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(54) **A VERSATILE METHOD FOR THE DETECTION OF MARKER-FREE PRECISION GENOME EDITING AND GENETIC VARIATION**

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(21) Appl. No.: **17/850,186**

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(57) **ABSTRACT**

The present disclosure provides, inter alia, specially designed DNA adaptors and methods of preparing the same. Methods and kits for carrying out and detecting marker-free precision genome editing and genetic variation using such adaptors are also provided.

Specification includes a Sequence Listing.

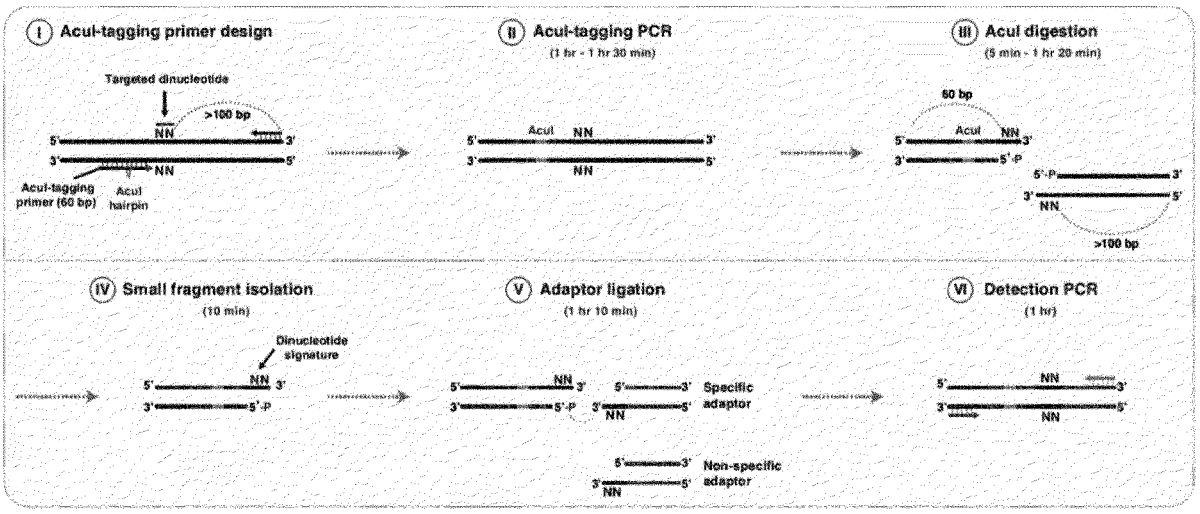
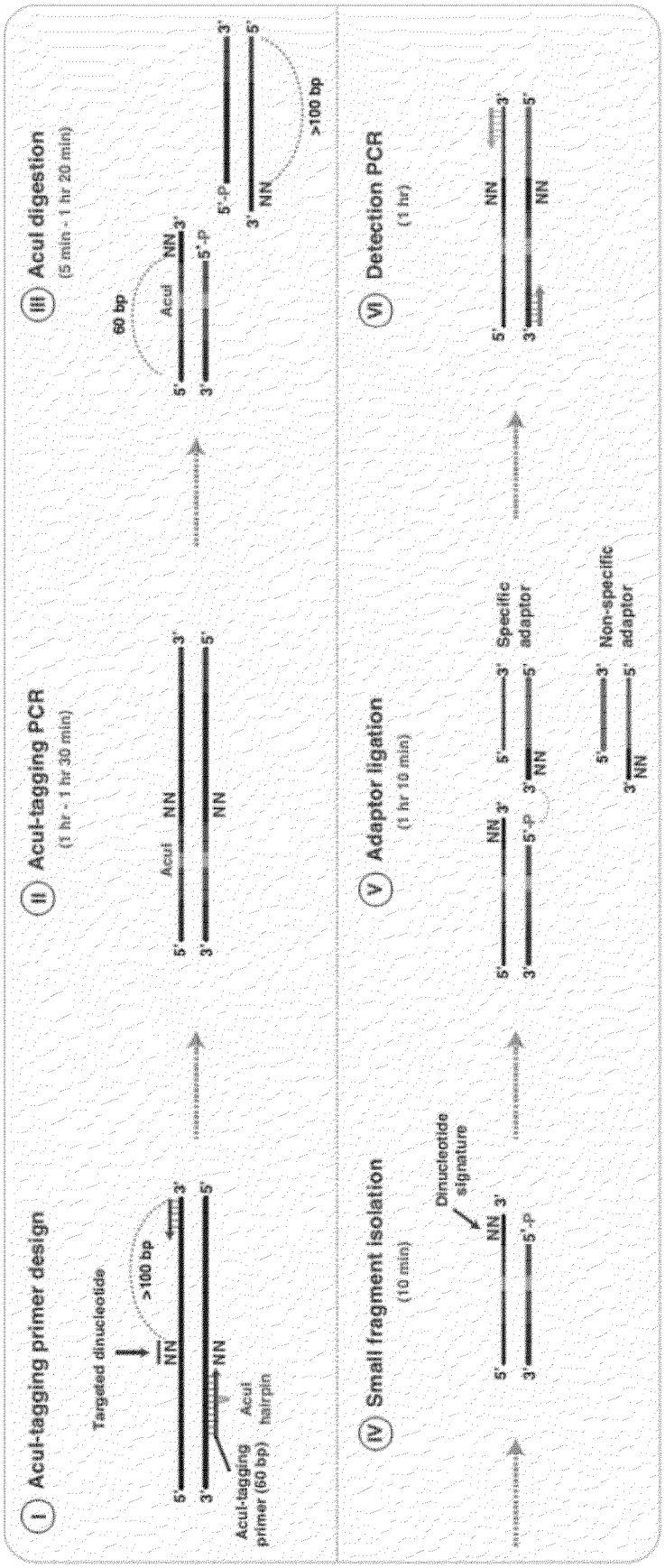
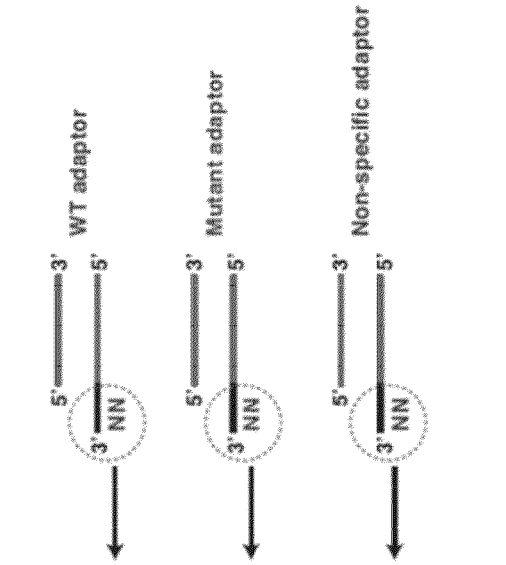
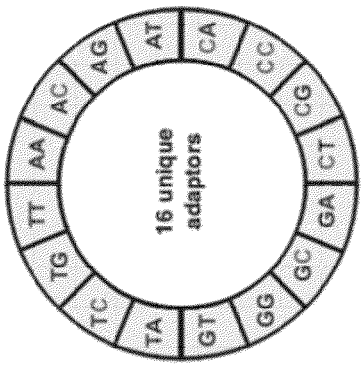


Figure 1A


I Acul-tagging primer design
Targeted dinucleotide
>100 bp
NN
5'
3'
Acul-tagging primer (60 bp)
Acul hairpin
II Acul-tagging PCR
(1 hr - 1 hr 30 min)
Acul NN
III Acul digestion
(5 min - 1 hr 20 min)
60 bp
Acul
5'-P
>100 bp
IV Small fragment isolation
(10 min)
Dinucleotide signature
V Adaptor ligation
(1 hr 10 min)
Specific adaptor
Non-specific adaptor
VI Detection PCR
(1 hr)





