TATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACT
AAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCATCAAA
TTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAA
GACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATT
GGCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCAT
GGAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACA
AACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTA
TTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTACTGAGGGAATGCGAAAACCAG
CATTTCTTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAA
GTAACCGTTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGA
AATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAGGCGCCTACCATGATTTGCTAAAAA
TTATTAAAGATAAAGATTTTTTGGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTT
TTAACATTGACCTTATTTGAAGATAGGGGGATGATTGAGGAAAGACTTAAAACATATGCTCA
CCTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTGGTTGGGGACGTT
TGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAATATTAGATTTT
TTGAAATCAGATGGTTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATAGTTTGAC

ATTTAAAGAAGATATTCAAAAAGCACAGGTGTCTGGACAAGGCCATAGTTTACATGAACAGA
TTGCTAACTTAGCTGGCAGTCCTGCTATTAAAAAAGGTATTTTACAGACTGTAAAAATTGTT
GATGAACTGGTCAAAGTAATGGGGCATAAGCCAGAAAATATCGTTATTGAAATGGCACGTGA
AAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAGAAG
GTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTCAATTGCAA
AATGAAAAGCTCTATCTCTATTATCTACAAAATGGAAGAGACATGTATGTGGACCAAGAATT
AGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTTCATTAAAG
ACGATTCAATAGACAATAAGGTACTAACGCGTTCTGATAAAAATCGTGGTAAATCGGATAAC
GTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGCCAA
GTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTGAAC
TTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTG
GCACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCGAGA
GGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAATTCT
ATAAAGTACGTGAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTCGTT
GGAACTGCTTTGATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAA
AGTTTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAA
AATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAATGGA
GAGATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGATAA
AGGGCGAGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGA
AAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCGGAC
AAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATATGGTGGTTTTGATAGTCCAAC
GGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAAAAT
CCGTTAAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAAATCCGATT
GACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGACTTAATCATTAAACTACCTAA
ATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAATTAC
AAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGTCAT
TATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTTGTGGAGCAGCA
TAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGTGTTATTTTAG
CAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAACCAATACGT
GAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGCTTT
TAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAGATG
CCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTA
GGAGGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQIYNQLFEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNS
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDRGMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGHSLHEQIANLAGSPAIKKGILQTVKIV
DELVKVMGHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFIKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

(single underline: HNH domain; double underline: RuvC domain)

In some embodiments, wild-type Cas9 corresponds to, or comprises the following nucleotide and/or amino acid sequences:

ATGGATAAAAAGTATTCTATTGGTTTAGACATCGGCACTAATTCCGTTGGATGGGCTGTCAT
AACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTCATT
CGATTAAAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGGCGACT
CGCCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTTACTTACA
AGAAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTTGGAAGAGT
CCTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATCTTTGGAAACATAGTAGAT
GAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTCAGAAAAAAGCTAGTTGACTC
AACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTGCCCATATGATAAAGTTCCGTG

GGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTCGGATGTCGACAAACTGTTCATC
CAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCTATAAATGCAAGTGGCGTGGA
TGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAATCCCGACGGCTAGAAAACCTGATCGCAC
AATTACCCGGAGAGAAGAAAAATGGGTTGTTCGGTAACCTTATAGCGCTCTCACTAGGCCTG
ACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAAGATGCCAAATTGCAGCTTAGTAAGGA
CACGTACGATGACGATCTCGACAATCTACTGGCACAAATTGGAGATCAGTATGCGGACTTAT
TTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCCTATCTGACATACTGAGAGTTAATACT
GAGATTACCAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGTACGATGAACATCACCAAGA
CTTGACACTTCTCAAGGCCCTAGTCCGTCAGCAACTGCCTGAGAAATATAAGGAAATATTCT
TTGATCAGTCGAAAAACGGGTACGCAGGTTATATTGACGGCGGAGCGAGTCAAGAGGAATTC
TACAAGTTTATCAAACCCATATTAGAGAAGATGGATGGGACGGAAGAGTTGCTTGTAAAACT
CAATCGCGAAGATCTACTGCGAAAGCAGCGGACTTTCGACAACGGTAGCATTCCACATCAAA
TCCACTTAGGCGAATTGCATGCTATACTTAGAAGGCAGGAGGATTTTTATCCGTTCCTCAAA
GACAATCGTGAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTACTATGTGGGACCCCT
GGCCCGAGGGAACTCTCGGTTCGCATGGATGACAAGAAAGTCCGAAGAAACGATTACTCCAT
GGAATTTTGAGGAAGTTGTCGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACC
AACTTTGACAAGAATTTACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTA
TTTCACAGTGTACAATGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCG
CCTTTCTAAGCGGAGAACAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAA
GTGACAGTTAAGCAATTGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGA
GATCTCCGGGGTAGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGA
TAATTAAAGATAAGGACTTCCTGGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTG
TTGACTCTTACCCTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCA
CCTGTTCGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGAT
TGTCGCGGAAACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATTTT
CTAAAGAGCGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTTAAC
CTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACGAACATA
TTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCAAAGTAGTG
GATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACATTGTAATCGAGATGGCACG
CGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGGATGAAGAGAATAGAAG
AGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGGAAAATACCCAATTG
CAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACATGTATGTTGATCAGGA
ACTGGACATAAACCGTTTATCTGATTACGACGTCGATCACATTGTACCCCAATCCTTTTTGA
AGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAAGAACCGAGGGAAAAGTGAC

AATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACTATTGGCGGCAGCTCCTAAATGC
GAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCTG
AACTTGACAAGGCCGGATTTATTAAACGTCAGCTCGTGGAAACCCGCCAAATCACAAAGCAT
GTTGCACAGATACTAGATTCCCGAATGAATACGAAATACGACGAGAACGATAAGCTGATTCG
GGAAGTCAAAGTAATCACTTTAAAGTCAAAATTGGTGTCGGACTTCAGAAAGGATTTTCAAT
TCTATAAAGTTAGGGAGATAAATAACTACCACCATGCGCACGACGCTTATCTTAATGCCGTC
GTAGGGACCGCACTCATTAAGAAATACCCGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTA
CAAAGTTTATGACGTCCGTAAGATGATCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAG
CCAAATACTTCTTTTATTCTAACATTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAAC
GGAGAGATACGCAAACGACCTTTAATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGA
TAAGGGCCGGGACTTCGCGACGGTGAGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAA
AGAAAACTGAGGTGCAGACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGT
GATAAGCTCATCGCTCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCC
TACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGA
AGTCAGTCAAAGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCC
ATCGACTTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACC
AAAGTATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGC
TTCAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCC
CATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAGCA
GCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTCATCC
TAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAACCCATA
CGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGCCGC
ATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAG
ACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGATTTGTCACAG
CTTGGGGGTGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAAGACCATGA
CGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGGCTGCAGGA

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK

DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

(single underline: HNH domain; double underline: RuvC domain).

In some embodiments, wild-type Cas9 corresponds to Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_002737.2 (nucleotide sequence as follows); and Uniprot Reference Sequence: Q99ZW2 (amino acid sequence as follows):

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGAT
CACTGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACA
GTATCAAAAAAAATCTTATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGACT
CGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACA
GGAGATTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGT
CTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGAT
GAAGTTGCTTATCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGTAGATTC
TACTGATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTG
GTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAAACTATTTATC
CAGTTGGTACAAACCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGA
TGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTC
AGCTCCCCGGTGAGAAGAAAAATGGCTTATTTGGGAATCTCATTGCTTTGTCATTGGGTTTG
ACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGA
TACTTACGATGATGATTTAGATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGT

TTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATACT
GAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCATCAAGA
CTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTATAAAGAAATCTTTT
TTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCCAAGAAGAATTT
TATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACT
AAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCATCAAA
TTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAA
GACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATT
GGCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCAT
GGAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACA
AACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTA
TTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTACTGAAGGAATGCGAAAACCAG
CATTTCTTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAA
GTAACCGTTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGA
AATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAGGTACCTACCATGATTTGCTAAAAA
TTATTAAAGATAAAGATTTTTTGGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTT
TTAACATTGACCTTATTTGAAGATAGGGAGATGATTGAGGAAAGACTTAAAACATATGCTCA
CCTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTGGTTGGGGACGTT
TGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAATATTAGATTTT
TTGAAATCAGATGGTTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATAGTTTGAC
ATTTAAAGAAGACATTCAAAAAGCACAAGTGTCTGGACAAGGCGATAGTTTACATGAACATA
TTGCAAATTTAGCTGGTAGCCCTGCTATTAAAAAAGGTATTTTACAGACTGTAAAAGTTGTT
GATGAATTGGTCAAAGTAATGGGGCGGCATAAGCCAGAAAATATCGTTATTGAAATGGCACG
TGAAAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAG
AAGGTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTCAATTG
CAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAAGAGACATGTATGTGGACCAAGA
ATTAGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTTCCTTA
AAGACGATTCAATAGACAATAAGGTCTTAACGCGTTCTGATAAAAATCGTGGTAAATCGGAT
AACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGC
CAAGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTG
AACTTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCAT
GTGGCACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCG
AGAGGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAAT
TCTATAAAGTACGTGAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTC

GTTGGAACTGCTTTGATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTA
TAAAGTTTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCG
CAAAATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAAT
GGAGAGATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGA
TAAAGGGCGAGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCA
AGAAAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCG
GACAAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATATGGTGGTTTTGATAGTCC
AACGGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAA
AATCCGTTAAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAAATCCG
ATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGACTTAATCATTAAACTACC
TAAATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAAT
TACAAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGT
CATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTTGTGGAGCA
GCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGTGTTATTT
TAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAACCAATA
CGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGC
TTTTAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAG
ATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAG
CTAGGAGGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD

NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD (single underline: HNH domain; double underline: RuvC domain)

In some embodiments, Cas9 refers to Cas9 from: *Corynebacterium ulcerans* (NCBI Refs: NC_015683.1, NC_017317.1); *Corynebacterium diphtheria* (NCBI Refs: NC_016782.1, NC_016786.1); *Spiroplasma syrphidicola* (NCBI Ref: NC_021284.1); *Prevotella intermedia* (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref: NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus torquisI* (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1), *Listeria innocua* (NCBI Ref: NP_472073.1), *Campylobacter jejuni* (NCBI Ref: YP_002344900.1) or *Neisseria meningitidis* (NCBI Ref: YP_002342100.1) or to a Cas9 from any other organism.

It should be appreciated that additional Cas9 proteins (*e.g.,* a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9), including variants and homologs thereof, are within the scope of this disclosure. Exemplary Cas9 proteins include, without limitation, those provided below. In some embodiments, the Cas9 protein is a nuclease dead Cas9 (dCas9). In some embodiments, the Cas9 protein is a Cas9 nickase (nCas9). In some embodiments, the Cas9 protein is a nuclease active Cas9.

In some embodiments, the Cas9 domain is a nuclease-inactive Cas9 domain (dCas9). For example, the dCas9 domain may bind to a duplexed nucleic acid molecule (*e.g.,* via a gRNA molecule) without cleaving either strand of the duplexed nucleic acid molecule. In some embodiments, the nuclease-inactive dCas9 domain comprises a D10X mutation and a H840X mutation of the amino acid sequence set forth herein, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid change. In some embodiments, the nuclease-inactive dCas9 domain comprises a D10A mutation and a H840A mutation of the amino acid sequence set forth herein, or a corresponding mutation in any of the amino acid sequences provided herein. As one example, a nuclease-inactive Cas9

domain comprises the amino acid sequence set forth in Cloning vector pPlatTET-gRNA2 (Accession No. BAV54124).

The amino acid sequence of an exemplary catalytically inactive Cas9 (dCas9) is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

(see, *e.g.*, Qi *et al.*, "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression." *Cell.* 2013; 152(5):1173-83, the entire contents of which are incorporated herein by reference).

Additional suitable nuclease-inactive dCas9 domains will be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure. Such additional exemplary suitable nuclease-inactive Cas9 domains include, but are not limited to, D10A/H840A, D10A/D839A/H840A, and D10A/D839A/H840A/N863A

mutant domains (See, *e.g.*, Prashant *et al.*, CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nature Biotechnology*. 2013; 31(9): 833-838, the entire contents of which are incorporated herein by reference).

In some embodiments, a Cas9 nuclease has an inactive (*e.g.*, an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase, referred to as an "nCas9" protein (for "nickase" Cas9). A nuclease-inactivated Cas9 protein may interchangeably be referred to as a "dCas9" protein (for nuclease-"dead" Cas9) or catalytically inactive Cas9. Methods for generating a Cas9 protein (or a fragment thereof) having an inactive DNA cleavage domain are known (*See, e.g.*, Jinek *et al., Science.* 337:816-821(2012); Qi *et al.*, "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression" (2013) *Cell.* 28;152(5):1173-83, the entire contents of each of which are incorporated herein by reference). For example, the DNA cleavage domain of Cas9 is known to include two subdomains, the HNH nuclease subdomain and the RuvC1 subdomain. The HNH subdomain cleaves the strand complementary to the gRNA, whereas the RuvC1 subdomain cleaves the non-complementary strand. Mutations within these subdomains can silence the nuclease activity of Cas9. For example, the mutations D10A and H840A completely inactivate the nuclease activity of *S. pyogenes* Cas9 (Jinek *et al., Science.* 337:816-821(2012); Qi *et al., Cell.* 28;152(5):1173-83 (2013)).

In some embodiments, the dCas9 domain comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the dCas9 domains provided herein. In some embodiments, the Cas9 domain comprises an amino acid sequences that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more or more mutations compared to any one of the amino acid sequences set forth herein. In some embodiments, the Cas9 domain comprises an amino acid sequence that has at least 10, at least 15, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, or at least 1200 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth herein.

In some embodiments, dCas9 corresponds to, or comprises in part or in whole, a Cas9 amino acid sequence having one or more mutations that inactivate the Cas9 nuclease activity.

For example, in some embodiments, a dCas9 domain comprises D10A and an H840A mutation or corresponding mutations in another Cas9.

In some embodiments, the dCas9 comprises the amino acid sequence of dCas9 (D10A and H840A):

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD (single underline: HNH domain; double underline: RuvC domain).

In some embodiments, the Cas9 domain comprises a D10A mutation, while the residue at position 840 remains a histidine in the amino acid sequence provided above, or at corresponding positions in any of the amino acid sequences provided herein.

In other embodiments, dCas9 variants having mutations other than D10A and H840A are provided, which, *e.g.*, result in nuclease inactivated Cas9 (dCas9). Such mutations, by way of example, include other amino acid substitutions at D10 and H840, or other substitutions within the nuclease domains of Cas9 (*e.g.*, substitutions in the HNH nuclease

subdomain and/or the RuvC1 subdomain). In some embodiments, variants or homologues of dCas9 are provided which are at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical. In some embodiments, variants of dCas9 are provided having amino acid sequences which are shorter, or longer, by about 5 amino acids, by about 10 amino acids, by about 15 amino acids, by about 20 amino acids, by about 25 amino acids, by about 30 amino acids, by about 40 amino acids, by about 50 amino acids, by about 75 amino acids, by about 100 amino acids or more.

In some embodiments, the Cas9 domain is a Cas9 nickase. The Cas9 nickase may be a Cas9 protein that is capable of cleaving only one strand of a duplexed nucleic acid molecule (*e.g.,* a duplexed DNA molecule). In some embodiments the Cas9 nickase cleaves the target strand of a duplexed nucleic acid molecule, meaning that the Cas9 nickase cleaves the strand that is base paired to (complementary to) a gRNA (*e.g.,* an sgRNA) that is bound to the Cas9. In some embodiments, a Cas9 nickase comprises a D10A mutation and has a histidine at position 840. In some embodiments the Cas9 nickase cleaves the non-target, non-base-edited strand of a duplexed nucleic acid molecule, meaning that the Cas9 nickase cleaves the strand that is not base paired to a gRNA (*e.g.,* an sgRNA) that is bound to the Cas9. In some embodiments, a Cas9 nickase comprises an H840A mutation and has an aspartic acid residue at position 10, or a corresponding mutation. In some embodiments the Cas9 nickase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the Cas9 nickases provided herein. Additional suitable Cas9 nickases will be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure.

The amino acid sequence of an exemplary catalytically Cas9 nickase (nCas9) is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT

NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

In some embodiments, Cas9 refers to a Cas9 from archaea (*e.g.,* nanoarchaea), which constitute a domain and kingdom of single-celled prokaryotic microbes. In some embodiments, the programmable nucleotide binding protein may be a CasX or CasY protein, which have been described in, for example, Burstein *et al.*, "New CRISPR-Cas systems from uncultivated microbes." Cell Res. 2017 Feb 21. doi: 10.1038/cr.2017.21, the entire contents of which is hereby incorporated by reference. Using genome-resolved metagenomics, a number of CRISPR-Cas systems were identified, including the first reported Cas9 in the archaeal domain of life. This divergent Cas9 protein was found in little-studied nanoarchaea as part of an active CRISPR-Cas system. In bacteria, two previously unknown systems were discovered, CRISPR-CasX and CRISPR-CasY, which are among the most compact systems yet discovered. In some embodiments, in a base editor system described herein Cas9 is replaced by CasX, or a variant of CasX. In some embodiments, in a base editor system described herein Cas9 is replaced by CasY, or a variant of CasY. It should be appreciated that other RNA-guided DNA binding proteins may be used as a nucleic acid programmable DNA binding protein (napDNAbp), and are within the scope of this disclosure.

In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) of any of the fusion proteins provided herein may be a CasX or CasY protein. In some embodiments, the napDNAbp is a CasX protein. In some embodiments, the napDNAbp is a CasY protein. In some embodiments, the napDNAbp comprises an amino

acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a naturally-occurring CasX or CasY protein. In some embodiments, the programmable nucleotide binding protein is a naturally-occurring CasX or CasY protein. In some embodiments, the programmable nucleotide binding protein comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to any CasX or CasY protein described herein. It should be appreciated that CasX and CasY from other bacterial species may also be used in accordance with the present disclosure.

In some embodiments, the Cas9 is a Neisseria menigitidis Cas9 (NmeCas9) or a variant thereof. NmeCas9 features and PAM sequences as described in Edraki et al. Mol. Cell. (2019) 73(4): 714-726 is incorporated herein by reference in its entirety.

An exemplary amino acid sequence of a Nme1Cas9 is provided below:

type II CRISPR RNA-guided endonuclease Cas9 [Neisseria meningitidis] WP_002235162.1

```
   1 maafkpnpin yilgldigia svgwamveid edenpiclid lgvrvferae vpktgdslam
  61 arrlarsvrr ltrrrahrll rarrllkreg vlqaadfden glikslpntp wqlraaaldr
 121 kltplewsav llhlikhrgy lsqrkneget adkelgallk gvadnahalq tgdfrtpael
 181 alnkfekesg hirnqrgdys htfsrkdlqa elillfekqk efgnphvsgg lkegietllm
 241 tqrpalsgda vqkmlghctf epaepkaakn tytaerfiwl tklnnlrile qgserpltdt
 301 eratlmdepy rkskltyaqa rkllgledta ffkglrygkd naeastlmem kayhaisral
 361 ekeglkdkks plnlspelqd eigtafslfk tdeditgrlk driqpeilea llkhisfdkf
 421 vqislkalrr ivplmeqgkr ydeacaeiyg dhygkkntee kiylppipad eirnpvvlra
 481 lsqarkving vvrrygspar ihietarevg ksfkdrkeie krqeenrkdr ekaaakfrey
 541 fpnfvgepks kdilklrlye qqhgkclysg keinlgrlne kgyveidhal pfsrtwddsf
 601 nnkvlvlgse nqnkgnqtpy eyfngkdnsr ewqefkarve tsrfprskkq rillqkfded
 661 gfkernlndt ryvnrflcqf vadrmrltgk gkkrvfasng qitnllrgfw glrkvraend
 721 rhhaldavvv acstvamqqk itrfvrykem nafdgktidk etgevlhqkt hfpqpweffa
 781 qevmirvfgk pdgkpefeea dtpeklrtll aeklssrpea vheyvtplfv srapnrkmsg
 841 qghmetvksa krldegvsvl rvpltqlklk dlekmvnrer epklyealka rleahkddpa
 901 kafaepfyky dkagnrtqqv kavrveqvqk tgvwvrnhng iadnatmvrv dvfekgdkyy
 961 lvpiyswqva kgilpdravv qgkdeedwql iddsfnfkfs lhpndlvevi tkkarmfgyf
1021 aschrgtgni nirihdldhk igkngilegi gvktalsfqk yqidelgkei rpcrlkkrpp
1081 vr
```

An exemplary amino acid sequence of a Nme2Cas9 is provided below:

type II CRISPR RNA-guided endonuclease Cas9 [Neisseria meningitidis] WP_002230835.1

```
   1 maafkpnpin yilgldigia svgwamveid eeenpirlid lgvrvferae vpktgdslam
  61 arrlarsvrr ltrrrahrll rarrllkreg vlqaadfden glikslpntp wqlraaaldr
 121 kltplewsav llhlikhrgy lsqrkneget adkelgallk gvannahalq tgdfrtpael
 181 alnkfekesg hirnqrgdys htfsrkdlqa elillfekqk efgnphvsgg lkegietllm
 241 tqrpalsgda vqkmlghctf epaepkaakn tytaerfiwl tklnnlrile qgserpltdt
```

```
 301 eratlmdepy rkskltyaqa rkllgledta ffkglrygkd naeastlmem kayhaisral
 361 ekeglkdkks plnlsselqd eigtafslfk tdeditgrlk drvqpeilea llkhisfdkf
 421 vqislkalrr ivplmeqgkr ydeacaeiyg dhygkkntee kiylppipad eirnpvvlra
 481 lsqarkving vvrrygspar ihietarevg ksfkdrkeie krqeenrkdr ekaaakfrey
 541 fpnfvgepks kdilklrlye qqhgkclysg keinlvrlne kgyveidhal pfsrtwddsf
 601 nnkvlvlgse nqnkgnqtpy eyfngkdnsr ewqefkarve tsrfprskkq rillqkfded
 661 gfkecnlndt ryvnrflcqf vadhilltgk gkrrvfasng qitnllrgfw glrkvraend
 721 rhhaldavvv acstvamqqk itrfvrykem nafdgktidk etgkvlhqkt hfpqpweffa
 781 qevmirvfgk pdgkpefeea dtpeklrtll aeklssrpea vheyvtplfv srapnrkmsg
 841 ahkdtlrsak rfvkhnekis vkrvwlteik ladlenmvny kngreielye alkarleayg
 901 gnakqafdpk dnpfykkggq lvkavrvekt qesgvllnkk naytiadngd mvrvdvfckv
 961 dkkgknqyfi vpiyawqvae nilpdidckg yriddsytfc fslhkydlia fqkdekskve
1021 fayyincdss ngrfylawhd kgskeqqfri stqnlvliqk yqvnelgkei rpcrlkkrpp
1081 vr
```

In some embodiments, the Cas protein is a CasX or CasY. An exemplary CasX ((uniprot.org/uniprot/F0NN87; uniprot.org/uniprot/F0NH53) tr|F0NN87|F0NN87_SULIHCRISPR-associatedCasx protein OS = Sulfolobus islandicus (strain HVE10/4) GN = SiH_0402 PE=4 SV=1) amino acid sequence is as follows:

MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGK
AKKKKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAP
SFVKPEFYEFGRSPGMVERTRRVKLEVEPHYLIIAAAGWVLTRLGKAKVSEGDYVGVNVFTP
TRGILYSLIQNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVRIYTISDAVGQNPTTIN
GGFSIDLTKLLEKRYLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTG SKRLEDLLY
FANRDLIMNLNSDDGKVRDLKLISAYVNGELIRGEG.

An exemplary CasX (>tr|F0NH53|F0NH53_SULIR CRISPR associated protein, Casx OS = *Sulfolobus islandicus* (strain REY15A) GN=SiRe_0771 PE=4 SV=1) amino acid sequence is as follows:

MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGK
AKKKKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAP
SFVKPEFYKFGRSPGMVERTRRVKLEVEPHYLIMAAAGWVLTRLGKAKVSEGDYVGVNVFTP
TRGILYSLIQNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVSIYTISDAVGQNPTTIN
GGFSIDLTKLLEKRDLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTGSKRLEDLLYF
ANRDLIMNLNSDDGKVRDLKLISAYVNGELIRGEG.

Deltaproteobacteria CasX

MEKRINKIRKKLSADNATKPVSRSGPMKTLLVRVMTDDLKKRLEKRRKKPEVMPQVISNNAA
NNLRMLLDDYTKMKEAILQVYWQEFKDDHVGLMCKFAQPASKKIDQNKLKPEMDEKGNLTTA
GFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPVKDSDEAVTYSLG
KFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGTIASFLSKYQDIII
EHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDfAYNEVIARVRMWVNLNLW

QKLKLSRDDAKPLLRLKGFPSFPVVERRENEVDWWNTINEVKKLIDAKRDMGRVFWSGVTAE
KRNTILEGYNYLPNENDHKKREGSLENPKKPAKRQFGDLLLYLEKKYAGDWGKVFDEAWERI
DKKIAGLTSHIEREEARNAEDAQSKAVLTDWLRAKASFVLERLKEMDEKEFYACEIQLQKWY
GDLRGNPFAVEAENRVVDISGFSIGSDGHSIQYRNLLAWKYLENGKREFYLLMNYGKKGRIR
FTDGTDIKKSGKWQGLLYGGGKAKVIDLTFDPDDEQLIILPLAFGTRQGREFIWNDLLSLET
GLIKLANGRVIEKTIYNKKIGRDEPALFVALTFERREVVDPSNIKPVNLIGVARGENIPAVI
ALTDPEGCPLPEFKDSSGGPTDILRIGEGYKEKQRAIQAAKEVEQRRAGGYSRKFASKSRNL
ADDMVRNSARDLFYHAVTHDAVLVFANLSRGFGRQGKRTFMTERQYTKMEDWLTAKLAYEGL
TSKTYLSKTLAQYTSKTCSNCGFTITYADMDVMLVRLKKTSDGWATTLNNKELKAEYQITYY
NRYKRQTVEKELSAELDRLSEESGNNDISKWTKGRRDEALFLLKKRFSHRPVQEQFVCLDCG
HEVHAAEQAALNIARSWLFLNSNSTEFKSYKSGKQPFVGAWQAFYKRRLKEVWKPNA

An exemplary CasY ((ncbi.nlm.nih.gov/protein/APG80656.1) >APG80656.1 CRISPR-associated protein CasY [uncultured Parcubacteria group bacterium]) amino acid sequence is as follows:

MSKRHPRISGVKGYRLHAQRLEYTGKSGAMRTIKYPLYSSPSGGRTVPREIVSAINDDYVGL
YGLSNFDDLYNAEKRNEEKVYSVLDFWYDCVQYGAVFSYTAPGLLKNVAEVRGGSYELTKTL
KGSHLYDELQIDKVIKFLNKKEISRANGSLDKLKKDIIDCFKAEYRERHKDQCNKLADDIKN
AKKDAGASLGERQKKLFRDFFGISEQSENDKPSFTNPLNLTCCLLPFDTVNNNRNRGEVLFN
KLKEYAQKLDKNEGSLEMWEYIGIGNSGTAFSNFLGEGFLGRLRENKITELKKAMMDITDAW
RGQEQEEELEKRLRILAALTIKLREPKFDNHWGGYRSDINGKLSSWLQNYINQTVKIKEDLK
GHKKDLKKAKEMINRFGESDTKEEAVVSSLLESIEKIVPDDSADDEKPDIPAIAIYRRFLSD
GRLTLNRFVQREDVQEALIKERLEAEKKKKPKKRKKKSDAEDEKETIDFKELFPHLAKPLKL
VPNFYGDSKRELYKKYKNAAIYTDALWKAVEKIYKSAFSSSLKNSFFDTDFDKDFFIKRLQK
IFSVYRRFNTDKWKPIVKNSFAPYCDIVSLAENEVLYKPKQSRSRKSAAIDKNRVRLPSTEN
IAKAGIALARELSVAGFDWKDLLKKEEHEEYIDLIELHKTALALLLAVTETQLDISALDFVE
NGTVKDFMKTRDGNLVLEGRFLEMFSQSIVFSELRGLAGLMSRKEFITRSAIQTMNGKQAEL
LYIPHEFQSAKITTPKEMSRAFLDLAPAEFATSLEPESLSEKSLLKLKQMRYYPHYFGYELT
RTGQGIDGGVAENALRLEKSPVKKREIKCKQYKTLGRGQNKIVLYVRSSYYQTQFLEWFLHR
PKNVQTDVAVSGSFLIDEKKVKTRWNYDALTVALEPVSGSERVFVSQPFTIFPEKSAEEEGQ
RYLGIDIGEYGIAYTALEITGDSAKILDQNFISDPQLKTLREEVKGLKLDQRRGTFAMPSTK
IARIRESLVHSLRNRIHHLALKHKAKIVYELEVSRFEEGKQKIKKVYATLKKADVYSEIDAD
KNLQTTVWGKLAVASEISASYTSQFCGACKKLWRAEMQVDETITTQELIGTVRVIKGGTLID
AIKDFMRPPIFDENDTPFPKYRDFCDKHHISKKMRGNSCLFICPFCRANADADIQASQTIAL
LRYVKEEKKVEDYFERFRKLKNIKVLGQMKKI.

The Cas9 nuclease has two functional endonuclease domains: RuvC and HNH. Cas9 undergoes a conformational change upon target binding that positions the nuclease domains to cleave opposite strands of the target DNA. The end result of Cas9-mediated DNA cleavage is a double-strand break (DSB) within the target DNA (~3-4 nucleotides upstream of the PAM sequence). The resulting DSB is then repaired by one of two general repair pathways: (1) the efficient but error-prone non-homologous end joining (NHEJ) pathway; or (2) the less efficient but high-fidelity homology directed repair (HDR) pathway.

The "efficiency" of non-homologous end joining (NHEJ) and/or homology directed repair (HDR) can be calculated by any convenient method. For example, in some embodiments, efficiency can be expressed in terms of percentage of successful HDR. For example, a surveyor nuclease assay can be used to generate cleavage products and the ratio of products to substrate can be used to calculate the percentage. For example, a surveyor nuclease enzyme can be used that directly cleaves DNA containing a newly integrated restriction sequence as the result of successful HDR. More cleaved substrate indicates a greater percent HDR (a greater efficiency of HDR). As an illustrative example, a fraction (percentage) of HDR can be calculated using the following equation [(cleavage products)/(substrate plus cleavage products)] (*e.g.*, (b+c)/(a+b+c), where "a" is the band intensity of DNA substrate and "b" and "c" are the cleavage products).

In some embodiments, efficiency can be expressed in terms of percentage of successful NHEJ. For example, a T7 endonuclease I assay can be used to generate cleavage products and the ratio of products to substrate can be used to calculate the percentage NHEJ. T7 endonuclease I cleaves mismatched heteroduplex DNA which arises from hybridization of wild-type and mutant DNA strands (NHEJ generates small random insertions or deletions (indels) at the site of the original break). More cleavage indicates a greater percent NHEJ (a greater efficiency of NHEJ). As an illustrative example, a fraction (percentage) of NHEJ can be calculated using the following equation: $(1-(1-(b+c)/(a+b+c))^{1/2})\times 100$, where "a" is the band intensity of DNA substrate and "b" and "c" are the cleavage products (Ran *et. al.*, Cell. 2013 Sep. 12; 154(6):1380-9; and Ran *et al.*, Nat Protoc. 2013 Nov.; 8(11): 2281–2308).

The NHEJ repair pathway is the most active repair mechanism, and it frequently causes small nucleotide insertions or deletions (indels) at the DSB site. The randomness of NHEJ-mediated DSB repair has important practical implications, because a population of cells expressing Cas9 and a gRNA or a guide polynucleotide can result in a diverse array of mutations. In most embodiments, NHEJ gives rise to small indels in the target DNA that result in amino acid deletions, insertions, or frameshift mutations leading to premature stop

codons within the open reading frame (ORF) of the targeted gene. The ideal end result is a loss-of-function mutation within the targeted gene.

While NHEJ-mediated DSB repair often disrupts the open reading frame of the gene, homology directed repair (HDR) can be used to generate specific nucleotide changes ranging from a single nucleotide change to large insertions like the addition of a fluorophore or tag.

In order to utilize HDR for gene editing, a DNA repair template containing the desired sequence can be delivered into the cell type of interest with the gRNA(s) and Cas9 or Cas9 nickase. The repair template can contain the desired edit as well as additional homologous sequence immediately upstream and downstream of the target (termed left & right homology arms). The length of each homology arm can be dependent on the size of the change being introduced, with larger insertions requiring longer homology arms. The repair template can be a single-stranded oligonucleotide, double-stranded oligonucleotide, or a double-stranded DNA plasmid. The efficiency of HDR is generally low (<10% of modified alleles) even in cells that express Cas9, gRNA and an exogenous repair template. The efficiency of HDR can be enhanced by synchronizing the cells, since HDR takes place during the S and G2 phases of the cell cycle. Chemically or genetically inhibiting genes involved in NHEJ can also increase HDR frequency.

In some embodiments, Cas9 is a modified Cas9. A given gRNA targeting sequence can have additional sites throughout the genome where partial homology exists. These sites are called off-targets and need to be considered when designing a gRNA. In addition to optimizing gRNA design, CRISPR specificity can also be increased through modifications to Cas9. Cas9 generates double-strand breaks (DSBs) through the combined activity of two nuclease domains, RuvC and HNH. Cas9 nickase, a D10A mutant of SpCas9, retains one nuclease domain and generates a DNA nick rather than a DSB. The nickase system can also be combined with HDR-mediated gene editing for specific gene edits.

In some embodiments, Cas9 is a variant Cas9 protein. A variant Cas9 polypeptide has an amino acid sequence that is different by one amino acid (*e.g.*, has a deletion, insertion, substitution, fusion) when compared to the amino acid sequence of a wild-type Cas9 protein. In some instances, the variant Cas9 polypeptide has an amino acid change (*e.g.*, deletion, insertion, or substitution) that reduces the nuclease activity of the Cas9 polypeptide. For example, in some instances, the variant Cas9 polypeptide has less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, or less than 1% of the nuclease activity of the corresponding wild-type Cas9 protein. In some embodiments, the variant Cas9

protein has no substantial nuclease activity. When a subject Cas9 protein is a variant Cas9 protein that has no substantial nuclease activity, it can be referred to as "dCas9."

In some embodiments, a variant Cas9 protein has reduced nuclease activity. For example, a variant Cas9 protein exhibits less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 1%, or less than about 0.1%, of the endonuclease activity of a wild-type Cas9 protein, *e.g.*, a wild-type Cas9 protein.

In some embodiments, a variant Cas9 protein can cleave the complementary strand of a guide target sequence but has reduced ability to cleave the non-complementary strand of a double stranded guide target sequence. For example, the variant Cas9 protein can have a mutation (amino acid substitution) that reduces the function of the RuvC domain. As a non-limiting example, in some embodiments, a variant Cas9 protein has a D10A (aspartate to alanine at amino acid position 10) and can therefore cleave the complementary strand of a double stranded guide target sequence but has reduced ability to cleave the non-complementary strand of a double stranded guide target sequence (thus resulting in a single strand break (SSB) instead of a double strand break (DSB) when the variant Cas9 protein cleaves a double stranded target nucleic acid) (see, for example, Jinek *et al.*, Science. 2012 Aug. 17; 337(6096):816-21).

In some embodiments, a variant Cas9 protein can cleave the non-complementary strand of a double stranded guide target sequence but has reduced ability to cleave the complementary strand of the guide target sequence. For example, the variant Cas9 protein can have a mutation (amino acid substitution) that reduces the function of the HNH domain (RuvC/HNH/RuvC domain motifs). As a non-limiting example, in some embodiments, the variant Cas9 protein has an H840A (histidine to alanine at amino acid position 840) mutation and can therefore cleave the non-complementary strand of the guide target sequence but has reduced ability to cleave the complementary strand of the guide target sequence (thus resulting in a SSB instead of a DSB when the variant Cas9 protein cleaves a double stranded guide target sequence). Such a Cas9 protein has a reduced ability to cleave a guide target sequence (*e.g.*, a single stranded guide target sequence) but retains the ability to bind a guide target sequence (*e.g.*, a single stranded guide target sequence).

In some embodiments, a variant Cas9 protein has a reduced ability to cleave both the complementary and the non-complementary strands of a double stranded target DNA. As a non-limiting example, in some embodiments, the variant Cas9 protein harbors both the D10A and the H840A mutations such that the polypeptide has a reduced ability to cleave both the complementary and the non-complementary strands of a double stranded target DNA. Such a

Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA).

As another non-limiting example, in some embodiments, the variant Cas9 protein harbors W476A and W1126A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA).

As another non-limiting example, in some embodiments, the variant Cas9 protein harbors P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA).

As another non-limiting example, in some embodiments, the variant Cas9 protein harbors H840A, W476A, and W1126A, mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). As another non-limiting example, in some embodiments, the variant Cas9 protein harbors H840A, D10A, W476A, and W1126A, mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). In some embodiments, the variant Cas9 has restored catalytic His residue at position 840 in the Cas9 HNH domain (A840H).

As another non-limiting example, in some embodiments, the variant Cas9 protein harbors, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). As another non-limiting example, in some embodiments, the variant Cas9 protein harbors D10A, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). In some embodiments, when a variant Cas9 protein harbors W476A and W1126A mutations or when the variant Cas9 protein harbors P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations, the variant Cas9 protein does

not bind efficiently to a PAM sequence. Thus, in some such embodiments, when such a variant Cas9 protein is used in a method of binding, the method does not require a PAM sequence. In other words, in some embodiments, when such a variant Cas9 protein is used in a method of binding, the method can include a guide RNA, but the method can be performed in the absence of a PAM sequence (and the specificity of binding is therefore provided by the targeting segment of the guide RNA). Other residues can be mutated to achieve the above effects (*i.e.*, inactivate one or the other nuclease portions). As non-limiting examples, residues D10, G12, G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or A987 can be altered (*i.e.*, substituted). Also, mutations other than alanine substitutions are suitable.

In some embodiments, a variant Cas9 protein that has reduced catalytic activity (*e.g.*, when a Cas9 protein has a D10, G12, G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or a A987 mutation, *e.g.*, D10A, G12A, G17A, E762A, H840A, N854A, N863A, H982A, H983A, A984A, and/or D986A), the variant Cas9 protein can still bind to target DNA in a site-specific manner (because it is still guided to a target DNA sequence by a guide RNA) as long as it retains the ability to interact with the guide RNA.

In some embodiments, the variant Cas protein can be spCas9, spCas9-VRQR, spCas9-VRER, xCas9 (sp), saCas9, saCas9-KKH, spCas9-MQKSER, spCas9-LRKIQK, or spCas9-LRVSQL.

In some embodiments, a modified SpCas9 including amino acid substitutions D1135M, S1136Q, G1218K, E1219F, A1322R, D1332A, R1335E, and T1337R (SpCas9-MQKFRAER) and having specificity for the altered PAM 5'-NGC-3' was used.

Alternatives to *S. pyogenes* Cas9 can include RNA-guided endonucleases from the Cpf1 family that display cleavage activity in mammalian cells. CRISPR from *Prevotella* and *Francisella 1* (CRISPR/Cpf1) is a DNA-editing technology analogous to the CRISPR/Cas9 system. Cpf1 is an RNA-guided endonuclease of a class II CRISPR/Cas system. This acquired immune mechanism is found in *Prevotella* and *Francisella* bacteria. Cpf1 genes are associated with the CRISPR locus, coding for an endonuclease that use a guide RNA to find and cleave viral DNA. Cpf1 is a smaller and simpler endonuclease than Cas9, overcoming some of the CRISPR/Cas9 system limitations. Unlike Cas9 nucleases, the result of Cpf1-mediated DNA cleavage is a double-strand break with a short 3′ overhang. Cpf1's staggered cleavage pattern can open up the possibility of directional gene transfer, analogous to traditional restriction enzyme cloning, which can increase the efficiency of gene editing. Like the Cas9 variants and orthologues described above, Cpf1 can also expand the number of sites that can be targeted by CRISPR to AT-rich regions or AT-rich genomes that lack the

NGG PAM sites favored by SpCas9. The Cpf1 locus contains a mixed alpha/beta domain, a RuvC-I followed by a helical region, a RuvC-II and a zinc finger-like domain. The Cpf1 protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9. Furthermore, Cpf1 does not have a HNH endonuclease domain, and the N-terminal of Cpf1 does not have the alpha-helical recognition lobe of Cas9. Cpf1 CRISPR-Cas domain architecture shows that Cpf1 is functionally unique, being classified as Class 2, type V CRISPR system. The Cpf1 loci encode Cas1, Cas2 and Cas4 proteins more similar to types I and III than from type II systems. Functional Cpf1 doesn't need the trans-activating CRISPR RNA (tracrRNA), therefore, only CRISPR (crRNA) is required. This benefits genome editing because Cpf1 is not only smaller than Cas9, but also it has a smaller sgRNA molecule (proximately half as many nucleotides as Cas9). The Cpf1-crRNA complex cleaves target DNA or RNA by identification of a protospacer adjacent motif 5'-YTN-3' in contrast to the G-rich PAM targeted by Cas9. After identification of PAM, Cpf1 introduces a sticky-end-like DNA double- stranded break of 4 or 5 nucleotides overhang.

Cas12 domains of Nucleobase Editors

Typically, microbial CRISPR-Cas systems are divided into Class 1 and Class 2 systems. Class 1 systems have multisubunit effector complexes, while Class 2 systems have a single protein effector. For example, Cas9 and Cpf1 are Class 2 effectors, albeit different types (Type II and Type V, respectively). In addition to Cpf1, Class 2, Type V CRISPR-Cas systems also comprise Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i). See, e.g., Shmakov et al., "Discovery and Functional Characterization of Diverse Class 2 CRISPR Cas Systems," Mol. Cell, 2015 Nov. 5; 60(3): 385-397; Makarova et al., "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" CRISPR Journal, 2018, 1(5): 325-336; and Yan et al., "Functionally Diverse Type V CRISPR-Cas Systems," Science, 2019 Jan. 4; 363: 88-91; the entire contents of each is hereby incorporated by reference. Type V Cas proteins contain a RuvC (or RuvC-like) endonuclease domain. While production of mature CRISPR RNA (crRNA) is generally tracrRNA-independent, Cas12b/C2c1, for example, requires tracrRNA for production of crRNA. Cas12b/C2c1 depends on both crRNA and tracrRNA for DNA cleavage.

Nucleic acid programmable DNA binding proteins contemplated in the present invention include Cas proteins that are classified as Class 2, Type V (Cas12 proteins). Non-limiting examples of Cas Class 2, Type V proteins include Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i, homologues

thereof, or modified versions thereof. As used herein, a Cas12 protein can also be referred to as a Cas12 nuclease, a Cas12 domain, or a Cas12 protein domain. In some embodiments, the Cas12 proteins of the present invention comprise an amino acid sequence interrupted by an internally fused protein domain such as a deaminase domain.

In some embodiments, the Cas12 domain is a nuclease inactive Cas12 domain or a Cas12 nickase. In some embodiments, the Cas12 domain is a nuclease active domain. For example, the Cas12 domain may be a Cas12 domain that nicks one strand of a duplexed nucleic acid (e.g., duplexed DNA molecule). In some embodiments, the Cas12 domain comprises any one of the amino acid sequences as set forth herein. In some embodiments the Cas12 domain comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth herein. In some embodiments, the Cas12 domain comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more mutations compared to any one of the amino acid sequences set forth herein. In some embodiments, the Cas12 domain comprises an amino acid sequence that has at least 10, at least 15, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, or at least 1200 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth herein.

In some embodiments, proteins comprising fragments of Cas12 are provided. For example, in some embodiments, a protein comprises one of two Cas12 domains: (1) the gRNA binding domain of Cas12; or (2) the DNA cleavage domain of Cas12. In some embodiments, proteins comprising Cas12 or fragments thereof are referred to as "Cas12 variants." A Cas12 variant shares homology to Cas12, or a fragment thereof. For example, a Cas12 variant is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to wild type Cas12. In some embodiments, the Cas12 variant may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more amino acid changes compared to wild type Cas12. In some

embodiments, the Cas12 variant comprises a fragment of Cas12 (e.g., a gRNA binding domain or a DNA cleavage domain), such that the fragment is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of wild type Cas12. In some embodiments, the fragment is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild type Cas12. In some embodiments, the fragment is at least 100 amino acids in length. In some embodiments, the fragment is at least 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, or at least 1300 amino acids in length.

In some embodiments, Cas12 corresponds to, or comprises in part or in whole, a Cas12 amino acid sequence having one or more mutations that alter the Cas12 nuclease activity. Such mutations, by way of example, include amino acid substitutions within the RuvC nuclease domain of Cas12. In some embodiments, variants or homologues of Cas12 are provided which are at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to a wild type Cas12. In some embodiments, variants of Cas12 are provided having amino acid sequences which are shorter, or longer, by about 5 amino acids, by about 10 amino acids, by about 15 amino acids, by about 20 amino acids, by about 25 amino acids, by about 30 amino acids, by about 40 amino acids, by about 50 amino acids, by about 75 amino acids, by about 100 amino acids or more.

In some embodiments, Cas12 fusion proteins as provided herein comprise the full-length amino acid sequence of a Cas12 protein, e.g., one of the Cas12 sequences provided herein. In other embodiments, however, fusion proteins as provided herein do not comprise a full-length Cas12 sequence, but only one or more fragments thereof. Exemplary amino acid sequences of suitable Cas12 domains are provided herein, and additional suitable sequences of Cas12 domains and fragments will be apparent to those of skill in the art.

Generally, the class 2, Type V Cas proteins have a single functional RuvC endonuclease domain (See, e.g., Chen et al., "CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity," Science 360:436-439 (2018)). In some cases,

the Cas12 protein is a variant Cas12b protein. (See Strecker et al., Nature Communications, 2019, 10(1): Art. No.: 212). In one embodiment, a variant Cas12 polypeptide has an amino acid sequence that is different by 1, 2, 3, 4, 5 or more amino acids (e.g., has a deletion, insertion, substitution, fusion) when compared to the amino acid sequence of a wild type Cas12 protein. In some instances, the variant Cas12 polypeptide has an amino acid change (e.g., deletion, insertion, or substitution) that reduces the activity of the Cas12 polypeptide. For example, in some instances, the variant Cas12 is a Cas12b polypeptide that has less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, or less than 1% of the nickase activity of the corresponding wild-type Cas12b protein. In some cases, the variant Cas12b protein has no substantial nickase activity.

In some cases, a variant Cas12b protein has reduced nickase activity. For example, a variant Cas12b protein exhibits less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 1%, or less than about 0.1%, of the nickase activity of a wild-type Cas12b protein.

In some embodiments, the Cas12 protein includes RNA-guided endonucleases from the Cas12a/Cpf1 family that displays activity in mammalian cells. CRISPR from Prevotella and Francisella 1 (CRISPR/Cpf1) is a DNA editing technology analogous to the CRISPR/Cas9 system. Cpf1 is an RNA-guided endonuclease of a class II CRISPR/Cas system. This acquired immune mechanism is found in Prevotella and Francisella bacteria. Cpf1 genes are associated with the CRISPR locus, coding for an endonuclease that use a guide RNA to find and cleave viral DNA. Cpf1 is a smaller and simpler endonuclease than Cas9, overcoming some of the CRISPR/Cas9 system limitations. Unlike Cas9 nucleases, the result of Cpf1-mediated DNA cleavage is a double-strand break with a short 3′ overhang. Cpf1's staggered cleavage pattern can open up the possibility of directional gene transfer, analogous to traditional restriction enzyme cloning, which can increase the efficiency of gene editing. Like the Cas9 variants and orthologues described above, Cpf1 can also expand the number of sites that can be targeted by CRISPR to AT-rich regions or AT-rich genomes that lack the NGG PAM sites favored by SpCas9. The Cpf1 locus contains a mixed alpha/beta domain, a RuvC-I followed by a helical region, a RuvC-II and a zinc finger-like domain. The Cpf1 protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9. Furthermore, Cpf1, unlike Cas9, does not have a HNH endonuclease domain, and the N-terminal of Cpf1 does not have the alpha-helical recognition lobe of Cas9. Cpf1 CRISPR-Cas domain architecture shows that Cpf1 is functionally unique, being classified as Class 2, type V CRISPR system. The Cpf1 loci encode Cas1, Cas2, and Cas4 proteins are more

similar to types I and III than type II systems. Functional Cpf1 does not require the trans-activating CRISPR RNA (tracrRNA), therefore, only CRISPR (crRNA) is required. This benefits genome editing because Cpf1 is not only smaller than Cas9, but also it has a smaller sgRNA molecule (approximately half as many nucleotides as Cas9). The Cpf1-crRNA complex cleaves target DNA or RNA by identification of a protospacer adjacent motif 5'-YTN-3' or 5'-TTTN-3' in contrast to the G-rich PAM targeted by Cas9. After identification of PAM, Cpf1 introduces a sticky-end-like DNA double-stranded break having an overhang of 4 or 5 nucleotides.

In some aspects of the present invention, a vector encodes a CRISPR enzyme that is mutated to with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence can be used. Cas12 can refer to a polypeptide with at least or at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild type exemplary Cas12 polypeptide (e.g., Cas12 from Bacillus hisashii). Cas12 can refer to a polypeptide with at most or at most about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild type exemplary Cas12 polypeptide (e.g., from Bacillus hisashii (BhCas12b), Bacillus sp. V3-13 (BvCas12b), and Alicyclobacillus acidiphilus (AaCas12b)). Cas12 can refer to the wild type or a modified form of the Cas12 protein that can comprise an amino acid change such as a deletion, insertion, substitution, variant, mutation, fusion, chimera, or any combination thereof.

Nucleic acid programmable DNA binding proteins

Some aspects of the disclosure provide fusion proteins comprising domains that act as nucleic acid programmable DNA binding proteins, which may be used to guide a protein, such as a base editor, to a specific nucleic acid (*e.g.*, DNA or RNA) sequence. In particular embodiments, a fusion protein comprises a nucleic acid programmable DNA binding protein domain and a deaminase domain. Non-limiting examples of nucleic acid programmable DNA binding proteins include, Cas9 (*e.g.*, dCas9 and nCas9), Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i. Non-limiting examples of Cas enzymes include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas8a, Cas8b, Cas8c, Cas9 (also known as Csn1 or Csx12), Cas10, Cas10d, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1,

Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csx11, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, homologues thereof, or modified or engineered versions thereof. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, although they may not be specifically listed in this disclosure. See, *e.g.*, Makarova et al. "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" CRISPR J. 2018 Oct;1:325-336. doi: 10.1089/crispr.2018.0033; Yan et al., "Functionally diverse type V CRISPR-Cas systems" Science. 2019 Jan 4;363(6422):88-91. doi: 10.1126/science.aav7271, the entire contents of each are hereby incorporated by reference.

One example of a nucleic acid programmable DNA-binding protein that has different PAM specificity than Cas9 is Clustered Regularly Interspaced Short Palindromic Repeats from *Prevotella* and *Francisella* 1 (Cpf1). Similar to Cas9, Cpf1 is also a class 2 CRISPR effector. It has been shown that Cpf1 mediates robust DNA interference with features distinct from Cas9. Cpf1 is a single RNA-guided endonuclease lacking tracrRNA, and it utilizes a T-rich protospacer-adjacent motif (TTN, TTTN, or YTN). Moreover, Cpf1 cleaves DNA via a staggered DNA double-stranded break. Out of 16 Cpf1-family proteins, two enzymes from *Acidaminococcus* and *Lachnospiraceae* are shown to have efficient genome-editing activity in human cells. Cpf1 proteins are known in the art and have been described previously, for example Yamano *et al.*, "Crystal structure of Cpf1 in complex with guide RNA and target DNA." *Cell* (165) 2016, p. 949-962; the entire contents of which is hereby incorporated by reference.

Useful in the present compositions and methods are nuclease-inactive Cpf1 (dCpf1) variants that may be used as a guide nucleotide sequence-programmable DNA-binding protein domain. The Cpf1 protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9 but does not have a HNH endonuclease domain, and the N-terminal of Cpf1 does not have the alfa-helical recognition lobe of Cas9. It was shown in Zetsche *et al.*, *Cell,* 163, 759-771, 2015 (which is incorporated herein by reference) that, the RuvC-like domain of Cpf1 is responsible for cleaving both DNA strands and inactivation of the RuvC-like domain inactivates Cpf1 nuclease activity. For example, mutations corresponding to D917A, E1006A, or D1255A in *Francisella novicida* Cpf1 inactivate Cpf1 nuclease activity. In some embodiments, the dCpf1 of the present disclosure comprises mutations

corresponding to D917A, E1006A, D1255A, D917A/E1006A, D917A/D1255A, E1006A/D1255A, or D917A/E1006A/D1255A. It is to be understood that any mutations, *e.g.*, substitution mutations, deletions, or insertions that inactivate the RuvC domain of Cpf1, may be used in accordance with the present disclosure.

In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) of any of the fusion proteins provided herein may be a Cpf1 protein. In some embodiments, the Cpf1 protein is a Cpf1 nickase (nCpf1). In some embodiments, the Cpf1 protein is a nuclease inactive Cpf1 (dCpf1). In some embodiments, the Cpf1, the nCpf1, or the dCpf1 comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a Cpf1 sequence disclosed herein. In some embodiments, the dCpf1 comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a Cpf1 sequence disclosed herein, and comprises mutations corresponding to D917A, E1006A, D1255A, D917A/E1006A, D917A/D1255A, E1006A/D1255A, or D917A/E1006A/D1255A. It should be appreciated that Cpf1 from other bacterial species may also be used in accordance with the present disclosure.

Wild-type *Francisella novicida* Cpf1 (D917, E1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI

KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D917A (A917, E1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 E1006A (D917, A1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN

QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D1255A (D917, E1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL

YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D917A/E1006A (A917, A1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D917A/D1255A (A917, E1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 E1006A/D1255A (D917, A1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI

LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D917A/E1006A/D1255A (A917, A1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL

KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDAAANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

In some embodiments, one of the Cas9 domains present in the fusion protein may be replaced with a guide nucleotide sequence-programmable DNA-binding protein domain that has no requirements for a PAM sequence.

In some embodiments, the Cas9 domain is a Cas9 domain from *Staphylococcus aureus* (SaCas9). In some embodiments, the SaCas9 domain is a nuclease active SaCas9, a nuclease inactive SaCas9 (SaCas9d), or a SaCas9 nickase (SaCas9n). In some embodiments, the SaCas9 comprises a N579A mutation, or a corresponding mutation in any of the amino acid sequences provided herein.

In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a NNGRRT or a NNGRRT PAM sequence. In some embodiments, the SaCas9 domain comprises one or more of a E781X, a N967X, and a R1014X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SaCas9 domain comprises one or more of a E781K, a N967K, and a R1014H mutation, or one or more corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SaCas9 domain comprises a E781K, a N967K, or a R1014H mutation, or corresponding mutations in any of the amino acid sequences provided herein.

Exemplary SaCas9 sequence

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHR
IQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVE
EDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQ
KAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKY
AYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIK
GYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNS
ELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKE
IPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKR
NRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHII
PRSVSFDNSFNNKVLVKQEENSKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKT
KKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSF
LRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIE

TEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIVNNLNG
LYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYS
KKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNL
DVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIE
VNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG

Residue N579 above, which is underlined and in bold, may be mutated (*e.g.*, to a A579) to yield a SaCas9 nickase.

Exemplary SaCas9n sequence

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHR
IQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVE
EDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQ
KAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKY
AYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIK
GYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNS
ELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKE
IPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKR
NRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHII
PRSVSFDNSFNNKVLVKQEE**A**SKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKT
KKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSF
LRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIE
TEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIVNNLNG
LYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYS
KKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNL
DVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIE
VNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG

Residue A579 above, which can be mutated from N579 to yield a SaCas9 nickase, is underlined and in bold.

Exemplary SaKKH Cas9

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHR
IQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVE
EDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQ
KAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKY
AYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIK
GYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNS

ELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKE
IPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKR
NRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHII
PRSVSFDNSFNNKVLVKQEE**A**SKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKT
KKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSF
LRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIE
TEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNR*K*LINDTLYSTRKDDKGNTLIVNNLNG
LYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYS
KKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNL
DVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFY*K*NDLIKINGELYRVIGVNNDLLNRIE
VNMIDITYREYLENMNDKRPP*H*IIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG.

Residue A579 above, which can be mutated from N579 to yield a SaCas9 nickase, is underlined and in bold. Residues K781, K967, and H1014 above, which can be mutated from E781, N967, and R1014 to yield a SaKKH Cas9 are underlined and in italics.

In some embodiments, the napDNAbp is a circular permutant. In the following sequences, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italics sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence.

CP5 (with MSP "NGC" PID and "D10A" nickase):

**EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRM
LASAKFLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYR
STKEVLDATLIHQSITGLYETRIDLSQLGGD***GGSGGSGGSGGSGGSGGSGGM***DKKYSIGLAI
GTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYT
RRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTI
YHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE
ENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLA
EDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASM
IKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM
DGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILT
FRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYF**

KKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM
IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF
MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK
PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQ
NGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKM
KNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNT
KYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPK
LESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) is a single effector of a microbial CRISPR-Cas system. Single effectors of microbial CRISPR-Cas systems include, without limitation, Cas9, Cpf1, Cas12b/C2c1, and Cas12c/C2c3. Typically, microbial CRISPR-Cas systems are divided into Class 1 and Class 2 systems. Class 1 systems have multisubunit effector complexes, while Class 2 systems have a single protein effector. For example, Cas9 and Cpf1 are Class 2 effectors. In addition to Cas9 and Cpf1, three distinct Class 2 CRISPR-Cas systems (Cas12b/C2c1, and Cas12c/C2c3) have been described by Shmakov *et al.*, "Discovery and Functional Characterization of Diverse Class 2 CRISPR Cas Systems", *Mol. Cell,* 2015 Nov. 5; 60(3): 385-397, the entire contents of which is hereby incorporated by reference. Effectors of two of the systems, Cas12b/C2c1, and Cas12c/C2c3, contain RuvC-like endonuclease domains related to Cpf1. A third system, contains an effector with two predicated HEPN RNase domains. Production of mature CRISPR RNA is tracrRNA-independent, unlike production of CRISPR RNA by Cas12b/C2c1. Cas12b/C2c1 depends on both CRISPR RNA and tracrRNA for DNA cleavage.

The crystal structure of *Alicyclobaccillus acidoterrastris* Cas12b/C2c1 (AacC2c1) has been reported in complex with a chimeric single-molecule guide RNA (sgRNA). See *e.g.*, Liu *et al.*, "C2c1-sgRNA Complex Structure Reveals RNA-Guided DNA Cleavage Mechanism", *Mol. Cell,* 2017 Jan. 19; 65(2):310-322, the entire contents of which are hereby incorporated by reference. The crystal structure has also been reported in *Alicyclobacillus acidoterrestris* C2c1 bound to target DNAs as ternary complexes. See *e.g.*, Yang *et al.*, "PAM-dependent Target DNA Recognition and Cleavage by C2C1 CRISPR-Cas endonuclease", *Cell,* 2016 Dec. 15; 167(7):1814-1828, the entire contents of which are hereby incorporated by reference. Catalytically competent conformations of AacC2c1, both with target and non-target DNA strands, have been captured independently positioned within a single RuvC catalytic pocket, with Cas12b/C2c1-mediated cleavage resulting in a staggered

seven-nucleotide break of target DNA. Structural comparisons between Cas12b/C2c1 ternary complexes and previously identified Cas9 and Cpf1 counterparts demonstrate the diversity of mechanisms used by CRISPR-Cas9 systems.

In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) of any of the fusion proteins provided herein may be a Cas12b/C2c1, or a Cas12c/C2c3 protein. In some embodiments, the napDNAbp is a Cas12b/C2c1 protein. In some embodiments, the napDNAbp is a Cas12c/C2c3 protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a naturally-occurring Cas12b/C2c1 or Cas12c/C2c3 protein. In some embodiments, the napDNAbp is a naturally-occurring Cas12b/C2c1 or Cas12c/C2c3 protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to any one of the napDNAbp sequences provided herein. It should be appreciated that Cas12b/C2c1 or Cas12c/C2c3 from other bacterial species may also be used in accordance with the present disclosure.

A Cas12b/C2c1 ((uniprot.org/uniprot/T0D7A2#2) sp|T0D7A2|C2C1_ALIAG CRISPR-associated endonuclease C2c1 OS = *Alicyclobacillus acido-terrestris* (strain ATCC 49025 / DSM 3922/ CIP 106132 / NCIMB 13137/GD3B) GN=c2c1 PE=1 SV=1) amino acid sequence is as follows:

MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYRRSPNGDGEQECD
KTAEECKAELLERLRARQVENGHRGPAGSDDELLQLARQLYELLVPQAIGAKGDAQQIARKF
LSPLADKDAVGGLGIAKAGNKPRWVRMREAGEPGWEEEKEKAETRKSADRTADVLRALADFG
LKPLMRVYTDSEMSSVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQEYAKLVEQ
KNRFEQKNFVGQEHLVHLVNQLQQDMKEASPGLESKEQTAHYVTGRALRGSDKVFEKWGKLA
PDAPFDLYDAEIKNVQRRNTRRFGSHDLFAKLAEPEYQALWREDASFLTRYAVYNSILRKLN
HAKMFATFTLPDATAHPIWTRFDKLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREV
DDVTVPISMSEQLDNLLPRDPNEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRRG
ARDVYLNVSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSDYLAEHPDDGKLGSE
GLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNSKGRVPFFFPIKGNDNLVAVHERSQLL
KLPGETESKDLRAIREERQRTLRQLRTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPVDAAN
HMTPDWREAFENELQKLKSLHGICSDKEWMDAVYESVRRVWRHMGKQVRDWRKDVRSGERPK
IRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRFAITLREHIDHAKE

DRLKKLADRIIMEALGYVYALDERGKGKWVAKYPPCQLILLEELSEYQFNNDRPPSENNQLM
QWSHRGVFQELINQAQVHDLLVGTMYAAFSSRFDARTGAPGIRCRRVPARCTQEHNPEPFPW
WLNKFVVEHTLDACPLRADDLIPTGEGEIFVSPFSAEEGDFHQIHADLNAAQNLQQRLWSDF
DISQIRLRCDWGEVDGELVLIPRLTGKRTADSYSNKVFYTNTGVTYYERERGKKRRKVFAQE
KLSEEEAELLVEADEAREKSVVLMRDPSGIINRGNWTRQKEFWSMV NQRIEGYLVKQIRSR
VPLQDSACENTGDI.

AacCas12b (*Alicyclobacillus acidiphilus*) - WP_067623834

MAVKSMKVKLRLDNMPEIRAGLWKLHTEVNAGVRYYTEWLSLLRQENLYRRSPNGDGEQECY
KTAEECKAELLERLRARQVENGHCGPAGSDDELLQLARQLYELLVPQAIGAKGDAQQIARKF
LSPLADKDAVGGLGIAKAGNKPRWVRMREAGEPGWEEEKAKAEARKSTDRTADVLRALADFG
LKPLMRVYTDSDMSSVQWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGEAYAKLVEQ
KSRFEQKNFVGQEHLVQLVNQLQQDMKEASHGLESKEQTAHYLTGRALRGSDKVFEKWEKLD
PDAPFDLYDTEIKNVQRRNTRRFGSHDLFAKLAEPKYQALWREDASFLTRYAVYNSIVRKLN
HAKMFATFTLPDATAHPIWTRFDKLGGNLHQYTFLFNEFGEGRHAIRFQKLLTVEDGVAKEV
DDVTVPISMSAQLDDLLPRDPHELVALYFQDYGAEQHLAGEFGGAKIQYRRDQLNHLHARRG
ARDVYLNLSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSDYLAEHPDDGKLGSE
GLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNSEGRVPFCFPIEGNENLVAVHERSQLL
KLPGETESKDLRAIREERQRTLRQLRTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPMDANQ
MTPDWREAFEDELQKLKSLYGICGDREWTEAVYESVRRVWRHMGKQVRDWRKDVRSGERPKI
RGYQKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRFAITLREHIDHAKED
RLKKLADRIIMEALGYVYALDDERGKGKWVAKYPPCQLILLEELSEYQFNNDRPPSENNQLM
QWSHRGVFQELLNQAQVHDLLVGTMYAAFSSRFDARTGAPGIRCRRVPARCAREQNPEPFPW
WLNKFVAEHKLDGCPLRADDLIPTGEGEFFVSPFSAEEGDFHQIHADLNAAQNLQRRLWSDF
DISQIRLRCDWGEVDGEPVLIPRTTGKRTADSYGNKVFYTKTGVTYYERERGKKRRKVFAQE
ELSEEEAELLVEADEAREKSVVLMRDPSGIINRGDWTRQKEFWSMVNQRIEGYLVKQIRSRV
RLQESACENTGDI

BhCas12b (*Bacillus hisashii*) NCBI Reference Sequence: WP_095142515

MAPKKKRKVGIHGVPAAATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAI
YEHHEQDPKNPKKVSKAEIQAELWDFVLKMQKCNSFTHEVDKDEVFNILRELYEELVPSSVE
KKGEANQLSNKFLYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEEEKKKWEEDKKKDP
LAKILGKLAEYGLIPLFIPYTDSNEPIVKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWES
WNLKVKEEYEKVEKEYKTLEERIKEDIQALKALEQYEKERQEQLLRDTLNTNEYRLSKRGLR
GWREIIQKWLKMDENEPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNHPEYPY
LYATFCEIDKKKKDAKQQATFTLADPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKL

TVQLDRLIYPTESGGWEEKGKVDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGT
LGGARVQFDRDHLRRYPHKVESGNVGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKVVNFKP
KELTEWIKDSKGKKLKSGIESLEIGLRVMSIDLGQRQAAAASIFEVVDQKPDIEGKLFFPIK
GTELYAVHRASFNIKLPGETLVKSREVLRKAREDNLKLMNQKLNFLRNVLHFQQFEDITERE
KRVTKWISRQENSDVPLVYQDELIQIRELMYKPYKDWVAFLKQLHKRLEVEIGKEVKHWRKS
LSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEPGEVRRLEPGQRFAIDQLNHLNALKED
RLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFEDLSNYNPYEERSRFENSKLMKWSR
REIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSVVTKEKLQDNRFFKNLQREGR
LTLDKIAVLKEGDLYPDKGGEKFISLSKDRKCVTTHADINAAQNLQKRFWTRTHGFYKVYCK
AYQVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVNAGKLKIKKGSSKQSSSELVDS
DILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFGKLERILISKLTNQYSISTIE
DDSSKQSMKRPAATKKAGQAKKKK

BvCas12b V4 (S893R/K846R/E837G changes rel. to wild type) is expressed as follows: 5' mRNA Cap---5'UTR---bhCas12b---STOP sequence --- 3'UTR --- 120polyA tail

5'UTR: GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC

3' UTR (TriLink standard UTR)

GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTT
CCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGA

Nucleic acid sequence of bhCas12b (V4)

ATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGCCACCAGATC
CTTCATCCTGAAGATCGAGCCCAACGAGGAAGTGAAGAAAGGCCTCTGGAAAACCCACGAGG
TGCTGAACCACGGAATCGCCTACTACATGAATATCCTGAAGCTGATCCGGCAAGAGGCCATC
TACGAGCACCACGAGCAGGACCCCAAGAATCCCAAGAAGGTGTCCAAGGCCGAGATCCAGGC
CGAGCTGTGGGATTTCGTGCTGAAGATGCAGAAGTGCAACAGCTTCACACACGAGGTGGACA
AGGACGAGGTGTTCAACATCCTGAGAGAGCTGTACGAGGAACTGGTGCCCAGCAGCGTGGAA
AAGAAGGGCGAAGCCAACCAGCTGAGCAACAAGTTTCTGTACCCTCTGGTGGACCCCAACAG
CCAGTCTGGAAAGGGAACAGCCAGCAGCGGCAGAAAGCCCAGATGGTACAACCTGAAGATTG
CCGGCGATCCCTCCTGGGAAGAAGAGAAGAAGAAGTGGGAAGAAGATAAGAAAAAGGACCCG
CTGGCCAAGATCCTGGGCAAGCTGGCTGAGTACGGACTGATCCCTCTGTTCATCCCCTACAC
CGACAGCAACGAGCCCATCGTGAAAGAAATCAAGTGGATGGAAAAGTCCCGGAACCAGAGCG
TGCGGCGGCTGGATAAGGACATGTTCATTCAGGCCCTGGAACGGTTCCTGAGCTGGGAGAGC
TGGAACCTGAAAGTGAAAGAGGAATACGAGAAGGTCGAGAAAGAGTACAAGACCCTGGAAGA
GAGGATCAAAGAGGACATCCAGGCTCTGAAGGCTCTGGAACAGTATGAGAAAGAGCGGCAAG
AACAGCTGCTGCGGGACACCCTGAACACCAACGAGTACCGGCTGAGCAAGAGAGGCCTTAGA

GGCTGGCGGGAAATCATCCAGAAATGGCTGAAAATGGACGAGAACGAGCCCTCCGAGAAGTA
CCTGGAAGTGTTCAAGGACTACCAGCGGAAGCACCCTAGAGAGGCCGGCGATTACAGCGTGT
ACGAGTTCCTGTCCAAGAAAGAGAACCACTTCATCTGGCGGAATCACCCTGAGTACCCCTAC
CTGTACGCCACCTTCTGCGAGATCGACAAGAAAAAGAAGGACGCCAAGCAGCAGGCCACCTT
CACACTGGCCGATCCTATCAATCACCCTCTGTGGGTCCGATTCGAGGAAAGAAGCGGCAGCA
ACCTGAACAAGTACAGAATCCTGACCGAGCAGCTGCACACCGAGAAGCTGAAGAAAAAGCTG
ACAGTGCAGCTGGACCGGCTGATCTACCCTACAGAATCTGGCGGCTGGGAAGAGAAGGGCAA
AGTGGACATTGTGCTGCTGCCCAGCCGGCAGTTCTACAACCAGATCTTCCTGGACATCGAGG
AAAAGGGCAAGCACGCCTTCACCTACAAGGATGAGAGCATCAAGTTCCCTCTGAAGGGCACA
CTCGGCGGAGCCAGAGTGCAGTTCGACAGAGATCACCTGAGAAGATACCCTCACAAGGTGGA
AAGCGGCAACGTGGGCAGAATCTACTTCAACATGACCGTGAACATCGAGCCTACAGAGTCCC
CAGTGTCCAAGTCTCTGAAGATCCACCGGGACGACTTCCCCAAGGTGGTCAACTTCAAGCCC
AAAGAACTGACCGAGTGGATCAAGGACAGCAAGGGCAAGAAACTGAAGTCCGGCATCGAGTC
CCTGGAAATCGGCCTGAGAGTGATGAGCATCGACCTGGGACAGAGACAGGCCGCTGCCGCCT
CTATTTTCGAGGTGGTGGATCAGAAGCCCGACATCGAAGGCAAGCTGTTTTTCCCAATCAAG
GGCACCGAGCTGTATGCCGTGCACAGAGCCAGCTTCAACATCAAGCTGCCCGGCGAGACACT
GGTCAAGAGCAGAGAAGTGCTGCGGAAGGCCAGAGAGGACAATCTGAAACTGATGAACCAGA
AGCTCAACTTCCTGCGGAACGTGCTGCACTTCCAGCAGTTCGAGGACATCACCGAGAGAGAG
AAGCGGGTCACCAAGTGGATCAGCAGACAAGAGAACAGCGACGTGCCCCTGGTGTACCAGGA
TGAGCTGATCCAGATCCGCGAGCTGATGTACAAGCCTTACAAGGACTGGGTCGCCTTCCTGA
AGCAGCTCCACAAGAGACTGGAAGTCGAGATCGGCAAAGAAGTGAAGCACTGGCGGAAGTCC
CTGAGCGACGGAAGAAAGGGCCTGTACGGCATCTCCCTGAAGAACATCGACGAGATCGATCG
GACCCGGAAGTTCCTGCTGAGATGGTCCCTGAGGCCTACCGAACCTGGCGAAGTGCGTAGAC
TGGAACCCGGCCAGAGATTCGCCATCGACCAGCTGAATCACCTGAACGCCCTGAAAGAAGAT
CGGCTGAAGAAGATGGCCAACACCATCATCATGCACGCCCTGGGCTACTGCTACGACGTGCG
GAAGAAGAAATGGCAGGCTAAGAACCCCGCCTGCCAGATCATCCTGTTCGAGGATCTGAGCA
ACTACAACCCCTACGAGGAAAGGTCCCGCTTCGAGAACAGCAAGCTCATGAAGTGGTCCAGA
CGCGAGATCCCCAGACAGGTTGCACTGCAGGGCGAGATCTATGGCCTGCAAGTGGGAGAAGT
GGGCGCTCAGTTCAGCAGCAGATTCCACGCCAAGACAGGCAGCCCTGGCATCAGATGTAGCG
TCGTGACCAAAGAGAAGCTGCAGGACAATCGGTTCTTCAAGAATCTGCAGAGAGAGGGCAGA
CTGACCCTGGACAAAATCGCCGTGCTGAAAGAGGGCGATCTGTACCCAGACAAAGGCGGCGA
GAAGTTCATCAGCCTGAGCAAGGATCGGAAGTGCGTGACCACACACGCCGACATCAACGCCG
CTCAGAACCTGCAGAAGCGGTTCTGGACAAGAACCCACGGCTTCTACAAGGTGTACTGCAAG
GCCTACCAGGTGGACGGCCAGACCGTGTACATCCCTGAGAGCAAGGACCAGAAGCAGAAGAT

CATCGAAGAGTTCGGCGAGGGCTACTTCATTCTGAAGGACGGGGTGTACGAATGGGTCAACG
CCGGCAAGCTGAAAATCAAGAAGGGCAGCTCCAAGCAGAGCAGCAGCGAGCTGGTGGATAGC
GACATCCTGAAAGACAGCTTCGACCTGGCCTCCGAGCTGAAAGGCGAAAAGCTGATGCTGTA
CAGGGACCCCAGCGGCAATGTGTTCCCCAGCGACAAATGGATGGCCGCTGGCGTGTTCTTCG
GAAAGCTGGAACGCATCCTGATCAGCAAGCTGACCAACCAGTACTCCATCAGCACCATCGAG
GACGACAGCAGCAAGCAGTCTATGAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAA
AAAGAAAAAG

In some embodiments, the Cas12b is BvCas12B, which is a variant of BhCas12b and comprises the following changes relative to BhCas12B: S893R, K846R, and E837G.

BvCas12b (Bacillus sp. V3-13) NCBI Reference Sequence: WP_101661451.1

MAIRSIKLKMKTNSGTDSIYLRKALWRTHQLINEGIAYYMNLLTLYRQEAIGDKTKEAYQAE
LINIIRNQQRNNGSSEEHGSDQEILALLRQLYELIIPSSIGESGDANQLGNKFLYPLVDPNS
QSGKGTSNAGRKPRWKRLKEEGNPDWELEKKKDEERKAKDPTVKIFDNLNKYGLLPLFPLFT
NIQKDIEWLPLGKRQSVRKWDKDMFIQAIERLLSWESWNRRVADEYKQLKEKTESYYKEHLT
GGEEWIEKIRKFEKERNMELEKNAFAPNDGYFITSRQIRGWDRVYEKWSKLPESASPEELWK
VVAEQQNKMSEGFGDPKVFSFLANRENRDIWRGHSERIYHIAAYNGLQKKLSRTKEQATFTL
PDAIEHPLWIRYESPGGTNLNLFKLEEKQKKNYYVTLSKIIWPSEEKWIEKENIEIPLAPSI
QFNRQIKLKQHVKGKQEISFSDYSSRISLDGVLGGSRIQFNRKYIKNHKELLGEGDIGPVFF
NLVVDVAPLQETRNGRLQSPIGKALKVISSDFSKVIDYKPKELMDWMNTGSASNSFGVASLL
EGMRVMSIDMGQRTSASVSIFEVVKELPKDQEQKLFYSINDTELFAIHKRSFLLNLPGEVVT
KNNKQQRQERRKKRQFVRSQIRMLANVLRLETKKTPDERKKAIHKLMEIVQSYDSWTASQKE
VWEKELNLLTNMAAFNDEIWKESLVELHHRIEPYVGQIVSKWRKGLSEGRKNLAGISMWNID
ELEDTRRLLISWSKRSRTPGEANRIETDEPFGSSLLQHIQNVKDDRLKQMANLIIMTALGFK
YDKEEKDRYKRWKETYPACQIILFENLNRYLFNLDRSRRENSRLMKWAHRSIPRTVSMQGEM
FGLQVGDVRSEYSSRFHAKTGAPGIRCHALTEEDLKAGSNTLKRLIEDGFINESELAYLKKG
DIIPSQGGELFVTLSKRYKKDSDNNELTVIHADINAAQNLQKRFWQQNSEVYRVPCQLARMG
EDKLYIPKSQTETIKKYFGKGSFVKNNTEQEVYKWEKSEKMKIKTDTTFDLQDLDGFEDISK
TIELAQEQQKKYLTMFRDPSGYFFNNETWRPQKEYWSIVNNIIKSCLKKKILSNKVEL

In some embodiments, the Cas12b is BTCas12b.BTCas12b (Bacillus thermoamylovorans) NCBI Reference Sequence: WP_041902512

MATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAIYEHHEQDPKNPKKV
SKAEIQAELWDFVLKMQKCNSFTHEVDKDVVFNILRELYEELVPSSVEKKGEANQLSNKF
LYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEEEKKKWEEDKKKDPLAKILGKLAE
YGLIPLFIPFTDSNEPIVKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWESWNLKVKEE

YEKVEKEHKTLEERIKEDIQAFKSLEQYEKERQEQLLRDTLNTNEYRLSKRGLRGWREII
QKWLKMDENEPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNHPEYPYLYAT
FCEIDKKKKDAKQQATFTLADPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKLTV
QLDRLIYPTESGGWEEKGKVDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGT
LGGARVQFDRDHLRRYPHKVESGNVGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKFVNF
KPKELTEWIKDSKGKKLKSGIESLEIGLRVMSIDLGQRQAAAASIFEVVDQKPDIEGKLF
FPIKGTELYAVHRASFNIKLPGETLVKSREVLRKAREDNLKLMNQKLNFLRNVLHFQQFE
DITEREKRVTKWISRQENSDVPLVYQDELIQIRELMYKPYKDWVAFLKQLHKRLEVEIGK
EVKHWRKSLSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEPGEVRRLEPGQRFAIDQ
LNHLNALKEDRLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFEDLSNYNPYEERS
RFENSKLMKWSRREIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSVVTKEKL
QDNRFFKNLQREGRLTLDKIAVLKEGDLYPDKGGEKFISLSKDRKLVTTHADINAAQNLQ
KRFWTRTHGFYKVYCKAYQVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWGNAGK
LKIKKGSSKQSSSELVDSDILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFG
KLERILISKLTNQYSISTIEDDSSKQSM

In some embodiments, a napDNAbp refers to Cas12c. In some embodiments, the Cas12c protein is a Cas12c1 or a variant of Cas12c1. In some embodiments, the Cas12 protein is a Cas12c2 or a variant of Cas12c2. In some embodiments, the Cas12 protein is a Cas12c protein from Oleiphilus sp. HI0009 (i.e., OspCas12c) or a variant of OspCas12c. These Cas12c molecules have been described in Yan et al., "Functionally Diverse Type V CRISPR-Cas Systems," Science, 2019 Jan. 4; 363: 88-91; the entire contents of which is hereby incorporated by reference. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring Cas12c1, Cas12c2, or OspCas12c protein. In some embodiments, the napDNAbp is a naturally-occurring Cas12c1, Cas12c2, or OspCas12c protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to any Cas12c1, Cas12c2, or OspCas12c protein described herein. It should be appreciated that Cas12c1, Cas12c2, or OspCas12c from other bacterial species may also be used in accordance with the present disclosure.

Cas12c1

MQTKKTHLHLISAKASRKYRRTIACLSDTAKKDLERRKQSGAADPAQELSCLKTIKFKLEVP
EGSKLPSFDRISQIYNALETIEKGSLSYLLFALILSGFRIFPNSSAAKTFASSSCYKNDQFA
SQIKEIFGEMVKNFIPSELESILKKGRRKNNKDWTEENIKRVLNSEFGRKNSEGSSALFDSF
LSKFSQELFRKFDSWNEVNKKYLEAAELLDSMLASYGPFDSVCKMIGDSDSRNSLPDKSTIA
FTNNAEITVDIESSVMPYMAIAALLREYRQSKSKAAPVAYVQSHLTTTNGNGLSWFFKFGLD
LIRKAPVSSKQSTSDGSKSLQELFSVPDDKLDGLKFIKEACEALPEASLLCGEKGELLGYQD
FRTSFAGHIDSWVANYVNRLFELIELVNQLPESIKLPSILTQKNHNLVASLGLQEAEVSHSL
ELFEGLVKNVRQTLKKLAGIDISSSPNEQDIKEFYAFSDVLNRLGSIRNQIENAVQTAKKDK
IDLESAIEWKEWKKLKKLPKLNGLGGGVPKQQELLDKALESVKQIRHYQRIDFERVIQWAVN
EHCLETVPKFLVDAEKKKINKESSTDFAAKENAVRFLLEGIGAAARGKTDSVSKAAYNWFVV
NNFLAKKDLNRYFINCQGCIYKPPYSKRRSLAFALRSDNKDTIEVVWEKFETFYKEISKEIE
KFNIFSQEFQTFLHLENLRMKLLLRRIQKPIPAEIAFFSLPQEYYDSLPPNVAFLALNQEIT
PSEYITQFNLYSSFLNGNLILLRRSRSYLRAKFSWVGNSKLIYAAKEARLWKIPNAYWKSDE
WKMILDSNVLVFDKAGNVLPAPTLKKVCEREGDLRLFYPLLRQLPHDWCYRNPFVKSVGREK
NVIEVNKEGEPKVASALPGSLFRLIGPAPFKSLLDDCFFNPLDKDLRECMLIVDQEISQKVE
AQKVEASLESCTYSIAVPIRYHLEEPKVSNQFENVLAIDQGEAGLAYAVFSLKSIGEAETKP
IAVGTIRIPSIRRLIHSVSTYRKKKQRLQNFKQNYDSTAFIMRENVTGDVCAKIVGLMKEFN
AFPVLEYDVKNLESGSRQLSAVYKAVNSHFLYFKEPGRDALRKQLWYGGDSWTIDGIEIVTR
ERKEDGKEGVEKIVPLKVFPGRSVSARFTSKTCSCCGRNVFDWLFTEKKAKTNKKFNVNSKG
ELTTADGVIQLFEADRSKGPKFYARRKERTPLTKPIAKGSYSLEEIERRVRTNLRRAPKSKQ
SRDTSQSQYFCVYKDCALHFSGMQADENAAINIGRRFLTALRKNRRSDFPSNVKISDRLLDN

Cas12c2

MTKHSIPLHAFRNSGADARKWKGRIALLAKRGKETMRTLQFPLEMSEPEAAAINTTPFAVAY
NAIEGTGKGTLFDYWAKLHLAGFRFFPSGGAATIFRQQAVFEDASWNAAFCQQSGKDWPWLV
PSKLYERFTKAPREVAKKDGSKKSIEFTQENVANESHVSLVGASITDKTPEDQKEFFLKMAG
ALAEKFDSWKSANEDRIVAMKVIDEFLKSEGLHLPSLENIAVKCSVETKPDNATVAWHDAPM
SGVQNLAIGVFATCASRIDNIYDLNGGKLSKLIQESATTPNVTALSWLFGKGLEYFRTTDID
TIMQDFNIPASAKESIKPLVESAQAIPTMTVLGKKNYAPFRPNFGGKIDSWIANYASRLMLL
NDILEQIEPGFELPQALLDNETLMSGIDMTGDELKELIEAVYAWVDAAKQGLATLLGRGGNV
DDAVQTFEQFSAMMDTLNGTLNTISARYVRAVEMAGKDEARLEKLIECKFDIPKWCKSVPKL
VGISGGLPKVEEEIKVMNAAFKDVRARMFVRFEEIAAYVASKGAGMDVYDALEKRELEQIKK
LKSAVPERAHIQAYRAVLHRIGRAVQNCSEKTKQLFSSKVIEMGVFKNPSHLNNFIFNQKGA

IYRSPFDRSRHAPYQLHADKLLKNDWLELLAEISATLMASESTEQMEDALRLERTRLQLQLS
GLPDWEYPASLAKPDIEVEIQTALKMQLAKDTVTSDVLQRAFNLYSSVLSGLTFKLLRRSFS
LKMRFSVADTTQLIYVPKVCDWAIPKQYLQAEGEIGIAARVVTESSPAKMVTEVEMKEPKAL
GHFMQQAPHDWYFDASLGGTQVAGRIVEKGKEVGKERKLVGYRMRGNSAYKTVLDKSLVGNT
ELSQCSMIIEIPYTQTVDADFRAQVQAGLPKVSINLPVKETITASNKDEQMLFDRFVAIDLG
ERGLGYAVFDAKTLELQESGHRPIKAITNLLNRTHHYEQRPNQRQKFQAKFNVNLSELRENT
VGDVCHQINRICAYYNAFPVLEYMVPDRLDKQLKSVYESVTNRYIWSSTDAHKSARVQFWLG
GETWEHPYLKSAKDKKPLVLSPGRGASGKGTSQTCSCCGRNPFDLIKDMKPRAKIAVVDGKA
KLENSELKLFERNLESKDDMLARRHRNERAGMEQPLTPGNYTVDEIKALLRANLRRAPKNRR
TKDTTVSEYHCVFSDCGKTMHADENAAVNIGGKFIADIEK

OspCas12c

MTKLRHRQKKLTHDWAGSKKREVLGSNGKLQNPLLMPVKKGQVTEFRKAFSAYARATKGEMT
DGRKNMFTHSFEPFKTKPSLHQCELADKAYQSLHSYLPGSLAHFLLSAHALGFRIFSKSGEA
TAFQASSKIEAYESKLASELACVDLSIQNLTISTLFNALTTSVRGKGEETSADPLIARFYTL
LTGKPLSRDTQGPERDLAEVISRKIASSFGTWKEMTANPLQSLQFFEEELHALDANVSLSPA
FDVLIKMNDLQGDLKNRTIVFDPDAPVFEYNAEDPADIIIKLTARYAKEAVIKNQNVGNYVK
NAITTTNANGLGWLLNKGLSLLPVSTDDELLEFIGVERSHPSCHALIELIAQLEAPELFEKN
VFSDTRSEVQGMIDSAVSNHIARLSSSRNSLSMDSEELERLIKSFQIHTPHCSLFIGAQSLS
QQLESLPEALQSGVNSADILLGSTQYMLTNSLVEESIATYQRTLNRINYLSGVAGQINGAIK
RKAIDGEKIHLPAAWSELISLPFIGQPVIDVESDLAHLKNQYQTLSNEFDTLISALQKNFDL
NFNKALLNRTQHFEAMCRSTKKNALSKPEIVSYRDLLARLTSCLYRGSLVLRRAGIEVLKKH
KIFESNSELREHVHERKHFVFVSPLDRKAKKLLRLTDSRPDLLHVIDEILQHDNLENKDRES
LWLVRSGYLLAGLPDQLSSSFINLPIITQKGDRRLIDLIQYDQINRDAFVMLVTSAFKSNLS
GLQYRANKQSFVVTRTLSPYLGSKLVYVPKDKDWLVPSQMFEGRFADILQSDYMVWKDAGRL
CVIDTAKHLSNIKKSVFSSEEVLAFLRELPHRTFIQTEVRGLGVNVDGIAFNNGDIPSLKTF
SNCVQVKVSRTNTSLVQTLNRWFEGGKVSPPSIQFERAYYKKDDQIHEDAAKRKIRFQMPAT
ELVHASDDAGWTPSYLLGIDPGEYGMGLSLVSINNGEVLDSGFIHINSLINFASKKSNHQTK
VVPRQQYKSPYANYLEQSKDSAAGDIAHILDRLIYKLNALPVFEALSGNSQSAADQVWTKVL
SFYTWGDNDAQNSIRKQHWFGASHWDIKGMLRQPPTEKKPKPYIAFPGSQVSSYGNSQRCSC
CGRNPIEQLREMAKDTSIKELKIRNSEIQLFDGTIKLFNPDPSTVIERRRHNLGPSRIPVAD
RTFKNISPSSLEFKELITIVSRSIRHSPEFIAKKRGIGSEYFCAYSDCNSSLNSEANAAANV
AQKFQKQLFFEL

In some embodiments, a napDNAbp refers to Cas12g, Cas12h, or Cas12i, which have been described in, for example, Yan et al., "Functionally Diverse Type V CRISPR-Cas Systems," Science, 2019 Jan. 4; 363: 88-91; the entire contents of each is hereby incorporated by reference. By aggregating more than 10 terabytes of sequence data, new classifications of Type V Cas proteins were identified that showed weak similarity to previously characterized Class V protein, including Cas12g, Cas12h, and Cas12i. In some embodiments, the Cas12 protein is a Cas12g or a variant of Cas12g. In some embodiments, the Cas12 protein is a Cas12h or a variant of Cas12h. In some embodiments, the Cas12 protein is a Cas12i or a variant of Cas12i. It should be appreciated that other RNA-guided DNA binding proteins may be used as a napDNAbp, and are within the scope of this disclosure. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring Cas12g, Cas12h, or Cas12i protein. In some embodiments, the napDNAbp is a naturally-occurring Cas12g, Cas12h, or Cas12i protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to any Cas12g, Cas12h, or Cas12i protein described herein. It should be appreciated that Cas12g, Cas12h, or Cas12i from other bacterial species may also be used in accordance with the present disclosure. In some embodiments, the Cas12i is a Cas12i1 or a Cas12i2.

Cas12g1

MAQASSTPAVSPRPRPRYREERTLVRKLLPRPGQSKQEFRENVKKLRKAFLQFNADVSGVCQ
WAIQFRPRYGKPAEPTETFWKFFLEPETSLPPNDSRSPEFRRLQAFEAAAGINGAAALDDPA
FTNELRDSILAVASRPKTKEAQRLFSRLKDYQPAHRMILAKVAAEWIESRYRRAHQNWERNY
EEWKKEKQEWEQNHPELTPEIREAFNQIFQQLEVKEKRVRICPAARLLQNKDNCQYAGKNKH
SVLCNQFNEFKKNHLQGKAIKFFYKDAEKYLRCGLQSLKPNVQGPFREDWNKYLRYMNLKEE
TLRGKNGGRLPHCKNLGQECEFNPHTALCKQYQQQLSSRPDLVQHDELYRKWRREYWREPRK
PVFRYPSVKRHSIAKIFGENYFQADFKNSVVGLRLDSMPAGQYLEFAFAPWPRNYRPQPGET
EISSVHLHFVGTRPRIGFRFRVPHKRSRFDCTQEELDELRSRTFPRKAQDQKFLEAARKRLL
ETFPGNAEQELRLLAVDLGTDSARAAFFIGKTFQQAFPLKIVKIEKLYEQWPNQKQAGDRRD
ASSKQPRPGLSRDHVGRHLQKMRAQASEIAQKRQELTGTPAPETTTDQAAKKATLQPFDLRG
LTVHTARMIRDWARLNARQIIQLAEENQVDLIVLESLRGFRPPGYENLDQEKKRRVAFFAHG

RIRRKVTEKAVERGMRVVTVPYLASSKVCAECRKKQKDNKQWEKNKKRGLFKCEGCGSQAQV
DENAARVLGRVFWGEIELPTAIP

Cas12h1

MKVHEIPRSQLLKIKQYEGSFVEWYRDLQEDRKKFASLLFRWAAFGYAAREDDGATYISPSQ
ALLERRLLLGDAEDVAIKFLDVLFKGGAPSSSCYSLFYEDFALRDKAKYSGAKREFIEGLAT
MPLDKIIERIRQDEQLSKIPAEEWLILGAEYSPEEIWEQVAPRIVNVDRSLGKQLRERLGIK
CRRPHDAGYCKILMEVVARQLRSHNETYHEYLNQTHEMKTKVANNLTNEFDLVCEFAEVLEE
KNYGLGWYVLWQGVKQALKEQKKPTKIQIAVDQLRQPKFAGLLTAKWRALKGAYDTWKLKKR
LEKRKAFPYMPNWDNDYQIPVGLTGLGVFTLEVKRTEVVVDLKEHGKLFCSHSHYFGDLTAE
KHPSRYHLKFRHKLKLRKRDSRVEPTIGPWIEAALREITIQKKPNGVFYLGLPYALSHGIDN
FQIAKRFFSAAKPDKEVINGLPSEMVVGAADLNLSNIVAPVKARIGKGLEGPLHALDYGYGE
LIDGPKILTPDGPRCGELISLKRDIVEIKSAIKEFKACQREGLTMSEETTTWLSEVESPSDS
PRCMIQSRIADTSRRLNSFKYQMNKEGYQDLAEALRLLDAMDSYNSLLESYQRMHLSPGEQS
PKEAKFDTKRASFRDLLRRRVAHTIVEYFDDCDIVFFEDLDGPSDSDSRNNALVKLLSPRTL
LLYIRQALEKRGIGMVEVAKDGTSQNNPISGHVGWRNKQNKSEIYFYEDKELLVMDADEVGA
MNILCRGLNHSVCPYSFVTKAPEKKNDEKKEGDYGKRVKRFLKDRYGSSNVRFLVASMGFVT
VTTKRPKDALVGKRLYYHGGELVTHDLHNRMKDEIKYLVEKEVLARRVSLSDSTIKSYKSFA
HV

Cas12i1

MSNKEKNASETRKAYTTKMIPRSHDRMKLLGNFMDYLMDGTPIFFELWNQFGGGIDRDIISG
TANKDKISDDLLLAVNWFKVMPINSKPQGVSPSNLANLFQQYSGSEPDIQAQEYFASNFDTE
KHQWKDMRVEYERLLAELQLSRSDMHHDLKLMYKEKCIGLSLSTAHYITSVMFGTGAKNNRQ
TKHQFYSKVIQLLEESTQINSVEQLASIILKAGDCDSYRKLRIRCSRKGATPSILKIVQDYE
LGTNHDDEVNVPSLIANLKEKLGRFEYECEWKCMEKIKAFLASKVGPYYLGSYSAMLENALS
PIKGMTTKNCKFVLKQIDAKNDIKYENEPFGKIVEGFFDSPYFESDTNVKWVLHPHHIGESN
IKTLWEDLNAIHSKYEEDIASLSEDKKEKRIKVYQGDVCQTINTYCEEVGKEAKTPLVQLLR
YLYSRKDDIAVDKIIDGITFLSKKHKVEKQKINPVIQKYPSFNFGNNSKLLGKIISPKDKLK
HNLKCNRNQVDNYIWIEIKVLNTKTMRWEKHHYALSSTRFLEEVYYPATSENPPDALAARFR
TKTNGYEGKPALSAEQIEQIRSAPVGLRKVKKRQMRLEAARQQNLLPRYTWGKDFNINICKR
GNNFEVTLATKVKKKKEKNYKVVLGYDANIVRKNTYAAIEAHANGDGVIDYNDLPVKPIESG
FVTVESQVRDKSYDQLSYNGVKLLYCKPHVESRRSFLEKYRNGTMKDNRGNNIQIDFMKDFE
AIADDETSLYYFNMKYCKLLQSSIRNHSSQAKEYREEIFELLRDGKLSVLKLSSLSNLSFVM

FKVAKSLIGTYFGHLLKKPKNSKSDVKAPPITDEDKQKADPEMFALRLALEEKRLNKVKSKK
EVIANKIVAKALELRDKYGPVLIKGENISDTTKKGKKSSTNSFLMDWLARGVANKVKEMVMM
HQGLEFVEVNPNFTSHQDPFVHKNPENTFRARYSRCTPSELTEKNRKEILSFLSDKPSKRPT
NAYYNEGAMAFLATYGLKKNDVLGVSLEKFKQIMANILHQRSEDQLLFPSRGGMFYLATYKL
DADATSVNWNGKQFWVCNADLVAAYNVGLVDIQKDFKKK

Cas12i2

MSSAIKSYKSVLRPNERKNQLLKSTIQCLEDGSAFFFKMLQGLFGGITPEIVRFSTEQEKQQ
QDIALWCAVNWFRPVSQDSLTHTIASDNLVEKFEEYYGGTASDAIKQYFSASIGESYYWNDC
RQQYYDLCRELGVEVSDLTHDLEILCREKCLAVATESNQNNSIISVLFGTGEKEDRSVKLRI
TKKILEAISNLKEIPKNVAPIQEIILNVAKATKETFRQVYAGNLGAPSTLEKFIAKDGQKEF
DLKKLQTDLKKVIRGKSKERDWCCQEELRSYVEQNTIQYDLWAWGEMFNKAHTALKIKSTRN
YNFAKQRLEQFKEIQSLNNLLVVKKLNDFFDSEFFSGEETYTICVHHLGGKDLSKLYKAWED
DPADPENAIVVLCDDLKNNFKKEPIRNILRYIFTIRQECSAQDILAAAKYNQQLDRYKSQKA
NPSVLGNQGFTWTNAVILPEKAQRNDRPNSLDLRIWLYLKLRHPDGRWKKHHIPFYDTRFFQ
EIYAAGNSPVDTCQFRTPRFGYHLPKLTDQTAIRVNKKHVKAAKTEARIRLAIQQGTLPVSN
LKITEISATINSKGQVRIPVKFDVGRQKGTLQIGDRFCGYDQNQTASHAYSLWEVVKEGQYH
KELGCFVRFISSGDIVSITENRGNQFDQLSYEGLAYPQYADWRKKASKFVSLWQITKKNKKK
EIVTVEAKEKFDAICKYQPRLYKFNKEYAYLLRDIVRGKSLVELQQIRQEIFRFIEQDCGVT
RLGSLSLSTLETVKAVKGIIYSYFSTALNASKNNPISDEQRKEFDPELFALLEKLELIRTRK
KKQKVERIANSLIQTCLENNIKFIRGEGDLSTTNNATKKKANSRSMDWLARGVFNKIRQLAP
MHNITLFGCGSLYTSHQDPLVHRNPDKAMKCRWAAIPVKDIGDWVLRKLSQNLRAKNIGTGE
YYHQGVKEFLSHYELQDLEEELLKWRSDRKSNIPCWVLQNRLAEKLGNKEAVVYIPVRGGRI
YFATHKVATGAVSIVFDQKQVWVCNADHVAAANIALTVKGIGEQSSDEENPDGSRIKLQLTS

Representative nucleic acid and protein sequences of the base editors follow:

BhCas12b GGSGGS-ABE8-Xten20 at P153

GCCACCATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGCCAC
CAGATCCTTCATCCTGAAGATCGAGCCCAACGAGGAAGTGAAGAAAGGCCTCTGGAAAACCC
ACGAGGTGCTGAACCACGGAATCGCCTACTACATGAATATCCTGAAGCTGATCCGGCAAGAG
GCCATCTACGAGCACCACGAGCAGGACCCCAAGAATCCCAAGAAGGTGTCCAAGGCCGAGAT
CCAGGCCGAGCTGTGGGATTTCGTGCTGAAGATGCAGAAGTGCAACAGCTTCACACACGAGG
TGGACAAGGACGAGGTGTTCAACATCCTGAGAGAGCTGTACGAGGAACTGGTGCCCAGCAGC
GTGGAAAAGAAGGGCGAAGCCAACCAGCTGAGCAACAAGTTTCTGTACCCTCTGGTGGACCC

CAACAGCCAGTCTGGAAAGGGAACAGCCAGCAGCGGCAGAAAGCCCAGATGGTACAACCTGA
AGATTGCCGGCGATCCCggaggctctggaggaagcTCCGAAGTCGAGTTTTCCCATGAGTAC
TGGATGAGACACGCATTGACTCTCGCAAAGAGGGCTCGAGATGAACGCGAGGTGCCCGTGGG
GGCAGTACTCGTGCTCAACAATCGCGTAATCGGCGAAGGTTGGAATAGGGCAATCGGACTCC
ACGACCCCACTGCACATGCGGAAATCATGGCCCTTCGACAGGGAGGGCTTGTGATGCAGAAT
TATCGACTTTATGATGCGACGCTGTACGTCACGTTTGAACCTTGCGTAATGTGCGCGGGAGC
TATGATTCACTCCCGCATTGGACGAGTTGTATTCGGTGTTCGCAACGCCAAGACGGGTGCCG
CAGGTTCACTGATGGACGTGCTGCATCATCCAGGCATGAACCACCGGGTAGAAATCACAGAA
GGCATATTGGCGGACGAATGTGCGGCGCTGTTGTGTCGTTTTTTTCGCATGCCCAGGCGGGT
CTTTAACGCCCAGAAAAAAGCACAATCCTCTACTGACGGCTCTTCTGGATCTGAAACACCTG
GCACAAGCGAGAGCGCCACCCCTGAGAGCTCTGGCTCCTGGGAAGAAGAGAAGAAGAAGTGG
GAAGAAGATAAGAAAAAGGACCCGCTGGCCAAGATCCTGGGCAAGCTGGCTGAGTACGGACT
GATCCCTCTGTTCATCCCCTACACCGACAGCAACGAGCCCATCGTGAAAGAAATCAAGTGGA
TGGAAAAGTCCCGGAACCAGAGCGTGCGGCGGCTGGATAAGGACATGTTCATTCAGGCCCTG
GAACGGTTCCTGAGCTGGGAGAGCTGGAACCTGAAAGTGAAAGAGGAATACGAGAAGGTCGA
GAAAGAGTACAAGACCCTGGAAGAGAGGATCAAAGAGGACATCCAGGCTCTGAAGGCTCTGG
AACAGTATGAGAAAGAGCGGCAAGAACAGCTGCTGCGGGACACCCTGAACACCAACGAGTAC
CGGCTGAGCAAGAGAGGCCTTAGAGGCTGGCGGGAAATCATCCAGAAATGGCTGAAAATGGA
CGAGAACGAGCCCTCCGAGAAGTACCTGGAAGTGTTCAAGGACTACCAGCGGAAGCACCCTA
GAGAGGCCGGCGATTACAGCGTGTACGAGTTCCTGTCCAAGAAAGAGAACCACTTCATCTGG
CGGAATCACCCTGAGTACCCCTACCTGTACGCCACCTTCTGCGAGATCGACAAGAAAAAGAA
GGACGCCAAGCAGCAGGCCACCTTCACACTGGCCGATCCTATCAATCACCCTCTGTGGGTCC
GATTCGAGGAAAGAAGCGGCAGCAACCTGAACAAGTACAGAATCCTGACCGAGCAGCTGCAC
ACCGAGAAGCTGAAGAAAAAGCTGACAGTGCAGCTGGACCGGCTGATCTACCCTACAGAATC
TGGCGGCTGGGAAGAGAAGGGCAAAGTGGACATTGTGCTGCTGCCCAGCCGGCAGTTCTACA
ACCAGATCTTCCTGGACATCGAGGAAAAGGGCAAGCACGCCTTCACCTACAAGGATGAGAGC
ATCAAGTTCCCTCTGAAGGGCACACTCGGCGGAGCCAGAGTGCAGTTCGACAGAGATCACCT
GAGAAGATACCCTCACAAGGTGGAAAGCGGCAACGTGGGCAGAATCTACTTCAACATGACCG
TGAACATCGAGCCTACAGAGTCCCCAGTGTCCAAGTCTCTGAAGATCCACCGGGACGACTTC
CCCAAGGTGGTCAACTTCAAGCCCAAAGAACTGACCGAGTGGATCAAGGACAGCAAGGGCAA
GAAACTGAAGTCCGGCATCGAGTCCCTGGAAATCGGCCTGAGAGTGATGAGCATCGACCTGG
GACAGAGACAGGCCGCTGCCGCCTCTATTTTCGAGGTGGTGGATCAGAAGCCCGACATCGAA
GGCAAGCTGTTTTTCCCAATCAAGGGCACCGAGCTGTATGCCGTGCACAGAGCCAGCTTCAA
CATCAAGCTGCCCGGCGAGACACTGGTCAAGAGCAGAGAAGTGCTGCGGAAGGCCAGAGAGG

ACAATCTGAAACTGATGAACCAGAAGCTCAACTTCCTGCGGAACGTGCTGCACTTCCAGCAG
TTCGAGGACATCACCGAGAGAGAGAAGCGGGTCACCAAGTGGATCAGCAGACAAGAGAACAG
CGACGTGCCCCTGGTGTACCAGGATGAGCTGATCCAGATCCGCGAGCTGATGTACAAGCCTT
ACAAGGACTGGGTCGCCTTCCTGAAGCAGCTCCACAAGAGACTGGAAGTCGAGATCGGCAAA
GAAGTGAAGCACTGGCGGAAGTCCCTGAGCGACGGAAGAAAGGGCCTGTACGGCATCTCCCT
GAAGAACATCGACGAGATCGATCGGACCCGGAAGTTCCTGCTGAGATGGTCCCTGAGGCCTA
CCGAACCTGGCGAAGTGCGTAGACTGGAACCCGGCCAGAGATTCGCCATCGACCAGCTGAAT
CACCTGAACGCCCTGAAAGAAGATCGGCTGAAGAAGATGGCCAACACCATCATCATGCACGC
CCTGGGCTACTGCTACGACGTGCGGAAGAAGAAATGGCAGGCTAAGAACCCCGCCTGCCAGA
TCATCCTGTTCGAGGATCTGAGCAACTACAACCCCTACGAGGAAAGGTCCCGCTTCGAGAAC
AGCAAGCTCATGAAGTGGTCCAGACGCGAGATCCCCAGACAGGTTGCACTGCAGGGCGAGAT
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SISTIEDDSSKQSMKRPAATKKAGQAKKKKGSYPYDVPDYAYPYDVPDYAYPYDVPDYA

For the sequences above, the Kozak sequence is bolded and underlined; marks the N-terminal nuclear localization signal (NLS); lower case characters denote the GGGSGGS linker; _ _ _ _ marks the sequence encoding ABE8, unmodified sequence encodes BhCas12b; double underling denotes the Xten20 linker; single underlining denotes the C-terminal NLS; GGATCC denotes the GS linker; and italicized characters represent the coding sequence of the 3x hemagglutinin (HA) tag.

Guide Polynucleotides

In an embodiment, the guide polynucleotide is a guide RNA. An RNA/Cas complex can assist in "guiding" Cas protein to a target DNA. Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3'-5' exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA," or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. *See, e.g.*, Jinek M. *et al.*, Science 337:816-821(2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. Cas9 nuclease sequences and structures are well known to those of skill in the art (see *e.g.*, "Complete genome sequence of an M1 strain of *Streptococcus pyogenes*." Ferretti, J.J. *et al.*, Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E. *et al.*, Nature 471:602-607(2011); and "Programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M.*et al*, Science 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences can be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in

Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference. In some embodiments, a Cas9 nuclease has an inactive (*e.g.*, an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase.

In some embodiments, the guide polynucleotide is at least one single guide RNA ("sgRNA" or "gNRA"). In some embodiments, the guide polynucleotide is at least one tracrRNA. In some embodiments, the guide polynucleotide does not require PAM sequence to guide the polynucleotide-programmable DNA-binding domain (*e.g.*, Cas9 or Cpf1) to the target nucleotide sequence.

The polynucleotide programmable nucleotide binding domain (*e.g.*, a CRISPR-derived domain) of the base editors disclosed herein can recognize a target polynucleotide sequence by associating with a guide polynucleotide. A guide polynucleotide (*e.g.*, gRNA) is typically single-stranded and can be programmed to site-specifically bind (*i.e.*, via complementary base pairing) to a target sequence of a polynucleotide, thereby directing a base editor that is in conjunction with the guide nucleic acid to the target sequence. A guide polynucleotide can be DNA. A guide polynucleotide can be RNA. In some embodiments, the guide polynucleotide comprises natural nucleotides (*e.g.*, adenosine). In some embodiments, the guide polynucleotide comprises non-natural (or unnatural) nucleotides (*e.g.*, peptide nucleic acid or nucleotide analogs). In some embodiments, the targeting region of a guide nucleic acid sequence can be at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. A targeting region of a guide nucleic acid can be between 10-30 nucleotides in length, or between 15-25 nucleotides in length, or between 15-20 nucleotides in length.

In some embodiments, a guide polynucleotide comprises two or more individual polynucleotides, which can interact with one another via for example complementary base pairing (*e.g.*, a dual guide polynucleotide). For example, a guide polynucleotide can comprise a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA). For example, a guide polynucleotide can comprise one or more trans-activating CRISPR RNA (tracrRNA).

In type II CRISPR systems, targeting of a nucleic acid by a CRISPR protein (*e.g.*, Cas9) typically requires complementary base pairing between a first RNA molecule (crRNA) comprising a sequence that recognizes the target sequence and a second RNA molecule (trRNA) comprising repeat sequences which forms a scaffold region that stabilizes the guide

RNA-CRISPR protein complex. Such dual guide RNA systems can be employed as a guide polynucleotide to direct the base editors disclosed herein to a target polynucleotide sequence.

In some embodiments, the base editor provided herein utilizes a single guide polynucleotide (*e.g.*, gRNA). In some embodiments, the base editor provided herein utilizes a dual guide polynucleotide (*e.g.*, dual gRNAs). In some embodiments, the base editor provided herein utilizes one or more guide polynucleotide (*e.g.*, multiple gRNA). In some embodiments, a single guide polynucleotide is utilized for different base editors described herein. For example, a single guide polynucleotide can be utilized for a cytidine base editor and an adenosine base editor.

In other embodiments, a guide polynucleotide can comprise both the polynucleotide targeting portion of the nucleic acid and the scaffold portion of the nucleic acid in a single molecule (*i.e.*, a single-molecule guide nucleic acid). For example, a single-molecule guide polynucleotide can be a single guide RNA (sgRNA or gRNA). Herein the term guide polynucleotide sequence contemplates any single, dual or multi-molecule nucleic acid capable of interacting with and directing a base editor to a target polynucleotide sequence.

Typically, a guide polynucleotide (*e.g.*, crRNA/trRNA complex or a gRNA) comprises a "polynucleotide-targeting segment" that includes a sequence capable of recognizing and binding to a target polynucleotide sequence, and a "protein-binding segment" that stabilizes the guide polynucleotide within a polynucleotide programmable nucleotide binding domain component of a base editor. In some embodiments, the polynucleotide targeting segment of the guide polynucleotide recognizes and binds to a DNA polynucleotide, thereby facilitating the editing of a base in DNA. In other embodiments, the polynucleotide targeting segment of the guide polynucleotide recognizes and binds to an RNA polynucleotide, thereby facilitating the editing of a base in RNA. Herein a "segment" refers to a section or region of a molecule, *e.g.*, a contiguous stretch of nucleotides in the guide polynucleotide. A segment can also refer to a region/section of a complex such that a segment can comprise regions of more than one molecule. For example, where a guide polynucleotide comprises multiple nucleic acid molecules, the protein-binding segment of can include all or a portion of multiple separate molecules that are for instance hybridized along a region of complementarity. In some embodiments, a protein-binding segment of a DNA-targeting RNA that comprises two separate molecules can comprise (i) base pairs 40-75 of a first RNA molecule that is 100 base pairs in length; and (ii) base pairs 10-25 of a second RNA molecule that is 50 base pairs in length. The definition of "segment," unless otherwise specifically defined in a particular context, is not limited to a specific number of total base

pairs, is not limited to any particular number of base pairs from a given RNA molecule, is not limited to a particular number of separate molecules within a complex, and can include regions of RNA molecules that are of any total length and can include regions with complementarity to other molecules.

A guide RNA or a guide polynucleotide can comprise two or more RNAs, *e.g.*, CRISPR RNA (crRNA) and transactivating crRNA (tracrRNA). A guide RNA or a guide polynucleotide can sometimes comprise a single-chain RNA, or single guide RNA (sgRNA) formed by fusion of a portion (*e.g.*, a functional portion) of crRNA and tracrRNA. A guide RNA or a guide polynucleotide can also be a dual RNA comprising a crRNA and a tracrRNA. Furthermore, a crRNA can hybridize with a target DNA.

As discussed above, a guide RNA or a guide polynucleotide can be an expression product. For example, a DNA that encodes a guide RNA can be a vector comprising a sequence coding for the guide RNA. A guide RNA or a guide polynucleotide can be transferred into a cell by transfecting the cell with an isolated guide RNA or plasmid DNA comprising a sequence coding for the guide RNA and a promoter. A guide RNA or a guide polynucleotide can also be transferred into a cell in other way, such as using virus-mediated gene delivery.

A guide RNA or a guide polynucleotide can be isolated. For example, a guide RNA can be transfected in the form of an isolated RNA into a cell or organism. A guide RNA can be prepared by *in vitro* transcription using any *in vitro* transcription system known in the art. A guide RNA can be transferred to a cell in the form of isolated RNA rather than in the form of plasmid comprising encoding sequence for a guide RNA.

A guide RNA or a guide polynucleotide can comprise three regions: a first region at the 5' end that can be complementary to a target site in a chromosomal sequence, a second internal region that can form a stem loop structure, and a third 3' region that can be single-stranded. A first region of each guide RNA can also be different such that each guide RNA guides a fusion protein to a specific target site. Further, second and third regions of each guide RNA can be identical in all guide RNAs.

A first region of a guide RNA or a guide polynucleotide can be complementary to sequence at a target site in a chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In some embodiments, a first region of a guide RNA can comprise from or from about 10 nucleotides to 25 nucleotides (*i.e.*, from 10 nucleotides to nucleotides; or from about 10 nucleotides to about 25 nucleotides; or from 10 nucleotides to about 25 nucleotides; or from about 10 nucleotides to 25 nucleotides) or more. For

example, a region of base pairing between a first region of a guide RNA and a target site in a chromosomal sequence can be or can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more nucleotides in length. Sometimes, a first region of a guide RNA can be or can be about 19, 20, or 21 nucleotides in length.

A guide RNA or a guide polynucleotide can also comprise a second region that forms a secondary structure. For example, a secondary structure formed by a guide RNA can comprise a stem (or hairpin) and a loop. A length of a loop and a stem can vary. For example, a loop can range from or from about 3 to 10 nucleotides in length, and a stem can range from or from about 6 to 20 base pairs in length. A stem can comprise one or more bulges of 1 to 10 or about 10 nucleotides. The overall length of a second region can range from or from about 16 to 60 nucleotides in length. For example, a loop can be or can be about 4 nucleotides in length and a stem can be or can be about 12 base pairs.

A guide RNA or a guide polynucleotide can also comprise a third region at the 3' end that can be essentially single-stranded. For example, a third region is sometimes not complementarity to any chromosomal sequence in a cell of interest and is sometimes not complementarity to the rest of a guide RNA. Further, the length of a third region can vary. A third region can be more than or more than about 4 nucleotides in length. For example, the length of a third region can range from or from about 5 to 60 nucleotides in length.

A guide RNA or a guide polynucleotide can target any exon or intron of a gene target. In some embodiments, a guide can target exon 1 or 2 of a gene; in other embodiments, a guide can target exon 3 or 4 of a gene. A composition can comprise multiple guide RNAs that all target the same exon or in some embodiments, multiple guide RNAs that can target different exons. An exon and an intron of a gene can be targeted.

A guide RNA or a guide polynucleotide can target a nucleic acid sequence of or of about 20 nucleotides. A target nucleic acid can be less than or less than about 20 nucleotides. A target nucleic acid can be at least or at least about 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, or anywhere between 1-100 nucleotides in length. A target nucleic acid can be at most or at most about 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, or anywhere between 1-100 nucleotides in length. A target nucleic acid sequence can be or can be about 20 bases immediately 5' of the first nucleotide of the PAM. A guide RNA can target a nucleic acid sequence. A target nucleic acid can be at least or at least about 1-10, 1-20, 1-30, 1-40, 1-50, 1-60, 1-70, 1-80, 1-90, or 1-100 nucleotides.

A guide polynucleotide, for example, a guide RNA, can refer to a nucleic acid that can hybridize to another nucleic acid, for example, the target nucleic acid or protospacer in a

genome of a cell. A guide polynucleotide can be RNA. A guide polynucleotide can be DNA. The guide polynucleotide can be programmed or designed to bind to a sequence of nucleic acid site-specifically. A guide polynucleotide can comprise a polynucleotide chain and can be called a single guide polynucleotide. A guide polynucleotide can comprise two polynucleotide chains and can be called a double guide polynucleotide. A guide RNA can be introduced into a cell or embryo as an RNA molecule. For example, an RNA molecule can be transcribed *in vitro* and/or can be chemically synthesized. An RNA can be transcribed from a synthetic DNA molecule, *e.g.*, a gBlocks® gene fragment. A guide RNA can then be introduced into a cell or embryo as an RNA molecule. A guide RNA can also be introduced into a cell or embryo in the form of a non-RNA nucleic acid molecule, *e.g.*, DNA molecule. For example, a DNA encoding a guide RNA can be operably linked to promoter control sequence for expression of the guide RNA in a cell or embryo of interest. A RNA coding sequence can be operably linked to a promoter sequence that is recognized by RNA polymerase III (Pol III). Plasmid vectors that can be used to express guide RNA include, but are not limited to, px330 vectors and px333 vectors. In some embodiments, a plasmid vector (*e.g.*, px333 vector) can comprise at least two guide RNA-encoding DNA sequences.

Methods for selecting, designing, and validating guide polynucleotides, *e.g.*, guide RNAs and targeting sequences are described herein and known to those skilled in the art. For example, to minimize the impact of potential substrate promiscuity of a deaminase domain in the nucleobase editor system (*e.g.*, an AID domain), the number of residues that could unintentionally be targeted for deamination (*e.g.*, off-target C residues that could potentially reside on ssDNA within the target nucleic acid locus) may be minimized. In addition, software tools can be used to optimize the gRNAs corresponding to a target nucleic acid sequence, *e.g.*, to minimize total off-target activity across the genome. For example, for each possible targeting domain choice using S. pyogenes Cas9, all off-target sequences (preceding selected PAMs, *e.g.*, NAG or NGG) may be identified across the genome that contain up to certain number (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of mismatched base-pairs. First regions of gRNAs complementary to a target site can be identified, and all first regions (*e.g.*, crRNAs) can be ranked according to its total predicted off-target score; the top-ranked targeting domains represent those that are likely to have the greatest on-target and the least off-target activity. Candidate targeting gRNAs can be functionally evaluated by using methods known in the art and/or as set forth herein.

As a non-limiting example, target DNA hybridizing sequences in crRNAs of a guide RNA for use with Cas9s may be identified using a DNA sequence searching algorithm.

gRNA design may be carried out using custom gRNA design software based on the public tool cas-offinder as described in Bae S., Park J., & Kim J.-S. Cas-OFFinder: A fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics 30, 1473-1475 (2014). This software scores guides after calculating their genome-wide off-target propensity. Typically matches ranging from perfect matches to 7 mismatches are considered for guides ranging in length from 17 to 24. Once the off-target sites are computationally-determined, an aggregate score is calculated for each guide and summarized in a tabular output using a web-interface. In addition to identifying potential target sites adjacent to PAM sequences, the software also identifies all PAM adjacent sequences that differ by 1, 2, 3 or more than 3 nucleotides from the selected target sites. Genomic DNA sequences for a target nucleic acid sequence, *e.g.*, a target gene may be obtained and repeat elements may be screened using publicly available tools, for example, the RepeatMasker program. RepeatMasker searches input DNA sequences for repeated elements and regions of low complexity. The output is a detailed annotation of the repeats present in a given query sequence.

Following identification, first regions of guide RNAs, *e.g.*, crRNAs, may be ranked into tiers based on their distance to the target site, their orthogonality and presence of 5' nucleotides for close matches with relevant PAM sequences (for example, a 5′ G based on identification of close matches in the human genome containing a relevant PAM *e.g.*, NGG PAM for S. pyogenes, NNGRRT or NNGRRV PAM for S. aureus). As used herein, orthogonality refers to the number of sequences in the human genome that contain a minimum number of mismatches to the target sequence. A "high level of orthogonality" or "good orthogonality" may, for example, refer to 20-mer targeting domains that have no identical sequences in the human genome besides the intended target, nor any sequences that contain one or two mismatches in the target sequence. Targeting domains with good orthogonality may be selected to minimize off-target DNA cleavage.

In some embodiments, a reporter system may be used for detecting base-editing activity and testing candidate guide polynucleotides. In some embodiments, a reporter system may comprise a reporter gene-based assay where base editing activity leads to expression of the reporter gene. For example, a reporter system may include a reporter gene comprising a deactivated start codon, *e.g.*, a mutation on the template strand from 3'-TAC-5' to 3'-CAC-5'. Upon successful deamination of the target C, the corresponding mRNA will be transcribed as 5'-AUG-3' instead of 5'-GUG-3', enabling the translation of the reporter gene. Suitable reporter genes will be apparent to those of skill in the art. Non-limiting examples of reporter

genes include gene encoding green fluorescence protein (GFP), red fluorescence protein (RFP), luciferase, secreted alkaline phosphatase (SEAP), or any other gene whose expression are detectable and apparent to those skilled in the art. The reporter system can be used to test many different gRNAs, *e.g.*, in order to determine which residue(s) with respect to the target DNA sequence the respective deaminase will target. sgRNAs that target non-template strand can also be tested in order to assess off-target effects of a specific base editing protein, *e.g.*, a Cas9 deaminase fusion protein. In some embodiments, such gRNAs can be designed such that the mutated start codon will not be base-paired with the gRNA. The guide polynucleotides can comprise standard ribonucleotides, modified ribonucleotides (*e.g.*, pseudouridine), ribonucleotide isomers, and/or ribonucleotide analogs. In some embodiments, the guide polynucleotide can comprise at least one detectable label. The detectable label can be a fluorophore (*e.g.*, FAM, TMR, Cy3, Cy5, Texas Red, Oregon Green, Alexa Fluors, Halo tags, or suitable fluorescent dye), a detection tag (*e.g.*, biotin, digoxigenin, and the like), quantum dots, or gold particles.

The guide polynucleotides can be synthesized chemically, synthesized enzymatically, or a combination thereof. For example, the guide RNA can be synthesized using standard phosphoramidite-based solid-phase synthesis methods. Alternatively, the guide RNA can be synthesized in vitro by operably linking DNA encoding the guide RNA to a promoter control sequence that is recognized by a phage RNA polymerase. Examples of suitable phage promoter sequences include T7, T3, SP6 promoter sequences, or variations thereof. In embodiments in which the guide RNA comprises two separate molecules (*e.g.*., crRNA and tracrRNA), the crRNA can be chemically synthesized and the tracrRNA can be enzymatically synthesized.

In some embodiments, a base editor system may comprise multiple guide polynucleotides, *e.g.*, gRNAs. For example, the gRNAs may target to one or more target loci (*e.g.*, at least 1 gRNA, at least 2 gRNA, at least 5 gRNA, at least 10 gRNA, at least 20 gRNA, at least 30 g RNA, at least 50 gRNA) comprised in a base editor system. The multiple gRNA sequences can be tandemly arranged and are preferably separated by a direct repeat.

A DNA sequence encoding a guide RNA or a guide polynucleotide can also be part of a vector. Further, a vector can comprise additional expression control sequences (*e.g.*, enhancer sequences, Kozak sequences, polyadenylation sequences, transcriptional termination sequences, etc.), selectable marker sequences (*e.g.*, GFP or antibiotic resistance genes such as puromycin), origins of replication, and the like. A DNA molecule encoding a

guide RNA can also be linear. A DNA molecule encoding a guide RNA or a guide polynucleotide can also be circular.

In some embodiments, one or more components of a base editor system may be encoded by DNA sequences. Such DNA sequences may be introduced into an expression system, *e.g.*, a cell, together or separately. For example, DNA sequences encoding a polynucleotide programmable nucleotide binding domain and a guide RNA may be introduced into a cell, each DNA sequence can be part of a separate molecule (*e.g.*, one vector containing the polynucleotide programmable nucleotide binding domain coding sequence and a second vector containing the guide RNA coding sequence) or both can be part of a same molecule (*e.g.*, one vector containing coding (and regulatory) sequence for both the polynucleotide programmable nucleotide binding domain and the guide RNA).

A guide polynucleotide can comprise one or more modifications to provide a nucleic acid with a new or enhanced feature. A guide polynucleotide can comprise a nucleic acid affinity tag. A guide polynucleotide can comprise synthetic nucleotide, synthetic nucleotide analog, nucleotide derivatives, and/or modified nucleotides.

In some embodiments, a gRNA or a guide polynucleotide can comprise modifications. A modification can be made at any location of a gRNA or a guide polynucleotide. More than one modification can be made to a single gRNA or a guide polynucleotide. A gRNA or a guide polynucleotide can undergo quality control after a modification. In some embodiments, quality control can include PAGE, HPLC, MS, or any combination thereof.

A modification of a gRNA or a guide polynucleotide can be a substitution, insertion, deletion, chemical modification, physical modification, stabilization, purification, or any combination thereof.

A gRNA or a guide polynucleotide can also be modified by 5'adenylate, 5' guanosine-triphosphate cap, 5'N7-Methylguanosine-triphosphate cap, 5'triphosphate cap, 3'phosphate, 3'thiophosphate, 5'phosphate, 5'thiophosphate, Cis-Syn thymidine dimer, trimers, C12 spacer, C3 spacer, C6 spacer, dSpacer, PC spacer, rSpacer, Spacer 18, Spacer 9,3'-3' modifications, 5'-5' modifications, abasic, acridine, azobenzene, biotin, biotin BB, biotin TEG, cholesteryl TEG, desthiobiotin TEG, DNP TEG, DNP-X, DOTA, dT-Biotin, dual biotin, PC biotin, psoralen C2, psoralen C6, TINA, 3'DABCYL, black hole quencher 1, black hole quencer 2, DABCYL SE, dT-DABCYL, IRDye QC-1, QSY-21, QSY-35, QSY-7, QSY-9, carboxyl linker, thiol linkers, 2'-deoxyribonucleoside analog purine, 2'-deoxyribonucleoside analog pyrimidine, ribonucleoside analog, 2'-O-methyl ribonucleoside

analog, sugar modified analogs, wobble/universal bases, fluorescent dye label, 2'-fluoro RNA, 2'-O-methyl RNA, methylphosphonate, phosphodiester DNA, phosphodiester RNA, phosphothioate DNA, phosphorothioate RNA, UNA, pseudouridine-5'-triphosphate, 5'-methylcytidine-5'-triphosphate, or any combination thereof.

In some embodiments, a modification is permanent. In other embodiments, a modification is transient. In some embodiments, multiple modifications are made to a gRNA or a guide polynucleotide. A gRNA or a guide polynucleotide modification can alter physiochemical properties of a nucleotide, such as their conformation, polarity, hydrophobicity, chemical reactivity, base-pairing interactions, or any combination thereof.

The PAM sequence can be any PAM sequence known in the art. Suitable PAM sequences include, but are not limited to, NGG, NGA, NGC, NGN, NGT, NGCG, NGAG, NGAN, NGNG, NGCN, NGCG, NGTN, NNGRRT, NNNRRT, NNGRR(N), TTTV, TYCV, TYCV, TATV, NNNNGATT, NNAGAAW, or NAAAAC. Y is a pyrimidine; N is any nucleotide base; W is A or T.

A modification can also be a phosphorothioate substitute. In some embodiments, a natural phosphodiester bond can be susceptible to rapid degradation by cellular nucleases and; a modification of internucleotide linkage using phosphorothioate (PS) bond substitutes can be more stable towards hydrolysis by cellular degradation. A modification can increase stability in a gRNA or a guide polynucleotide. A modification can also enhance biological activity. In some embodiments, a phosphorothioate enhanced RNA gRNA can inhibit RNase A, RNase T1, calf serum nucleases, or any combinations thereof. These properties can allow the use of PS-RNA gRNAs to be used in applications where exposure to nucleases is of high probability *in vivo* or *in vitro*. For example, phosphorothioate (PS) bonds can be introduced between the last 3-5 nucleotides at the 5'- or ''-end of a gRNA which can inhibit exonuclease degradation. In some embodiments, phosphorothioate bonds can be added throughout an entire gRNA to reduce attack by endonucleases.

Different Cas12b orthologs (e.g., BhCas12b, BvCas12b, and AaCas12b) use different scaffold sequences (also referred to as tracrRNA). In some embodiments, the scaffold sequence is optimized for use with a BhCas12b protein and has the following sequence: (where the T's are replaced by uridines (U's) in the actual gRNA).

BhCas12b sgRNA scaffold (underlined) + 20nt to 23nt guide sequence (denoted by Ns).

5'GTTCTGTCTTTTGGTCAGGACAACCGTCTAGCTATAAGTGCTGCAGGGTGTGAGAAACTC
CTATTGCTGGACGATGTCTCTTACGAGGCATTAGCACNNNNNNNNNNNNNNNNNNNN-3'

In some embodiments, the scaffold sequence is optimized for use with a BvCas12b protein and has the following sequence: (where the T's are replaced by uridines (U's) in the actual gRNA).

BvCas12b sgRNA scaffold (underlined) + 20nt to 23nt guide sequence (denoted by Ns)

5′GACCTATAGGGTCAATGAATCTGTGCGTGTGCCATAAGTAATTAAAAATTACCCACCACA GGAGCACCTGAAAACAGGTGCTTGGCACNNNNNNNNNNNNNNNNNNNN-3′

In some embodiments, the scaffold sequence is optimized for use with a AaCas12b protein and has the following sequence: (where the T's are replaced by uridines (U's) in the actual gRNA).

AaCas12b sgRNA scaffold (underlined) + 20nt to 23nt guide sequence (denoted by Ns)

5′GTCTAAAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCCAGGTGGCAAAGCC CGTTGAACTTCTCAAAAAGAACGATCTGAGAAGTGGCACNNNNNNNNNNNNNNNNNNNN-3′

Thus, a skilled artisan can change the genomic target of the Cas protein specificity is partially determined by how specific the gRNA targeting sequence is for the genomic target compared to the rest of the genome.

Protospacer Adjacent Motif

The term "protospacer adjacent motif (PAM)" or PAM-like motif refers to a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease in the CRISPR bacterial adaptive immune system. In some embodiments, the PAM can be a 5' PAM (*i.e.*, located upstream of the 5' end of the protospacer). In other embodiments, the PAM can be a 3' PAM (*i.e.*, located downstream of the 5' end of the protospacer).

The PAM sequence is essential for target binding, but the exact sequence depends on a type of Cas protein.

A base editor provided herein can comprise a CRISPR protein-derived domain that is capable of binding a nucleotide sequence that contains a canonical or non-canonical protospacer adjacent motif (PAM) sequence. A PAM site is a nucleotide sequence in proximity to a target polynucleotide sequence. Some aspects of the disclosure provide for base editors comprising all or a portion of CRISPR proteins that have different PAM specificities.

For example, typically Cas9 proteins, such as Cas9 from *S. pyogenes* (spCas9), require a canonical NGG PAM sequence to bind a particular nucleic acid region, where the "N" in "NGG" is adenine (A), thymine (T), guanine (G), or cytosine (C), and the G is guanine. A PAM can be CRISPR protein-specific and can be different between different base editors

comprising different CRISPR protein-derived domains. A PAM can be 5' or 3' of a target sequence. A PAM can be upstream or downstream of a target sequence. A PAM can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides in length. Often, a PAM is between 2-6 nucleotides in length. Several PAM variants are described in Table 1 below.

Table 1. Cas9 proteins and corresponding PAM sequences

Variant	PAM
spCas9	NGG
spCas9-VRQR	NGA
spCas9-VRER	NGCG
xCas9 (sp)	NGN
saCas9	NNGRRT
saCas9-KKH	NNNRRT
spCas9-MQKSER	NGCG
spCas9-MQKSER	NGCN
spCas9-LRKIQK	NGTN
spCas9-LRVSQK	NGTN
spCas9-LRVSQL	NGTN
spCas9-MQKFRAER	NGC
Cpf1	5' (TTTV)
SpyMac	5'-NAA-3'

In some embodiments, the PAM is NGC. In some embodiments, the NGC PAM is recognized by a Cas9 variant. In some embodiments, the NGC PAM variant includes one or more amino acid substitutions selected from D1135M, S1136Q, G1218K, E1219F, A1322R, D1332A, R1335E, and T1337R (collectively termed "MQKFRAER").

In some embodiments, the PAM is NGT. In some embodiments, the NGT PAM is recognized by a Cas9 variant. In some embodiments, the NGT PAM variant is generated through targeted mutations at one or more residues 1335, 1337, 1135, 1136, 1218, and/or 1219. In some embodiments, the NGT PAM variant is created through targeted mutations at one or more residues 1219, 1335, 1337, 1218. In some embodiments, the NGT PAM variant is created through targeted mutations at one or more residues 1135, 1136, 1218, 1219, and 1335. In some embodiments, the NGT PAM variant is selected from the set of targeted mutations provided in **Table 2** and **Table 3** below.

Table 2: NGT PAM Variant Mutations at residues 1219, 1335, 1337, 1218

Variant	E1219V	R1335Q	T1337	G1218
1	F	V	T	
2	F	V	R	
3	F	V	Q	
4	F	V	L	
5	F	V	T	R
6	F	V	R	R
7	F	V	Q	R
8	F	V	L	R
9	L	L	T	
10	L	L	R	
11	L	L	Q	
12	L	L	L	
13	F	I	T	
14	F	I	R	
15	F	I	Q	
16	F	I	L	
17	F	G	C	
18	H	L	N	
19	F	G	C	A
20	H	L	N	V
21	L	A	W	
22	L	A	F	
23	L	A	Y	

Variant	E1219V	R1335Q	T1337	G1218
24	I	A	W	
25	I	A	F	
26	I	A	Y	

Table 3: NGT PAM Variant Mutations at residues 1135, 1136, 1218, 1219, and 1335

Variant	D1135L	S1136R	G1218S	E1219V	R1335Q
27	G				
28	V				
29	I				
30		A			
31		W			
32		H			
33		K			
34			K		
35			R		
36			Q		
37			T		
38			N		
39				I	
40				A	
41				N	
42				Q	
43				G	
44				L	
45				S	
46				T	
47					L
48					I
49					V
50					N
51					S
52					T

Variant	D1135L	S1136R	G1218S	E1219V	R1335Q
53					F
54					Y
55	N1286Q	I1331F			

In some embodiments, the NGT PAM variant is selected from variant 5, 7, 28, 31, or 36 in Tables 2 and 3. In some embodiments, the variants have improved NGT PAM recognition.

In some embodiments, the NGT PAM variants have mutations at residues 1219, 1335, 1337, and/or 1218. In some embodiments, the NGT PAM variant is selected with mutations for improved recognition from the variants provided in Table 4 below.

Table 4: NGT PAM Variant Mutations at residues 1219, 1335, 1337, and 1218

Variant	E1219V	R1335Q	T1337	G1218
1	F	V	T	
2	F	V	R	
3	F	V	Q	
4	F	V	L	
5	F	V	T	R
6	F	V	R	R
7	F	V	Q	R
8	F	V	L	R

In some embodiments, base editors with specificity for NGT PAM may be generated as provided in **Table 5** below.

Table 5A. NGT PAM variants

	NGTN variant	D1135	S1136	G1218	E1219	A1322R	R1335	T1337
Variant 1	LRKIQK	L	R	K	I	-	Q	K
Variant 2	LRSVQK	L	R	S	V	-	Q	K
Variant 3	LRSVQL	L	R	S	V	-	Q	L
Variant 4	LRKIRQK	L	R	K	I	R	Q	K
Variant 5	LRSVRQK	L	R	S	V	R	Q	K

	NGTN variant	D1135	S1136	G1218	E1219	A1322R	R1335	T1337
Variant 6	LRSVRQL	L	R	S	V	R	Q	L

In some embodiments the NGTN variant is variant 1. In some embodiments, the NGTN variant is variant 2. In some embodiments, the NGTN variant is variant 3. In some embodiments, the NGTN variant is variant 4. In some embodiments, the NGTN variant is variant 5. In some embodiments, the NGTN variant is variant 6.

In some embodiments, the Cas9 domain is a Cas9 domain from *Streptococcus pyogenes* (SpCas9). In some embodiments, the SpCas9 domain is a nuclease active SpCas9, a nuclease inactive SpCas9 (SpCas9d), or a SpCas9 nickase (SpCas9n). In some embodiments, the SpCas9 comprises a D10X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, as numbered in SEQ ID NO: 1, wherein X is any amino acid except for D. In some embodiments, the SpCas9 comprises a D10A mutation, as numbered in SEQ ID NO: 1, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SpCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having an NGG, a NGA, or a NGCG PAM sequence. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a T1337X mutation, as numbered in SEQ ID NO: 1, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1136E, R1335Q, and T1337R mutation, as numbered in SEQ ID NO: 1, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135E, a R1335Q, and a T1337R mutation, as numbered in SEQ ID NO: 1, or corresponding mutations in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a T1337X mutation, as numbered in SEQ ID NO: 1, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135V, a R1335Q, and a T1337R mutation, as numbered in SEQ ID NO: 1, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135V, a R1335Q, and a T1337R mutation, as numbered in SEQ ID NO: 1, or corresponding mutations in any of the amino acid

sequences provided herein. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a G1218X, as numbered in SEQ ID NO: 1, a R1335X, and a T1337X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135V, a G1218R, a R1335Q, and a T1337R mutation, as numbered in SEQ ID NO: 1, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135V, a G1218R, a R1335Q, and a T1337R mutation, as numbered in SEQ ID NO: 1, or corresponding mutations in any of the amino acid sequences provided herein.

In some embodiments, the Cas9 is a Cas9 variant having specificity for an altered PAM sequence. In some embodiments, the Additional Cas9 variants and PAM sequences are described in Miller et al., Continuous evolution of SpCas9 variants compatible with non-G PAMs. Nat Biotechnol (2020). https://doi.org/10.1038/s41587-020-0412-8, the entirety of which is incorporated herein by reference. in some embodiments, a Cas9 variate have no specific PAM requirements. In some embodiments, a Cas9 variant, e.g. a SpCas9 variant has specificity for a NRNH PAM, wherein R is A or G and H is A, C, or T. In some embodiments, the SpCas9 variant has specificity for a PAM sequence AAA, TAA, CAA, GAA, TAT, GAT, or CAC. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1134, 1135, 1137, 1139, 1151, 1180, 1188, 1211, 1218, 1219, 1221, 1249, 1256, 1264, 1290, 1318, 1317, 1320, 1321, 1323, 1332, 1333, 1335, 1337, or 1339 as numbered in SEQ ID NO: 1 or a corresponding position thereof. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1135, 1218, 1219, 1221, 1249, 1320, 1321, 1323, 1332, 1333, 1335, or 1337 as numbered in SEQ ID NO: 1 or a corresponding position thereof. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1134, 1135, 1137, 1139, 1151, 1180, 1188, 1211, 1219, 1221, 1256, 1264, 1290, 1318, 1317, 1320, 1323, 1333 as numbered in SEQ ID NO: 1 or a corresponding position thereof. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1131, 1135, 1150, 1156, 1180, 1191, 1218, 1219, 1221, 1227, 1249, 1253, 1286, 1293, 1320, 1321, 1332, 1335, 1339 as numbered in SEQ ID NO: 1 or a corresponding position thereof. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1127, 1135, 1180, 1207, 1219, 1234, 1286, 1301, 1332, 1335, 1337, 1338, 1349 as numbered in SEQ ID NO: 1 or a corresponding position thereof. Exemplary amino acid substitutions and PAM specificity of SpCas9 variants are shown in Tables 5B, 5C, 5D, and 5E below.

Table 5B. Additional variant mutations and PAMs

SpCas9 /PAM	SpCas9 amino acid position												
	1114	1135	1218	1219	1221	1249	1320	1321	1323	1332	1333	1335	1337
	R	D	G	E	Q	P	A	P	A	D	R	R	T
AAA		N		V	H						G		
AAA		N		V	H						G		
AAA				V							G		
TAA	G	N		V							I		
TAA		N		V							I		A
TAA	G	N		V							I		A
CAA				V							K		
CAA		N		V							K		
CAA		N		V							K		
GAA				V	H		V				K		
GAA		N		V			V				K		
GAA				V	H		V				K		
TAT			S	V	H	S		S				L	
TAT			S	V	H	S		S				L	
TAT			S	V	H	S		S				L	
GAT				V							I		
GAT				V					D			Q	
GAT				V					D			Q	
CAC				V						N		Q	N
CAC		N		V								Q	N
CAC				V						N		Q	N

Table 5C. Additional variant mutations and PAMs

SpCas9 /PAM	SpCas9 amino acid position																	
	1114	1134	1135	1137	1139	1151	1180	1188	1211	1219	1221	1256	1264	1290	1318	1317	1320	1323	1333
	R	F	D	P	V	K	D	K	K	E	Q	Q	H	V	L	N	A	A	R
GAA										V	H						V		K
GAA			N	S						V							V	D	K
GAA			N							V	H		Y				V		K
CAA			N							V	H		Y				V		K
CAA	G		N	S						V	H		Y				V		K
CAA			N					R		V	H						V		K
CAA			N				G		R	V	H		Y				V		K
CAA			N							V	H		Y				V		K
AAA			N				G			V	H	R	Y				V	D	K
CAA	G		N				G			V	H		Y				V	D	K
CAA		L	N				G			V	H		Y			T	V	D	K
TAA	G		N				G			V	H		Y	G	S		V	D	K
TAA	G		N			E	G			V	H		Y		S		V		K
TAA	G		N				G			V	H		Y		S		V	D	K
TAA	G		N				G		R	V	H						V		K

SpCas9/PAM	SpCas9 amino acid position																		
	1114	1134	1135	1137	1139	1151	1180	1188	1211	1219	1221	1256	1264	1290	1318	1317	1320	1323	1333
	R	F	D	P	V	K	D	K	K	E	Q	Q	H	V	L	N	A	A	R
TAA			N				G		R	V	H		Y				V		K
TAA	G		N		A		G			V	H						V		K
TAA	G		N							V	H						V		K

Table 5D. Additional variant mutations and PAMs

SpCas9/PAM	SpCas9 amino acid position																		
	1114	1131	1135	1150	1156	1180	1191	1218	1219	1221	1227	1249	1253	1286	1293	1320	1321	1332	1335	1339
	R	Y	D	E	K	D	K	G	E	Q	A	P	E	N	A	A	P	D	R	T
SacB.TAT			N				N		V	H						V	S		L	
SacB.TAT			N					S	V	H		S					S	G	L	
AAT			N					S	V	H	V	S		K	T		S	G	L	I
TAT	G		N			G		S	V	H		S	K				S	G	L	
TAT	G		N			G		S	V	H		S					S	G	L	
TAT	G	C	N			G		S	V	H		S					S	G	L	
TAT	G	C	N			G		S	V	H		S					S	G	L	
TAT	G	C	N			G		S	V	H		S					S	G	L	
TAT	G	C	N		E	G		S	V	H		S					S	G	L	
TAT	G	C	N	V		G		S	V	H		S					S	G	L	
TAT		C	N			G		S	V	H		S					S	G	L	
TAT	G	C	N			G		S	V	H		S					S	G	L	

Table 5E. Additional variant mutations and PAM.

SpCas9	SpCas9 amino acid position												
	1114	1127	1135	1180	1207	1219	1234	1286	1301	1332	1335	1337	1338	1349
	R	D	D	D	E	E	N	N	P	D	R	T	S	H
SacB.CAC			N			V				N	Q	N		
AAC	G		N			V				N	Q	N		
AAC	G		N			V				N	Q	N		
TAC	G		N			V				N	Q	N		
TAC	G		N			V		H		N	Q	N		
TAC	G		N		G	V	D	H		N	Q	N		
TAC	G		N			V				N	Q	N		
TAC	G	G	N	E		V		H		N	Q	N		
TAC	G		N			V		H		N	Q	N		
TAC	G		N			V				N	Q	N	T	R

In some embodiments, the Cas9 is a Neisseria menigitidis Cas9 (NmeCas9) or a variant thereof. In some embodiments, the NmeCas9 has specificity for a NNNNGAYW PAM, wherein Y is C or T and W is A or T. In some embodiments, the NmeCas9 has

specificity for a NNNNGYTT PAM, wherein Y is C or T. In some embodiments, the NmeCas9 has specificity for a NNNNGTCT PAM. In some embodiments, the NmeCas9 is a Nme1 Cas9. In some embodiments, the NmeCas9 has specificity for a NNNNGATT PAM, a NNNNCCTA PAM, a NNNNCCTC PAM, a NNNNCCTT PAM, a NNNNCCTG PAM, a NNNNCCGT PAM, a NNNNCCGGPAM, a NNNNCCCA PAM, a NNNNCCCT PAM, a NNNNCCCC PAM, a NNNNCCAT PAM, a NNNNCCAG PAM, a NNNNCCAT PAM, or a NNNGATT PAM. In some embodiments, the Nme1Cas9 has specificity for a NNNNGATT PAM, a NNNNCCTA PAM, a NNNNCCTC PAM, a NNNNCCTT PAM, or a NNNNCCTG PAM. In some embodiments, the NmeCas9 has specificity for a CAA PAM, a CAAA PAM, or a CCA PAM. In some embodiments, the NmeCas9 is a Nme2 Cas9. In some embodiments, the NmeCas9 has specificity for a NNNNCC (N4CC) PAM, wherein N is any one of A, G, C, or T. in some embodiments, the NmeCas9 has specificity for a NNNNCCGT PAM, a NNNNCCGGPAM, a NNNNCCCA PAM, a NNNNCCCT PAM, a NNNNCCCC PAM, a NNNNCCAT PAM, a NNNNCCAG PAM, a NNNNCCAT PAM, or a NNNGATT PAM. In some embodiments, the NmeCas9 is a Nme3Cas9. In some embodiments, the NmeCas9 has specificity for a NNNNCAAA PAM, a NNNNCC PAM, or a NNNNCNNN PAM. Additional NmeCas9 features and PAM sequences as described in Edraki et al. Mol. Cell. (2019) 73(4): 714-726 is incorporated herein by reference in its entirety.

An exemplary amino acid sequence of a Nme1Cas9 is provided below:

type II CRISPR RNA-guided endonuclease Cas9 [Neisseria meningitidis] WP_002235162.1

```
   1 maafkpnpin yilgldigia svgwamveid edenpiclid lgvrvferae vpktgdslam
  61 arrlarsvrr ltrrrahrll rarrllkreg vlqaadfden glikslpntp wqlraaaldr
 121 kltplewsav llhlikhrgy lsqrkneget adkelgallk gvadnahalq tgdfrtpael
 181 alnkfekesg hirnqrgdys htfsrkdlqa elillfekqk efgnphvsgg lkegietllm
 241 tqrpalsgda vqkmlghctf epaepkaakn tytaerfiwl tklnnlrile qgserpltdt
 301 eratlmdepy rkskltyaqa rkllgledta ffkglrygkd naeastlmem kayhaisral
 361 ekeglkdkks plnlspelqd eigtafslfk tdeditgrlk driqpeilea llkhisfdkf
 421 vqislkalrr ivplmeqgkr ydeacaeiyg dhygkkntee kiylppipad eirnpvvlra
 481 lsqarkving vvrrygspar ihietarevg ksfkdrkeie krqeenrkdr ekaaakfrey
 541 fpnfvgepks kdilklrlye qqhgkclysg keinlgrlne kgyveidhal pfsrtwddsf
 601 nnkvlvlgse nqnkgnqtpy eyfngkdnsr ewqefkarve tsrfprskkq rillqkfded
 661 gfkernlndt ryvnrflcqf vadrmrltgk gkkrvfasng qitnllrgfw glrkvraend
 721 rhhaldavvv acstvamqqk itrfvrykem nafdgktidk etgevlhqkt hfpqpweffa
 781 qevmirvfgk pdgkpefeea dtpeklrtll aeklssrpea vheyvtplfv srapnrkmsg
 841 qghmetvksa krldegvsvl rvpltqlklk dlekmvnrer epklyealka rleahkddpa
 901 kafaepfyky dkagnrtqqv kavrveqvqk tgvwvrnhng iadnatmvrv dvfekgdkyy
 961 lvpiyswqva kgilpdravv qgkdeedwql iddsfnfkfs lhpndlvevi tkkarmfgyf
1021 aschrgtgni nirihdldhk igkngilegi gvktalsfqk yqidelgkei rpcrlkkrpp
1081 vr
```

An exemplary amino acid sequence of a Nme2Cas9 is provided below:

type II CRISPR RNA-guided endonuclease Cas9 [Neisseria meningitidis] WP_002230835.1

```
   1 maafkpnpin yilgldigia svgwamveid eeenpirlid lgvrvferae vpktgdslam
  61 arrlarsvrr ltrrrahrll rarrllkreg vlqaadfden glikslpntp wqlraaaldr
 121 kltplewsav llhlikhrgy lsqrkneget adkelgallk gvannahalq tgdfrtpael
 181 alnkfekesg hirnqrgdys htfsrkdlqa elillfekqk efgnphvsgg lkegietllm
 241 tqrpalsgda vqkmlghctf epaepkaakn tytaerfiwl tklnnlrile qgserpltdt
 301 eratlmdepy rkskltyaqa rkllgledta ffkglrygkd naeastlmem kayhaisral
 361 ekeglkdkks plnlsselqd eigtafslfk tdeditgrlk drvqpeilea llkhisfdkf
 421 vqislkalrr ivplmeqgkr ydeacaeiyg dhygkkntee kiylppipad eirnpvvlra
 481 lsqarkving vvrrygspar ihietarevg ksfkdrkeie krqeenrkdr ekaaakfrey
 541 fpnfvgepks kdilklrlye qqhgkclysg keinlvrlne kgyveidhal pfsrtwddsf
 601 nnkvlvlgse nqnkgnqtpy eyfngkdnsr ewqefkarve tsrfprskkq rillqkfded
 661 gfkecnlndt ryvnrflcqf vadhilltgk gkrrvfasng qitnllrgfw glrkvraend
 721 rhhaldavvv acstvamqqk itrfvrykem nafdgktidk etgkvlhqkt hfpqpweffa
 781 qevmirvfgk pdgkpefeea dtpeklrtll aeklssrpea vheyvtplfv srapnrkmsg
 841 ahkdtlrsak rfvkhnekis vkrvwlteik ladlenmvny kngreielye alkarleayg
 901 gnakqafdpk dnpfykkggq lvkavrvekt qesgvllnkk naytiadngd mvrvdvfckv
 961 dkkgknqyfi vpiyawqvae nilpdidckg yriddsytfc fslhkydlia fqkdekskve
1021 fayyincdss ngrfylawhd kgskeqqfri stqnlvliqk yqvnelgkei rpcrlkkrpp
1081 vr
```

In some embodiments, the Cas9 domains of any of the fusion proteins provided herein comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a Cas9 polypeptide described herein. In some embodiments, the Cas9 domains of any of the fusion proteins provided herein comprises the amino acid sequence of any Cas9 polypeptide described herein. In some embodiments, the Cas9 domains of any of the fusion proteins provided herein consists of the amino acid sequence of any Cas9 polypeptide described herein.

In some examples, a PAM recognized by a CRISPR protein-derived domain of a base editor disclosed herein can be provided to a cell on a separate oligonucleotide to an insert (*e.g.*, an AAV insert) encoding the base editor. In such embodiments, providing PAM on a separate oligonucleotide can allow cleavage of a target sequence that otherwise would not be able to be cleaved, because no adjacent PAM is present on the same polynucleotide as the target sequence.

In an embodiment, *S. pyogenes* Cas9 (SpCas9) can be used as a CRISPR endonuclease for genome engineering. However, others can be used. In some embodiments, a different endonuclease can be used to target certain genomic targets. In some embodiments, synthetic SpCas9-derived variants with non-NGG PAM sequences can be used. Additionally, other Cas9 orthologues from various species have been identified and these "non-SpCas9s" can bind a variety of PAM sequences that can also be useful for the

present disclosure. For example, the relatively large size of SpCas9 (approximately 4kb coding sequence) can lead to plasmids carrying the SpCas9 cDNA that cannot be efficiently expressed in a cell. Conversely, the coding sequence for *Staphylococcus aureus* Cas9 (SaCas9) is approximately 1 kilobase shorter than SpCas9, possibly allowing it to be efficiently expressed in a cell. Similar to SpCas9, the SaCas9 endonuclease is capable of modifying target genes in mammalian cells *in vitro* and in mice *in vivo*. In some embodiments, a Cas protein can target a different PAM sequence. In some embodiments, a target gene can be adjacent to a Cas9 PAM, 5'-NGG, for example. In other embodiments, other Cas9 orthologs can have different PAM requirements. For example, other PAMs such as those of *S. thermophilus* (5'-NNAGAA for CRISPR1 and 5'-NGGNG for CRISPR3) and *Neisseria meningiditis* (5'-NNNNGATT) can also be found adjacent to a target gene.

In some embodiments, for a *S. pyogenes* system, a target gene sequence can precede (*i.e.*, be 5' to) a 5'-NGG PAM, and a 20-nt guide RNA sequence can base pair with an opposite strand to mediate a Cas9 cleavage adjacent to a PAM. In some embodiments, an adjacent cut can be or can be about 3 base pairs upstream of a PAM. In some embodiments, an adjacent cut can be or can be about 10 base pairs upstream of a PAM. In some embodiments, an adjacent cut can be or can be about 0-20 base pairs upstream of a PAM. For example, an adjacent cut can be next to, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 base pairs upstream of a PAM. An adjacent cut can also be downstream of a PAM by 1 to 30 base pairs. The sequences of exemplary SpCas9 proteins capable of binding a PAM sequence follow:

The amino acid sequence of an exemplary PAM-binding SpCas9 is as follows:

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV

DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

The amino acid sequence of an exemplary PAM-binding SpCas9n is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI

REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

The amino acid sequence of an exemplary PAM-binding SpEQR Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGF**E**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQ
LGGD

In the above sequence, residues E1134, Q1334, and R1336, which can be mutated from D1134, R1334, and T1336 to yield a SpEQR Cas9, are underlined and in bold.

The amino acid sequence of an exemplary PAM-binding SpVQR Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT

EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQ
LGGD

In the above sequence, residues V1134, Q1334, and R1336, which can be mutated from D1134, R1334, and T1336 to yield a SpVQR Cas9, are underlined and in bold.

The amino acid sequence of an exemplary PAM-binding SpVRER Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL

QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASA**R**ELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**E**Y**R**STKEVLDATLIHQSITGLYETRIDLSQ
LGGD.

In the above sequence, residues V1134, R1217, Q1334, and R1336, which can be mutated from D1134, G1217, R1334, and T1336 to yield a SpVRER Cas9, are underlined and in bold.

In some embodiments, the Cas9 domain is a recombinant Cas9 domain. In some embodiments, the recombinant Cas9 domain is a SpyMacCas9 domain. In some embodiments, the SpyMacCas9 domain is a nuclease active SpyMacCas9, a nuclease inactive SpyMacCas9 (SpyMacCas9d), or a SpyMacCas9 nickase (SpyMacCas9n). In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SpyMacCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having a NAA PAM sequence.

The sequence of an exemplary Cas9 A homolog of Spy Cas9 in *Streptococcus macacae* with native 5'-NAAN-3' PAM specificity is known in the art and described, for example, by Jakimo *et al.*, (www.biorxiv.org/content/biorxiv/early/2018/09/27/429654.full.pdf), and is provided below.

SpyMacCas9
MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAE
ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFG
NIVDEVAYHEKYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSD
VDKLFIQLVQIYNQLFEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGN
LIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNSEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELH
AILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE

VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFL
SGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGAYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDRGMIEERLKTYAHLFDDKVMKQLKRRRYTGWG
RLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGHSL
HEQIANLAGSPAIKKGILQTVKIVDELVKVMGHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHI
VPQSFIKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT
KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSK
LVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKM
IAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFA
TVRKVLSMPQVNIVKKTEIQTVGQNGGLFDDNPKSPLEVTPSKLVPLKKELNPKKYGGYQ
KPTTAYPVLLITDTKQLIPISVMNKKQFEQNPVKFLRDRGYQQVGKNDFIKLPKYTLVDI
GDGIKRLWASSKEIHKGNQLVVSKKSQILLYHAHHLDSDLSNDYLQNHNQQFDVLFNEII
SFSKKCKLGKEHIQKIENVYSNKKNSASIEELAESFIKLLGFTQLGATSPFNFLGVKLNQ
KQYKGKKDYILPCTEGTLIRQSITGLYETRVDLSKIGED.

In some embodiments, a variant Cas9 protein harbors, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1218A mutations such that the polypeptide has a reduced ability to cleave a target DNA or RNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). As another non-limiting example, in some embodiments, the variant Cas9 protein harbors D10A, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1218A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). In some embodiments, when a variant Cas9 protein harbors W476A and W1126A mutations or when the variant Cas9 protein harbors P475A, W476A, N477A, D1125A, W1126A, and D1218A mutations, the variant Cas9 protein does not bind efficiently to a PAM sequence. Thus, in some such cases, when such a variant Cas9 protein is used in a method of binding, the method does not require a PAM sequence. In other words, in some embodiments, when such a variant Cas9 protein is used in a method of binding, the method can include a guide RNA, but the method can be performed in the absence of a PAM sequence (and the specificity of binding is therefore provided by the targeting segment of the guide RNA). Other residues can be mutated to achieve the above effects (*i.e.*, inactivate one or the other nuclease portions). As non-limiting examples, residues D10, G12, G17, E762,

H840, N854, N863, H982, H983, A984, D986, and/or A987 can be altered (*i.e.*, substituted). Also, mutations other than alanine substitutions are suitable.

In some embodiments, a CRISPR protein-derived domain of a base editor can comprise all or a portion of a Cas9 protein with a canonical PAM sequence (NGG). In other embodiments, a Cas9-derived domain of a base editor can employ a non-canonical PAM sequence. Such sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., et al., "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" Nature 523, 481-485 (2015); and Kleinstiver, B. P., et al., "Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition" Nature Biotechnology 33, 1293-1298 (2015); the entire contents of each are hereby incorporated by reference.

Cas9 Domains with Reduced PAM Exclusivity

Typically, Cas9 proteins, such as Cas9 from *S. pyogenes* (spCas9), require a canonical NGG PAM sequence to bind a particular nucleic acid region, where the "N" in "NGG" is adenosine (A), thymidine (T), or cytosine (C), and the G is guanosine. This may limit the ability to edit desired bases within a genome. In some embodiments, the base editing fusion proteins provided herein may need to be placed at a precise location, for example a region comprising a target base that is upstream of the PAM. See *e.g.*, Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" *Nature* 533, 420-424 (2016), the entire contents of which are hereby incorporated by reference. Accordingly, in some embodiments, any of the fusion proteins provided herein may contain a Cas9 domain that is capable of binding a nucleotide sequence that does not contain a canonical (*e.g.,* NGG) PAM sequence. Cas9 domains that bind to non-canonical PAM sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., *et al.*, "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" *Nature* 523, 481-485 (2015); and Kleinstiver, B. P., *et al.*, "Broadening the targeting range of *Staphylococcus aureus* CRISPR-Cas9 by modifying PAM recognition" *Nature Biotechnology* 33, 1293-1298 (2015); the entire contents of each are hereby incorporated by reference.

High fidelity Cas9 domains

Some aspects of the disclosure provide high fidelity Cas9 domains. In some embodiments, high fidelity Cas9 domains are engineered Cas9 domains comprising one or

more mutations that decrease electrostatic interactions between the Cas9 domain and a sugar-phosphate backbone of a DNA, as compared to a corresponding wild-type Cas9 domain. Without wishing to be bound by any particular theory, high fidelity Cas9 domains that have decreased electrostatic interactions with a sugar-phosphate backbone of DNA may have less off-target effects. In some embodiments, a Cas9 domain (*e.g.*, a wild-type Cas9 domain) comprises one or more mutations that decreases the association between the Cas9 domain and a sugar-phosphate backbone of a DNA. In some embodiments, a Cas9 domain comprises one or more mutations that decreases the association between the Cas9 domain and a sugar-phosphate backbone of a DNA by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70%.

In some embodiments, any of the Cas9 fusion proteins provided herein comprise one or more of a N497X, a R661X, a Q695X, and/or a Q926X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, any of the Cas9 fusion proteins provided herein comprise one or more of a N497A, a R661A, a Q695A, and/or a Q926A mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the Cas9 domain comprises a D10A mutation, or a corresponding mutation in any of the amino acid sequences provided herein. Cas9 domains with high fidelity are known in the art and would be apparent to the skilled artisan. For example, Cas9 domains with high fidelity have been described in Kleinstiver, B.P., *et al.* "High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects." *Nature* 529, 490-495 (2016); and Slaymaker, I.M., *et al.* "Rationally engineered Cas9 nucleases with improved specificity." *Science* 351, 84-88 (2015); the entire contents of each are incorporated herein by reference.

In some embodiments, the modified Cas9 is a high fidelity Cas9 enzyme. In some embodiments, the high fidelity Cas9 enzyme is SpCas9(K855A), eSpCas9(1.1), SpCas9-HF1, or hyper accurate Cas9 variant (HypaCas9). The modified Cas9 eSpCas9(1.1) contains alanine substitutions that weaken the interactions between the HNH/RuvC groove and the non-target DNA strand, preventing strand separation and cutting at off-target sites. Similarly, SpCas9-HF1 lowers off-target editing through alanine substitutions that disrupt Cas9's interactions with the DNA phosphate backbone. HypaCas9 contains mutations (SpCas9 N692A/M694A/Q695A/H698A) in the REC3 domain that increase Cas9 proofreading and target discrimination. All three high fidelity enzymes generate less off-target editing than wildtype Cas9.

An exemplary high fidelity Cas9 is provided below.

High Fidelity Cas9 domain mutations relative to Cas9 are shown in bold and underlined.

DKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR
LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDE
VAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQ
LVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLT
PNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTE
ITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFY
KFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD
NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT**A**
FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKV
TVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVL
TLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWG**A**LSRKLINGIRDKQSGKTILDFL
KSDGFANRNFM**A**LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVD
ELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETR**A**ITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

Fusion proteins comprising a Cas9 domain and a Cytidine Deaminase and/or Adenosine Deaminase

Some aspects of the disclosure provide fusion proteins comprising a napDNAbp (*e.g.*, a Cas9 domain) and one or more adenosine deaminase, cytidine deaminase domains, and/or DNA glycosylase domains. In some embodiments, the fusion protein comprises a Cas9 domain and an adenosine deaminase domain (*e.g.*, TadA*A). It should be appreciated that the Cas9 domain may be any of the Cas9 domains or Cas9 proteins (*e.g.*, dCas9 or nCas9) provided herein. In some embodiments, any of the Cas9 domains or Cas9 proteins (*e.g.*,

dCas9 or nCas9) provided herein may be fused with any of the cytidine deaminases and/or adenosine deaminases (*e.g.*, TadA*A) provided herein. For example, and without limitation, in some embodiments, the fusion protein comprises the structure:

NH_2-[cytidine deaminase]-[Cas9 domain]-[adenosine deaminase]-COOH;
NH_2-[adenosine deaminase]-[Cas9 domain]-[cytidine deaminase]-COOH;
NH_2-[adenosine deaminase]-[cytidine deaminase]-[Cas9 domain]-COOH;
NH_2-[cytidine deaminase]-[adenosine deaminase]-[Cas9 domain]-COOH;
NH_2-[Cas9 domain]-[adenosine deaminase]-[cytidine deaminase]-COOH;
NH_2-[Cas9 domain]-[cytidine deaminase]-[adenosine deaminase]-COOH;
NH_2-[adenosine deaminase]-[Cas9 domain]-COOH;
NH_2-[Cas9 domain]-[adenosine deaminase]-COOH;
NH_2-[cytidine deaminase]-[Cas9 domain]-COOH; or
NH_2-[Cas9 domain]-[cytidine deaminase]-COOH.

In some embodiments, the fusion proteins comprising a cytidine deaminase, abasic editor, and adenosine deaminase and a napDNAbp (*e.g.*, Cas9 domain) do not include a linker sequence. In some embodiments, a linker is present between the cytidine deaminase and/or adenosine deaminase domains and the napDNAbp. In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker. In some embodiments, the cytidine deaminase and adenosine deaminase and the napDNAbp are fused via any of the linkers provided herein. For example, in some embodiments the cytidine deaminase and/or adenosine deaminase and the napDNAbp are fused via any of the linkers provided herein.

Fusion proteins comprising a nuclear localization sequence (NLS)

In some embodiments, the fusion proteins provided herein further comprise one or more (*e.g.*, 2, 3, 4, 5) nuclear targeting sequences, for example a nuclear localization sequence (NLS). In one embodiment, a bipartite NLS is used. In some embodiments, a NLS comprises an amino acid sequence that facilitates the importation of a protein, that comprises an NLS, into the cell nucleus (*e.g.*, by nuclear transport). In some embodiments, any of the fusion proteins provided herein further comprise a nuclear localization sequence (NLS). In some embodiments, the NLS is fused to the N-terminus of the fusion protein. In some embodiments, the NLS is fused to the C-terminus of the fusion protein. In some embodiments, the NLS is fused to the N-terminus of the Cas9 domain. In some embodiments, the NLS is fused to the C-terminus of an nCas9 domain or a dCas9 domain. In some embodiments, the NLS is fused to the N-terminus of the deaminase. In some embodiments, the NLS is fused to the C-terminus of the deaminase. In some embodiments,

the NLS is fused to the fusion protein via one or more linkers. In some embodiments, the NLS is fused to the fusion protein without a linker. In some embodiments, the NLS comprises an amino acid sequence of any one of the NLS sequences provided or referenced herein. Additional nuclear localization sequences are known in the art and would be apparent to the skilled artisan. For example, NLS sequences are described in Plank *et al.*, PCT/EP2000/011690, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences. In some embodiments, an NLS comprises the amino acid sequence PKKKRKVEGADKRTADGSEFESPKKKRKV, KRTADGSEFESPKKKRKV, KRPAATKKAGQAKKKK, KKTELQTTNAENKTKKL, KRGINDRNFWRGENGRKTR, RKSGKIAAIVVKRPRKPKKKRKV, or MDSLLMNRRKFLYQFKNVRWAKGRRETYLC.

In some embodiments, the NLS is present in a linker or the NLS is flanked by linkers, for example, the linkers described herein. In some embodiments, the N-terminus or C-terminus NLS is a bipartite NLS. A bipartite NLS comprises two basic amino acid clusters, which are separated by a relatively short spacer sequence (hence bipartite - 2 parts, while monopartite NLSs are not). The NLS of nucleoplasmin, KR[PAATKKAGQA]KKKK, is the prototype of the ubiquitous bipartite signal: two clusters of basic amino acids, separated by a spacer of about 10 amino acids. The sequence of an exemplary bipartite NLS follows: PKKKRKVEGADKRTADGSEFESPKKKRKV

In some embodiments, the fusion proteins of the invention do not comprise a linker sequence. In some embodiments, linker sequences between one or more of the domains or proteins are present. In some embodiments, the general architecture of exemplary Cas9 fusion proteins with an adenosine deaminase or cytidine deaminase and a Cas9 domain comprises any one of the following structures, where NLS is a nuclear localization sequence (*e.g.*, any NLS provided herein), NH_2 is the N-terminus of the fusion protein, and COOH is the C-terminus of the fusion protein:

NH_2-NLS-[adenosine deaminase]-[Cas9 domain]-COOH;
NH_2-NLS [Cas9 domain]-[adenosine deaminase]-COOH;
NH_2-[adenosine deaminase]-[Cas9 domain]-NLS-COOH;
NH_2-[Cas9 domain]-[adenosine deaminase]-NLS-COOH.;
NH_2-NLS-[cytidine deaminase]-[Cas9 domain]-COOH;
NH_2-NLS [Cas9 domain]-[cytidine deaminase]-COOH;
NH_2-[cytidine deaminase]-[Cas9 domain]-NLS-COOH; or

NH_2-[Cas9 domain]-[cytidine deaminase]-NLS-COOH.

It should be appreciated that the fusion proteins of the present disclosure may comprise one or more additional features. For example, in some embodiments, the fusion protein may comprise inhibitors, cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags that are useful for solubilization, purification, or detection of the fusion proteins. Suitable protein tags provided herein include, but are not limited to, biotin carboxylase carrier protein (BCCP) tags, myc-tags, calmodulin-tags, FLAG-tags, hemagglutinin (HA)-tags, polyhistidine tags, also referred to as histidine tags or His-tags, maltose binding protein (MBP)-tags, nus-tags, glutathione-S-transferase (GST)-tags, green fluorescent protein (GFP)-tags, thioredoxin-tags, S-tags, Softags (*e.g.*, Softag 1, Softag 3), strep-tags , biotin ligase tags, FlAsH tags, V5 tags, and SBP-tags. Additional suitable sequences will be apparent to those of skill in the art. In some embodiments, the fusion protein comprises one or more His tags.

A vector that encodes a CRISPR enzyme comprising one or more nuclear localization sequences (NLSs) can be used. For example, there can be or be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 NLSs used. A CRISPR enzyme can comprise the NLSs at or near the ammo-terminus, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 NLSs at or near the carboxy-terminus, or any combination of these (*e.g.*, one or more NLS at the ammo-terminus and one or more NLS at the carboxy terminus). When more than one NLS is present, each can be selected independently of others, such that a single NLS can be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies.

CRISPR enzymes used in the methods can comprise about 6 NLSs. An NLS is considered near the N- or C-terminus when the nearest amino acid to the NLS is within about 50 amino acids along a polypeptide chain from the N- or C-terminus, *e.g.*, within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, or 50 amino acids.

Nucleobase Editing Domain

Described herein are base editors comprising a fusion protein that includes a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain (*e.g.*, a deaminase domain). The base editor can be programmed to edit one or more bases in a target polynucleotide sequence by interacting with a guide polynucleotide capable of recognizing the target sequence. Once the target sequence has been recognized, the base editor is anchored on the polynucleotide where editing is to occur and the deaminase domain components of the base editor can then edit a target base.

In some embodiments, the nucleobase editing domain includes a deaminase domain. As particularly described herein, the deaminase domain includes a cytosine deaminase or an adenosine deaminase. In some embodiments, the terms "cytosine deaminase" and "cytidine deaminase" can be used interchangeably. In some embodiments, the terms "adenine deaminase" and "adenosine deaminase" can be used interchangeably. Details of nucleobase editing proteins are described in International PCT Application Nos. PCT/2017/045381 (WO2018/027078) and PCT/US2016/058344 (WO2017/070632), each of which is incorporated herein by reference for its entirety. Also see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

A to G Editing

In some embodiments, a base editor described herein can comprise a deaminase domain which includes an adenosine deaminase. Such an adenosine deaminase domain of a base editor can facilitate the editing of an adenine (A) nucleobase to a guanine (G) nucleobase by deaminating the A to form inosine (I), which exhibits base pairing properties of G. Adenosine deaminase is capable of deaminating (*i.e.*, removing an amine group) adenine of a deoxyadenosine residue in deoxyribonucleic acid (DNA).

In some embodiments, the nucleobase editors provided herein can be made by fusing together one or more protein domains, thereby generating a fusion protein. In certain embodiments, the fusion proteins provided herein comprise one or more features that improve the base editing activity (*e.g.*, efficiency, selectivity, and specificity) of the fusion proteins. For example, the fusion proteins provided herein can comprise a Cas9 domain that has reduced nuclease activity. In some embodiments, the fusion proteins provided herein can have a Cas9 domain that does not have nuclease activity (dCas9), or a Cas9 domain that cuts one strand of a duplexed DNA molecule, referred to as a Cas9 nickase (nCas9). Without wishing to be bound by any particular theory, the presence of the catalytic residue (*e.g.*, H840) maintains the activity of the Cas9 to cleave the non-edited (*e.g.*, non-deaminated) strand containing a T opposite the targeted A. Mutation of the catalytic residue (*e.g.*, D10 to A10) of Cas9 prevents cleavage of the edited strand containing the targeted A residue. Such

Cas9 variants are able to generate a single-strand DNA break (nick) at a specific location based on the gRNA-defined target sequence, leading to repair of the non-edited strand, ultimately resulting in a T to C change on the non-edited strand. In some embodiments, an A-to-G base editor further comprises an inhibitor of inosine base excision repair, for example, a uracil glycosylase inhibitor (UGI) domain or a catalytically inactive inosine specific nuclease. Without wishing to be bound by any particular theory, the UGI domain or catalytically inactive inosine specific nuclease can inhibit or prevent base excision repair of a deaminated adenosine residue (*e.g.*, inosine), which can improve the activity or efficiency of the base editor.

A base editor comprising an adenosine deaminase can act on any polynucleotide, including DNA, RNA and DNA-RNA hybrids. In certain embodiments, a base editor comprising an adenosine deaminase can deaminate a target A of a polynucleotide comprising RNA. For example, the base editor can comprise an adenosine deaminase domain capable of deaminating a target A of an RNA polynucleotide and/or a DNA-RNA hybrid polynucleotide. In an embodiment, an adenosine deaminase incorporated into a base editor comprises all or a portion of adenosine deaminase acting on RNA (ADAR, *e.g.*, ADAR1 or ADAR2). In another embodiment, an adenosine deaminase incorporated into a base editor comprises all or a portion of adenosine deaminase acting on tRNA (ADAT). A base editor comprising an adenosine deaminase domain can also be capable of deaminating an A nucleobase of a DNA polynucleotide. In an embodiment an adenosine deaminase domain of a base editor comprises all or a portion of an ADAT comprising one or more mutations which permit the ADAT to deaminate a target A in DNA. For example, the base editor can comprise all or a portion of an ADAT from *Escherichia coli* (EcTadA) comprising one or more of the following mutations: D108N, A106V, D147Y, E155V, L84F, H123Y, I156F, or a corresponding mutation in another adenosine deaminase.

The adenosine deaminase can be derived from any suitable organism (*e.g.*, *E. coli*). In some embodiments, the adenine deaminase is a naturally-occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (*e.g.*, mutations in ecTadA). The corresponding residue in any homologous protein can be identified by *e.g.*, sequence alignment and determination of homologous residues. The mutations in any naturally-occurring adenosine deaminase (*e.g.*, having homology to ecTadA) that corresponds to any of the mutations described herein (*e.g.*, any of the mutations identified in ecTadA) can be generated accordingly.

Adenosine deaminases

In some embodiments, a base editor described herein can comprise a deaminase domain which includes an adenosine deaminase. Such an adenosine deaminase domain of a base editor can facilitate the editing of an adenine (A) nucleobase to a guanine (G) nucleobase by deaminating the A to form inosine (I), which exhibits base pairing properties of G. Adenosine deaminase is capable of deaminating (*i.e.*, removing an amine group) adenine of a deoxyadenosine residue in deoxyribonucleic acid (DNA).

In some embodiments, the adenosine deaminases provided herein are capable of deaminating adenine. In some embodiments, the adenosine deaminases provided herein are capable of deaminating adenine in a deoxyadenosine residue of DNA. In some embodiments, the adenine deaminase is a naturally-occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (*e.g.*, mutations in ecTadA). One of skill in the art will be able to identify the corresponding residue in any homologous protein, *e.g.*, by sequence alignment and determination of homologous residues. Accordingly, one of skill in the art would be able to generate mutations in any naturally-occurring adenosine deaminase (*e.g.*, having homology to ecTadA) that corresponds to any of the mutations described herein, *e.g.*, any of the mutations identified in ecTadA. In some embodiments, the adenosine deaminase is from a prokaryote. In some embodiments, the adenosine deaminase is from a bacterium. In some embodiments, the adenosine deaminase is from *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shewanella putrefaciens, Haemophilus influenzae, Caulobacter crescentus,* or *Bacillus subtilis*. In some embodiments, the adenosine deaminase is from *E. coli*.

The invention provides adenosine deaminase variants that have increased efficiency (>50-60%) and specificity. In particular, the adenosine deaminase variants described herein are more likely to edit a desired base within a polynucleotide, and are less likely to edit bases that are not intended to be altered (*i.e.*, "bystanders").

In particular embodiments, the TadA is any one of the TadA described in PCT/US2017/045381 (WO 2018/027078), which is incorporated herein by reference in its entirety.

In some embodiments, the nucleobase editors of the invention are adenosine deaminase variants comprising an alteration in the following sequence: MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (also termed TadA*7.10).

In particular embodiments, the fusion proteins comprise a single (*e.g.*, provided as a monomer) TadA*8 variant. In some embodiments, the TadA*8 is linked to a Cas9 nickase. In some embodiments, the fusion proteins of the invention comprise as a heterodimer of a wild-type TadA (TadA(wt)) linked to a TadA*8 variant. In other embodiments, the fusion proteins of the invention comprise as a heterodimer of a TadA*7.10 linked to a TadA*8 variant. In some embodiments, the base editor is ABE8 comprising a TadA*8 variant monomer. In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant and a TadA(wt). In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant and TadA*7.10. In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant. In some embodiments, the TadA*8 variant is selected from Table 7. In some embodiments, the ABE8 is selected from Table 7. The relevant sequences follow:

Wild-type TadA (TadA(wt)) or "the TadA reference sequence"

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHP
GMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD (SEQ ID NO:2)

TadA*7.10:

MSEVEFSHEYW MRHALTLAKR ARDEREVPVG AVLVLNNRVI GEGWNRAIGL
HDPTAHAEIM ALRQGGLVMQ NYRLIDATLY VTFEPCVMCA GAMIHSRIGR
VVFGVRNAKT GAAGSLMDVL HYPGMNHRVE ITEGILADEC AALLCYFFRM
PRQVFNAQKK AQSSTD

In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth in any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (*e.g.*, any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to a reference sequence, or any of the adenosine deaminases provided herein. In some embodiments, the

adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences known in the art or described herein.

In some embodiments the TadA deaminase is a full-length *E. coli* TadA deaminase. For example, in certain embodiments, the adenosine deaminase comprises the amino acid sequence:

MRRAFITGVFFLSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGR
HDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGA
AGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD.

It should be appreciated, however, that additional adenosine deaminases useful in the present application would be apparent to the skilled artisan and are within the scope of this disclosure. For example, the adenosine deaminase may be a homolog of adenosine deaminase acting on tRNA (ADAT). Without limitation, the amino acid sequences of exemplary AD AT homologs include the following:

Staphylococcus aureus TadA:

MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAHAEHIA
IERAAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCSGS
LMNLLQQSNFNHRAIVDKGVLKEACSTLLTTFFKNLRANKKSTN

Bacillus subtilis TadA:

MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEMLVIDEA
CKALGTWRLEGATLYVTLEPCPMCAGAVVLSRVEKVVFGAFDPKGGCSGTLMNLLQEERFNH
QAEVVSGVLEEECGGMLSAFFRELRKKKKAARKNLSE

Salmonella typhimurium (*S. typhimurium*) TadA:

MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNHRVIGEGWNRPIGR
HDPTAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAMVHSRIGRVVFGARDAKTGA
AGSLIDVLHHPGMNHRVEIIEGVLRDECATLLSDFFRMRRQEIKALKKADRAEGAGPAV

Shewanella putrefaciens (*S. putrefaciens*) TadA:

MDEYWMQVAMQMAEKAEAAGEVPVGAVLVKDGQQIATGYNLSISQHDPTAHAEILCLRSAGK
KLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGTVVNLLQHPAFNHQV
EVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE

Haemophilus influenzae F3031 (*H. influenzae*) TadA:

MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQSDPTAHA
EIIALRNGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVFGASDYKTGAIGSRFHF
FDDYKMNHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKALLKSLSDK

Caulobacter crescentus (*C. crescentus*) TadA:

MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAHDPTAHA
EIAAMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGRVVFGADDPKGGAVVHGPKF
FAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI

Geobacter sulfurreducens (*G. sulfurreducens*) TadA:

MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSNDPSAHA
EMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVVFGCYDPKGGAAGSLYDL
SADPRLNHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAKATPALFIDERKVPPEP

An embodiment of *E. Coli* TadA (ecTadA) includes the following:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

In some embodiments, the adenosine deaminase is from a prokaryote. In some embodiments, the adenosine deaminase is from a bacterium. In some embodiments, the adenosine deaminase is from *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shewanella putrefaciens*, *Haemophilus influenzae*, *Caulobacter crescentus*, or *Bacillus subtilis*. In some embodiments, the adenosine deaminase is from *E. coli*.

In one embodiment, a fusion protein of the invention comprises a wild-type TadA linked to TadA7.10, which is linked to Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA7.10 domain (*e.g.*, provided as a monomer). In other embodiments, the ABE7.10 editor comprises TadA7.10 and TadA(wt), which are capable of forming heterodimers.

In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at

least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth in any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (*e.g.*, any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to a reference sequence, or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences known in the art or described herein.

It should be appreciated that any of the mutations provided herein (*e.g.*, based on the TadA reference sequence) can be introduced into other adenosine deaminases, such as *E. coli* TadA (ecTadA), *S. aureus* TadA (saTadA), or other adenosine deaminases (*e.g.*, bacterial adenosine deaminases). It would be apparent to the skilled artisan that additional deaminases may similarly be aligned to identify homologous amino acid residues that can be mutated as provided herein. Thus, any of the mutations identified in the TadA reference sequence can be made in other adenosine deaminases (*e.g.*, ecTadA) that have homologous amino acid residues. It should also be appreciated that any of the mutations provided herein can be made individually or in any combination in the TadA reference sequence or another adenosine deaminase. It should be appreciated that the amino acid substitutions in a TadA variant are as numbered in the TadA reference sequence (SEQ ID NO: 2), and can be a corresponding amino acid substitution or position in any other TadA variant that have homologous amino acid residues. It should be appreciated that the numbering of the specific positions or residues in the respective sequences depends on the particular protein and numbering scheme used; numbering might be different in a TadA varaiant sharing homology with the TadA reference sequence, and differences in sequences from species to species may affect numbering. One of skill in the art will be able to identify the respective residue in any homologous protein and in the respective encoding nucleic acid by methods well known in the art, e.g., by sequence alignment and determination of homologous residues.

In some embodiments, the adenosine deaminase comprises a D108X mutation in the TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D108G, D108N, D108V, D108A, or D108Y mutation, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an A106X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A106V mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, wild-type TadA or ecTadA).

In some embodiments, the adenosine deaminase comprises a E155X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a E155D, E155G, or E155V mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises a D147X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D147Y, mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an A106X, E155X, or D147X, mutation in the TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an E155D, E155G, or E155V mutation. In some embodiments, the adenosine deaminase comprises a D147Y.

For example, an adenosine deaminase can contain a D108N, a A106V, a E155V, and/or a D147Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, an adenosine deaminase comprises the following group of mutations (groups of mutations are separated by a ";") in

TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA): D108N and A106V; D108N and E155V; D108N and D147Y; A106V and E155V; A106V and D147Y; E155V and D147Y; D108N, A106V, and E155V; D108N, A106V, and D147Y; D108N, E155V, and D147Y; A106V, E155V, and D 147Y; and D108N, A106V, E155V, and D147Y. It should be appreciated, however, that any combination of corresponding mutations provided herein can be made in an adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a H8X, T17X, L18X, W23X, L34X, W45X, R51X, A56X, E59X, E85X, M94X, I95X, V102X, F104X, A106X, R107X, D108X, K110X, M118X, N127X, A138X, F149X, M151X, R153X, Q154X, I156X, and/or K157X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H8Y, T17S, L18E, W23L, L34S, W45L, R51H, A56E, or A56S, E59G, E85K, or E85G, M94L, I95L, V102A, F104L, A106V, R107C, or R107H, or R107P, D108G, or D108N, or D108V, or D108A, or D108Y, K110I, M118K, N127S, A138V, F149Y, M151V, R153C, Q154L, I156D, and/or K157R mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a H8X, D108X, and/or N127X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid. In some embodiments, the adenosine deaminase comprises one or more of a H8Y, D108N, and/or N127S mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of H8X, R26X, M61X, L68X, M70X, A106X, D108X, A109X, N127X, D147X, R152X, Q154X, E155X, K161X, Q163X, and/or T166X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H8Y, R26W, M61I, L68Q, M70V, A106T, D108N, A109T, N127S, D147Y, R152C, Q154H or Q154R, E155G or E155V or E155D, K161Q, Q163H, and/or T166P mutation in

TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8X, D108X, N127X, D147X, R152X, and Q154X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8X, M61X, M70X, D108X, N127X, Q154X, E155X, and Q163X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, D108X, N127X, E155X, and T166X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8X, A106X, D108X, mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8X, R26X, L68X, D108X, N127X, D147X, and E155X, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, D108X, A109X, N127X, and E155X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8Y, D108N, N127S, D147Y, R152C, and Q154H in TadA reference sequence, or a corresponding mutation or mutations in another

adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8Y, M61I, M70V, D108N, N127S, Q154R, E155G and Q163H in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, D108N, N127S, E155V, and T166P in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8Y, A106T, D108N, N127S, E155D, and K161Q in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8Y, R26W, L68Q, D108N, N127S, D147Y, and E155V in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, D108N, A109T, N127S, and E155G in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA).

Any of the mutations provided herein and any additional mutations (*e.g.*, based on the ecTadA amino acid sequence) can be introduced into any other adenosine deaminases. Any of the mutations provided herein can be made individually or in any combination in TadA reference sequence or another adenosine deaminase (*e.g.*, ecTadA).

Details of A to G nucleobase editing proteins are described in International PCT Application No. PCT/2017/045381 (WO2018/027078) and Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature, 551, 464-471 (2017), the entire contents of which are hereby incorporated by reference.

In some embodiments, the adenosine deaminase comprises one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a D108N, D108G, or D108V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a A106V and D108N mutation in TadA reference sequence, or corresponding mutations in another adenosine

deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises R107C and D108N mutations in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, N127S, D147Y, and Q154H mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, N127S, D147Y, and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a D108N, D147Y, and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, and N127S mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a A106V, D108N, D147Y and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a S2X, H8X, I49X, L84X, H123X, N127X, I156X and/or K160X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of S2A, H8Y, I49F, L84F, H123Y, N127S, I156F and/or K160S mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an L84X mutation adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an L84F mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an H123X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an H123Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an I156X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an I156F mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, or seven mutations selected from the group consisting of L84X, A106X, D108X, H123X, D147X, E155X, and I156X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of S2X, I49X, A106X, D108X, D147X, and E155X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, A106X, D108X, N127X, and K160X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, or seven mutations selected from the group consisting of L84F, A106V, D108N, H123Y, D147Y, E155V, and I156F in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of S2A, I49F, A106V, D108N, D147Y, and E155V in TadA reference sequence.

In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, A106T, D108N, N127S, and K160S in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a E25X, R26X, R107X, A142X, and/or A143X mutation in TadA reference sequence, or one or more

corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of E25M, E25D, E25A, E25R, E25V, E25S, E25Y, R26G, R26N, R26Q, R26C, R26L, R26K, R107P, R107K, R107A, R107N, R107W, R107H, R107S, A142N, A142D, A142G, A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one or more of the mutations described herein corresponding to TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an E25X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an E25M, E25D, E25A, E25R, E25V, E25S, or E25Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an R26X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises R26G, R26N, R26Q, R26C, R26L, or R26K mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an R107X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an R107P, R107K, R107A, R107N, R107W, R107H, or R107S mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an A142X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A142N, A142D, A142G, mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an A143X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a H36X, N37X, P48X, I49X, R51X, M70X, N72X, D77X, E134X, S 146X, Q154X, K157X, and/or K161X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H36L, N37T, N37S, P48T, P48L, I49V, R51H, R51L, M70L, N72S, D77G, E134G, S146R, S146C, Q154H, K157N, and/or K161T mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an H36X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an H36L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an N37X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an N37T, or N37S mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an P48X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an P48T, or P48L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an R51X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an R51H, or R51L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an S146X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an S146R, or S146C mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an K157X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a K157N mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an P48X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a P48S, P48T, or P48A mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an A142X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a A142N mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an W23X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a

W23R, or W23L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA).

In some embodiments, the adenosine deaminase comprises an R152X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a R152P, or R52H mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA).

In one embodiment, the adenosine deaminase may comprise the mutations H36L, R51L, L84F, A106V, D108N, H123Y, S146C, D147Y, E155V, I156F, and K157N. In some embodiments, the adenosine deaminase comprises the following combination of mutations relative to TadA reference sequence, where each mutation of a combination is separated by a "_" and each combination of mutations is between parentheses:

(A106V_D108N),
(R107C_D108N),
(H8Y_D108N_N127S_D147Y_Q154H),
(H8Y_D108N_N127S_D147Y_E155V),
(D108N_D147Y_E155V),
(H8Y_D108N_N127S),
(H8Y_D108N_N127S_D147Y_Q154H),
(A106V_D108N_D147Y_E155V),
(D108Q_D147Y_E155V),
(D108M_D147Y_E155V),
(D108L_D147Y_E155V),
(D108K_D147Y_E155V),
(D108I_D147Y_E155V),
(D108F_D147Y_E155V),
(A106V_D108N_D147Y),
(A106V_D108M_D147Y_E155V),
(E59A_A106V_D108N_D147Y_E155V),
(E59A cat dead_A106V_D108N_D147Y_E155V),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156Y),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),

(E25G_R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(E25D_R26G_L84F_A106V_R107K_D108N_H123Y_A142N_A143G_D147Y_E155V_I156F),
(R26Q_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(E25M_R26G_L84F_A106V_R107P_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(R26C_L84F_A106V_R107H_D108N_H123Y_A142N_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_A142N_A143L_D147Y_E155V_I156F),
(R26G_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(E25A_R26G_L84F_A106V_R107N_D108N_H123Y_A142N_A143E_D147Y_E155V_I156F),
(R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(A106V_D108N_A142N_D147Y_E155V),
(R26G_A106V_D108N_A142N_D147Y_E155V),
(E25D_R26G_A106V_R107K_D108N_A142N_A143G_D147Y_E155V),
(R26G_A106V_D108N_R107H_A142N_A143D_D147Y_E155V),
(E25D_R26G_A106V_D108N_A142N_D147Y_E155V),
(A106V_R107K_D108N_A142N_D147Y_E155V),
(A106V_D108N_A142N_A143G_D147Y_E155V),
(A106V_D108N_A142N_A143L_D147Y_E155V),
(H36L_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F _K157N),
(N37T_P48T_M70L_L84F_A106V_D108N_H123Y_D147Y_I49V_E155V_I156F),
(N37S_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K161T),
(H36L_L84F_A106V_D108N_H123Y_D147Y_Q154H_E155V_I156F),
(N72S_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F),
(H36L_P48L_L84F_A106V_D108N_H123Y_E134G_D147Y_E155V_I156F),
(H36L_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K157N),
(H36L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F_K161T),
(N37S_R51H_D77G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R51L_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K157N),
(D24G_Q71R_L84F_H96L_A106V_D108N_H123Y_D147Y_E155V_I156F_K160E),
(H36L_G67V_L84F_A106V_D108N_H123Y_S146T_D147Y_E155V_I156F),

(Q71L_L84F_A106V_D108N_H123Y_L137M_A143E_D147Y_E155V_I156F),
(E25G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_Q159L),
(L84F_A91T_F104I_A106V_D108N_H123Y_D147Y_E155V_I156F),
(N72D_L84F_A106V_D108N_H123Y_G125A_D147Y_E155V_I156F),
(P48S_L84F_S97C_A106V_D108N_H123Y_D147Y_E155V_I156F),
(W23G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(D24G_P48L_Q71R_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_Q159L),
(L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(H36L_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V_I156F
_K157N), (N37S_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F_K161T),
(L84F_A106V_D108N_D147Y_E155V_I156F),
(R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K160E_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K160E),
(R74Q_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R74A_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R74Q_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_R98Q_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_R129Q_D147Y_E155V_I156F),
(P48S_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(P48S_A142N),
(P48T_I49V_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F_L157N),
(P48T_I49V_A142N),
(H36L_P48S_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N
),
(H36L_P48S_R51L_L84F_A106V_D108N_H123Y_S146C_A142N_D147Y_E155V_I156F
(H36L_P48T_I49V_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F
_K157N),
(H36L_P48T_I49V_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V
_I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N
),

(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V_I156F_K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_A142N_D147Y_E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N),
(W23R_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F_K161T),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152H_E155V_I156F_K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142A_S146C_D147Y_E155V
_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142A_S146C_D147Y_R152P _E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F_K161T),
(W23R_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V_I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_R152P_E155V
_I156F _K157N).

In certain embodiments, the fusion proteins provided herein comprise one or more features that improve the base editing activity of the fusion proteins. For example, any of the fusion proteins provided herein may comprise a Cas9 domain that has reduced nuclease activity. In some embodiments, any of the fusion proteins provided herein may have a Cas9 domain that does not have nuclease activity (dCas9), or a Cas9 domain that cuts one strand of a duplexed DNA molecule, referred to as a Cas9 nickase (nCas9).

In some embodiments, the adenosine deaminase is TadA*7.10. In some embodiments, TadA*7.10 comprises at least one alteration. In particular embodiments, TadA*7.10 comprises one or more of the following alterations or additional alterations to TadA*7.10: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. The alteration Y123H is also referred to herein as H123H (the alteration H123Y in TadA*7.10 reverted back to Y123H (wt)). In other embodiments, the TadA*7.10 comprises a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In particular embodiments, an adenosine deaminase variant comprises a deletion of the C terminus beginning at residue 149, 150, 151, 152, 153, 154, 155, 156, and 157.

In some embodiments, a TadA variant comprises at least one alteration relative to TadA7.10. In some embodiments, a TadA variant comprises at least one alteration relative to a wild type TadA. Amino acid alterations in a TadA variant may be any one of the amino acid subsitutions as described herein relative to TadA7.10 or wild type TadA. In some embodiments, a TadA variant, e.g. a TadA8, comprises an amino acid alteration at amino acid position 23, 26, 36, 37, 48, 49, 51, 72, 84, 87, 105, 108, 123, 125, 142, 145, 147, 152, 155, 16, 157, 161, or any combination thereof. In some embodiments, the TadA variant comprises amino acid alteration V82X relative to TadA7.10, wherein X is any amino acid other than V. In some embodiments, the TadA variant comprises a V82S alteration relative to TadA7.10. In some embodiments, amino acid X is an acidic amino acid, a basic amino acid, or a neutral amino acid. In some embodiments, a TadA variant comprises amino acid alteration T166X relative to TadA7.10, wherein X is any amino acid other than T. In some embodiments, amino acid X is an acidic amino acid, a basic amino acid, or a neutral amino acid. In some embodiments, a TadA variant comrpsies amino acid alteration V82X, Y147X, Q154X, I76X, Y123X, R23X, L36X, A48X, L51X, F84X, V106X, N108X, Y123X, C146X, Y147X, P152X, Q154X, V155X, F156X, N157X, T166X relative to TadA7.10, or any combination thereof, wherein X is any amino acid other than the amino acid in TadA7.10. In some embodiments, X is an acidic amino acid, a basic amino acid, or a neutral amino acid. In some embodiments, X reverts the amino acid to a wild type amino acid in the TadA reference sequence.

In other embodiments, a base editor of the invention is a monomer comprising an adenosine deaminase variant (*e.g.*, TadA*8) comprising one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R compared to TadA*7.10 or the reference TadA sequence. In other embodiments, the adenosine deaminase variant (TadA*8) is a monomer comprising a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In other embodiments, a base editor is a heterodimer comprising a wild-type adenosine deaminase and an adenosine deaminase variant (*e.g.*, TadA*8) comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In other embodiments, the base editor is a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

In one embodiment, an adenosine deaminase is a TadA*8 that comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMD
VLHYPGMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD

In some embodiments, the TadA*8 is a truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8.

In some embodiments the TadA*8 is TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12,

TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, TadA*8.24.

In one embodiment, a fusion protein of the invention comprises a wild-type TadA is linked to an adenosine deaminase variant described herein (*e.g.*, TadA*8), which is linked to Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA*8 domain (*e.g.*, provided as a monomer). In other embodiments, the base editor comprises TadA*8 and TadA(wt), which are capable of forming heterodimers. Exemplary sequences follow:

TadA(wt):

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHP
GMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD

TadA*7.10:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

TadA*8:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth in any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (*e.g.*, any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to a reference sequence, or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least

60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences known in the art or described herein.

In particular embodiments, a TadA*8 comprises one or more mutations at any of the following positions shown in bold. In other embodiments, a TadA*8 comprises one or more mutations at any of the positions shown with underlining:

MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG 50
LHDPTAHAEI MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG 100
RVVFGVRNAK TGAAGSLMDV LHYPGMNHRV EITEGILADE CAALLCYFFR 150
MPRQVFNAQK KAQSSTD

For example, the TadA*8 comprises alterations at amino acid position 82 and/or 166 (*e.g.*, V82S, T166R) alone or in combination with any one or more of the following Y147T, Y147R, Q154S, Y123H, and/or Q154R. In particular embodiments, a combination of alterations is selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

In some embodiments, the adenosine deaminase is TadA*8, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG
LHDPTAHAEI MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG
RVVFGVRNAK TGAAGSLMDV LHYPGMNHRV EITEGILADE CAALLCTFFR
MPRQVFNAQK KAQSSTD

In some embodiments, the TadA*8 is truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8.