Adaptors complementary to WT and mutant dinucleotide signatures

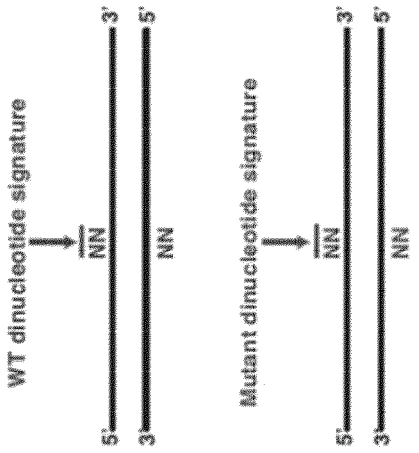


Figure 1B

Figure 1C

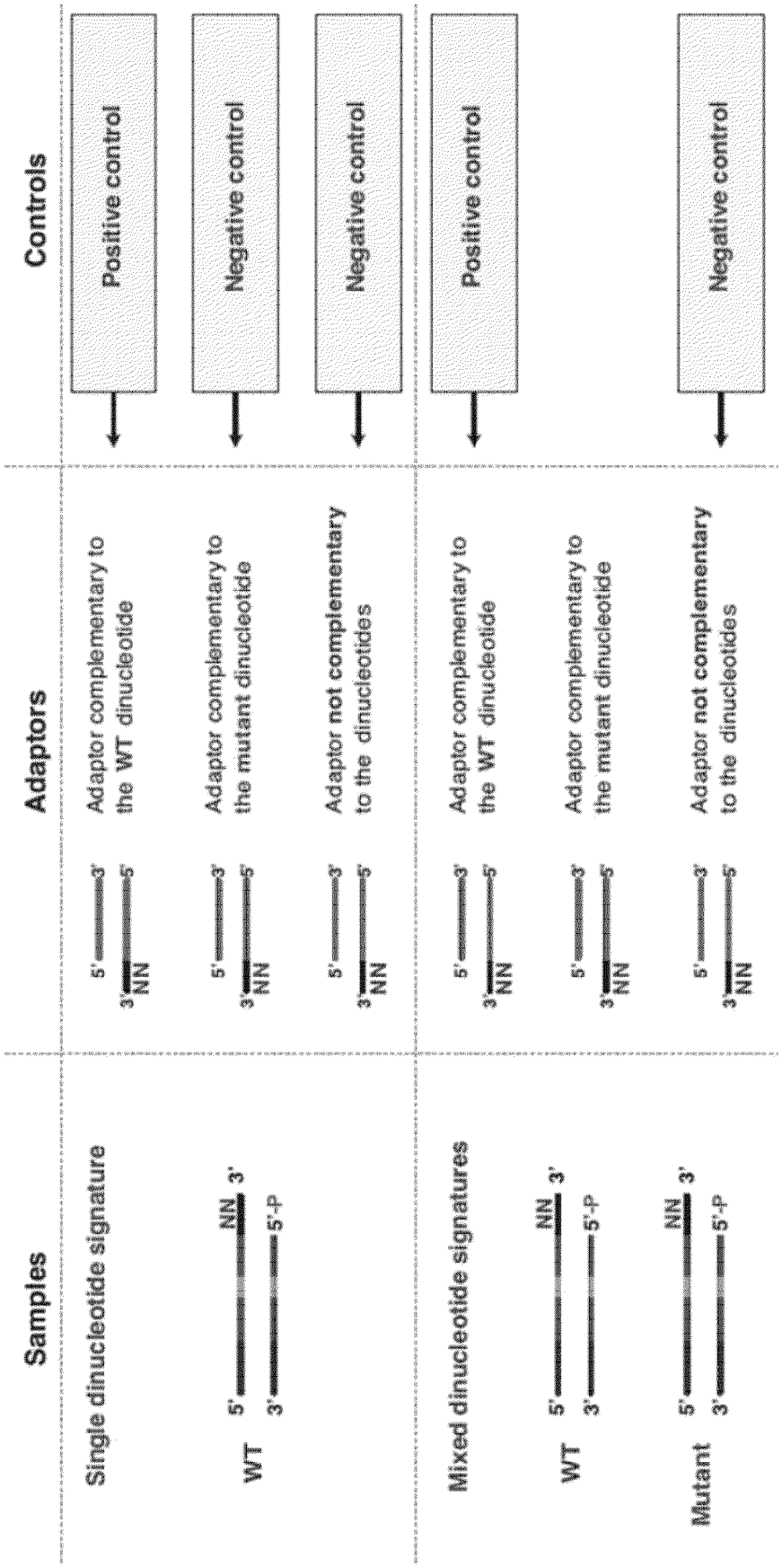

Samples
Adaptors
Controls
Single dinucleotide signature
WT
Adaptor complementary to the WT dinucleotide
Positive control
Adaptor complementary to the mutant dinucleotide
Negative control
Adaptor not complementary to the dinucleotides
Negative control
Mixed dinucleotide signatures
WT
Adaptor complementary to the WT dinucleotide
Positive control
Adaptor complementary to the mutant dinucleotide
Mutant
Adaptor not complementary to the dinucleotides
Negative control

Figure 2A

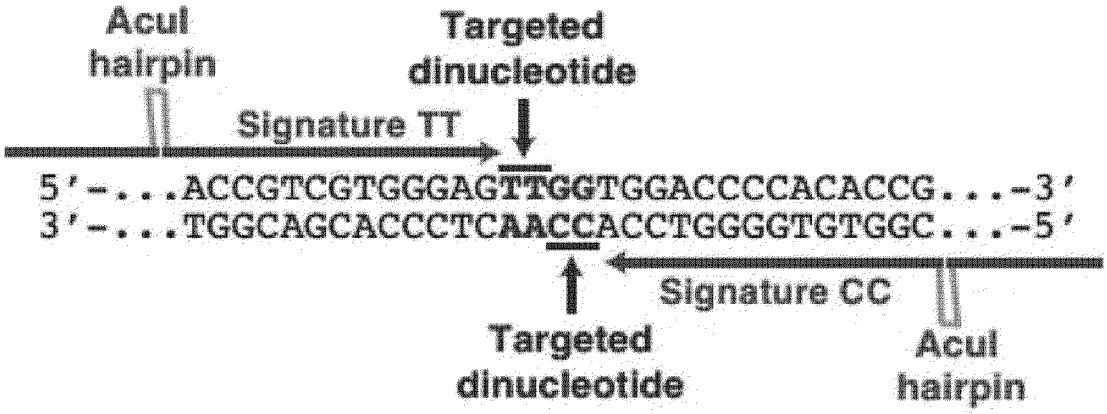


Figure 2B

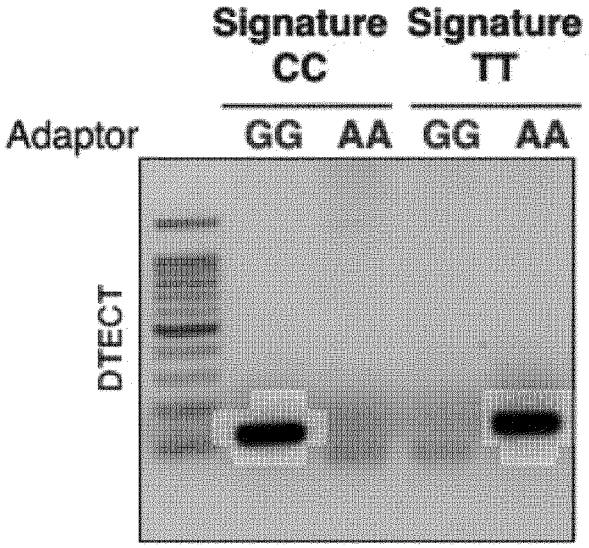


Figure 2C

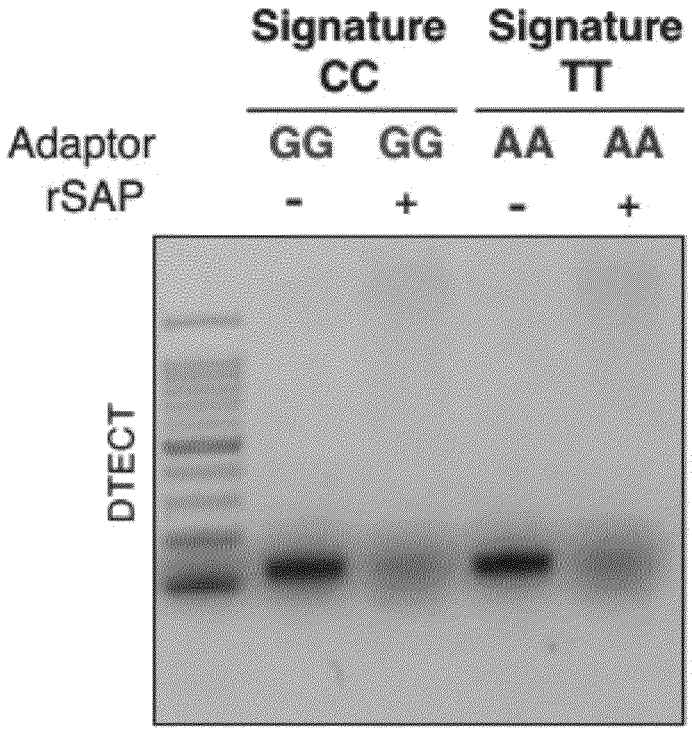


Figure 2D

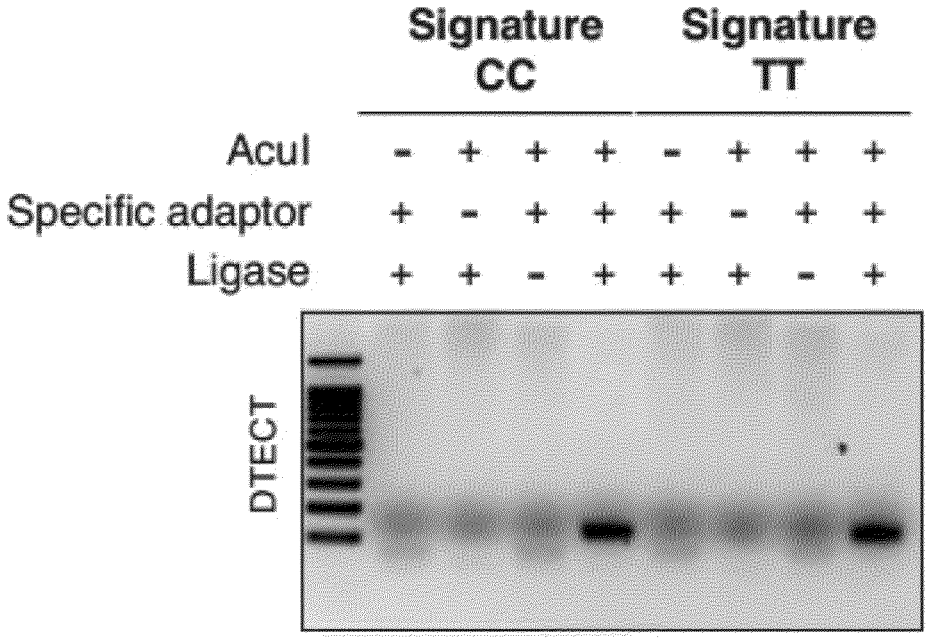


Figure 2E

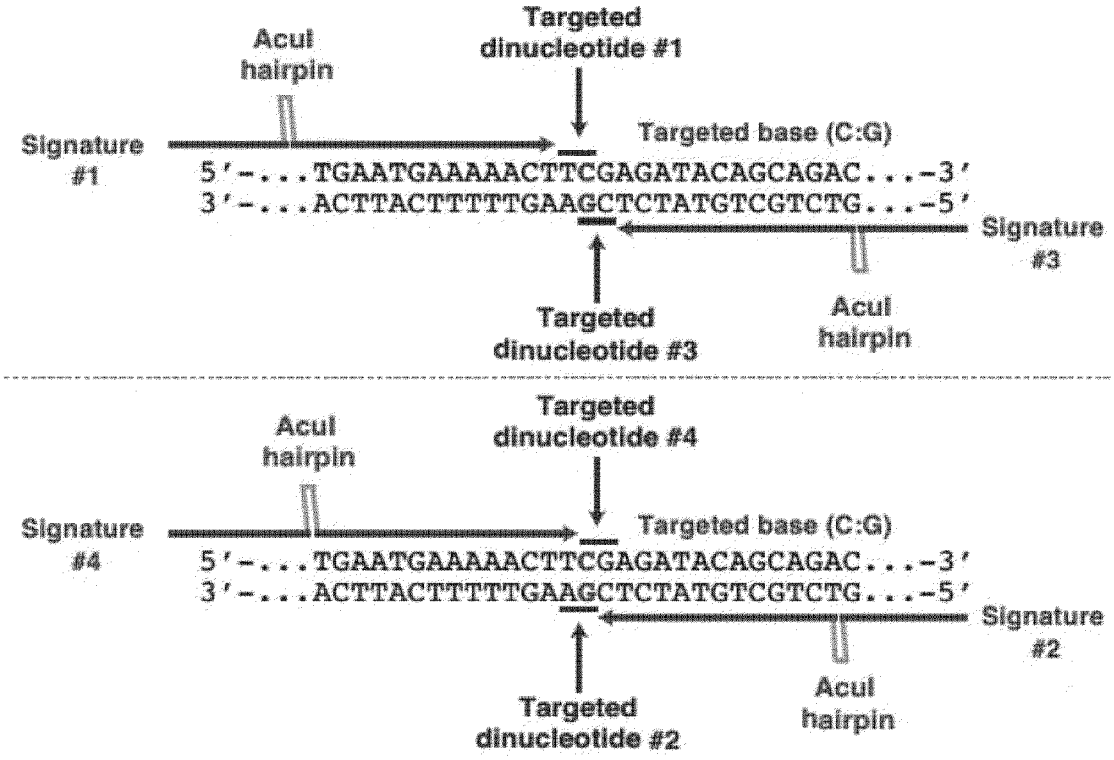


Figure 2F

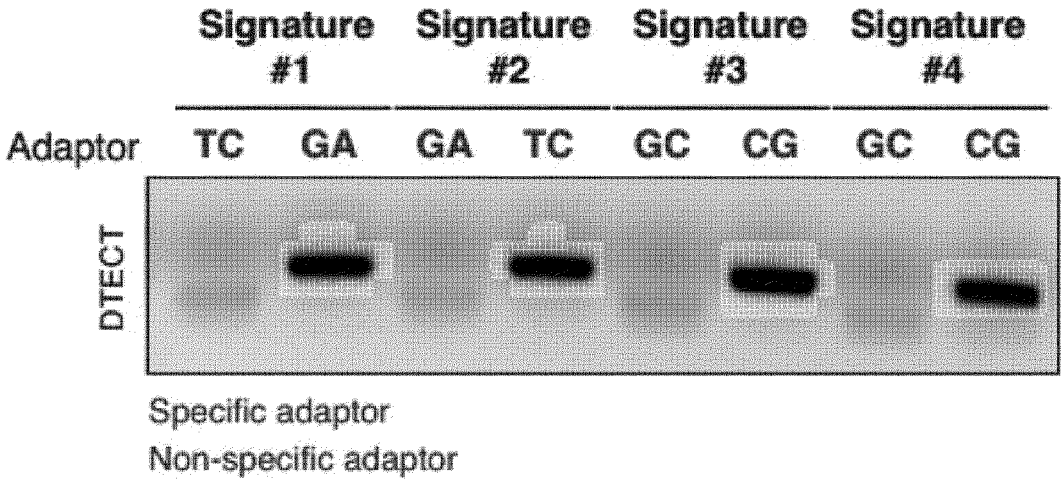


Figure 2G

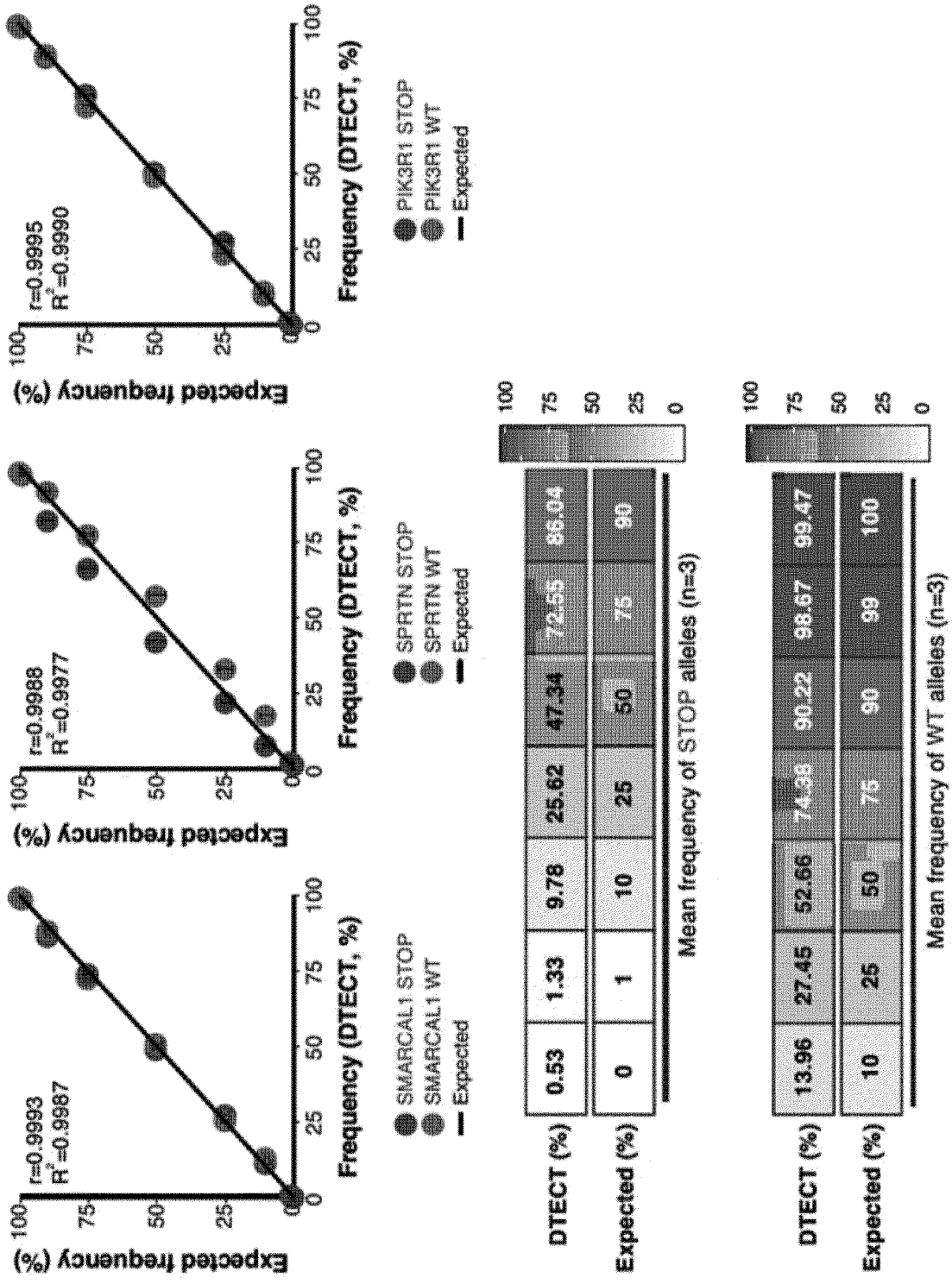

r=0.9993
R²=0.9987
Expected frequency (%)
Frequency (DTECT, %)