In one embodiment, a fusion protein of the invention comprises a wild-type TadA is linked to an adenosine deaminase variant described herein (*e.g.*, TadA*8), which is linked to Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA*8 domain (*e.g.*, provided as a monomer). In other embodiments, the base editor comprises TadA*8 and TadA(wt), which are capable of forming heterodimers.

In some embodiments, a synthetic library of adenosine deaminases alleles, *e.g.*, TadA alleles can be utilized to generate an adenosine base editor with modified base editing efficiency and/or specificity. In some embodiments, an adenosine base editor generated from a synthetic library comprises higher base editing efficiency and/or specificity. In some embodimetns, an adenosine base editor generated from a synthetic library exhibits increased base editing efficiency, increased base editing specificity, reduced off-target editing, reduced bystander editing, reduced indel formation, and/or reduced spuriours editing compared to an adenosine base editor with a wild type TadA. In some embodiments, an adenosine base editor generated from a synthetic library exhibits increased base editing efficiency, increased base editing specificity, reduced off-target editing, reduced bystander editing, reduced indel formation, and/or reduced spuriours editing compared to an adenosine base editor with a TadA*7.10. In some embodiments, the synthetic library comprises randomized TadA portion of ABE. In some embodiments, the synthetic library comprises all 20 canonical amino acid substitutions at each position of TadA. In some embodiments, the synthetic library comprises an average frequency of 1-2 nucleotide substitution mutations per library member. In some embodiments, the synthetic library comprises background mutations found in TadA*7.10.

In some embodiments, the base editing system described herein comprises an ABE with TadA inserted into a Cas9. Sequences of relevant ABEs with TadA inserted into a Cas9 are provided.

101 Cas9 TadAins 1015

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVGSSGSETPGTSESATPESSGSEVEFSHEYWMRHAL
TLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQG
GLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGS
LMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSST
DYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

102 Cas9 TadAins 1022

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIGSSGSETPGTSESATPESSGSEVEFSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

103 Cas9 TadAins 1029

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGSSGSETPGTSESATPESSGS
EVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLH
DPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRV
VFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMP
RQVFNAQKKAQSSTDGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

103 Cas9 TadAins 1040

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSGSSGSETPGT
SESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVI
GEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCA
GAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADEC
AALLCYFFRMPRQVFNAQKKAQSSTDNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

105 Cas9 TadAins 1068

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGEGSSGSETPGTSESATPESSGSEVEFSHEYWMR
HALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMAL
RQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGA
AGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQ
SSTDTGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

106 Cas9 TadAins 1247

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGGSS
GSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVL
VLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTF
EPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

107 Cas9 TadAins 1054

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID
ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

108 Cas9 TadAins 1026

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEGSSGSETPGTSESATPESSGSEVE
FSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG
VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQV
FNAQKKAQSSTDQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

109 Cas9 TadAins 768

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQGSSGSETPGTSESATPESSGSEVEFSHEYWMR
HALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMAL
RQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGA
AGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRTTQKGQKNSR
ERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL
DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK
MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQIT
KHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREI
NNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQE
IGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGR
DFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDP
KKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSK
RVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD
TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.1 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY

YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGA
VLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYV
TFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEI
TEGILADECAALLCYFFRMPREDNEQKQLFVEQHKHYLDEIIEQISEFSK
RVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD
TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.2 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK

NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVP
VGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDAT
LYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHR
VEITEGILADECAALLCYFFRMPREDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFK
YFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.3 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD

LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDER
EVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLI
DATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGM
NHRVEITEGILADECAALLCYFFRMPREDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.4 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI

IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDER
EVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLI
DATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGM
NHRVEITEGILADECAALLCYFFRMRREDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.5 Cas9 TadAins 1249

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ

LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSGS
SGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID
ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCYFFRMRRPEDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.5 Cas9 TadAins delta 59-66 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD

SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESGSSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFE
PCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEG
ILADECAALLCYFFRMPRQVFNAQKKAQSSTDEDNEQKQLFVEQHKHYLD
EIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTN
LGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGG
D

110.6 Cas9 TadAins 1251

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD

SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
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GSSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDE
REVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRL
IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG
MNHRVEITEGILADECAALLCYFFRMRRDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.7 Cas9 TadAins 1252

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
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NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
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IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV

MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
DGSSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARD
EREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYR
LIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMRRNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.8 Cas9 TadAins delta 59-66 C-truncate 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP

VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGA
VLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMC
AGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADE
CAALLCYFFRMPRQEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADA
NLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR
YTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

111.1 Cas9 TadAins 997

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
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LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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CONTENANT LES PAGES 1 À 259

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 3
CONTAINING PAGES 1 TO 259

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS

What is claimed is:

1. A method of treating a neurological disorder in a subject, the method comprising: administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide,
wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a target gene or a regulatory element thereof associated with the neurological disorder in the subject, thereby treating the neurological disorder in the subject.

2. The method of claim 1, wherein the target gene is an alpha-L-iduronidase (IDUA) gene and the neurological disease is Hurler syndrome.

3. A method of treating Hurler syndrome in a subject, the method comprising administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,
wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in an alpha-L-iduronidase (IDUA) gene or a regulatory element thereof in the subject, thereby treating Hurler syndrome in the subject.

4. The method of claim 2 or 3, wherein the administration ameliorates at least one symptom related to Hurler syndrome.

5. The method of claim 4, wherein the administration results in faster amelioration of at least one symptom related to Hurler syndrome as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

6. The method of any one of claims 2-5, wherein the IDUA gene or regulatory element thereof comprises a SNP associated with Hurler syndrome.

7. The method of any one of claims 2-6, wherein the A-to-G nucleobase alteration is at the SNP associated with Hurler syndrome.

8. The method of claim 6 or 7, wherein the SNP associated with Hurler syndrome results in a W402X or a W401X amino acid mutation in an IDUA polypeptide as numbered in SEQ ID NO: 4, or a variant thereof, encoded by the IDUA gene, wherein X is a stop codon.

9. The method of any one of claims 6-8, wherein the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a wild type nucleobase.

10. The method of any one of claims 6-8, wherein the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Hurler syndrome.

11. The method of any one of claims 6-10, wherein the A-to-G alteration at the SNP associated with Hurler Syndrome changes a stop codon to a tryptophan in an IDUA polypeptide encoded by the IDUA gene.

12. The method of any one of claims 6-10, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *IDUA* gene or regulatory element thereof comprising the SNP associated with Hurler syndrome.

13. The method of any one of claims 6-12, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the IDUA gene or regulatory element thereof comprising the SNP associated with Hurler syndrome.

14. The method of claim 13, wherein the sgRNA comprises a nucleic acid sequence selected from the group consisting of: 5′- GACUCUAGGCAGAGGUCUCAA -3′, 5′- ACUCUAGGCAGAGGUCUCAA-3′, 5′- CUCUAGGCCGAAGUGUCGC -3′, and 5′- GCUCUAGGCCGAAGUGUCGC-3′.

15. The method of claim 1, wherein the target gene is a leucine-rich repeat kinase-2 (LRRK2) gene and the neurological disease is Parkinson's disease.

16. A method of treating Parkinson's disease in a subject, the method comprising: administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration a leucine-rich repeat kinase-2 (LRRK2) gene or a regulatory element thereof in the subject, thereby treating Parkinson's disease in the subject.

17. The method of any one of claims 1, 15, or 16 wherein the administration ameliorates at least one symptom related to Parkinson's disease.

18. The method of claim 17, wherein the administration results in faster amelioration of at least one symptom related to Parkinson's disease as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

19. The method of any one of claims 15-18, wherein the LRRK2 gene or regulatory element thereof comprises a SNP associated with Parkinson's disease.

20. The method of claim 19, wherein the A-to-G nucleobase alteration is at the SNP associated with Parkinson's disease.

21. The method of claim 19 or 20, wherein the SNP associated with Parkinson Disease results in a A419V, a R1441C, a R1441H, or a G2019S amino acid mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

22. The method of any one of claims 19-21, wherein the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a wild type nucleobase.

23. The method of any one of claims 19-21, wherein the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a non-wild type nucleobase that results in one or more ameliorated symptoms of Parkinson's disease.

24. The method of any one of claims 19-22, wherein the A-to-G nucleobase alteration changes a Cysteine or Histidine to an Arginine in a LRRK2 polypeptide encoded by the LRRK2 gene.

25. The method of any one of claims 19-22, wherein the A-to-G alteration changes a Serine to a Glycine in a LRRK2 polypeptide encoded by the LRRK2 gene.

26. The method of any one of claims 19-22, wherein the A-to-G alteration replaces the Cysteine (C) or Histidine (H) with an Arginine (R) at position 144 or replaces the Serine with a Glycine (G) at position 2019 of a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

27. A method of treating Parkinson's disease in a subject, the method comprising: administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration at a SNP in a LRRK2 gene associated with Parkinson's disease, wherein the SNP does not encode a G2019S mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof.

28. The method of claim 27, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof.

29. The method of any one of claims 15-28, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson's Disease.

30. The method of any one of claims 15-28, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson Disease.

31. The method of claim 30, wherein the sgRNA comprises a nucleic acid sequence: 5′-AAGCGCAAGCCUGGAGGGAA -3′; or 5′-ACUACAGCAUUGCUCAGUAC-3′.

32. The method of claim 1, wherein the target gene is a methyl CpG binding protein 2 (MECP2) gene and the neurological disease is Rett syndrome.

33. A method of treating Rett syndrome in a subject, the method comprising administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,
wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a methyl CpG binding protein 2 (MECP2) gene or a regulatory element thereof in the subject, thereby treating Rett syndrome in the subject.

34. The method of claim 32 or 33, wherein the administration ameliorates at least one symptom related to Rett syndrome.

35. The method of claim 34, wherein the administration results in faster amelioration of at least one symptom related to Rett syndrome as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

36. The method of any one of claims 32-35, wherein the MECP2 gene or regulatory element thereof comprises a SNP associated with Rett syndrome.

37. The method of claim 36, wherein the A-to-G nucleobase alteration is at the SNP associated with Rett Syndrome.

38. The method of claim 36 or 37, wherein the SNP associated with Rett syndrome results in a R106W or a T158M amino acid mutation in a MECP2 polypeptide as numbered in SEQ ID NO: 5, or a variant thereof, encoded by the MECP2 gene.

39. The method of claim 36 or 37, wherein the SNP associated with Rett syndrome results in a R255X or a R270X amino acid mutation in a MECP2 polypeptide encoded by the MECP2 gene, wherein X is a stop codon.

40. The method of any one of claims 36-39, wherein the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a wild type nucleobase.

41. The method of any one of claims 36-39, wherein the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a non-wild type nucleobase that results in ameliorated Rett syndrome symptoms.

42. The method of any one of claims 36-39, wherein the A-to-G nucleobase alteration at the SNP associated with Rett Syndrome changes a stop codon to tryptophan in MECP2 polypeptide.

43. The method of any one of claims 36-42 wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome.

44. The method of any one of claims 36-42, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome.

45. The method of claim 44, wherein the guide polynucleotide comprises a nucleic acid sequence selected from the group consisting of: 5′- CUUUUCACUUCCUGCCGGGG-3′, 5′-AGCUUCCAUGUCCAGCCUUC-3′, 5′- ACCAUGAAGUCAAAAUCAUU-3′, and 5′- GCUUUCAGCCCCGUUUCUUG-3′.

46. The method of claim 1, wherein the target gene is an ATP-binding cassette subfamily member 4 (ABCA4) gene and the neurological disease is Stargardt disease.

47. A method of treating Stargardt disease in a subject, the method comprising administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,
wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in an ATP-binding cassette subfamily member 4 (ABCA4) gene or a regulatory element thereof in the subject, thereby treating Stargardt disease in the subject.

48. The method of claim 46 or 47, wherein the administration ameliorates at least one symptom related to Stargardt disease.

49. The method of claim 48, wherein the administration results in faster amelioration of at least one symptom related to Stargardt disease as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

50. The method of any one of claims 46-49, wherein the ABCA4 gene comprises a SNP associated with Stargardt disease.

51. The method of claim 50, wherein the A-to-G nucleobase alteration is at the SNP associated with Stargardt disease.

52. The method of claim 50 or 51, wherein the SNP associated with Stargardt disease results in a A1038V or a G1961E amino acid mutation in an ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof, encoded by the ABCA4 gene.

53. The method of claim 52, wherein the SNP associated with Stargardt disease results in a G1961E amino acid mutation in the ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof.

54. The method of any one of claims 51-53, wherein the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a wild type nucleobase.

55. The method of any one of claims 51-53, wherein the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a non-wild type nucleobase that results in one or more ameliorated symptoms of Stargardt disease.

56. The method of any one of claims 51-55, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt disease.

57. The method of any one of claims 51-56, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease.

58. The method of claim 57, wherein the sgRNA comprises the sequence 5′-CUCCAGGGCGAACUUCGACACACAGC-3′.

59. The method of any one of the preceding claims wherein the treatment results in ameliorated symptoms of the neurological disorder compared to treatment with a base editor comprising an adenosine deaminase domain without the amino acid substitutions.

60. A method of editing a target gene or regulatory element thereof associated with a neurological disorder, the method comprising contacting the target gene or regulatory element thereof with (i) an adenosine base editor and (ii) a guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a target gene or a regulatory element thereof associated with the neurological disorder.

61. The method of claim 60, wherein the target gene is a leucine-rich repeat kinase-2 (LRRK2) gene and the neurological disease is Parkinson's disease.

62. A method of editing a leucine-rich repeat kinase-2 (LRRK2) gene or a regulatory element thereof, the method comprising contacting the LRRK2 gene or regulatory element thereof with (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the LRRK2 gene a regulatory element thereof.

63. The method of claim 62, wherein the A-to-G nucleobase alteration is at the SNP associated with Parkinson's disease.

64. The method of claim 62 or 63, wherein the SNP associated with Parkinson Disease results in a A419V, a R1441C, a R1441H, or a G2019S amino acid mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

65. The method of any one of claims 62-64, wherein the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a wild type nucleobase.

66. The method of any one of claims 62-64, wherein the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a non-wild type nucleobase that results in one or more ameliorated symptoms of Parkinson's disease.

67. The method of any one of claims 62-66, wherein the A-to-G nucleobase alteration changes a Cysteine or Histidine to an Arginine in a LRRK2 polypeptide encoded by the LRRK2 gene.

68. The method of any one of claims 62-66, wherein the A-to-G alteration changes a Serine to a Glycine in a LRRK2 polypeptide encoded by the LRRK2 gene.

69. The method of any one of claims 62-66, wherein the A-to-G alteration replaces the Cysteine (C) or Histidine (H) with an Arginine (R) at position 144 or replaces the Serine with a Glycine (G) at position 2019 of a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

70. A method of editing a leucine-rich repeat kinase-2 (LRRK2) gene or a regulatory element thereof, the method comprising contacting the LRRK2 gene or regulatory element thereof with (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration at a SNP in a LLRK2 gene, wherein the SNP does not encode a G2019S mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof.

71. The method of claim 70, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof.

72. The method of any one of claims 61-71, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson's Disease.

73. The method of any one of claims 61-71, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson Disease.

74. The method of claim 73, wherein the sgRNA comprises a nucleic acid sequence: 5′-AAGCGCAAGCCUGGAGGGAA -3′; or 5′-ACUACAGCAUUGCUCAGUAC-3′.

75. The method of claim 60, wherein the target gene is an alpha-L-iduronidase (IDUA) gene and the neurological disease is Hurler syndrome.

76. A method of editing an alpha-L-iduronidase (IDUA) gene or a regulatory element thereof, the method comprising contacting the IDUA gene or regulatory element thereof with (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,
wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the IDUA gene or a regulatory element thereof.

77. The method of any one of claims 75 or 76, wherein the IDUA gene or regulatory element thereof comprises a SNP associated with Hurler syndrome.

78. The method of any one of claims 75-77, wherein the A-to-G nucleobase alteration is at the SNP associated with Hurler syndrome.

79. The method of claim 77 or 78, wherein the SNP associated with Hurler syndrome results in a W402X or a W401X amino acid mutation in an IDUA polypeptide as numbered in SEQ ID NO: 4, or a variant thereof, encoded by the IDUA gene, wherein X is a stop codon.

80. The method of any one of claims 77-79, wherein the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a wild type nucleobase.

81. The method of any one of claims 77-79, wherein the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Hurler syndrome.

82. The method of any one of claims 77-81, wherein the A-to-G alteration at the SNP associated with Hurler Syndrome changes a stop codon to a tryptophan in an IDUA polypeptide encoded by the IDUA gene.

83. The method of any one of claims 77-81, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *IDUA* gene or regulatory element thereof comprising the SNP associated with Hurler syndrome.

84. The method of any one of claims 77-83, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the IDUA gene or regulatory element thereof comprising the SNP associated with Hurler syndrome.

85. The method of claim 84, wherein the sgRNA comprises a nucleic acid sequence selected from the group consisting of: 5′- GACUCUAGGCAGAGGUCUCAA -3′,5′-ACUCUAGGCAGAGGUCUCAA-3′, 5′- CUCUAGGCCGAAGUGUCGC -3′, and 5′-GCUCUAGGCCGAAGUGUCGC-3′.

86. The method of claim 60, wherein the target gene is a methyl CpG binding protein 2 (MECP2) gene and the neurological disease is Rett syndrome.

87. A method of editing a methyl CpG binding protein 2 (MECP2) gene or regulatory element thereof, the method comprising administering to the subject (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the MECP2 gene or a regulatory element thereof.

88. The method of claim 86 or 87, wherein the MECP2 gene or regulatory element thereof comprises a SNP associated with Rett syndrome.

89. The method of claim 88, wherein the A-to-G nucleobase alteration is at the SNP associated with Rett Syndrome.

90. The method of claim 88 or 89, wherein the SNP associated with Rett syndrome results in a R106W or a T158M amino acid mutation in a MECP2 polypeptide as numbered in SEQ ID NO: 5, or a variant thereof, encoded by the MECP2 gene.

91. The method of claim 88 or 89, wherein the SNP associated with Rett syndrome results in a R255X or a R270X amino acid mutation in a MECP2 polypeptide encoded by the MECP2 gene, wherein X is a stop codon.

92. The method of any one of claims 88-81, wherein the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a wild type nucleobase.

93. The method of any one of claims 88-91, wherein the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Rett syndrome.

94. The method of any one of claims 88-91, wherein the A-to-G nucleobase alteration at the SNP associated with Rett Syndrome changes a stop codon to tryptophan in MECP2 polypeptide.

95. The method of any one of claims 88-94, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome.

96. The method of any one of claims 88-94, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome.

97. The method of claim 96, wherein the guide polynucleotide comprises a nucleic acid sequence selected from the group consisting of: 5'- CUUUUCACUUCCUGCCGGGG-3', 5'-AGCUUCCAUGUCCAGCCUUC-3', 5'- ACCAUGAAGUCAAAAUCAUU-3', and 5'- GCUUUCAGCCCCGUUUCUUG-3'.

98. The method of claim 60, wherein the target gene is an ATP-binding cassette subfamily member 4 (ABCA4) gene and the neurological disease is Stargardt disease.

99. A method of editing an ATP binding cassette subfamily member 4 (ABCA4) gene or regulatory element thereof, the method comprising contacting the ABCA4 gene or regulatory element thereof with (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the ABCA4 gene or a regulatory element thereof.

100. The method of claim 98 or 99, wherein the administration ameliorates at least one symptom related to Stargardt disease.

101. The method of claim 100, wherein the administration results in faster amelioration of at least one symptom related to Stargardt disease as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

102. The method of any one of claims 98-101, wherein the ABCA4 gene comprises a SNP associated with Stargardt disease.

103. The method of claim 102, wherein the A-to-G nucleobase alteration is at the SNP associated with Stargardt disease.

104. The method of claim 102 or 103, wherein the SNP associated with Stargardt disease results in a A1038V, or a G1961E amino acid mutation in an ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof, encoded by the ABCA4 gene.

105. The method of claim 104, wherein the SNP associated with Stargardt disease results in a G1961E amino acid mutation in the ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof.

106. The method of any one of claims 103-105, wherein the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a wild type nucleobase.

107. The method of any one of claims 103-105, wherein the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a non-wild type nucleobase that results in one or more ameliorated symptoms of Stargardt disease.

108. The method of any one of claims 103-107, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease.

109. The method of any one of claims 103-108, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease.

110. The method of claim 109, wherein the sgRNA comprises the sequence 5′-CUCCAGGGCGAACUUCGACACACAGC-3′.

111. The method of any one of claims 60-110, wherein the contacting is in a cell.

112. The method of claim 111, wherein the contacting results in less than 10% indels in a genome in the cell, wherein indel rate is measured by mismatch frequency between sequences flanking the single nucleotide modification and an unmodified sequence.

113. The method of claim 111, wherein the contacting results in less than 5% indels in a genome in the cell, wherein indel rate is measured by mismatch frequency between sequences flanking the single nucleotide modification and an unmodified sequence.

114. The method of claim 111, wherein the contacting results in less than 1% indels in a genome in the cell, wherein indel rate is measured by mismatch frequency between sequences flanking the single nucleotide modification and an unmodified sequence.

115. The method of any one of claims 111-114, wherein the cell is a neuron.

116. The method of any one of claims 60-110, wherein the contacting is in a population of cells.

117. The method of claim 116, wherein the contacting results in the A-to-G nucleobase alteration in at least 40% of the population of cells after the contacting step.

118. The method of claim 116, wherein the contacting results in the A-to-G nucleobase alteration in at least 50% of the population of cells after the contacting step.

119. The method of claim 116, wherein the contacting results in the A-to-G nucleobase alteration in at least 70% of the population of cells after the contacting step.

120. The method of any one of claims 116-119, wherein at least 90% of the cells are viable after the contacting step.

121. The method of any one of claims 116-120, wherein the population of cells was not enriched after the contacting step.

122. The method of any one of claims 116-121, wherein the population of cells are neurons.

123. The method of any one of claims 111-122, wherein the contacting is in vivo or ex vivo.

124. The method of any one of claims 1-123, wherein the polynucleotide programmable DNA binding domain is a Cas9.

125. The method of claim 124, wherein the Cas9 is a SpCas9, a SaCas9, or a variant thereof.

126. The method of claim 124 or 125, wherein the polynucleotide programmable DNA binding domain comprises a modified SpCas9 having an altered protospacer-adjacent motif (PAM) specificity.

127. The method of claim 126, wherein the Cas9 has specificity for a PAM sequence selected from the group consisting of NGG, NGA, NGCG, NGN, NNGRRT, NNNRRT, NGCG, NGCN, NGTN, and NGC; wherein N is A, G, C, or T; and wherein R is A or G.

128. The method of any one of claims 124-127, wherein the polynucleotide programmable DNA binding domain is a nuclease inactive variant.

129. The method of any one of claims 124-127, wherein the polynucleotide programmable DNA binding domain is a nickase variant.

130. The method of claim 129, wherein the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

131. The method of any one of claims 1-130, wherein the adenosine deaminase domain comprises a TadA domain.

132. The method of claim 131, wherein the adenosine deaminase comprises a TadA deaminase comprising a V82S alteration and/or a T166R alteration.

133. The method of any one of claims 1-132, wherein the adenosine deaminase further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, Q154R, or a combination thereof.

134. The method of any one of claims 1-133, wherein the adenosine deaminase comprises a combination of alterations selected from the group consisting of: Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y147T + Q154R; Y147T + Q154S; and Y123H + Y147R + Q154R + I76Y.

135. The method of any one of claims 1-134, wherein the adenosine base editor domain comprises an adenosine deaminase monomer.

136. The method of any one of claims 1-135, wherein the adenosine base editor comprises an adenosine deaminase dimer.

137. The method of any one of claims 131-136, wherein the TadA deaminase is a TadA*8 variant.

138. The method of claim 137, wherein the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, and TadA*8.13.

139. The method of claim 138, wherein the adenosine base editor is an ABE8 base editor selected from the group consisting of: ABE8.1, ABE8.2, ABE8.3, ABE8.4, ABE8.5, ABE8.6, ABE8.7, ABE8.8, ABE8.9, ABE8.10, ABE8.11, ABE8.12, and ABE8.13.

140. A cell produced by the method of any one of claims 111-115.

141. A population of cells produced by the method of any one of claims 116-122.

142. A base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a target gene or a regulatory element thereof associated with the neurological disorder.

143. The base editor system of claim 142, wherein the target gene is a leucine-rich repeat kinase-2 (LRRK2) gene and the neurological disease is Parkinson's disease.

144. A base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,
wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a LRRK2 gene a regulatory element thereof.

145. The base editor system of claim 144, wherein the A-to-G nucleobase alteration is at a SNP associated with Parkinson's disease in the LRRK2 gene or regulatory element thereof.

146. The base editor system of claim 144 or 145, wherein the SNP associated with Parkinson Disease results in a A419V, a R1441C, a R1441H, or a G2019S amino acid mutation in a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

147. The base editor system of any one of claims 145 or 146, wherein the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a wild type nucleobase.

148. The base editor system of any one of claims 145 or 146, wherein the A-to-G nucleobase alteration changes the SNP associated with Parkinson's disease to a non-wild type nucleobase that results in ameliorated Parkinson's symptoms.

149. The base editor system of any one of claims 145-148, wherein the A-to-G nucleobase alteration changes a Cysteine or Histidine to an Arginine in a LRRK2 polypeptide encoded by the LRRK2 gene.

150. The base editor system of any one of claims 145-148, wherein the A-to-G alteration changes a Serine to a Glycine in a LRRK2 polypeptide encoded by the LRRK2 gene.

151. The base editor system of any one of claims 145-148, wherein the A-to-G alteration replaces the Cysteine (C) or Histidine (H) with an Arginine (R) at position 144 or replaces the Serine with a Glycine (G) at position 2019 of a LRRK2 polypeptide as numbered in SEQ ID NO: 3, or a variant thereof, encoded by the LRRK2 gene.

152. The base editor system of claim 151, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof.

153. The base editor system of any one of claims 143-152, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson's Disease.

154. The base editor system of any one of claims 143-152, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *LRRK2* gene or regulatory element thereof comprising the SNP associated with Parkinson Disease.

155. The base editor system of claim 154, wherein the sgRNA comprises a nucleic acid sequence: 5′-AAGCGCAAGCCUGGAGGGAA -3′; or 5′-ACUACAGCAUUGCUCAGUAC-3′.

156. The base editor system of claim 142, wherein the target gene is an alpha-L-iduronidase (IDUA) gene and the neurological disease is Hurler syndrome.

157. A base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,
wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in an alpha-L-iduronidase (IDUA) gene or a regulatory element thereof.

158. The base editor system of claim 156 or 157, wherein the IDUA gene or regulatory element thereof comprises a SNP associated with Hurler syndrome.

159. The base editor system of any one of claims 156-158, wherein the A-to-G nucleobase alteration is at the SNP associated with Hurler syndrome.

160. The base editor system of claim 158 or 159, wherein the SNP associated with Hurler syndrome results in a W402X or a W401X amino acid mutation in an IDUA polypeptide as numbered in SEQ ID NO: 4, or a variant thereof, encoded by the IDUA gene, wherein X is a stop codon.

161. The base editor system of any one of claims 158-160, wherein the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a wild type nucleobase.

162. The base editor system of any one of claims 158-160, wherein the A-to-G nucleobase alteration changes the SNP associated with Hurler syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Hurler syndrome.

163. The base editor system of any one of claims 158-162, wherein the A-to-G alteration at the SNP associated with Hurler Syndrome changes a stop codon to a tryptophan in an IDUA polypeptide encoded by the IDUA gene.

164. The base editor system of any one of claims 158-162, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *IDUA* gene or regulatory element thereof comprising the SNP associated with Hurler syndrome.

165. The base editor system of any one of claims 158-164, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the IDUA gene or regulatory element thereof comprising the SNP associated with Hurler syndrome.

166. The base editor system of claim 165, wherein the sgRNA comprises a nucleic acid sequence selected from the group consisting of: 5′- GACUCUAGGCAGAGGUCUCAA -3′, 5′- ACUCUAGGCAGAGGUCUCAA-3′, 5′- CUCUAGGCCGAAGUGUCGC -3′, and 5′-GCUCUAGGCCGAAGUGUCGC-3′.

167. The base editor system of claim 142, wherein the target gene is a methyl CpG binding protein 2 (MECP2) gene and the neurological disease is Rett syndrome.

168. A base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a methyl CpG binding protein 2 (MECP2) gene or regulatory element thereof.

169. The base editor system of claim 167 or 168, wherein the MECP2 gene or regulatory element thereof comprises a SNP associated with Rett syndrome.

170. The base editor system of claim 169, wherein the A-to-G nucleobase alteration is at the SNP associated with Rett Syndrome.

171. The base editor system of claim 169 or 170, wherein the SNP associated with Rett syndrome results in a R106W or a T158M amino acid mutation in a MECP2 polypeptide as numbered in SEQ ID NO: 5, or a variant thereof, encoded by the MECP2 gene.

172. The base editor system of claim 169 or 170, wherein the SNP associated with Rett syndrome results in a R255X or a R270X amino acid mutation in a MECP2 polypeptide encoded by the MECP2 gene, wherein X is a stop codon.

173. The base editor system of any one of claims 169-172, wherein the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a wild type nucleobase.

174. The base editor system of any one of claims 169-172, wherein the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a non-wild type nucleobase that results in one or more ameliorated symptoms of Rett syndrome.

175. The base editor system of any one of claims 169-172, wherein the A-to-G nucleobase alteration at the SNP associated with Rett Syndrome changes a stop codon to tryptophan in MECP2 polypeptide.

176. The base editor system of any one of claims 169-175, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome.

177. The base editor system of any one of claims 169-175, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with Rett syndrome.

178. The base editor system of claim 177, wherein the guide polynucleotide comprises a nucleic acid sequence selected from the group consisting of: 5′-CUUUUCACUUCCUGCCGGGG-3′, 5′-AGCUUCCAUGUCCAGCCUUC-3′, 5′-ACCAUGAAGUCAAAAUCAUU-3′, and 5′- GCUUUCAGCCCCGUUUCUUG-3′.

179. The base editor system of claim 142, wherein the target gene is an ATP binding cassette subfamily member 4 (ABCA4) gene and the neurological disease is Stargardt disease.

180. A base editor system comprising contacting (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain,

wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 as numbered in SEQ ID NO: 2 or a corresponding position thereof,

and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in an ATP binding cassette subfamily member 4 (ABCA4) gene or regulatory element thereof.

181. The base editor system of claim 179 or 180, wherein the administration ameliorates at least one symptom related to Stargardt disease.

182. The base editor system of claim 181, wherein the administration results in faster amelioration of at least one symptom related to Stargardt disease as compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

183. The base editor system of any one of claims 179-182, wherein the ABCA4 gene comprises a SNP associated with Stargardt disease.

184. The base editor system of claim 183, wherein the A-to-G nucleobase alteration is at the SNP associated with Stargardt disease.

185. The base editor system of claim 183 or 184, wherein the SNP associated with Stargardt disease results in a A1038V, or a G1961E amino acid mutation in an ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof, encoded by the ABCA4 gene.

186. The base editor system of claim 185, wherein the SNP associated with Stargardt disease results in a G1961E amino acid mutation in the ABCA4 polypeptide as numbered in SEQ ID NO: 6, or a variant thereof.

187. The base editor system of any one of claims 184-186, wherein the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a wild type nucleobase.

188. The base editor system of any one of claims 184-186, wherein the A-to-G nucleobase alteration changes the SNP associated with Stargardt disease to a non-wild type nucleobase that results in ameliorated Stargardt disease symptoms.

189. The base editor system of any one of claims 184-188, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease.

190. The base editor system of any one of claims 184-189, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *ABCA4* gene or regulatory element thereof comprising the SNP associated with Stargardt Disease.

191. The base editor system of claim 190, wherein the sgRNA comprises the sequence 5′-CUCCAGGGCGAACUUCGACACACAGC-3′.

192. The base editor system of any one of claims 142-191, wherein the polynucleotide programmable DNA binding domain is a Cas9.

193. The base editor system of claim 192, wherein the Cas9 is a SpCas9, a SaCas9, or a variant thereof.

194. The base editor system of claim 192 or 193, wherein the polynucleotide programmable DNA binding domain comprises a modified SpCas9 having an altered protospacer-adjacent motif (PAM) specificity.

195. The base editor system of claim 194, wherein the Cas9 has specificity for a PAM sequence selected from the group consisting of NGG, NGA, NGCG, NGN, NNGRRT, NNNRRT, NGCG, NGCN, NGTN, and NGC, wherein N is A, G, C, or T and wherein R is A or G.

196. The base editor system of any one of claims 192-195, wherein the polynucleotide programmable DNA binding domain is a nuclease inactive variant.

197. The base editor system of any one of claims 192-195, wherein the polynucleotide programmable DNA binding domain is a nickase variant.

198. The base editor system of claim 197, wherein the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

199. The base editor system of any one of claims 142-198, wherein the adenosine deaminase domain comprises a TadA domain.

200. The base editor system of claim 199, wherein the adenosine deaminase comprises a TadA deaminase comprising a V82S alteration and/or a T166R alteration.

201. The base editor system of any one of claims 142-200, wherein the adenosine deaminase further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, Q154R, or a combination thereof.

202. The base editor system of any one of claims 142-201, wherein the adenosine deaminase comprises a combination of alterations selected from the group consisting of: Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y147T + Q154R; Y147T + Q154S; and Y123H + Y147R + Q154R + I76Y.

203. The base editor system of any one of claims 142-202, wherein the adenosine base editor domain comprises an adenosine deaminase monomer.

204. The base editor system of any one of claims 142-203, wherein the adenosine base editor comprises an adenosine deaminase dimer.

205. The base editor system of any one of claims 199-204, wherein the TadA deaminase is a TadA*8 variant.

206. The base editor system of claim 205, wherein the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, and TadA*8.13.

207. The base editor system of claim 206, wherein the adenosine base editor is an ABE8 base editor selected from the group consisting of: ABE8.1, ABE8.2, ABE8.3, ABE8.4, ABE8.5, ABE8.6, ABE8.7, ABE8.8, ABE8.9, ABE8.10, ABE8.11, ABE8.12, and ABE8.13.

208. A vector comprising the nucleic acid sequence encoding the adenosine base editor of any one of claims 142-207.

209. A vector comprising the nucleic acid sequence encoding the adenosine base editor and the guide polynucleotide of any one of claims 142-207.

210. The vector of claim or 208 or 209, wherein the vector is a viral vector, a lentiviral vector, or an AAV vector.

211. A cell comprising the base editor system of any one of claims 142-207, or the vector of any one of claims 208-210.

212. The cell of claim 211, wherein the cell is a central nervous system cell.

213. The cell of claim 211, wherein the cell is a neuron.

214. The cell of claim 211, wherein the cell is a photoreceptor.

215. The cell of any one of claims 211-214, wherein the cell is *in vitro*, *in vivo*, or *ex vivo*.

216. A pharmaceutical composition comprising the base editor of any one of claims 142-207, the vector of any one of claims 208-210, or the cell of any one of claims 211-215 and a pharmaceutically acceptable carrier.

217. The pharmaceutical composition of claim 216, further comprising a lipid.

218. The pharmaceutical composition of claim 216, further comprising a virus.

219. A kit comprising the base editor of any one of claims 142-207 or the vector of any one of claims 208-210.

220. The method of any one of claims 1-139, wherein at least one nucleotide of the guide polynucleotide comprises a non-naturally occurring modification.

221. The method of any one of claims 14, 31, 45, 58, 74, 85, 97, or 110, wherein at least one nucleotide of the nucleic acid sequence comprises a non-naturally occurring modification.

222. The base editor system of any one of claims 155, 166, 178, or 191, wherein at least one nucleotide of the nucleic acid sequence comprises a non-naturally occurring modification.

223. The base editor system of claim 222, wherein the non-naturally occurring modification is a chemical modification.

224. The base editor system of claim 223, wherein the chemical modification is a 2'-O-methylation.

225. The base editor system of claim 223, wherein the nucleic acid sequence comprises a phosphorothioate.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 145

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 145

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

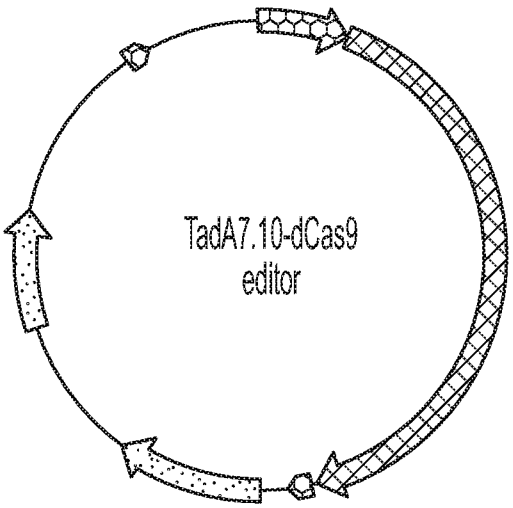


FIG. 1A

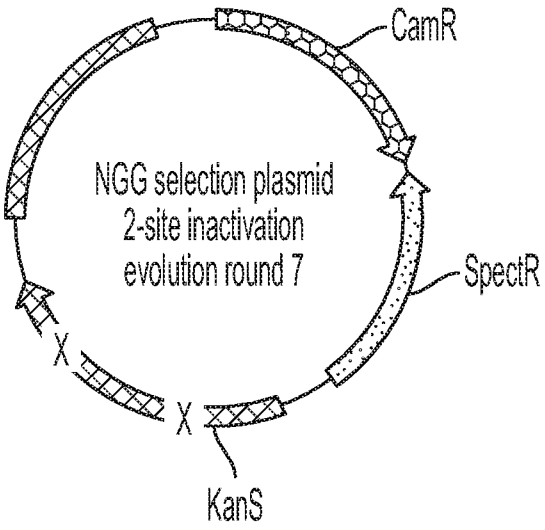


FIG. 1B

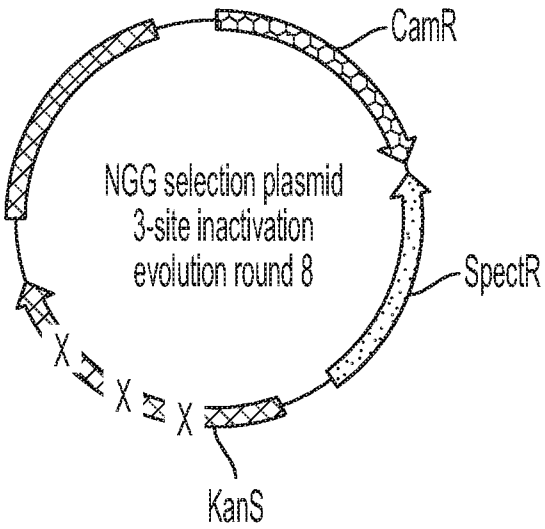


FIG. 1C

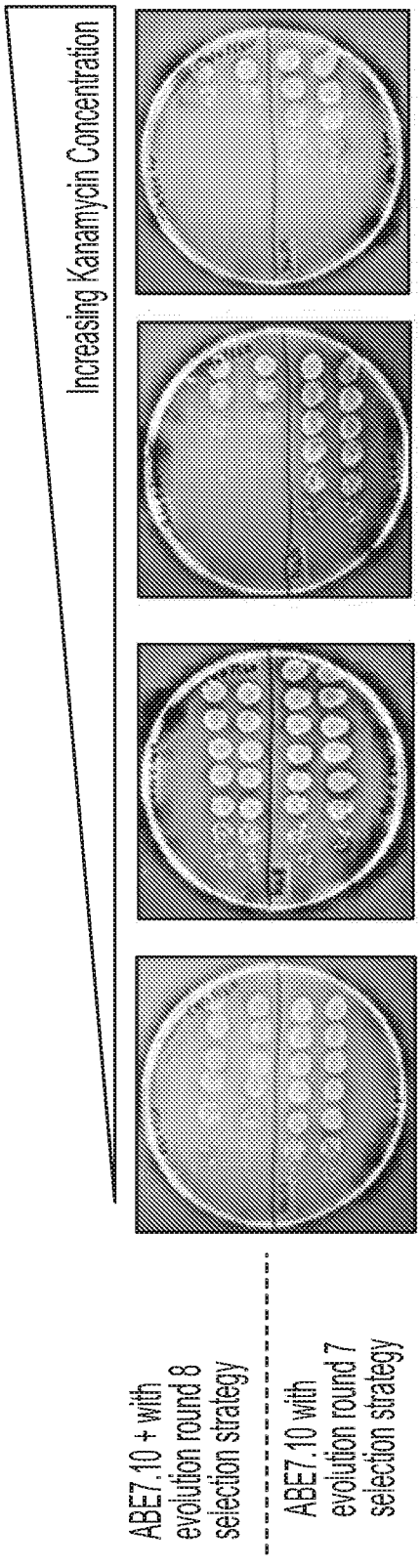

Increasing Kanamycin Concentration
ABE7.10 + with evolution round 8 selection strategy
ABE7.10 with evolution round 7 selection strategy

FIG. 2

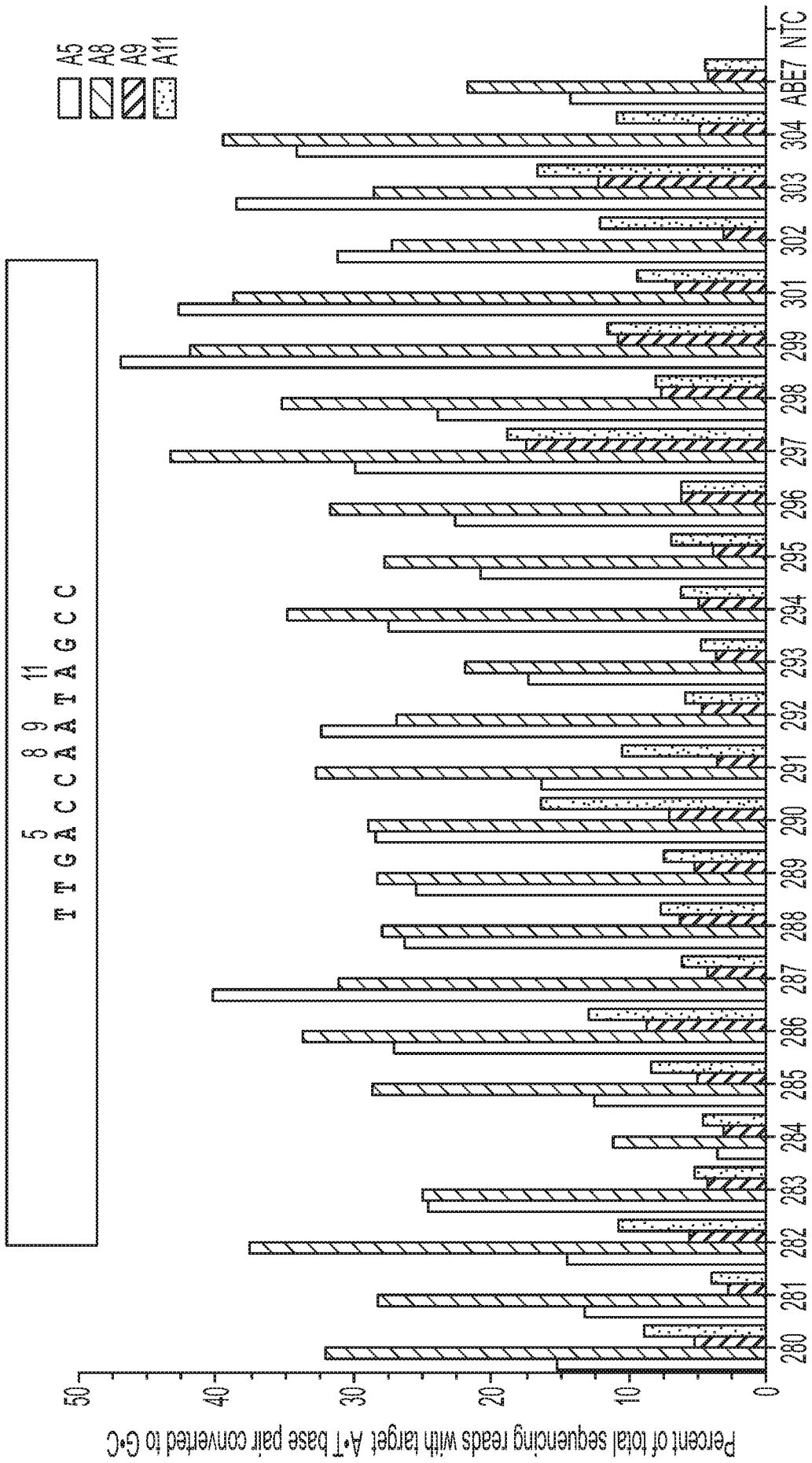

A5
A8
A9
A11
5 8 9 11
TTGACCAATAGCC
Percent of total sequencing reads with target A•T base pair converted to G•C
0
10
20
30
40
50
NTC
ABE7
304
303
302
301
299
298
297
296
295
294
293
292
291
290
289
288
287
286
285
284
283
282
281
280
ABE8 construct

FIG. 3A

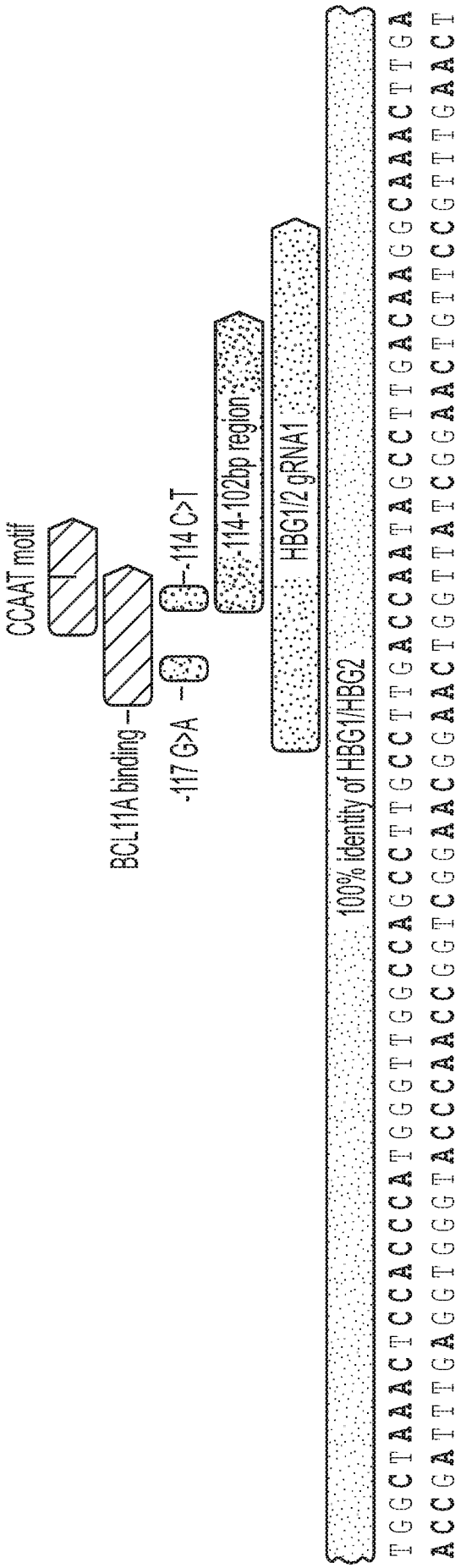

CCAAT motif
BCL11A binding
-117 G>A
-114 C>T
-114-102bp region
HBG1/2 gRNA1
100% identity of HBG1/HBG2
TGGCTAAACTCCACCCATGGGTTGGCCAGCCTTGCCTTGACCAATAGCCTTGACAAGGCAAACTTGA
ACCGATTTGAGGTGGGTACCCAACCGGTCGGAACGGAACTGGTTATCGGAACTGTTCCGTTTGAACT

FIG. 3B

	His		Leu			Thr			Pro→Pro			Val→Ala			Glu			Lys			Ser→Pro		
P	A	M	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
G	C	A	t	C	T	G	A	C	T	C	C	T	G	T	G	G	A	G	A	A	G	T	C	T

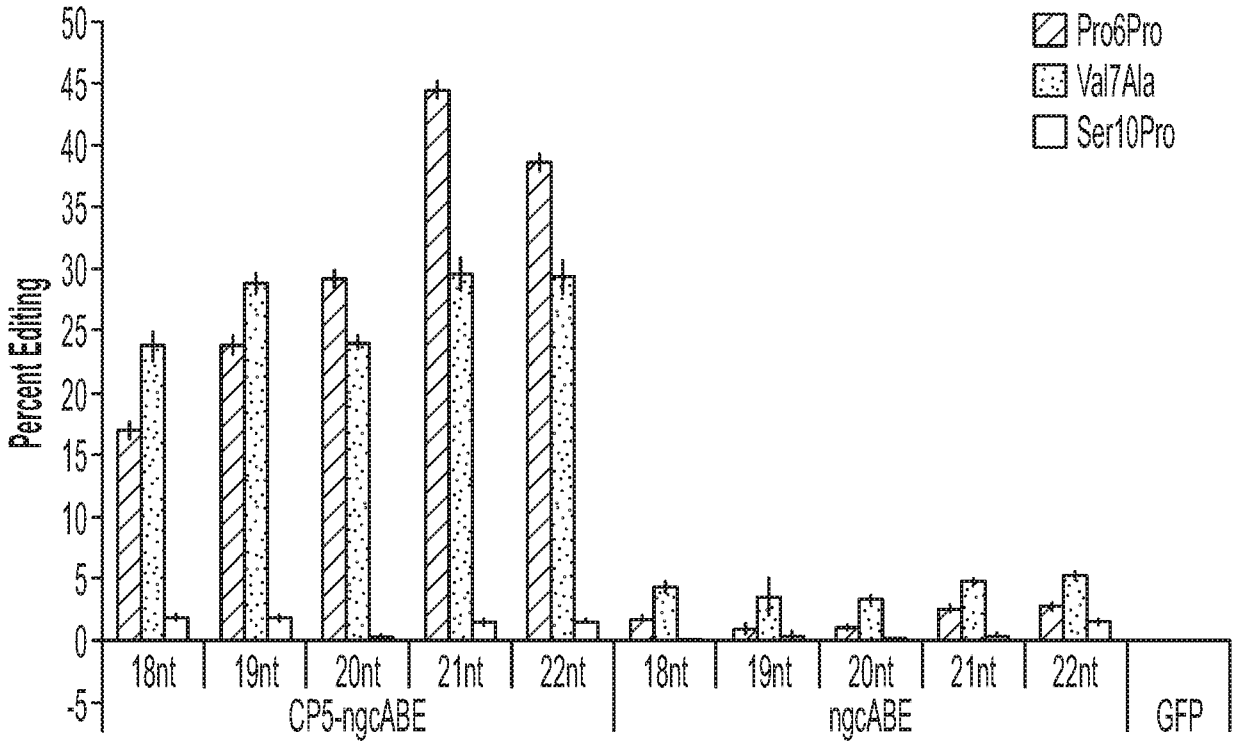


FIG. 4

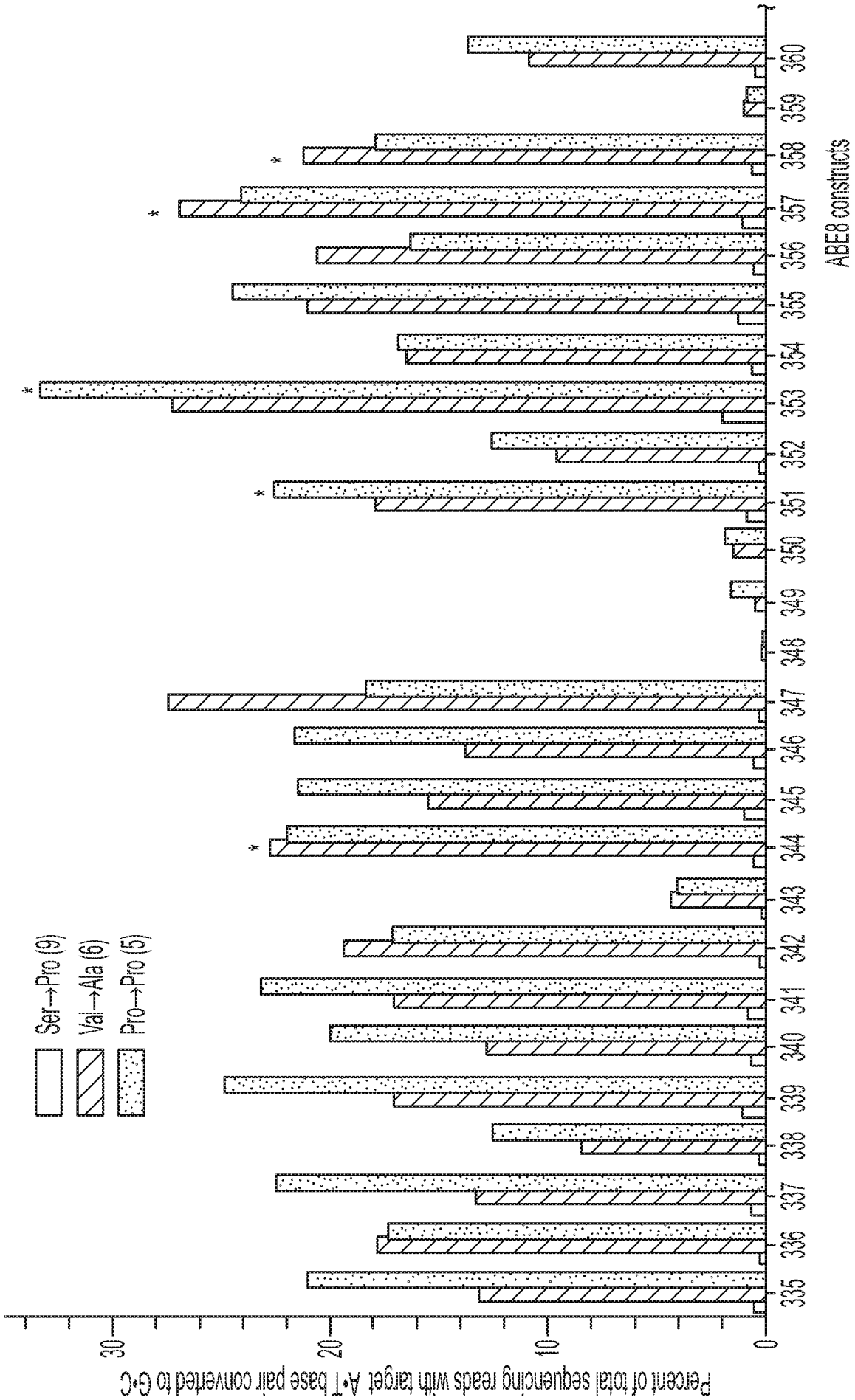

Ser→Pro (9)
Val→Ala (6)
Pro→Pro (5)
Percent of total sequencing reads with target A•T base pair converted to G•C
0
10
20
30
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
ABE8 constructs
*
*
*
*

FIG. 5

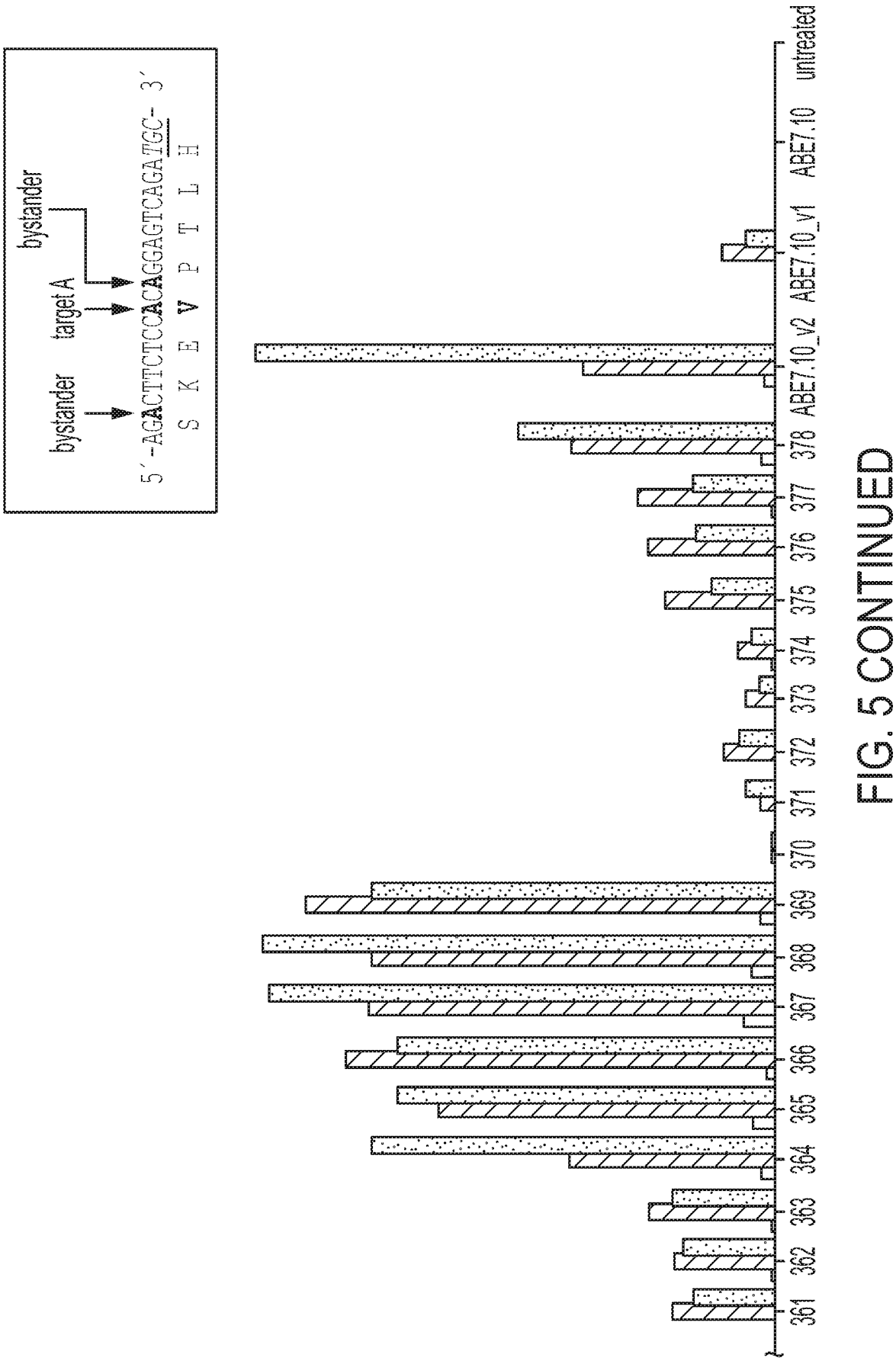


FIG. 5 CONTINUED

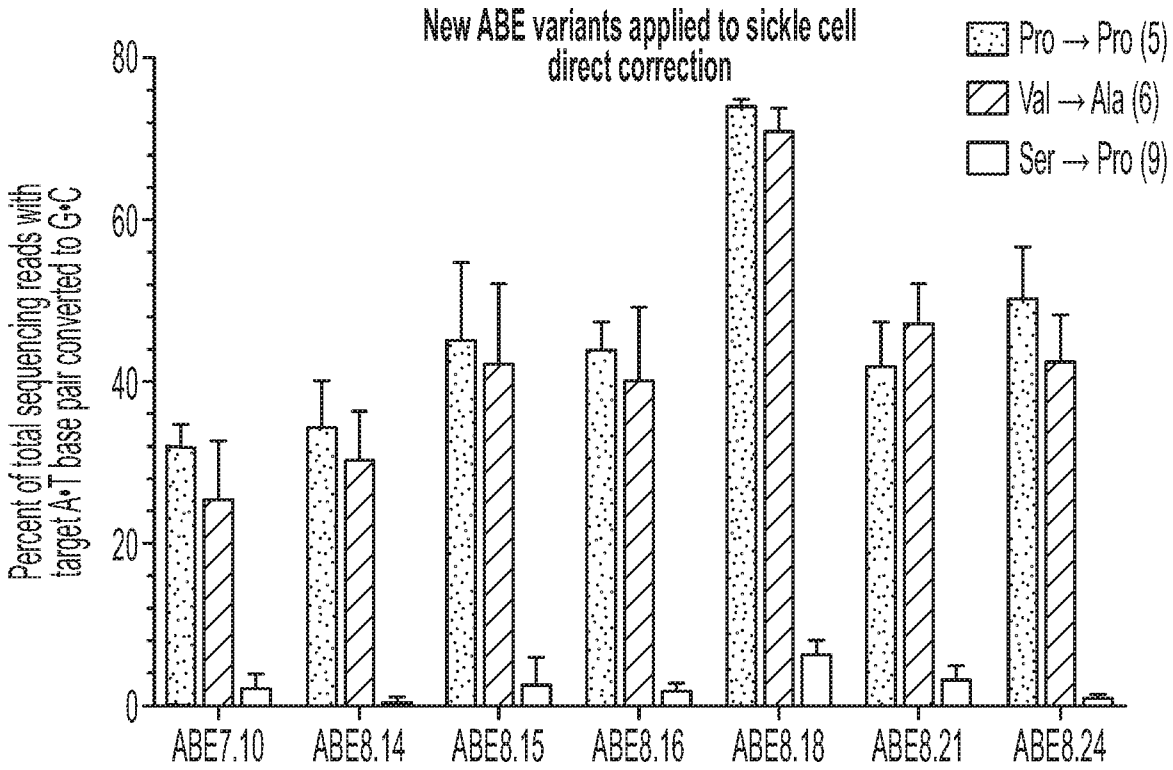

New ABE variants applied to sickle cell direct correction
Pro → Pro (5)
Val → Ala (6)
Ser → Pro (9)
Percent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
ABE7.10
ABE8.14
ABE8.15
ABE8.16
ABE8.18
ABE8.21
ABE8.24

FIG. 6

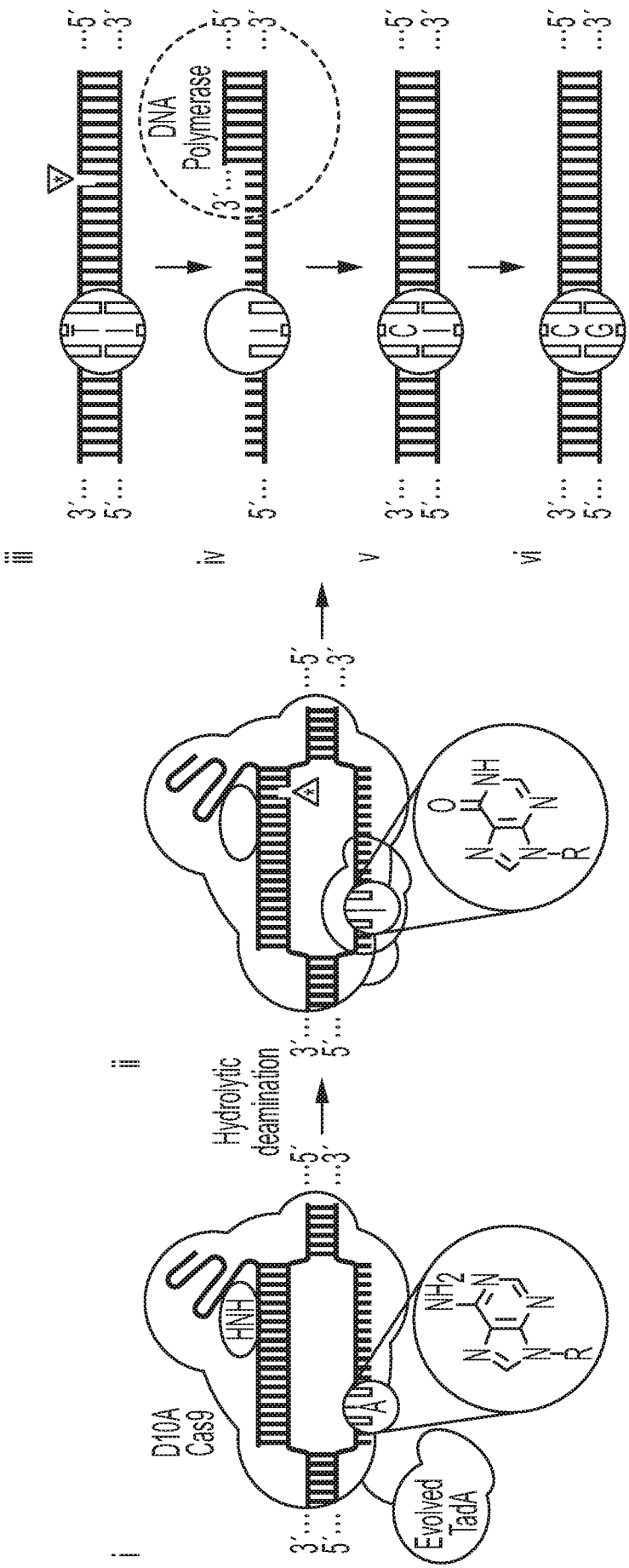

i
ii
iii
iv
v
vi
D10A Cas9
HNH
RuvC
Evolved TadA
Hydrolytic deamination
DNA Polymerase
5′
3′
NH2
NH
O
N
R

FIG. 7A

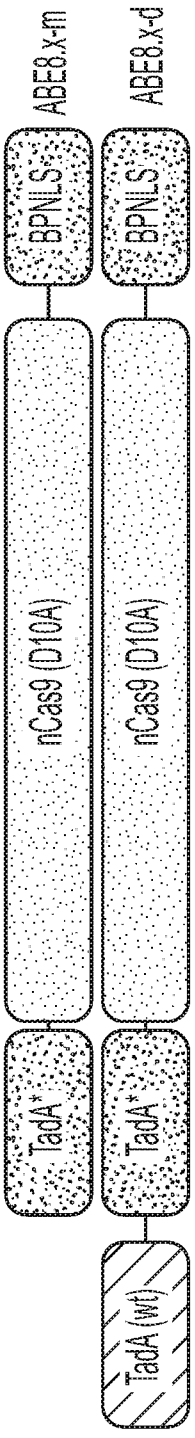

ABE8.x-m
ABE8.x-d
BPNLS
nCas9 (D10A)
TadA*
TadA (wt)

FIG. 7B

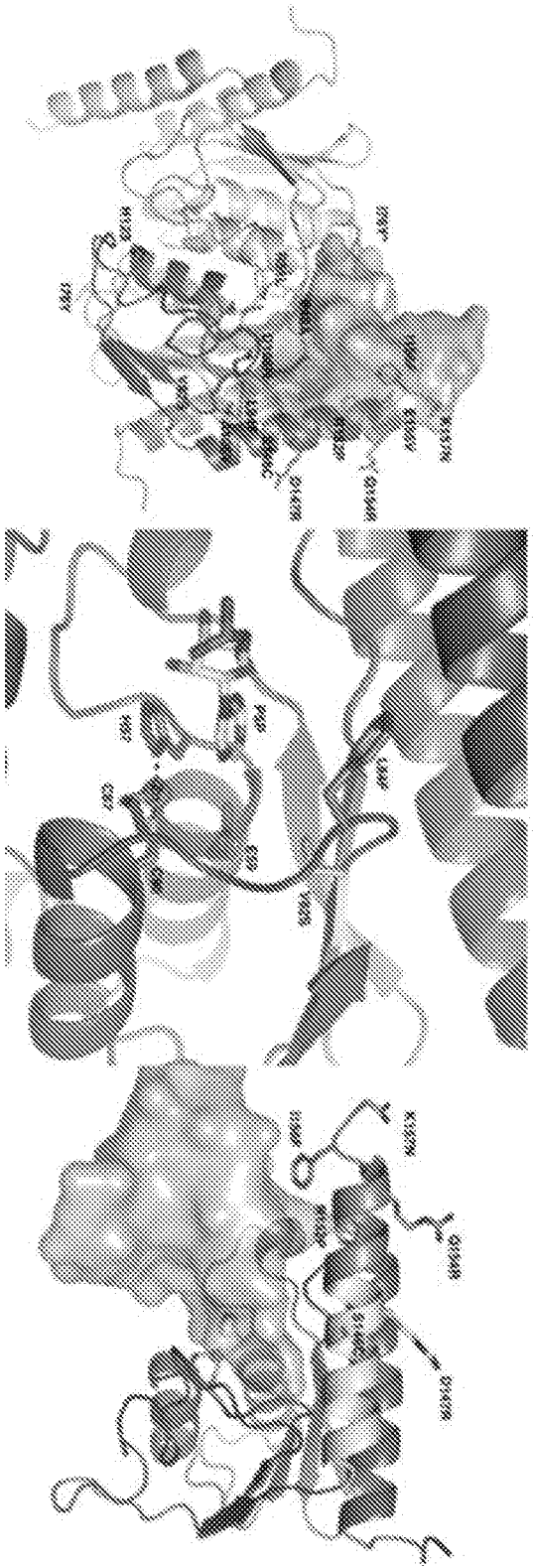


FIG. 7C

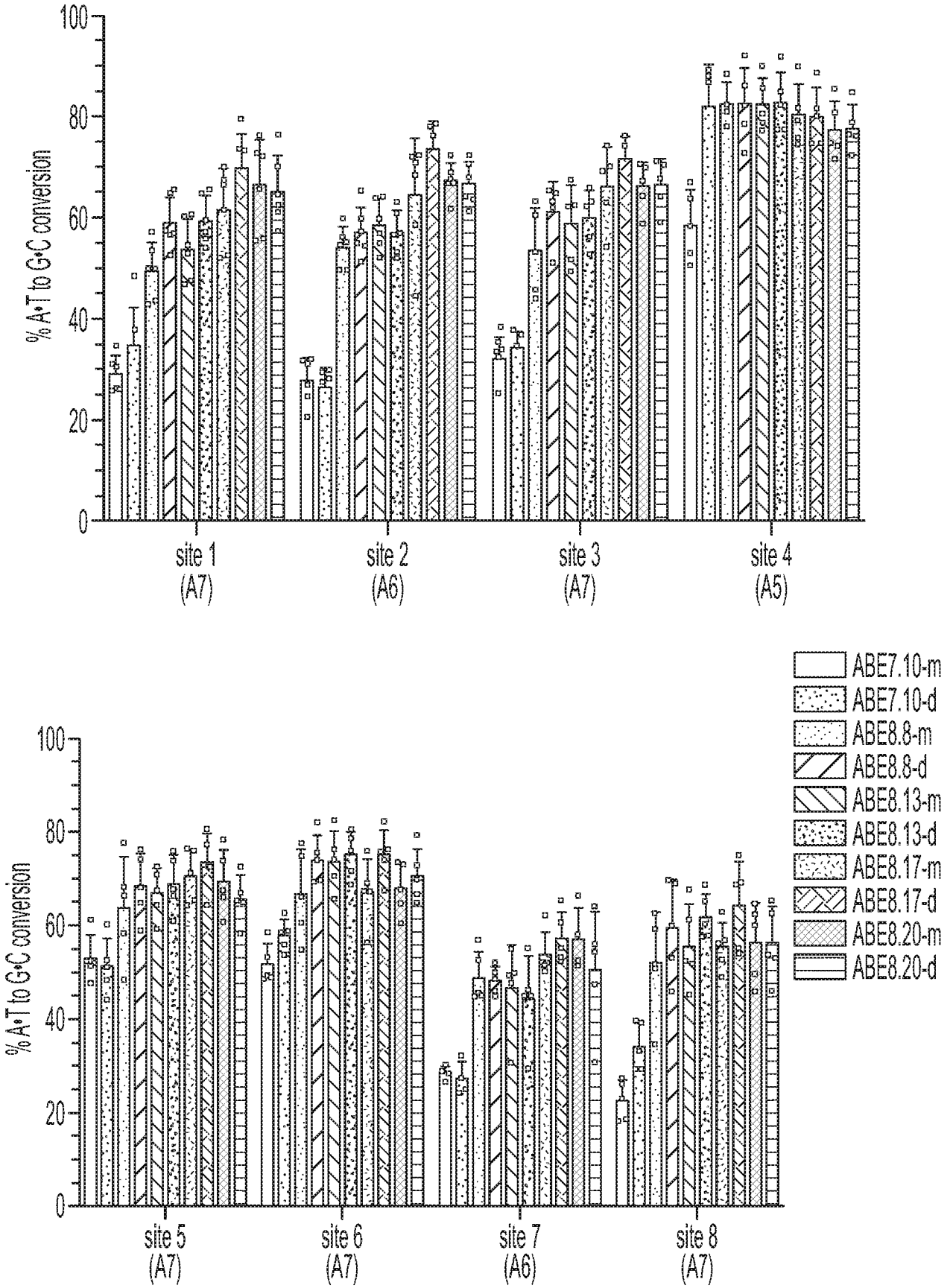

% A•T to G•C conversion
100
80
60
40
20
0
site 1
(A7)
site 2
(A6)
site 3
(A7)
site 4
(A5)
site 5
(A7)
site 6
(A7)
site 7
(A6)
site 8
(A7)
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d

FIG. 7D

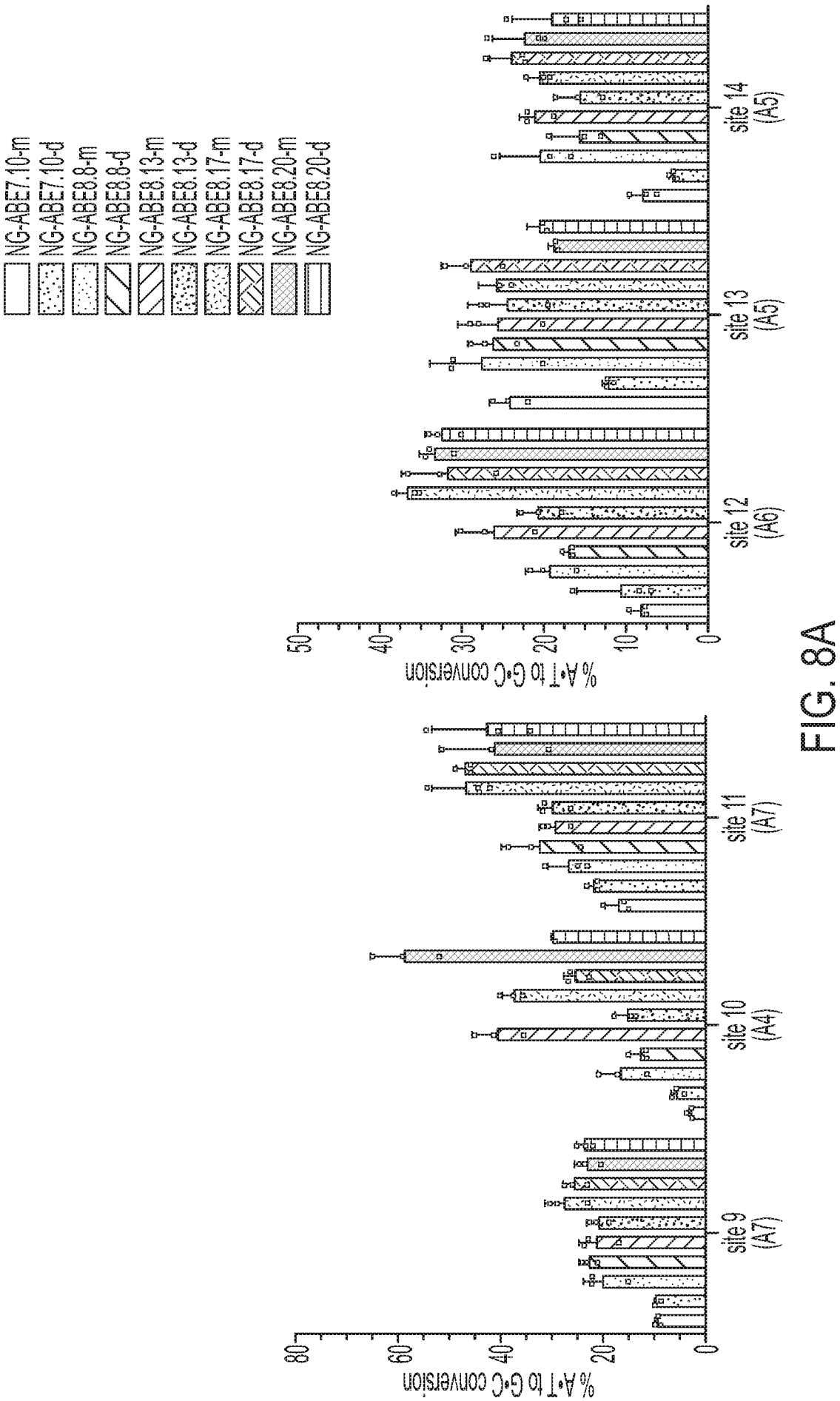

NG-ABE7.10-m
NG-ABE7.10-d
NG-ABE8.8-m
NG-ABE8.8-d
NG-ABE8.13-m
NG-ABE8.13-d
NG-ABE8.17-m
NG-ABE8.17-d
NG-ABE8.20-m
NG-ABE8.20-d
% A•T to G•C conversion
0
10
20
30
40
50
site 12 (A6)
site 13 (A5)
site 14 (A5)
% A•T to G•C conversion
0
20
40
60
80
site 9 (A7)
site 10 (A4)
site 11 (A7)

FIG. 8A

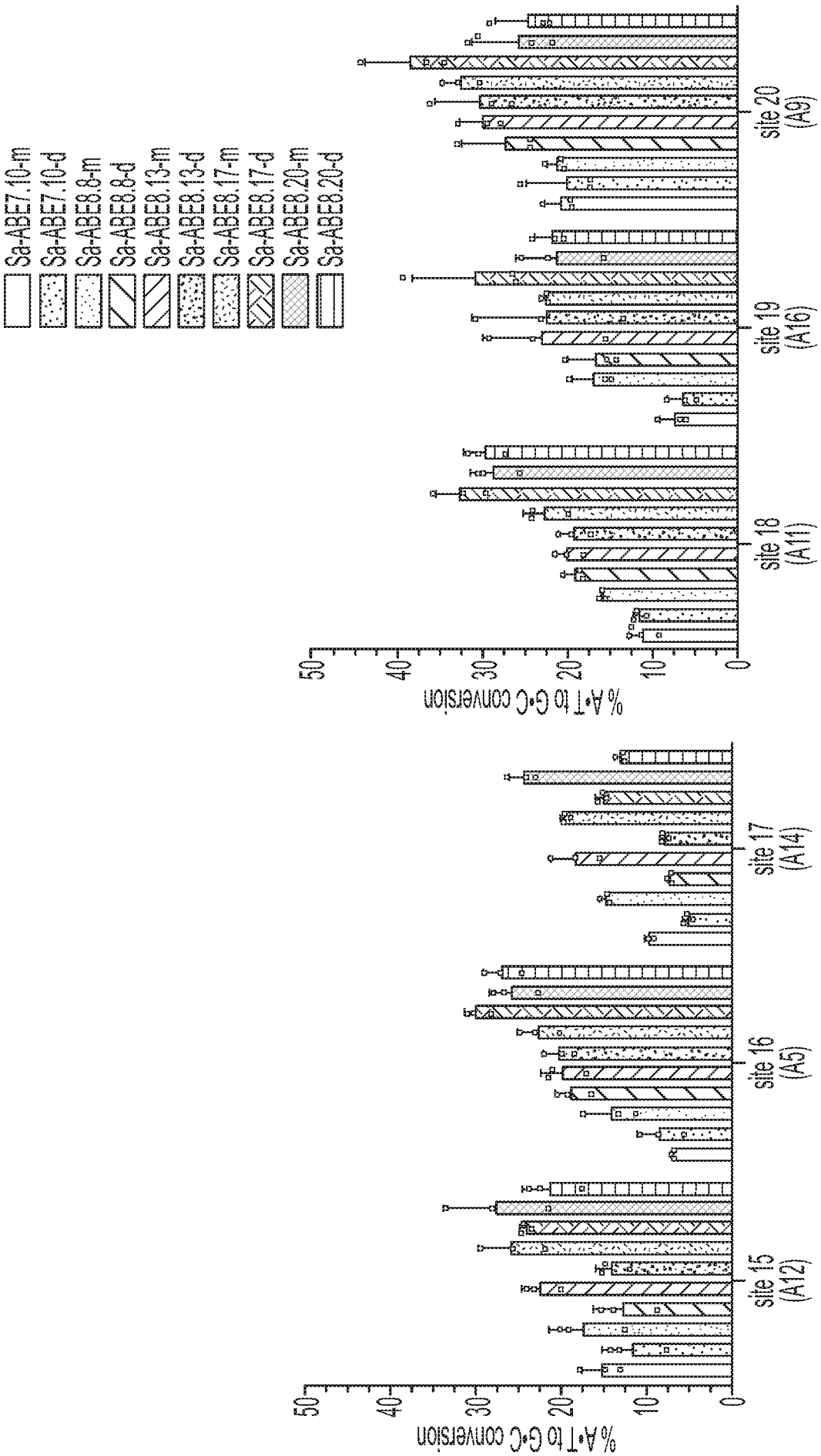

Sa-ABE7.10-m
Sa-ABE7.10-d
Sa-ABE8.8-m
Sa-ABE8.8-d
Sa-ABE8.13-m
Sa-ABE8.13-d
Sa-ABE8.17-m
Sa-ABE8.17-d
Sa-ABE8.20-m
Sa-ABE8.20-d
% A•T to G•C conversion
0
10
20
30
40
50
site 15 (A12)
site 16 (A5)
site 17 (A14)
site 18 (A11)
site 19 (A16)
site 20 (A9)

FIG. 8B

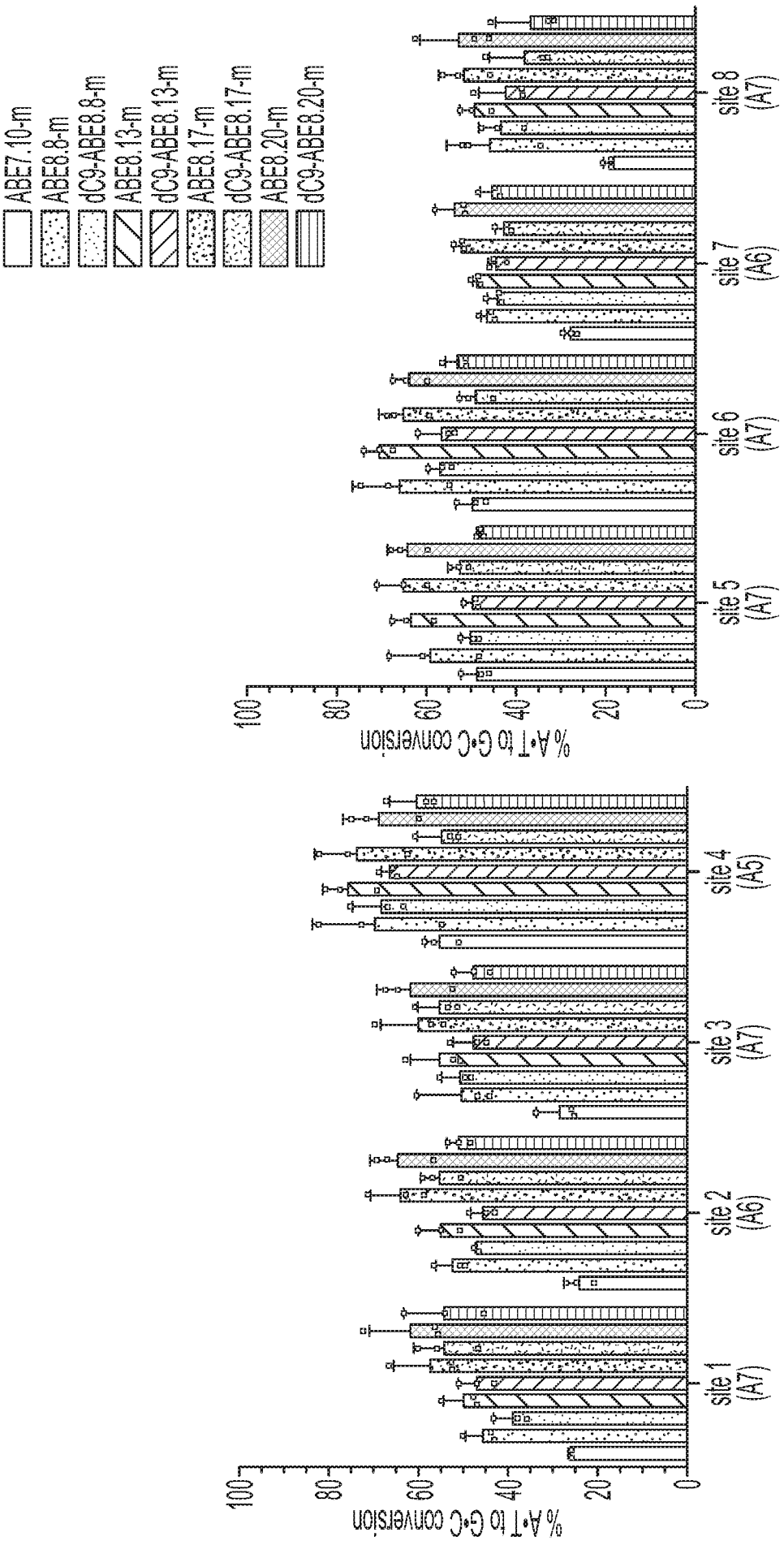

ABE7.10-m
ABE8.8-m
dC9-ABE8.8-m
ABE8.13-m
dC9-ABE8.13-m
ABE8.17-m
dC9-ABE8.17-m
ABE8.20-m
dC9-ABE8.20-m
% A•T to G•C conversion
0
20
40
60
80
100
site 1 (A7)
site 2 (A6)
site 3 (A7)
site 4 (A5)
site 5 (A7)
site 6 (A7)
site 7 (A6)
site 8 (A7)

FIG. 8C

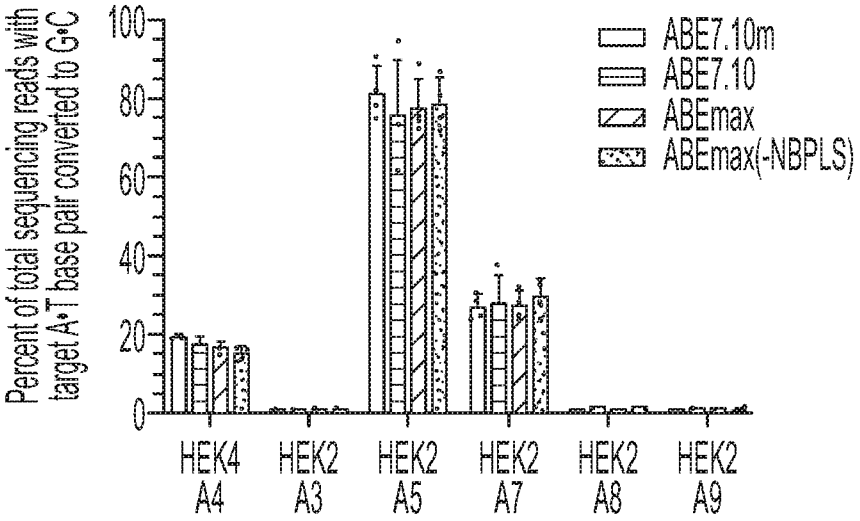


FIG. 9A

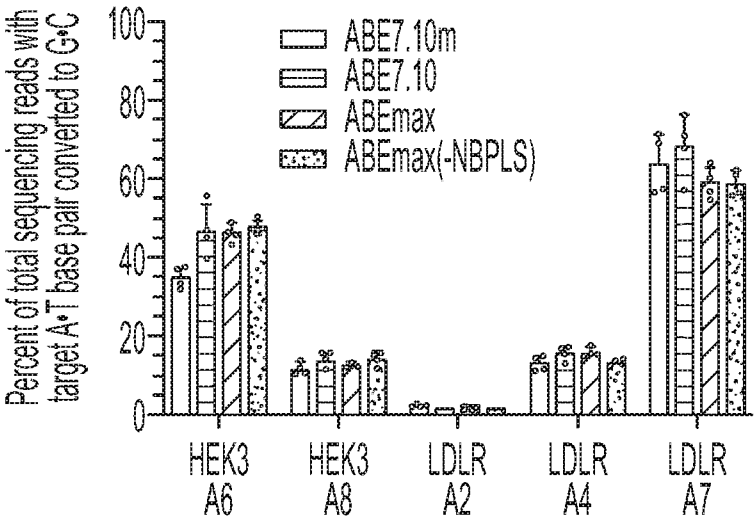


FIG. 9B

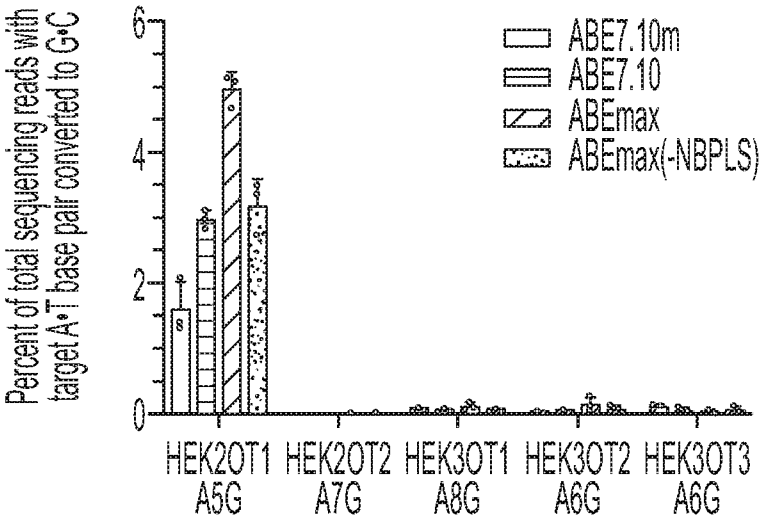


FIG. 9C

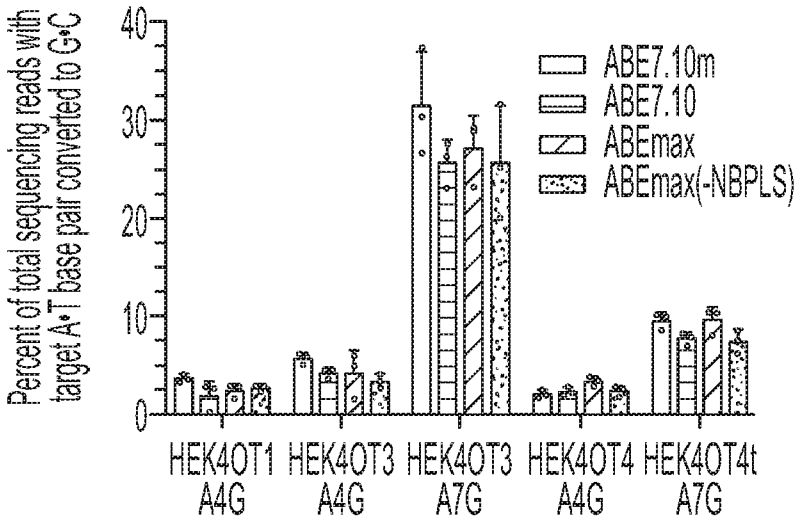

Percent of total sequencing reads with target A•T base pair converted to G•C
40
30
20
10
0
ABE7.10m
ABE7.10
ABEmax
ABEmax(-NBPLS)
HEK4OT1 A4G
HEK4OT3 A4G
HEK4OT3 A7G
HEK4OT4 A4G
HEK4OT4t A7G

FIG. 9D

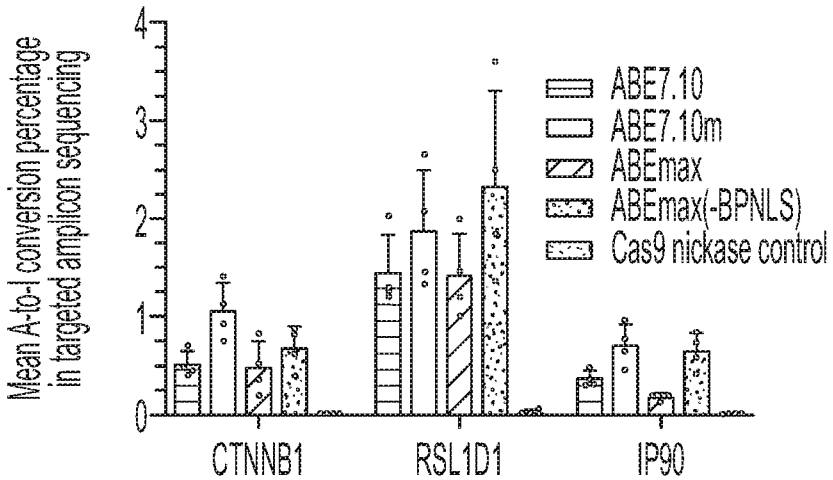

Mean A-to-I conversion percentage in targeted amplicon sequencing
4
3
2
1
0
ABE7.10
ABE7.10m
ABEmax
ABEmax(-BPNLS)
Cas9 nickase control
CTNNB1
RSL1D1
IP90

FIG. 9E

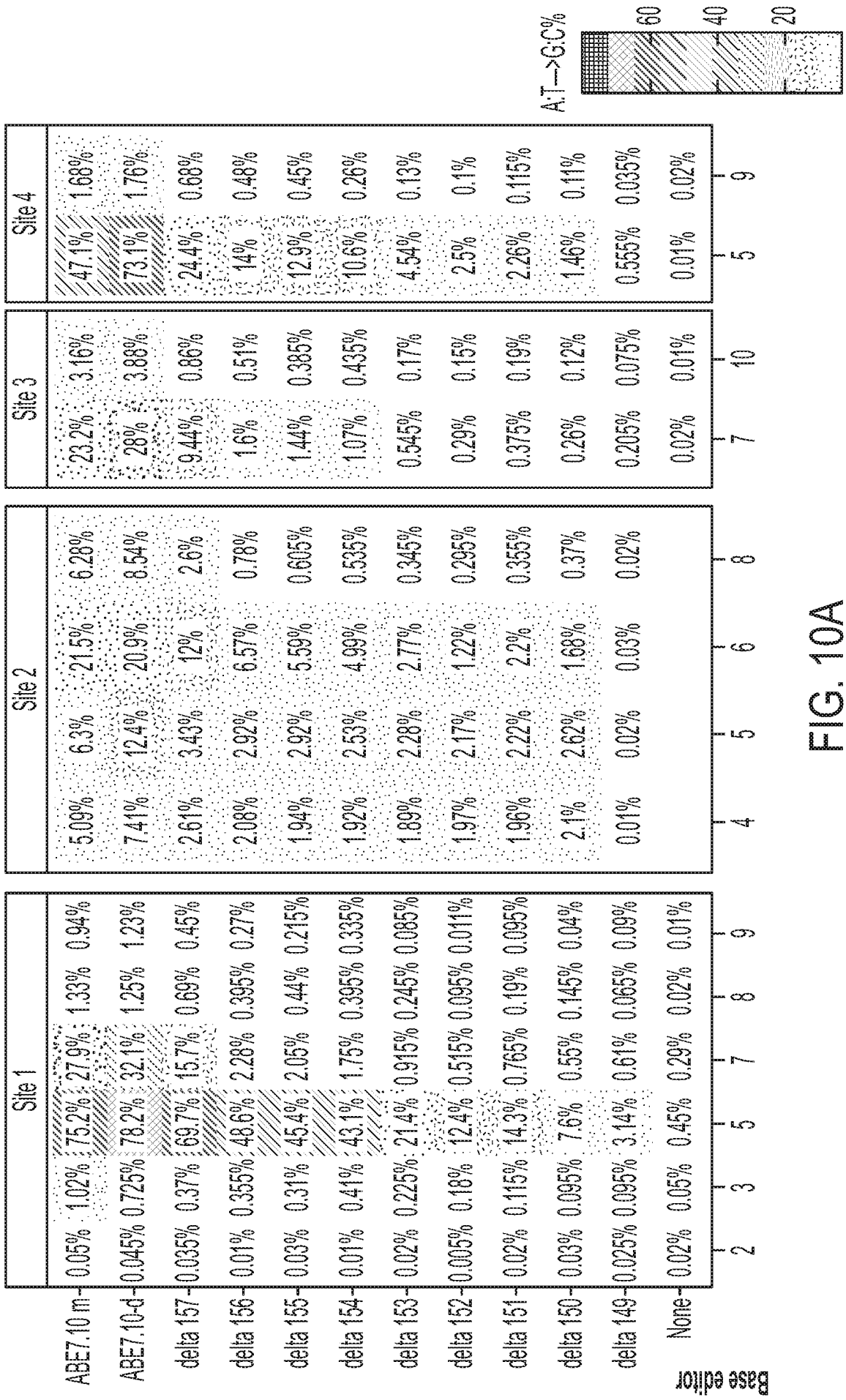

Base editor
ABE7.10 m
ABE7.10-d
delta 157
delta 156
delta 155
delta 154
delta 153
delta 152
delta 151
delta 150
delta 149
None
Site 1
Site 2
Site 3
Site 4
A:T—>G:C%
60
40
20

FIG. 10A

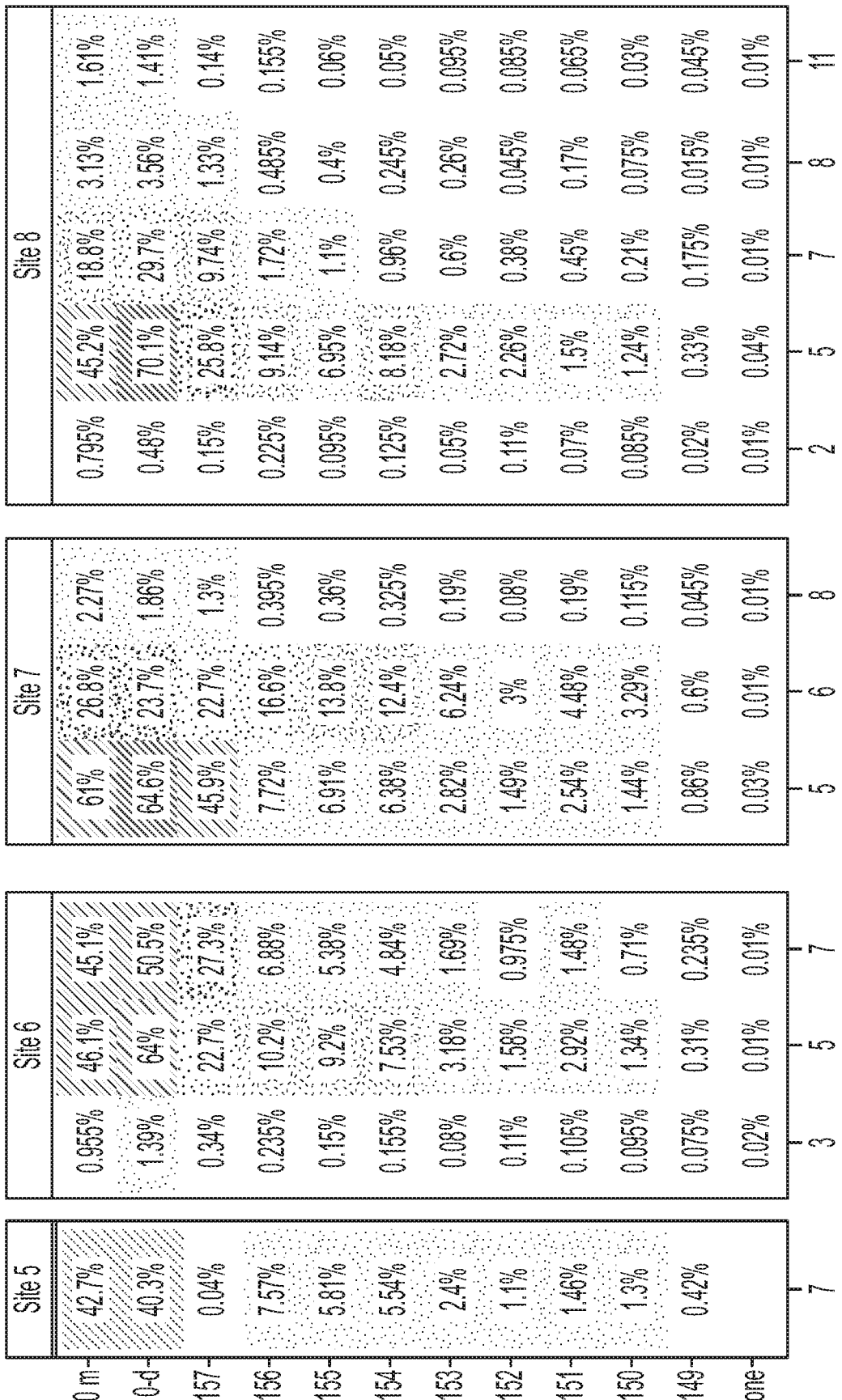


FIG. 10A CONTINUED

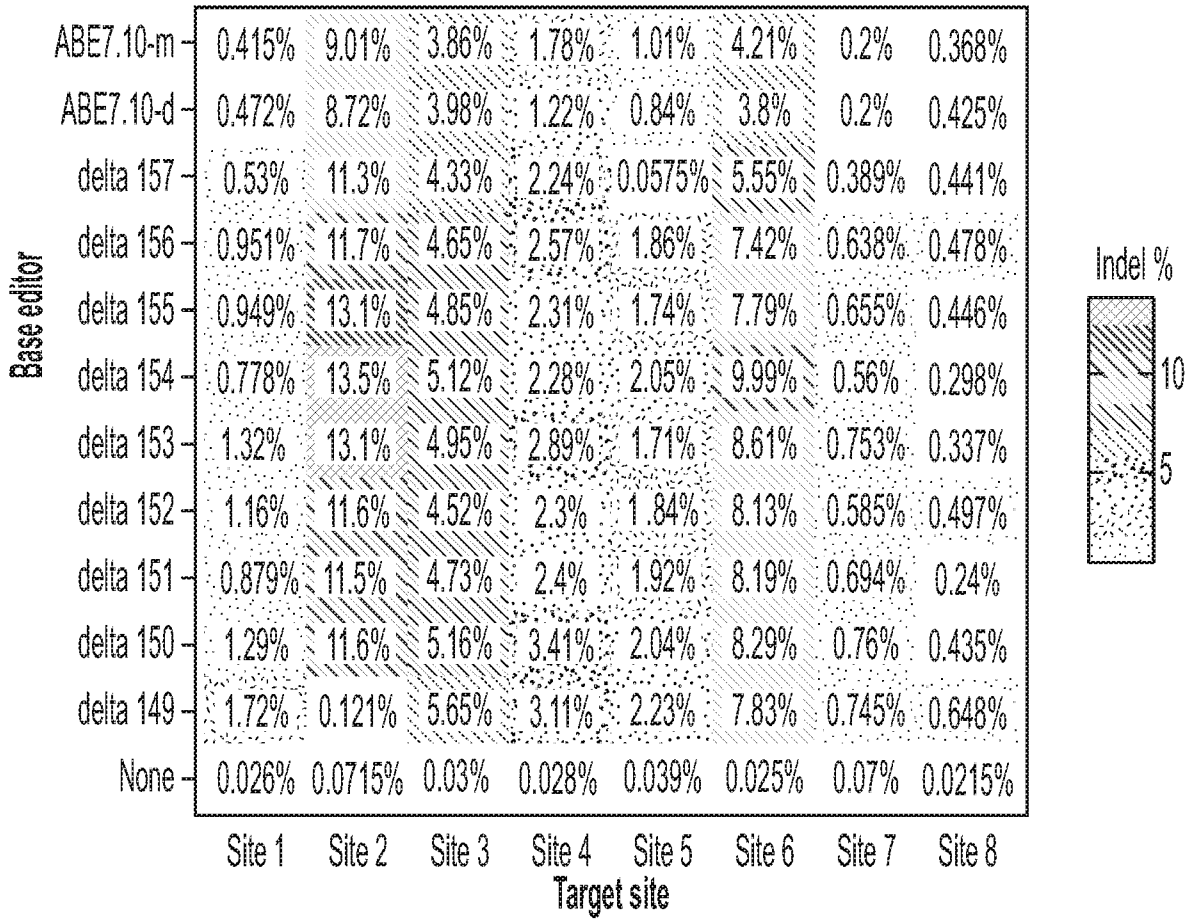

Base editor
ABE7.10-m 0.415% 9.01% 3.86% 1.78% 1.01% 4.21% 0.2% 0.368%
ABE7.10-d 0.472% 8.72% 3.98% 1.22% 0.84% 3.8% 0.2% 0.425%
delta 157 0.53% 11.3% 4.33% 2.24% 0.0575% 5.55% 0.389% 0.441%
delta 156 0.951% 11.7% 4.65% 2.57% 1.86% 7.42% 0.638% 0.478%
delta 155 0.949% 13.1% 4.85% 2.31% 1.74% 7.79% 0.655% 0.446%
delta 154 0.778% 13.5% 5.12% 2.28% 2.05% 9.99% 0.56% 0.298%
delta 153 1.32% 13.1% 4.95% 2.89% 1.71% 8.61% 0.753% 0.337%
delta 152 1.16% 11.6% 4.52% 2.3% 1.84% 8.13% 0.585% 0.497%
delta 151 0.879% 11.5% 4.73% 2.4% 1.92% 8.19% 0.694% 0.24%
delta 150 1.29% 11.6% 5.16% 3.41% 2.04% 8.29% 0.76% 0.435%
delta 149 1.72% 0.121% 5.65% 3.11% 2.23% 7.83% 0.745% 0.648%
None 0.026% 0.0715% 0.03% 0.028% 0.039% 0.025% 0.07% 0.0215%
Site 1 Site 2 Site 3 Site 4 Site 5 Site 6 Site 7 Site 8
Target site
Indel %
10
5

FIG. 10B

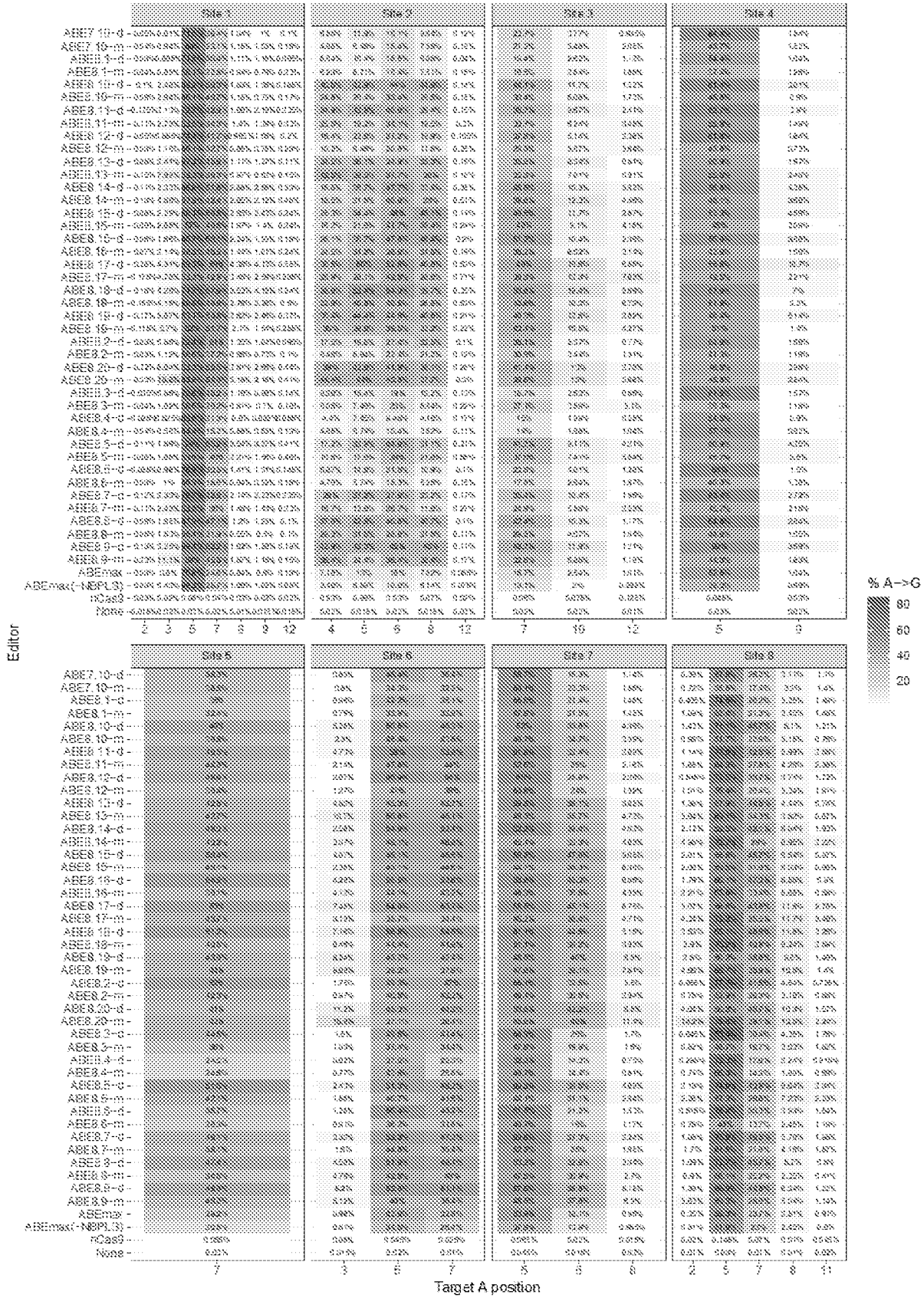

Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 7
Site 8
Editor
ABE7.10-d
ABE7.10-m
ABE8.1-d
ABE8.1-m
ABE8.10-d
ABE8.10-m
ABE8.11-d
ABE8.11-m
ABE8.12-d
ABE8.12-m
ABE8.13-d
ABE8.13-m
ABE8.14-d
ABE8.14-m
ABE8.15-d
ABE8.15-m
ABE8.16-d
ABE8.16-m
ABE8.17-d
ABE8.17-m
ABE8.18-d
ABE8.18-m
ABE8.19-d
ABE8.19-m
ABE8.2-d
ABE8.2-m
ABE8.20-d
ABE8.20-m
ABE8.3-d
ABE8.3-m
ABE8.4-d
ABE8.4-m
ABE8.5-d
ABE8.5-m
ABE8.6-d
ABE8.6-m
ABE8.7-d
ABE8.7-m
ABE8.8-d
ABE8.8-m
ABE8.9-d
ABE8.9-m
ABEmax
ABEmax(-NBPLS)
nCas9
None
Target A position
% A->G
80
60
40
20

FIG. 11

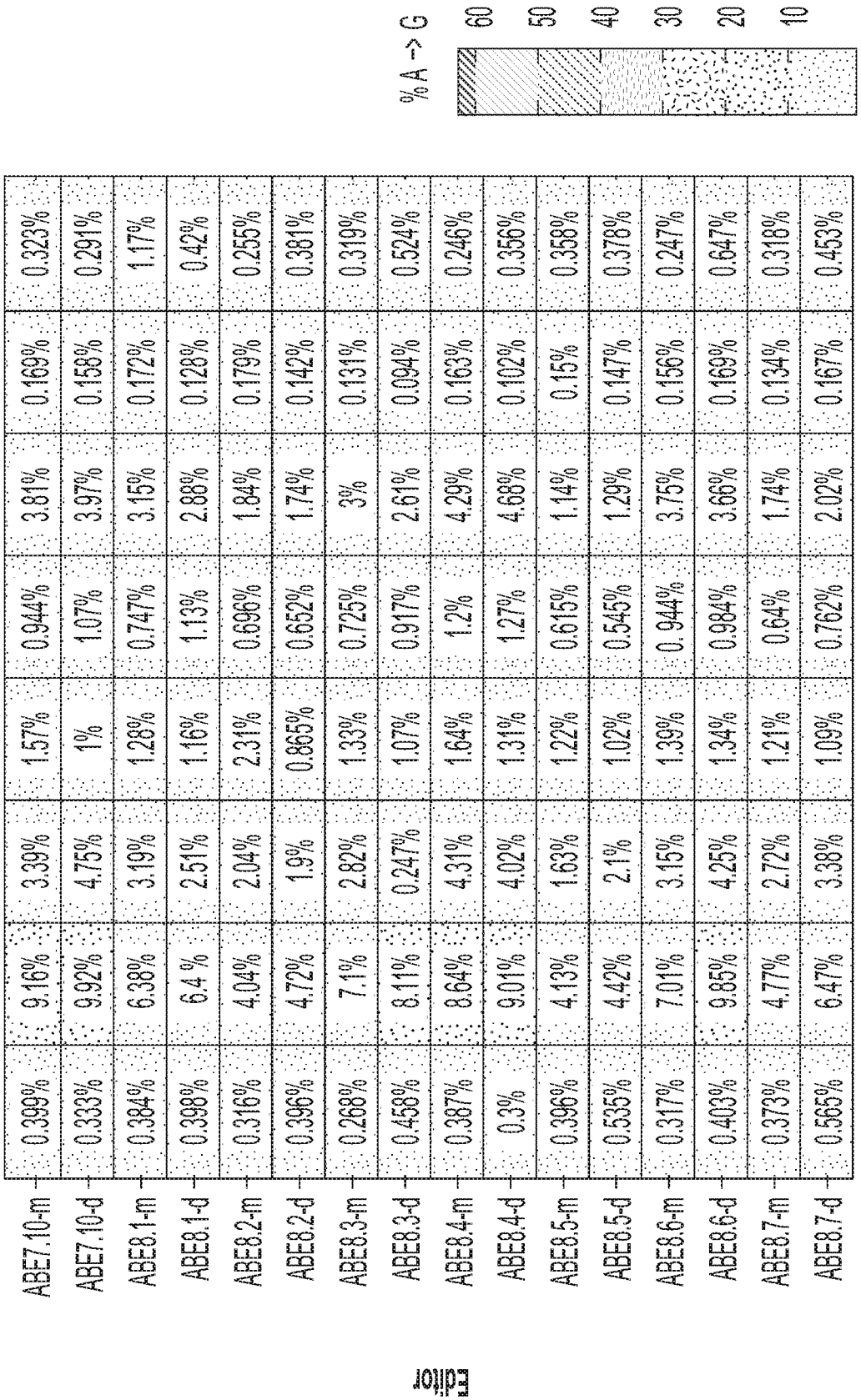

% A -> G
60
50
40
30
20
10
editor
ABE7.10-m
ABE7.10-d
ABE8.1-m
ABE8.1-d
ABE8.2-m
ABE8.2-d
ABE8.3-m
ABE8.3-d
ABE8.4-m
ABE8.4-d
ABE8.5-m
ABE8.5-d
ABE8.6-m
ABE8.6-d
ABE8.7-m
ABE8.7-d
0.399% 9.16% 3.39% 1.57% 0.944% 3.81% 0.169% 0.323%
0.333% 9.92% 4.75% 1% 1.07% 3.97% 0.158% 0.291%
0.384% 6.38% 3.19% 1.28% 0.747% 3.15% 0.172% 1.17%
0.398% 6.4 % 2.51% 1.16% 1.13% 2.88% 0.128% 0.42%
0.316% 4.04% 2.04% 2.31% 0.696% 1.84% 0.179% 0.255%
0.396% 4.72% 1.9% 0.865% 0.652% 1.74% 0.142% 0.381%
0.268% 7.1% 2.82% 1.33% 0.725% 3% 0.131% 0.319%
0.458% 8.11% 0.247% 1.07% 0.917% 2.61% 0.094% 0.524%
0.387% 8.64% 4.31% 1.64% 1.2% 4.29% 0.163% 0.246%
0.3% 9.01% 4.02% 1.31% 1.27% 4.68% 0.102% 0.356%
0.396% 4.13% 1.63% 1.22% 0.615% 1.14% 0.15% 0.358%
0.535% 4.42% 2.1% 1.02% 0.545% 1.29% 0.147% 0.378%
0.317% 7.01% 3.15% 1.39% 0.944% 3.75% 0.156% 0.247%
0.403% 9.85% 4.25% 1.34% 0.984% 3.66% 0.169% 0.647%
0.373% 4.77% 2.72% 1.21% 0.64% 1.74% 0.134% 0.318%
0.565% 6.47% 3.38% 1.09% 0.762% 2.02% 0.167% 0.453%

FIG. 12

ABE8.8-m	0.375%	2.76%	2.05%	0.744%	0.592%	0.549%	0.134%	0.232%
ABE8.8-d	0.554%	3.48%	2.17%	0.751%	0.59%	0.898%	0.128%	0.395%
ABE8.9-m	0.447%	1.97%	1.64%	0.802%	0.588%	0.501%	0.192%	0.31%
ABE8.9-d	0.462%	3.07%	147%	0.744%	0.478%	0.608%	0.133%	0.321%
ABE8.10-m	0.39%	2.74%	1.41%	1.01%	0.568%	0.476%	0.144%	0.284%
ABE8.10-d	0.311%	2.9%	1.59%	0.702%	0.494%	0.598%	0.18%	0.413%
ABE8.11-m	0.468%	4.95%	2.47%	1.1%	0.79%	1.19%	0.124%	0.344%
ABE8.11-d	0.584%	4.87%	2.5%	1.07%	1.31%	1.03%	0.142%	0.32%
ABE8.12-m	0.404%	6.58%	2.71%	1.33%	0.76%	2.04%	0.164%	0.278%
ABE8.12-d	0.517%	9.34%	3.23%	0.763%	0.767%	2.63%	0.142%	0.363%
ABE8.13-m	0.479%	1.9%	1.84%	0.833%	0.746%	0.423%	0.146%	0.288%
ABE8.13-d	0.39%	3.19%	1.8%	0.715%	0.556%	0.886%	0.14%	0.266%
ABE8.14-m	0.376%	2.66%	1.48%	0.86%	0.541%	0.57%	0.139%	0.327%
ABE8.14-d	0.476%	4.52%	1.63%	0.76%	0.519%	1.02%	0.143%	0.344%
ABE8.15-m	0.24%	1.81%	1.04%	0.818%	0.522%	0.499%	0.155%	0.276%
ABE8.15-d	0.412%	2%	1.06%	0.666%	0.494%	0.58%	0.164%	0.29%

FIG. 12 CONTINUED

FIG. 12 CONTINUED

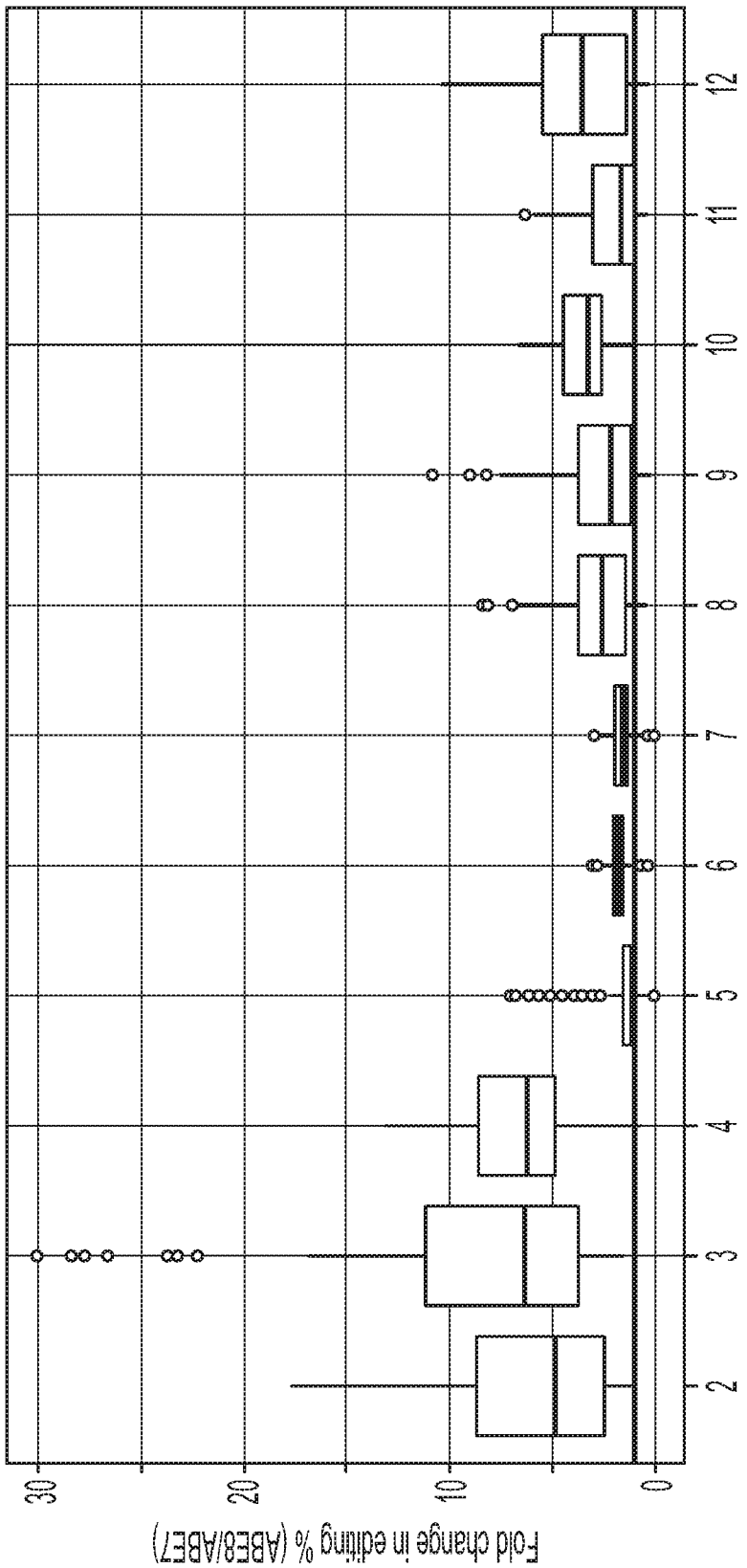

Fold change in editing % (ABE8/ABE7)
30
20
10
0
2
3
4
5
6
7
8
9
10
11
12
Target site position

FIG. 13

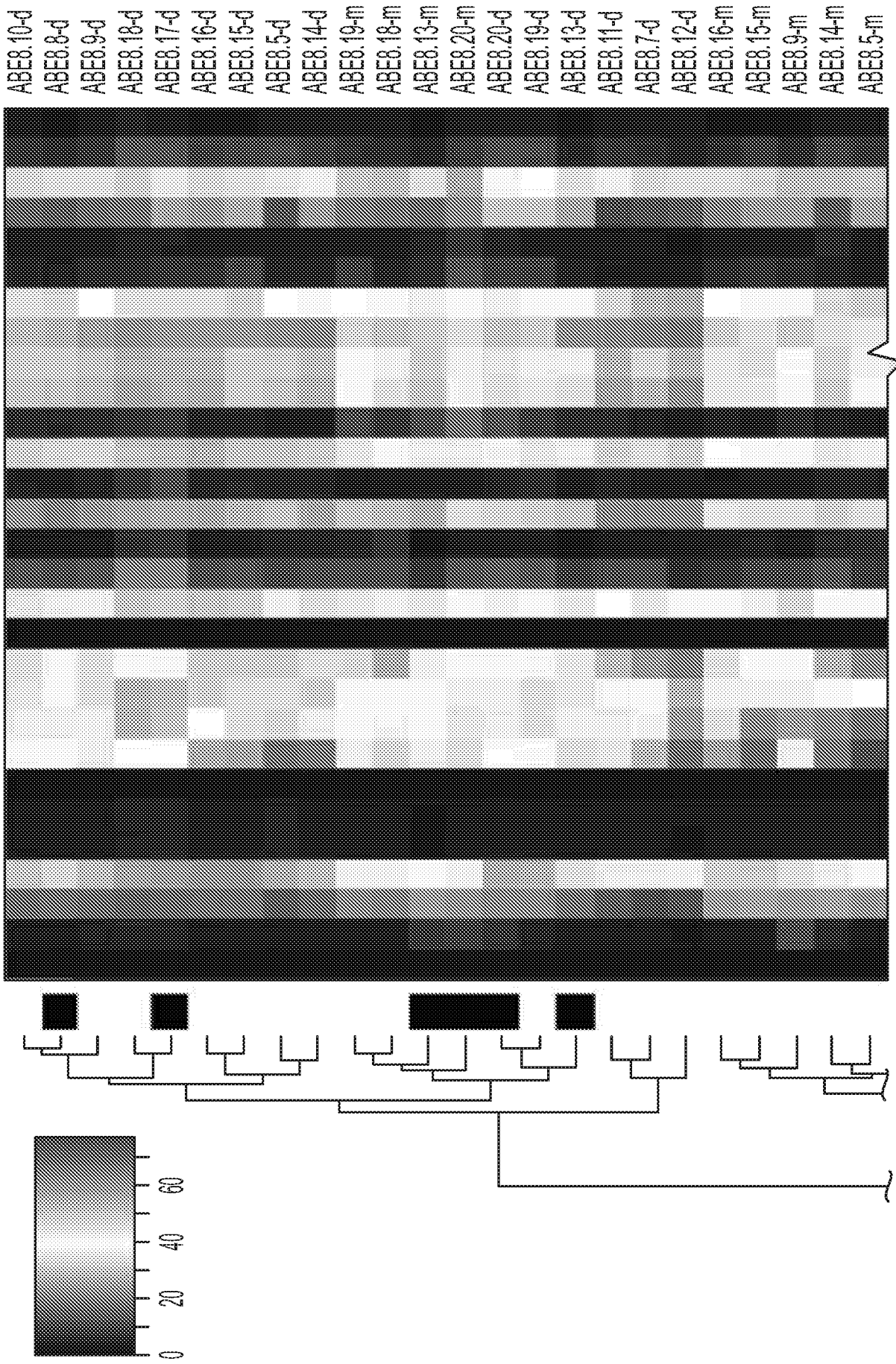

ABE8.10-d
ABE8.8-d
ABE8.9-d
ABE8.18-d
ABE8.17-d
ABE8.16-d
ABE8.15-d
ABE8.5-d
ABE8.14-d
ABE8.19-m
ABE8.18-m
ABE8.13-m
ABE8.20-m
ABE8.20-d
ABE8.19-d
ABE8.13-d
ABE8.11-d
ABE8.7-d
ABE8.12-d
ABE8.16-m
ABE8.15-m
ABE8.9-m
ABE8.14-m
ABE8.5-m
0
20
40
60

FIG. 14

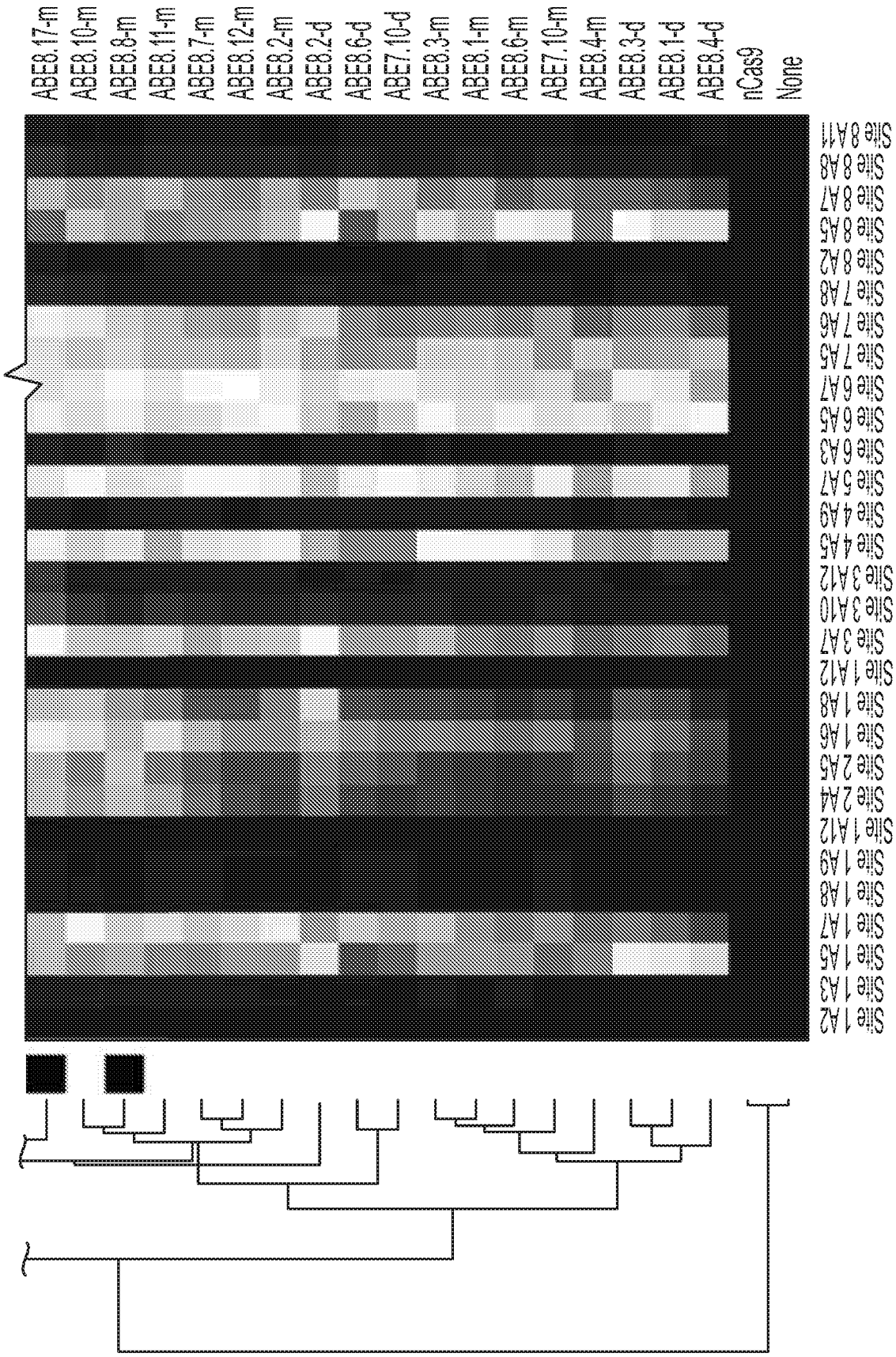


FIG. 14 CONTINUED

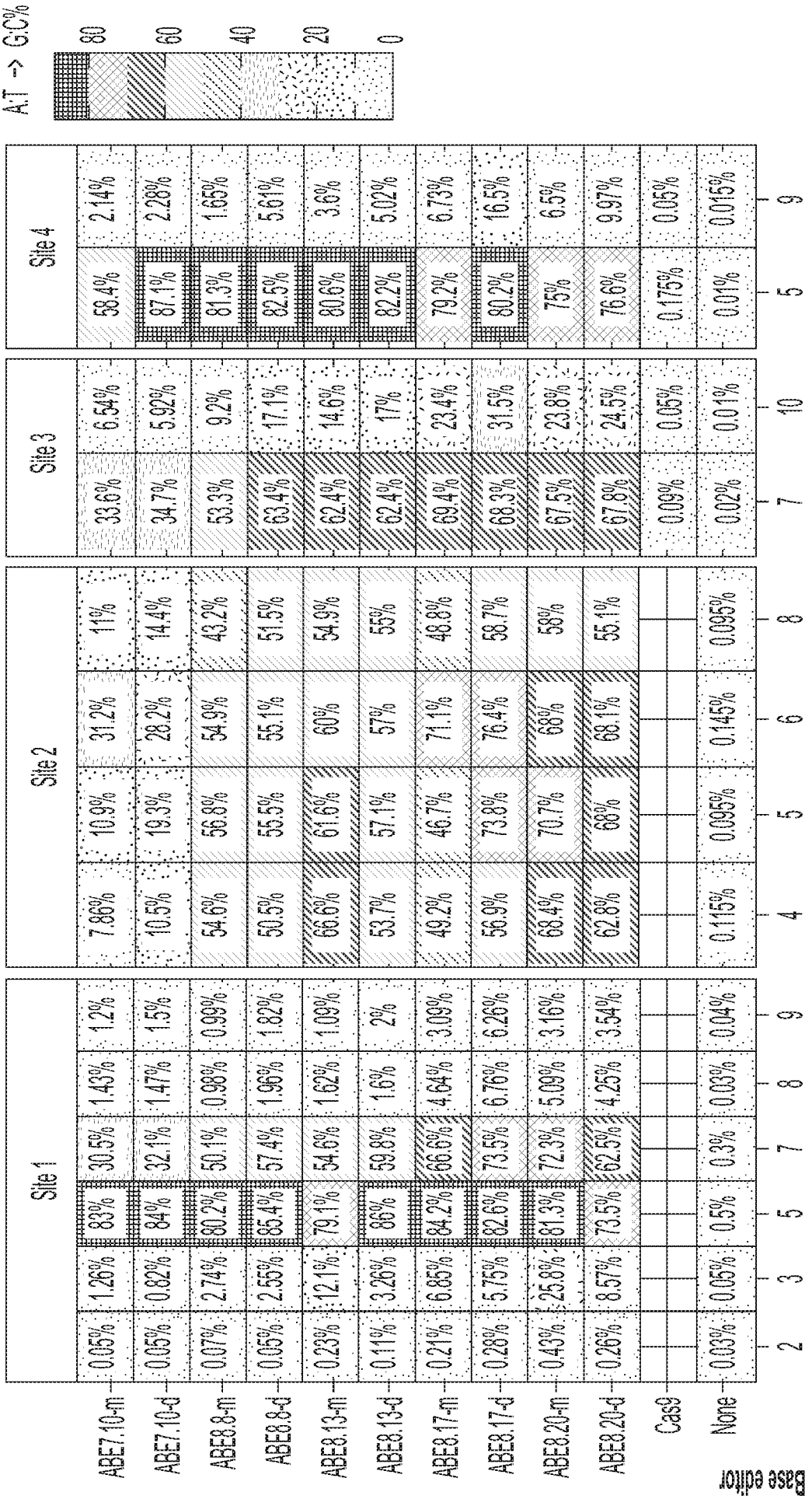

A:T -> G:C%
80
60
40
20
0
Site 1
Site 2
Site 3
Site 4
2
3
5
7
8
9
4
5
6
8
7
10
9
Base editor
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
Cas9
None

FIG. 15

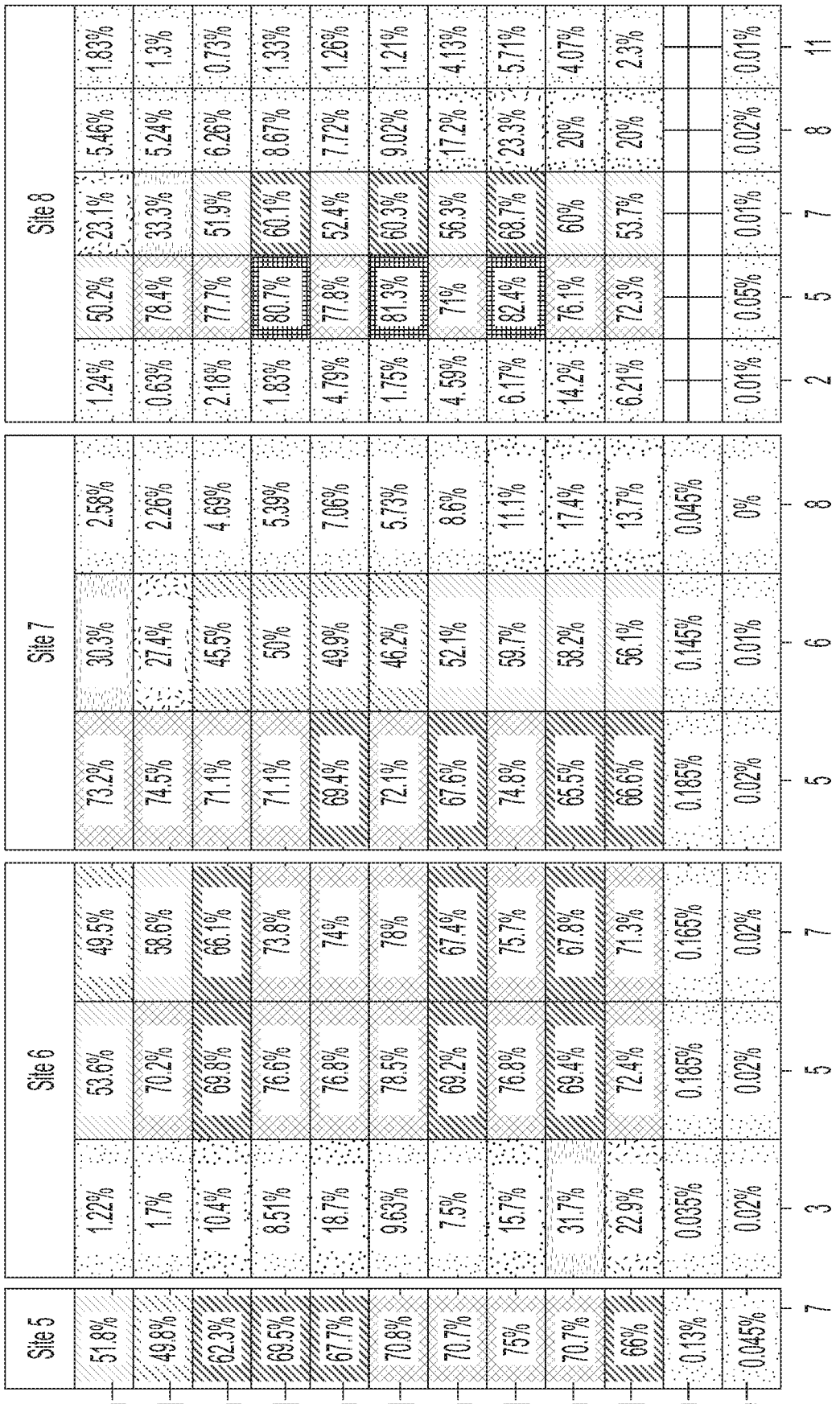


FIG. 15 CONTINUED

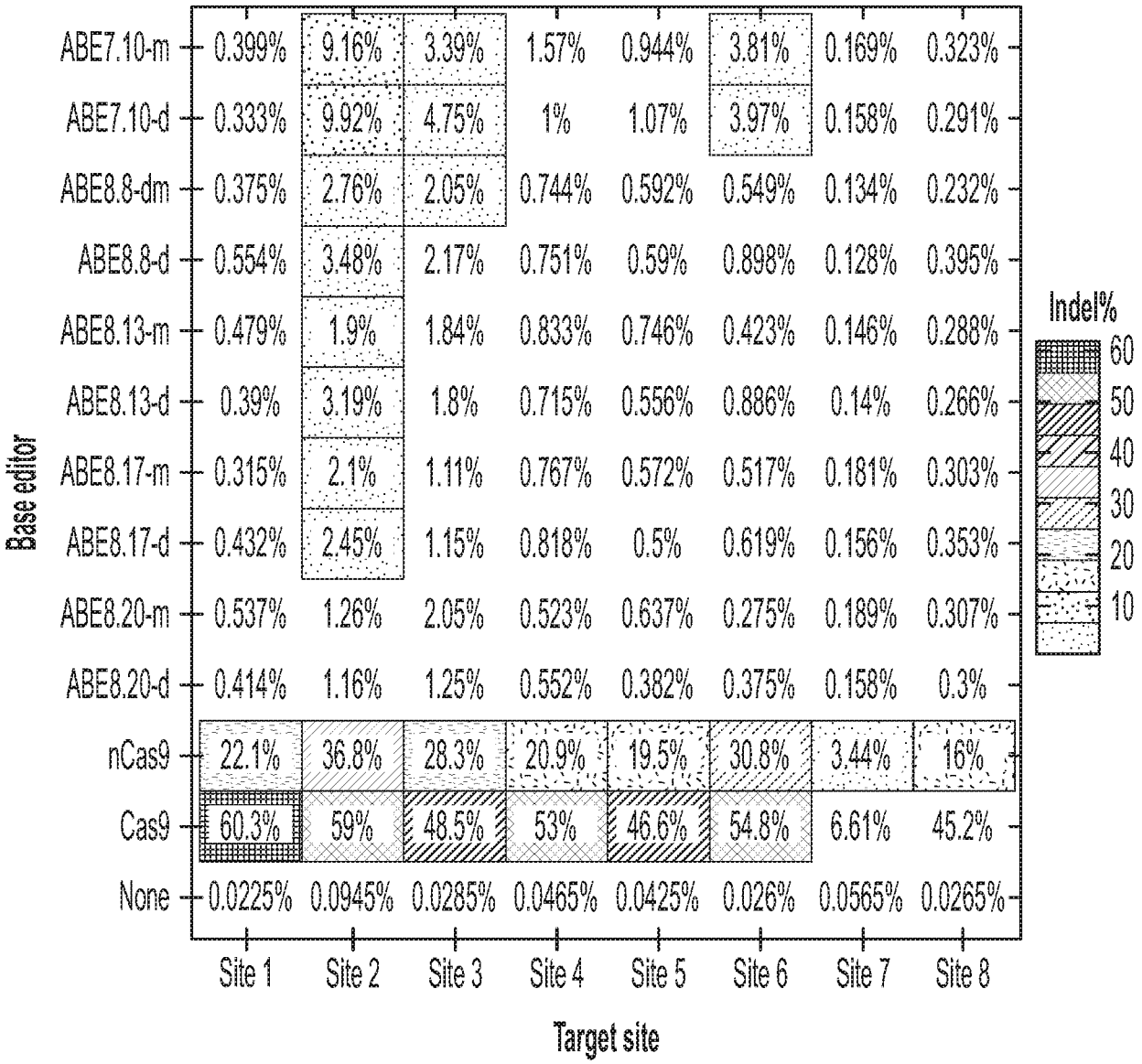

Base editor
ABE7.10-m 0.399% 9.16% 3.39% 1.57% 0.944% 3.81% 0.169% 0.323%
ABE7.10-d 0.333% 9.92% 4.75% 1% 1.07% 3.97% 0.158% 0.291%
ABE8.8-dm 0.375% 2.76% 2.05% 0.744% 0.592% 0.549% 0.134% 0.232%
ABE8.8-d 0.554% 3.48% 2.17% 0.751% 0.59% 0.898% 0.128% 0.395%
ABE8.13-m 0.479% 1.9% 1.84% 0.833% 0.746% 0.423% 0.146% 0.288%
ABE8.13-d 0.39% 3.19% 1.8% 0.715% 0.556% 0.886% 0.14% 0.266%
ABE8.17-m 0.315% 2.1% 1.11% 0.767% 0.572% 0.517% 0.181% 0.303%
ABE8.17-d 0.432% 2.45% 1.15% 0.818% 0.5% 0.619% 0.156% 0.353%
ABE8.20-m 0.537% 1.26% 2.05% 0.523% 0.637% 0.275% 0.189% 0.307%
ABE8.20-d 0.414% 1.16% 1.25% 0.552% 0.382% 0.375% 0.158% 0.3%
nCas9 22.1% 36.8% 28.3% 20.9% 19.5% 30.8% 3.44% 16%
Cas9 60.3% 59% 48.5% 53% 46.6% 54.8% 6.61% 45.2%
None 0.0225% 0.0945% 0.0285% 0.0465% 0.0425% 0.026% 0.0565% 0.0265%
Site 1 Site 2 Site 3 Site 4 Site 5 Site 6 Site 7 Site 8
Target site
Indel%
60
50
40
30
20
10

FIG. 16

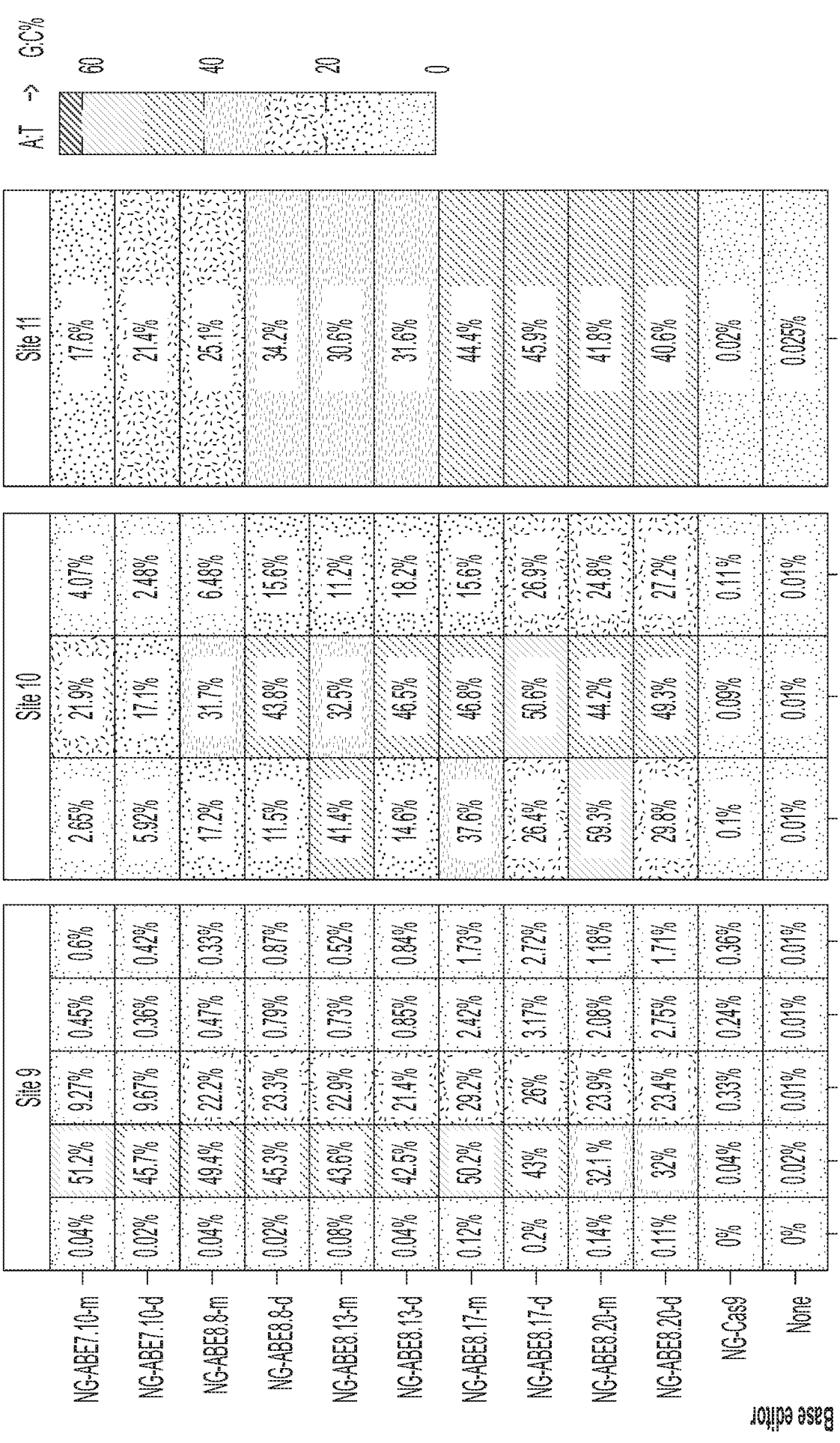

A:T -> G:C%
60
40
20
0
Site 9
Site 10
Site 11
2
5
7
8
9
4
7
9
7
Base editor
NG-ABE7.10-m
NG-ABE7.10-d
NG-ABE8.8-m
NG-ABE8.8-d
NG-ABE8.13-m
NG-ABE8.13-d
NG-ABE8.17-m
NG-ABE8.17-d
NG-ABE8.20-m
NG-ABE8.20-d
NG-Cas9
None
0.04% 51.2% 9.27% 0.45% 0.6% 2.65% 21.9% 4.07% 17.6%
0.02% 45.7% 9.67% 0.36% 0.42% 5.92% 17.1% 2.48% 21.4%
0.04% 49.4% 22.2% 0.47% 0.33% 17.2% 31.7% 6.48% 25.1%
0.02% 45.3% 23.3% 0.79% 0.87% 11.5% 43.8% 15.6% 34.2%
0.08% 43.6% 22.9% 0.73% 0.52% 41.4% 32.5% 11.2% 30.6%
0.04% 42.5% 21.4% 0.85% 0.84% 14.6% 46.5% 18.2% 31.6%
0.12% 50.2% 29.2% 2.42% 1.73% 37.6% 46.8% 15.6% 44.4%
0.2% 43% 26% 3.17% 2.72% 26.4% 50.6% 26.9% 45.9%
0.14% 32.1% 23.9% 2.08% 1.18% 59.3% 44.2% 24.8% 41.8%
0.11% 32% 23.4% 2.75% 1.71% 29.8% 49.3% 27.2% 40.6%
0% 0.04% 0.33% 0.24% 0.36% 0.1% 0.09% 0.11% 0.02%
0% 0.02% 0.01% 0.01% 0.01% 0.01% 0.01% 0.01% 0.025%

FIG. 17

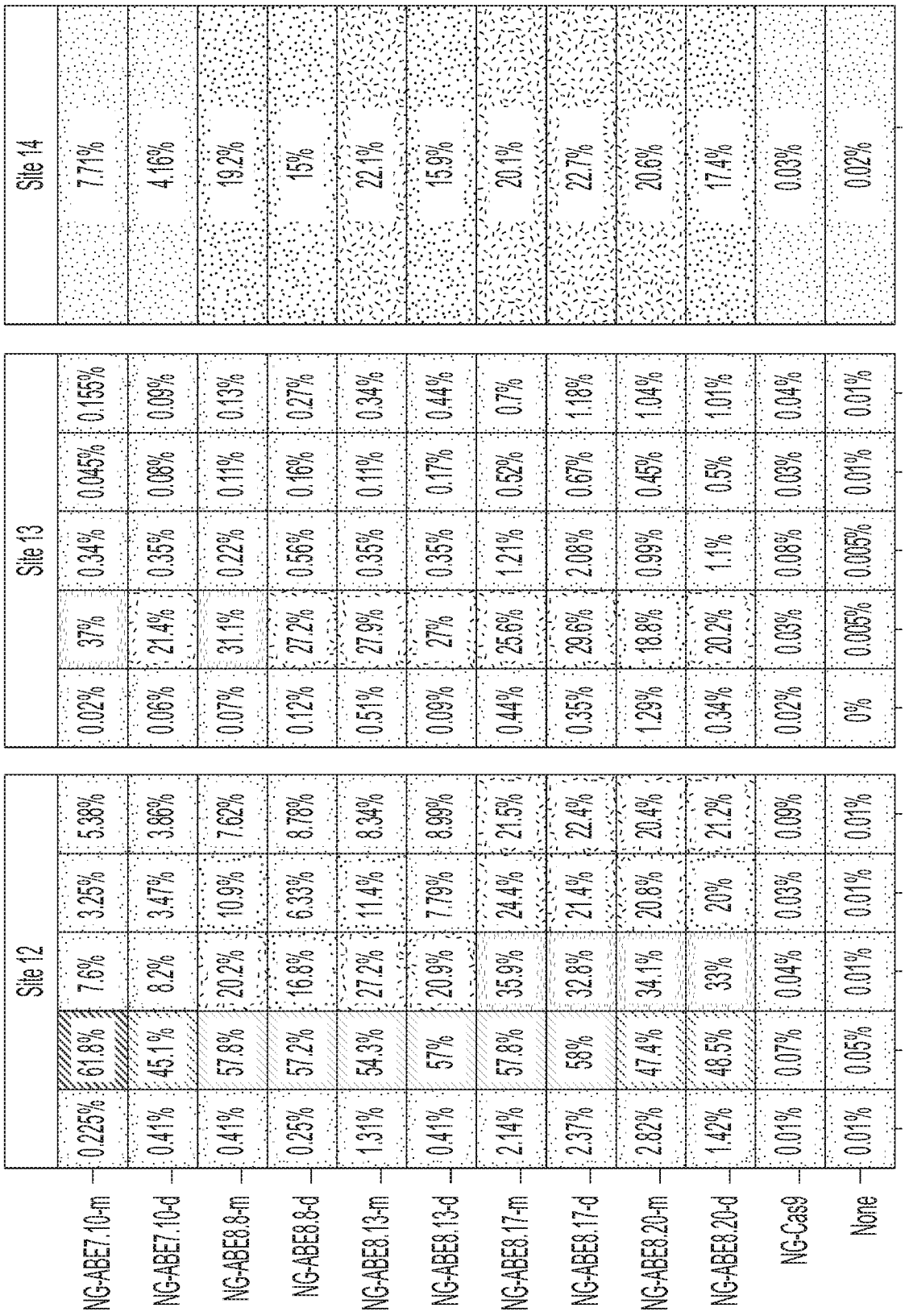


FIG. 17 CONTINUED

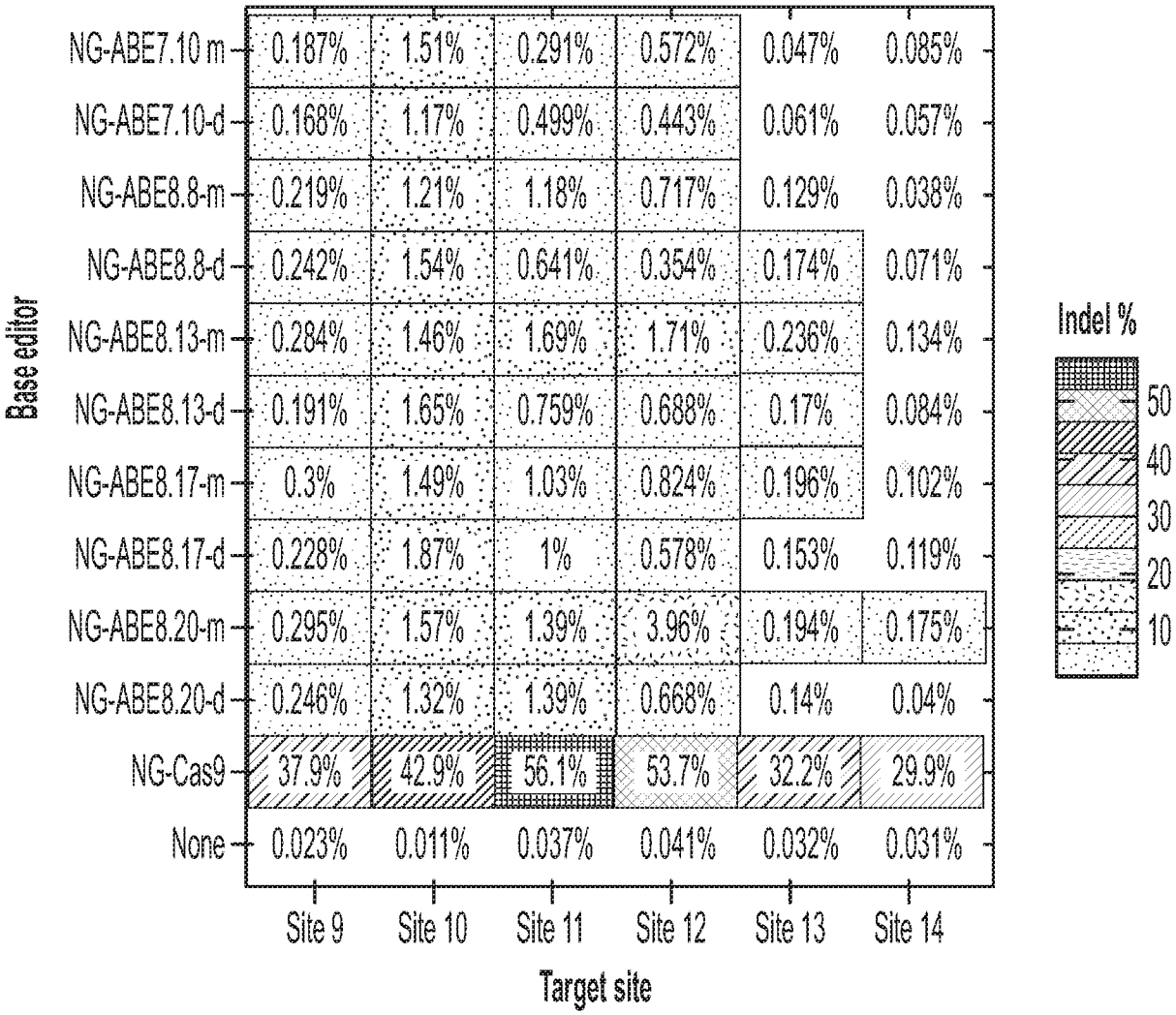

Base editor
NG-ABE7.10 m
NG-ABE7.10-d
NG-ABE8.8-m
NG-ABE8.8-d
NG-ABE8.13-m
NG-ABE8.13-d
NG-ABE8.17-m
NG-ABE8.17-d
NG-ABE8.20-m
NG-ABE8.20-d
NG-Cas9
None
0.187% 1.51% 0.291% 0.572% 0.047% 0.085%
0.168% 1.17% 0.499% 0.443% 0.061% 0.057%
0.219% 1.21% 1.18% 0.717% 0.129% 0.038%
0.242% 1.54% 0.641% 0.354% 0.174% 0.071%
0.284% 1.46% 1.69% 1.71% 0.236% 0.134%
0.191% 1.65% 0.759% 0.688% 0.17% 0.084%
0.3% 1.49% 1.03% 0.824% 0.196% 0.102%
0.228% 1.87% 1% 0.578% 0.153% 0.119%
0.295% 1.57% 1.39% 3.96% 0.194% 0.175%
0.246% 1.32% 1.39% 0.668% 0.14% 0.04%
37.9% 42.9% 56.1% 53.7% 32.2% 29.9%
0.023% 0.011% 0.037% 0.041% 0.032% 0.031%
Site 9
Site 10
Site 11
Site 12
Site 13
Site 14
Target site
Indel %
50
40
30
20
10

FIG. 18

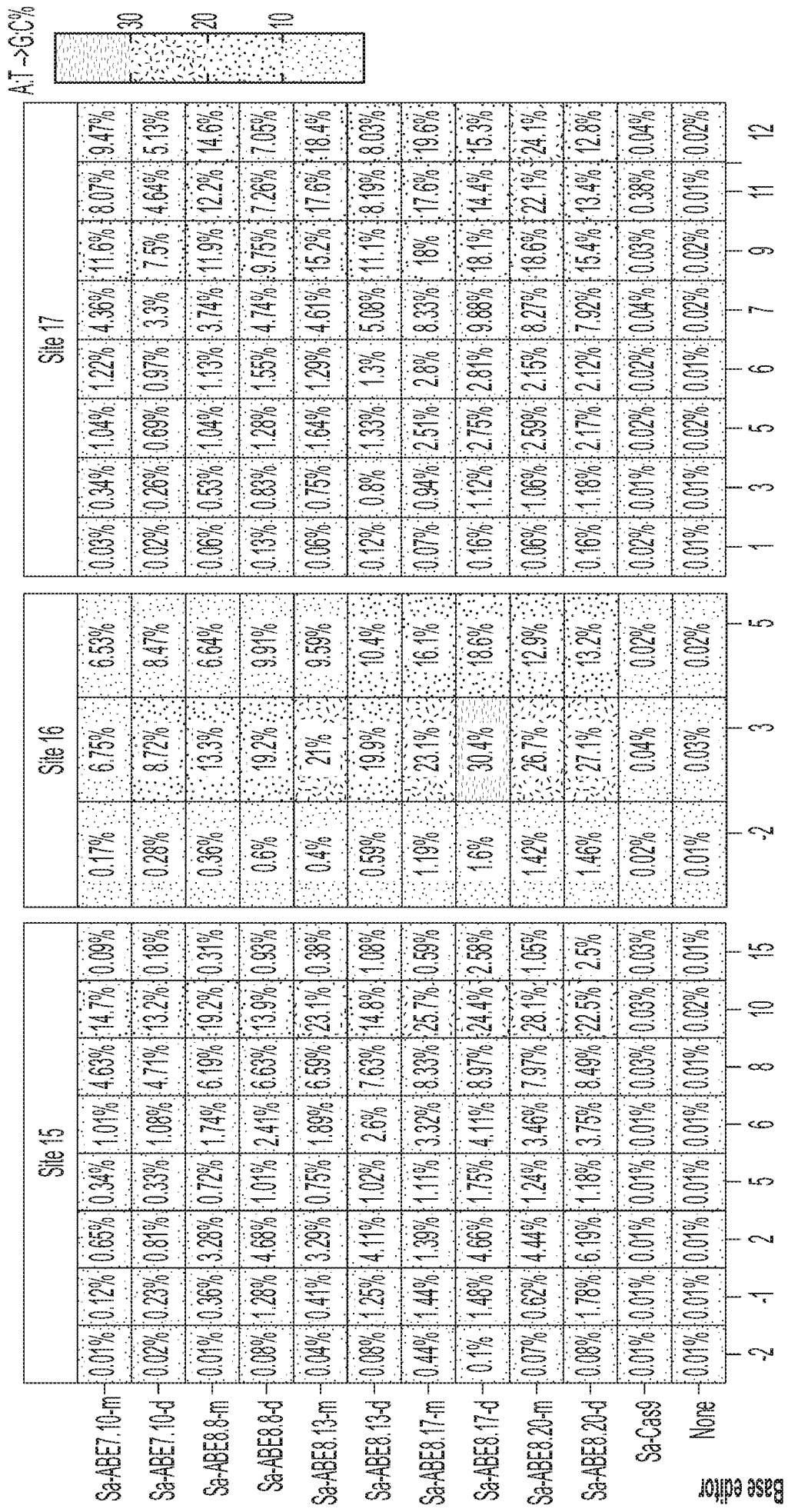

A:T ->G:C%
30
20
10
Site 15
Site 16
Site 17
-2
-1
1
2
3
5
6
8
10
11
12
15
base editor
Sa-ABE7.10-m
Sa-ABE7.10-d
Sa-ABE8.8-m
Sa-ABE8.8-d
Sa-ABE8.13-m
Sa-ABE8.13-d
Sa-ABE8.17-m
Sa-ABE8.17-d
Sa-ABE8.20-m
Sa-ABE8.20-d
Sa-Cas9
None

FIG. 19

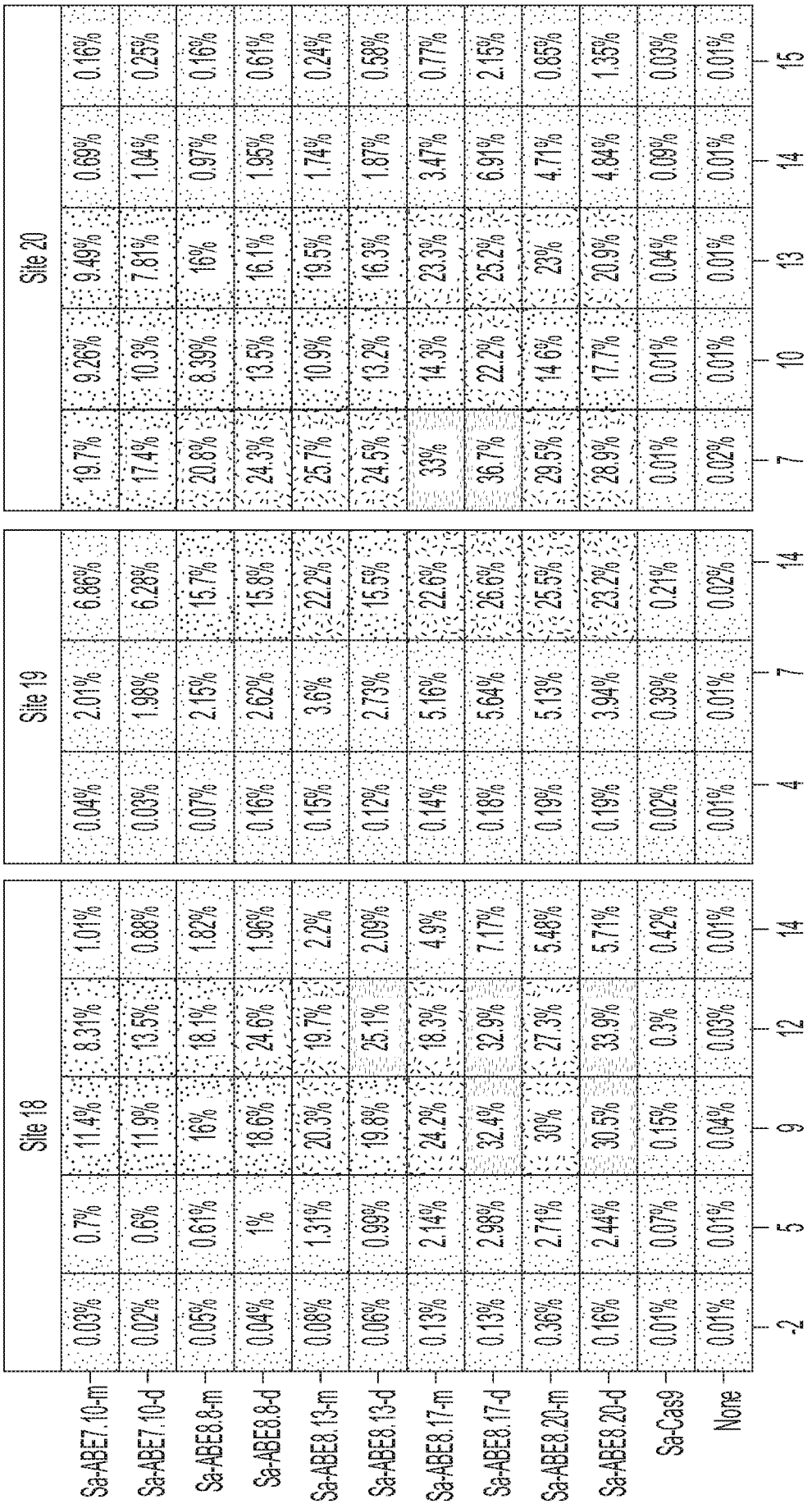


FIG. 19 CONTINUED

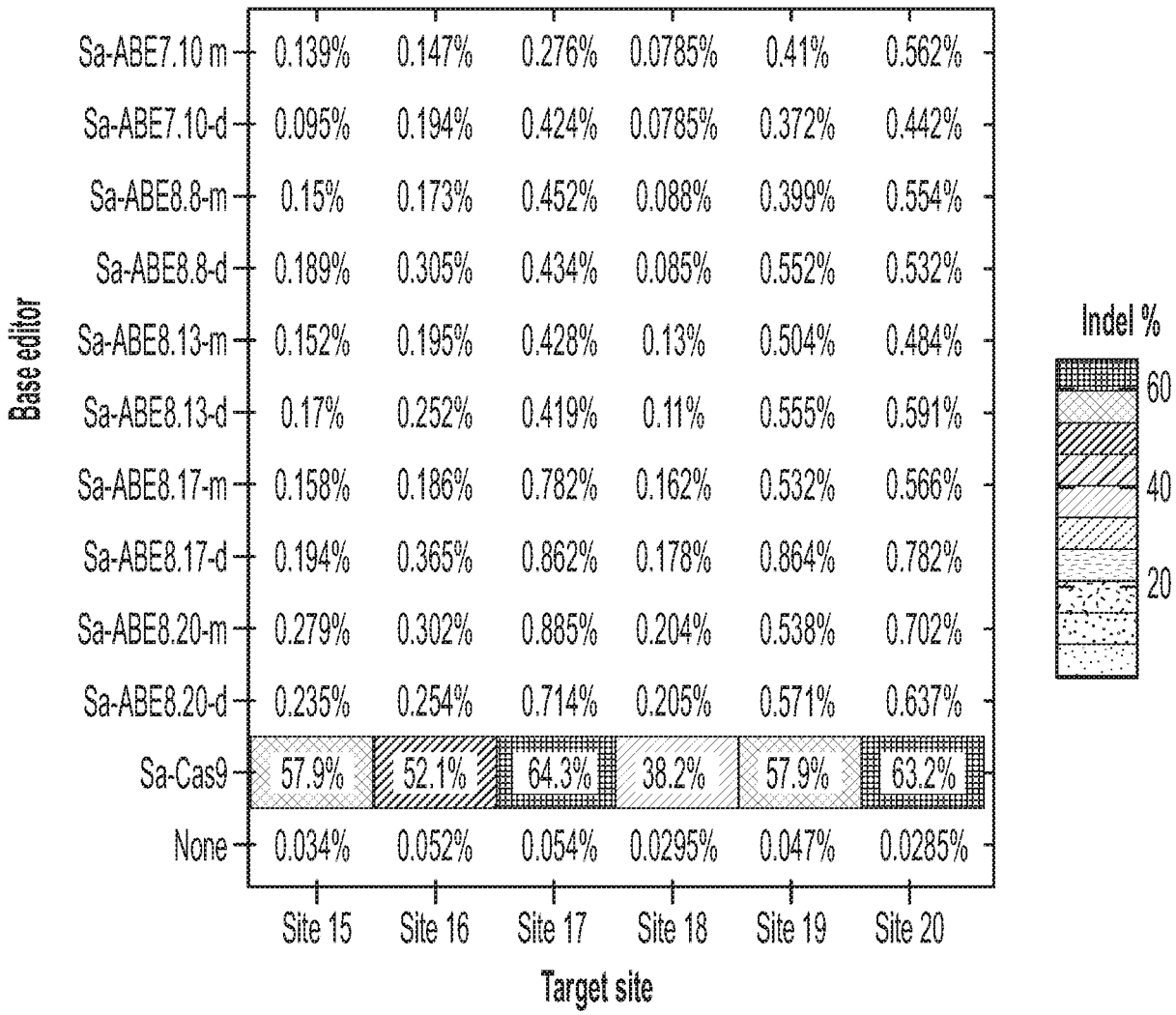

Base editor
Sa-ABE7.10 m
Sa-ABE7.10-d
Sa-ABE8.8-m
Sa-ABE8.8-d
Sa-ABE8.13-m
Sa-ABE8.13-d
Sa-ABE8.17-m
Sa-ABE8.17-d
Sa-ABE8.20-m
Sa-ABE8.20-d
Sa-Cas9
None
0.139% 0.147% 0.276% 0.0785% 0.41% 0.562%
0.095% 0.194% 0.424% 0.0785% 0.372% 0.442%
0.15% 0.173% 0.452% 0.088% 0.399% 0.554%
0.189% 0.305% 0.434% 0.085% 0.552% 0.532%
0.152% 0.195% 0.428% 0.13% 0.504% 0.484%
0.17% 0.252% 0.419% 0.11% 0.555% 0.591%
0.158% 0.186% 0.782% 0.162% 0.532% 0.566%
0.194% 0.365% 0.862% 0.178% 0.864% 0.782%
0.279% 0.302% 0.885% 0.204% 0.538% 0.702%
0.235% 0.254% 0.714% 0.205% 0.571% 0.637%
57.9% 52.1% 64.3% 38.2% 57.9% 63.2%
0.034% 0.052% 0.054% 0.0295% 0.047% 0.0285%
Site 15 Site 16 Site 17 Site 18 Site 19 Site 20
Target site
Indel %
60
40
20

FIG. 20

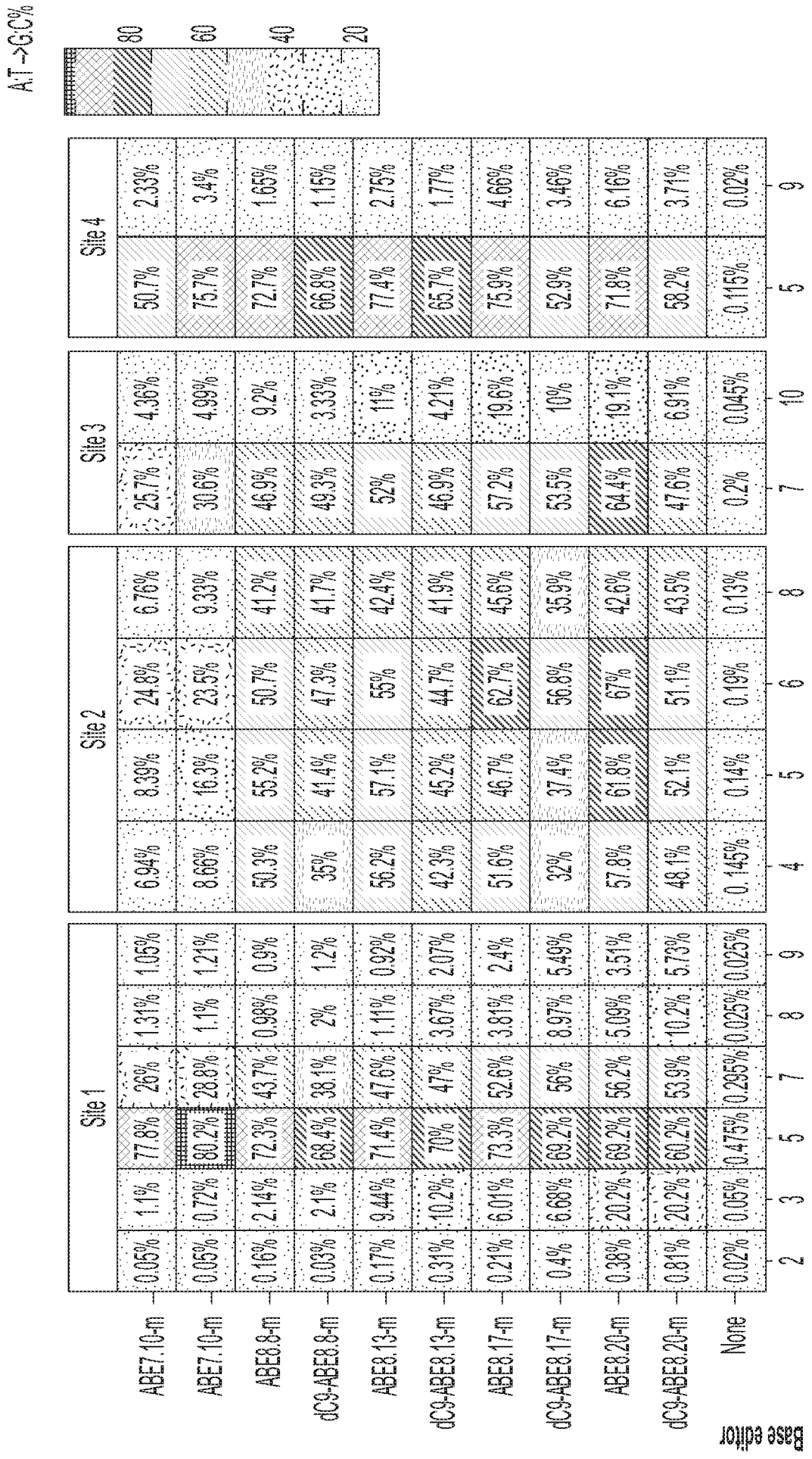

A:T ->G:C%
80
60
40
20
Site 1
Site 2
Site 3
Site 4
2 3 5 7 8 9 4 5 6 8 7 10 5 9
Base editor
ABE7.10-m
ABE7.10-m
ABE8.8-m
dC9-ABE8.8-m
ABE8.13-m
dC9-ABE8.13-m
ABE8.17-m
dC9-ABE8.17-m
ABE8.20-m
dC9-ABE8.20-m
None
0.05% 1.1% 77.8% 26% 1.31% 1.05% 6.94% 8.39% 24.8% 6.76% 25.7% 4.36% 50.7% 2.33%
0.05% 0.72% 80.2% 28.8% 1.1% 1.21% 8.66% 16.3% 23.5% 9.33% 30.6% 4.99% 75.7% 3.4%
0.16% 2.14% 72.3% 43.7% 0.98% 0.9% 50.3% 55.2% 50.7% 41.2% 46.9% 9.2% 72.7% 1.65%
0.03% 2.1% 68.4% 38.1% 2% 1.2% 35% 41.4% 47.3% 41.7% 49.3% 3.33% 66.8% 1.15%
0.17% 9.44% 71.4% 47.6% 1.11% 0.92% 56.2% 57.1% 55% 42.4% 52% 11% 77.4% 2.75%
0.31% 10.2% 70% 47% 3.67% 2.07% 42.3% 45.2% 44.7% 41.9% 46.9% 4.21% 65.7% 1.77%
0.21% 6.01% 73.3% 52.6% 3.81% 2.4% 51.6% 46.7% 62.7% 45.6% 57.2% 19.6% 75.9% 4.66%
0.4% 6.68% 69.2% 56% 8.97% 5.49% 32% 37.4% 56.8% 35.9% 53.5% 10% 52.9% 3.46%
0.38% 20.2% 69.2% 56.2% 5.09% 3.51% 57.8% 61.8% 67% 42.6% 64.4% 19.1% 71.8% 6.16%
0.81% 20.2% 60.2% 53.9% 10.2% 5.73% 48.1% 52.1% 51.1% 43.5% 47.6% 6.91% 58.2% 3.71%
0.02% 0.05% 0.475% 0.295% 0.025% 0.025% 0.145% 0.14% 0.19% 0.13% 0.2% 0.045% 0.115% 0.02%

FIG. 21

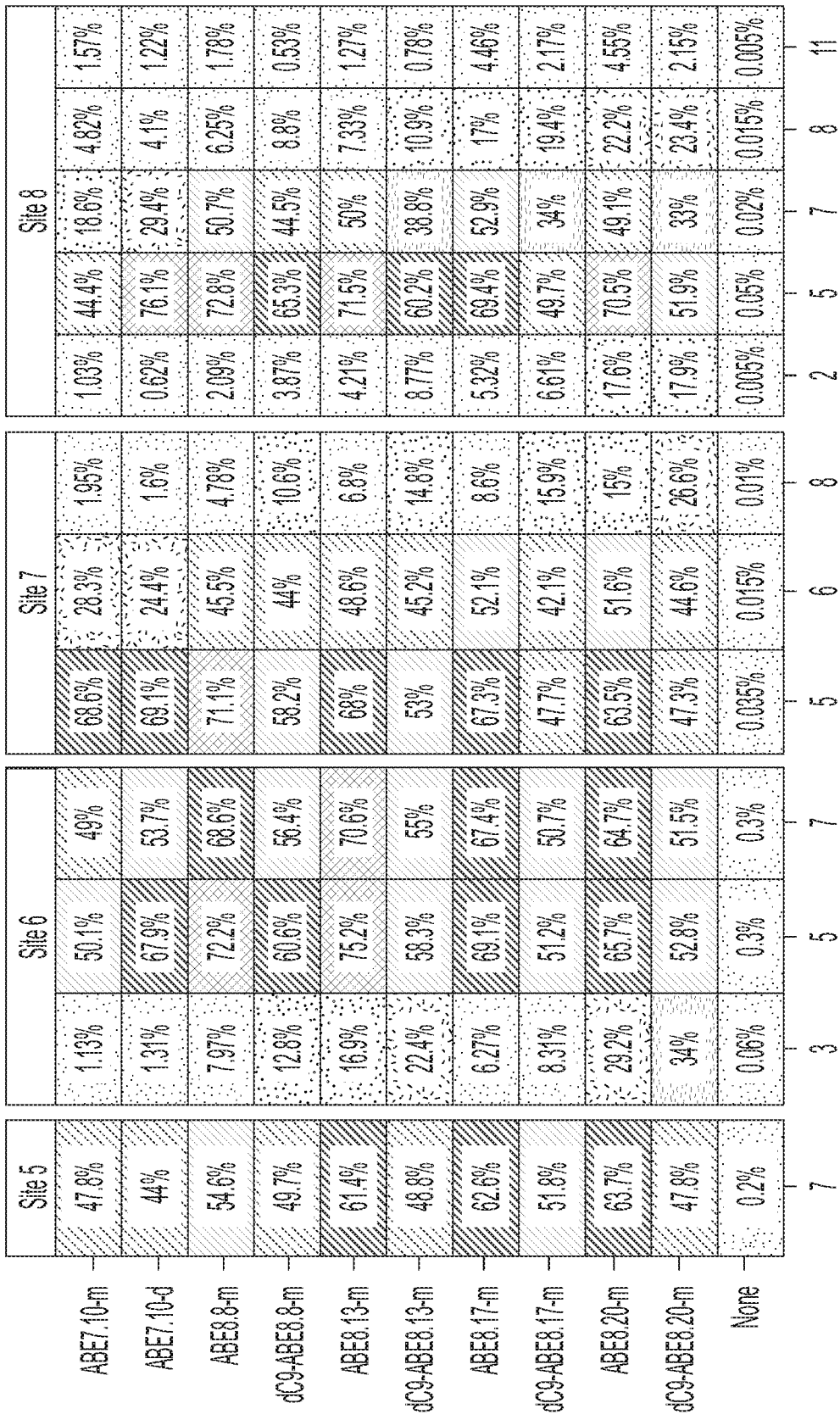


FIG. 21 CONTINUED

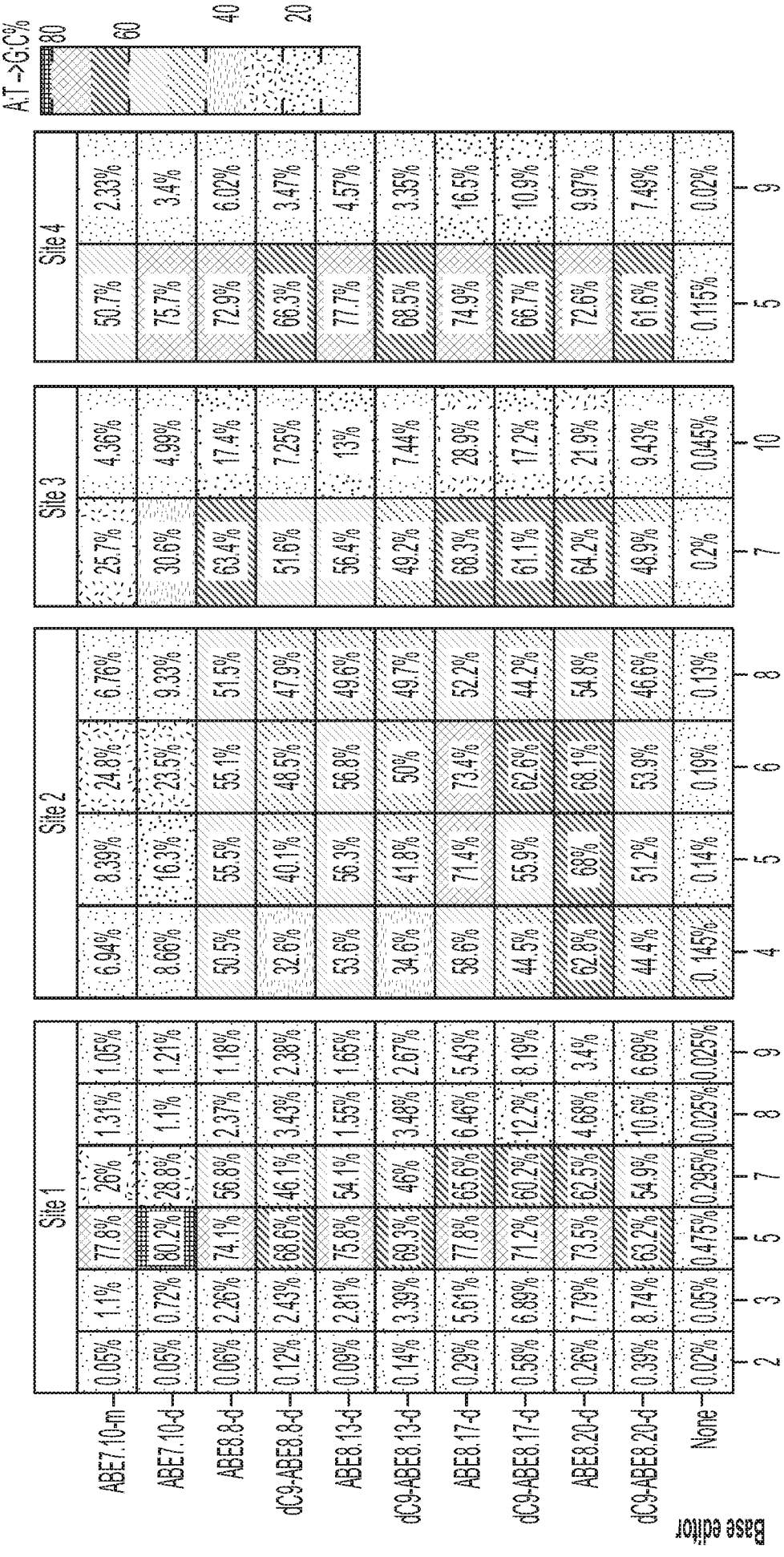

A:T->G:C%
80
60
40
20
Site 1
Site 2
Site 3
Site 4
2
3
5
7
8
9
4
5
6
8
7
10
5
9
Base editor
ABE7.10-m
ABE7.10-d
ABE8.8-d
dC9-ABE8.8-d
ABE8.13-d
dC9-ABE8.13-d
ABE8.17-d
dC9-ABE8.17-d
ABE8.20-d
dC9-ABE8.20-d
None
0.05% 1.1% 77.8% 26% 1.31% 1.05%
0.05% 0.72% 80.2% 28.8% 1.1% 1.21%
0.06% 2.26% 74.1% 56.8% 2.37% 1.18%
0.12% 2.43% 68.6% 46.1% 3.43% 2.38%
0.09% 2.81% 75.8% 54.1% 1.55% 1.65%
0.14% 3.39% 69.3% 46% 3.48% 2.67%
0.29% 5.61% 77.8% 65.6% 6.46% 5.43%
0.58% 6.89% 71.2% 60.2% 12.2% 8.19%
0.26% 7.79% 73.5% 62.5% 4.68% 3.4%
0.39% 8.74% 63.2% 54.9% 10.6% 6.69%
0.02% 0.05% 0.475% 0.285% 0.025% 0.025%
6.94% 8.39% 24.8% 6.76%
8.66% 16.3% 23.5% 9.33%
50.5% 55.5% 55.1% 51.5%
32.6% 40.1% 48.5% 47.9%
53.6% 56.3% 56.8% 49.6%
34.6% 41.8% 50% 49.7%
58.6% 71.4% 73.4% 52.2%
44.5% 55.9% 62.6% 44.2%
62.8% 68% 68.1% 54.8%
44.4% 51.2% 53.9% 46.6%
0.145% 0.14% 0.19% 0.13%
25.7% 4.36%
30.6% 4.99%
63.4% 17.4%
51.6% 7.25%
56.4% 13%
49.2% 7.44%
68.3% 28.9%
61.1% 17.2%
64.2% 21.9%
48.9% 9.43%
0.2% 0.045%
50.7% 2.33%
75.7% 3.4%
72.9% 6.02%
66.3% 3.47%
77.7% 4.57%
68.5% 3.35%
74.9% 16.5%
66.7% 10.9%
72.6% 9.97%
61.6% 7.49%
0.115% 0.02%

FIG. 22

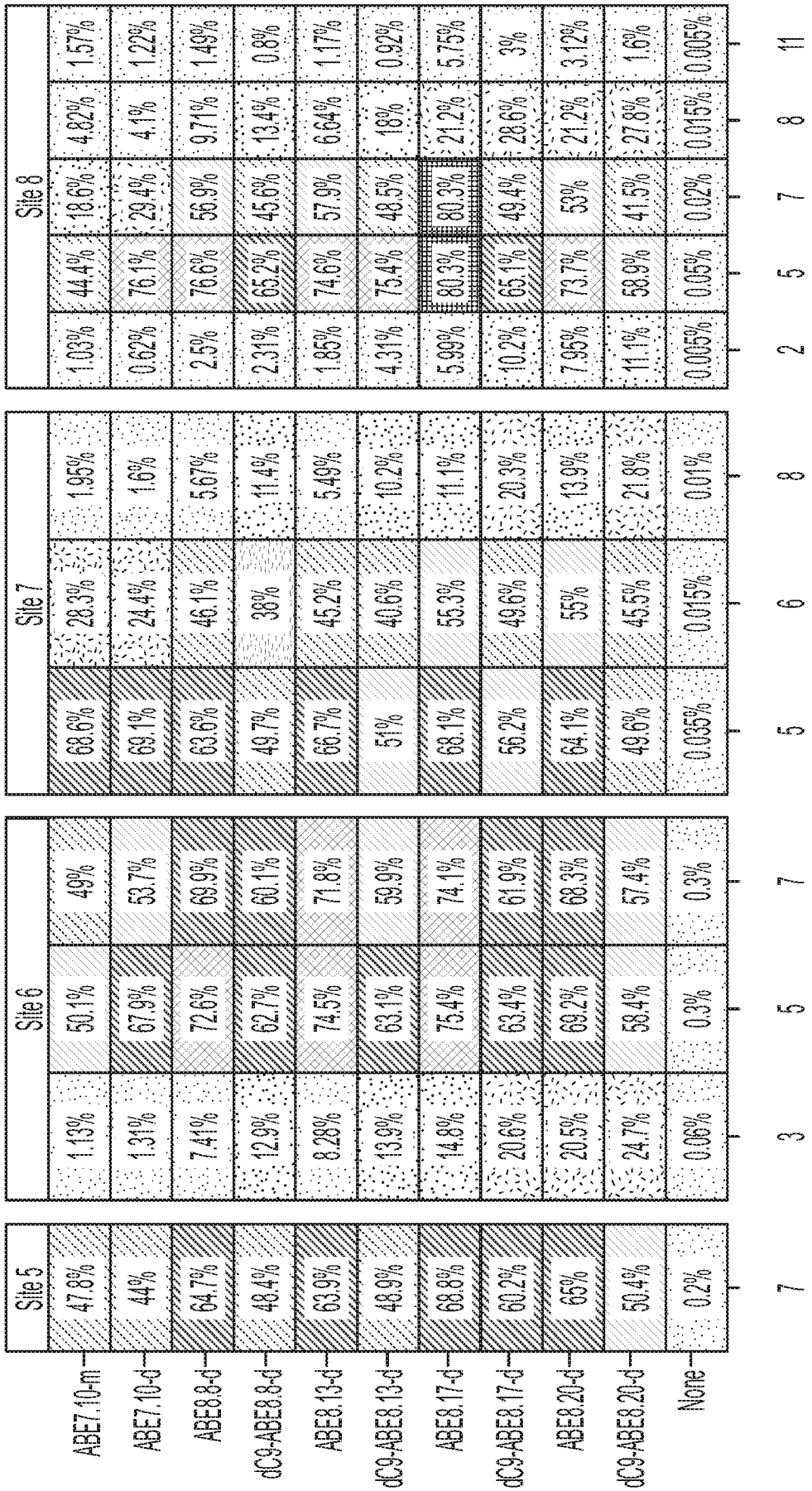


FIG. 22 CONTINUED

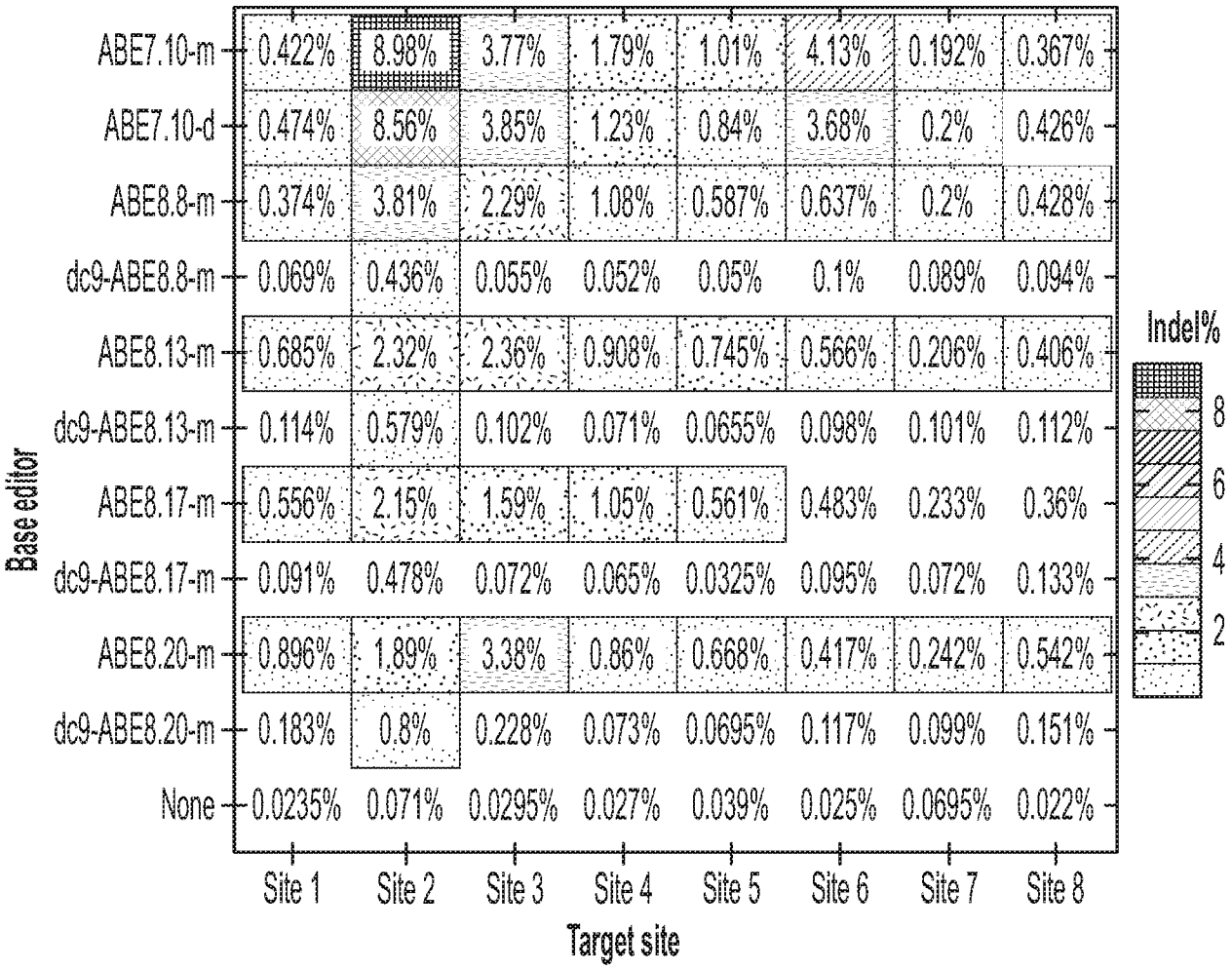

ABE7.10-m 0.422% 8.98% 3.77% 1.79% 1.01% 4.13% 0.192% 0.367%
ABE7.10-d 0.474% 8.56% 3.85% 1.23% 0.84% 3.68% 0.2% 0.426%
ABE8.8-m 0.374% 3.81% 2.29% 1.08% 0.587% 0.637% 0.2% 0.428%
dc9-ABE8.8-m 0.069% 0.436% 0.055% 0.052% 0.05% 0.1% 0.089% 0.094%
ABE8.13-m 0.685% 2.32% 2.36% 0.908% 0.745% 0.566% 0.206% 0.406%
dc9-ABE8.13-m 0.114% 0.579% 0.102% 0.071% 0.0655% 0.098% 0.101% 0.112%
ABE8.17-m 0.556% 2.15% 1.59% 1.05% 0.561% 0.483% 0.233% 0.36%
dc9-ABE8.17-m 0.091% 0.478% 0.072% 0.065% 0.0325% 0.095% 0.072% 0.133%
ABE8.20-m 0.896% 1.89% 3.38% 0.86% 0.668% 0.417% 0.242% 0.542%
dc9-ABE8.20-m 0.183% 0.8% 0.228% 0.073% 0.0695% 0.117% 0.099% 0.151%
None 0.0235% 0.071% 0.0295% 0.027% 0.039% 0.025% 0.0695% 0.022%
Base editor
Site 1 Site 2 Site 3 Site 4 Site 5 Site 6 Site 7 Site 8
Target site
Indel%
8
6
4
2