SMARCAL1 STOP
SMARCAL1 WT
Expected
r=0.9988
R²=0.9977
SPRTN STOP
SPRTN WT
r=0.9995
R²=0.9990
PIK3R1 STOP
PIK3R1 WT
DTECT (%) 0.53 1.33 9.78 25.62 47.34 72.55 86.04
Expected (%) 0 1 10 25 50 75 90
Mean frequency of STOP alleles (n=3)
DTECT (%) 13.96 27.45 52.66 74.38 90.22 98.67 99.47
Expected (%) 10 25 50 75 90 99 100
Mean frequency of WT alleles (n=3)

Figure 2H

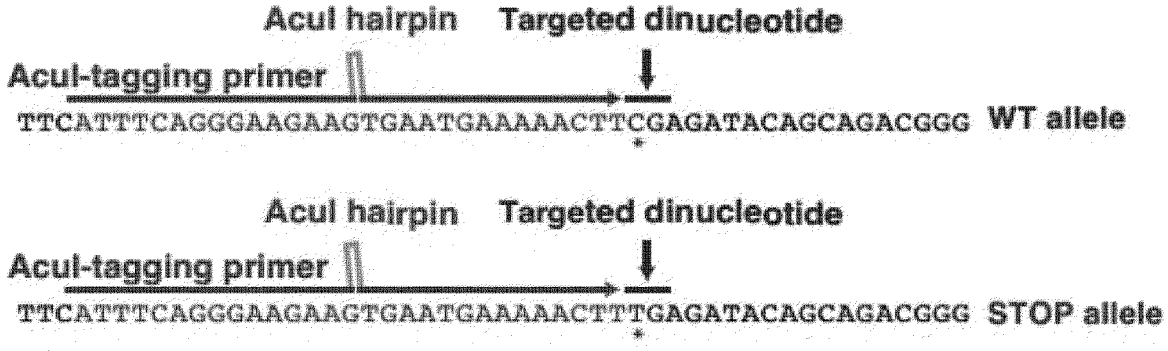
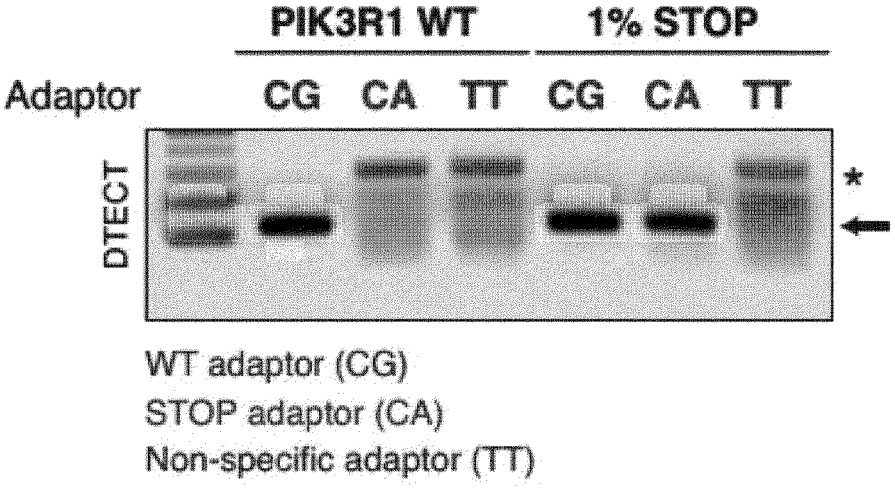