FIG. 23A

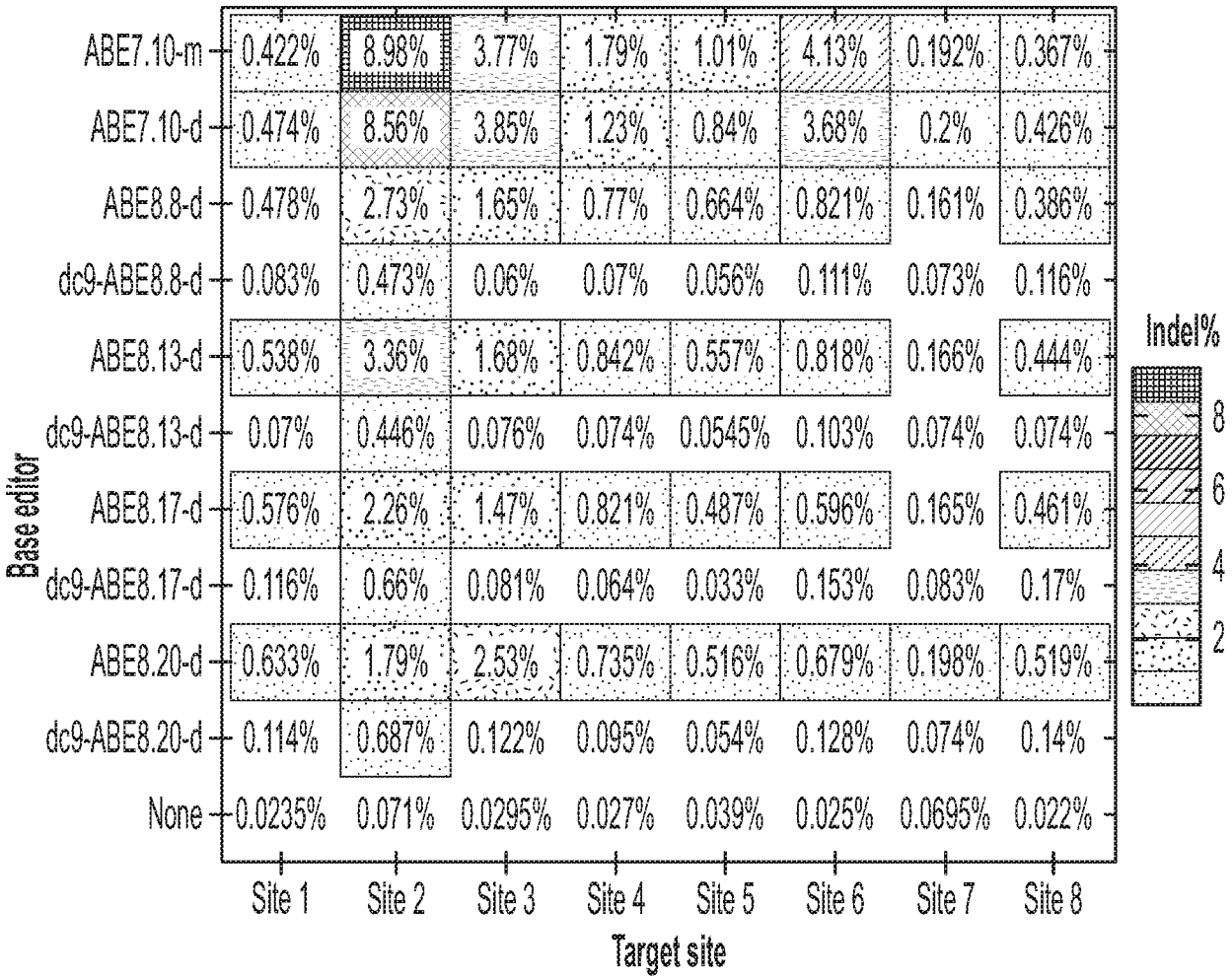
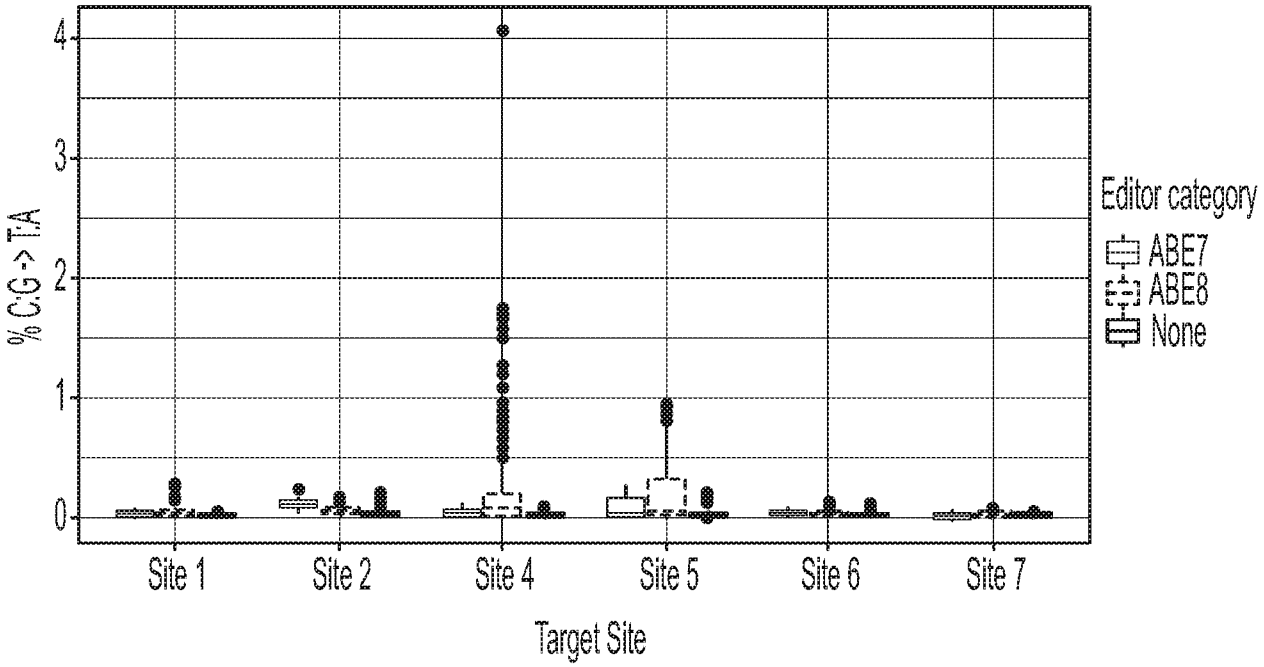

ABE7.10-m 0.422% 8.98% 3.77% 1.79% 1.01% 4.13% 0.192% 0.367%
ABE7.10-d 0.474% 8.56% 3.85% 1.23% 0.84% 3.68% 0.2% 0.426%
ABE8.8-d 0.478% 2.73% 1.65% 0.77% 0.664% 0.821% 0.161% 0.386%
dc9-ABE8.8-d 0.083% 0.473% 0.06% 0.07% 0.056% 0.111% 0.073% 0.116%
ABE8.13-d 0.538% 3.36% 1.68% 0.842% 0.557% 0.818% 0.166% 0.444%
dc9-ABE8.13-d 0.07% 0.446% 0.076% 0.074% 0.0545% 0.103% 0.074% 0.074%
ABE8.17-d 0.576% 2.26% 1.47% 0.821% 0.487% 0.596% 0.165% 0.461%
dc9-ABE8.17-d 0.116% 0.66% 0.081% 0.064% 0.033% 0.153% 0.083% 0.17%
ABE8.20-d 0.633% 1.79% 2.53% 0.735% 0.516% 0.679% 0.198% 0.519%
dc9-ABE8.20-d 0.114% 0.687% 0.122% 0.095% 0.054% 0.128% 0.074% 0.14%
None 0.0235% 0.071% 0.0295% 0.027% 0.039% 0.025% 0.0695% 0.022%
Base editor
Site 1 Site 2 Site 3 Site 4 Site 5 Site 6 Site 7 Site 8
Target site
Indel%
8
6
4
2

FIG. 23B



Site	target DNA sequence
1	GAACACAAAGCATAGACTGC
2	GGGAAAGACCCAGCATCCGT
3	GCTCCCATCACATCAACCGG
5	GGCTTCAGGTTCTAAATGAG
6	GCAGAGAGTCGCCGTCTCCA
7	GTGTAAGACCTCAAAAGCAC

FIG. 24

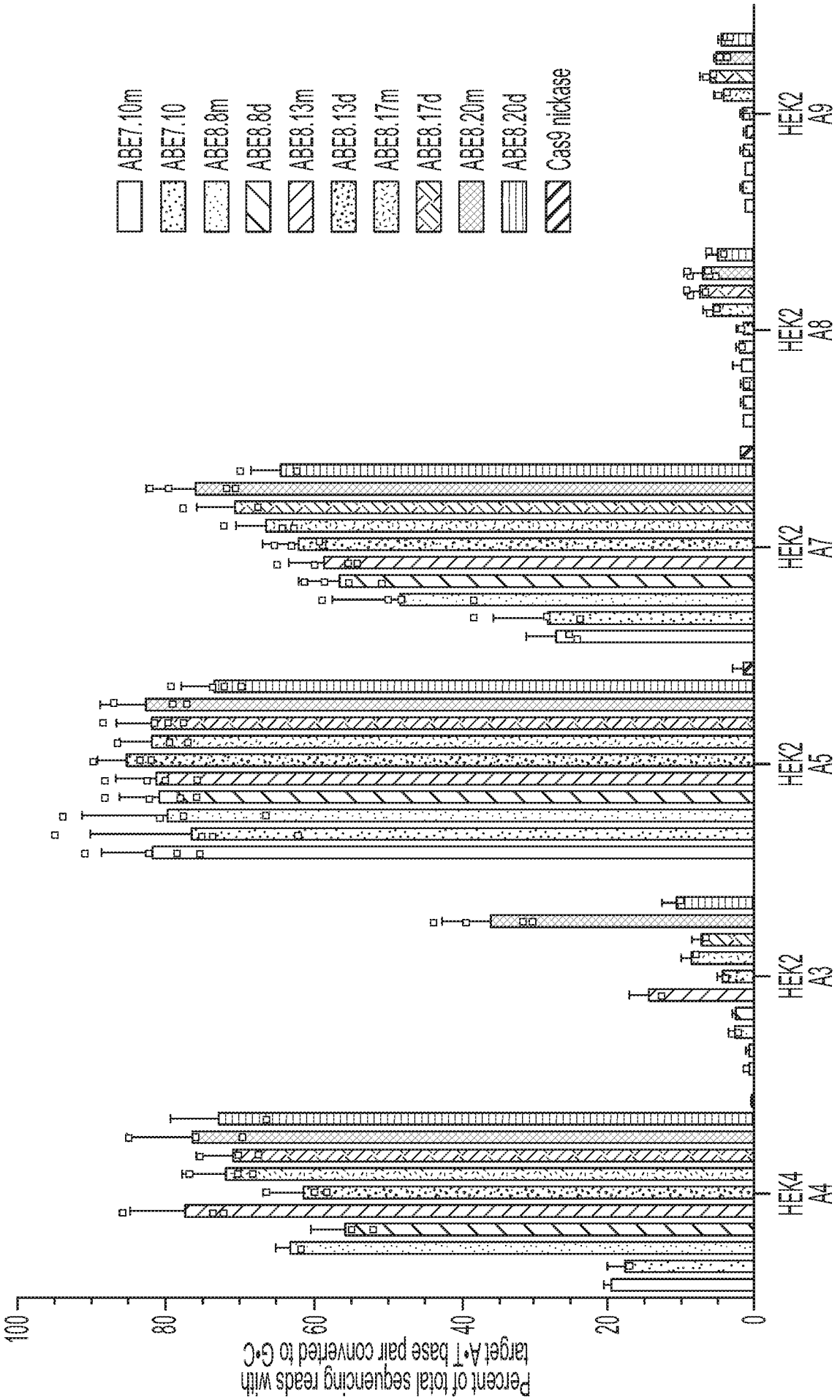

ABE7.10m
ABE7.10
ABE8.8m
ABE8.8d
ABE8.13m
ABE8.13d
ABE8.17m
ABE8.17d
ABE8.20m
ABE8.20d
Cas9 nickase
HEK4 A4
HEK2 A3
HEK2 A5
HEK2 A7
HEK2 A8
HEK2 A9
0
20
40
60
80
100
Percent of total sequencing reads with target A•T base pair converted to G•C

FIG. 25A

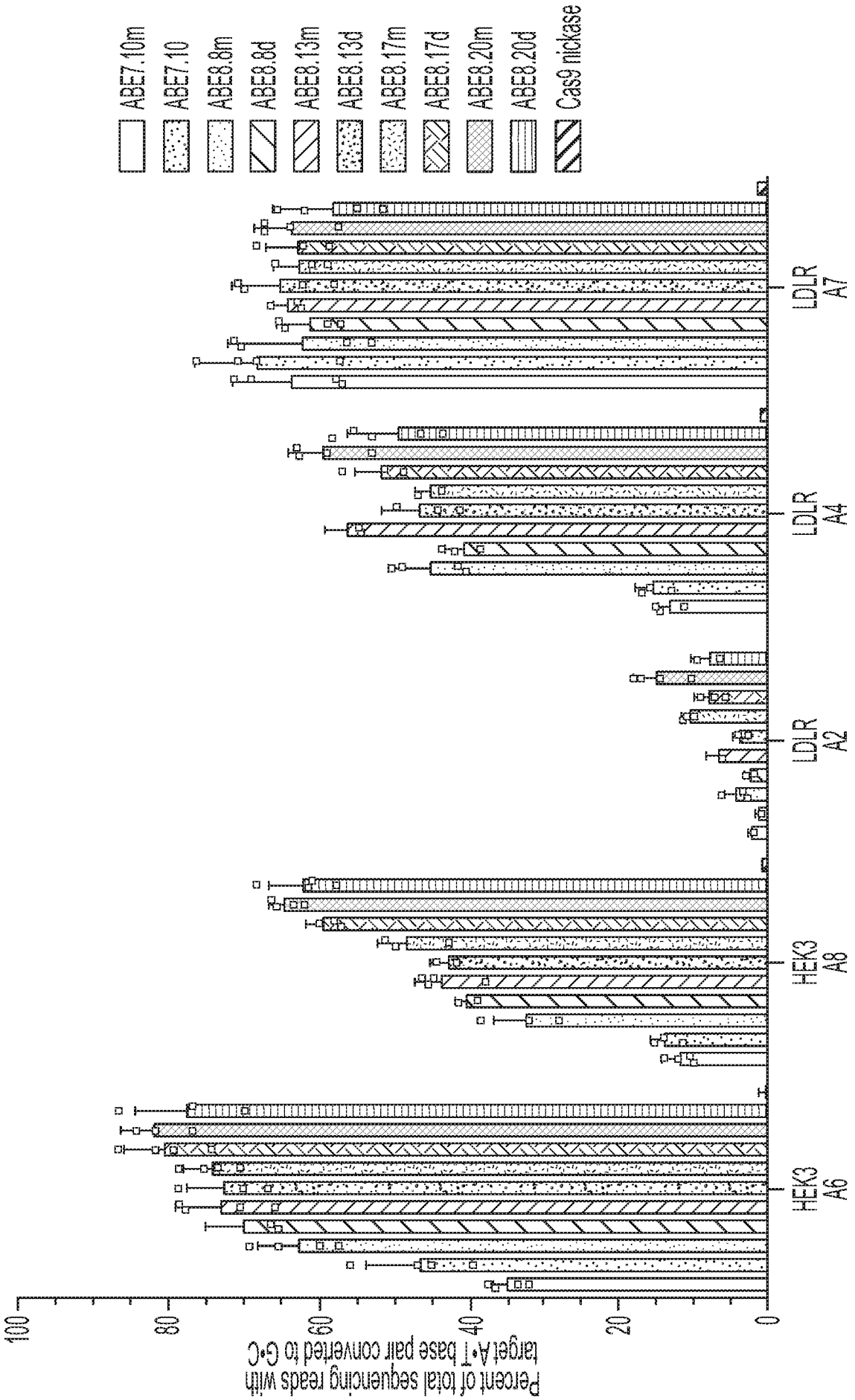

ABE7.10m
ABE7.10
ABE8.8m
ABE8.8d
ABE8.13m
ABE8.13d
ABE8.17m
ABE8.17d
ABE8.20m
ABE8.20d
Cas9 nickase
LDLR A7
LDLR A4
LDLR A2
HEK3 A8
HEK3 A6
100
80
60
40
20
0
Percent of total sequencing reads with target A•T base pair converted to G•C

FIG. 25B

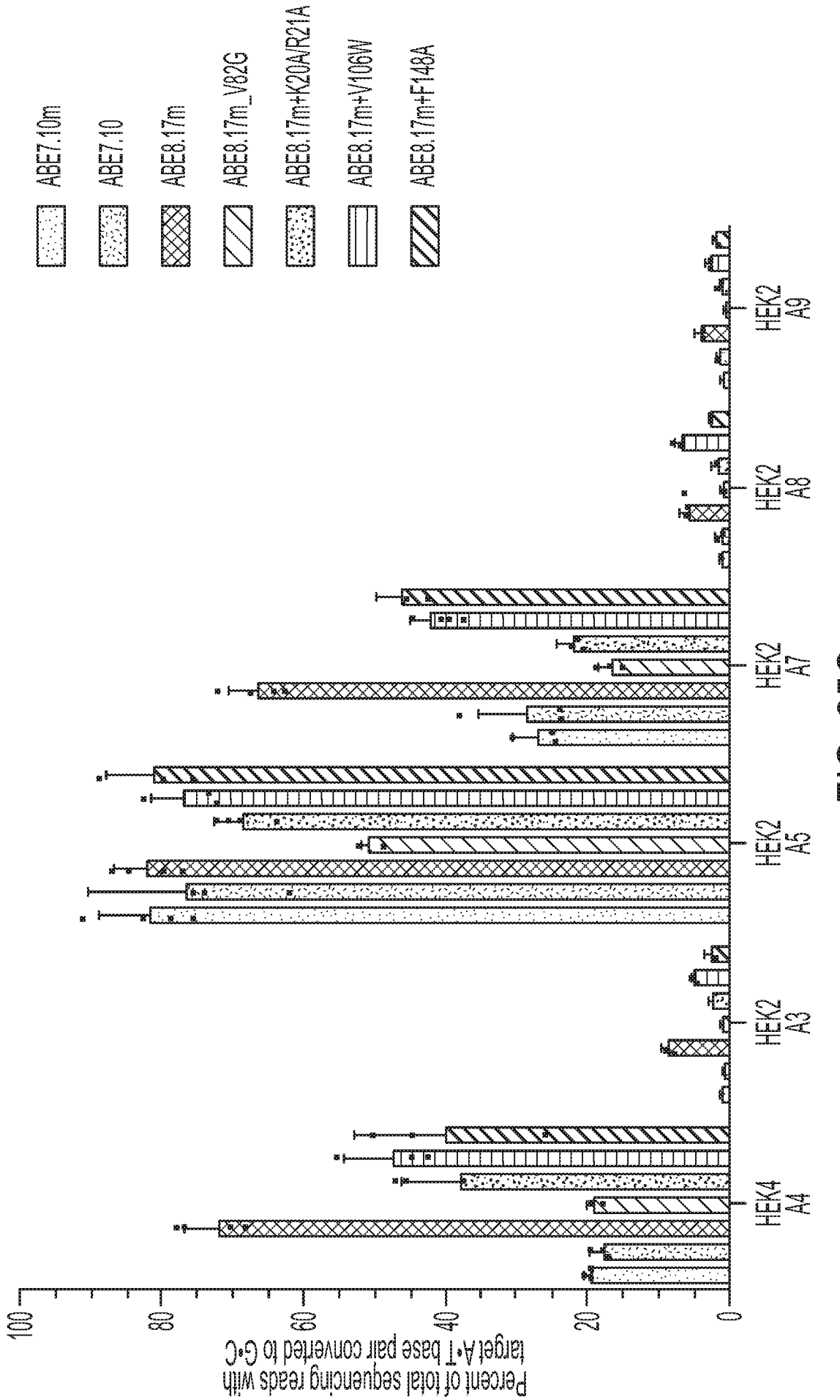

ABE7.10m
ABE7.10
ABE8.17m
ABE8.17m_V82G
ABE8.17m+K20A/R21A
ABE8.17m+V106W
ABE8.17m+F148A
Percent of total sequencing reads with target A•T base pair converted to G•C
100
80
60
40
20
0
HEK4 A4
HEK2 A3
HEK2 A5
HEK2 A7
HEK2 A8
HEK2 A9

FIG. 25C

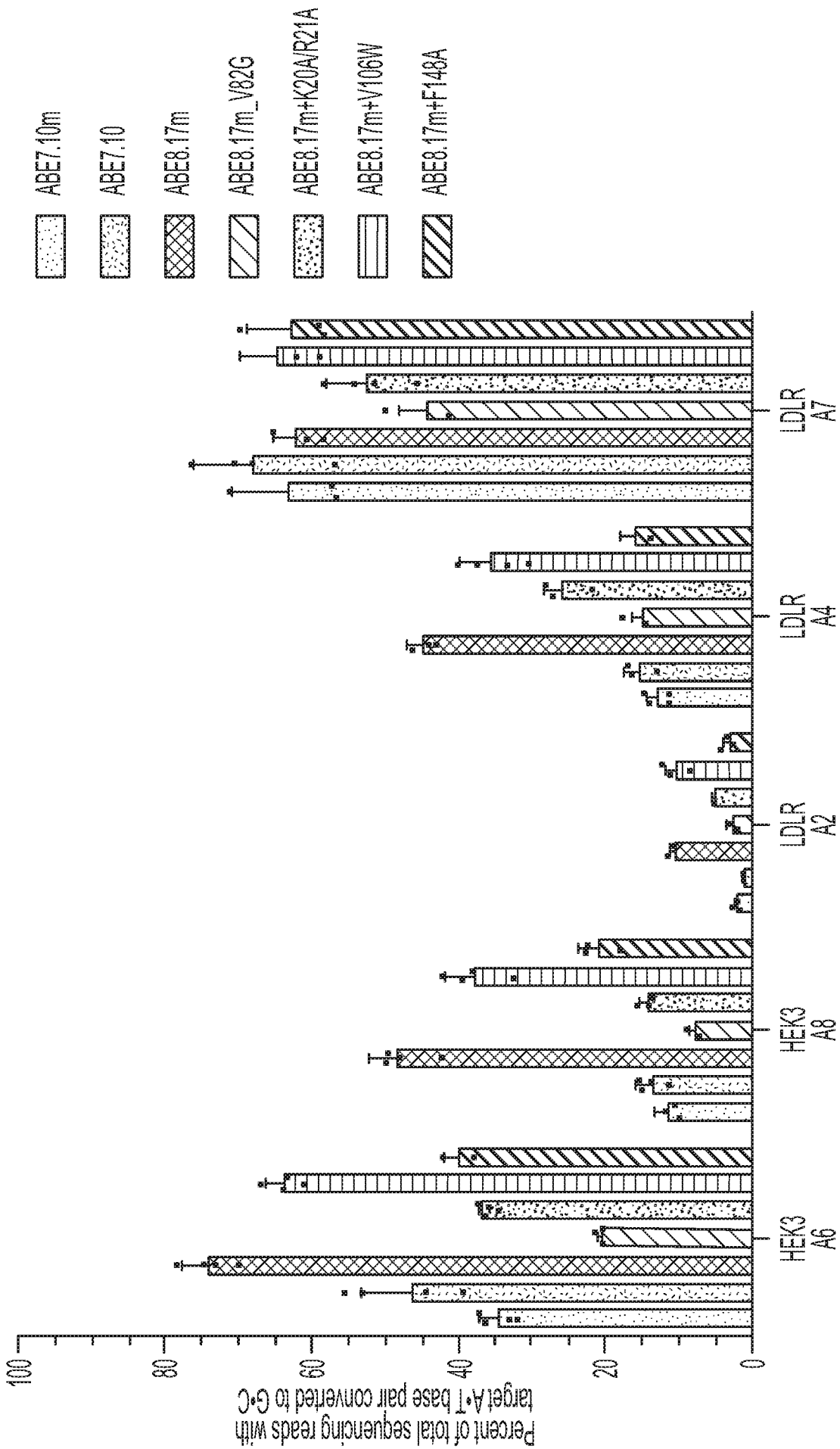

ABE7.10m
ABE7.10
ABE8.17m
ABE8.17m_V82G
ABE8.17m+K20A/R21A
ABE8.17m+V106W
ABE8.17m+F148A
Percent of total sequencing reads with target A•T base pair converted to G•C
100
80
60
40
20
0
HEK3 A6
HEK3 A8
LDLR A2
LDLR A4
LDLR A7

FIG. 25D

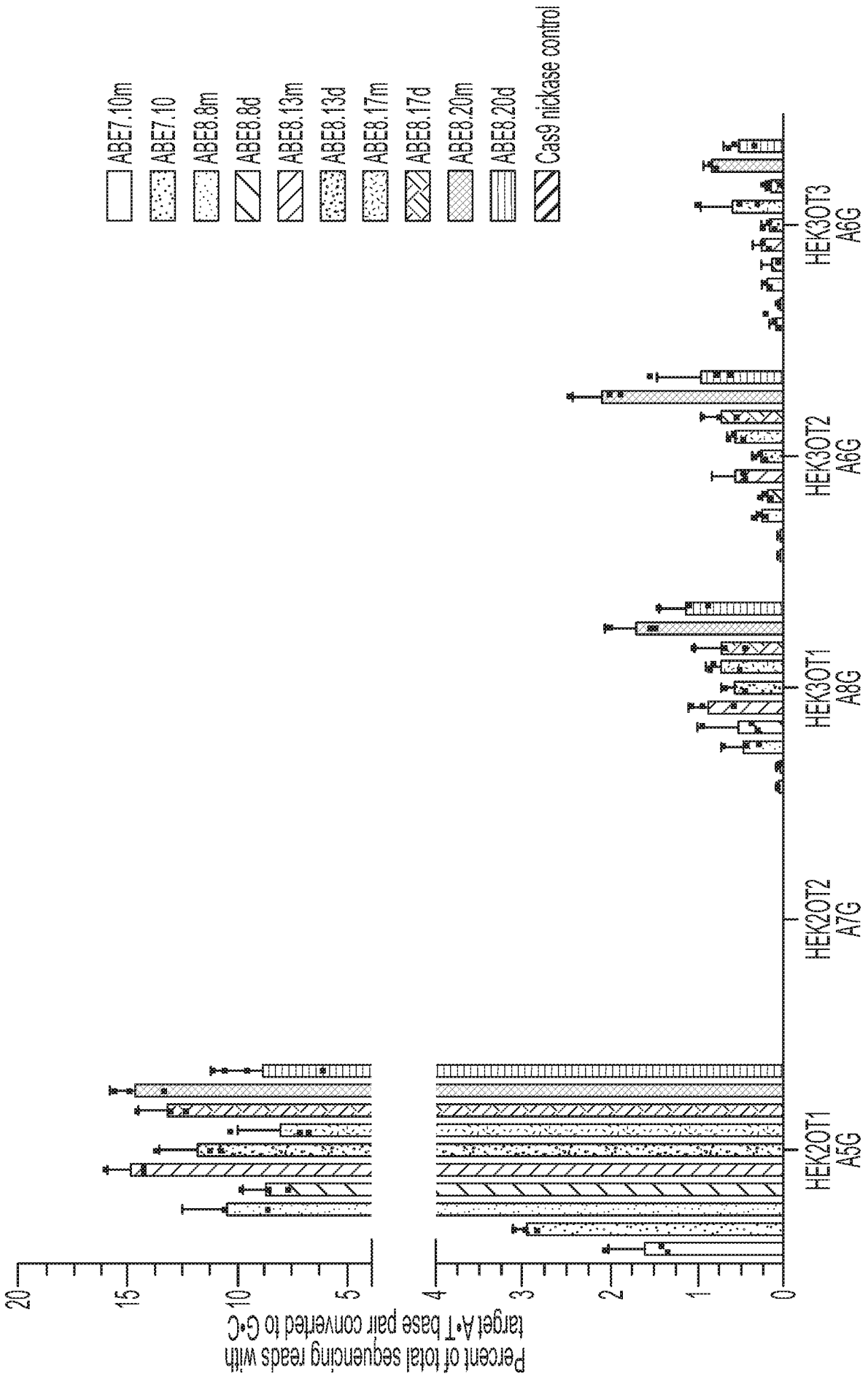

ABE7.10m
ABE7.10
ABE8.8m
ABE8.8d
ABE8.13m
ABE8.13d
ABE8.17m
ABE8.17d
ABE8.20m
ABE8.20d
Cas9 nickase control
HEK2OT1 A5G
HEK2OT2 A7G
HEK3OT1 A8G
HEK3OT2 A6G
HEK3OT3 A6G
Percent of total sequencing reads with target A•T base pair converted to G•C
0
1
2
3
4
5
10
15
20

FIG. 25E

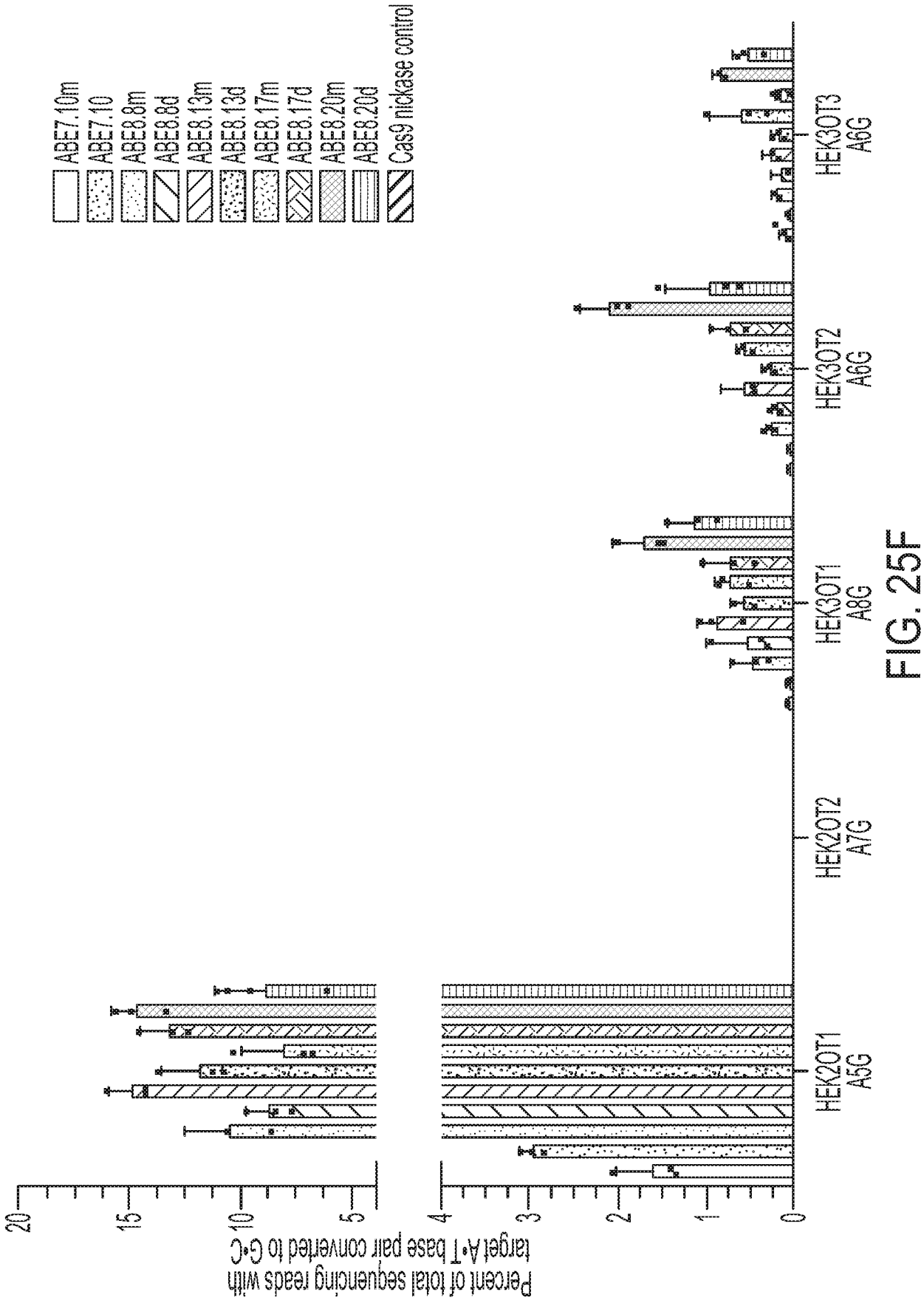

ABE7.10m
ABE7.10
ABE8.8m
ABE8.8d
ABE8.13m
ABE8.13d
ABE8.17m
ABE8.17d
ABE8.20m
ABE8.20d
Cas9 nickase control
Percent of total sequencing reads with target A•T base pair converted to G•C
20
15
10
5
4
3
2
1
0
HEK2OT1 A5G
HEK2OT2 A7G
HEK3OT1 A8G
HEK3OT2 A6G
HEK3OT3 A6G

FIG. 25F

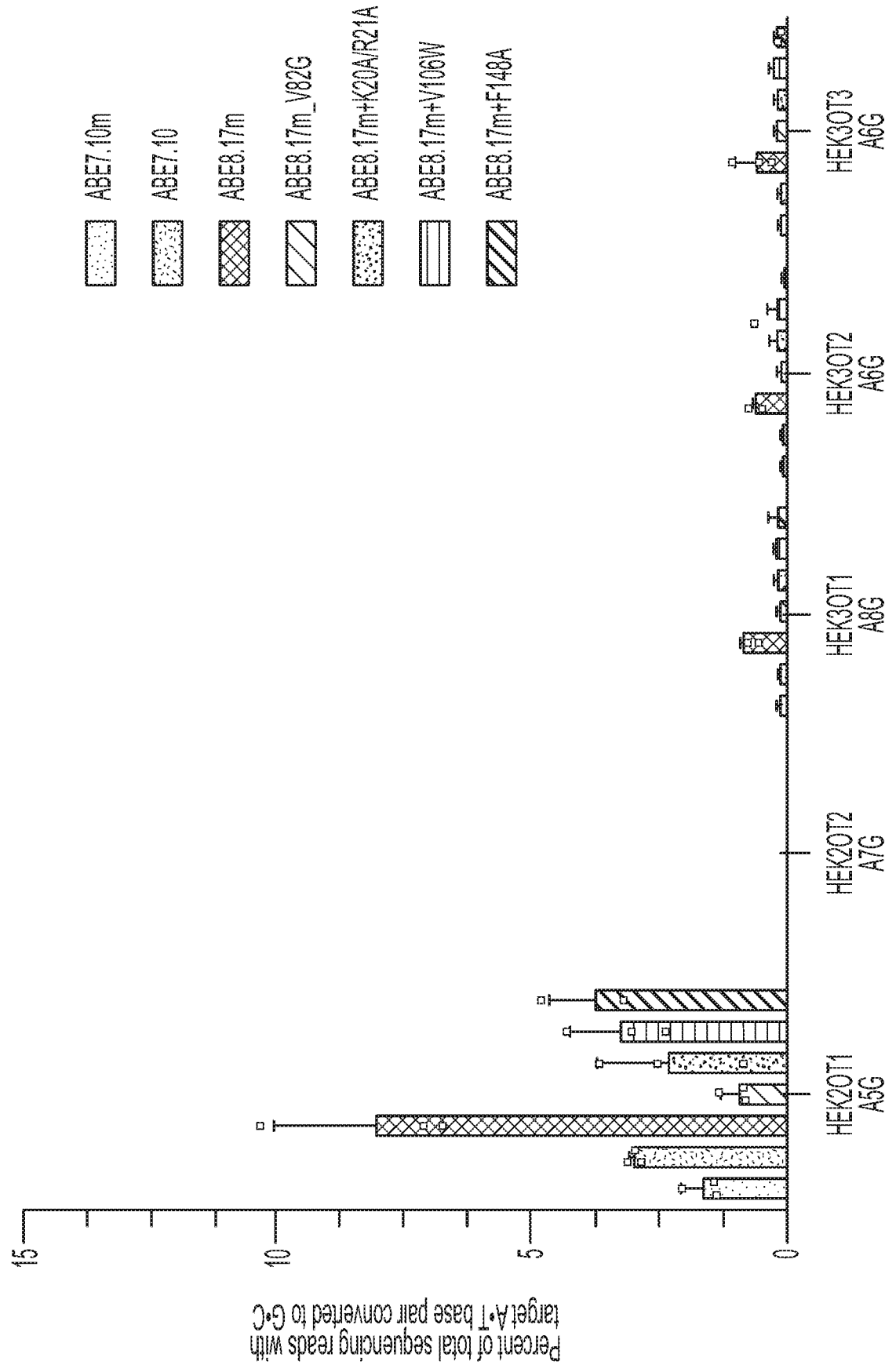

ABE7.10m
ABE7.10
ABE8.17m
ABE8.17m_V82G
ABE8.17m+K20A/R21A
ABE8.17m+V106W
ABE8.17m+F148A
HEK2OT1 A5G
HEK2OT2 A7G
HEK3OT1 A8G
HEK3OT2 A6G
HEK3OT3 A6G
0
5
10
15
Percent of total sequencing reads with target A•T base pair converted to G•C

FIG. 25G

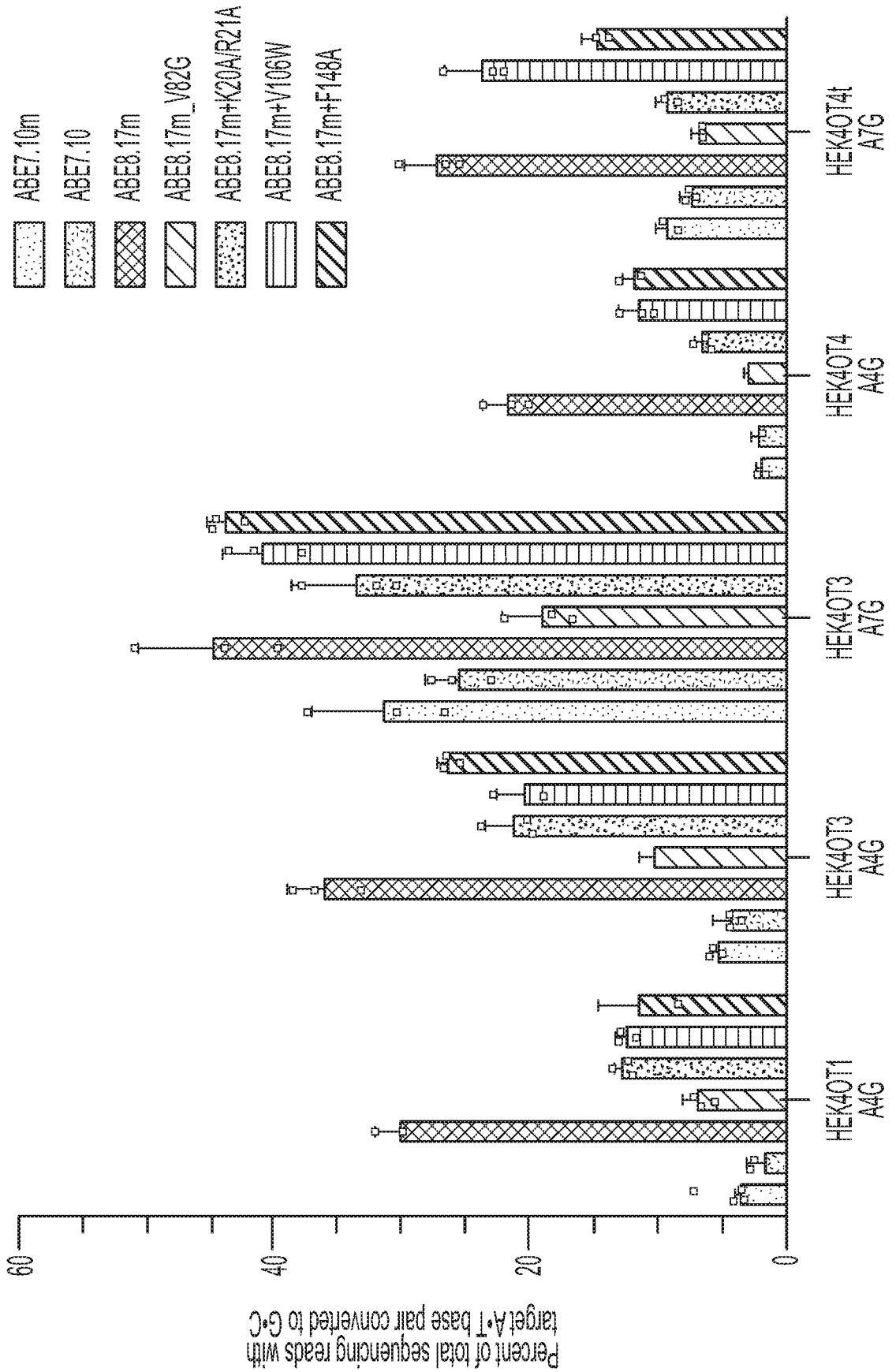

ABE7.10m
ABE7.10
ABE8.17m
ABE8.17m_V82G
ABE8.17m+K20A/R21A
ABE8.17m+V106W
ABE8.17m+F148A
HEK4OT1 A4G
HEK4OT3 A4G
HEK4OT3 A7G
HEK4OT4 A4G
HEK4OT4t A7G
0
20
40
60
Percent of total sequencing reads with target A•T base pair converted to G•C

FIG. 25H

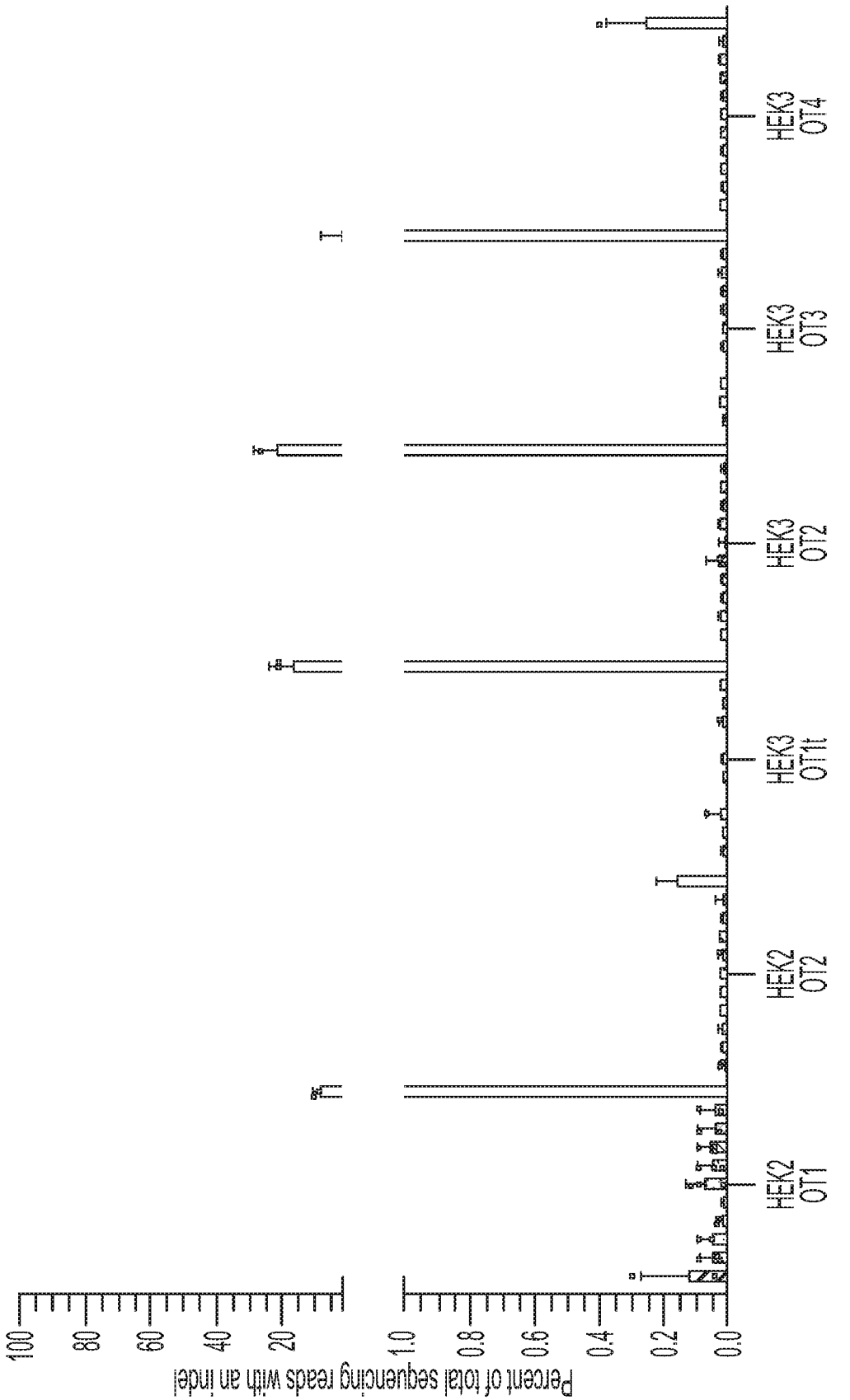

Percent of total sequencing reads with an indel
100
80
60
40
20
1.0
0.8
0.6
0.4
0.2
0.0
HEK2 OT1
HEK2 OT2
HEK3 OT1t
HEK3 OT2
HEK3 OT3
HEK3 OT4

FIG. 26

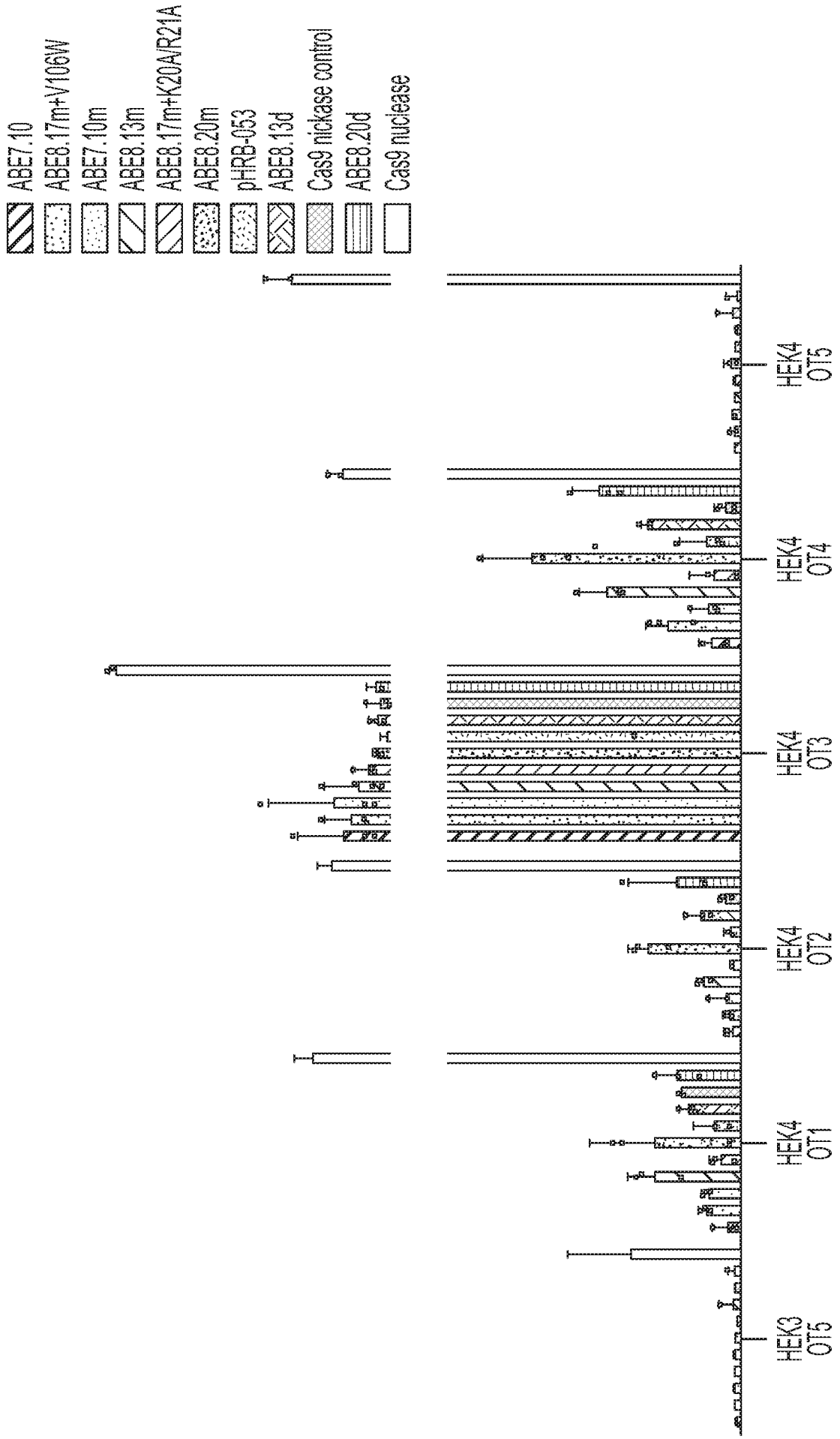


FIG. 26 CONTINUED

-198 target sequence:
GTGGGGA7AGGGGCCCCCAAGAGG
% A•T to G•C conversion
100
80
60
40
20
0
48h
144h
donor 1
48h
144h
donor 2

FIG. 27A

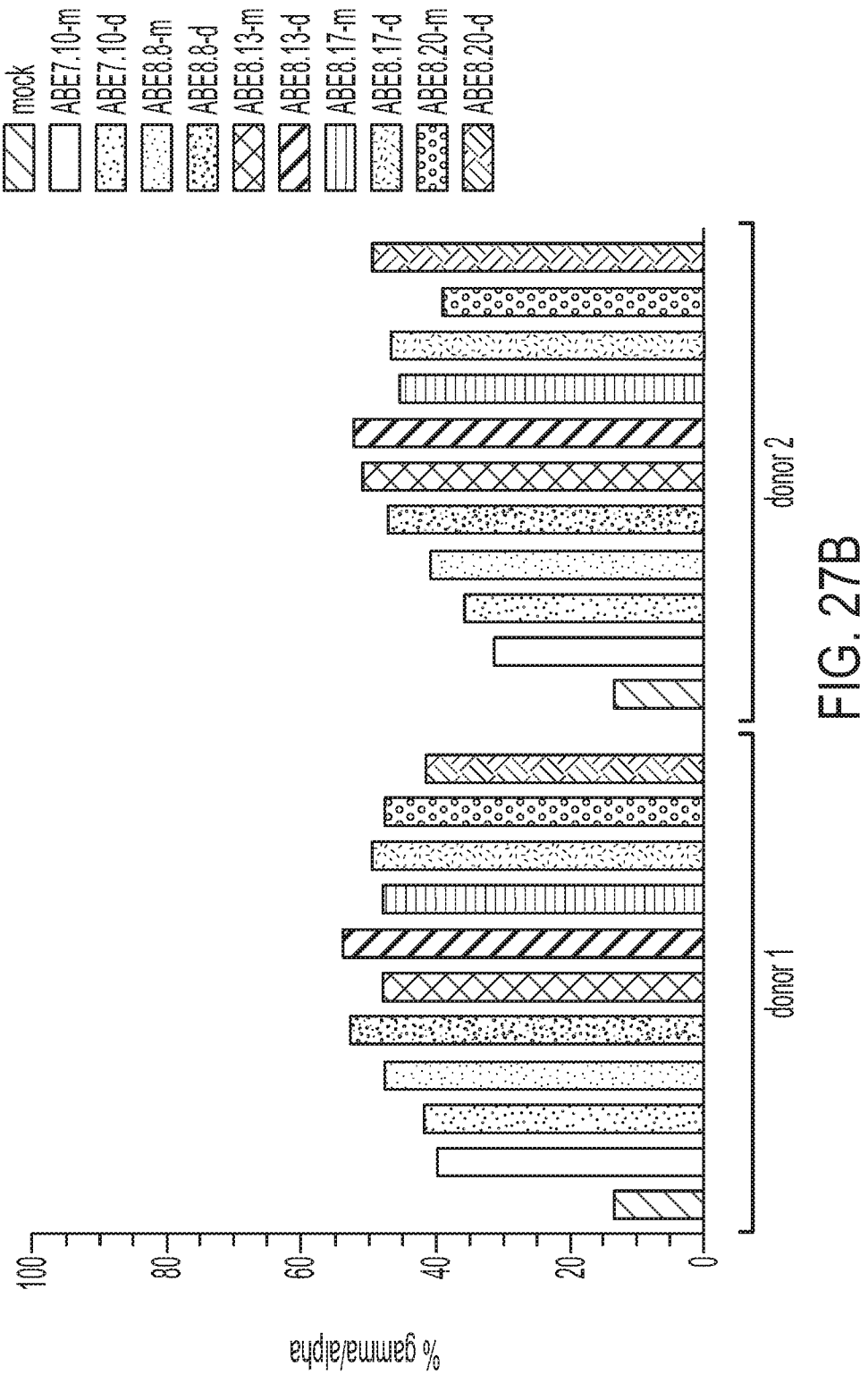

mock
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
donor 1
donor 2
% gamma/alpha
0
20
40
60
80
100

FIG. 27B

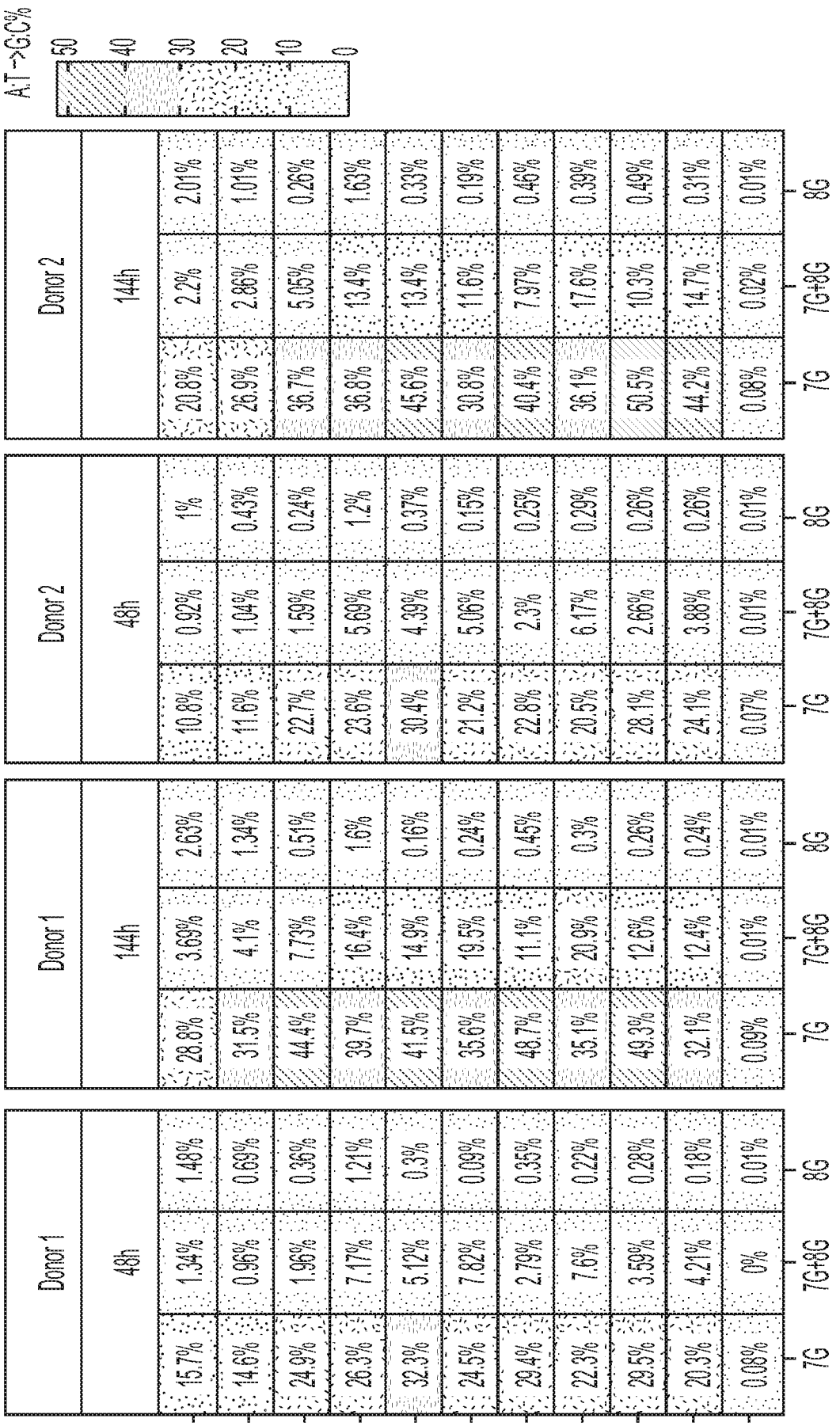

A.T ->G.C%
50
40
30
20
10
0
Donor 1
48h
144h
Donor 2
48h
144h
Donor 1
48h
144h
Donor 2
48h
144h
7G
7G+8G
8G
Target site edit
Treatment
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
AdE8.20-m
ABE8.20-d
Mock
15.7% 1.34% 1.48%
14.6% 0.96% 0.69%
24.9% 1.96% 0.36%
26.3% 7.17% 1.21%
32.3% 5.12% 0.3%
24.5% 7.82% 0.09%
29.4% 2.79% 0.35%
22.3% 7.6% 0.22%
29.5% 3.59% 0.28%
20.3% 4.21% 0.18%
0.08% 0% 0.01%
28.8% 3.69% 2.63%
31.5% 4.1% 1.34%
44.4% 7.73% 0.51%
39.7% 16.4% 1.6%
41.5% 14.9% 0.16%
35.6% 19.5% 0.24%
48.7% 11.1% 0.45%
35.1% 20.9% 0.3%
49.3% 12.6% 0.26%
32.1% 12.4% 0.24%
0.09% 0.01% 0.01%
10.8% 0.92% 1%
11.6% 1.04% 0.43%
22.7% 1.59% 0.24%
23.6% 5.69% 1.2%
30.4% 4.39% 0.37%
21.2% 5.06% 0.15%
22.8% 2.3% 0.25%
20.5% 6.17% 0.29%
28.1% 2.66% 0.26%
24.1% 3.88% 0.26%
0.07% 0.01% 0.01%
20.8% 2.2% 2.01%
26.9% 2.86% 1.01%
36.7% 5.05% 0.26%
36.8% 13.4% 1.63%
45.6% 13.4% 0.33%
30.8% 11.6% 0.19%
40.4% 7.97% 0.46%
36.1% 17.6% 0.39%
50.5% 10.3% 0.49%
44.2% 14.7% 0.31%
0.08% 0.02% 0.01%

FIG. 28A

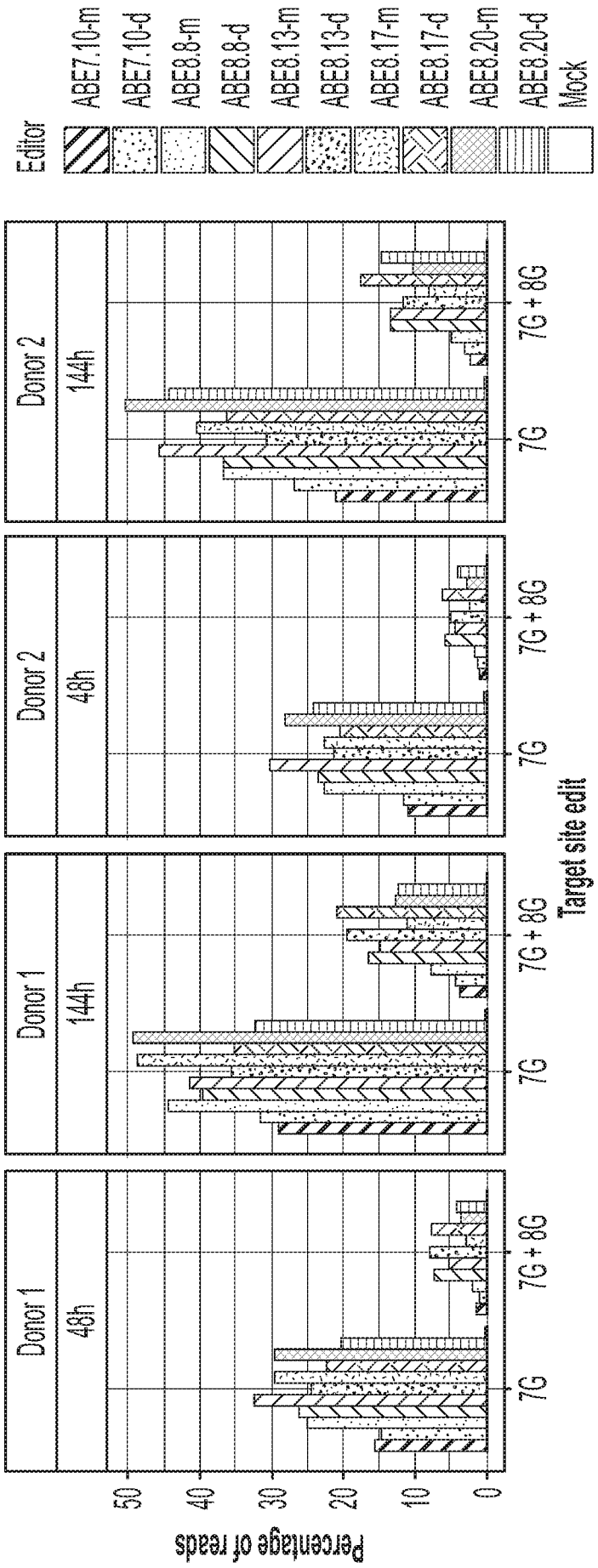

Editor
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
Mock
Donor 1
48h
Donor 1
144h
Donor 2
48h
Donor 2
144h
7G
7G + 8G
Target site edit
Percentage of reads
0
10
20
30
40
50

FIG. 28B

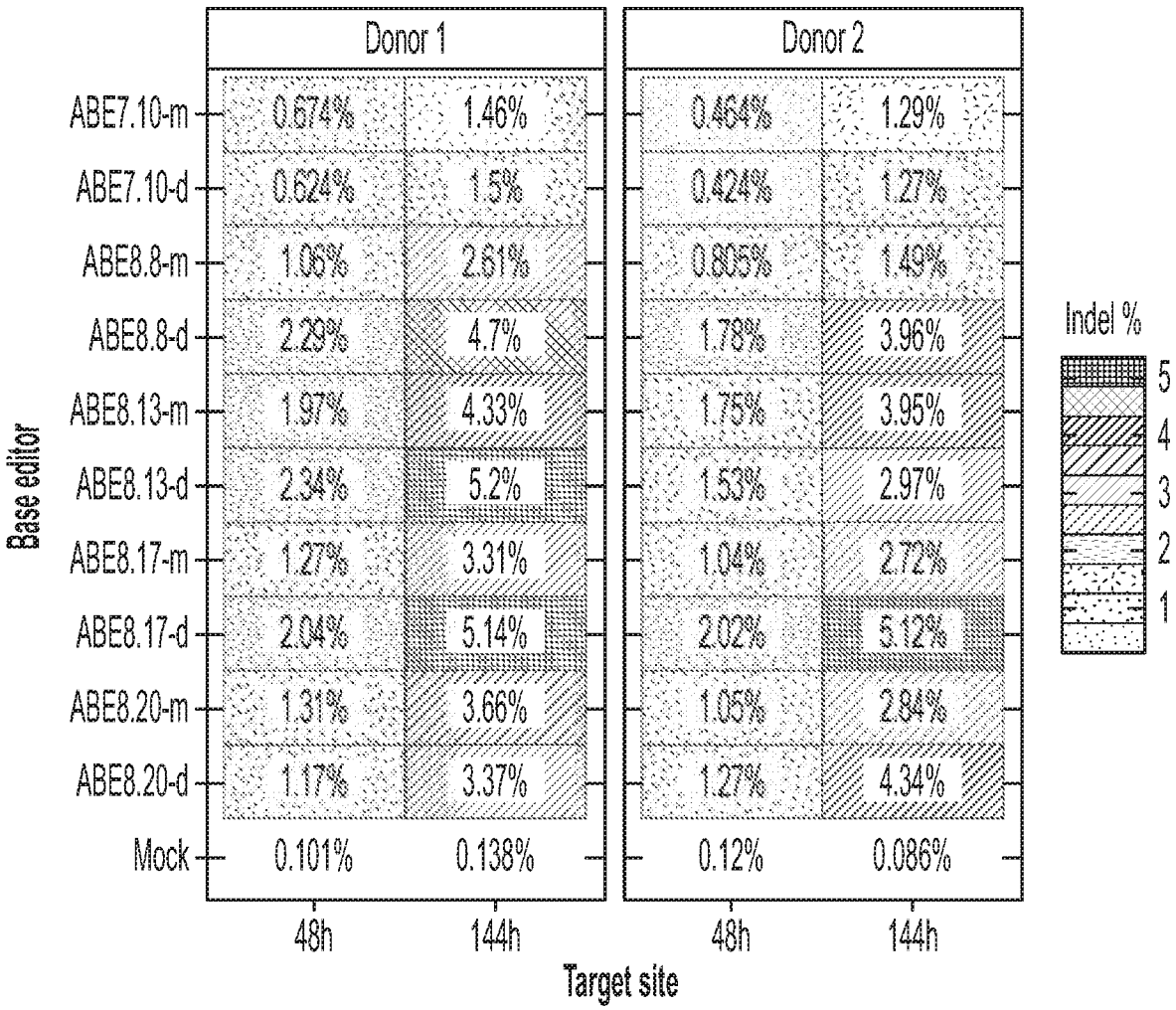
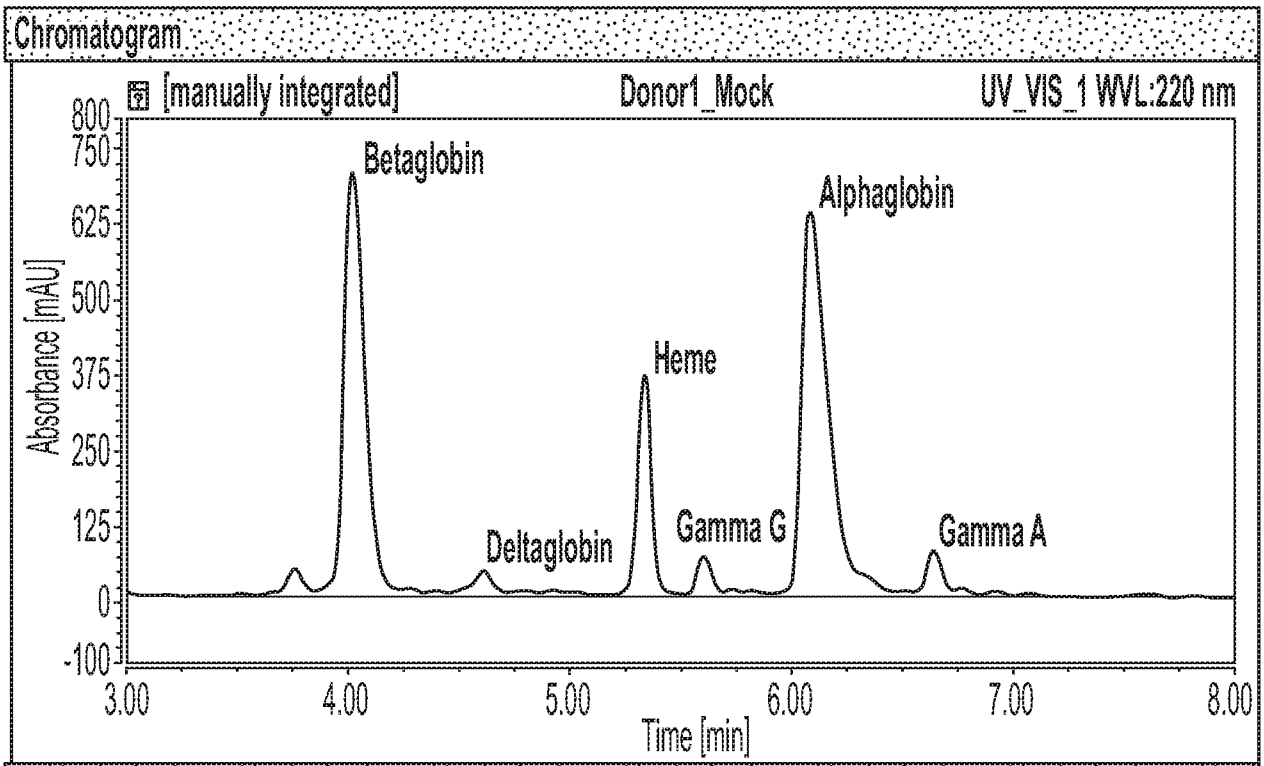

Donor 1
Donor 2
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
Mock
0.674%
1.46%
0.464%
1.29%
0.624%
1.5%
0.424%
1.27%
1.06%
2.61%
0.805%
1.49%
2.29%
4.7%
1.78%
3.96%
1.97%
4.33%
1.75%
3.95%
2.34%
5.2%
1.53%
2.97%
1.27%
3.31%
1.04%
2.72%
2.04%
5.14%
2.02%
5.12%
1.31%
3.66%
1.05%
2.84%
1.17%
3.37%
1.27%
4.34%
0.101%
0.138%
0.12%
0.086%
Base editor
48h
144h
48h
144h
Target site
Indel %
5
4
3
2
1

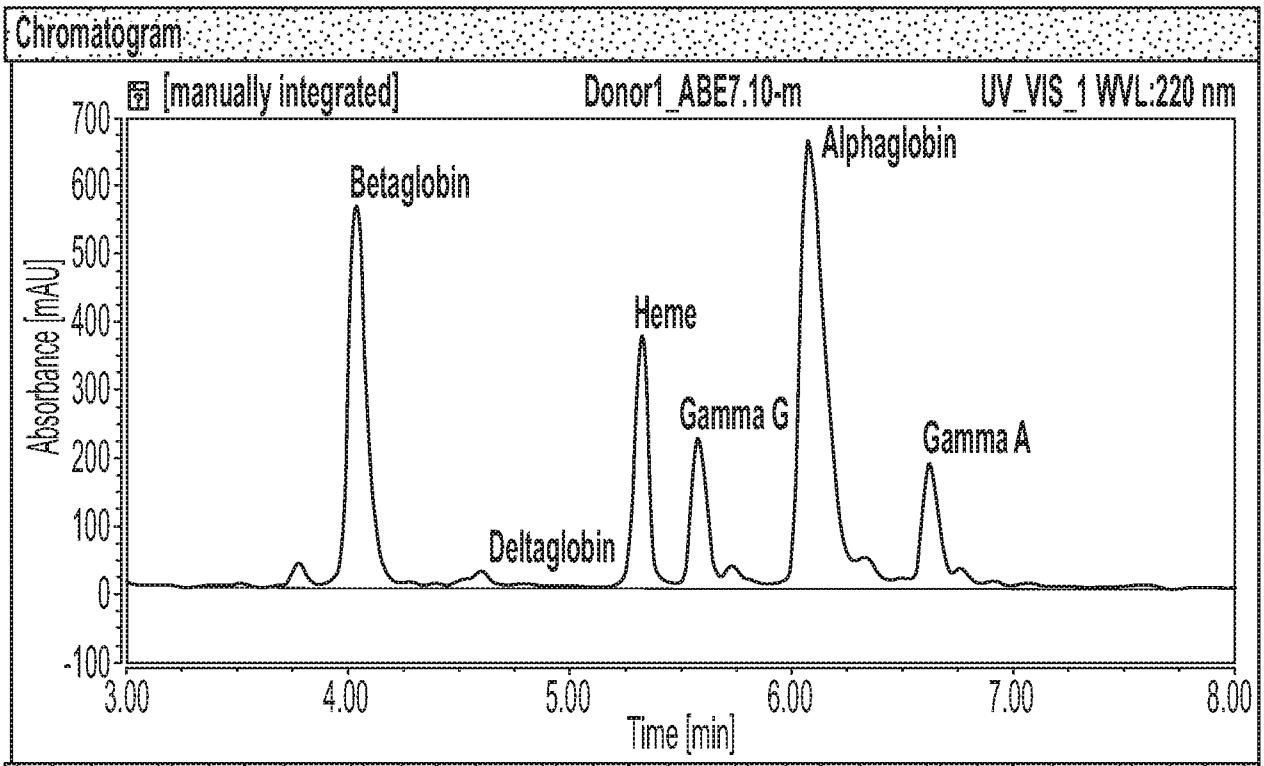
FIG. 29



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.020	77.631	701.634	33.61	32.98
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
10	Deltaglobin	4.610	3.780	42.943	1.64	2.02
14	Heme	5.337	27.191	366.542	11.77	17.23
15	Gamma G	5.603	5.248	66.622	2.27	3.13
18	Alphaglobin	6.083	88.477	636.811	38.31	29.94
21	Gamma A	6.637	6.751	77.427	2.92	3.64

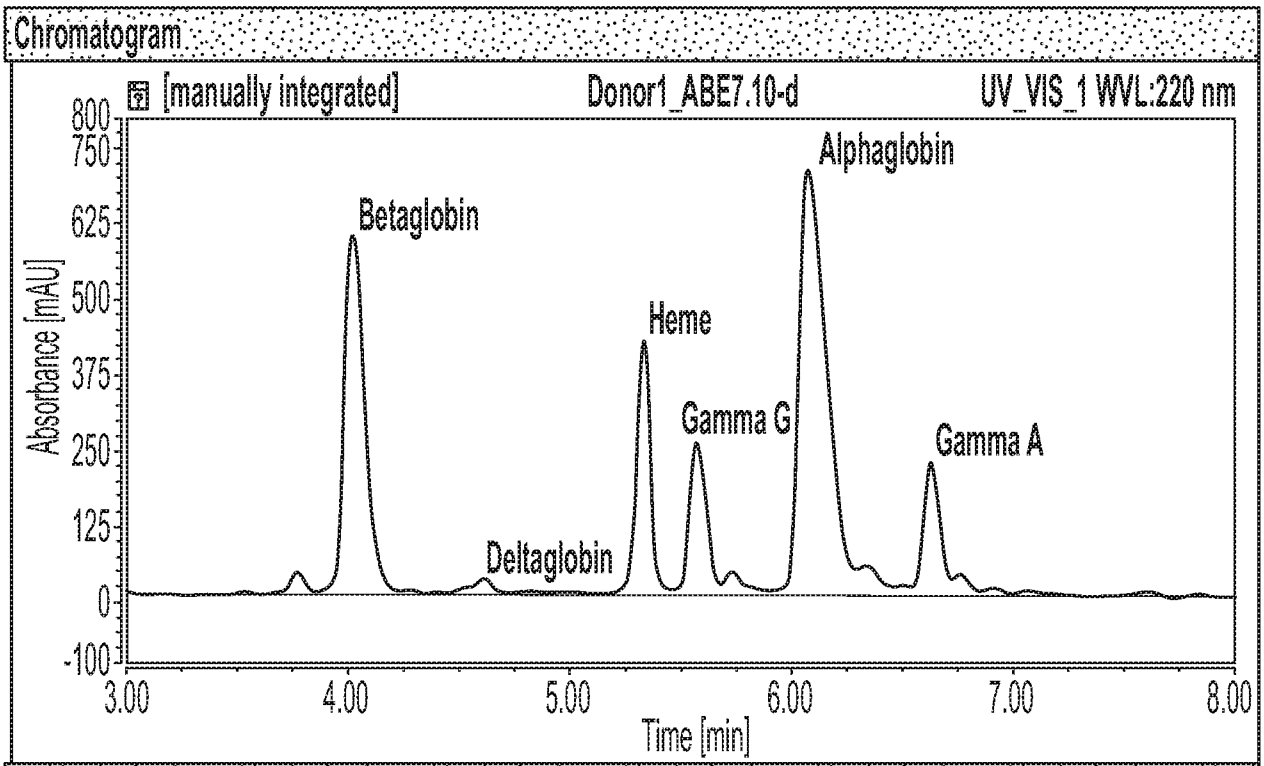
FIG. 30



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.043	56.404	561.657	24.21	24.80
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.607	2.463	24.382	1.06	1.08
13	Heme	5.330	27.334	371.802	11.73	16.42
14	Gamma G	5.583	18.764	222.410	8.06	9.82
16	Alphaglobin	6.080	88.236	657.695	37.88	29.04
19	Gamma A	6.627	16.470	185.055	7.07	8.17

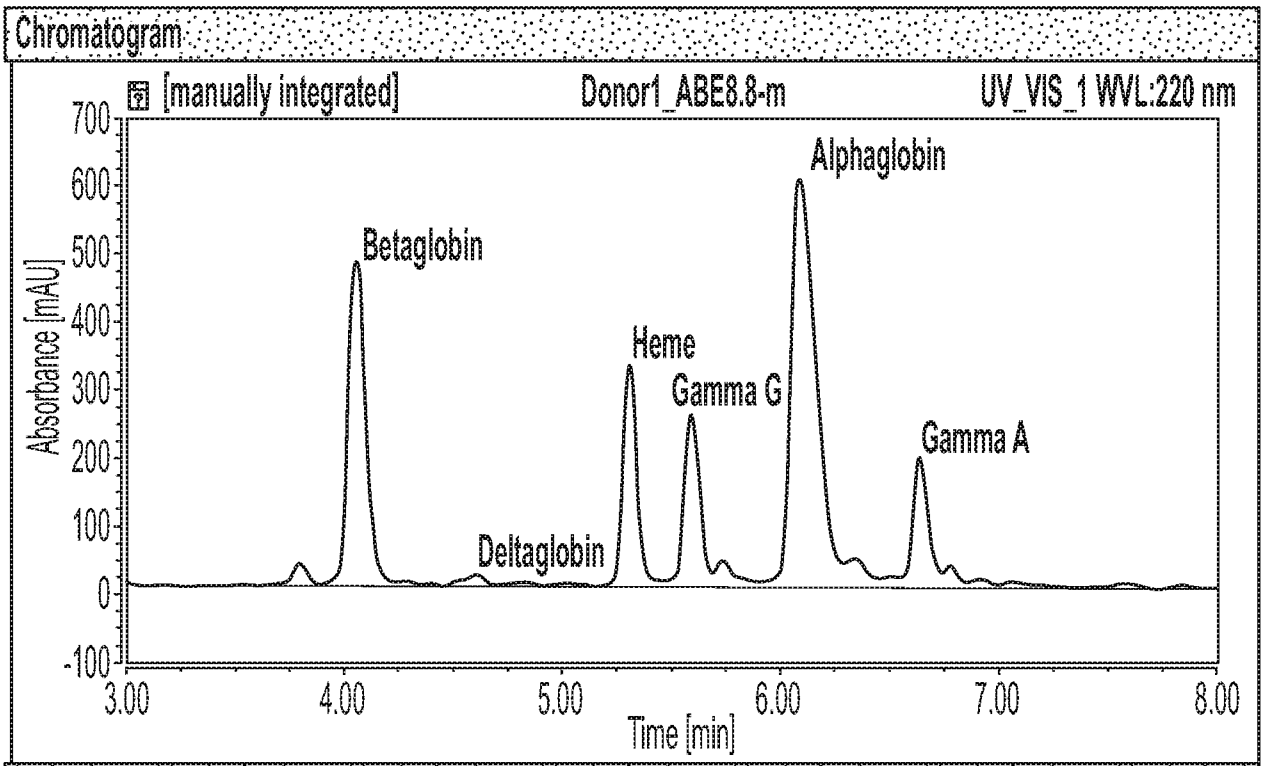
FIG. 31



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.020	63.540	596.818	23.88	23.74
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
10	Deltaglobin	4.613	2.822	27.354	1.06	1.09
14	Heme	5.333	31.163	421.735	11.71	16.77
15	Gamma G	5.570	22.558	253.399	8.48	10.08
18	Alphaglobin	6.070	100.612	704.910	37.81	28.03
21	Gamma A	6.623	19.220	220.846	7.22	8.78

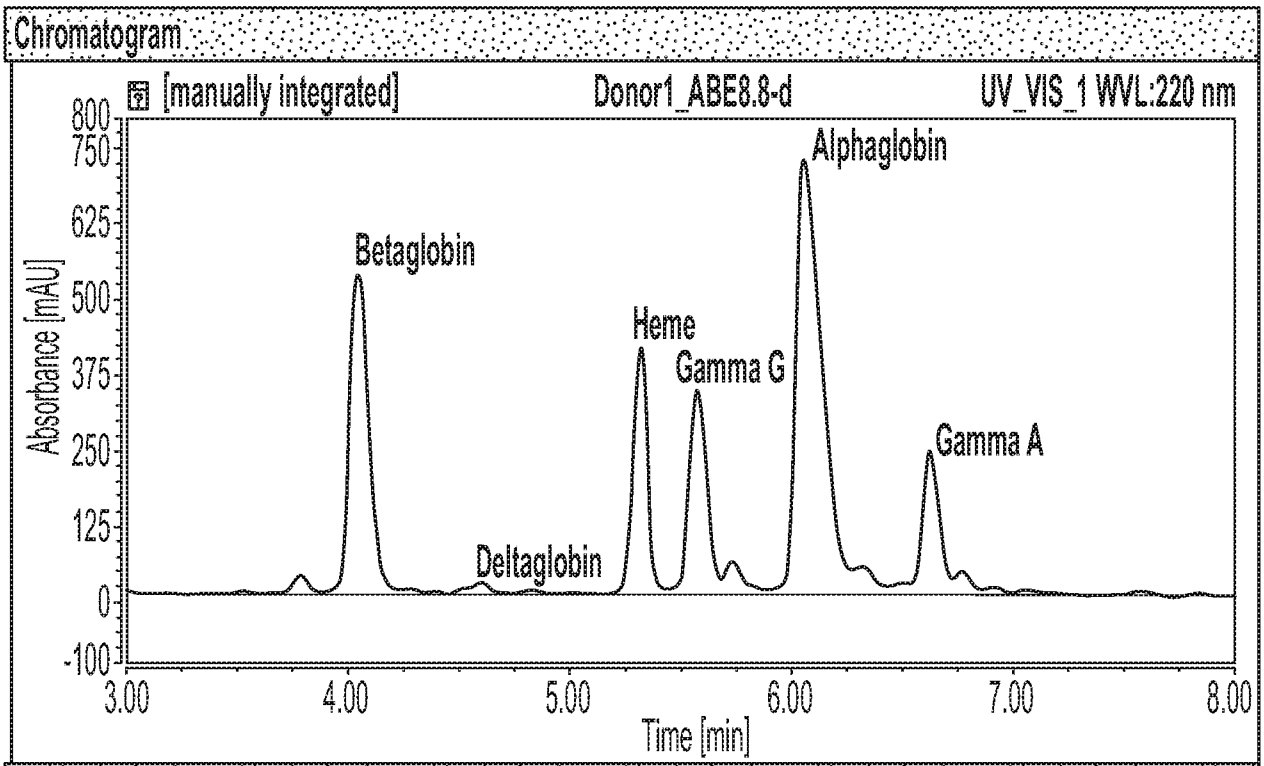
FIG. 32



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.053	44.227	479.648	21.03	22.78
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.607	1.704	17.056	0.81	0.81
13	Heme	5.310	24.221	326.068	11.52	15.48
14	Gamma G	5.590	21.346	256.462	10.15	12.18
16	Alphaglobin	6.087	79.706	602.792	37.90	28.62
19	Gamma A	6.640	16.631	192.016	7.91	9.12

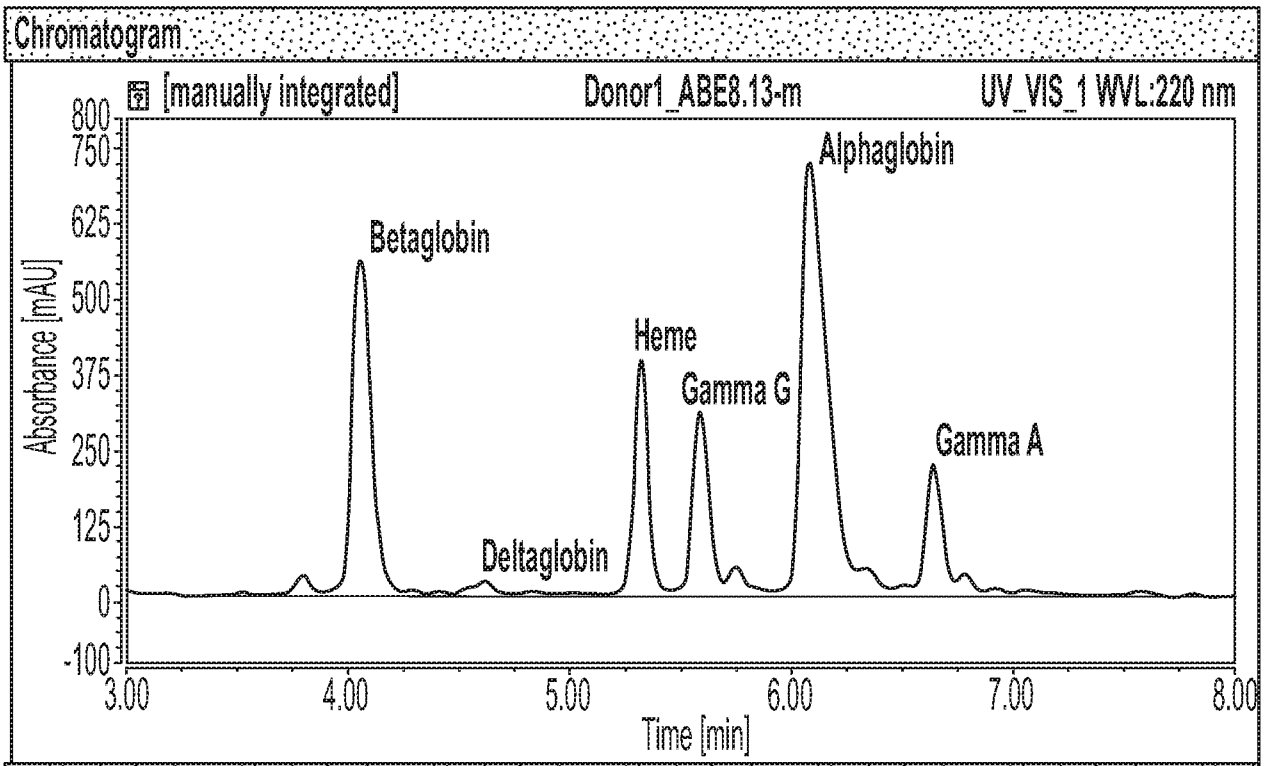
FIG. 33



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.043	51.802	530.669	19.60	20.80
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.597	2.269	21.040	0.86	0.82
13	Heme	5.323	30.637	410.481	11.59	16.09
14	Gamma G	5.573	30.747	340.883	11.63	13.36
16	Alphaglobin	6.060	98.417	721.350	37.24	28.28
19	Gamma A	6.627	21.595	239.647	8.17	9.39

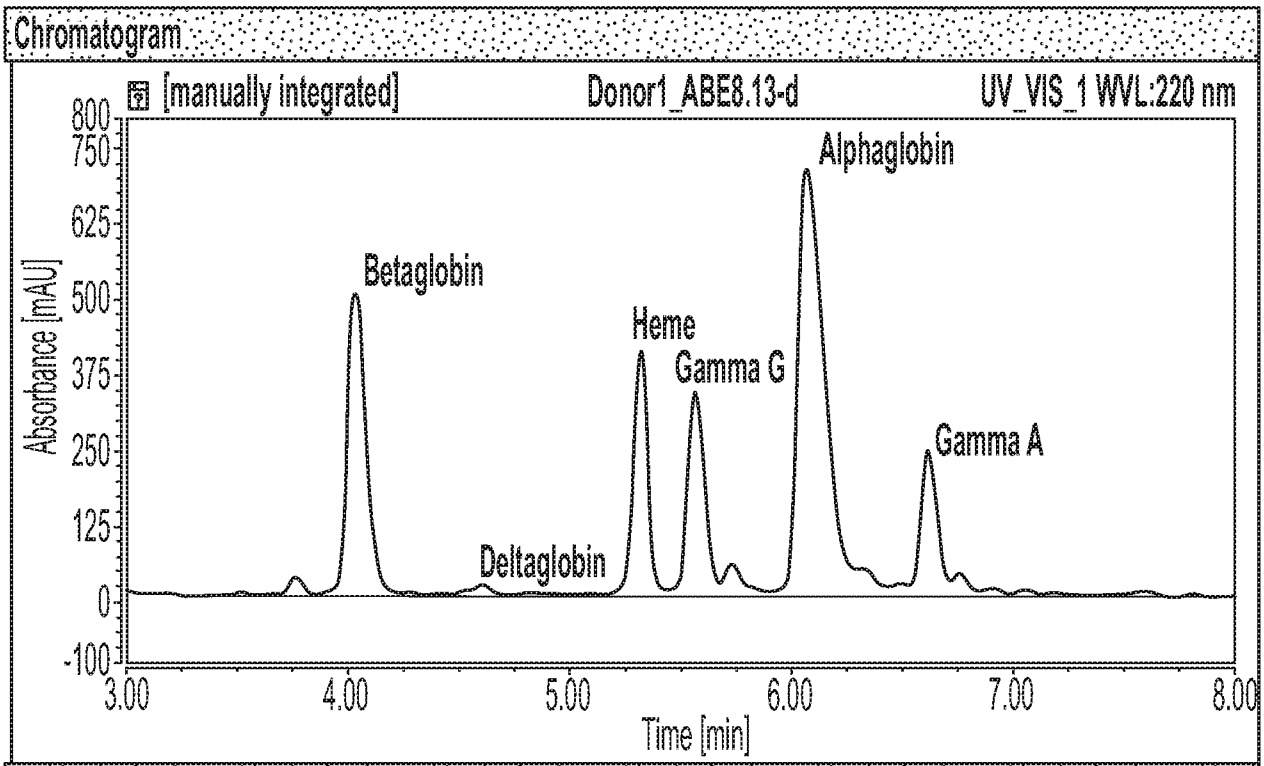
FIG. 34



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.047	55.084	554.598	21.23	22.22
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.613	2.417	24.372	0.93	0.98
13	Heme	5.320	29.500	393.066	11.37	15.75
14	Gamma G	5.580	27.177	306.655	10.48	12.29
16	Alphaglobin	6.073	98.048	719.982	37.79	28.85
19	Gamma A	6.630	19.949	219.869	7.69	8.81

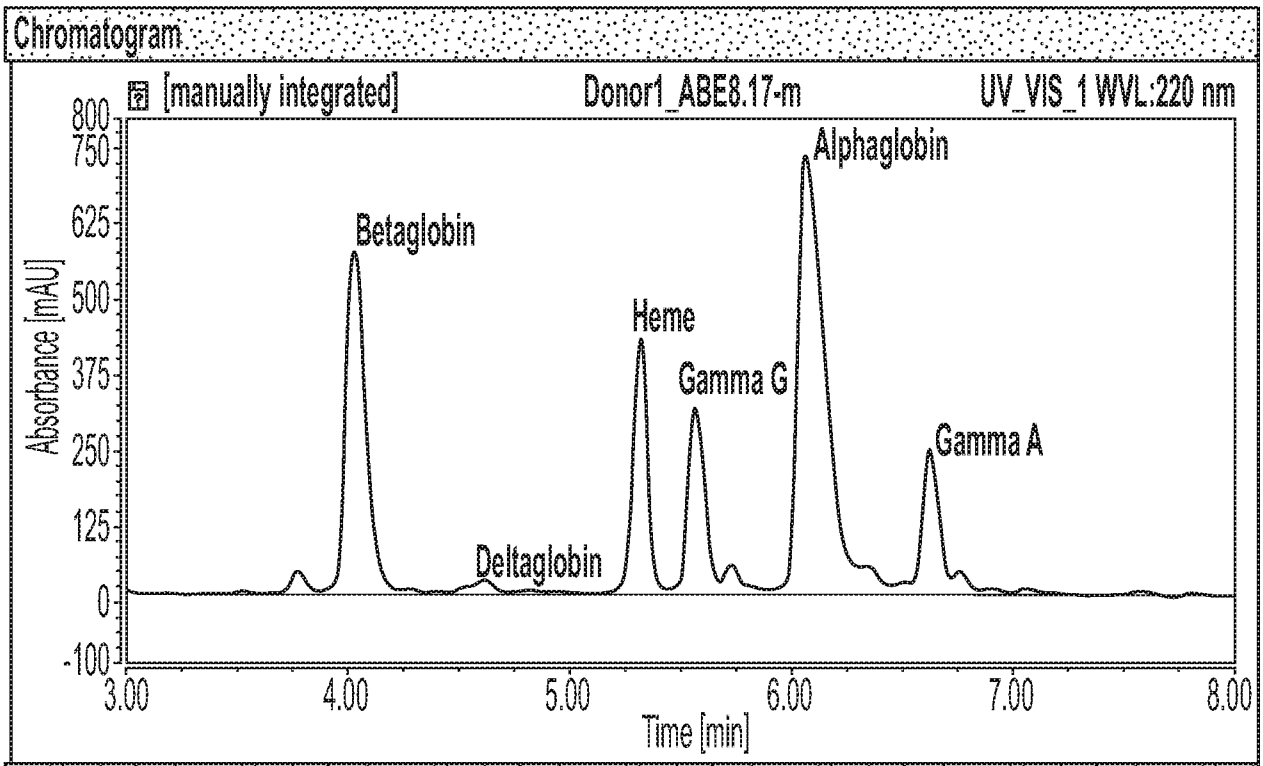
FIG. 35



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.037	49.284	501.306	19.04	20.14
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.610	1.960	18.855	0.76	0.76
13	Heme	5.327	30.274	407.530	11.69	16.37
14	Gamma G	5.570	31.059	339.163	12.00	13.63
16	Alphaglobin	6.070	98.109	707.464	37.89	28.42
19	Gamma A	6.617	21.869	243.715	8.45	9.79

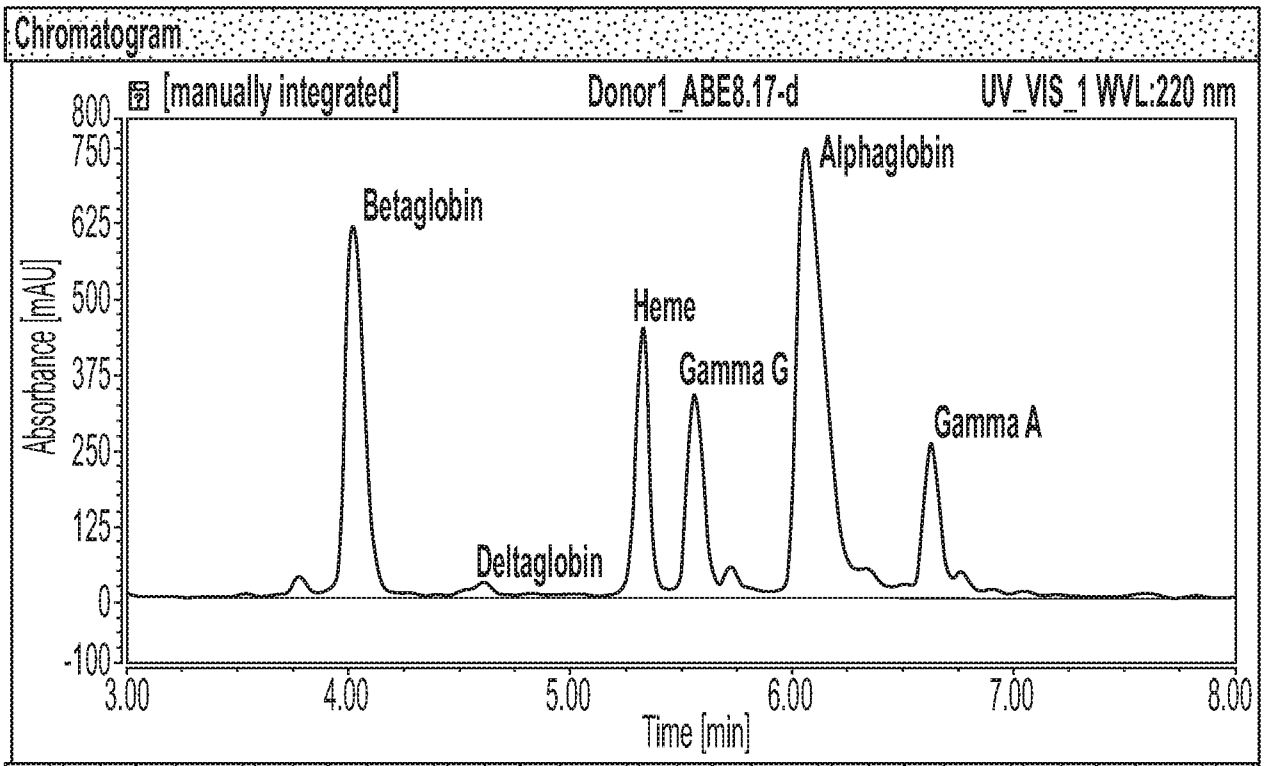
FIG. 36



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.030	59.000	569.475	21.35	21.91
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.617	2.483	24.660	0.90	0.95
13	Heme	5.323	31.792	426.360	11.50	16.40
14	Gamma G	5.567	28.258	310.274	10.22	11.94
16	Alphaglobin	6.067	104.586	728.120	37.84	28.01
19	Gamma A	6.627	21.512	243.710	7.78	9.37

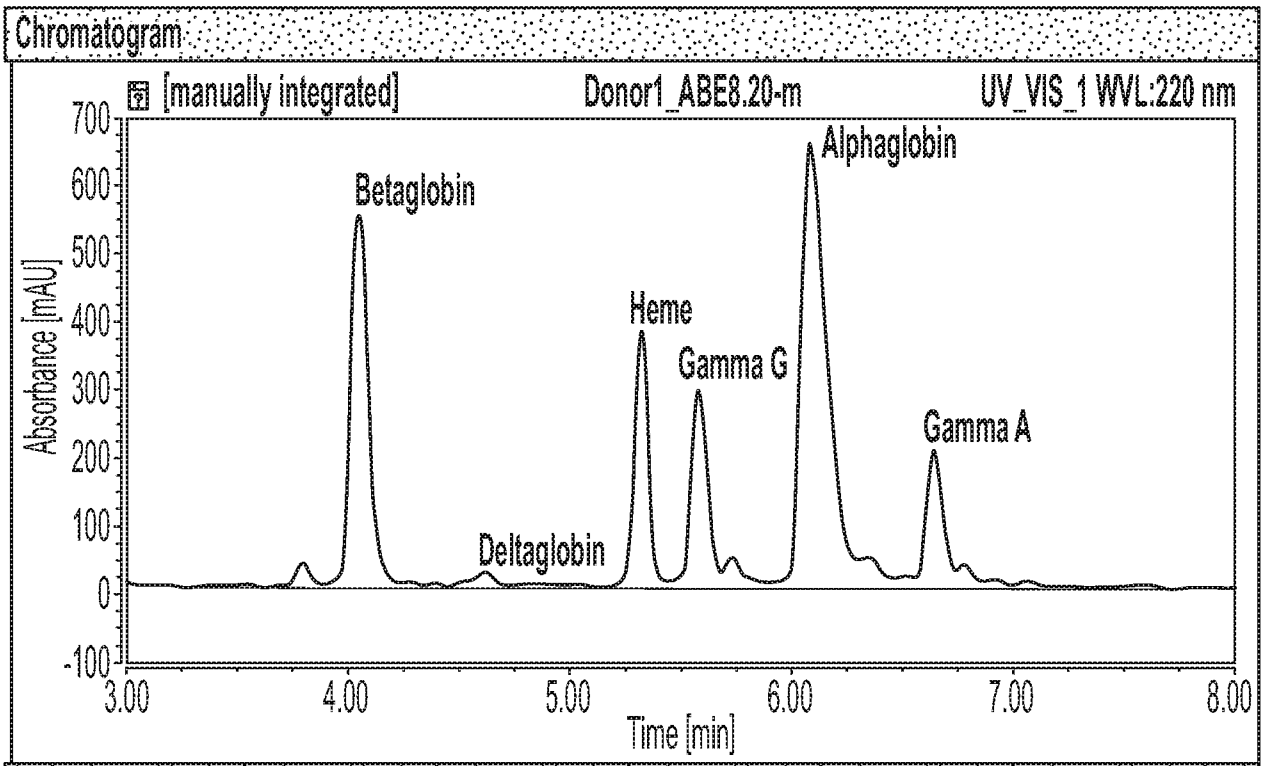
FIG. 37



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.023	60.046	613.639	21.18	22.63
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
10	Deltaglobin	4.610	2.491	24.450	0.88	0.90
14	Heme	5.330	33.236	446.899	11.72	16.48
15	Gamma G	5.560	29.840	335.709	10.52	12.38
17	Alphaglobin	6.063	106.077	742.712	37.41	27.38
20	Gamma A	6.627	22.570	257.472	7.96	9.49

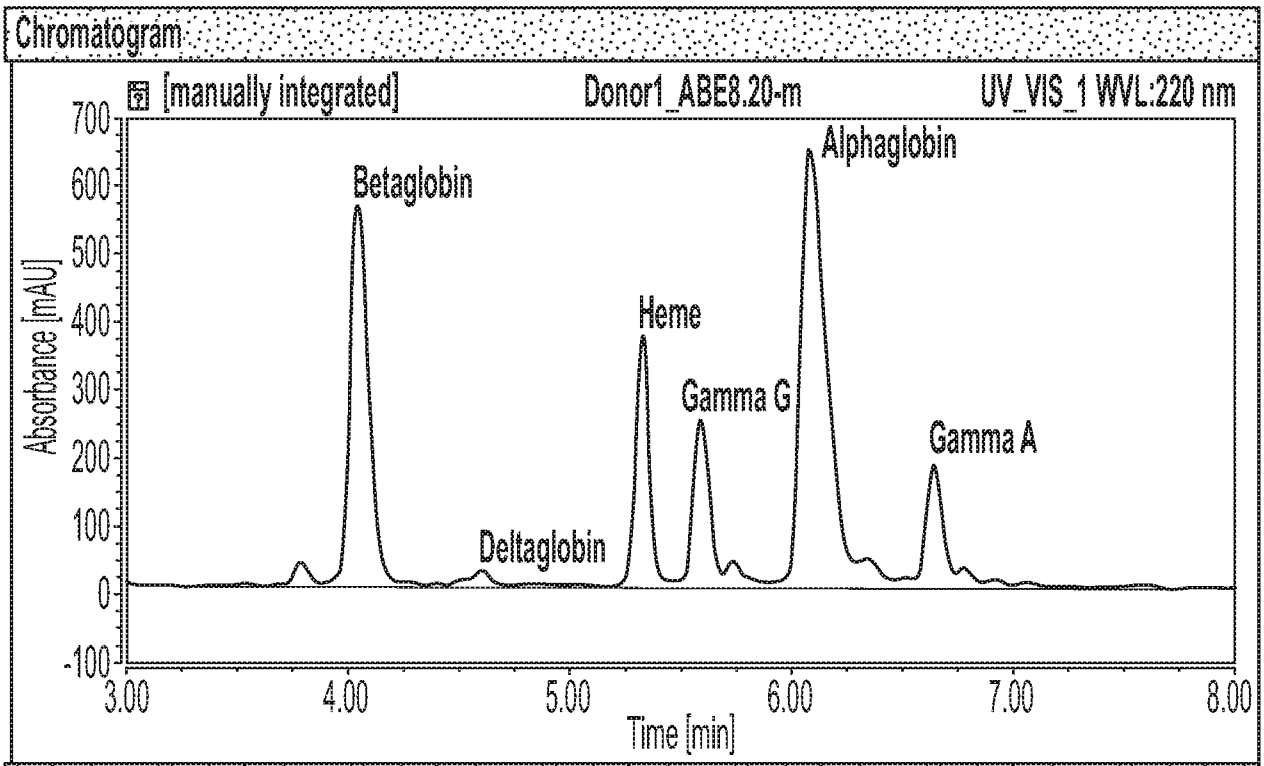
FIG. 38



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.040	51.610	548.050	21.57	23.31
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.610	2.233	21.329	0.93	0.91
13	Heme	5.320	27.916	377.668	11.67	16.06
14	Gamma G	5.573	24.931	291.767	10.42	12.41
16	Alphaglobin	6.077	90.099	653.942	37.66	27.81
19	Gamma A	6.637	17.798	203.835	7.44	8.67

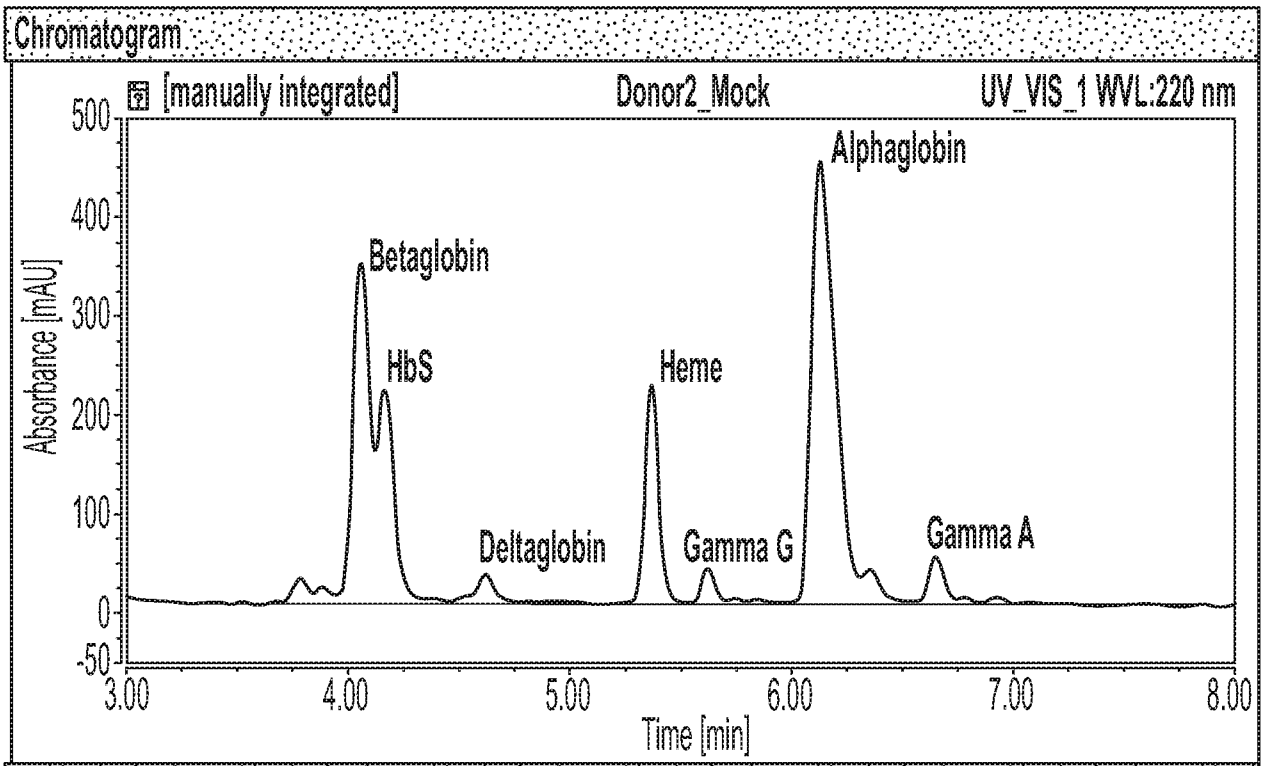
FIG. 39



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.040	55.490	562.369	23.82	24.71
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
10	Deltaglobin	4.600	2.399	23.713	1.03	1.04
14	Heme	5.327	27.308	371.229	11.72	16.31
15	Gamma G	5.587	20.808	248.715	8.93	10.93
17	Alphaglobin	6.073	87.517	645.669	37.56	28.38
20	Gamma A	6.637	15.700	180.637	6.74	7.94

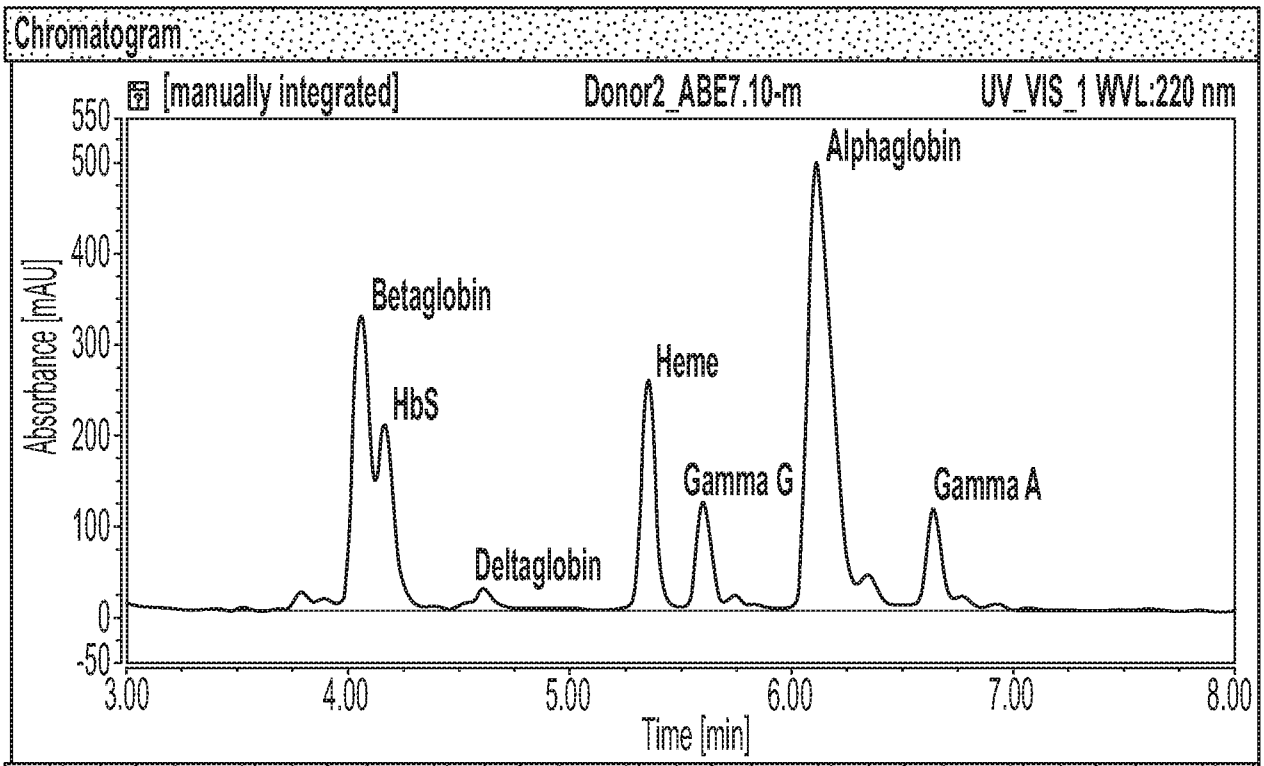
FIG. 40



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.057	28.943	345.410	20.25	23.19
6	HbS	4.160	19.794	216.879	13.85	14.56
9	Deltaglobin	4.617	3.170	30.593	2.22	2.05
11	Heme	5.367	16.498	222.155	11.54	14.91
12	Gamma G	5.620	2.805	37.002	1.96	2.48
15	Alphaglobin	6.127	54.386	448.385	38.05	30.10
18	Gamma A	6.647	4.170	49.520	2.92	3.32

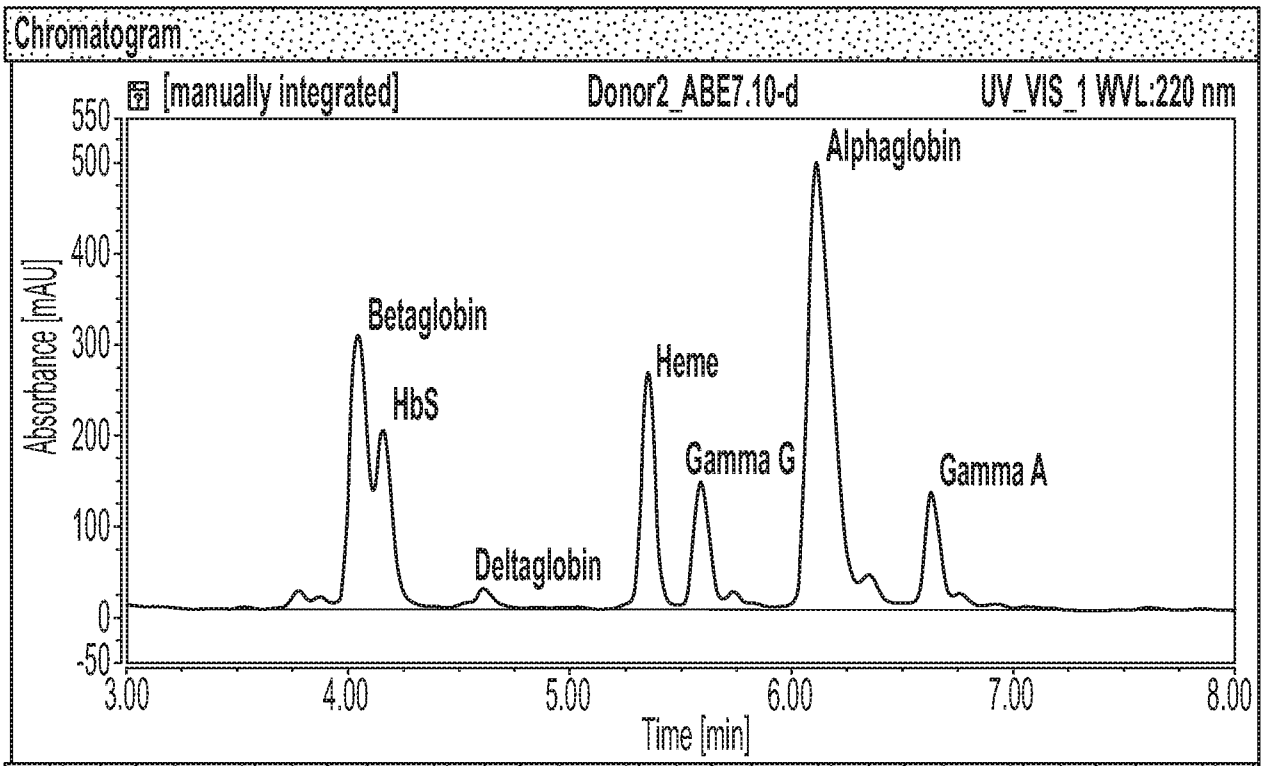
FIG. 41



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.057	27.210	325.305	16.96	19.17
6	HbS	4.163	18.038	204.594	11.24	12.05
9	Deltaglobin	4.610	2.458.	24.147	1.53	1.42
14	Heme	5.353	18.788	253.663	11.71	14.94
15	Gamma G	5.600	9.475	120.558	5.91	7.10
18	Alphaglobin	6.110	60.688	493.180	37.83	29.06
21	Gamma A	6.637	9.526	113.714	5.94	6.70

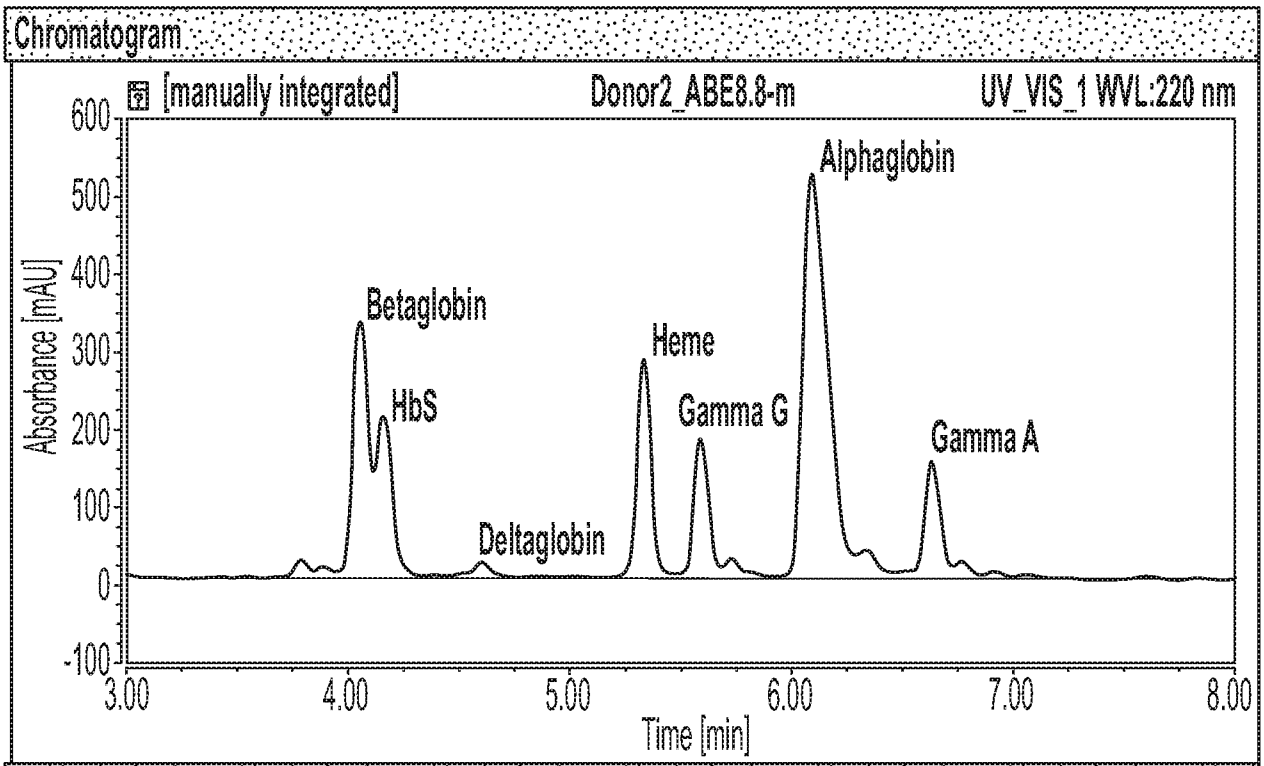
FIG. 42



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.050	26.525	303.461	15.99	17.61
6	HbS	4.160	17.741	200.547	10.70	11.64
9	Deltaglobin	4.620	2.209	22.450	1.33	1.30
14	Heme	5.357	19.493	262.669	11.75	15.24
15	Gamma G	5.593	11.528	141.729	6.95	8.23
18	Alphaglobin	6.113	62.859	497.019	37.90	28.85
21	Gamma A	6.630	10.827	131.062	6.53	7.61

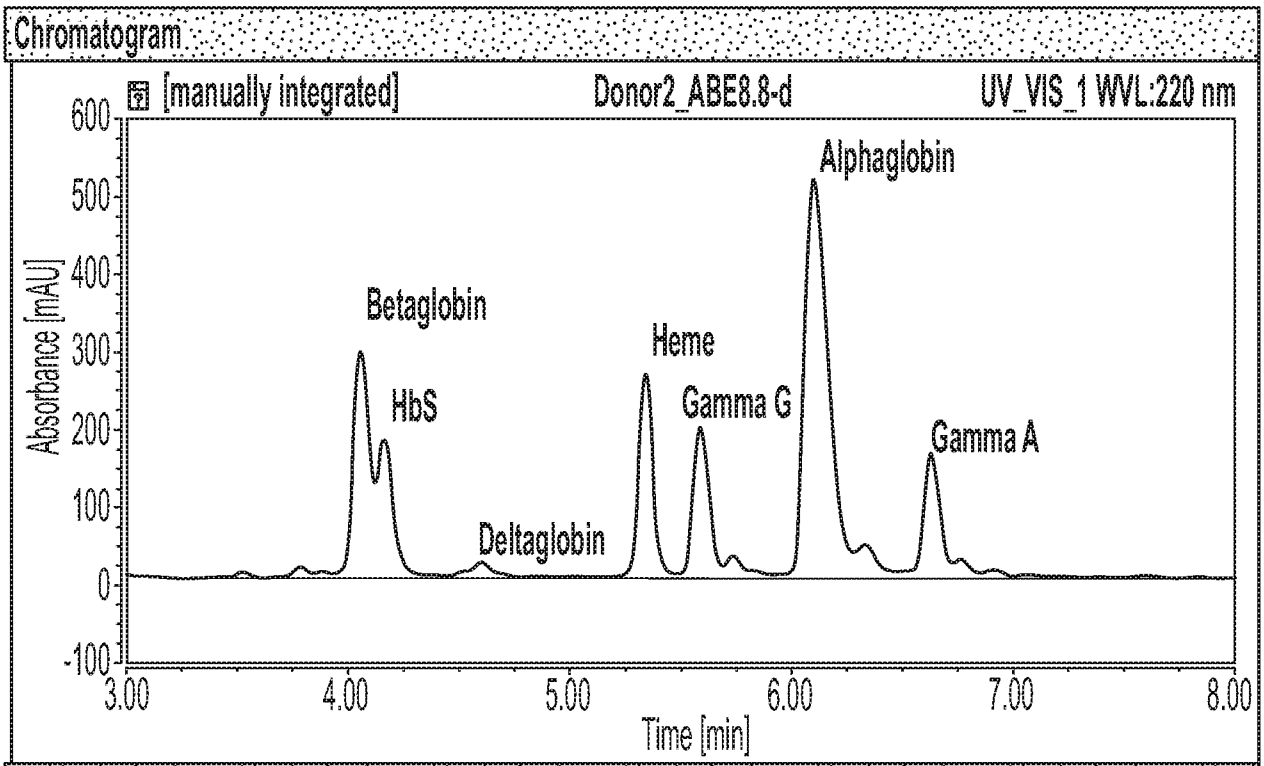
FIG. 43



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.057	26.492	330.353	14.76	17.48
6	HbS	4.160	17.650	209.154	9.84	11.07
9	Deltaglobin	4.603	2.190	21.674	1.22	1.15
14	Heme	5.340	20.930	280.930	11.66	14.87
15	Gamma G	5.593	14.357	180.573	8.00	9.56
18	Alphaglobin	6.097	67.616	524.013	37.68	27.73
21	Gamma A	6.637	12.935	152.286	7.21	8.06

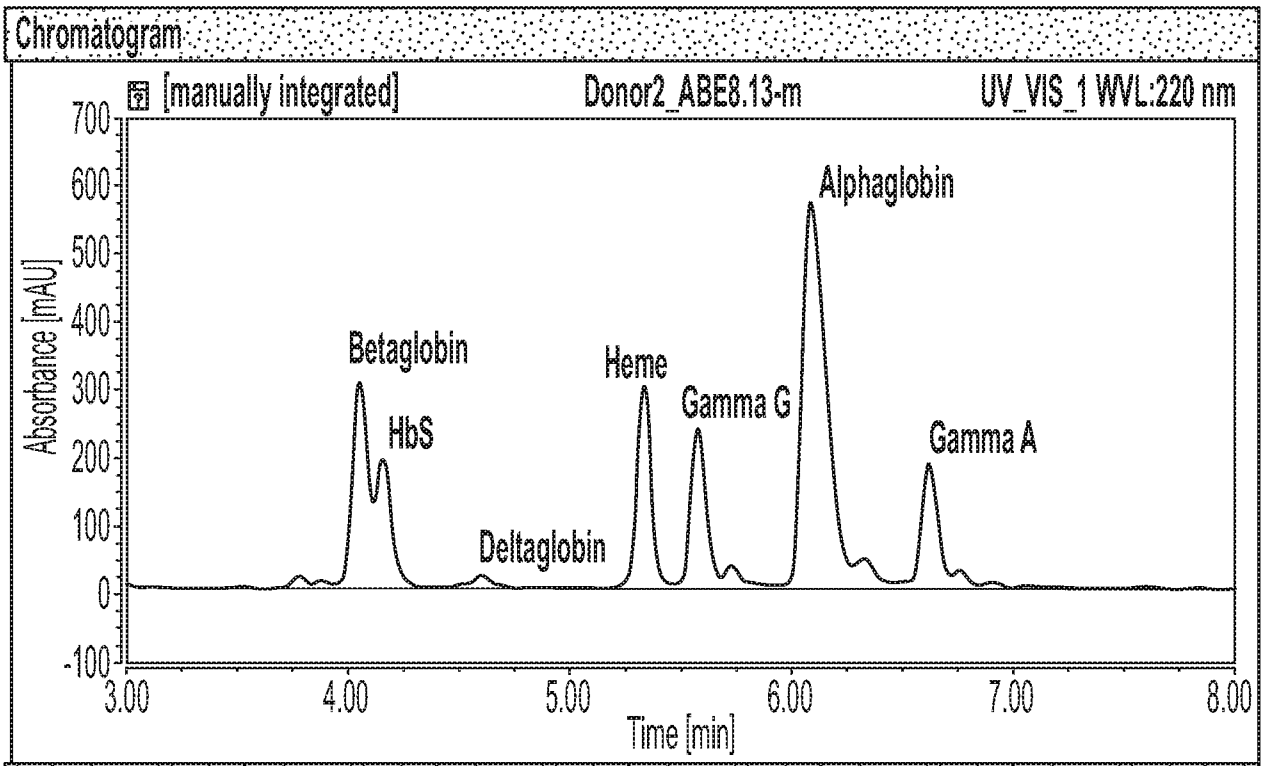
FIG. 44



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.063	23.888	291.371	13.90	16.06
7	HbS	4.167	15.512	178.139	9.02	9.82
10	Deltaglobin	4.607	2.249	20.296	1.31	1.12
15	Heme	5.343	19.534	262.940	11.36	14.49
16	Gamma G	5.593	16.034	195.308	9.33	10.77
19	Alphaglobin	6.103	63.055	512.839	36.68	28.27
22	Gamma A	6.633	13.860	161.449	8.06	8.90

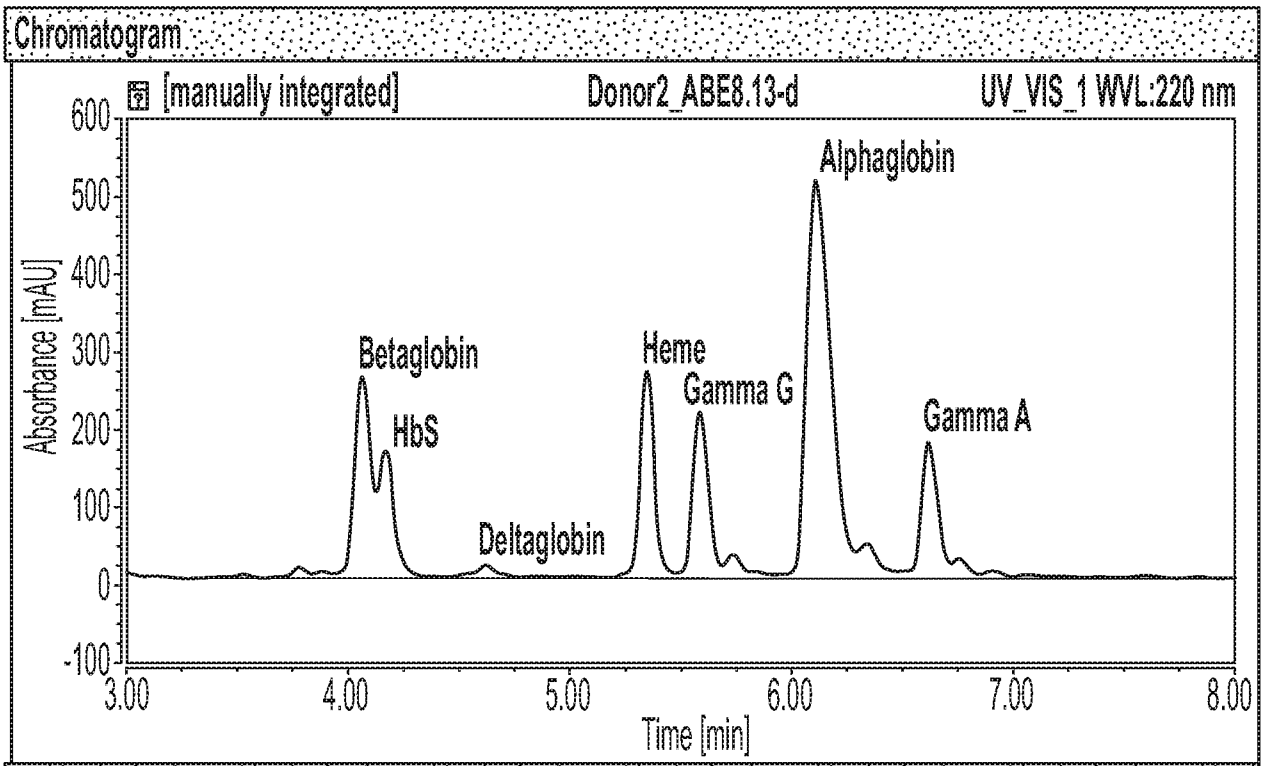
FIG. 45



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.057	24.649	303.680	12.77	15.05
7	HbS	4.163	16.705	190.174	8.65	9.43
10	Deltaglobin	4.607	2.114	20.478	1.10	1.01
15	Heme	5.340	22.158	298.385	11.48	14.79
16	Gamma G	5.583	20.107	236.242	10.41	11.71
19	Alphaglobin	6.093	71.587	568.640	37.08	28.18
22	Gamma A	6.623	16.259	184.774	8.42	9.16

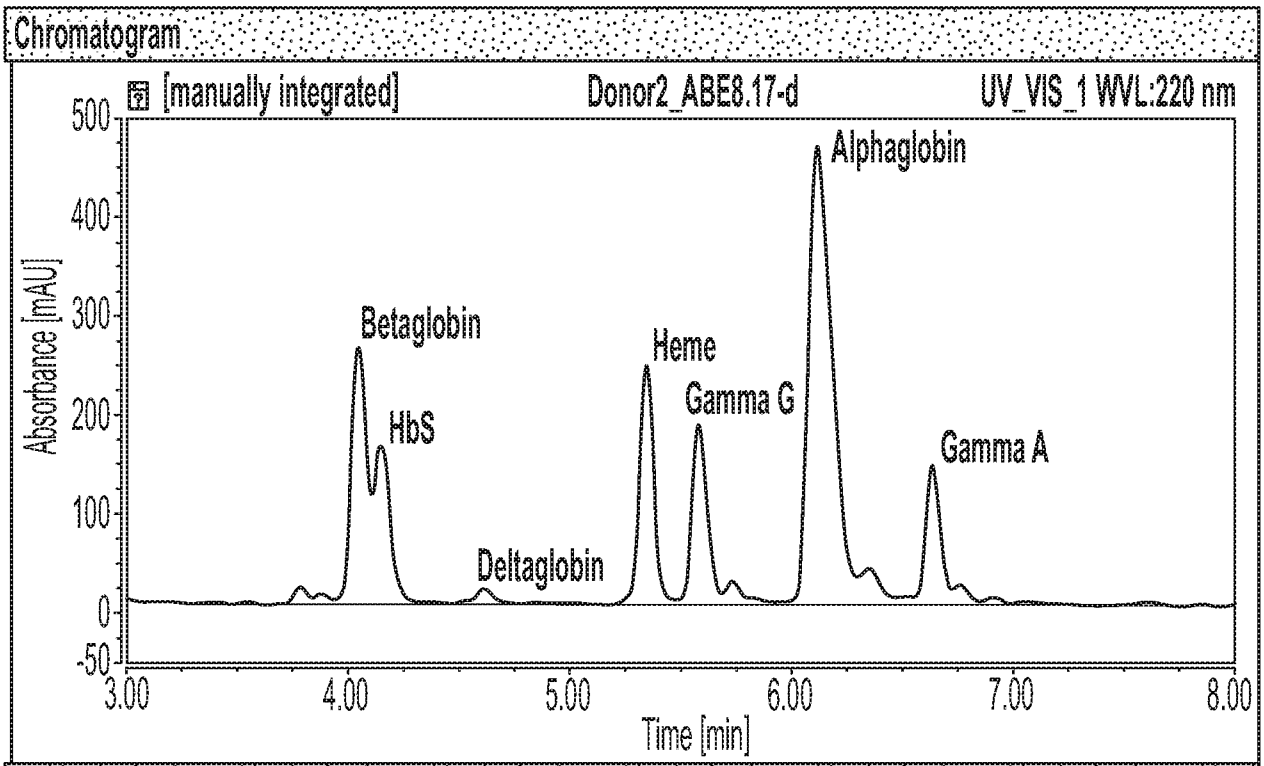
FIG. 46



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.057	21.912	260.274	12.79	14.49
6	HbS	4.163	13.772	163.531	8.04	9.10
9	Deltaglobin	4.617	1.827	16.318	1.07	0.91
13	Heme	5.347	19.932	268.078	11.64	14.92
14	Gamma G	5.587	18.026	215.798	10.52	12.01
17	Alphaglobin	6.110	64.064	513.833	37.40	28.61
20	Gamma A	6.620	15.335	177.074	8.95	9.86

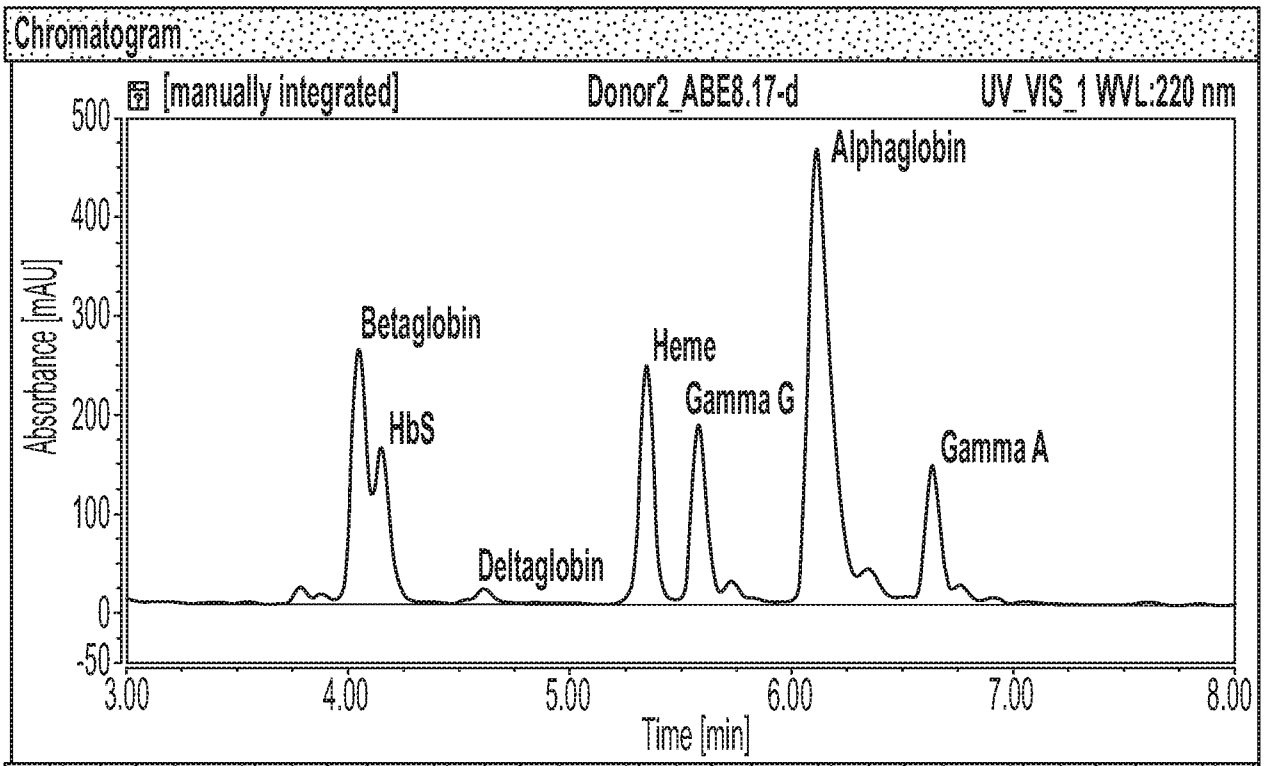
FIG. 47



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.050	20.688	260.391	13.65	15.97
6	HbS	4.150	13.530	159.994	8.93	9.81
9	Deltaglobin	4.617	1.590	15.794	1.05	0.97
14	Heme	5.350	18.069	242.095	11.92	14.85
15	Gamma G	5.583	14.825	182.475	9.78	11.19
18	Alphaglobin	6.117	57.055	464.210	37.65	28.48
21	Gamma A	6.637	11.691	142.851	7.72	8.76

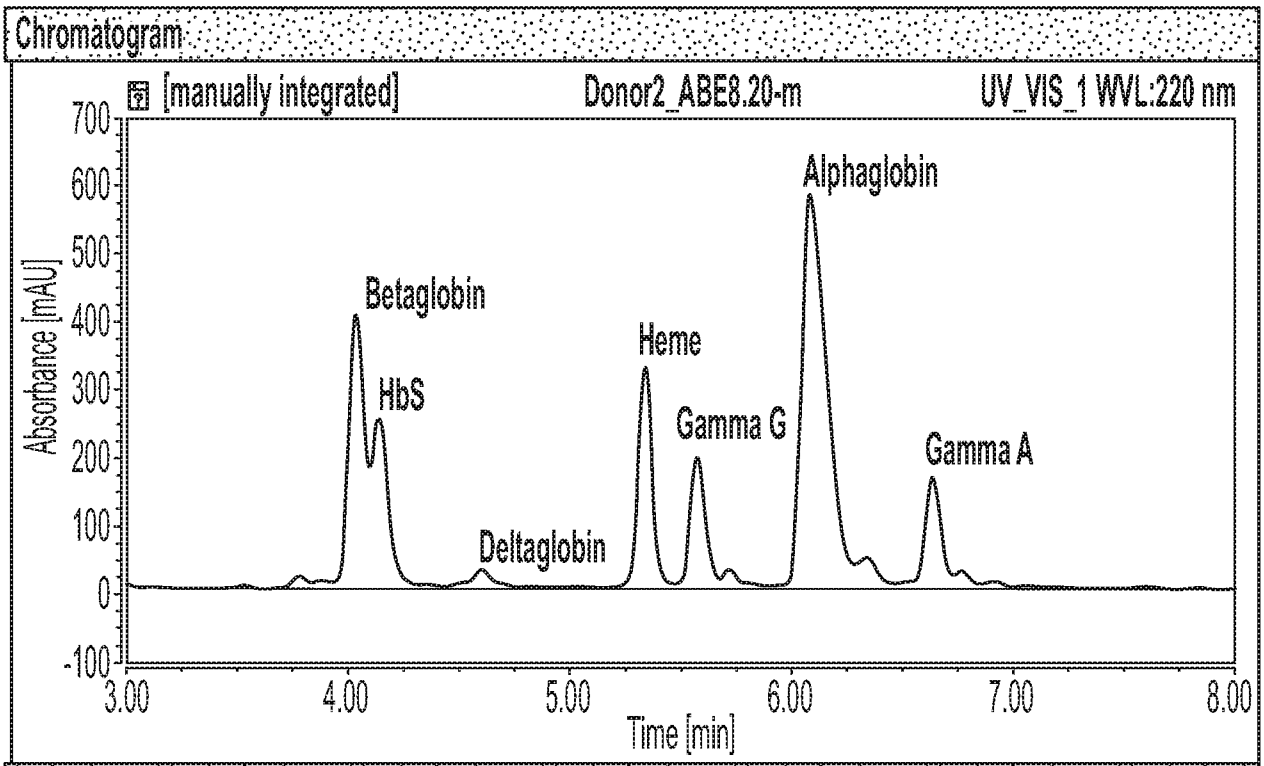
FIG. 48



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.050	20.688	260.391	13.65	15.97
6	HbS	4.150	13.530	159.994	8.93	9.81
9	Deltaglobin	4.617	1.590	15.794	1.05	0.97
14	Heme	5.350	18.069	242.095	11.92	14.85
15	Gamma G	5.583	14.825	182.475	9.78	11.19
18	Alphaglobin	6.117	57.055	464.210	37.65	28.48
21	Gamma A	6.637	11.691	142.851	7.72	8.76

FIG. 49



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.040	33.339	402.775	15.75	18.52
7	HbS	4.143	22.030	252.466	10.41	11.61
10	Deltaglobin	4.607	3.256	29.080	1.54	1.34
14	Heme	5.343	24.399	328.365	11.53	15.10
15	Gamma G	5.580	15.959	197.001	7.54	9.06
18	Alphaglobin	6.087	78.149	581.487	36.93	26.74
21	Gamma A	6.637	14.315	166.369	6.76	7.65

FIG. 50

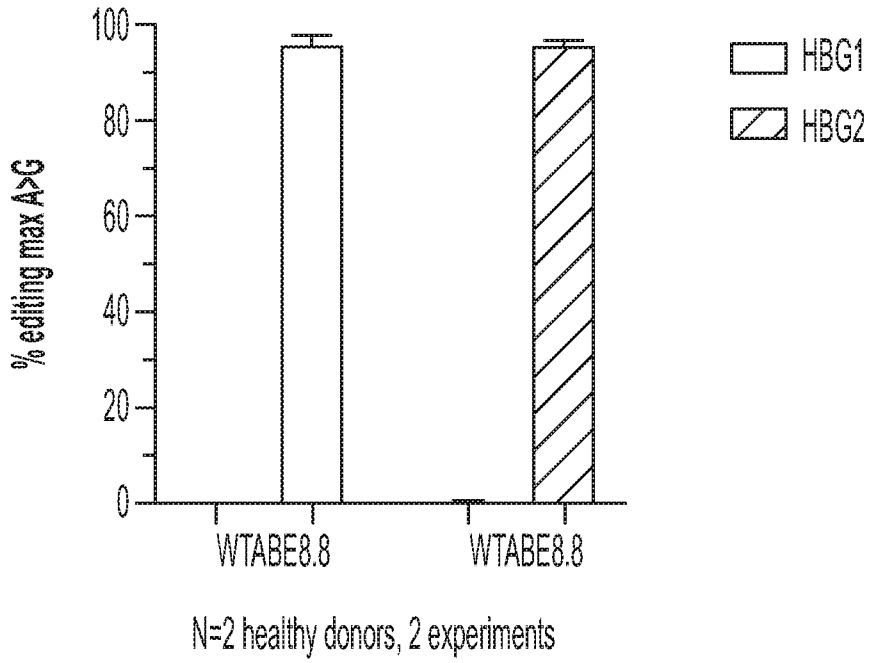


FIG. 51A

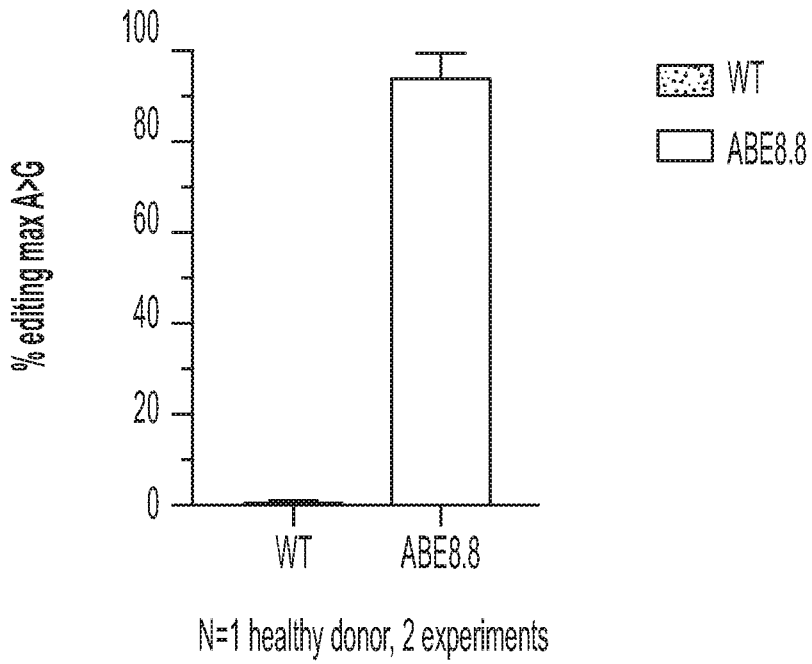


FIG. 51B

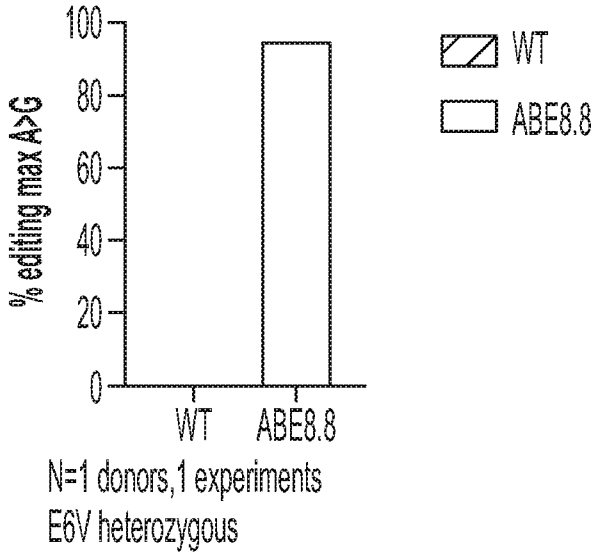


FIG. 51C

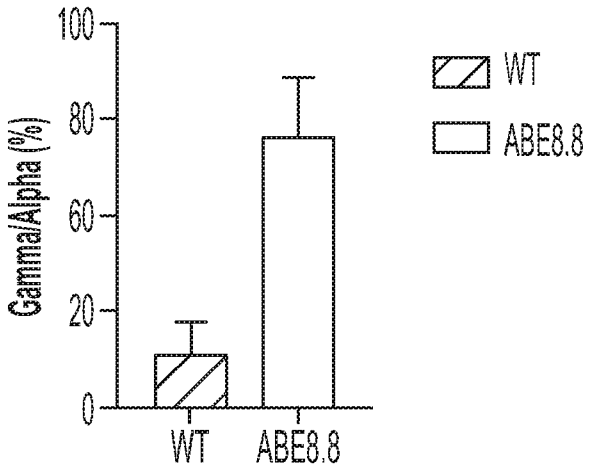


FIG. 51D

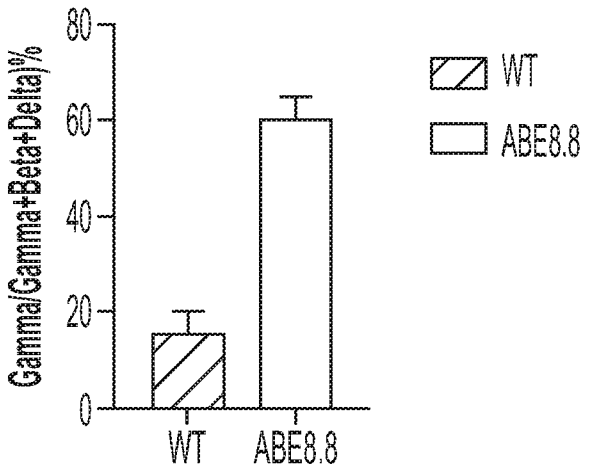


FIG. 51E

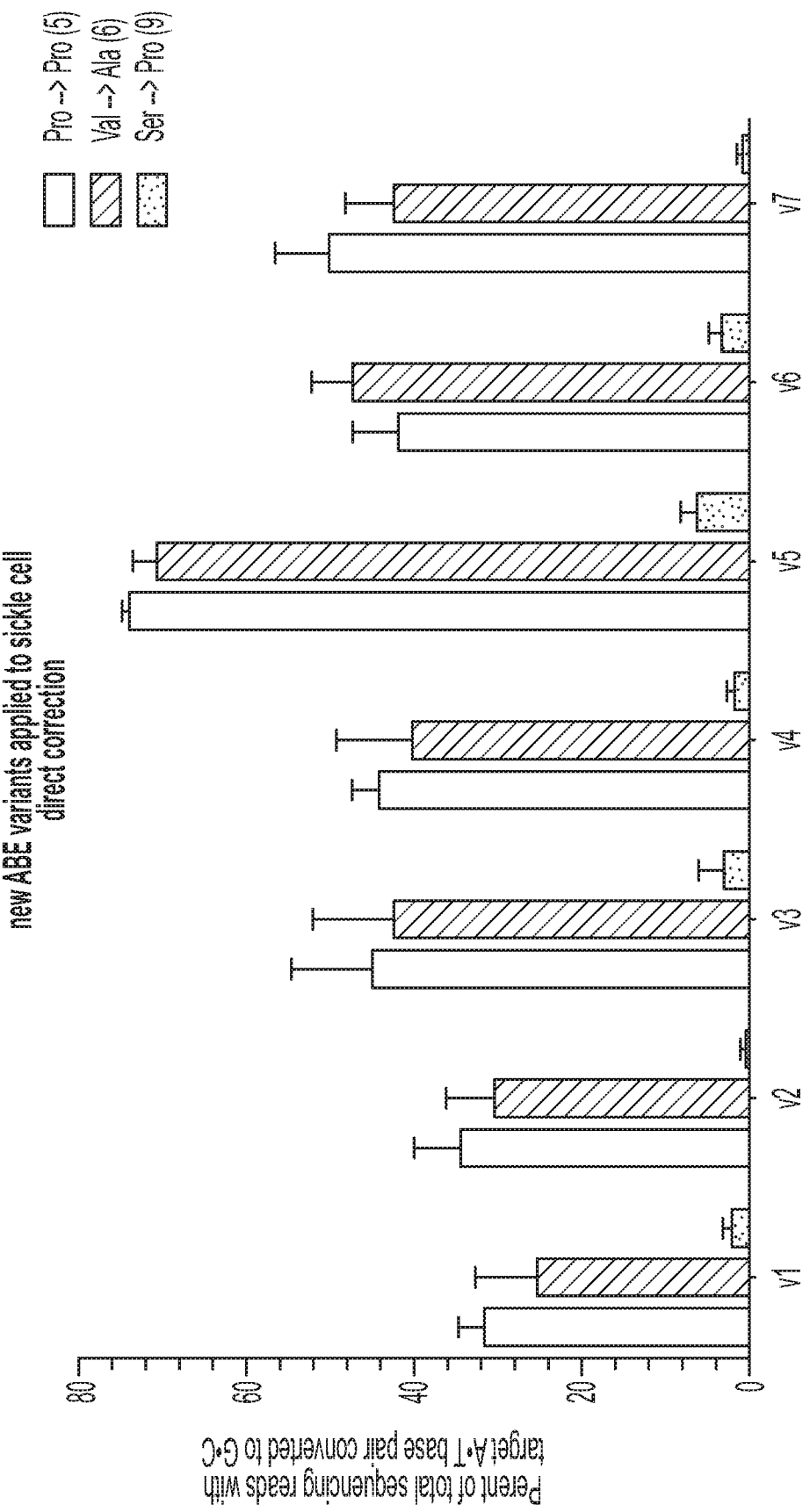

Pro --> Pro (5)
Val --> Ala (6)
Ser --> Pro (9)
new ABE variants applied to sickle cell direct correction
Percent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
v1
v2
v3
v4
v5
v6
v7

FIG. 52A

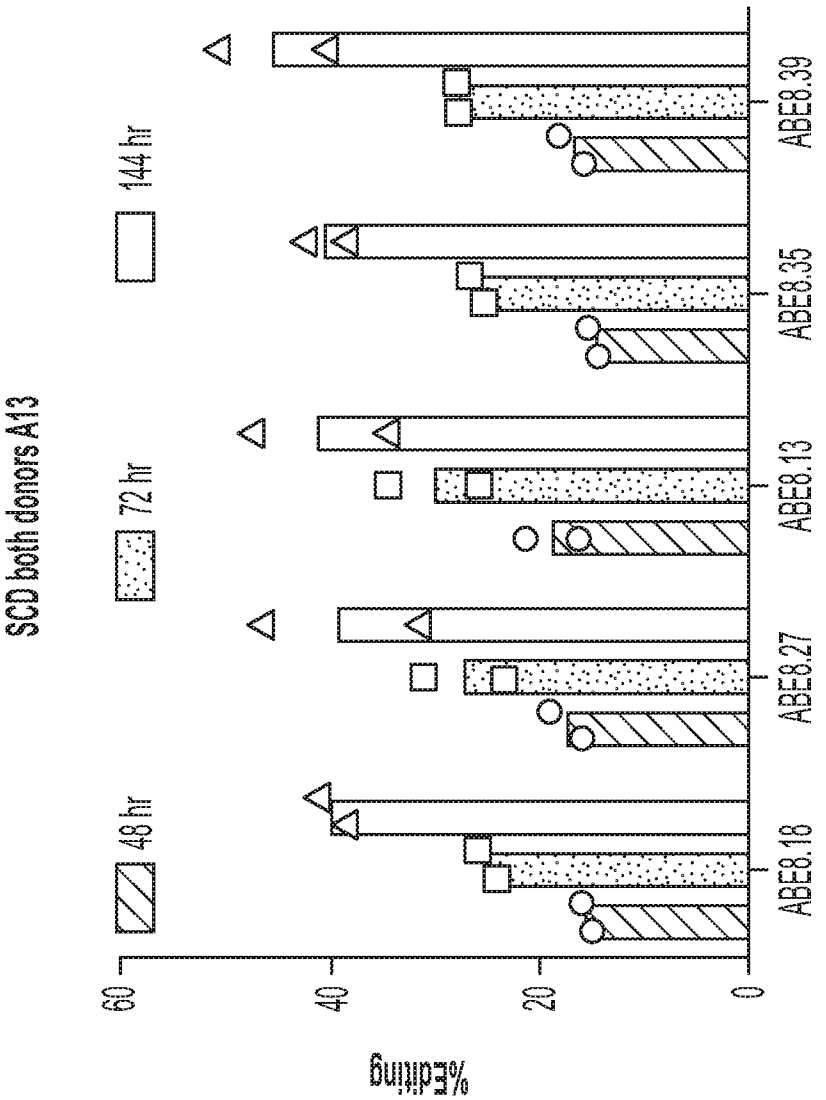

SCD both donors A13
48 hr
72 hr
144 hr
%Editing
60
40
20
0
ABE8.18
ABE8.27
ABE8.13
ABE8.35
ABE8.39

FIG. 52B

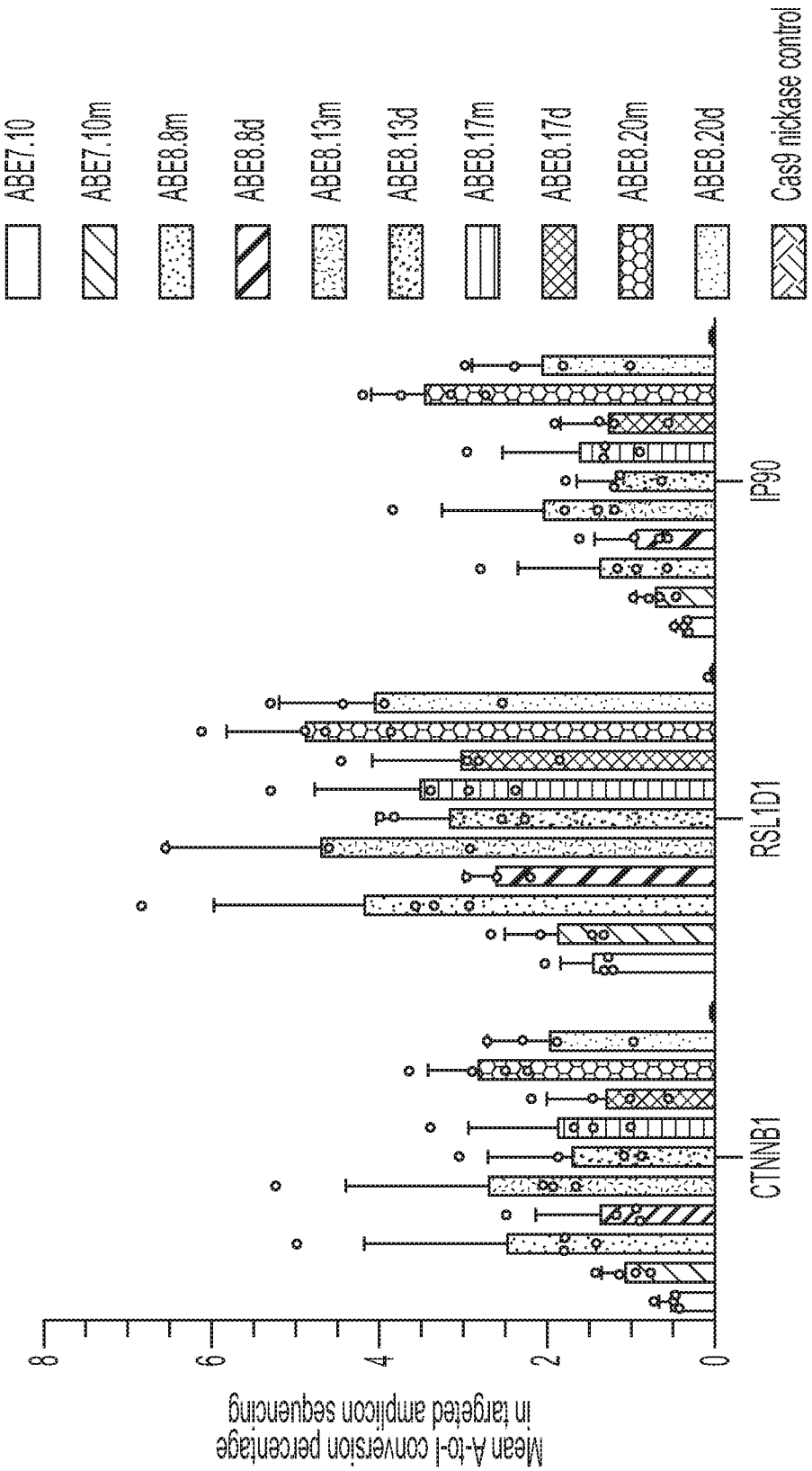

ABE7.10
ABE7.10m
ABE8.8m
ABE8.8d
ABE8.13m
ABE8.13d
ABE8.17m
ABE8.17d
ABE8.20m
ABE8.20d
Cas9 nickase control
IP90
RSL1D1
CTNNB1
0
2
4
6
8
Mean A-to-I conversion percentage
in targeted amplicon sequencing

FIG. 53A

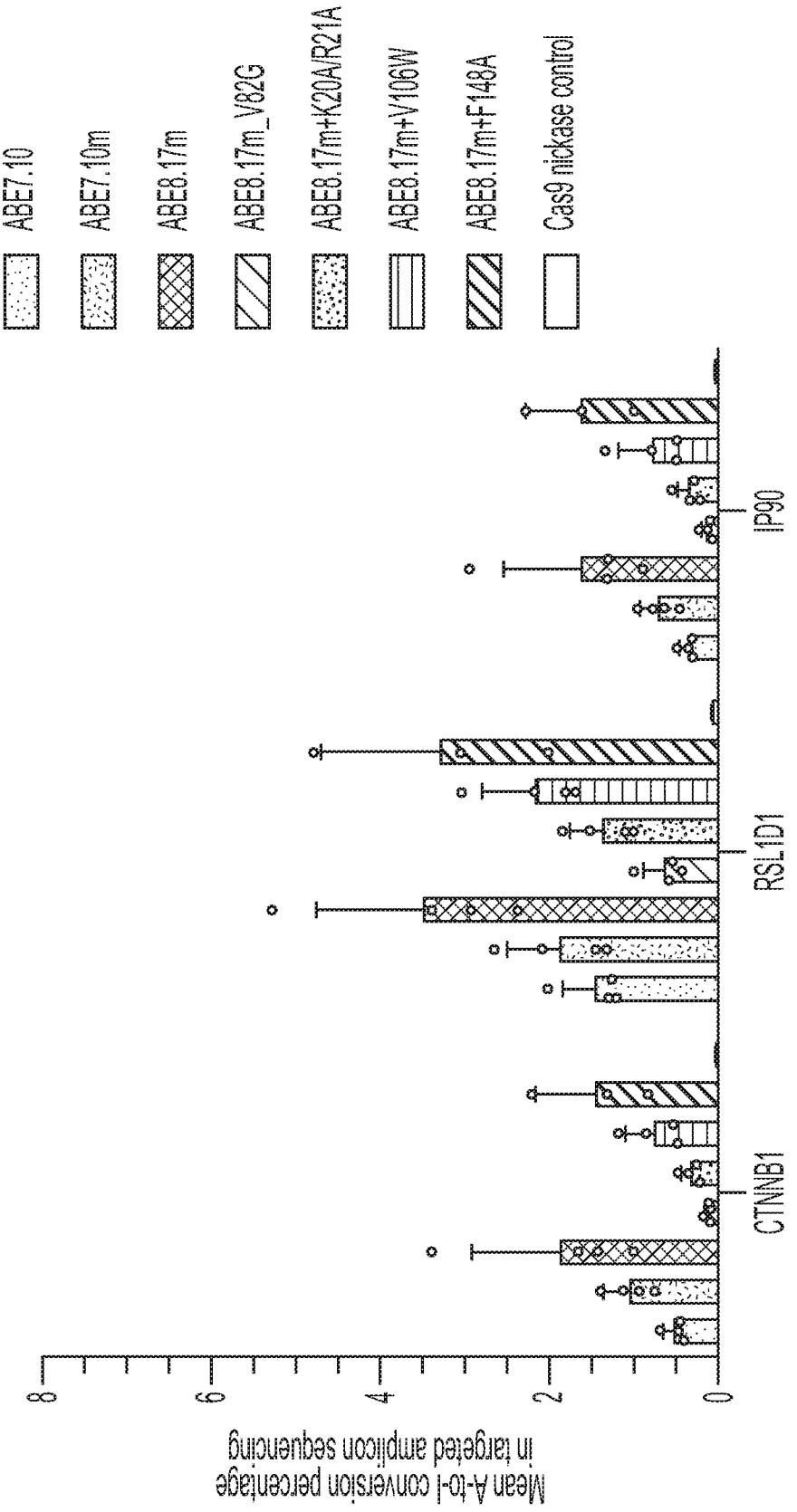

ABE7.10
ABE7.10m
ABE8.17m
ABE8.17m_V82G
ABE8.17m+K20A/R21A
ABE8.17m+V106W
ABE8.17m+F148A
Cas9 nickase control
Mean A-to-I conversion percentage in targeted amplicon sequencing
0
2
4
6
8
CTNNB1
RSL1D1
IP90

FIG. 53B

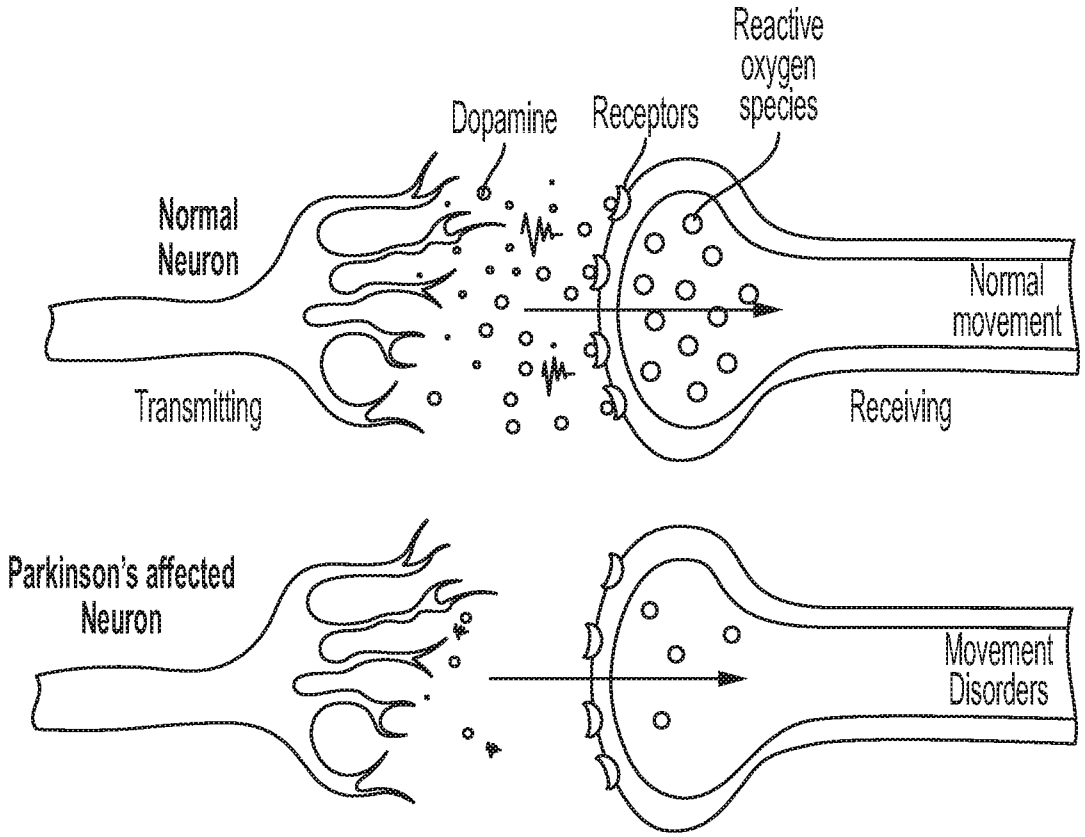

Reactive oxygen species
Dopamine
Receptors
Normal Neuron
Normal movement
Transmitting
Receiving
Parkinson's affected Neuron
Movement Disorders

FIG. 54

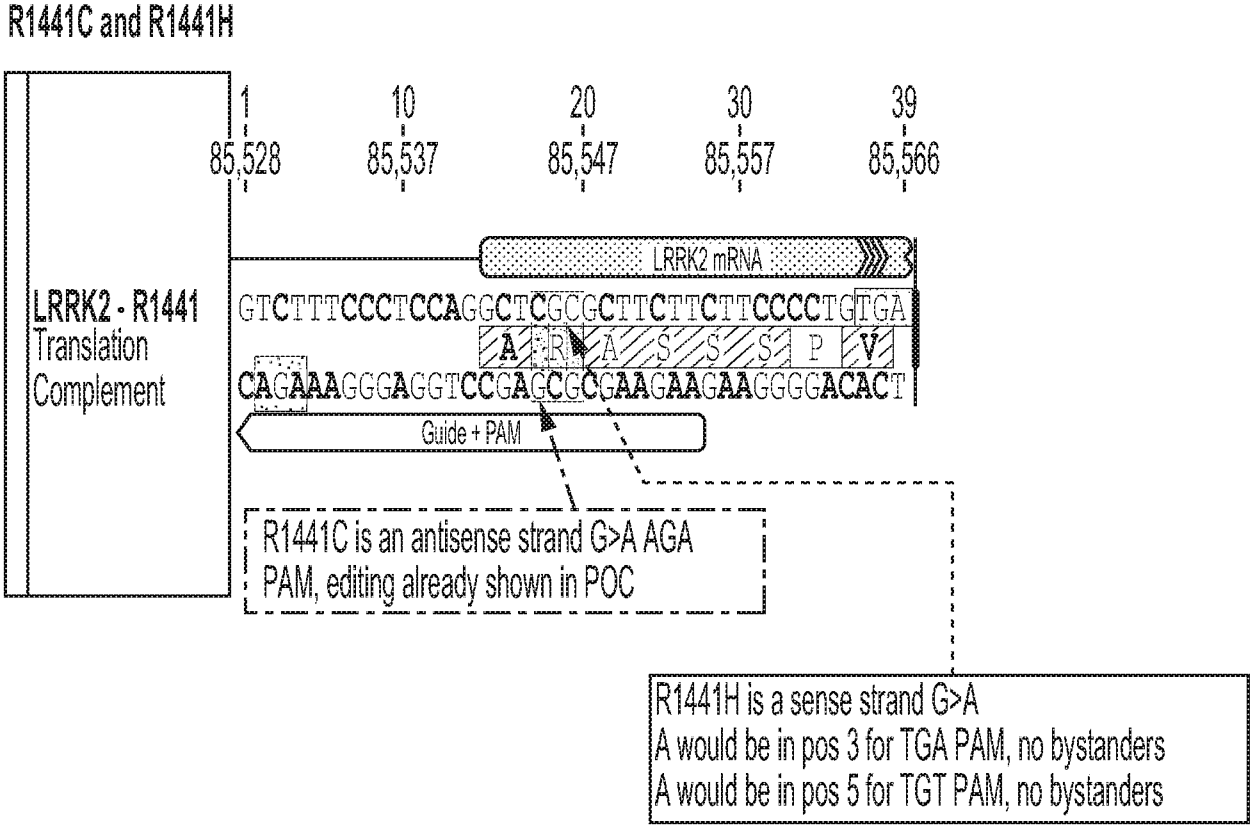

R1441C and R1441H
1
10
20
30
39
85,528
85,537
85,547
85,557
85,566
LRRK2 mRNA
LRRK2 - R1441
Translation
Complement
GTCTTTCCCTCCAGGCTCGCGCTTCTTCTTCCCCTGTGA
A R A S S S P V
CAGAAAGGGAGGTCCGAGCGCGAAGAAGAAGGGGACACT
Guide + PAM
R1441C is an antisense strand G>A AGA PAM, editing already shown in POC
R1441H is a sense strand G>A
A would be in pos 3 for TGA PAM, no bystanders
A would be in pos 5 for TGT PAM, no bystanders

FIG. 55

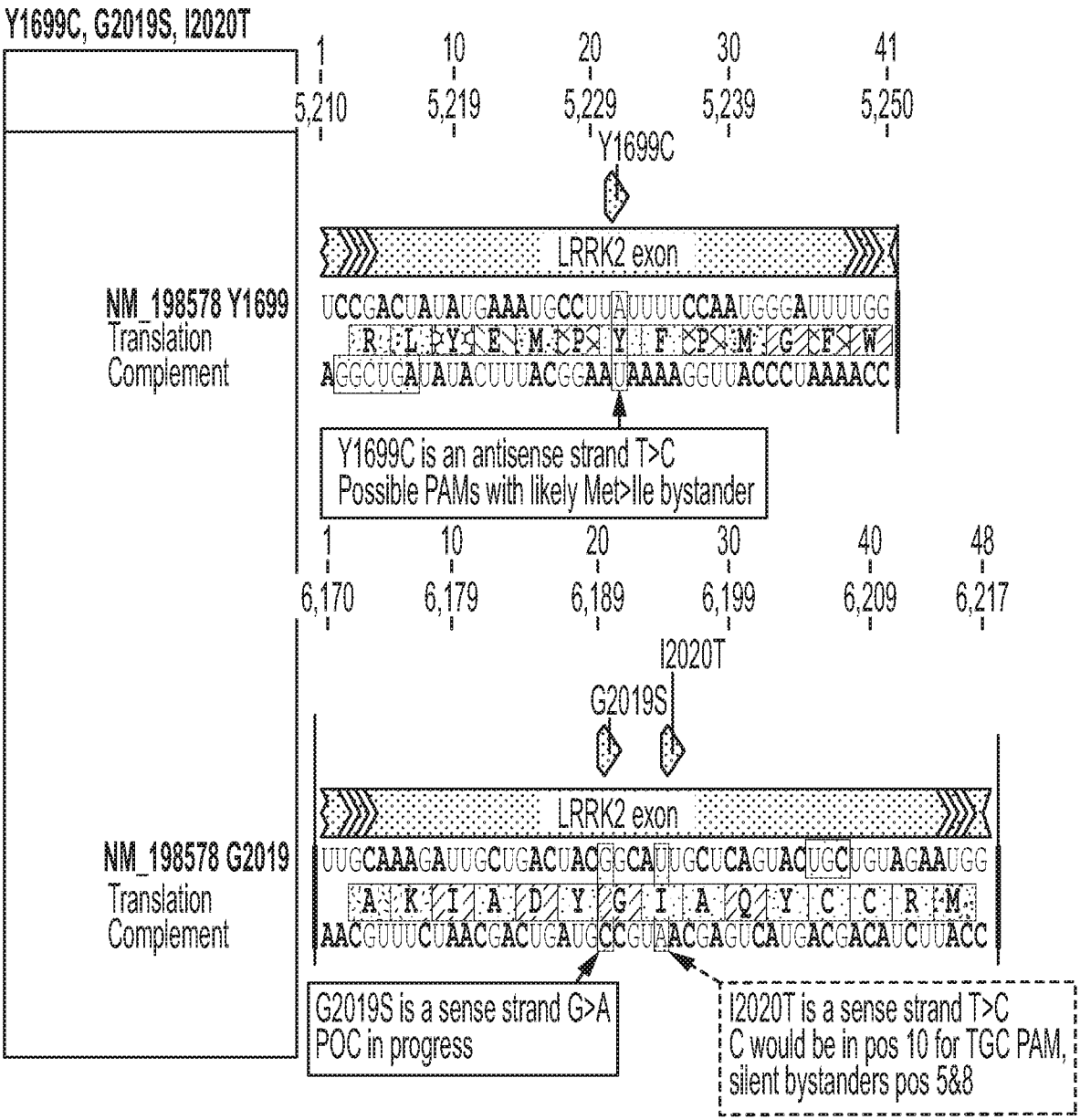

Y1699C, G2019S, I2020T
1
10
20
30
41
5,210
5,219
5,229
5,239
5,250
Y1699C
LRRK2 exon
NM_198578 Y1699
Translation
Complement
UCCGACUAUAUGAAAUGCCUUAUUUUCCAAUGGGAUUUUGG
R L Y E M P Y F P M G F W
AGGCUGAUAUACUUUACGGAAUAAAAGGUUACCCUAAAACC
Y1699C is an antisense strand T>C
Possible PAMs with likely Met>Ile bystander
1
10
20
30
40
48
6,170
6,179
6,189
6,199
6,209
6,217
I2020T
G2019S
LRRK2 exon
NM_198578 G2019
Translation
Complement
UUGCAAAGAUUGCUGACUACGGCAUUGCUCAGUACUGCUGUAGAAUGG
A K I A D Y G I A Q Y C C R M
AACGUUUCUAACGACUGAUGCCGUAACGAGUCAUGACGACAUCUUACC
G2019S is a sense strand G>A
POC in progress
I2020T is a sense strand T>C
C would be in pos 10 for TGC PAM,
silent bystanders pos 5&8

FIG. 56

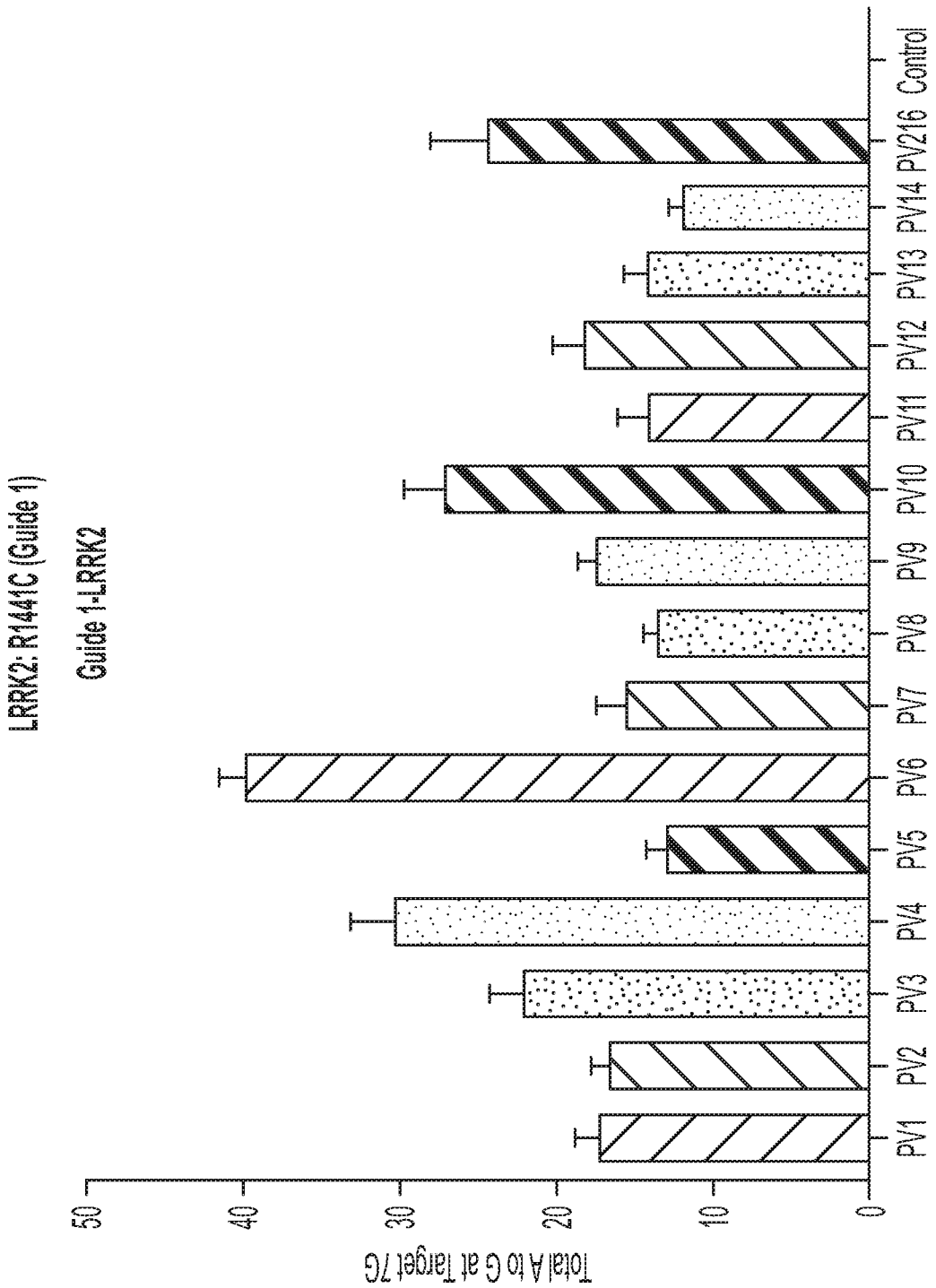

LRRK2: R1441C (Guide 1)
Guide 1-LRRK2
Total A to G at Target 7G
0
10
20
30
40
50
PV1
PV2
PV3
PV4
PV5
PV6
PV7
PV8
PV9
PV10
PV11
PV12
PV13
PV14
PV216
Control
NGA PAM

FIG. 57A

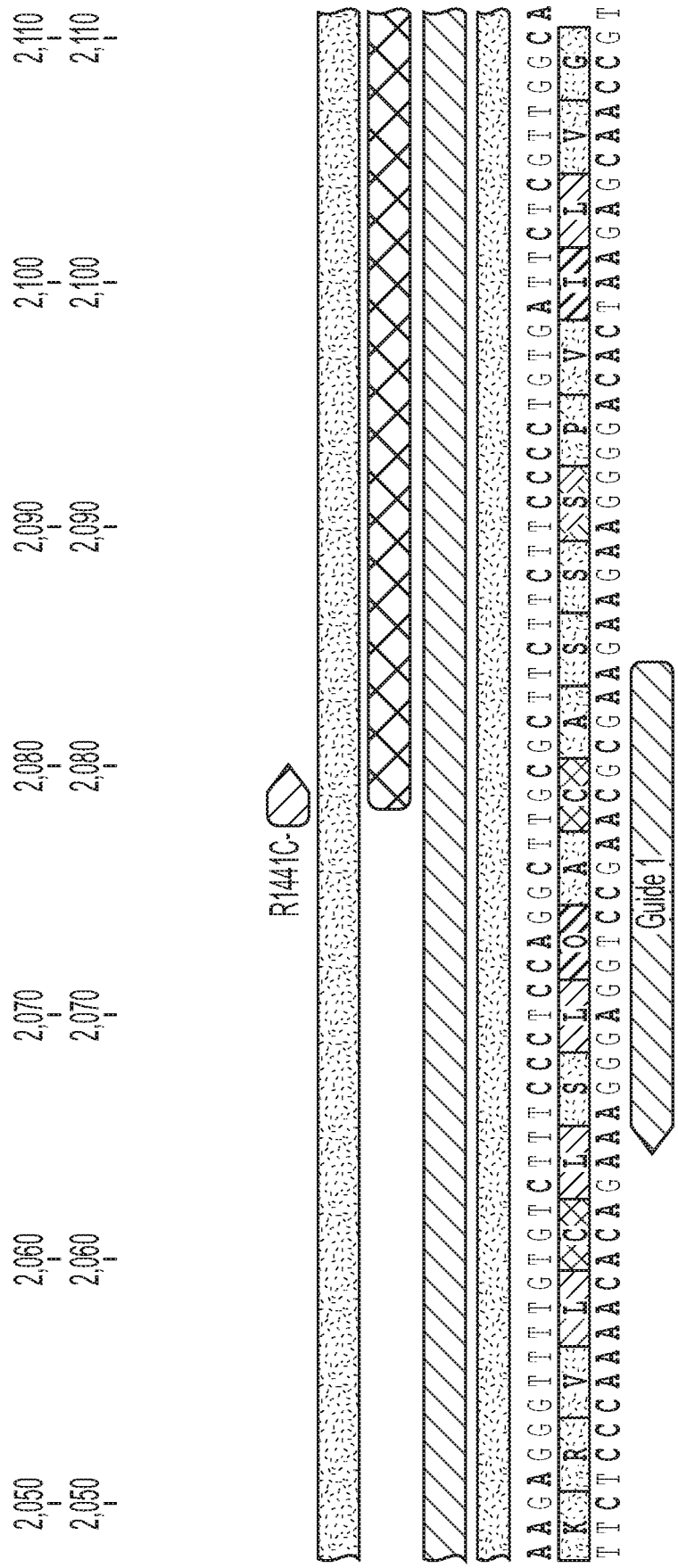


FIG. 57B

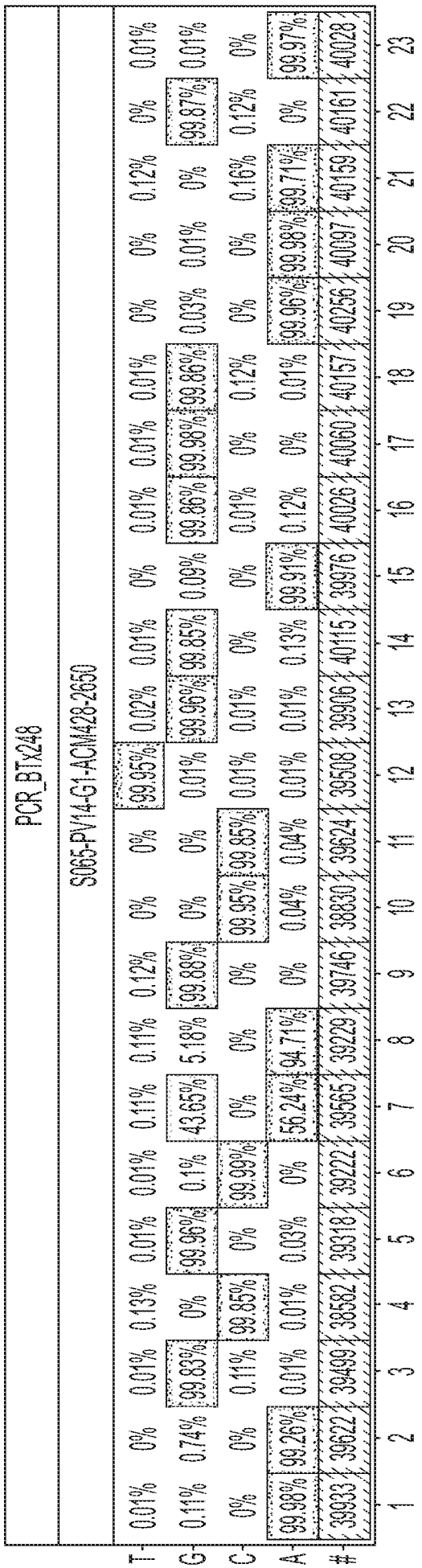

PCR_BTx248
S065-PV14-G1-ACM428-2650
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 57C

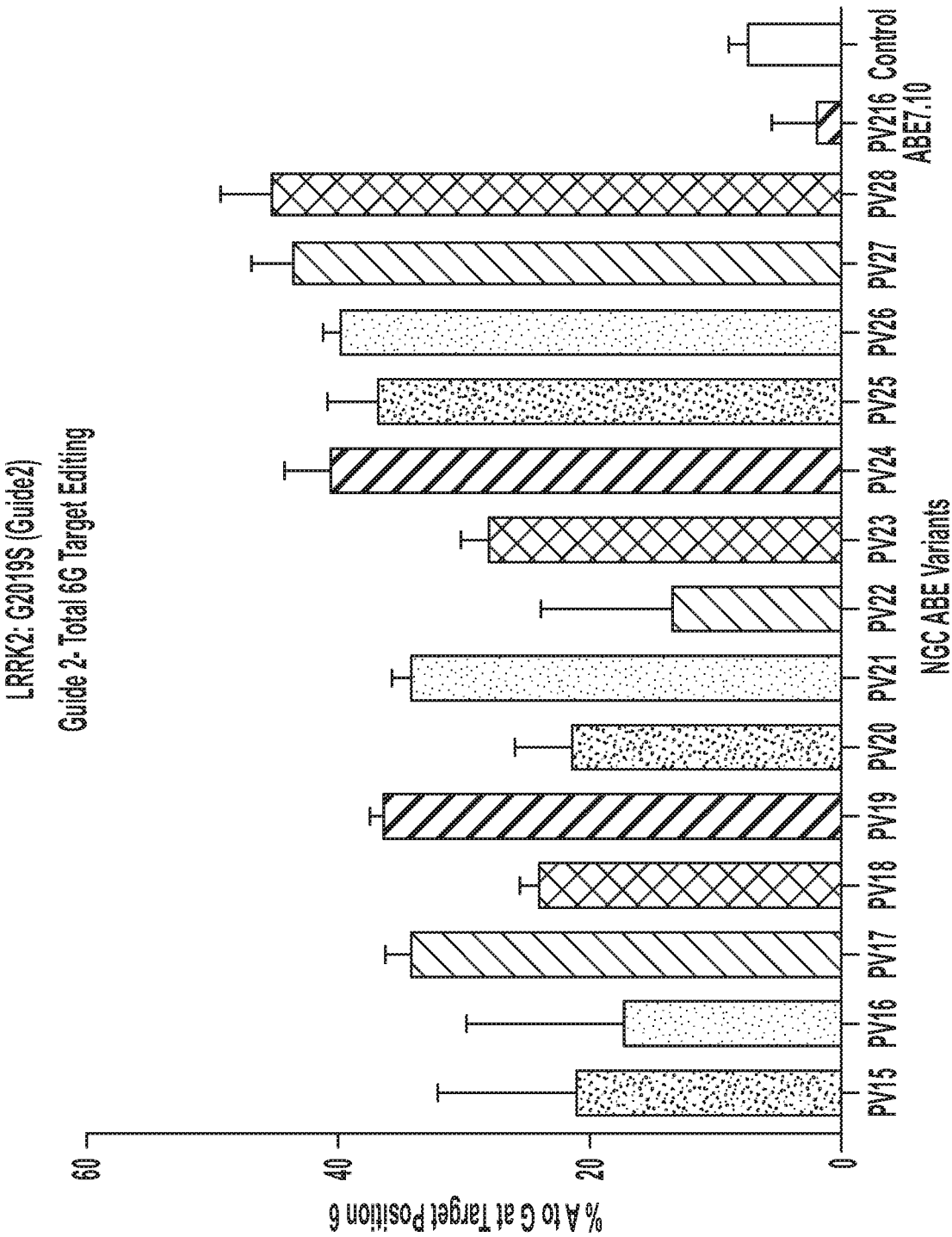

LRRK2: G2019S (Guide2)
Guide 2- Total 6G Target Editing
% A to G at Target Position 6
0
20
40
60
PV15
PV16
PV17
PV18
PV19
PV20
PV21
PV22
PV23
PV24
PV25
PV26
PV27
PV28
PV216
ABE7.10
Control
NGC ABE Variants

FIG. 58A

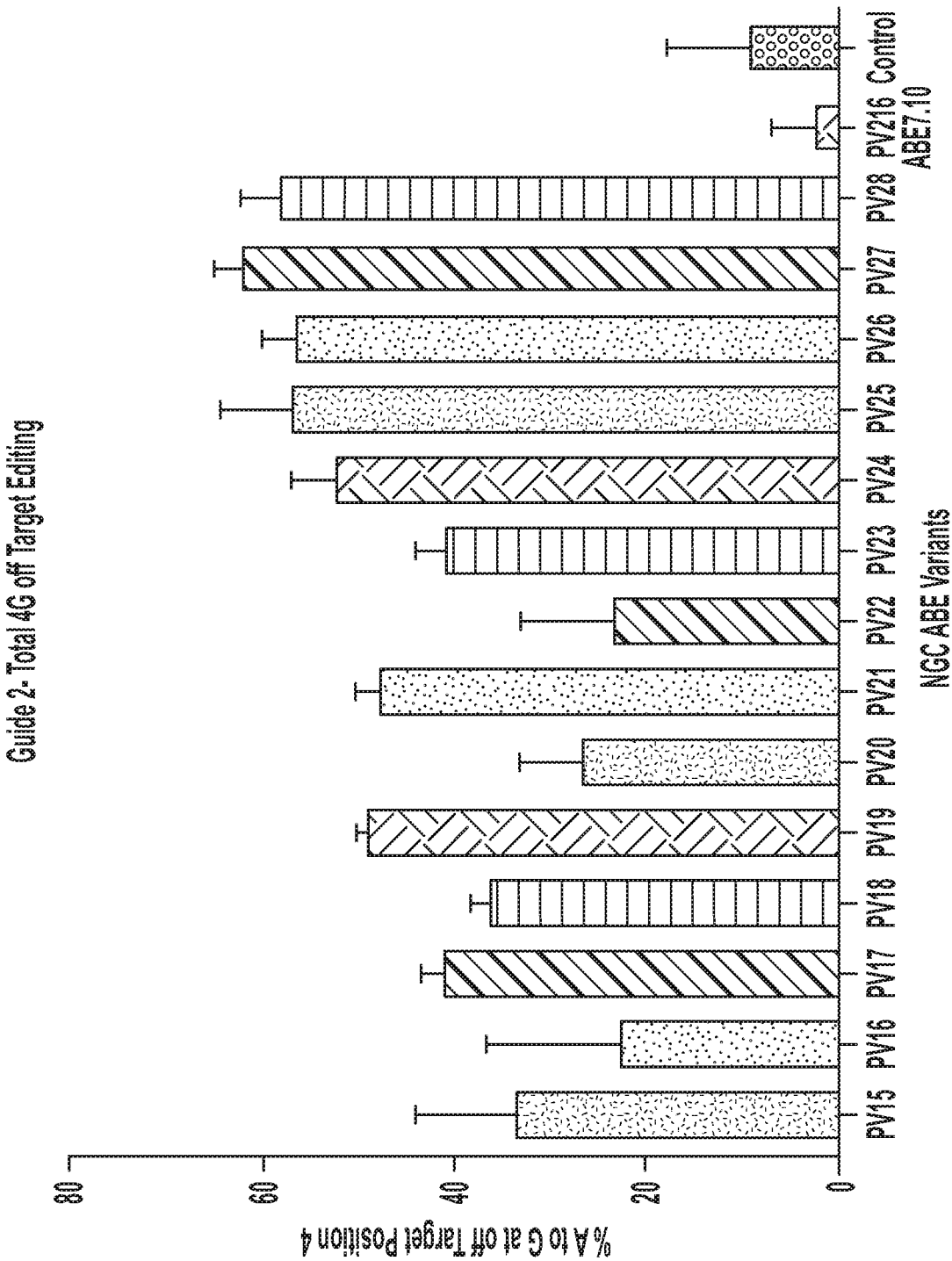


FIG. 58A CONTINUED

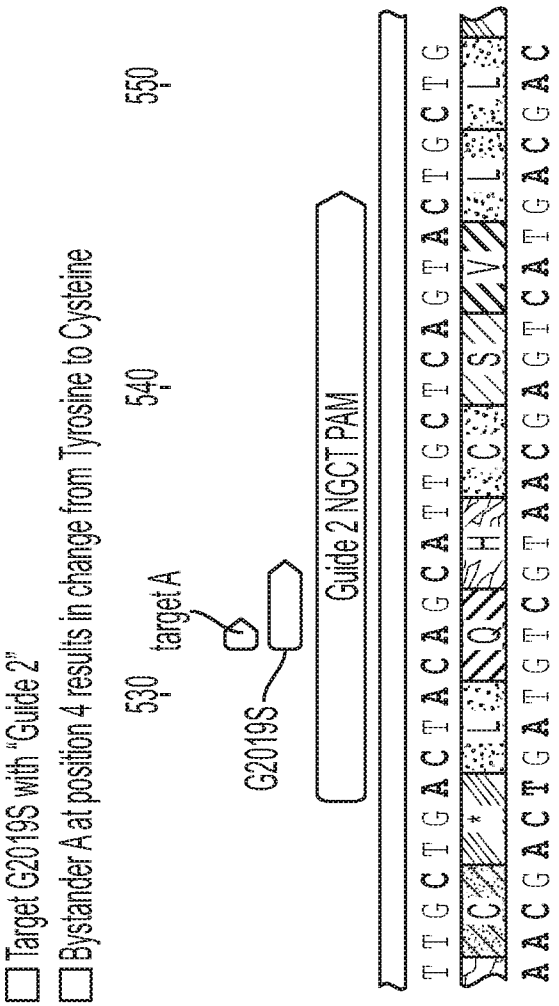

Target G2019S with "Guide 2"
Bystander A at position 4 results in change from Tyrosine to Cysteine
530
target A
540
550
G2019S
Guide 2 NGCT PAM
TTGCTGACTACAGCATTGCTCAGTACTGCTG
AACGACTGATGTCGTAACGAGTCATGACGAC

FIG. 58B

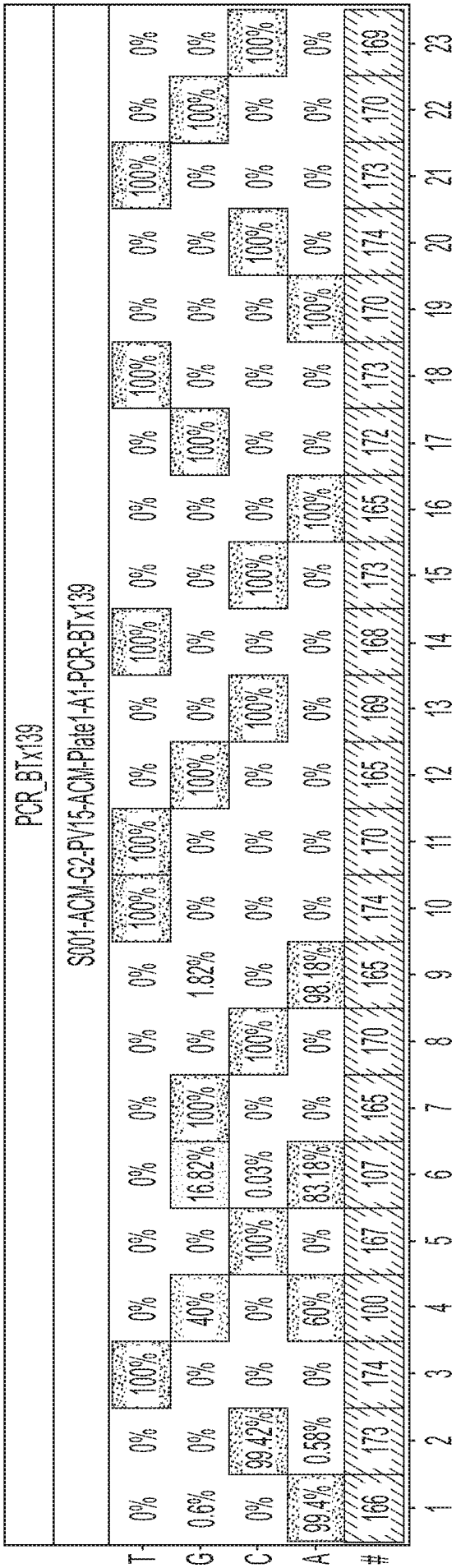

PCR_BTx139
S001-ACM-G2-PV15-ACM-Plate1-A1-PCR-BTx139
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 58C

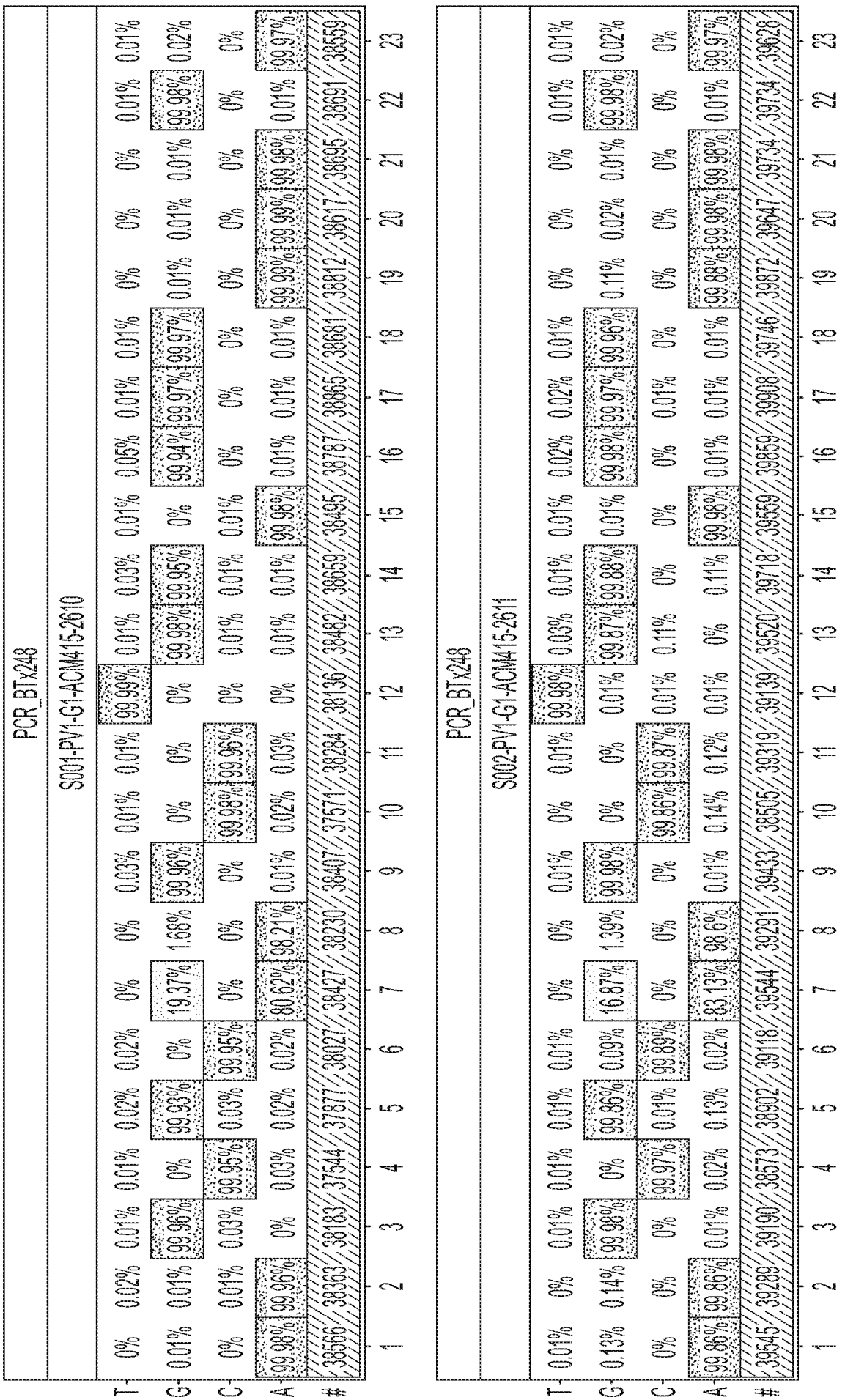

PCR_BTx248
S001-PV1-G1-ACM415-2610
PCR_BTx248
S002-PV1-G1-ACM415-2611
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 59A