Figure 2I



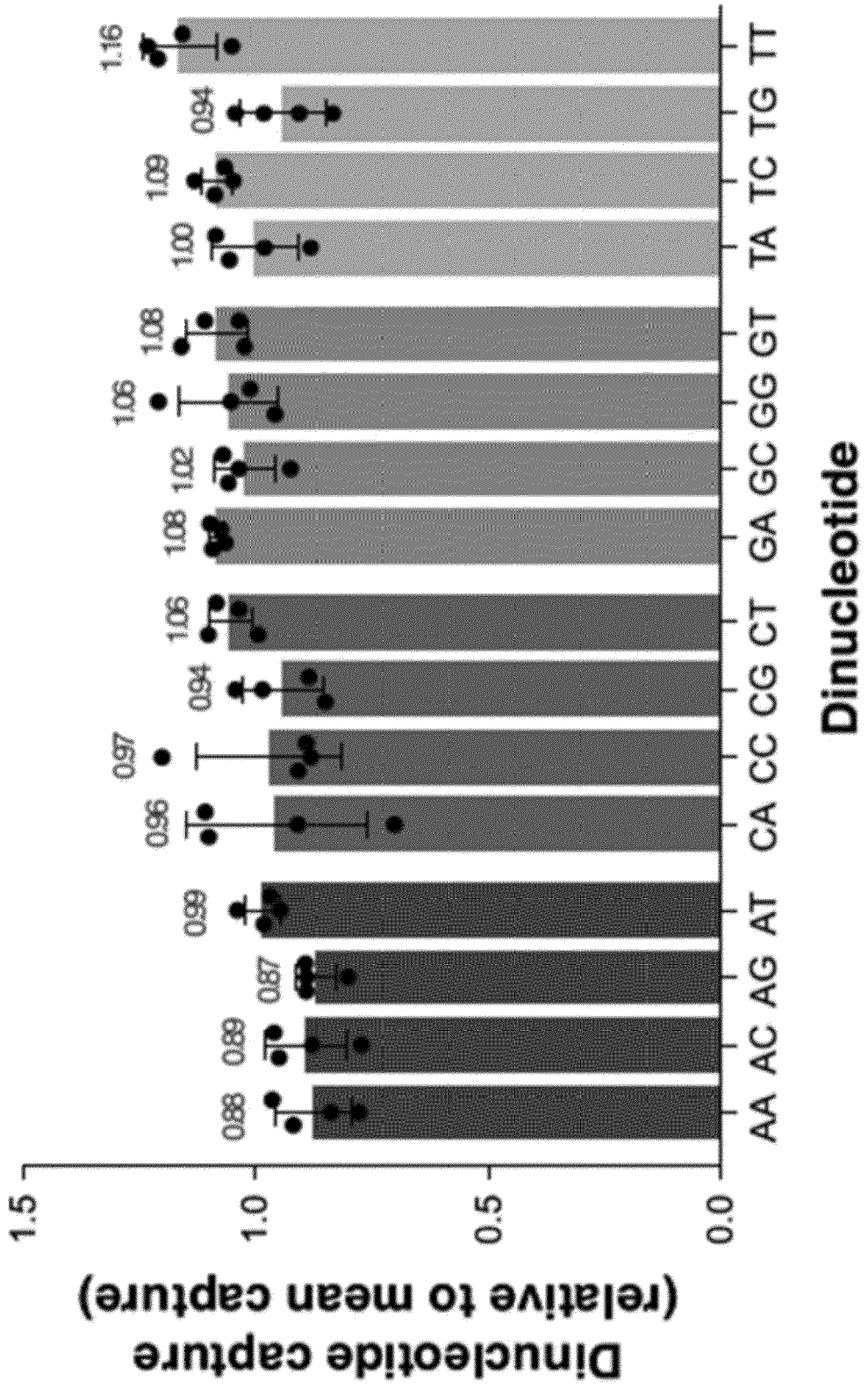

Dinucleotide capture
(relative to mean capture)
1.5
1.0
0.5
0.0
0.88
0.89
0.87
0.99
0.96
0.97
0.94
1.06
1.08
1.02
1.06
1.08
1.00
1.09
0.94
1.16
AA AC AG AT CA CC CG CT GA GC GG GT TA TC TG TT
Dinucleotide

Figure 2J

Figure 2K

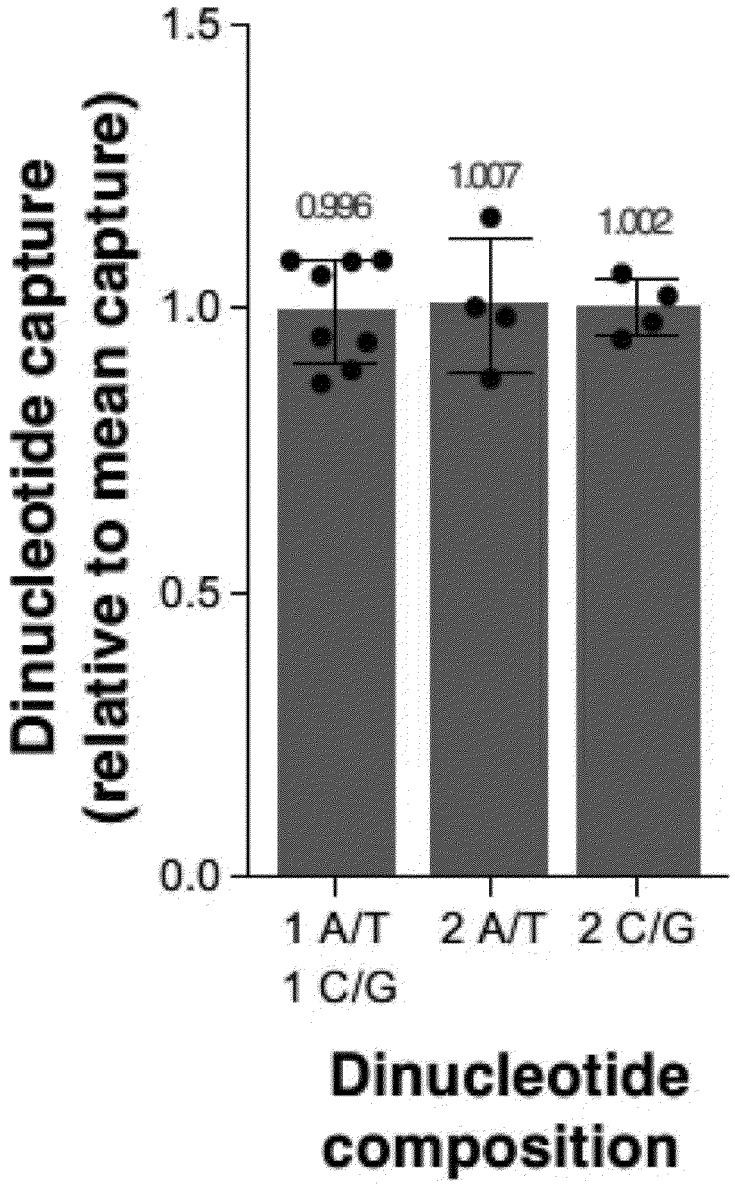

Dinucleotide capture
(relative to mean capture)
1.5
1.0
0.5
0.0
0.996
1.007
1.002
1 A/T
1 C/G
2 A/T
2 C/G
Dinucleotide
composition