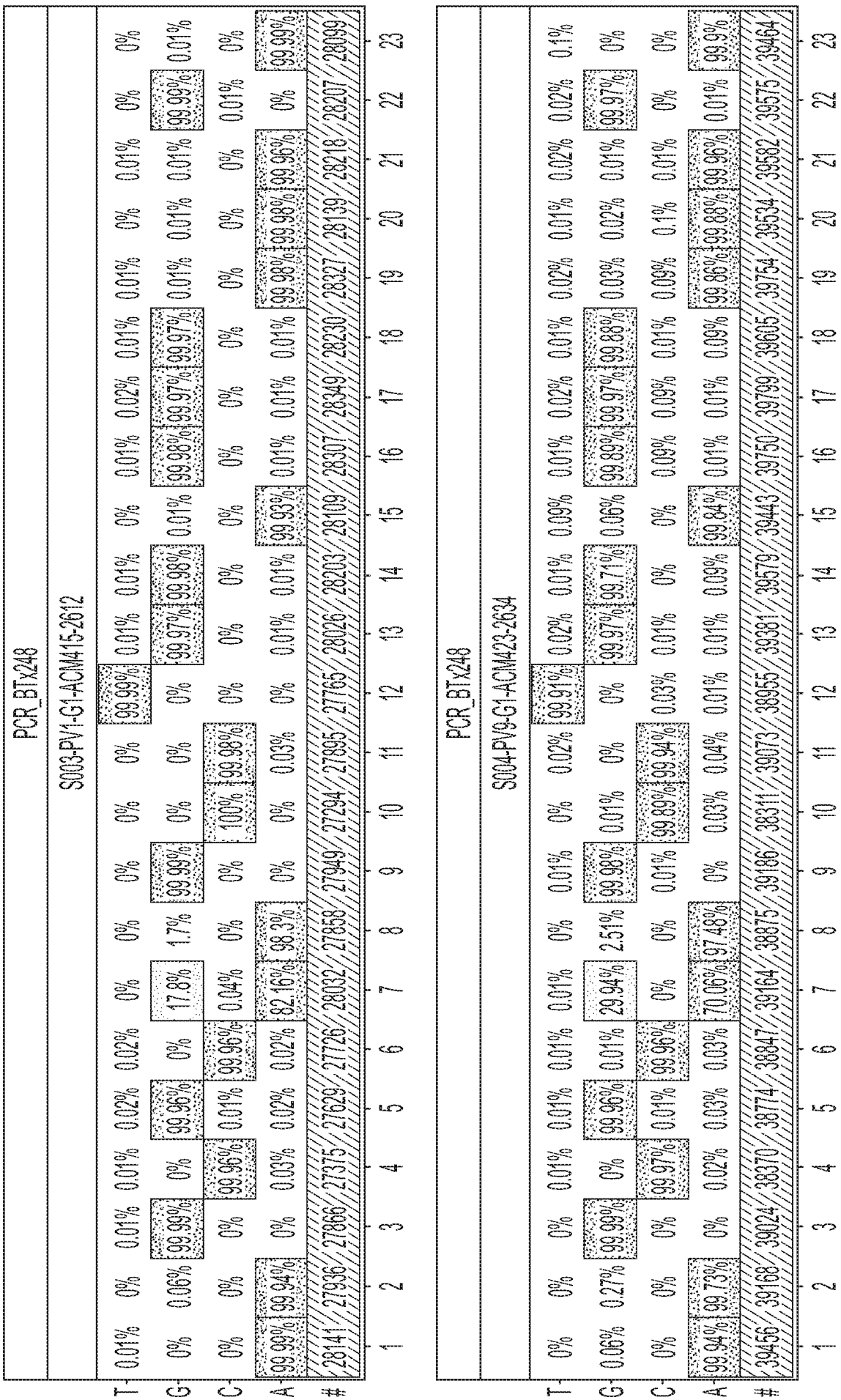


FIG. 59A CONTINUED

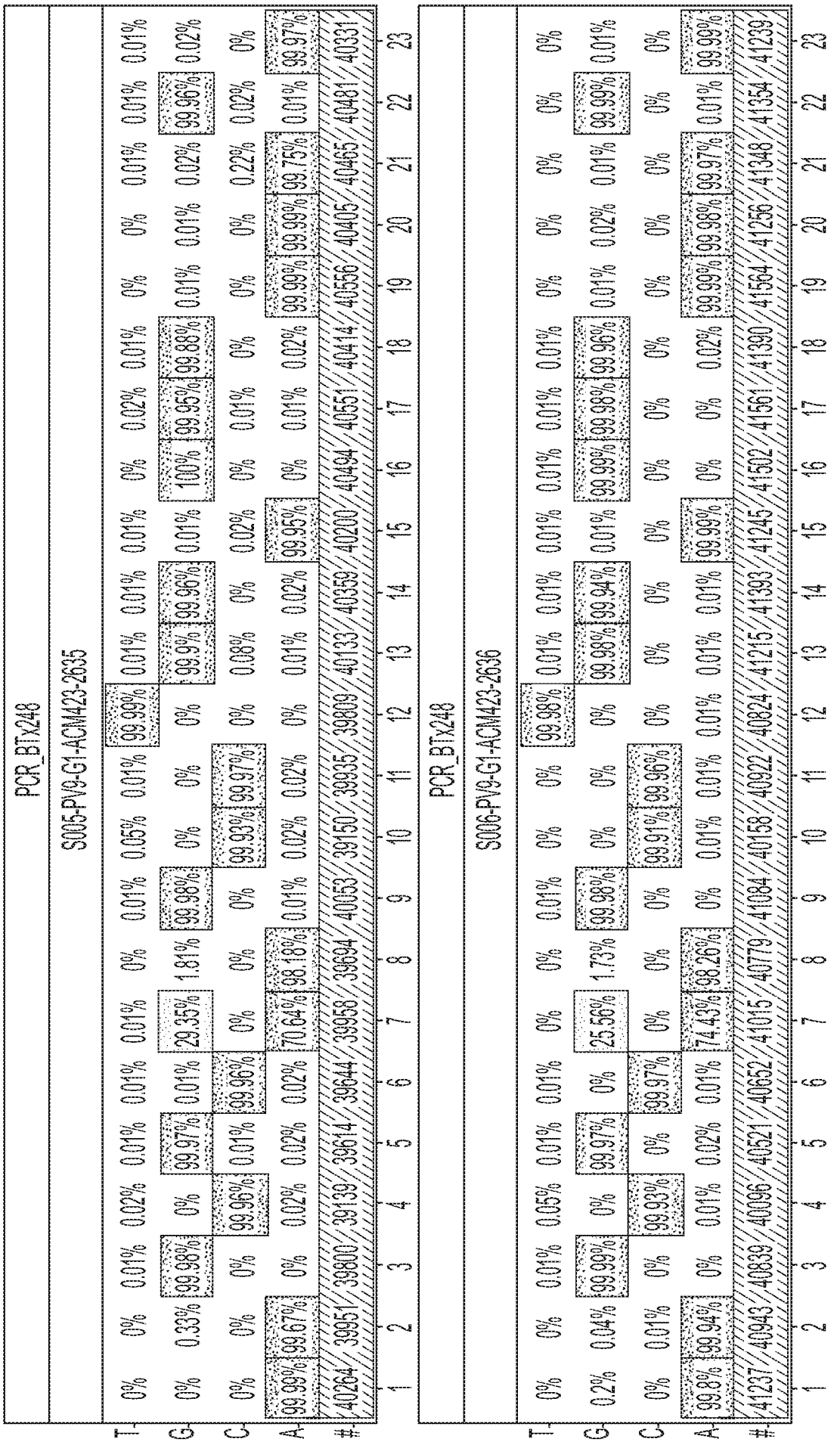

PCR_BTx248
S005-PV9-G1-ACM423-2635
PCR_BTx248
S006-PV9-G1-ACM423-2636

FIG. 59B

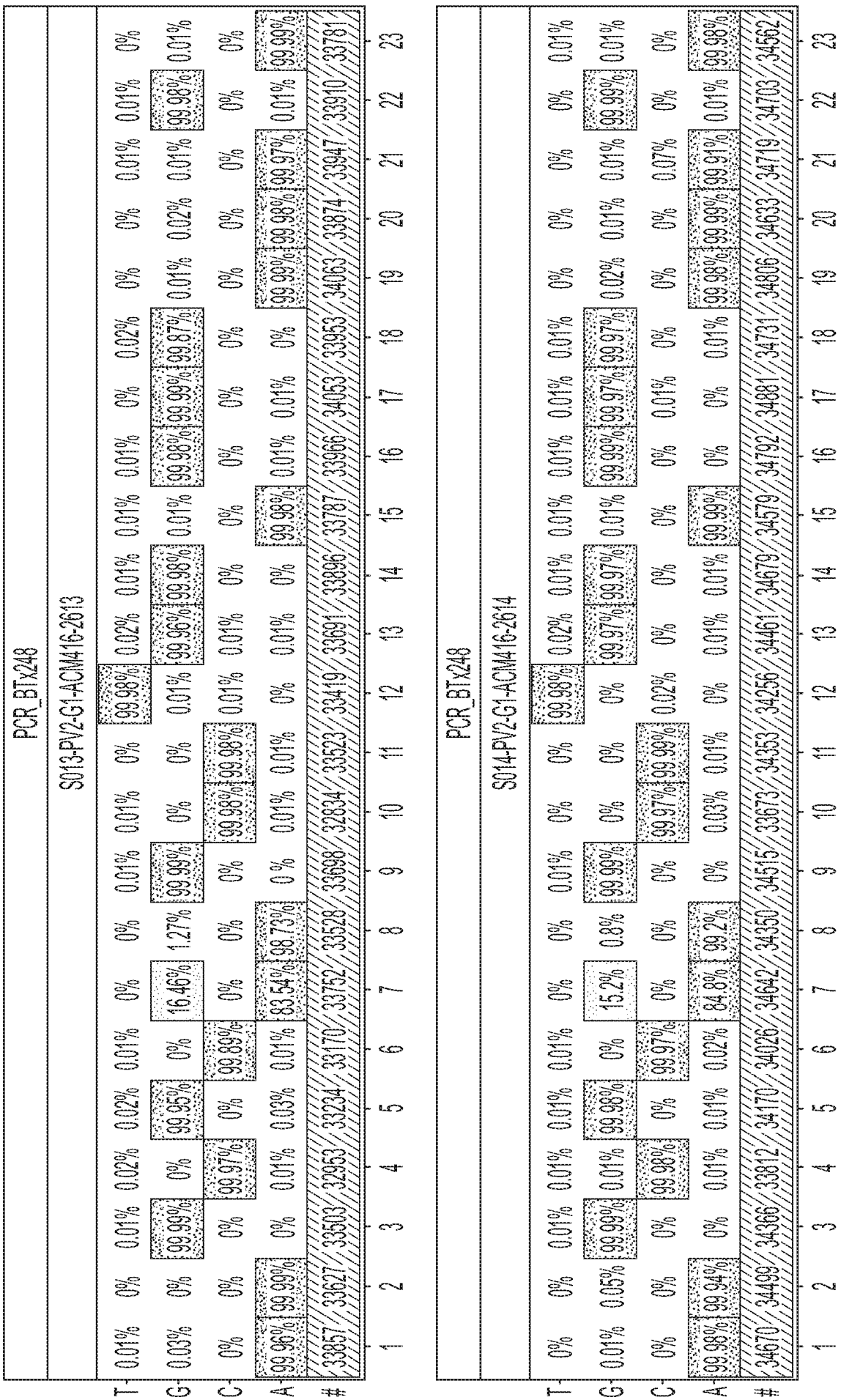


FIG. 59B CONTINUED

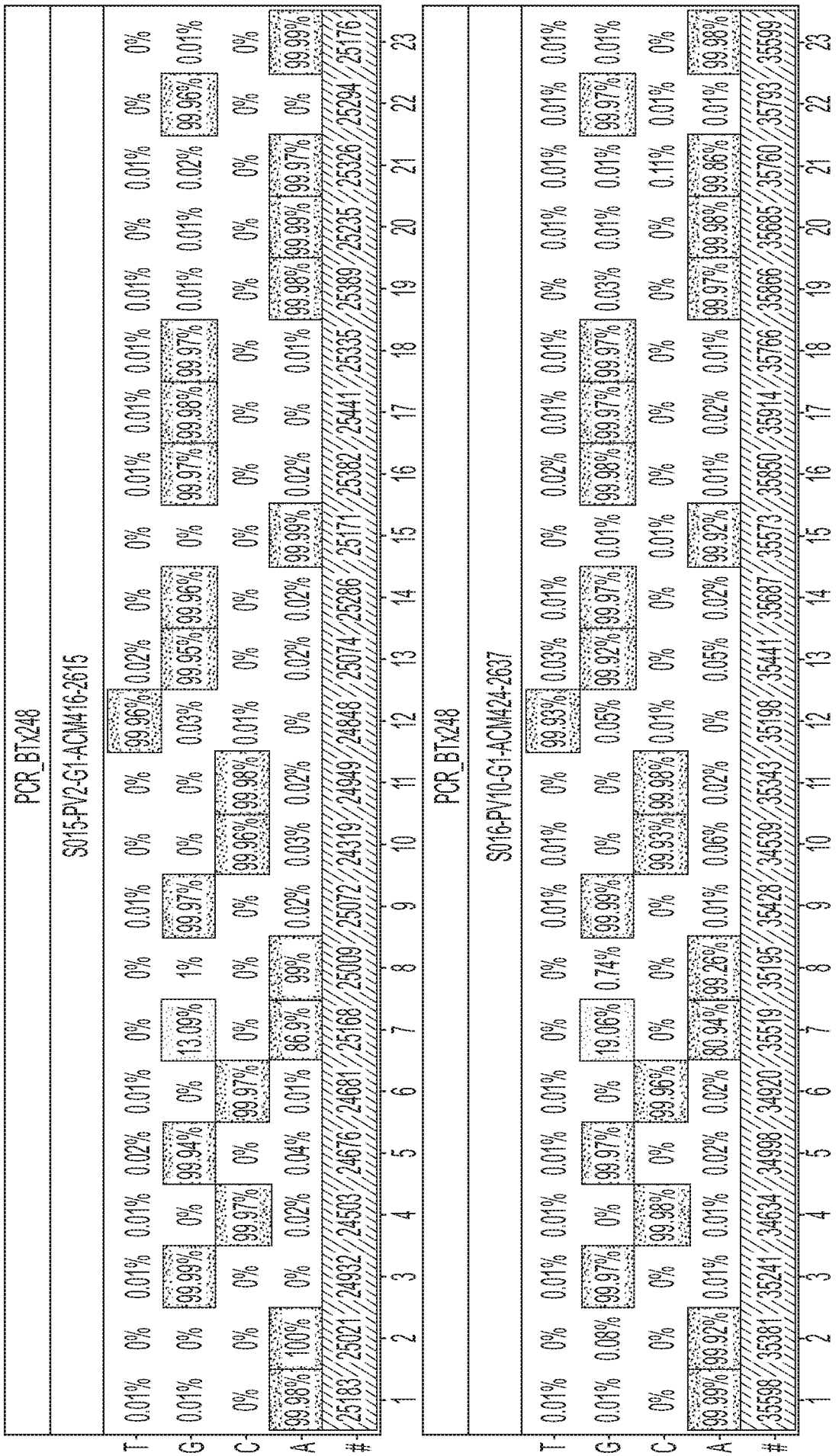

PCR_BTx248
S015-PV2-G1-ACM416-2615
PCR_BTx248
S016-PV10-G1-ACM424-2637

FIG. 59C

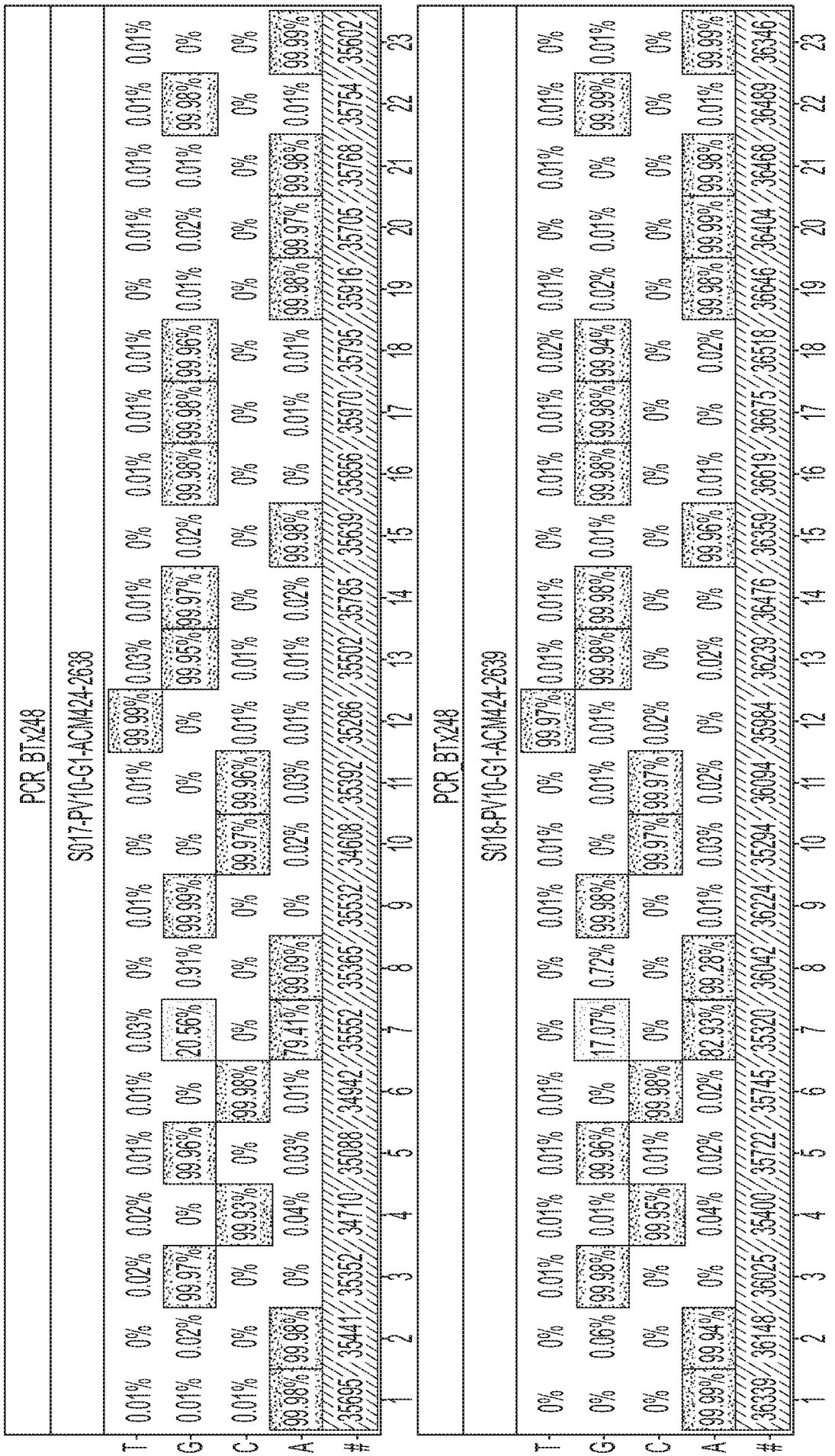


FIG. 59C CONTINUED

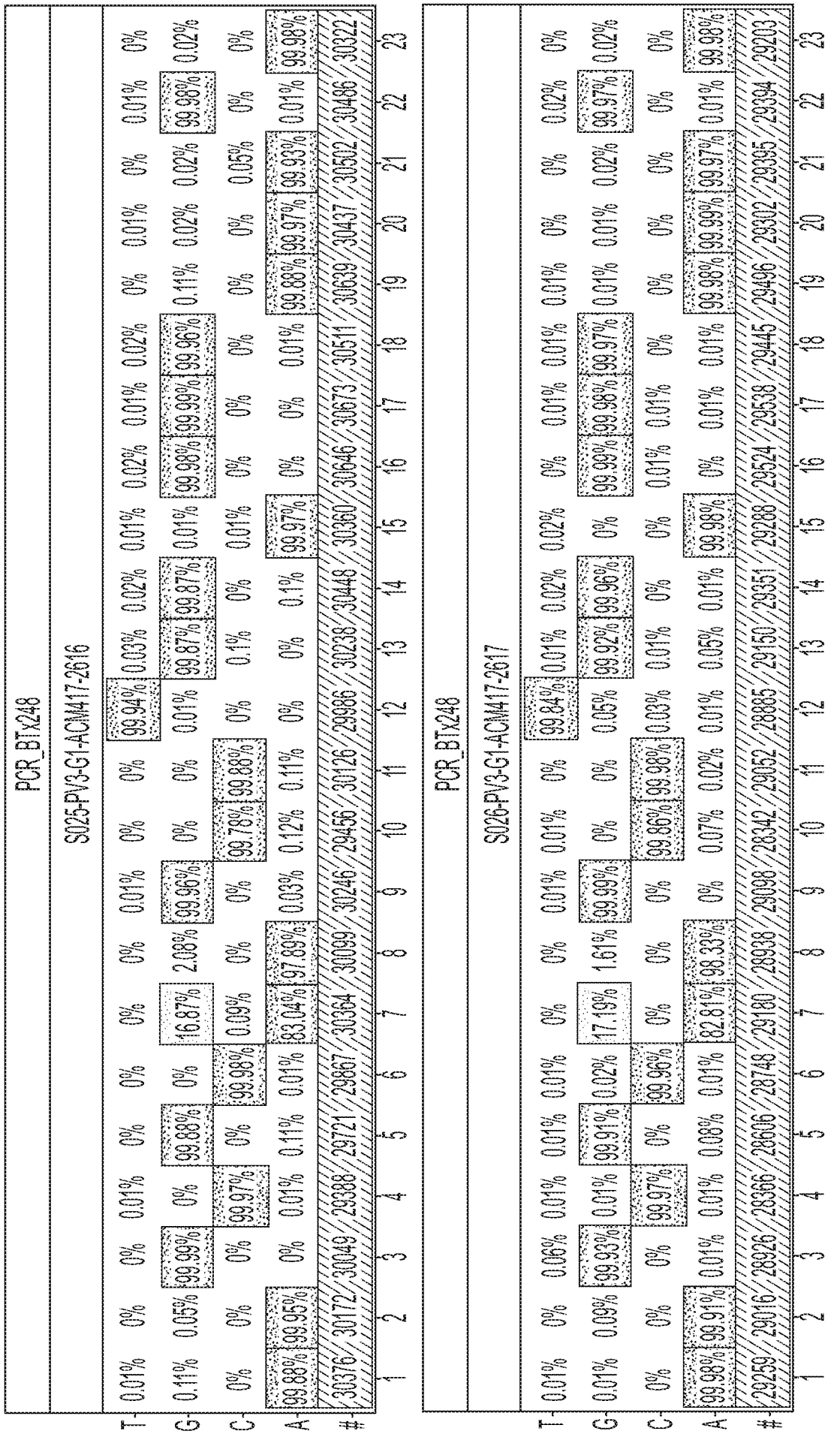

PCR_BTx248
S025-PV3-G1-ACM417-2616
PCR_BTx248
S026-PV3-G1-ACM417-2617

FIG. 59D

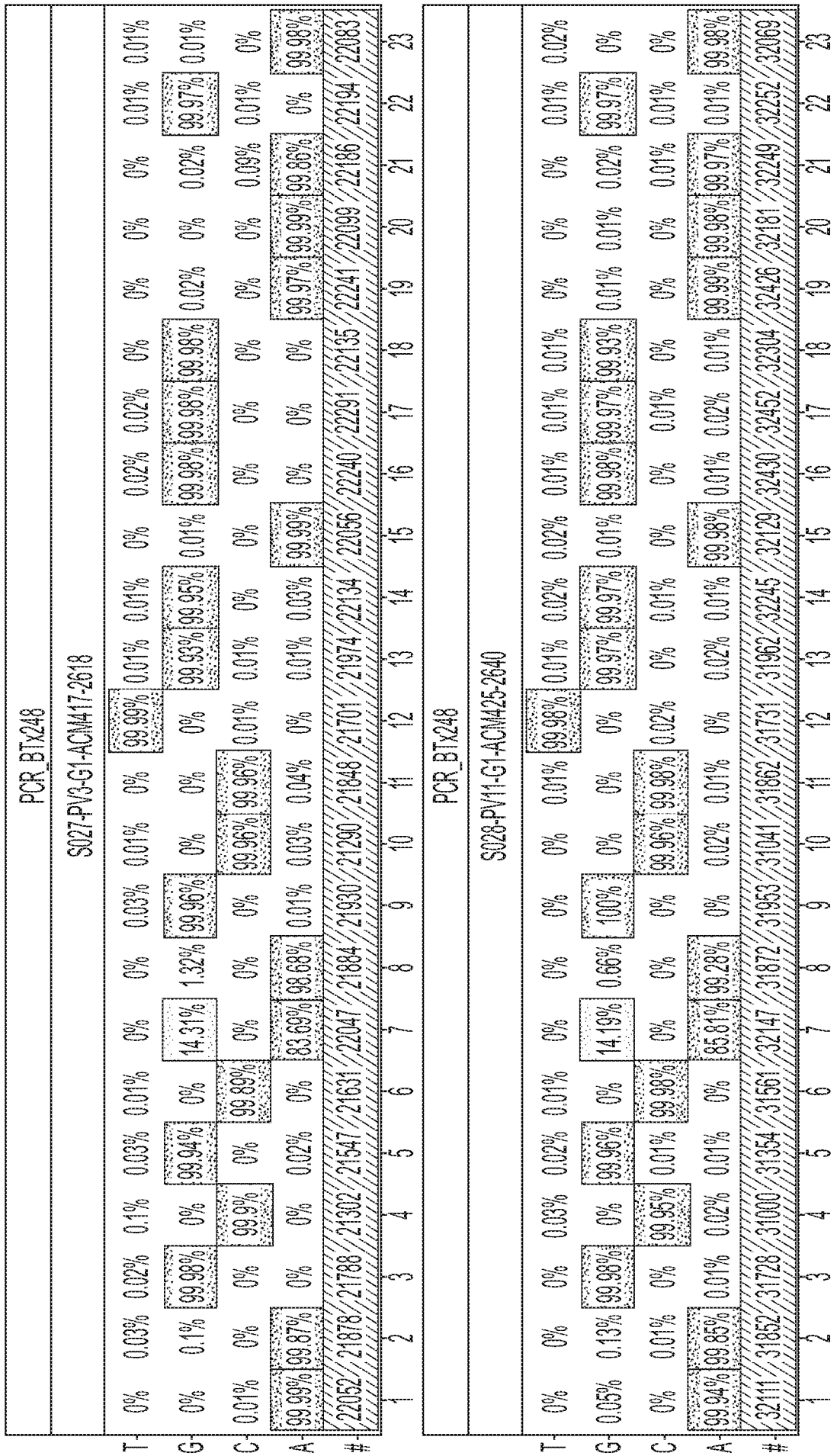


FIG. 59D CONTINUED

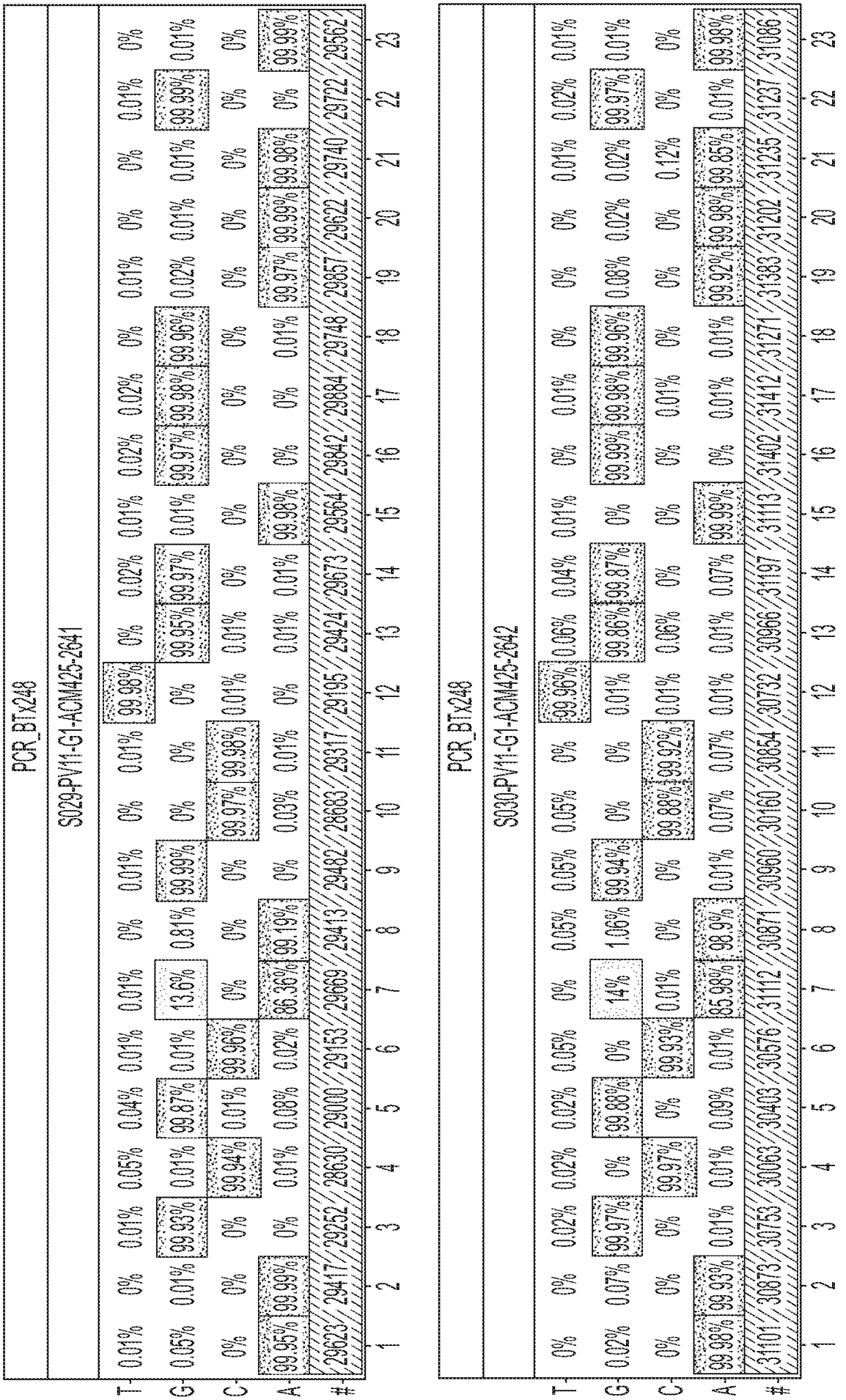

PCR_BTx248
S029-PV11-G1-ACM425-2641
PCR_BTx248
S030-PV11-G1-ACM425-2642

FIG. 59E

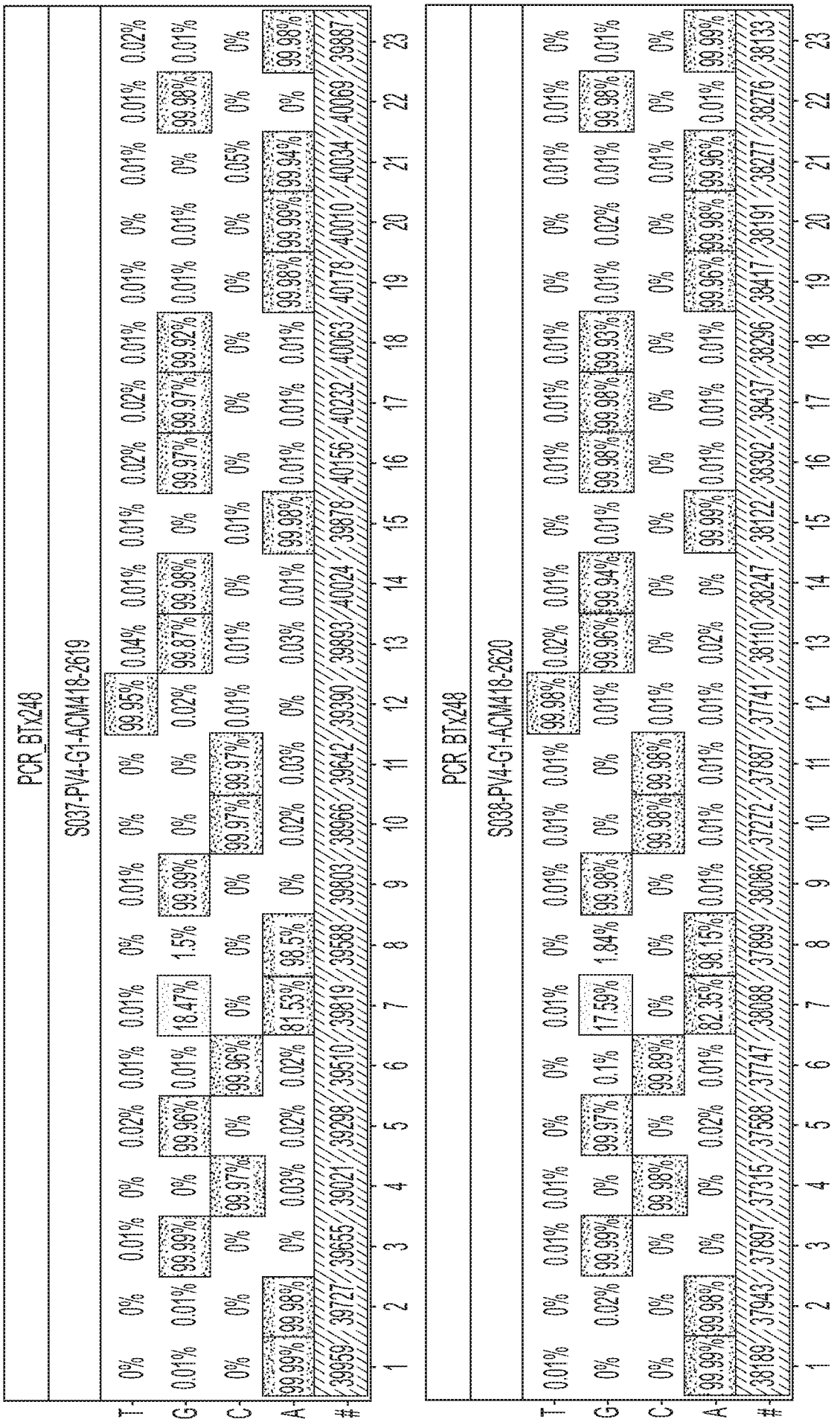


FIG. 59E CONTINUED

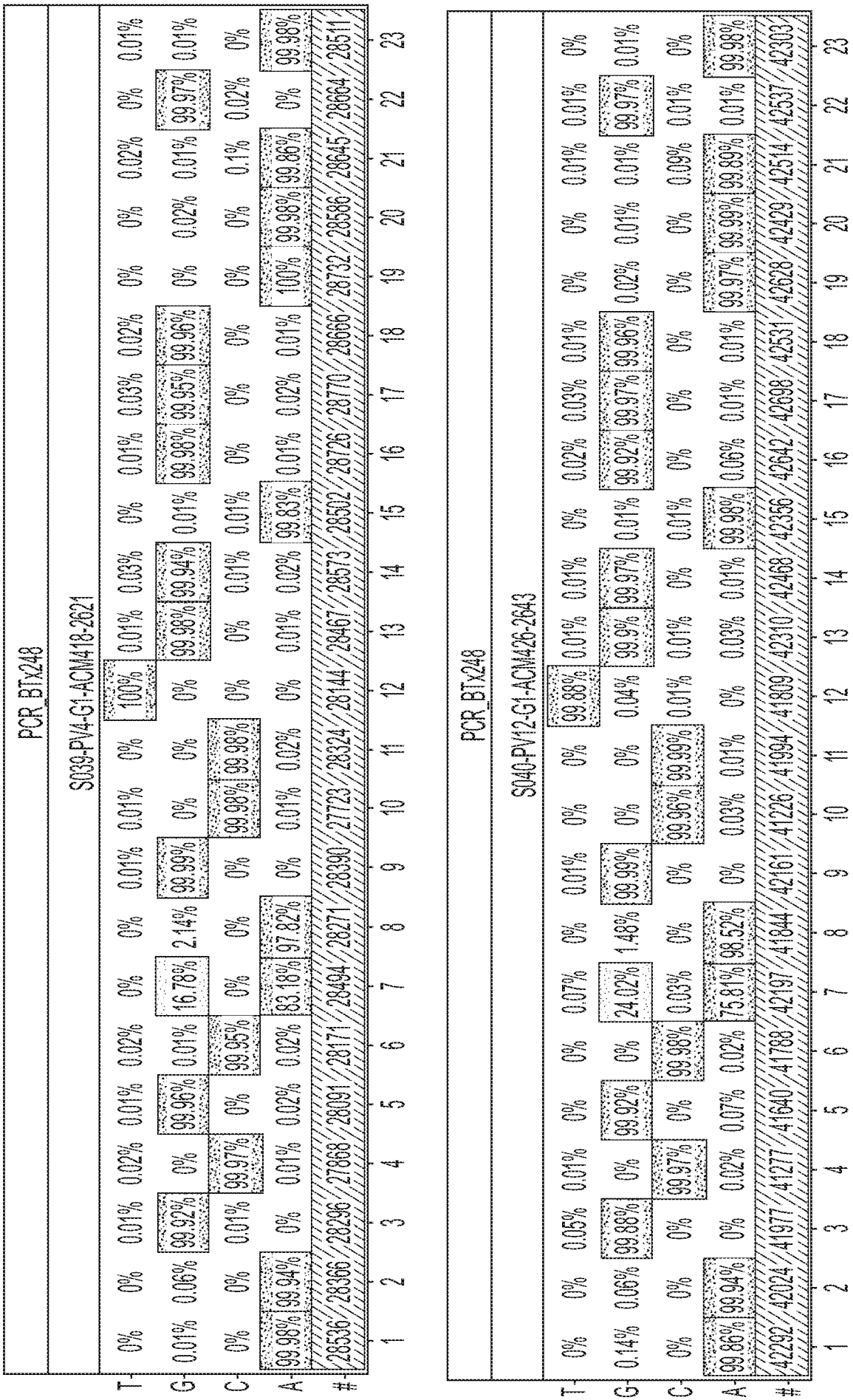

PCR_BTx248
S039-PV4-G1-ACM418-2621
PCR_BTx248
S040-PV12-G1-ACM426-2643

FIG. 59F

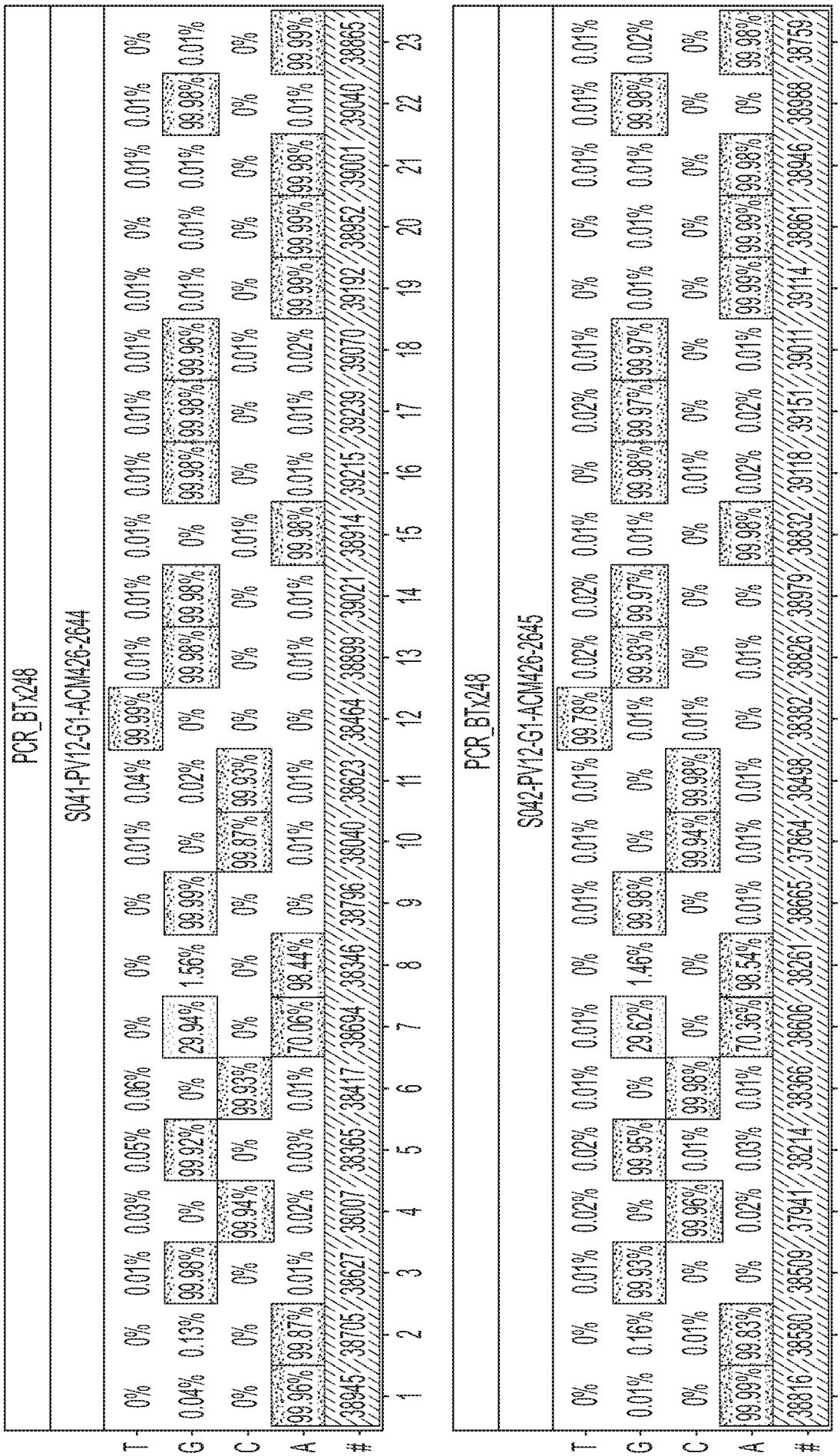


FIG. 59F CONTINUED

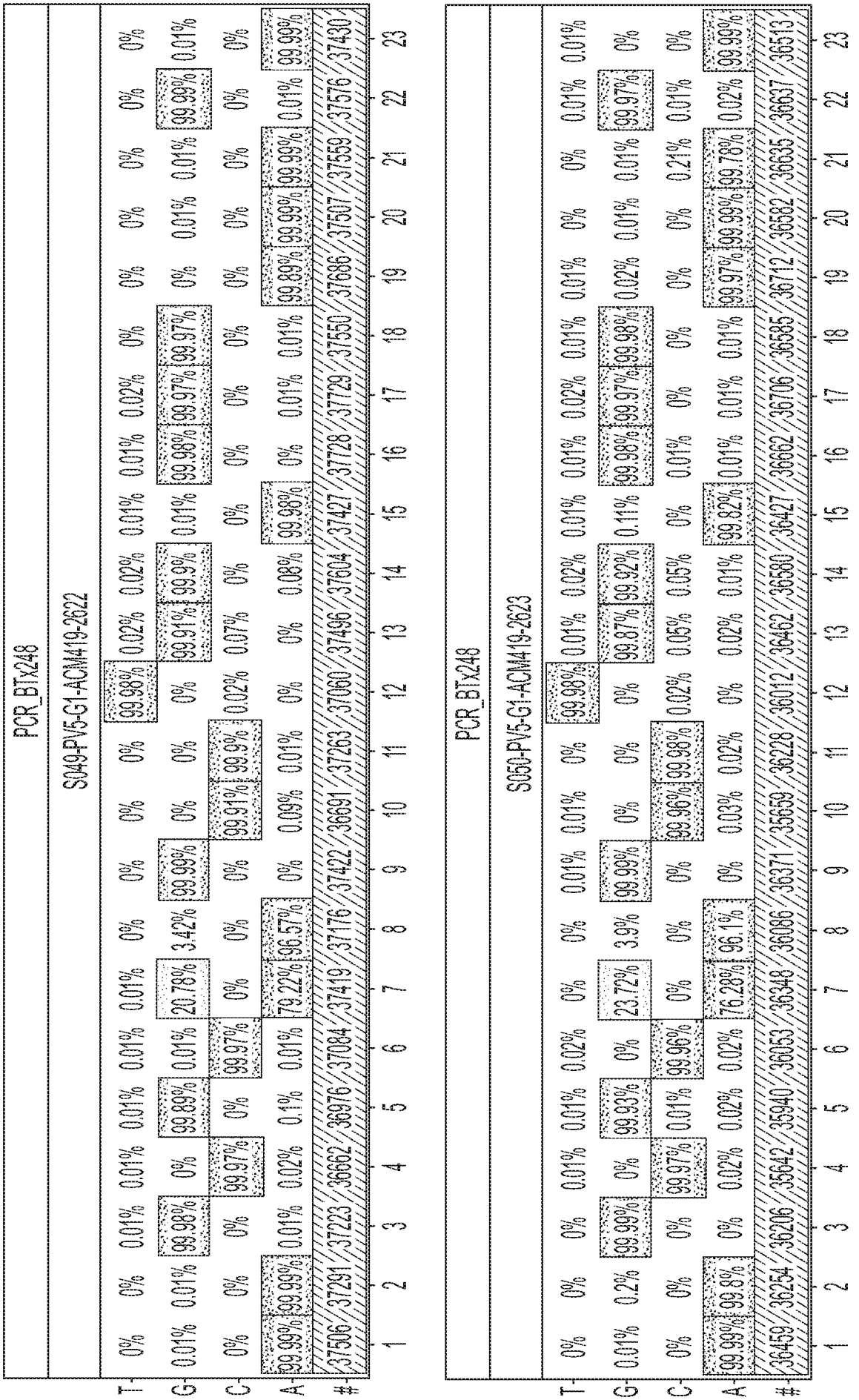

PCR_BTx248
S049-PV5-G1-ACM419-2622
PCR_BTx248
S050-PV5-G1-ACM419-2623

FIG. 59G

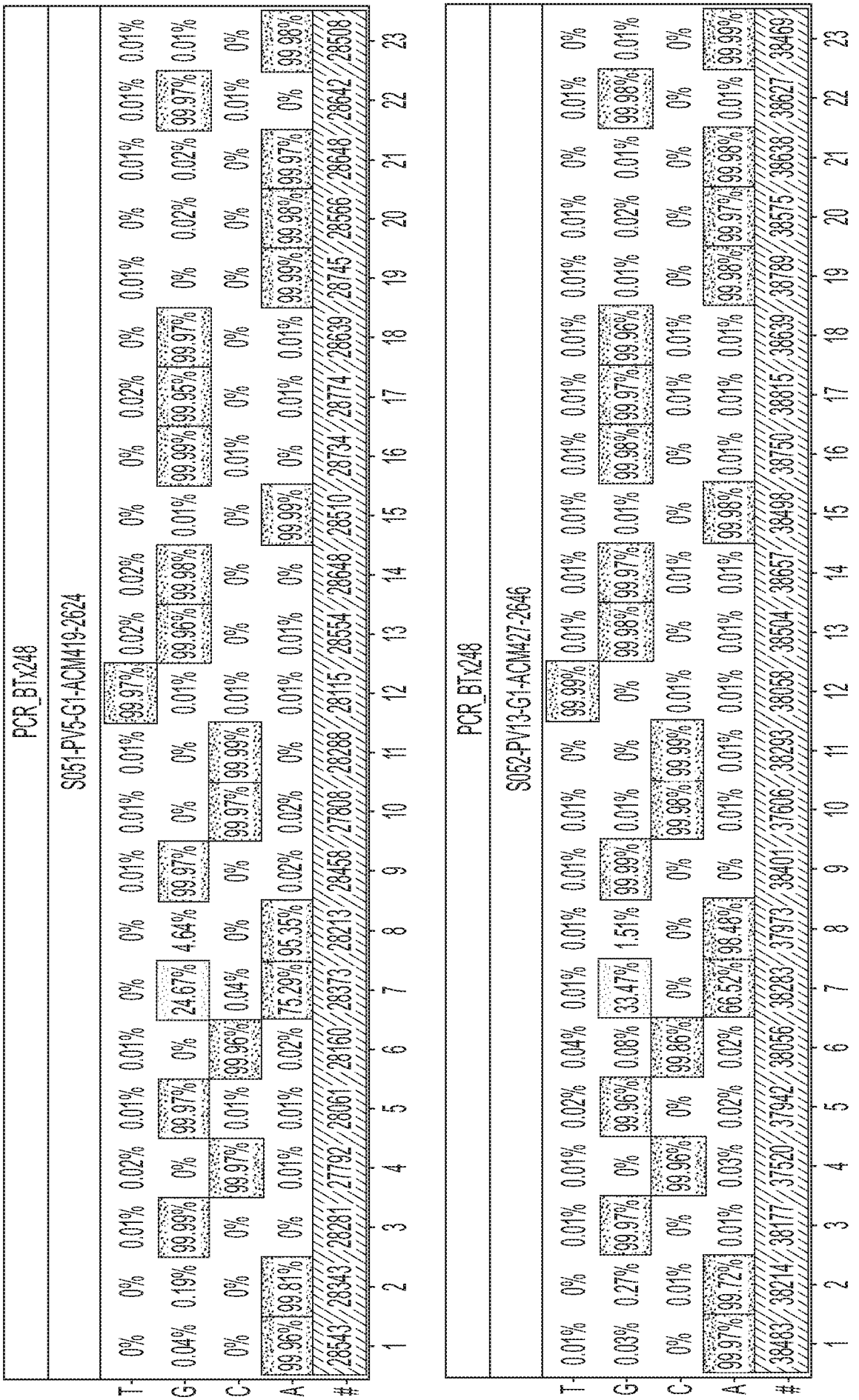


FIG. 59G CONTINUED

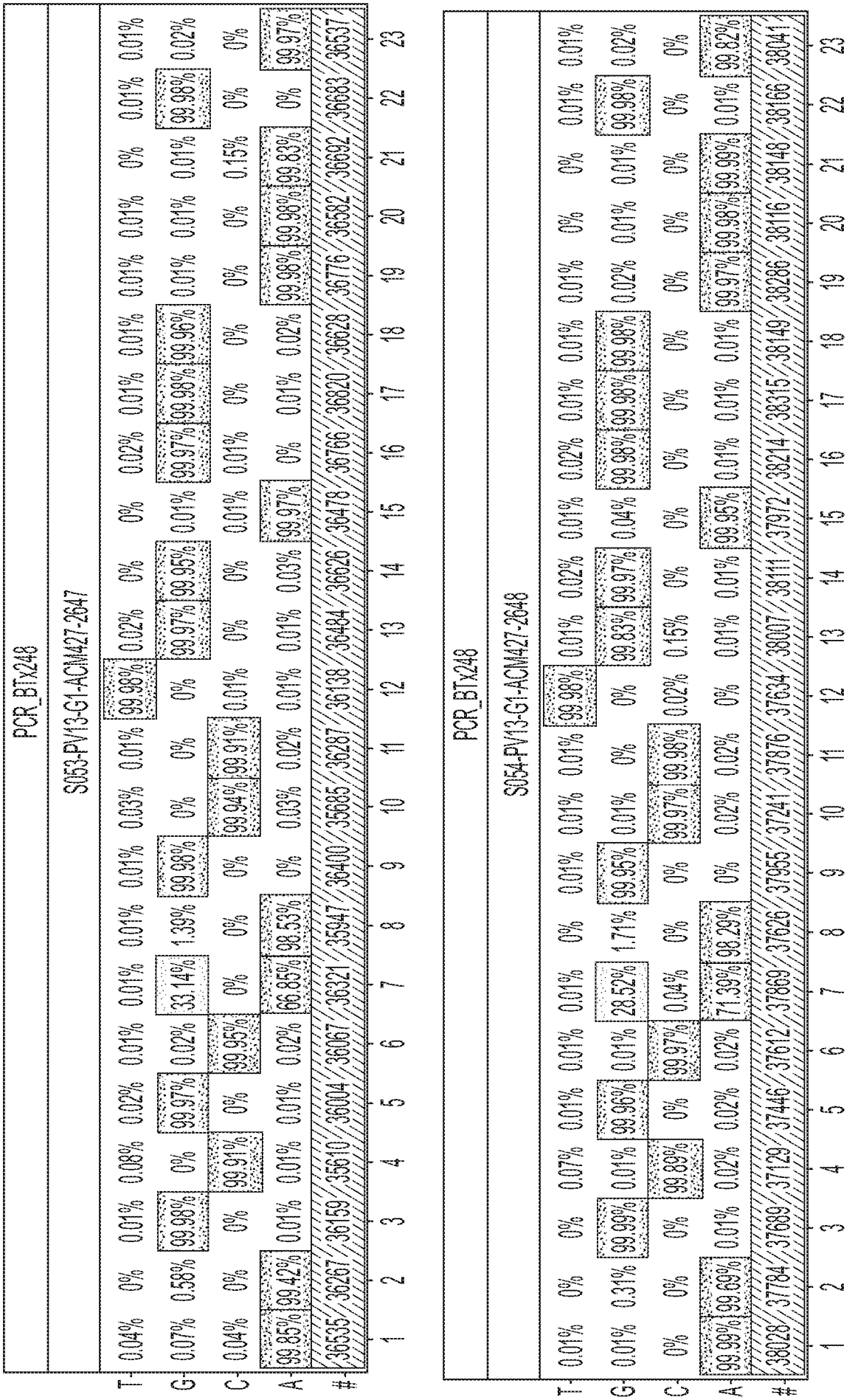

PCR_BTx248
S053-PV13-G1-ACM427-2647
PCR_BTx248
S054-PV13-G1-ACM427-2648
T
G
C
A
#

FIG. 59H

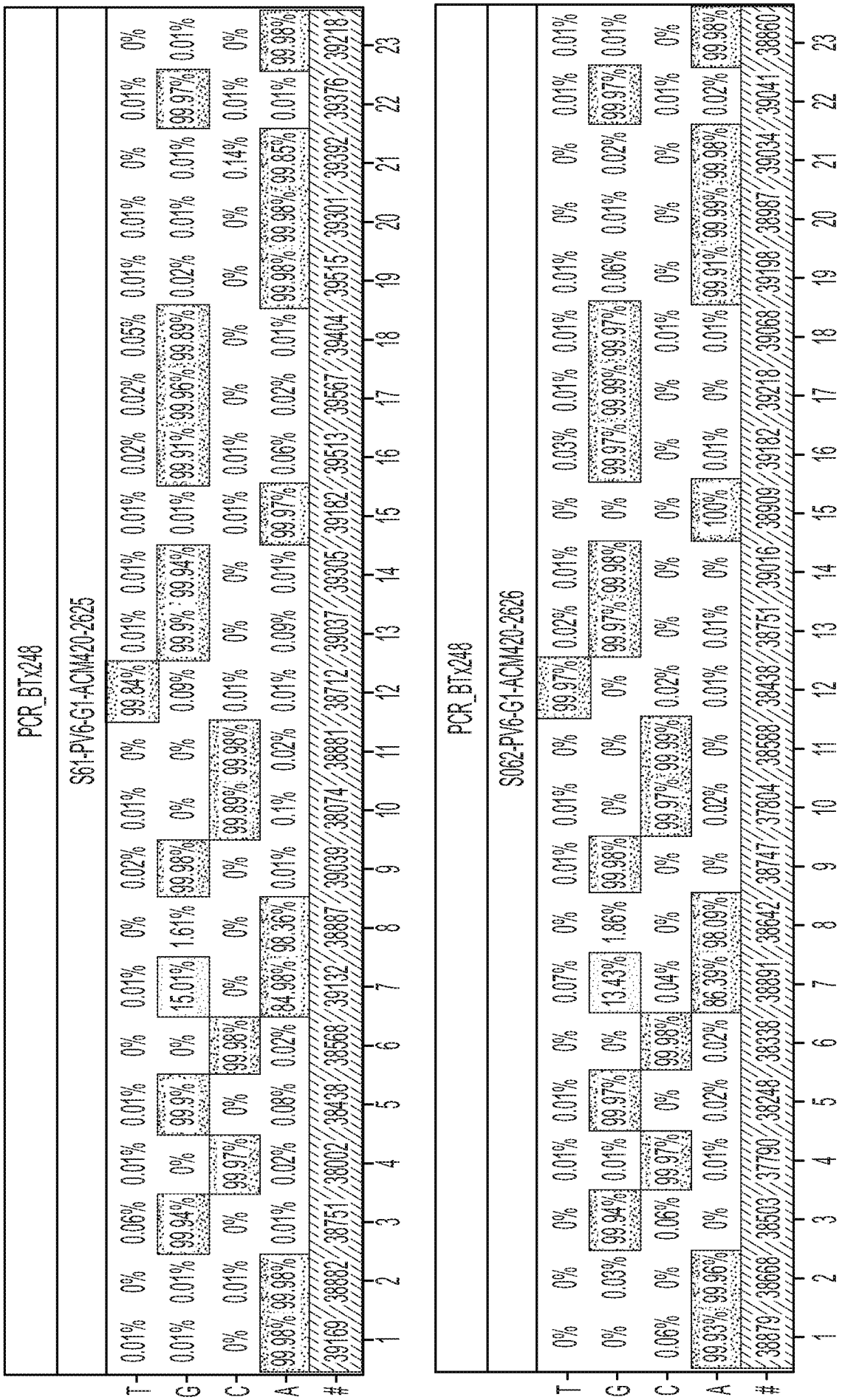


FIG. 59H CONTINUED

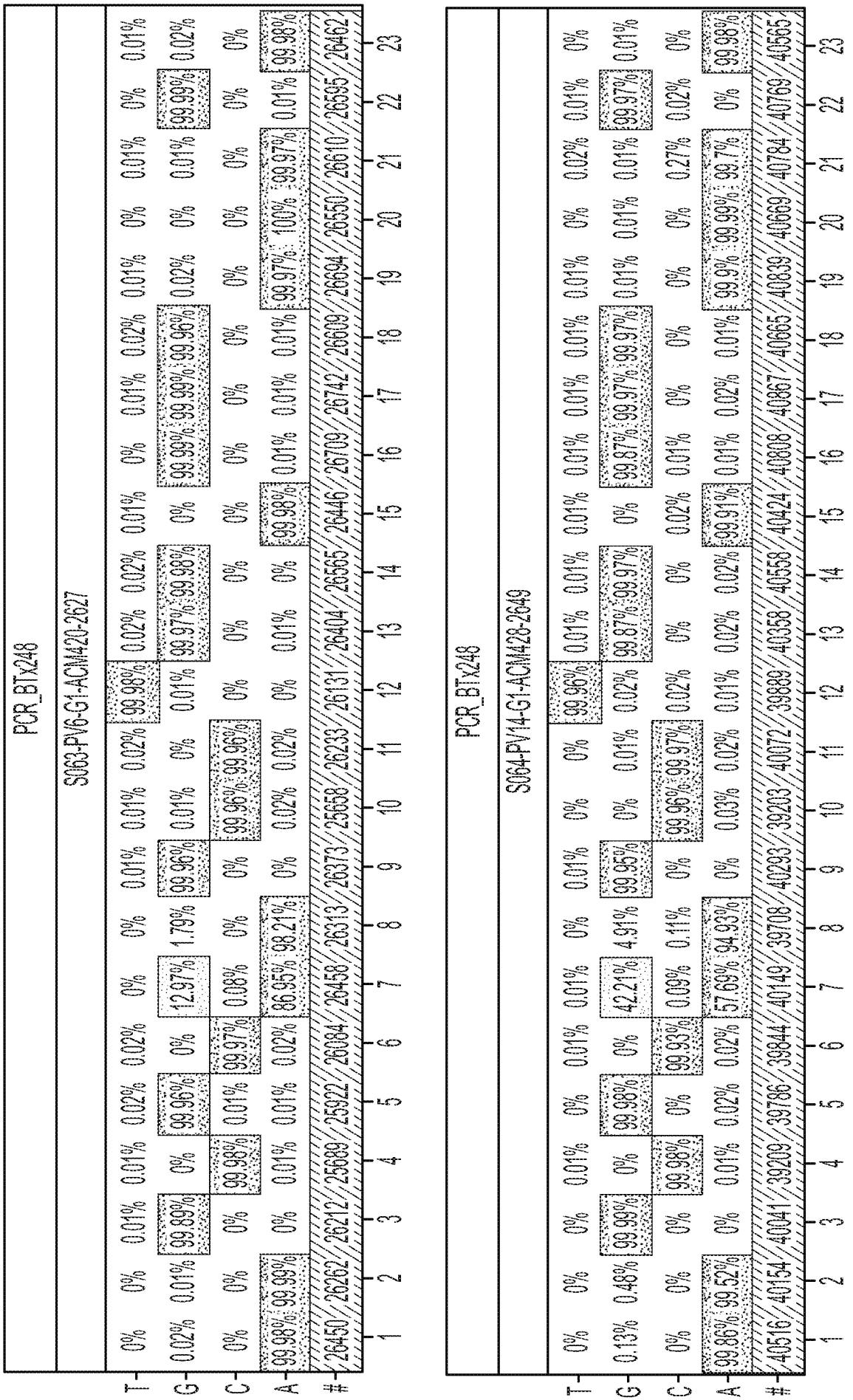

PCR_BTx248
S063-PV6-G1-ACM420-2627
PCR_BTx248
S064-PV14-G1-ACM428-2649

FIG. 59I

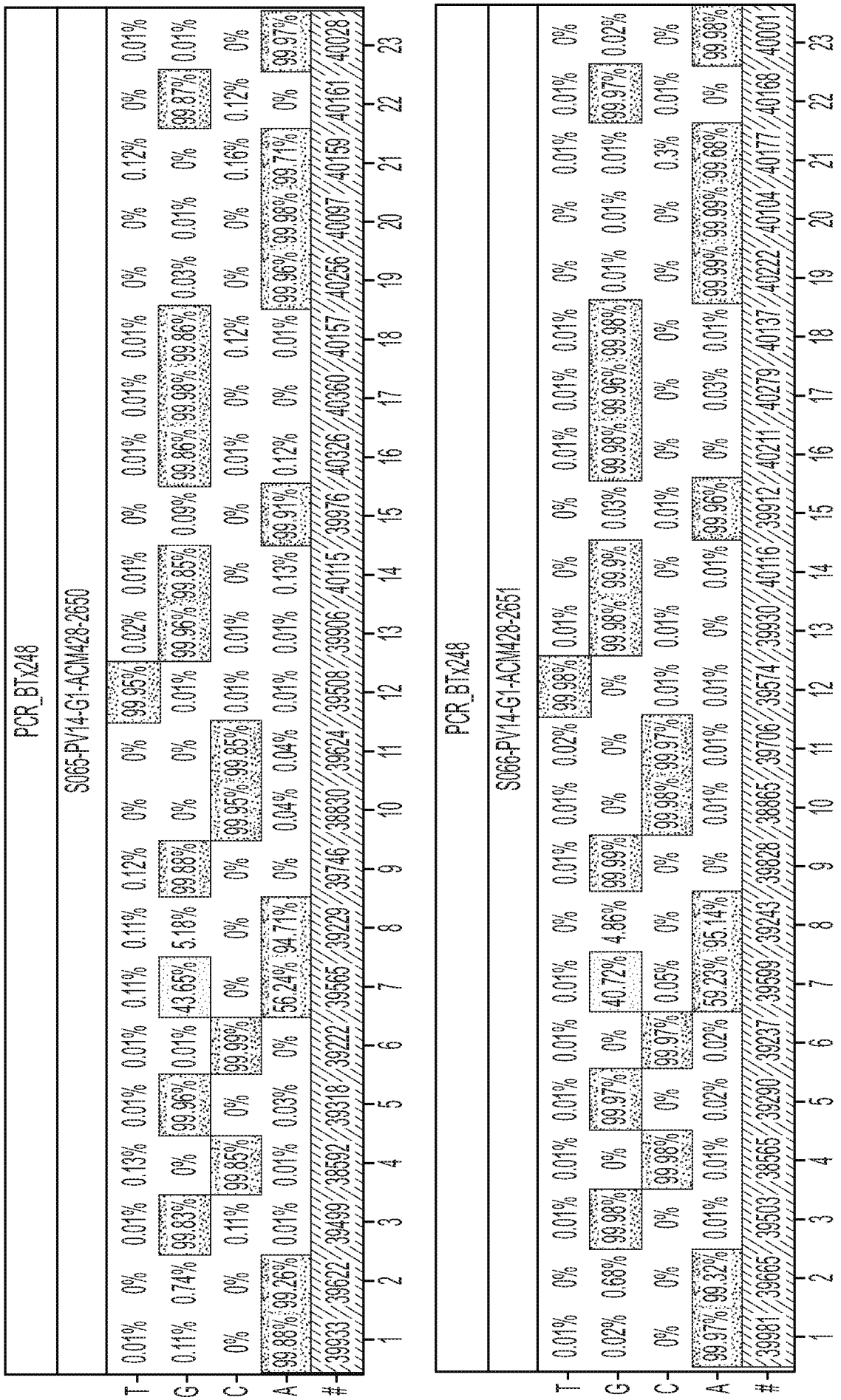


FIG. 59I CONTINUED

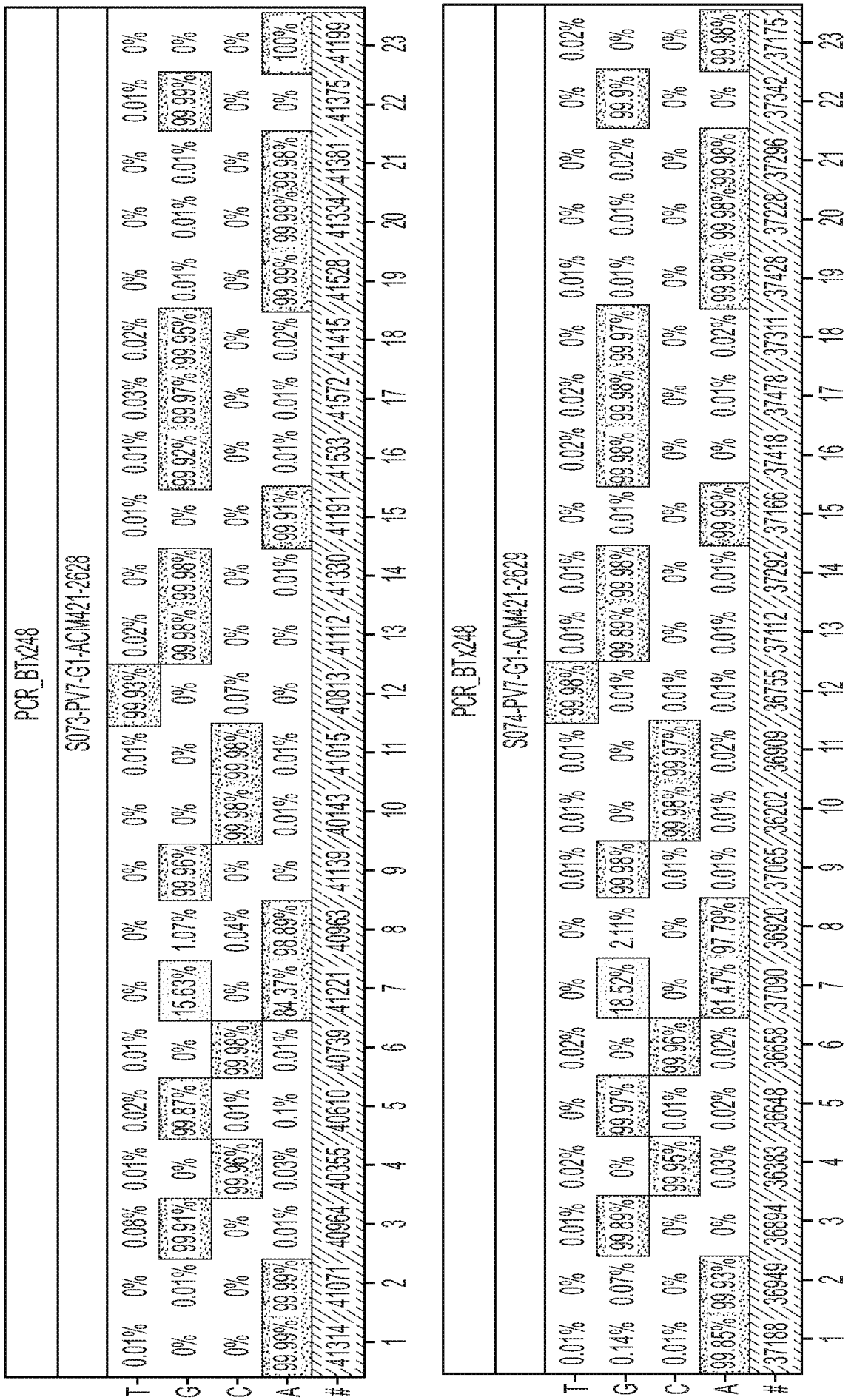

PCR_BTx248
S073-PV7-G1-ACM421-2628
PCR_BTx248
S074-PV7-G1-ACM421-2629

FIG. 59J

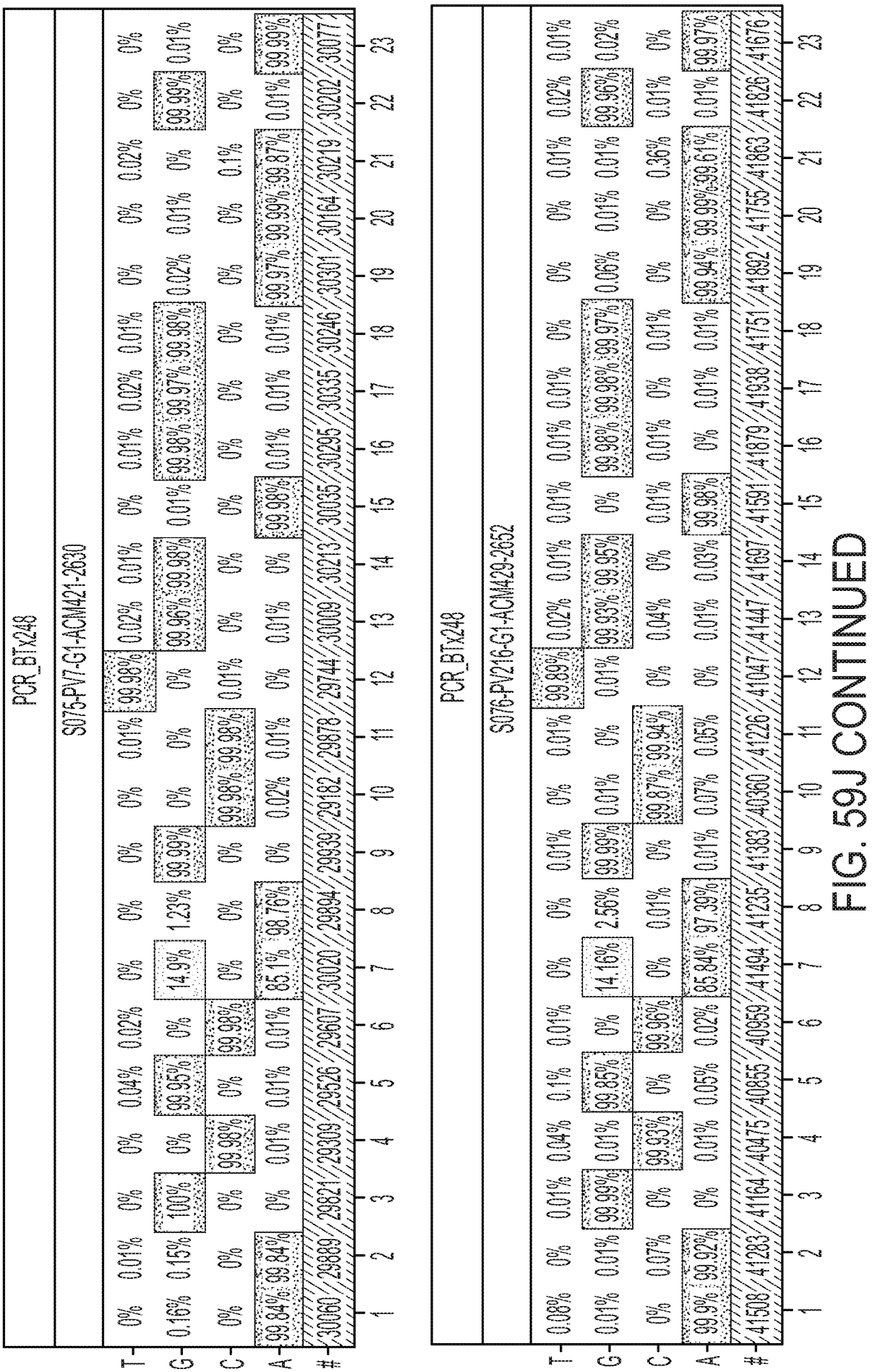


FIG. 59J CONTINUED

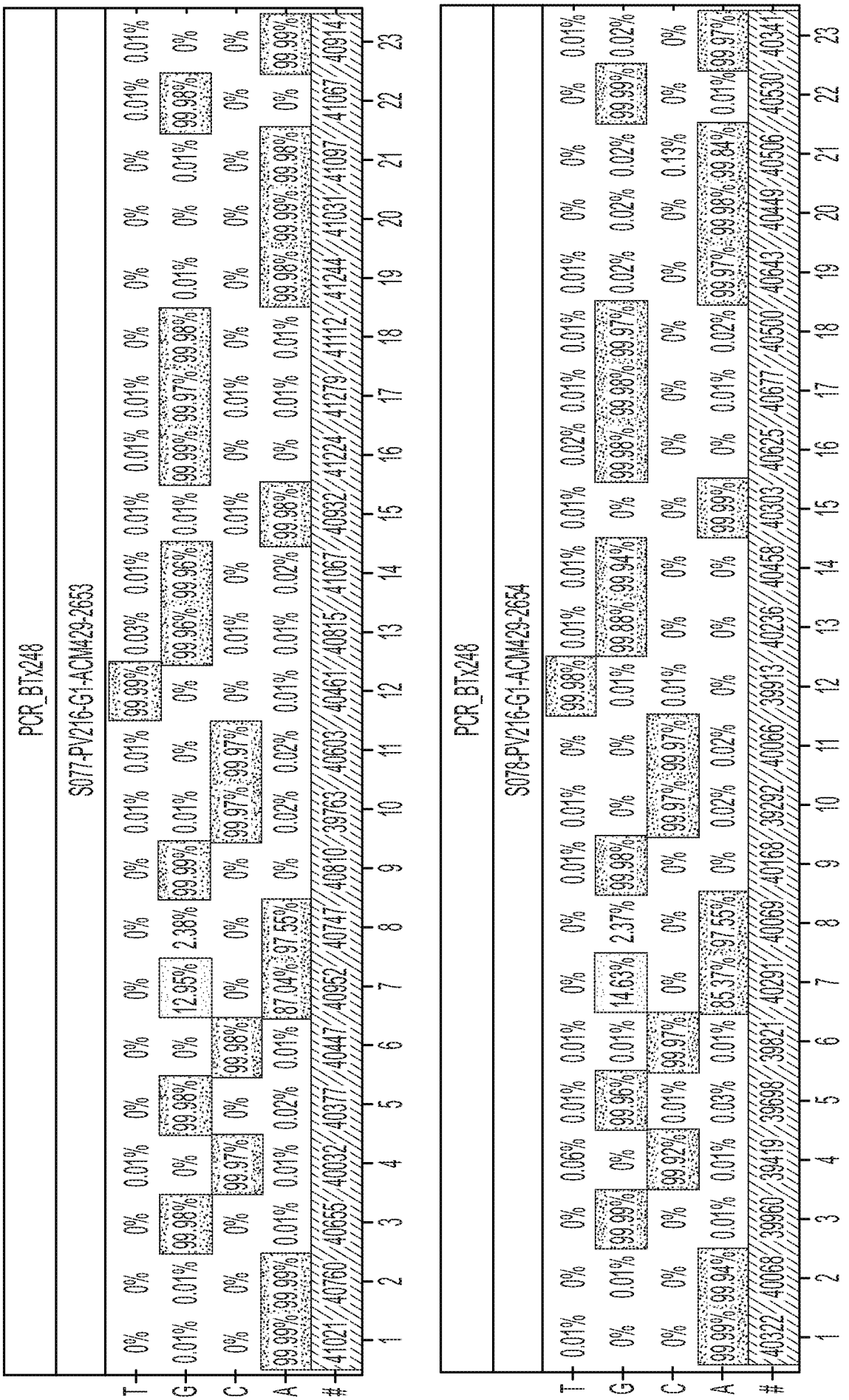

POR_BTx248
S077-PV216-G1-ACM429-2653
POR_BTx248
S078-PV216-G1-ACM429-2654
T
G
C
A
#

FIG. 59K

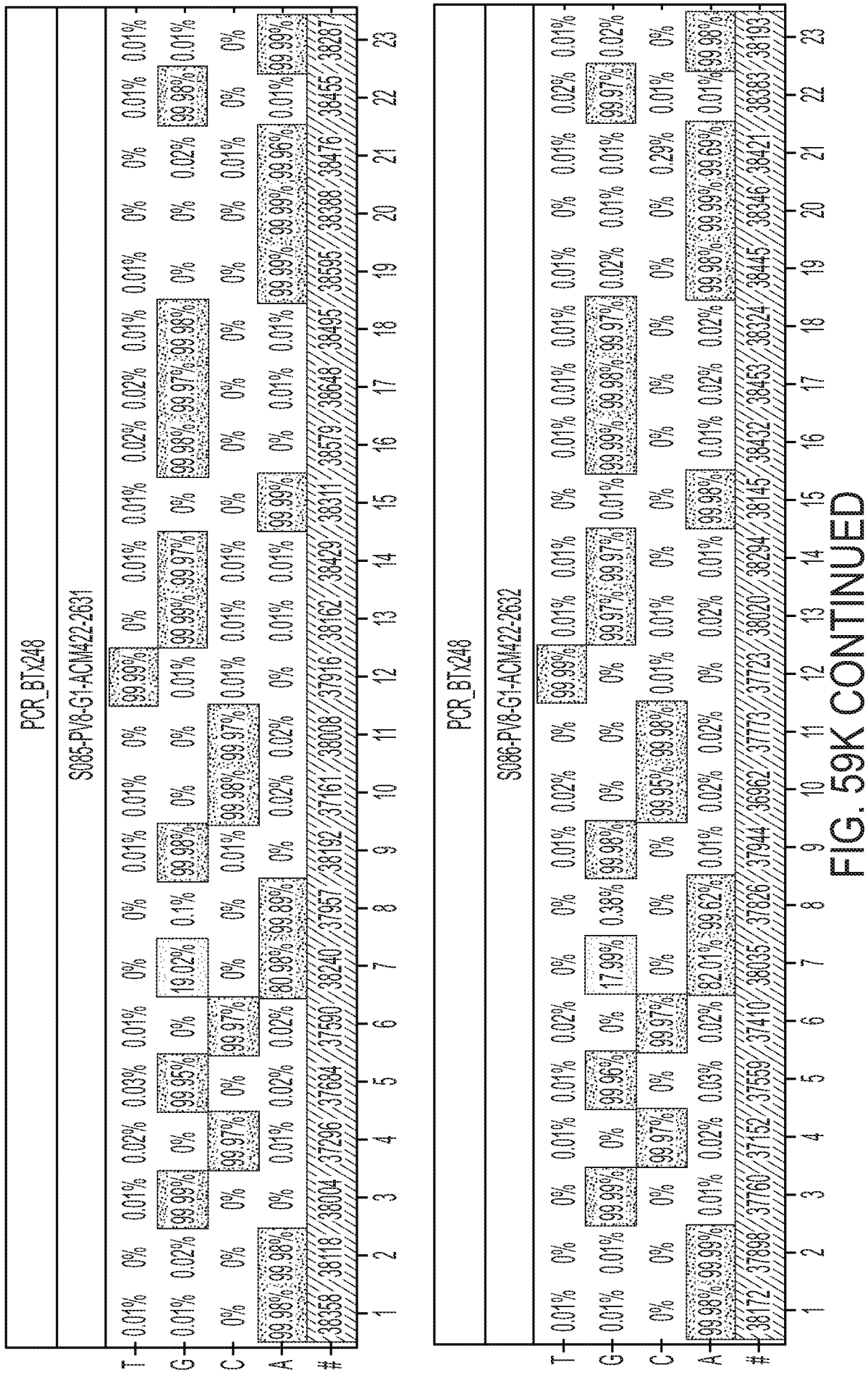


FIG. 59K CONTINUED

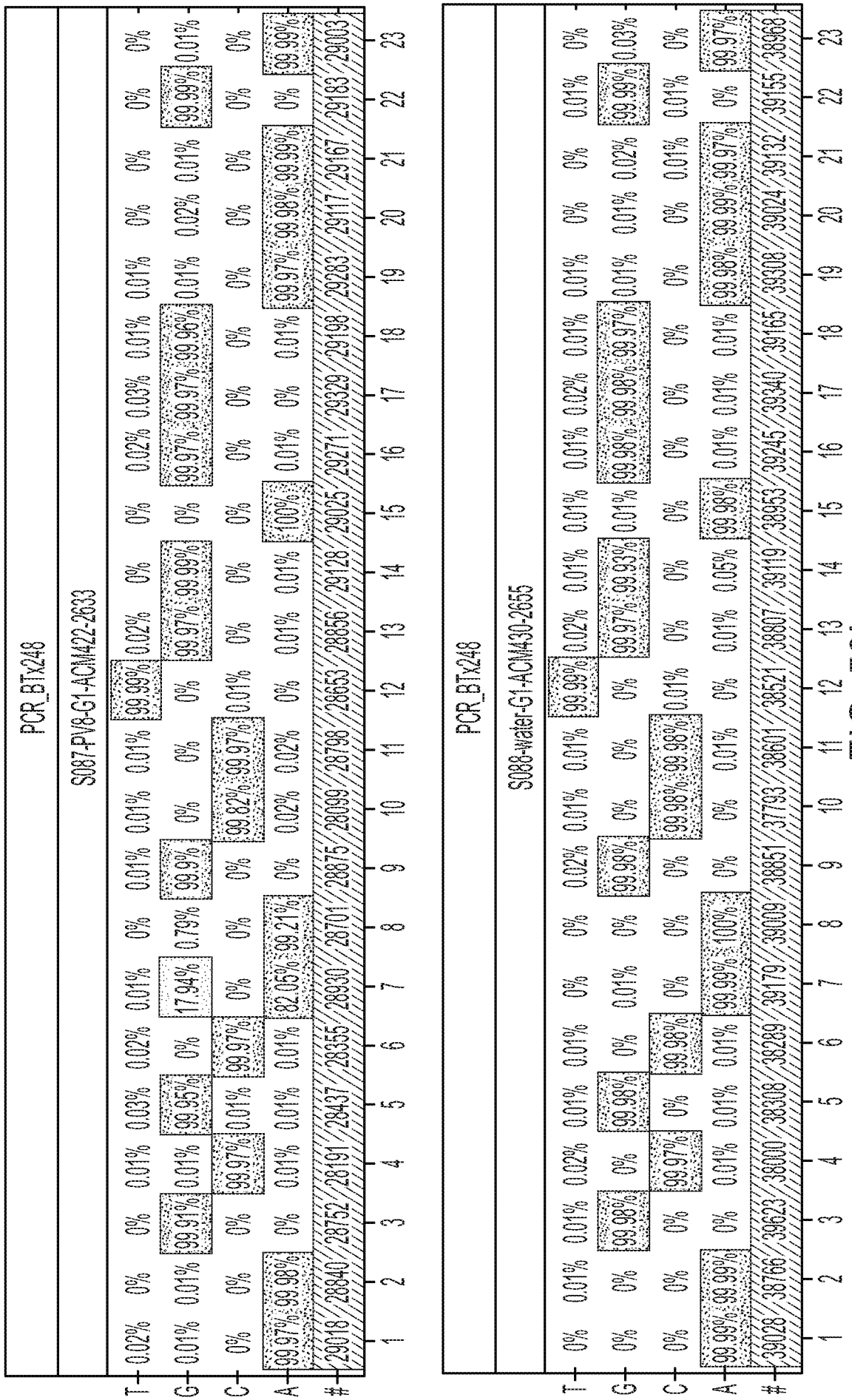

PCR_BTx248
S087-PV8-G1-ACM422-2633
PCR_BTx248
S088-water-G1-ACM430-2655
T
G
C
A
#

FIG. 59L

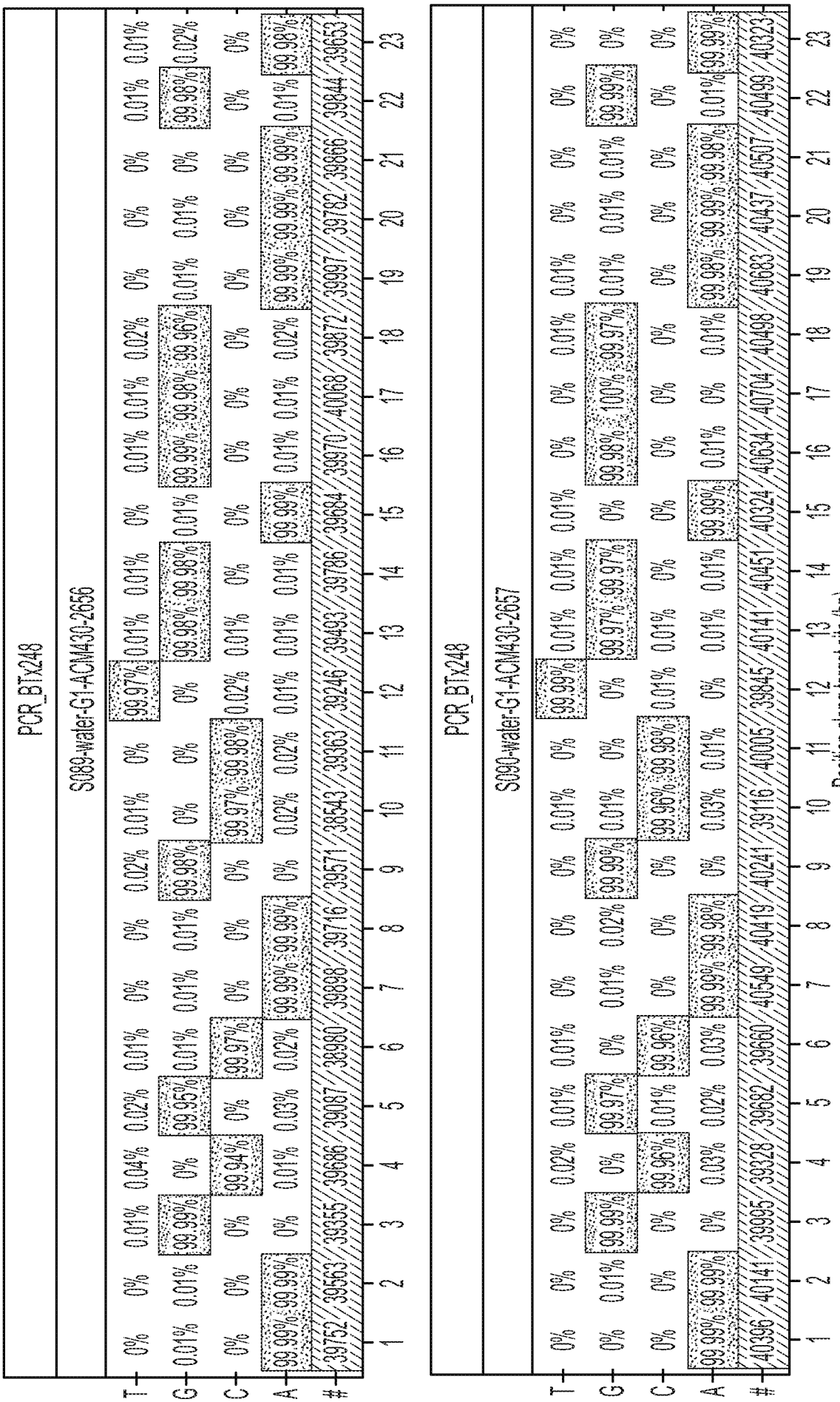


FIG. 59L CONTINUED

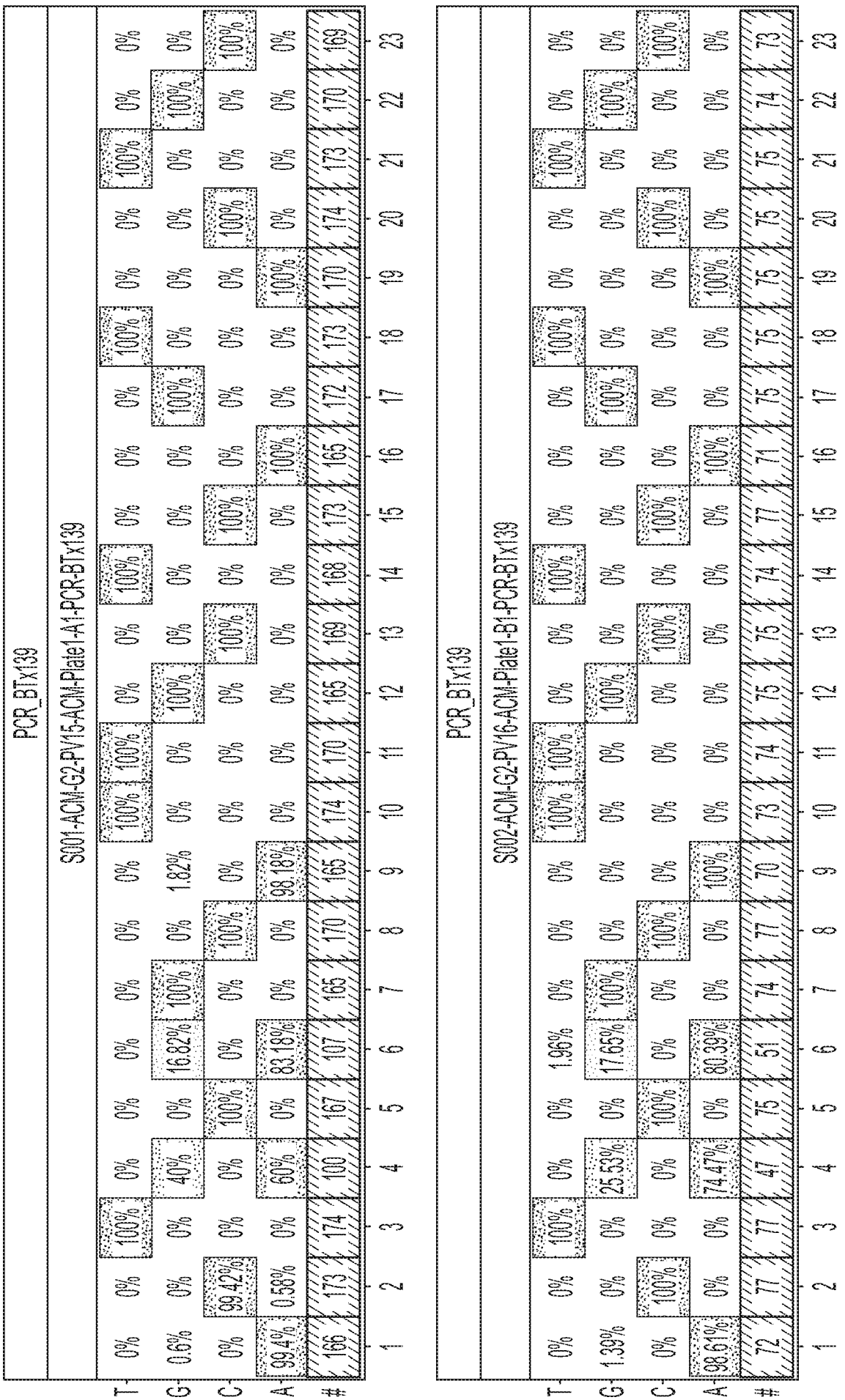

PCR_BTx139
S001-ACM-G2-PV15-ACM-Plate1-A1-PCR-BTx139
PCR_BTx139
S002-ACM-G2-PV16-ACM-Plate1-B1-PCR-BTx139
T
G
C
A
#

FIG. 60A

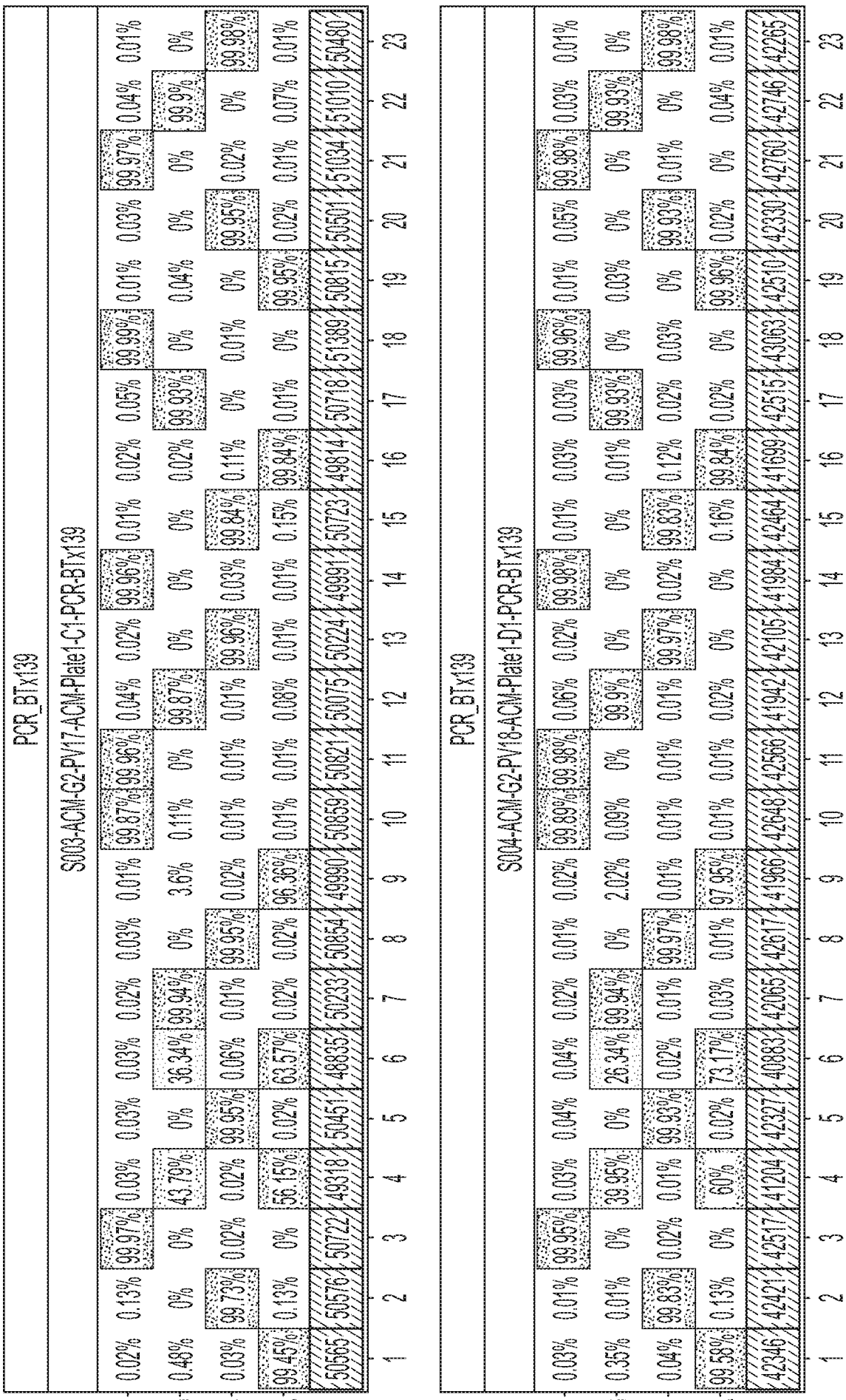


FIG. 60A CONTINUED

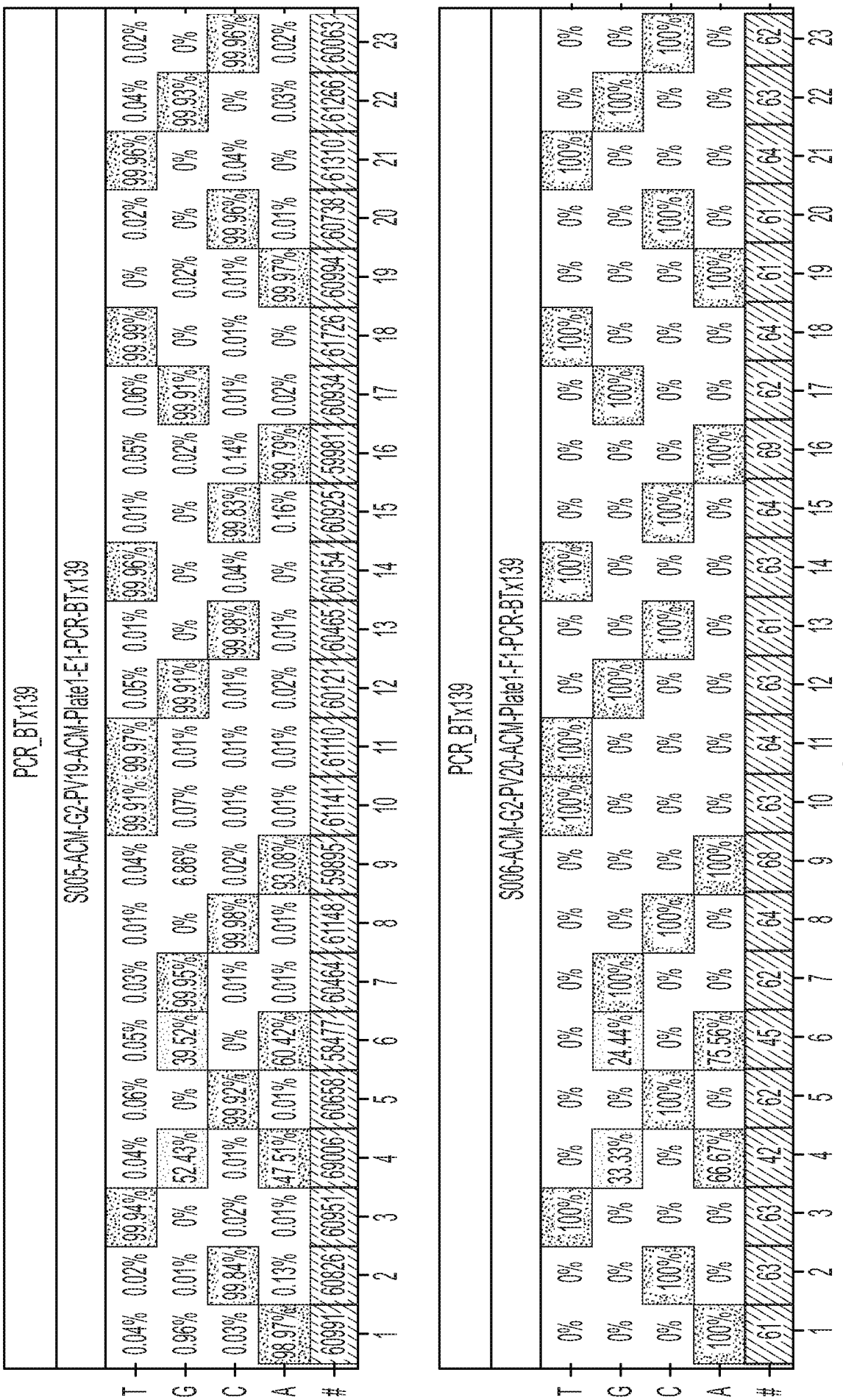

PCR_BTx139
S005-ACM-G2-PV19-ACM-Plate1-F1-PCR-BTx139
PCR_BTx139
S006-ACM-G2-PV20-ACM-Plate1-F1-PCR-BTx139
T
G
C
A
#