Figure 3A

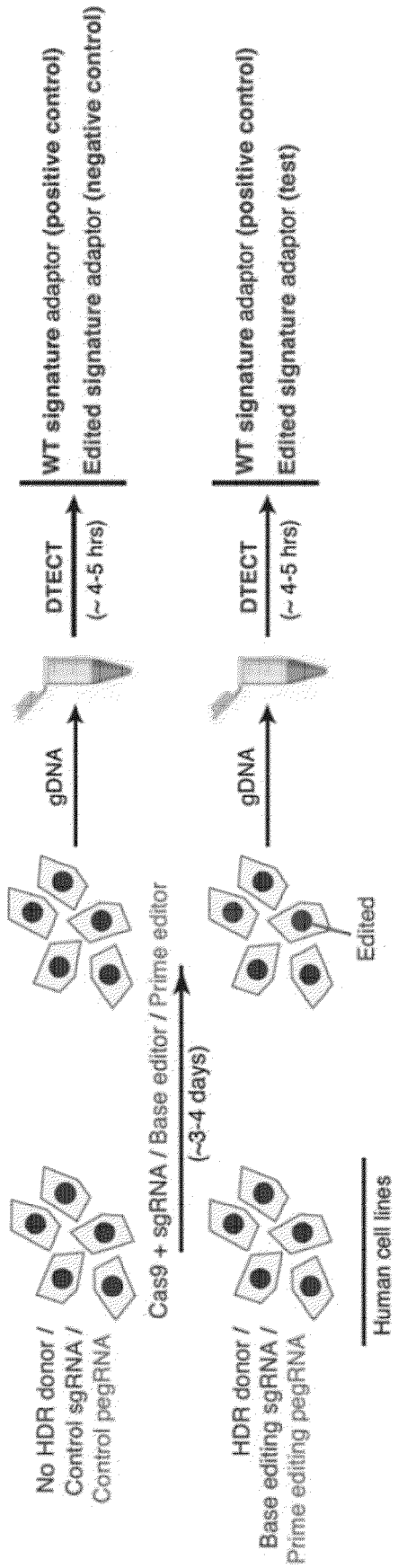
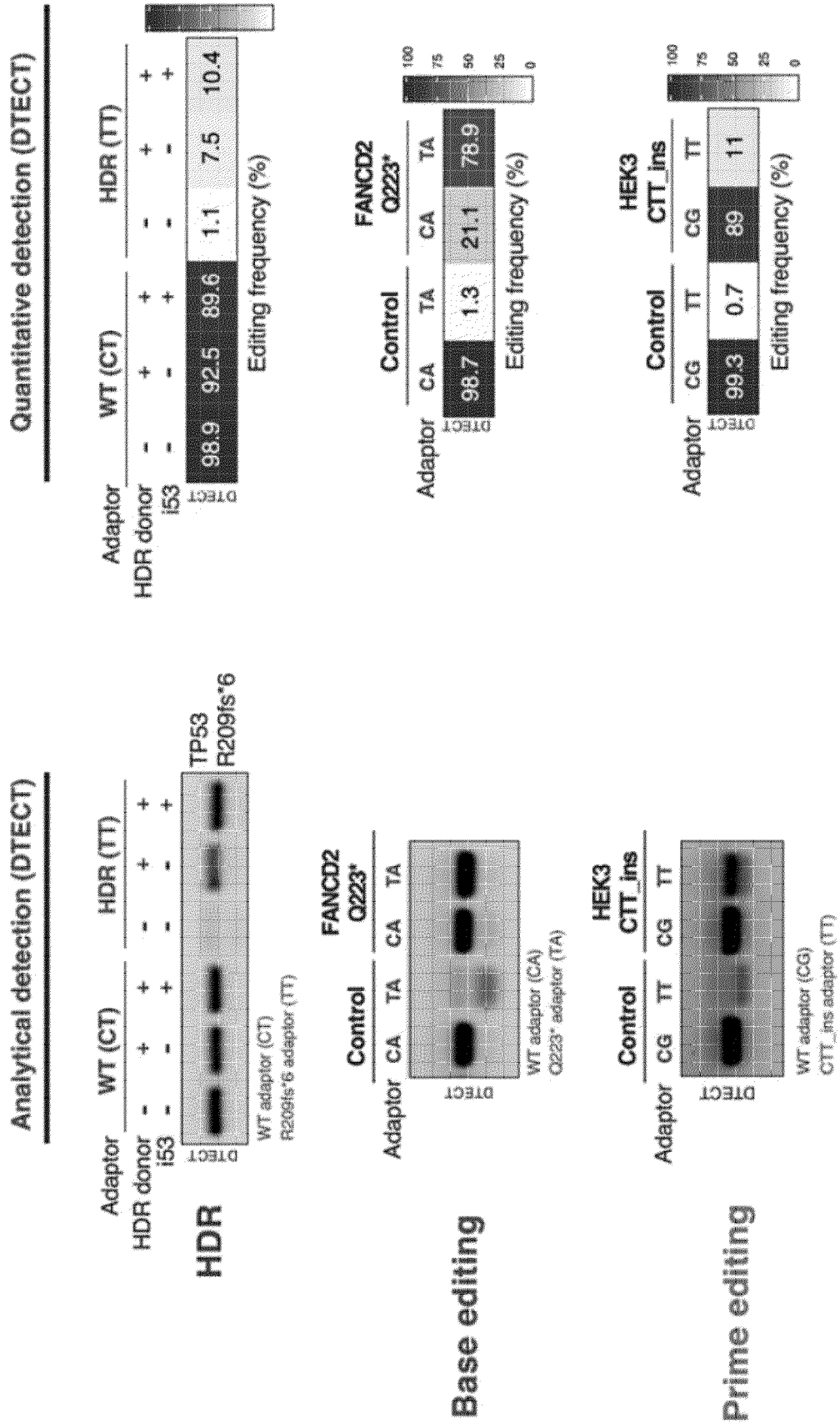

No HDR donor /
Control sgRNA /
Control pegRNA
HDR donor /
Base editing sgRNA /
Prime editing pegRNA
Human cell lines
Cas9 + sgRNA / Base editor / Prime editor
(~3-4 days)
gDNA
gDNA
Edited
DTECT
(~ 4-5 hrs)
DTECT
(~ 4-5 hrs)
WT signature adaptor (positive control)
Edited signature adaptor (negative control)
WT signature adaptor (positive control)
Edited signature adaptor (test)

Figure 3B


Analytical detection (DTECT)
Quantitative detection (DTECT)
HDR
Base editing
Prime editing
Adaptor
HDR donor
i53
WT (CT)
HDR (TT)
DTECT
TP53 R209fs*6
WT adaptor (CT)
R209fs*6 adaptor (TT)
Editing frequency (%)
FANCD2 Q223*
Control
CA
TA
98.9
92.5
89.6
1.1
7.5
10.4
98.7
1.3
21.1
78.9
HEK3 CTT_ins
CG
TT
99.3
0.7
89
11
WT adaptor (CA)
Q223* adaptor (TA)
WT adaptor (CG)
CTT_ins adaptor (TT)
100
75
50
25
0

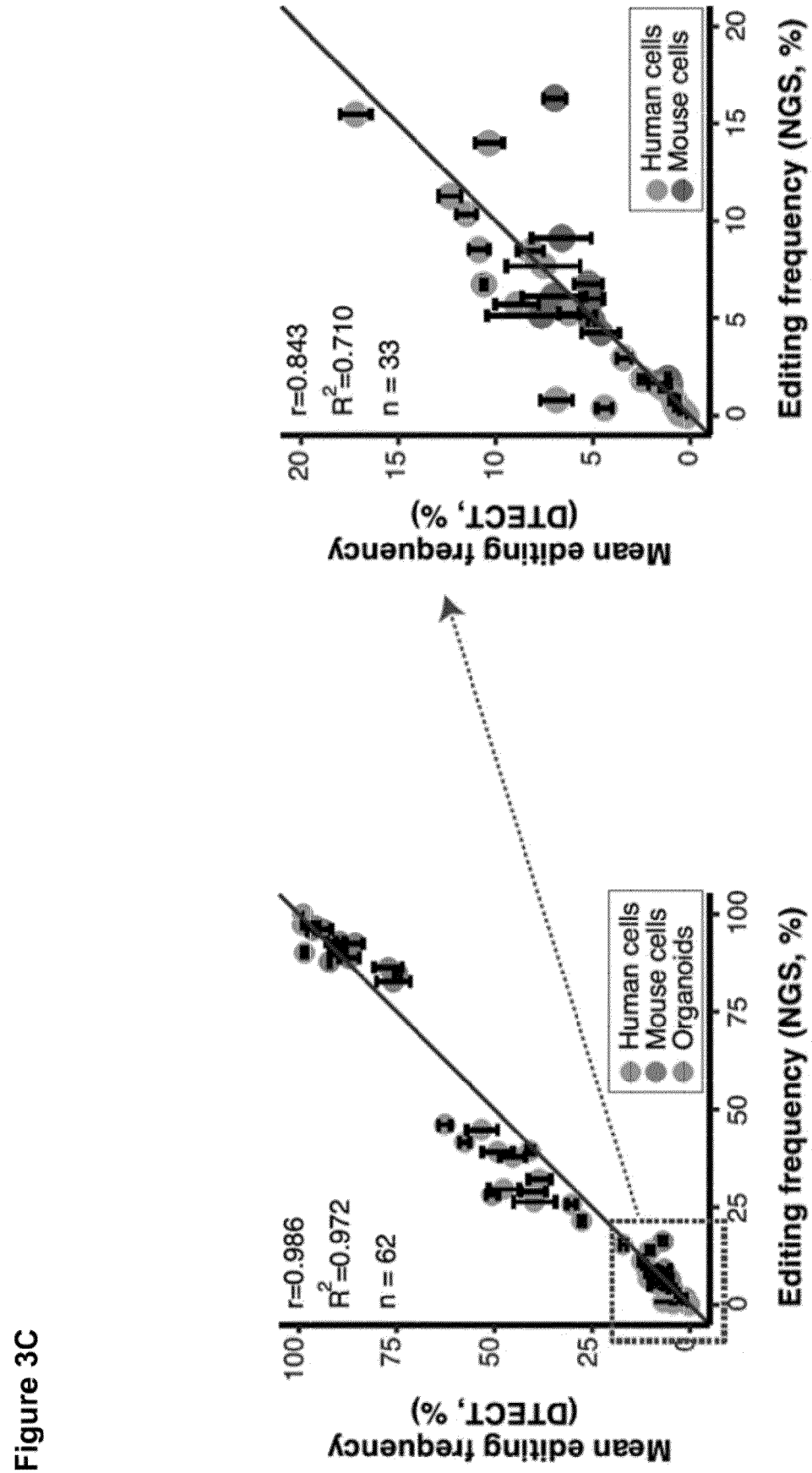

Mean editing frequency (DTECT, %)
Editing frequency (NGS, %)
r=0.986
R2=0.972
n = 62
Human cells
Mouse cells
Organoids
Mean editing frequency (DTECT, %)
Editing frequency (NGS, %)
r=0.843
R2=0.710
n = 33
Human cells
Mouse cells

Figure 3C

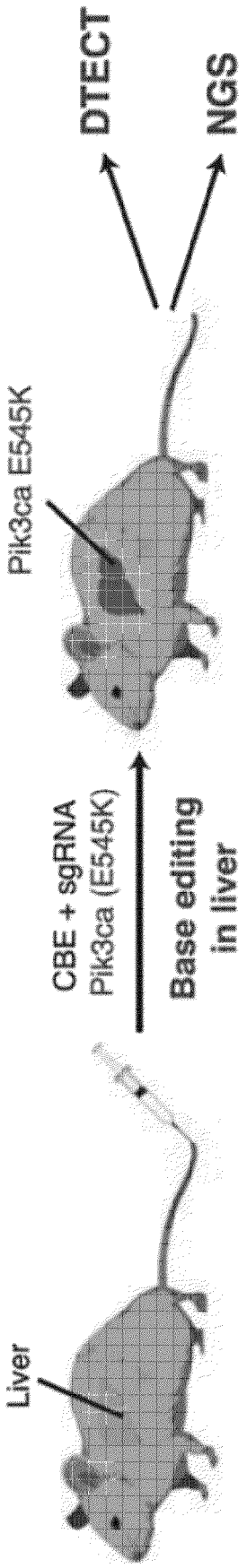

Liver
CBE + sgRNA
Pik3ca (E545K)
Base editing
in liver
Pik3ca E545K
DTECT
NGS

Figure 3D

Figure 3E

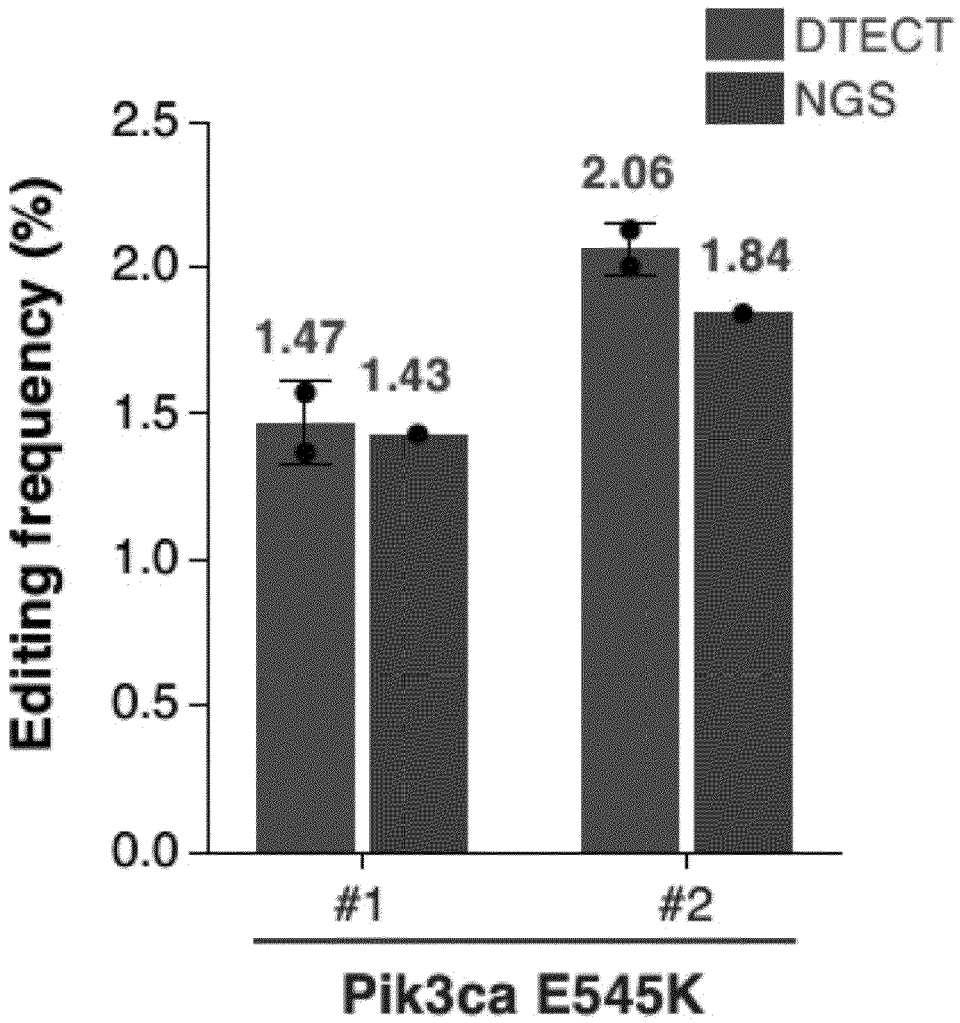
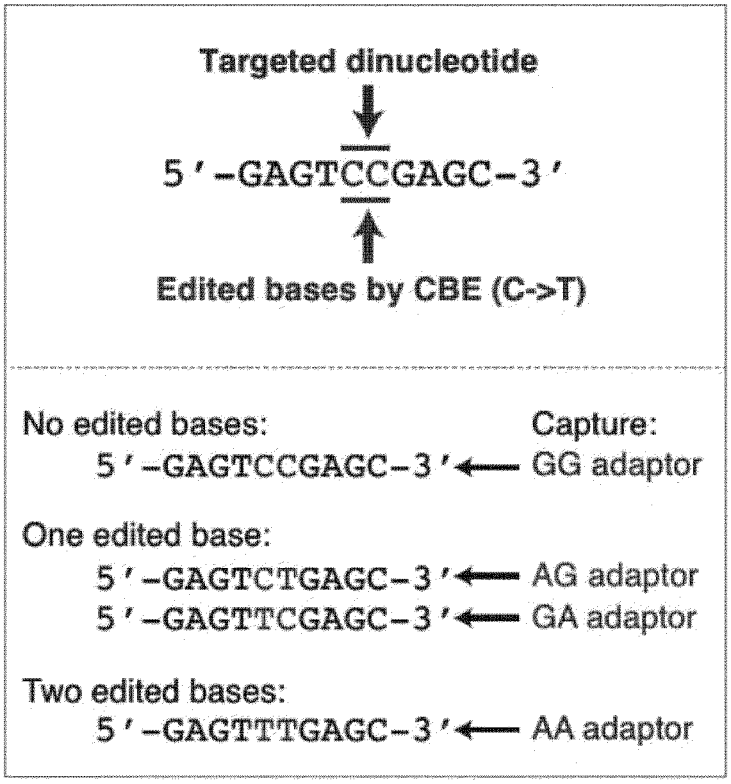

DTECT
NGS
Editing frequency (%)
2.5
2.0
1.5
1.0
0.5
0.0
1.47
1.43
2.06
1.84
#1
#2
Pik3ca E545K