FIG. 60B

FIG. 60B CONTINUED

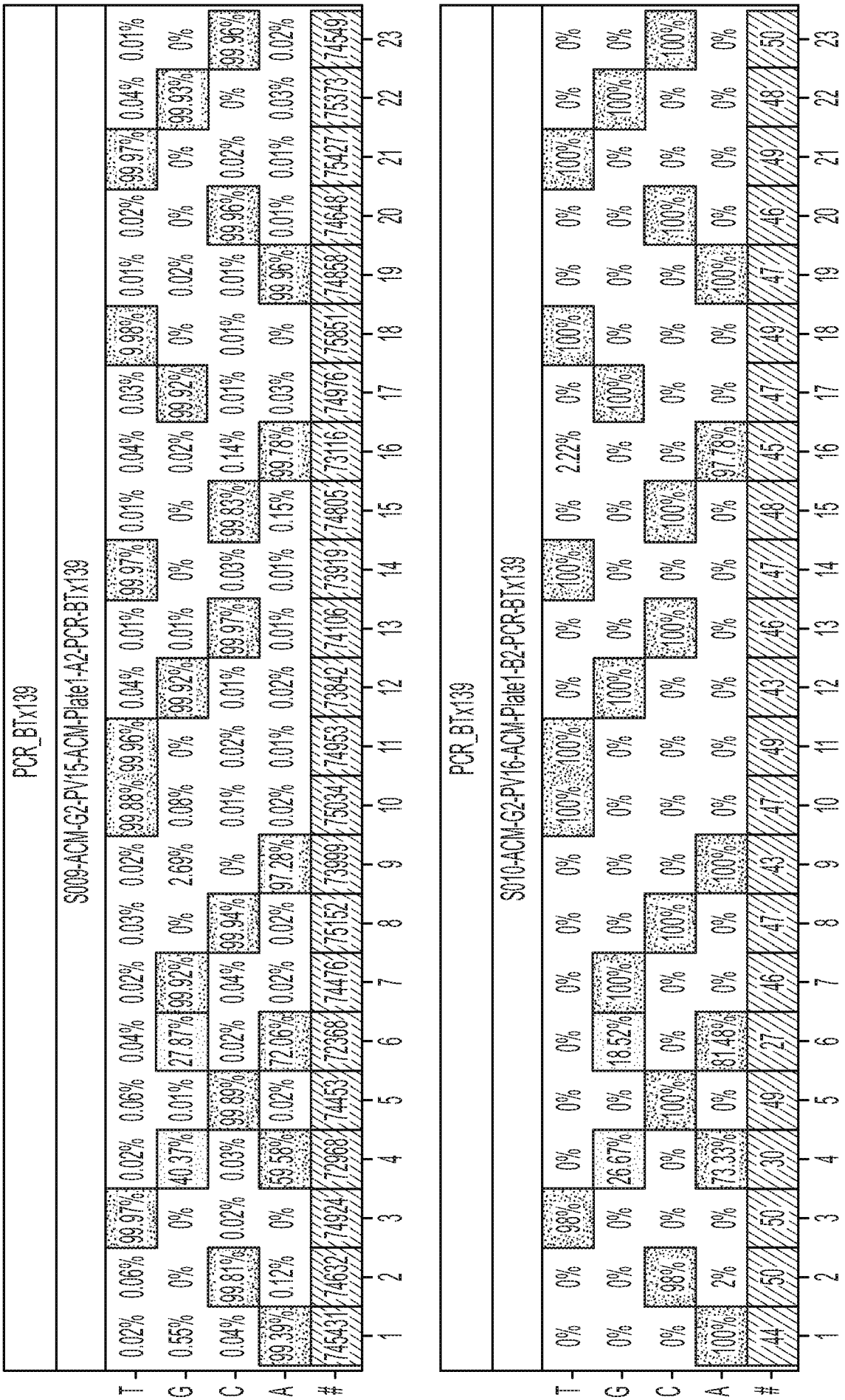

PCR_BTx139
S009-ACM-G2-PV15-ACM-Plate1-A2-PCR-BTx139
PCR_BTx139
S010-ACM-G2-PV16-ACM-Plate1-B2-PCR-BTx139
T
G
C
A
#

FIG. 60C

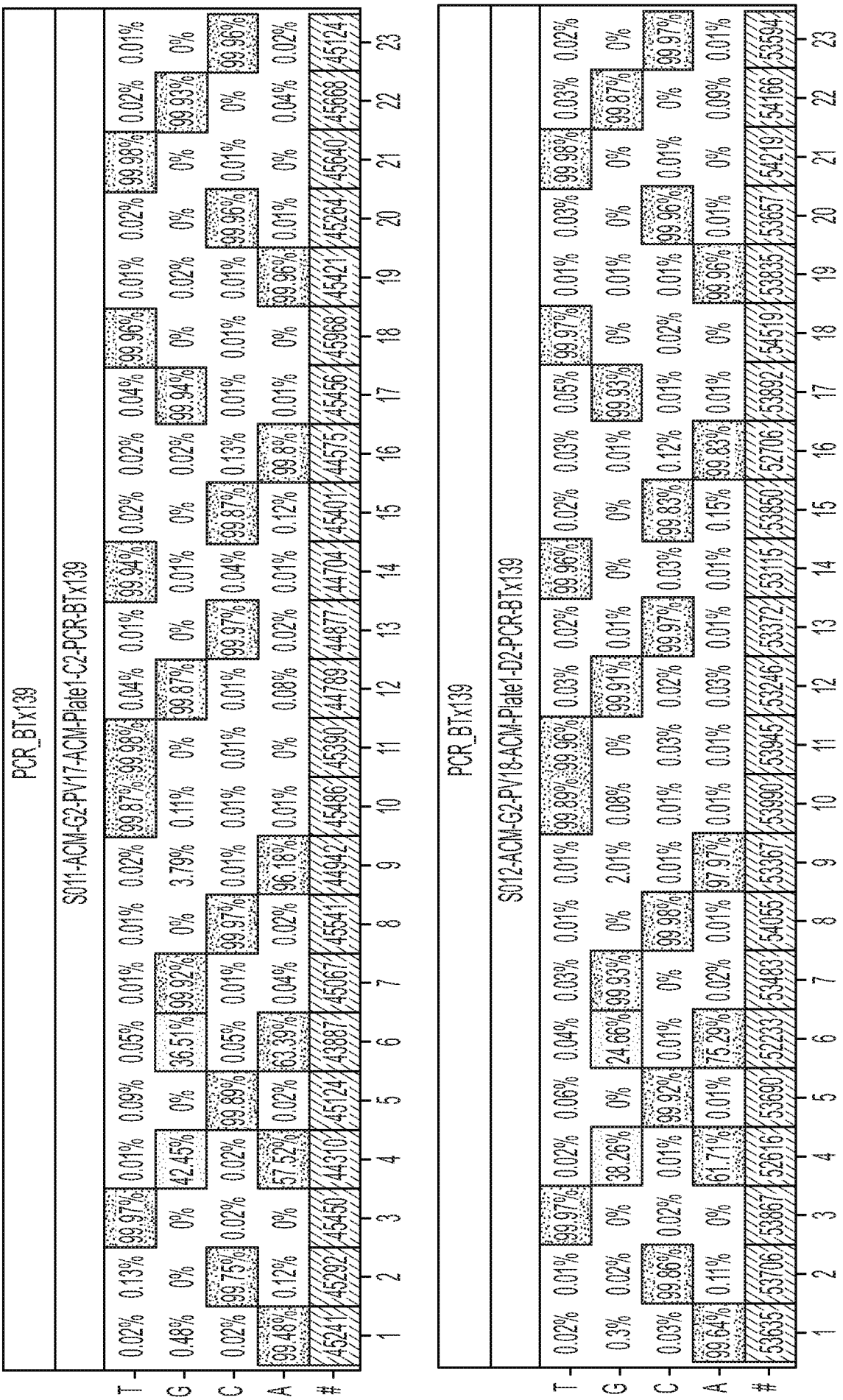


FIG. 60C CONTINUED

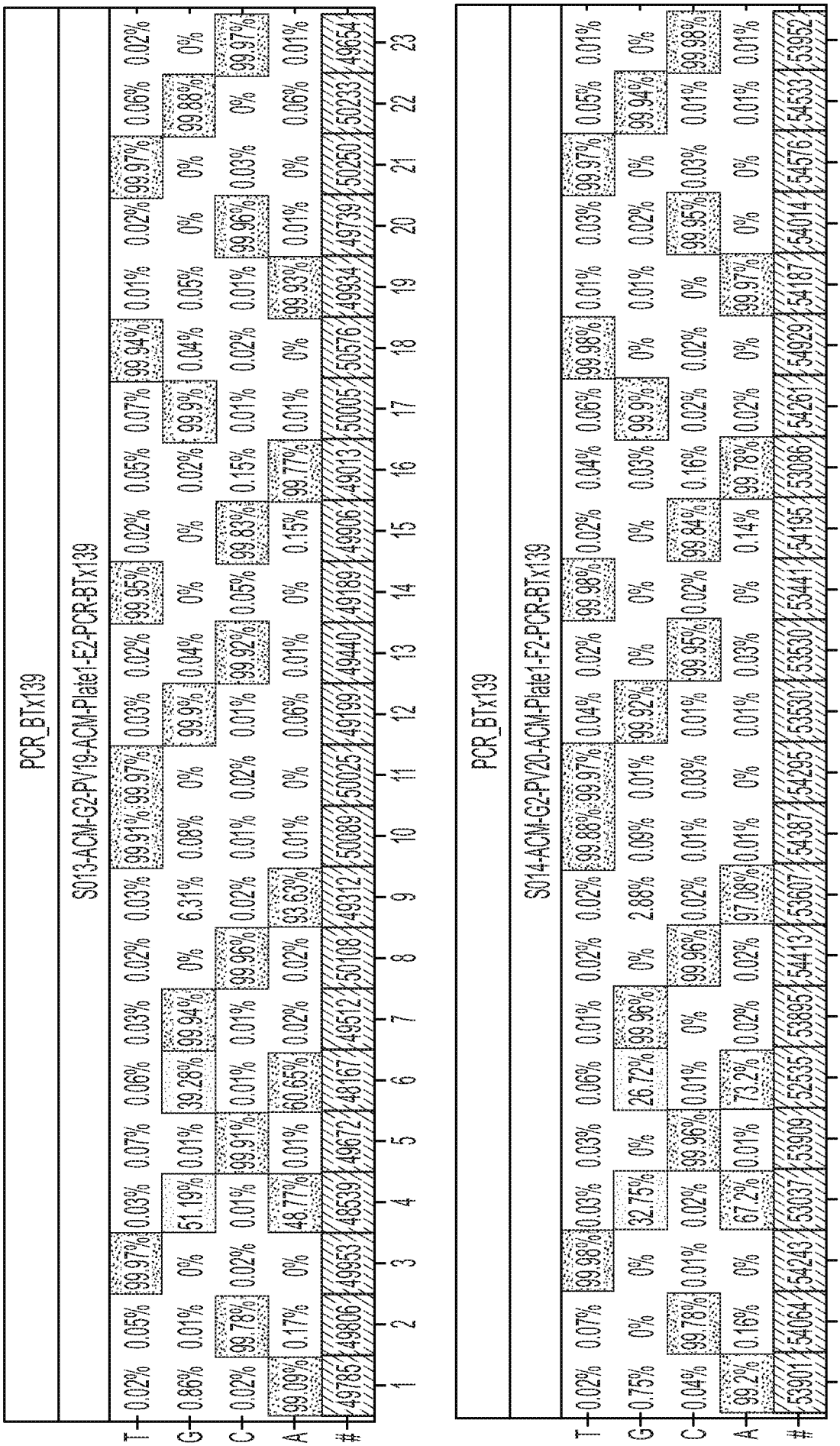

PCR_BTx139
S013-ACM-G2-PV19-ACM-Plate1-F2-PCR-BTx139
PCR_BTx139
S014-ACM-G2-PV20-ACM-Plate1-F2-PCR-BTx139
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 60D

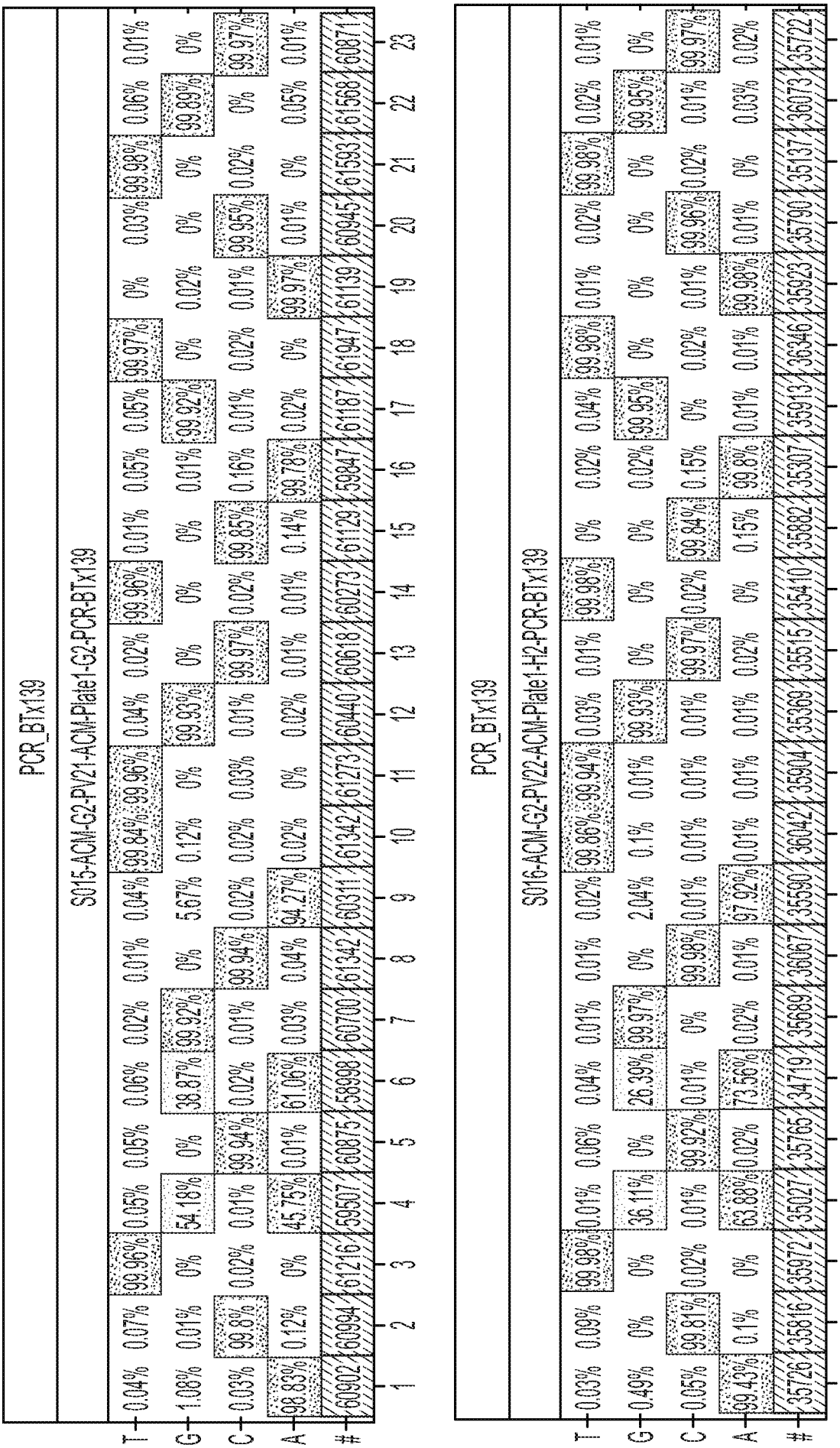


FIG. 60D CONTINUED

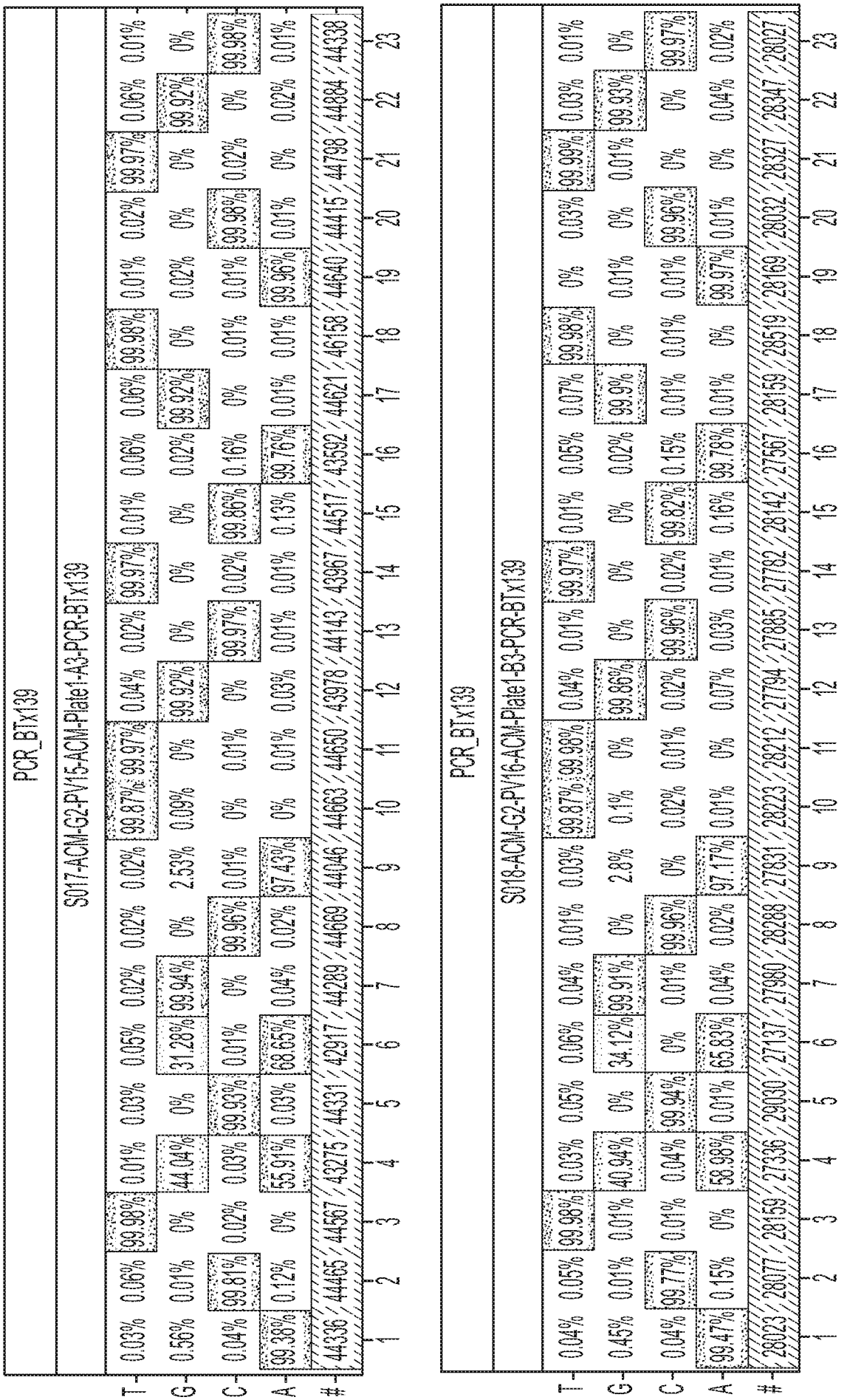

PCR_BTx139
S017-ACM-G2-PV15-ACM-Plate1-A3-PCR-BTx139
PCR_BTx139
S018-ACM-G2-PV16-ACM-Plate1-B3-PCR-BTx139

FIG. 60E

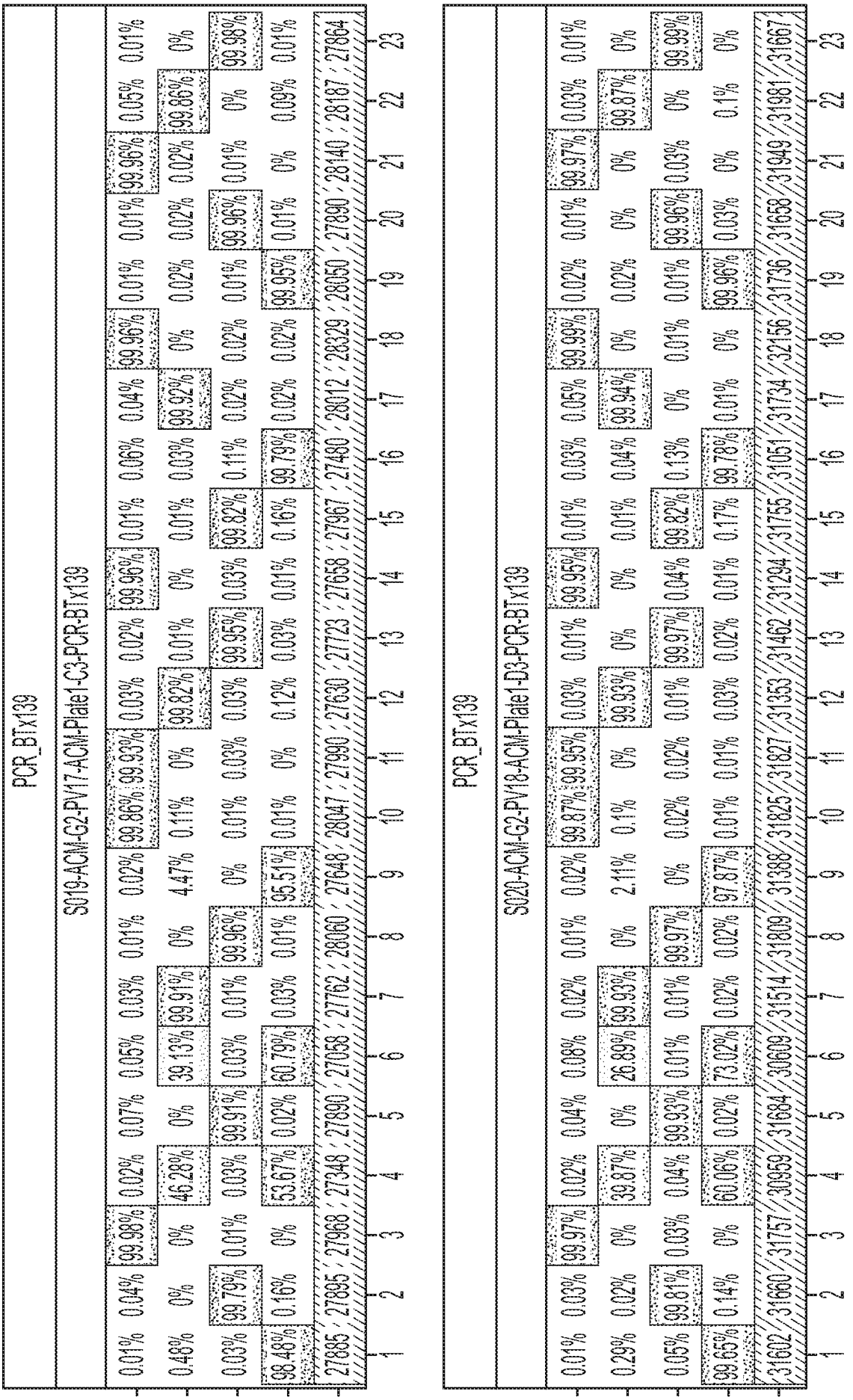


FIG. 60E CONTINUED

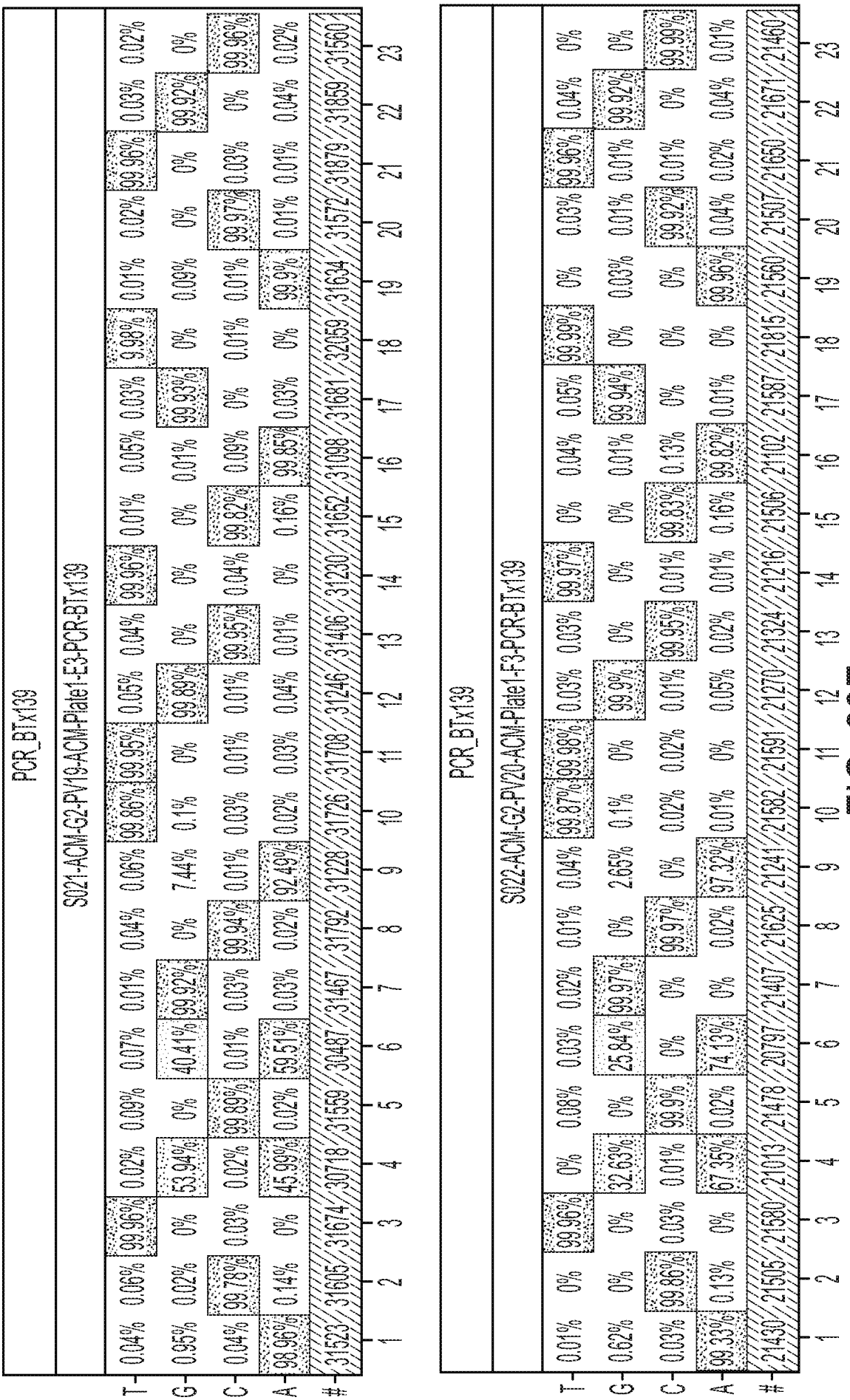

PCR_BTx139
S021-ACM-G2-PV19-ACM-Plate1-E3-PCR-BTx139
PCR_BTx139
S022-ACM-G2-PV20-ACM-Plate1-F3-PCR-BTx139
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 60F

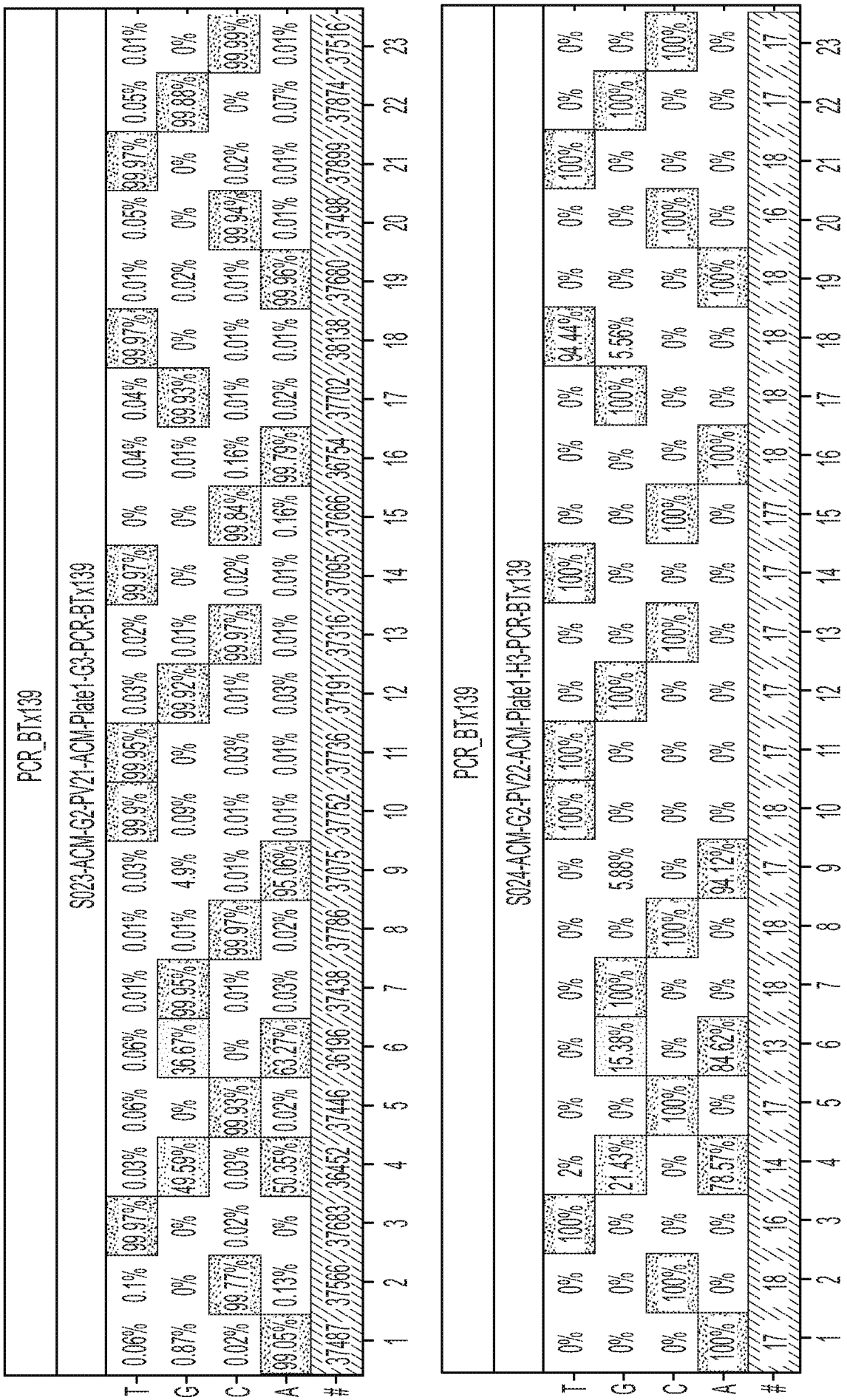


FIG. 60F CONTINUED

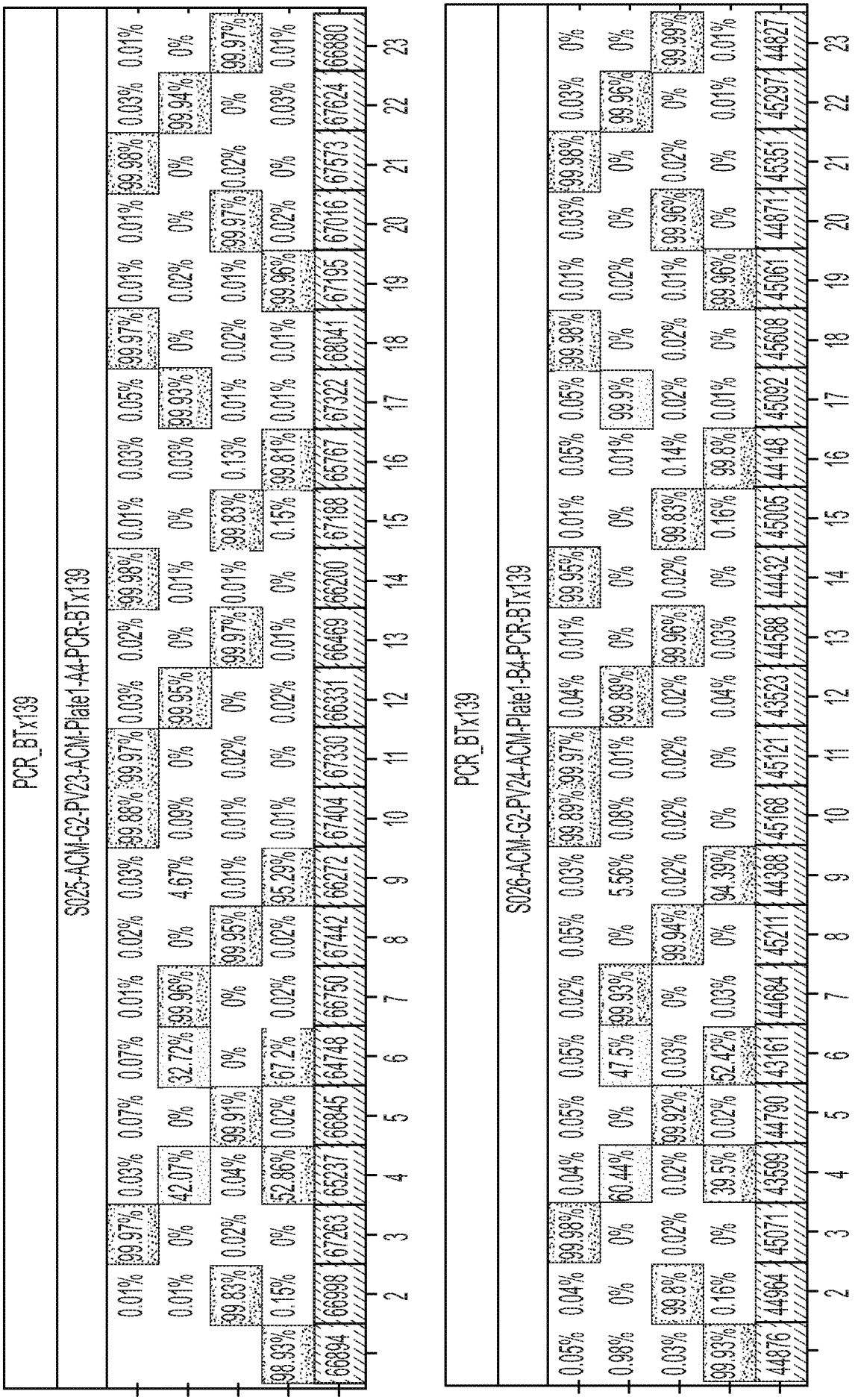

PCR_BTx139
S025-ACM-G2-PV23-ACM-Plate1-A4-PCR-BTx139
PCR_BTx139
S026-ACM-G2-PV24-ACM-Plate1-B4-PCR-BTx139

FIG. 60G

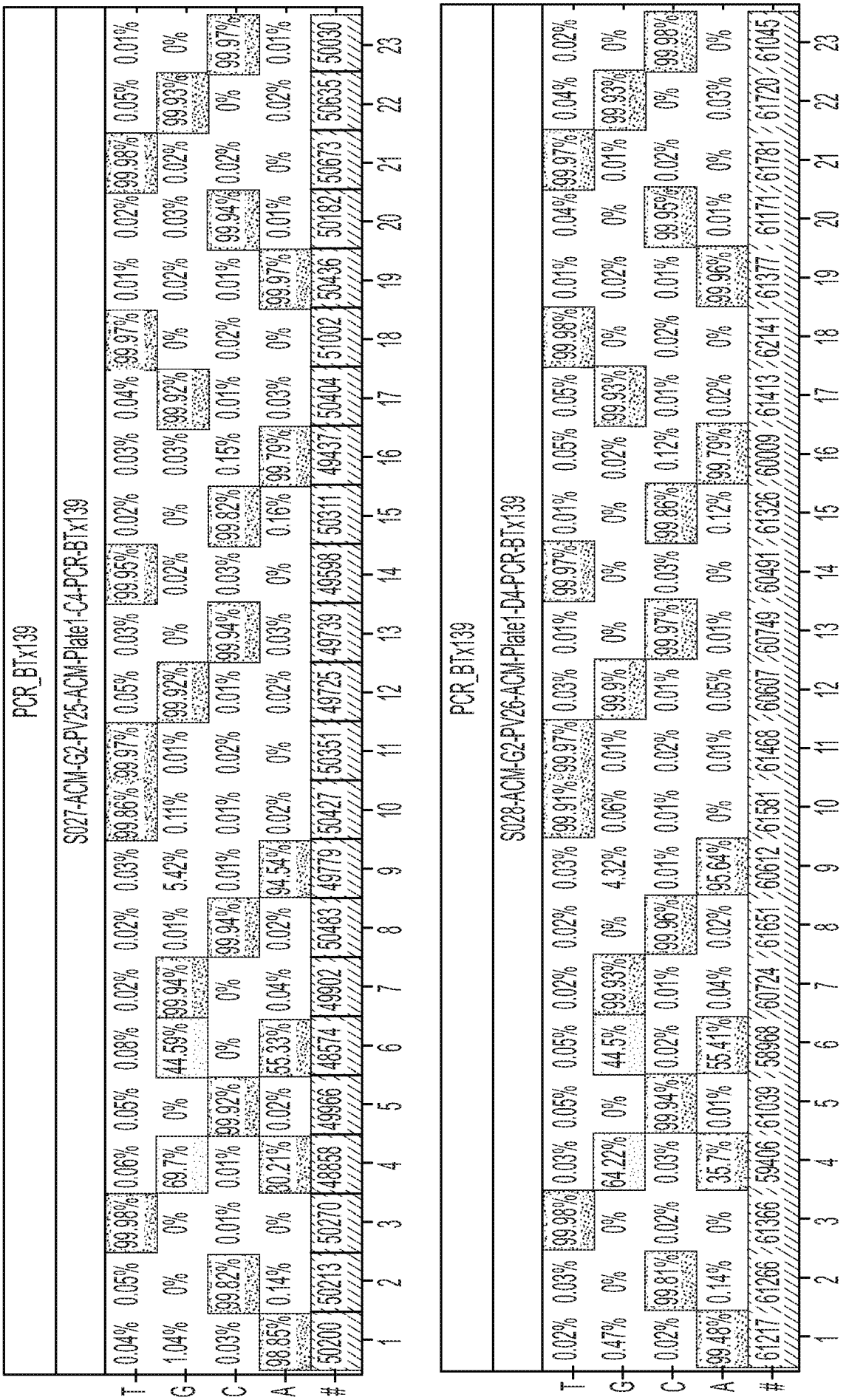


FIG. 60G CONTINUED

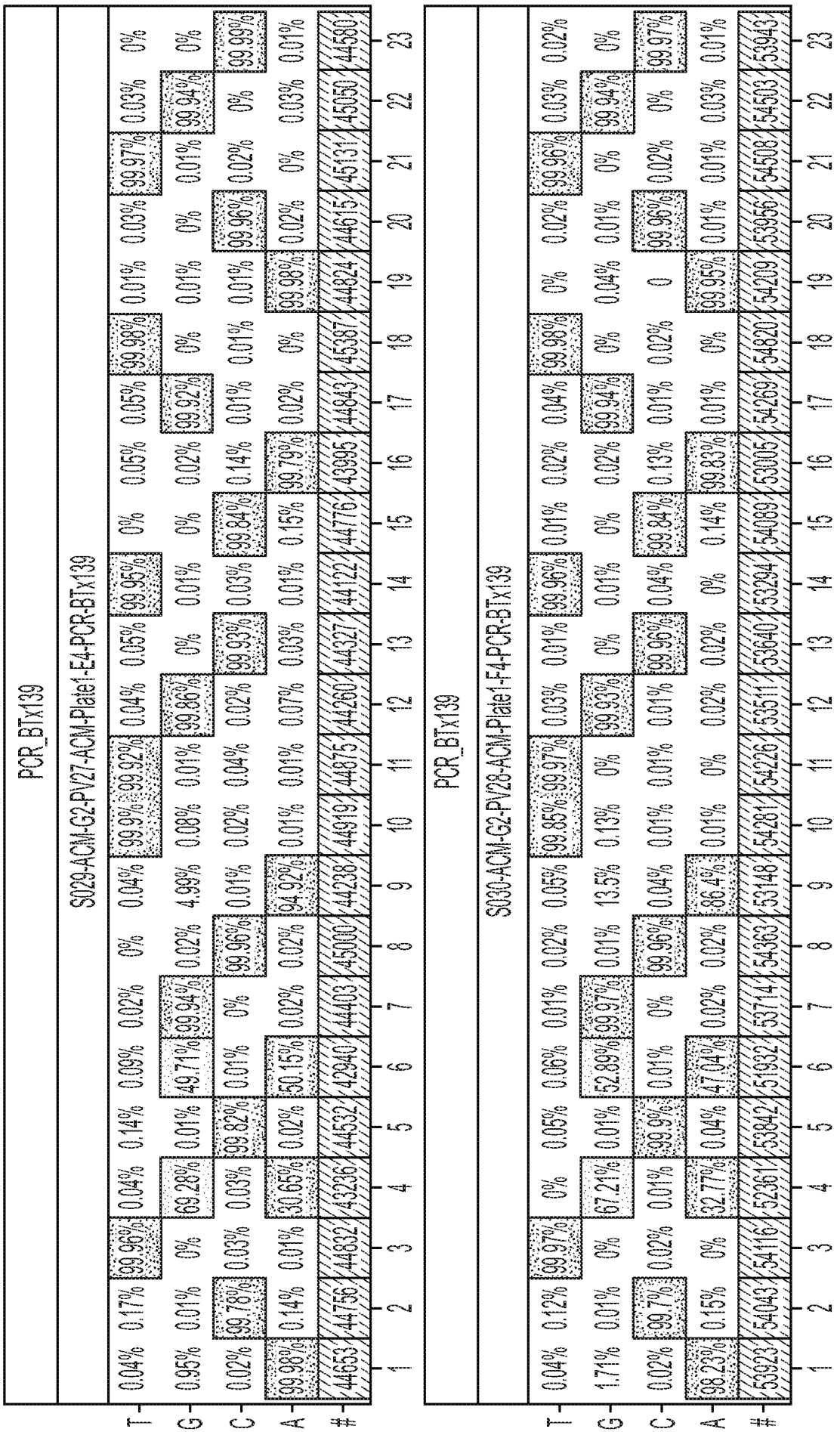

PCR_BTx139
S029-ACM-G2-PV27-ACM-Plate1-E4-PCR-BTx139
PCR_BTx139
S030-ACM-G2-PV28-ACM-Plate1-F4-PCR-BTx139
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 60H

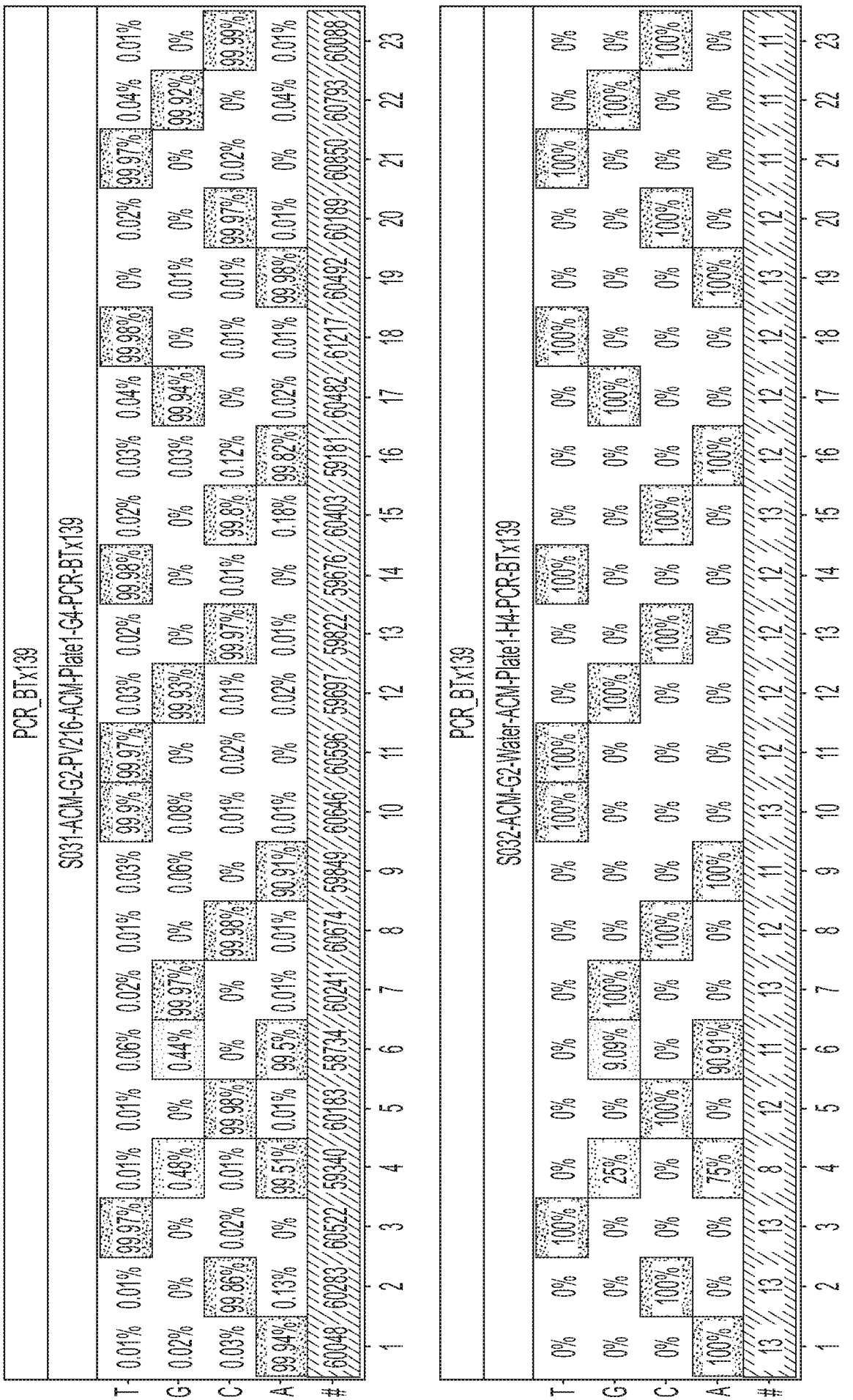


FIG. 60H CONTINUED

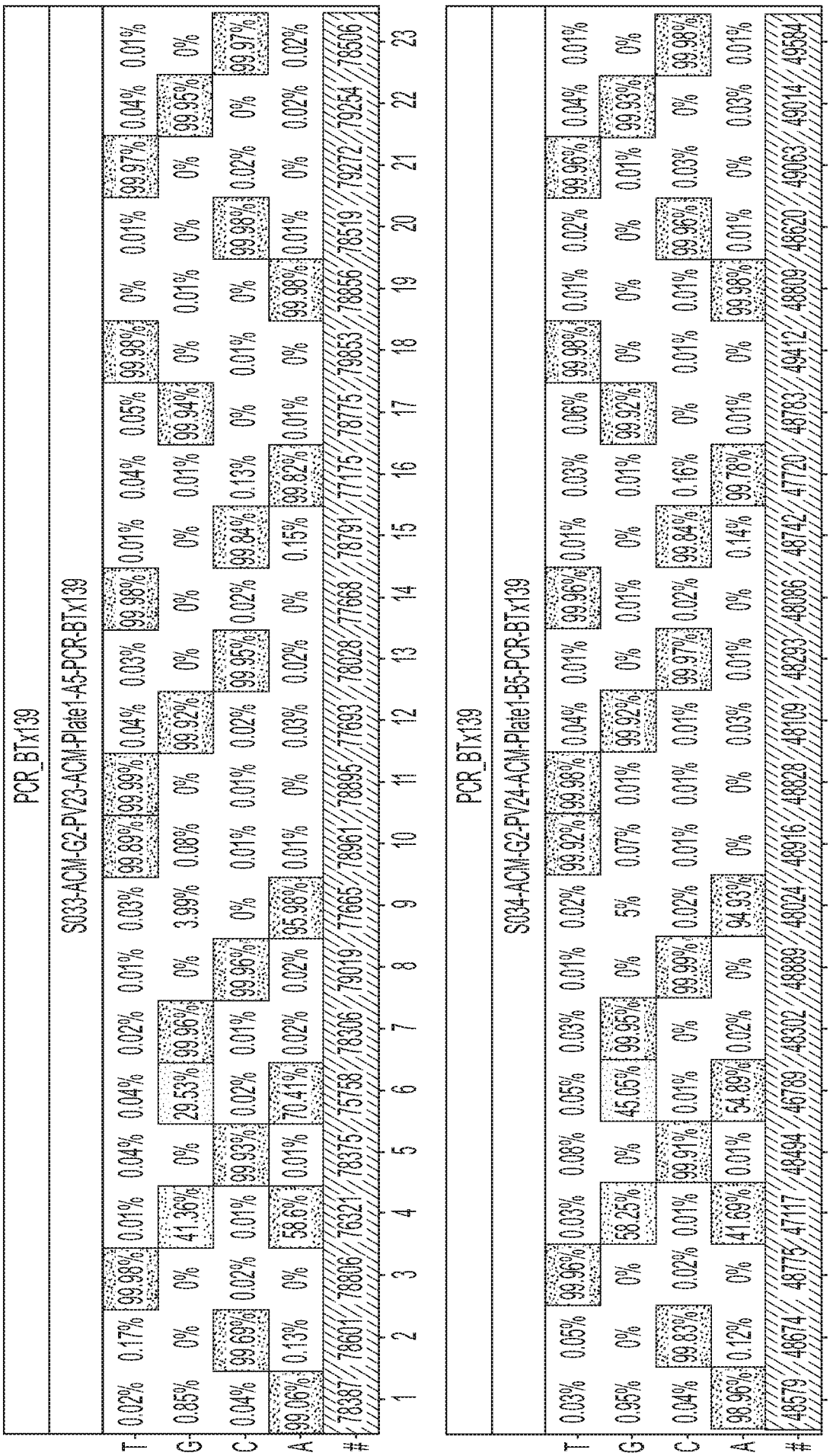

PCR_BTx139
S033-ACM-G2-PV23-ACM-Plate1-A5-PCR-BTx139
PCR_BTx139
S034-ACM-G2-PV24-ACM-Plate1-B5-PCR-BTx139
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 6OI

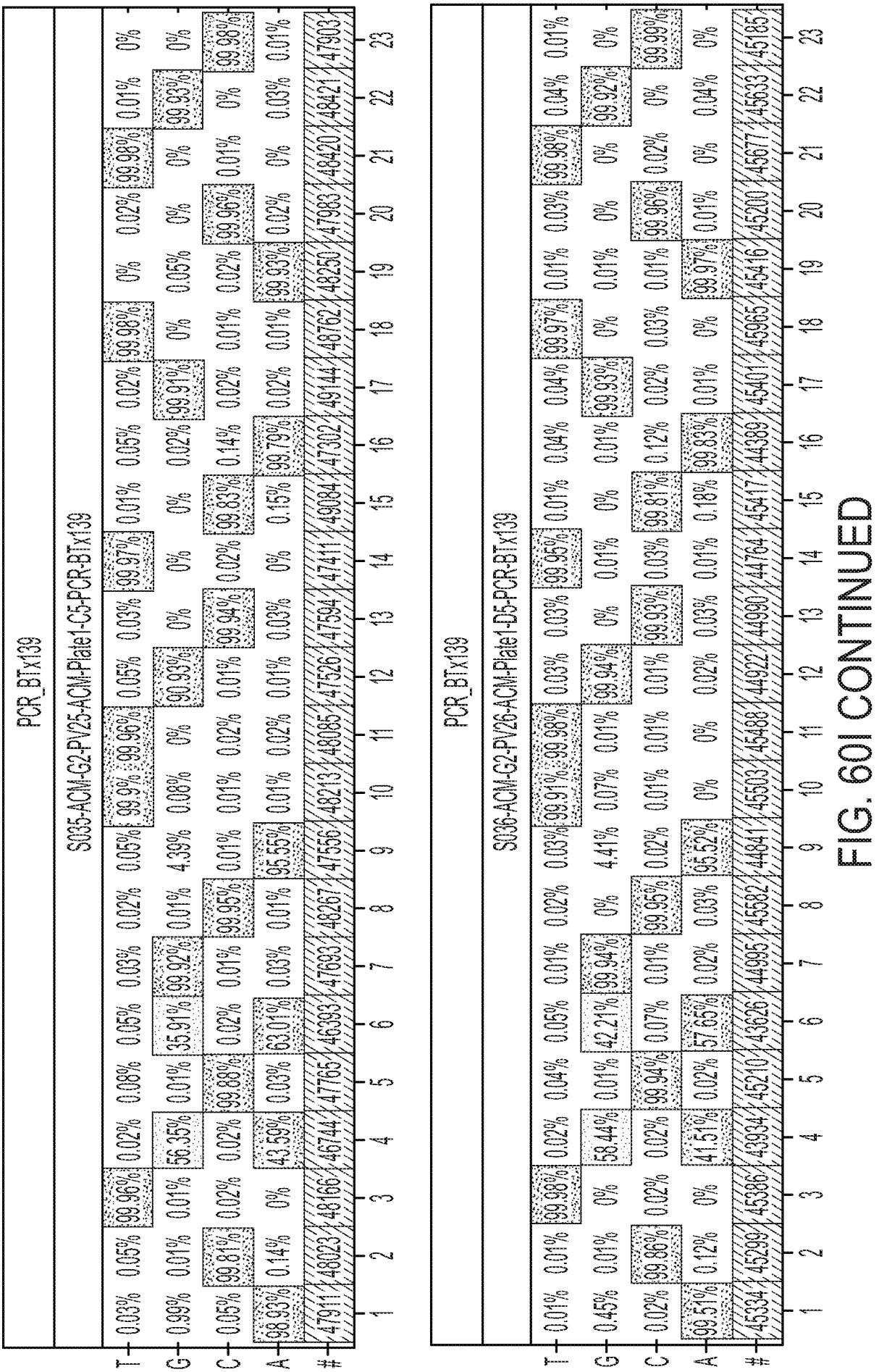


FIG. 60I CONTINUED

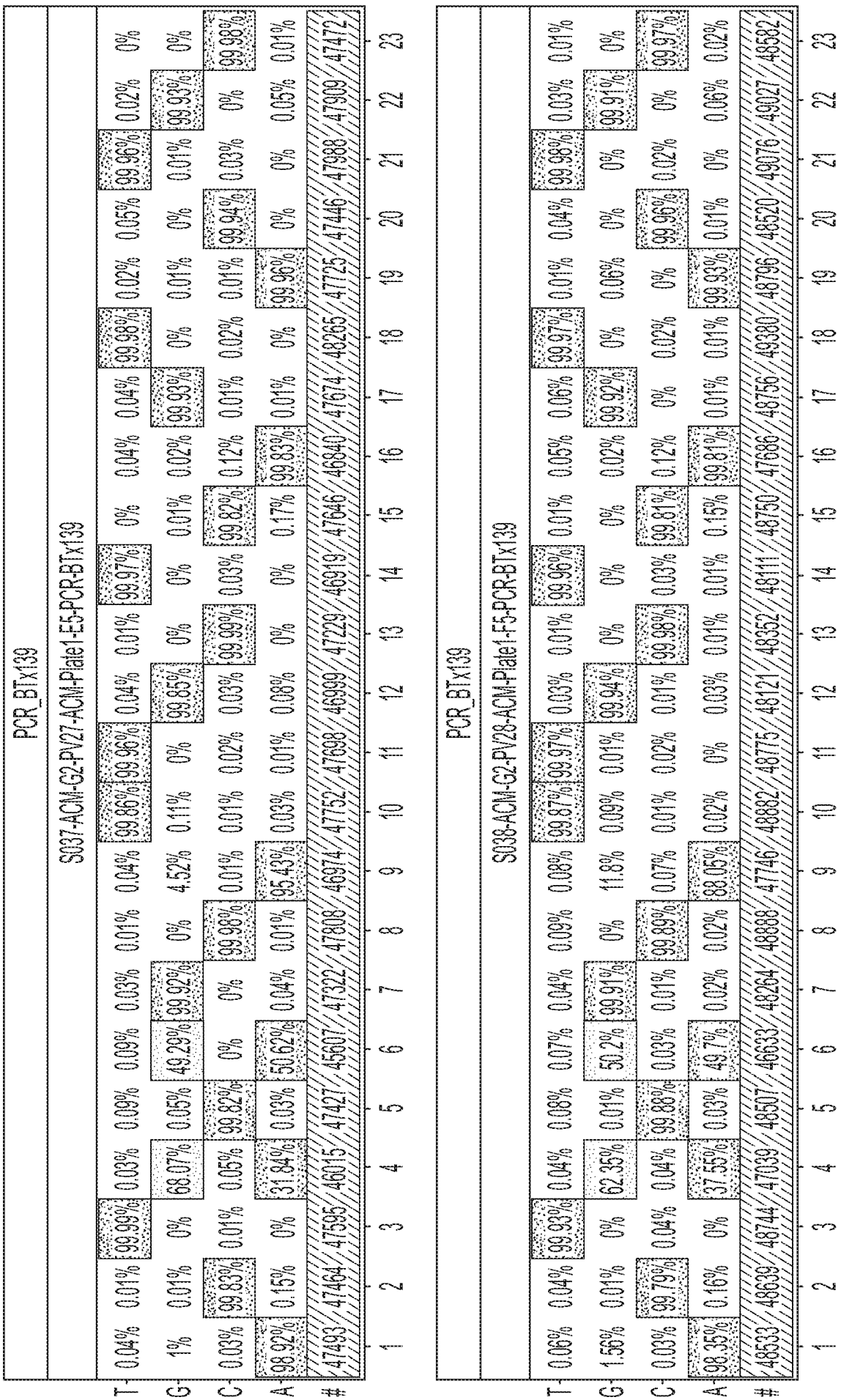

PCR_BTx139
S037-ACM-G2-PV27-ACM-Plate1-F5-PCR-BTx139
PCR_BTx139
S038-ACM-G2-PV28-ACM-Plate1-F5-PCR-BTx139

FIG. 60J

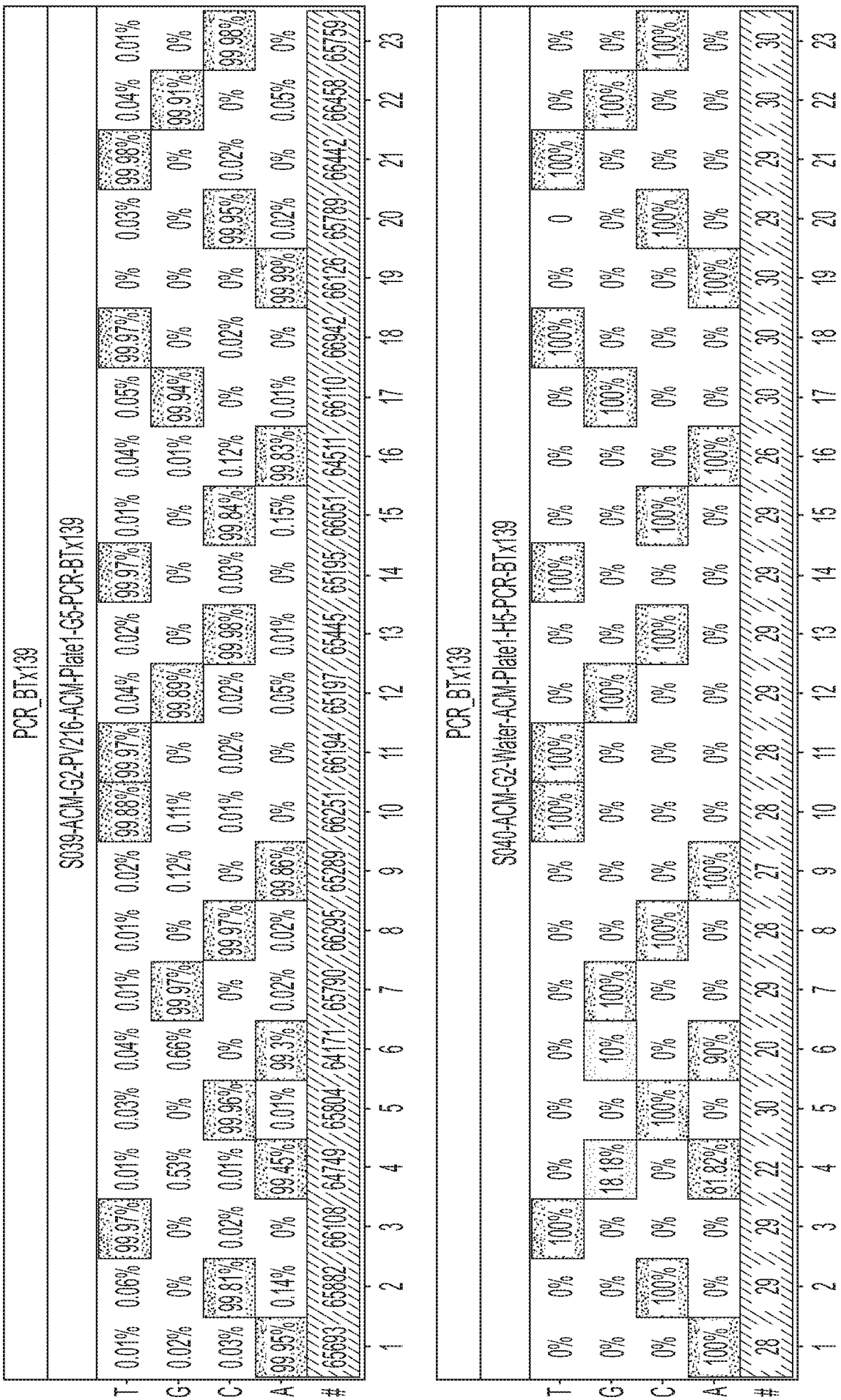


FIG. 60J CONTINUED

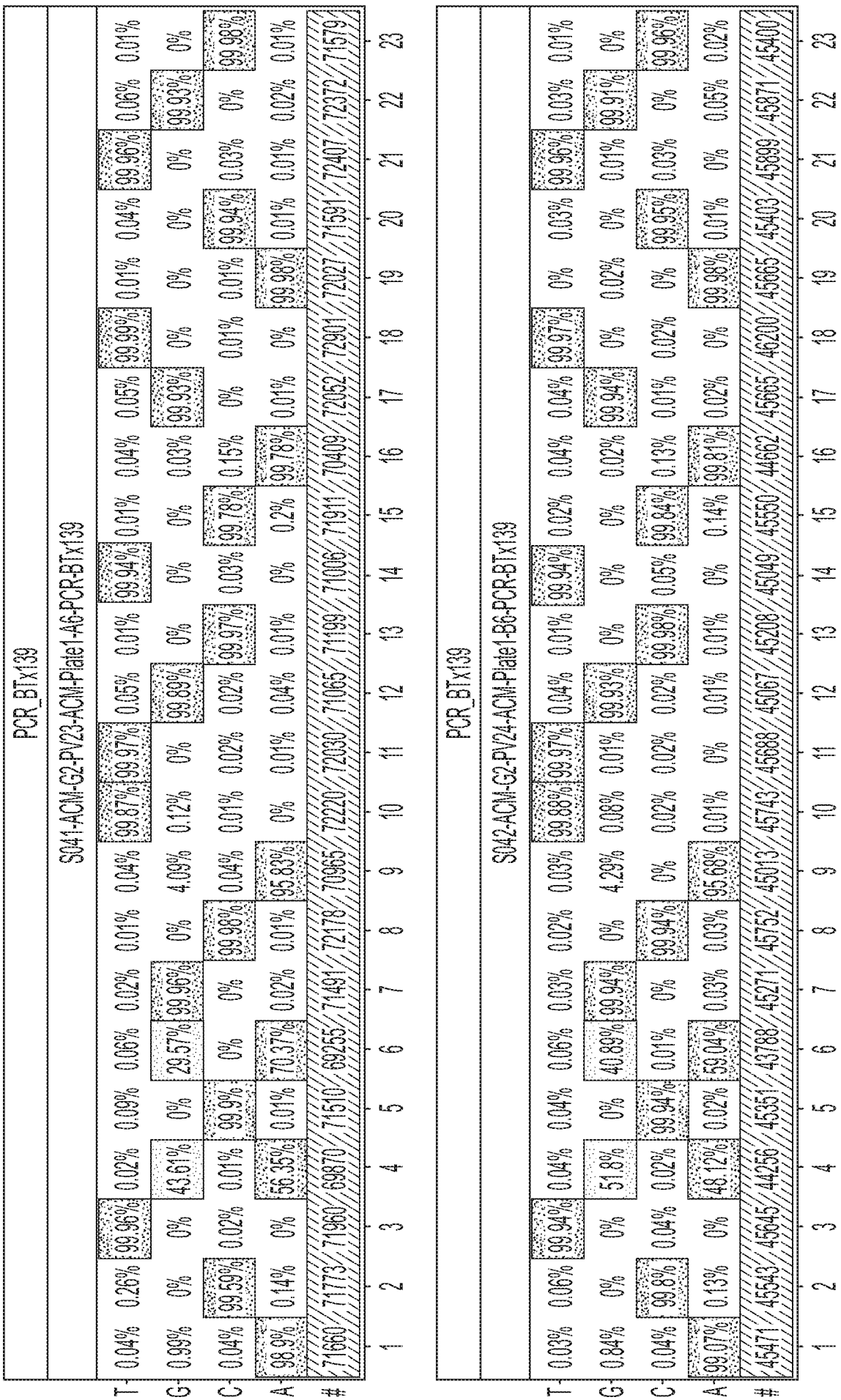

PCR_BTx139
S041-ACM-G2-PV23-ACM-Plate1-A6-PCR-BTx139
PCR_BTx139
S042-ACM-G2-PV24-ACM-Plate1-B6-PCR-BTx139
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 60K

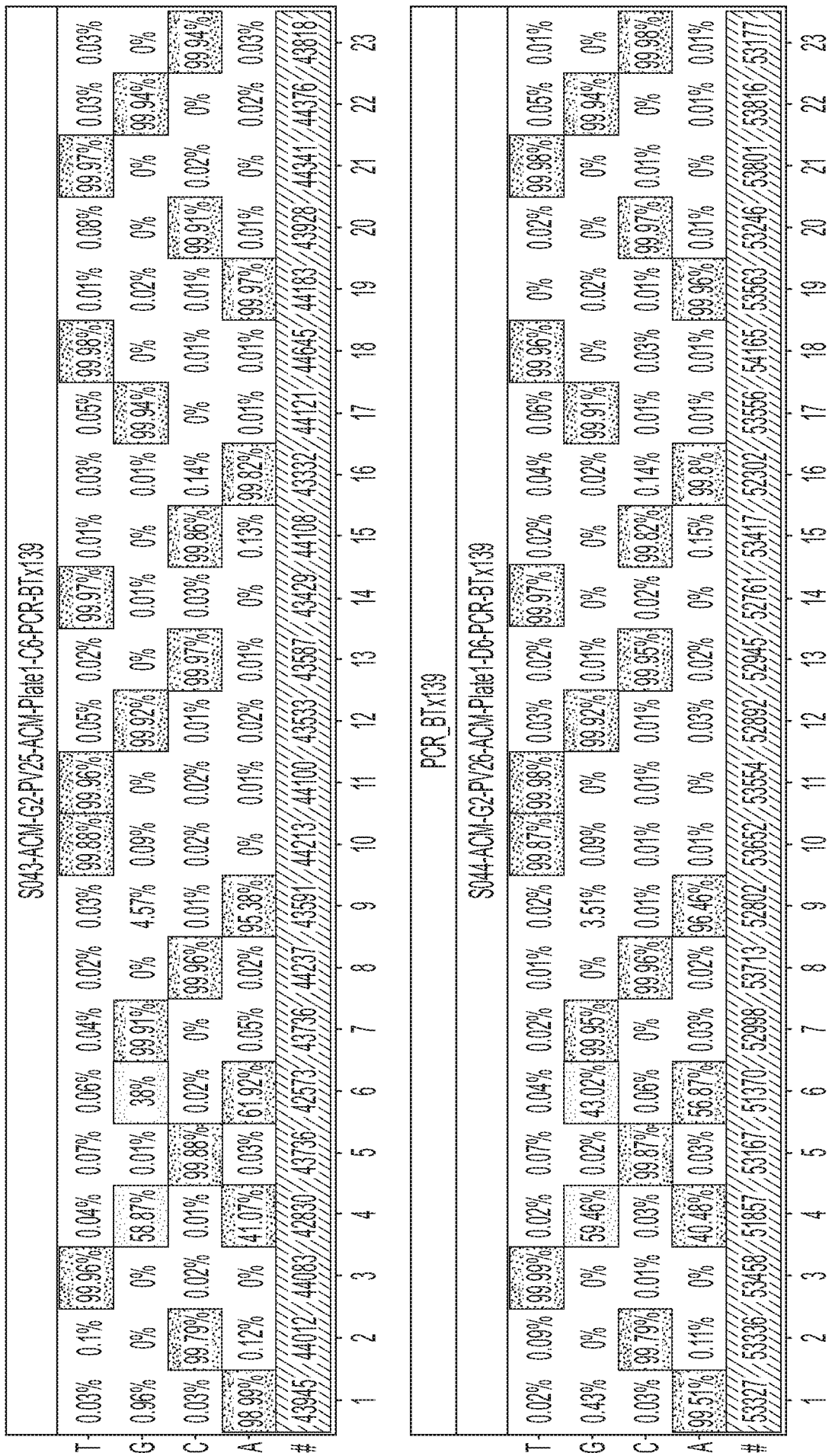


FIG. 60K CONTINUED

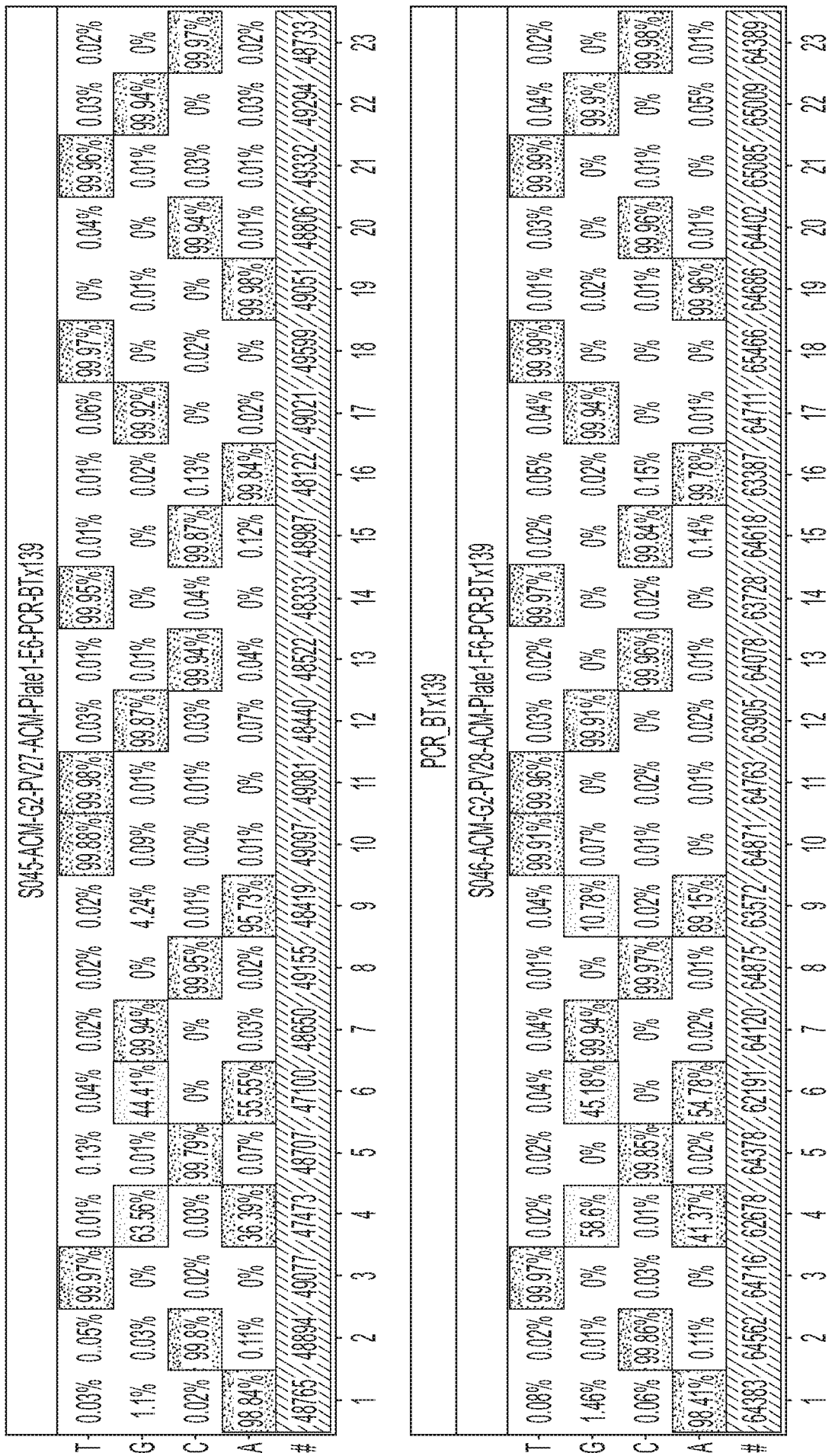

PCR_BTx139
S045-ACM-G2-PV27-ACM-Plate1-F6-PCR-BTx139
PCR_BTx139
S046-ACM-G2-PV28-ACM-Plate1-F6-PCR-BTx139
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 60L

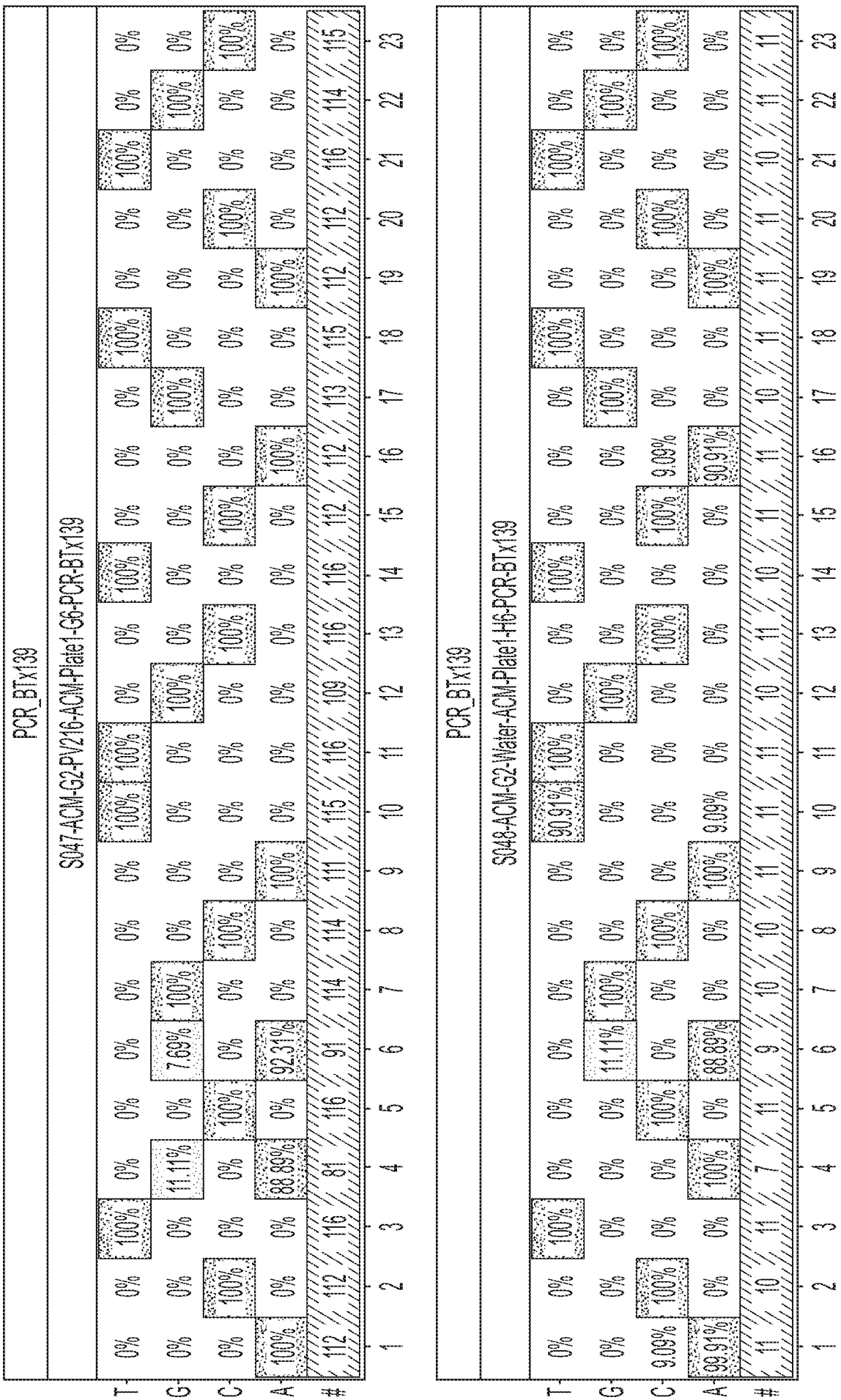


FIG. 60L CONTINUED

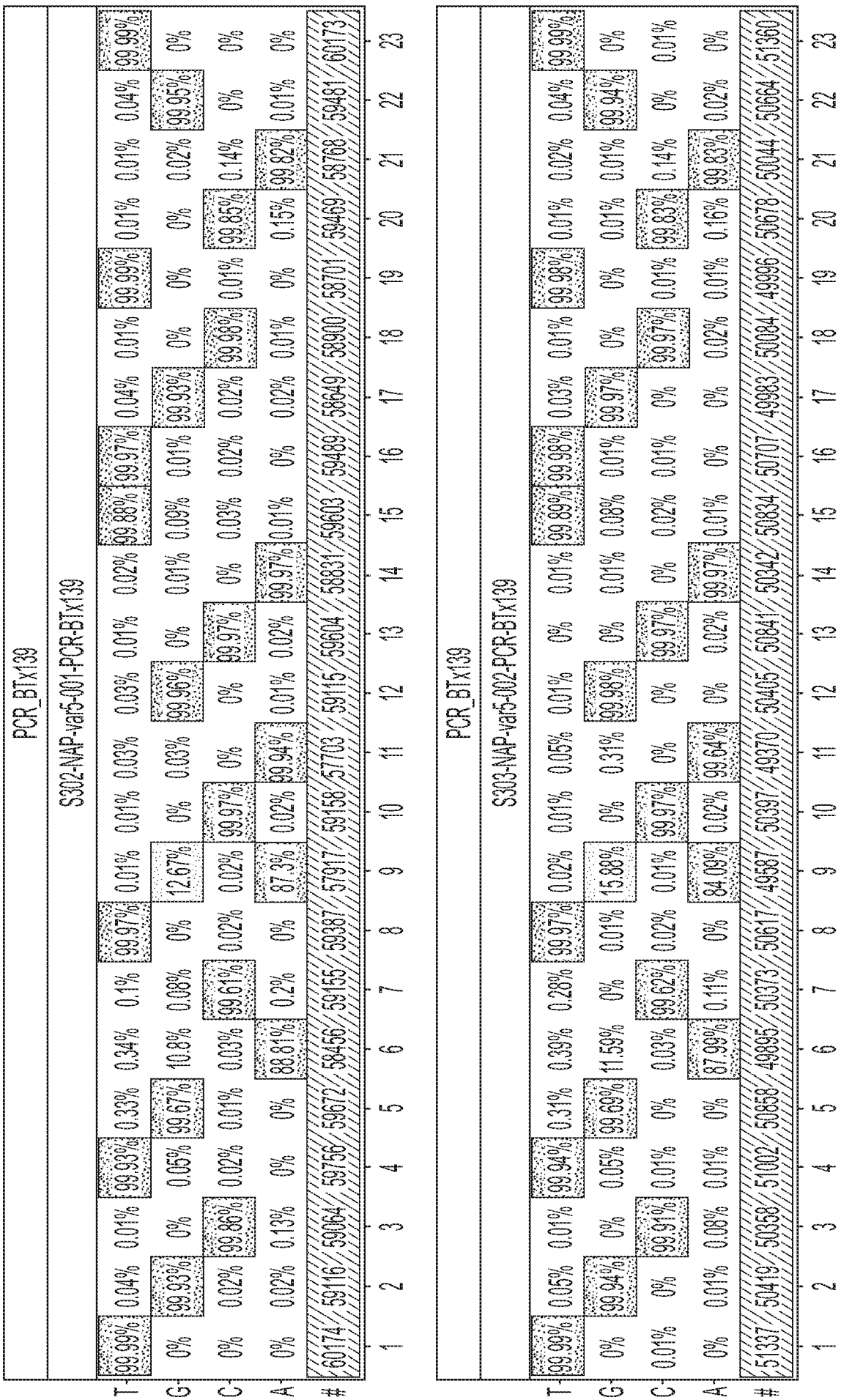

PCR_BTx139
S302-NAP-var5-001-PCR-BTx139
PCR_BTx139
S303-NAP-var5-002-PCR-BTx139
T
G
C
A
#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIG. 60M

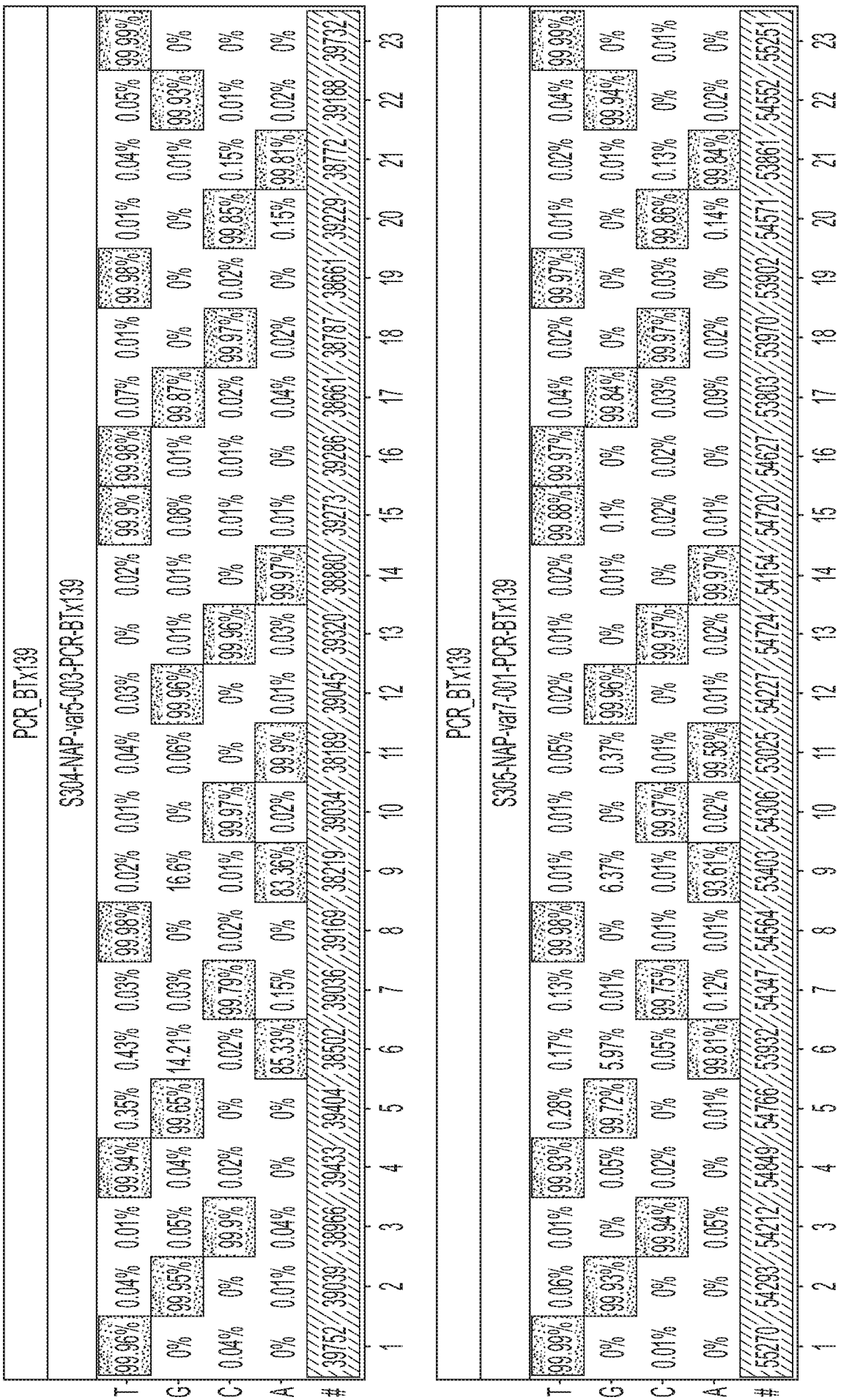


FIG. 60M CONTINUED

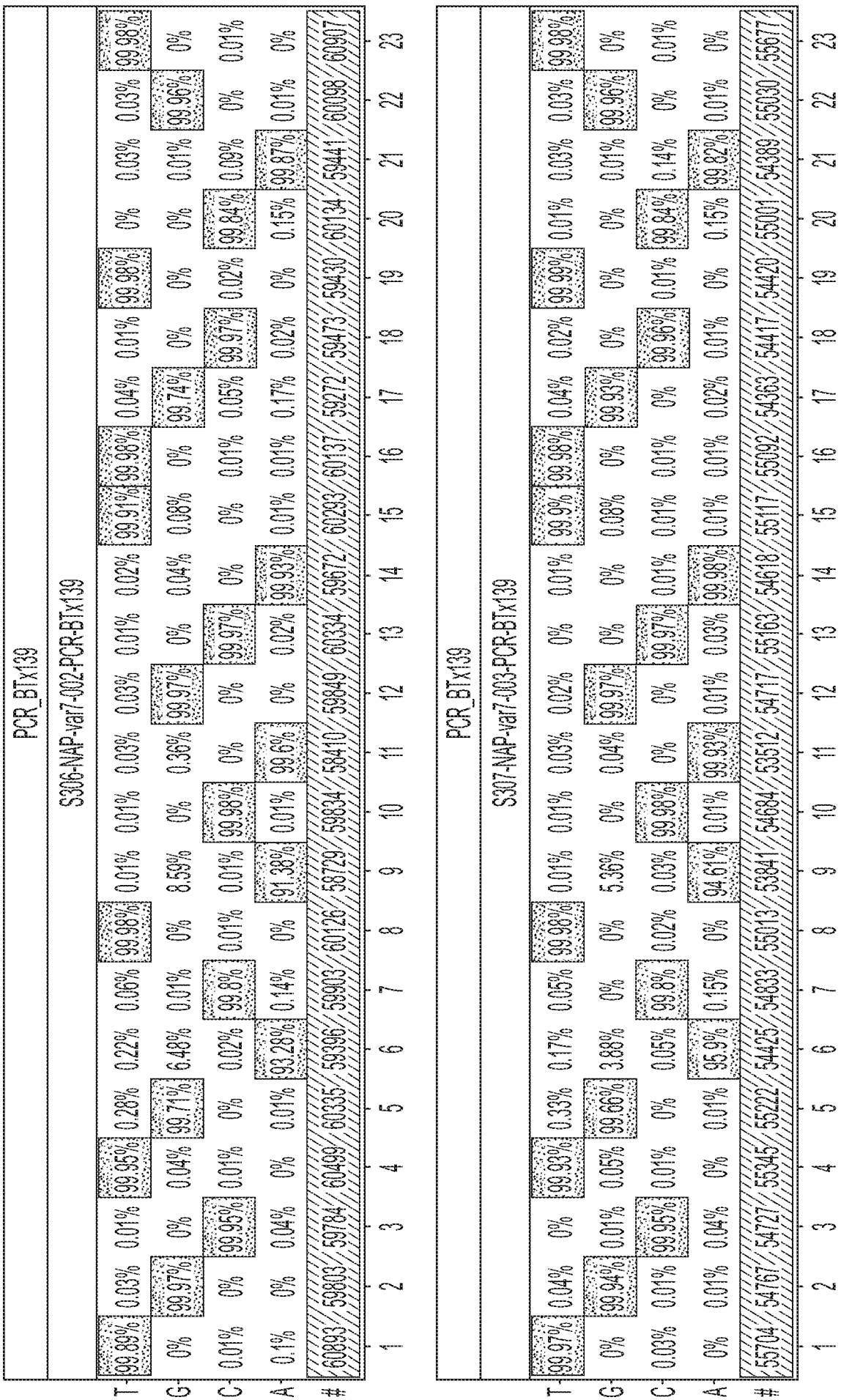

PCR_BTx139
S306-NAP-var7-002-PCR-BTx139
PCR_BTx139
S307-NAP-var7-003-PCR-BTx139
T
G
C
A
#

FIG. 60N

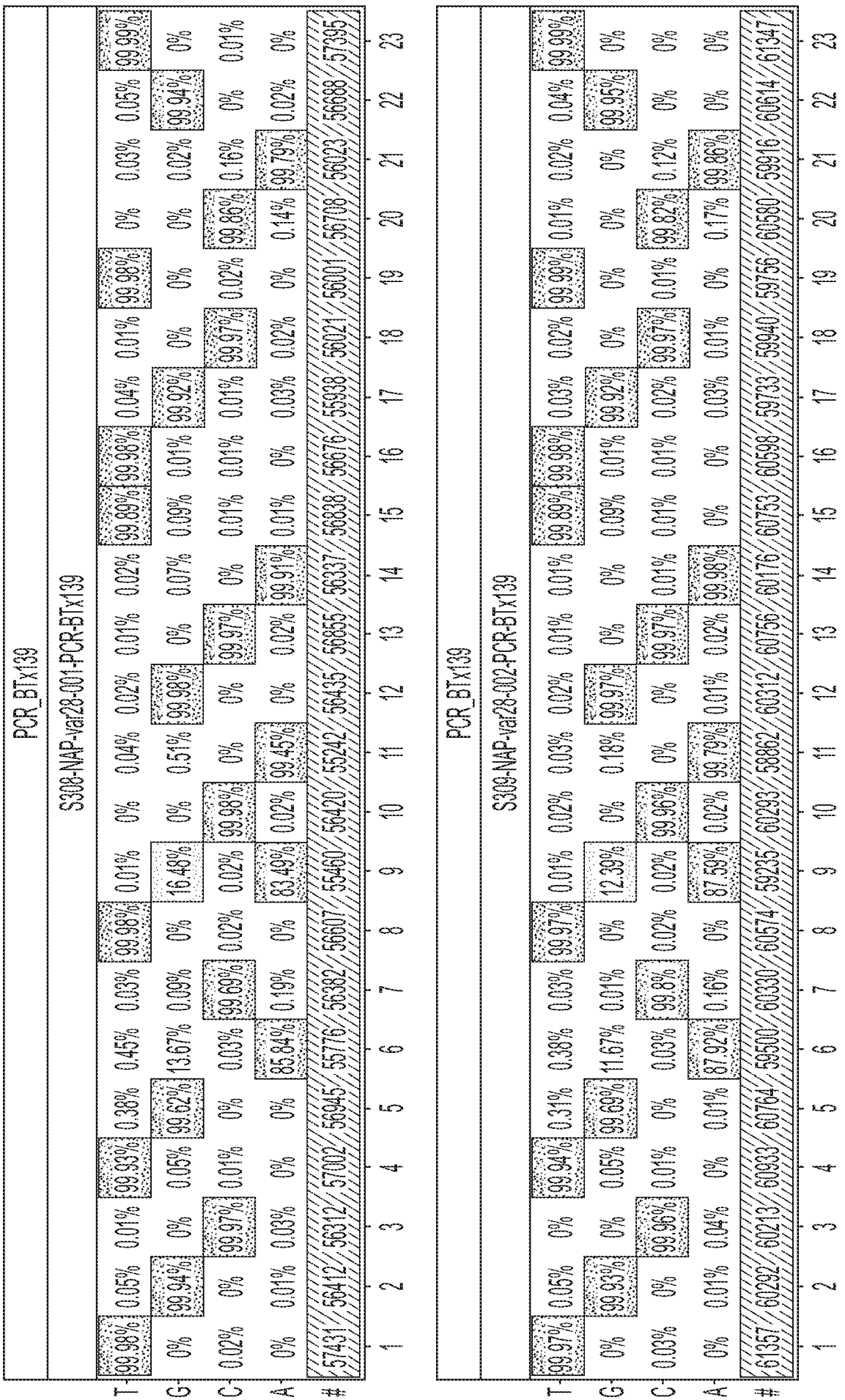


FIG. 60N CONTINUED

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 145

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

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THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 145

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NOTE POUR LE TOME / VOLUME NOTE:

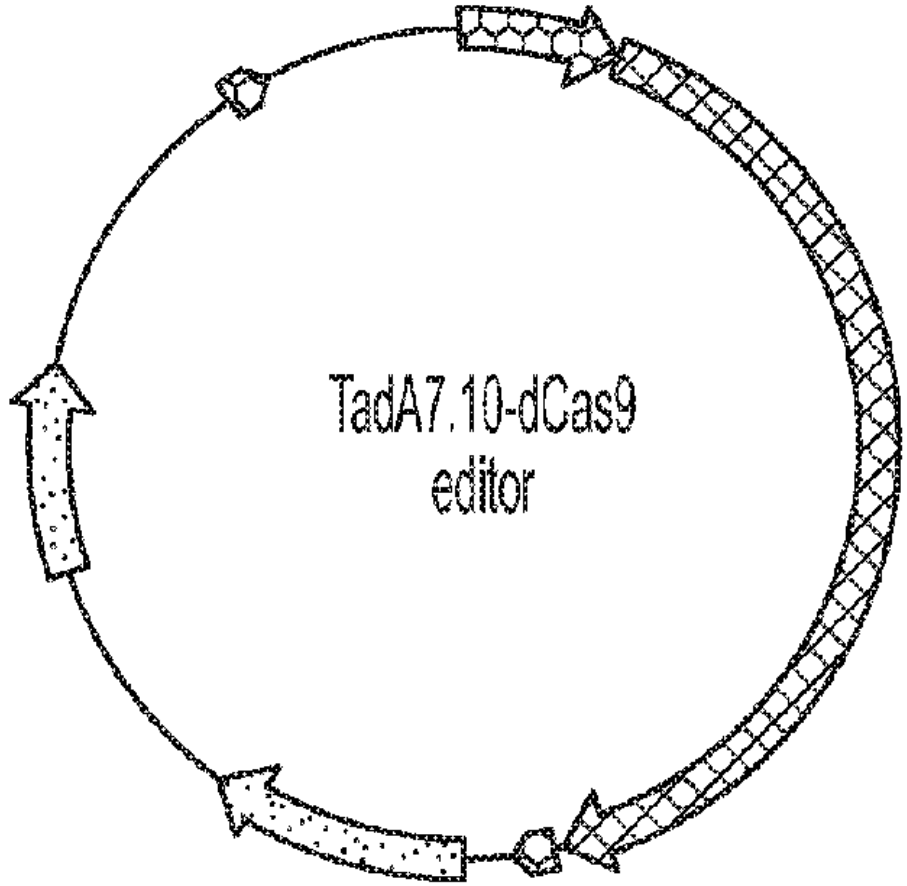

FIG. 1A