Figure 4A



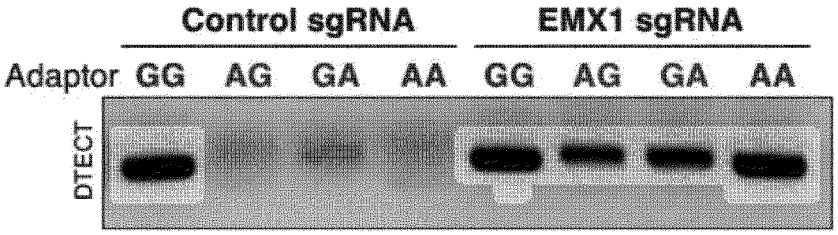


Figure 4B

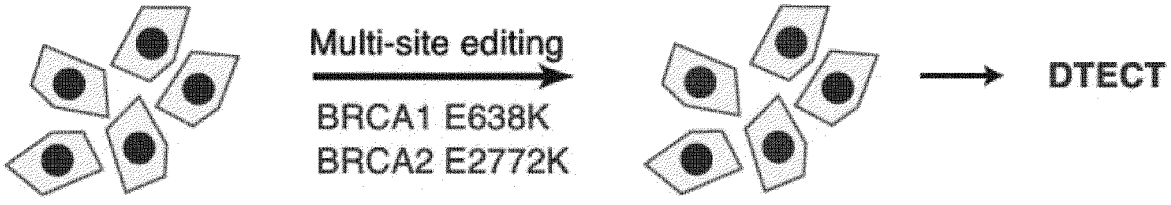
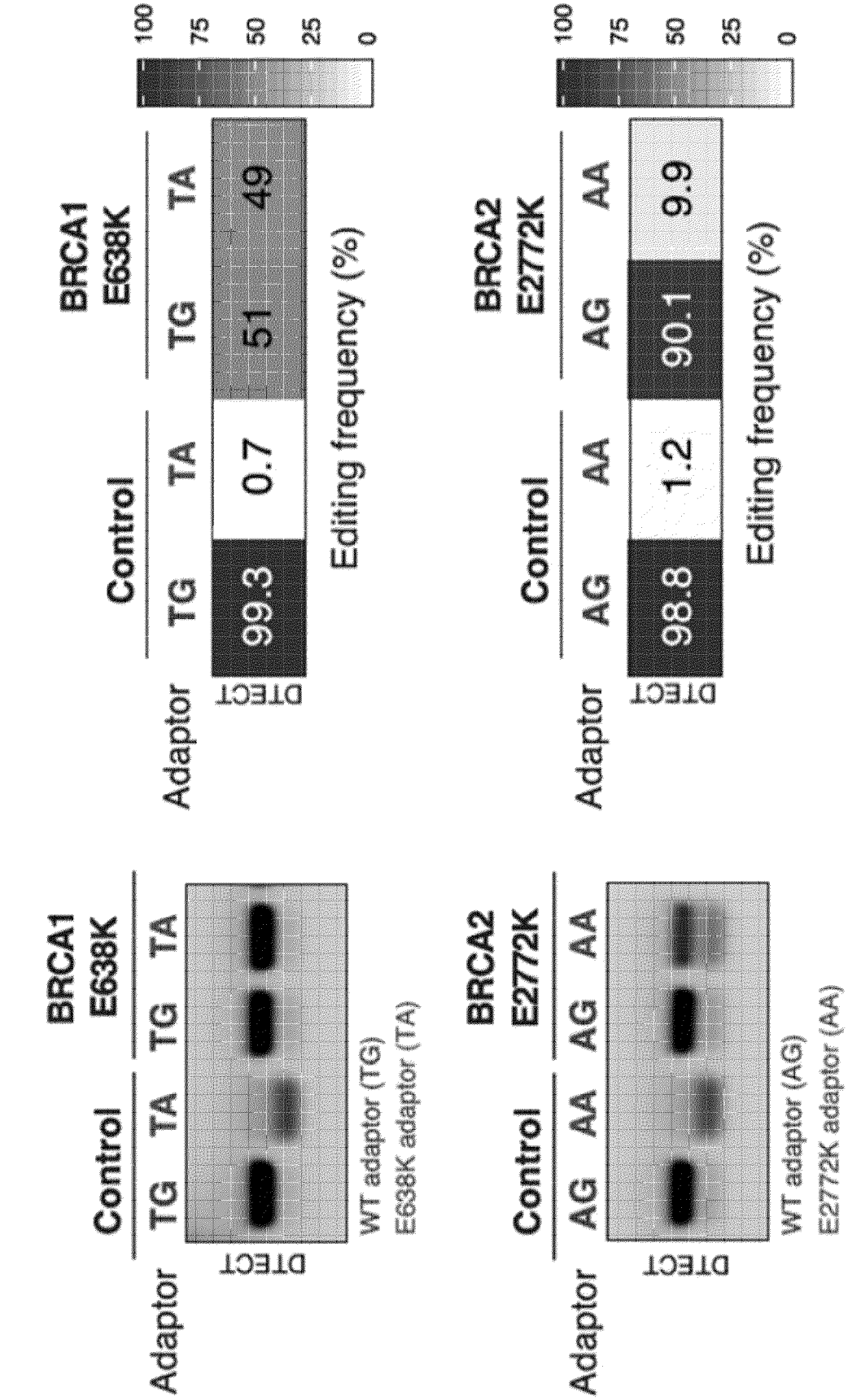


Figure 4C


BRCA1
E638K
Control
Adaptor
TG
TA
TG
TA
DTECT
WT adaptor (TG)
E638K adaptor (TA)
BRCA1
E638K
Control
Adaptor
TG
TA
TG
TA
DTECT
99.3
0.7
51
49
100
75
50
25
0
Editing frequency (%)
BRCA2
E2772K
Control
Adaptor
AG
AA
AG
AA
DTECT
WT adaptor (AG)
E2772K adaptor (AA)
BRCA2
E2772K
Control
Adaptor
AG
AA
AG
AA
DTECT
98.8
1.2
90.1
9.9
100
75
50
25
0
Editing frequency (%)

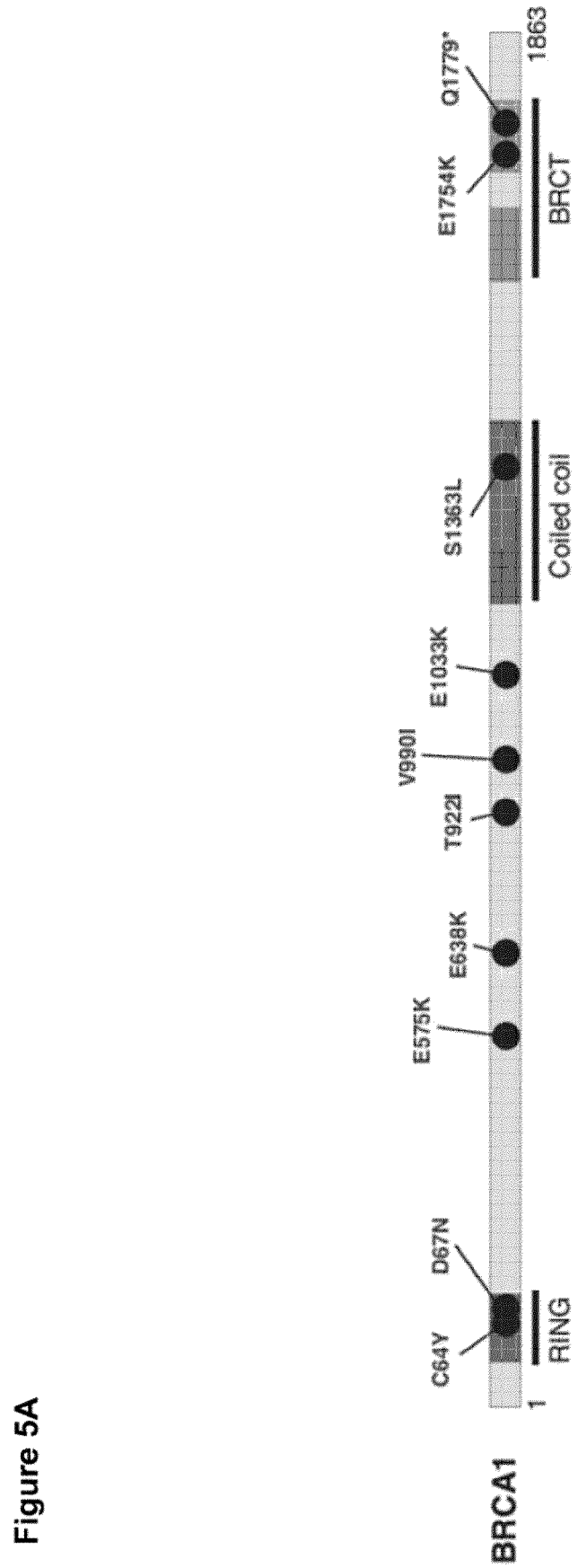

BRCA1
1
RING
1863
BRCT
Coiled coil
C64Y
D67N
E575K
E638K
T922I
V990I
E1033K
S1363L
E1754K
Q1779*

Figure 5A

Figure 5B

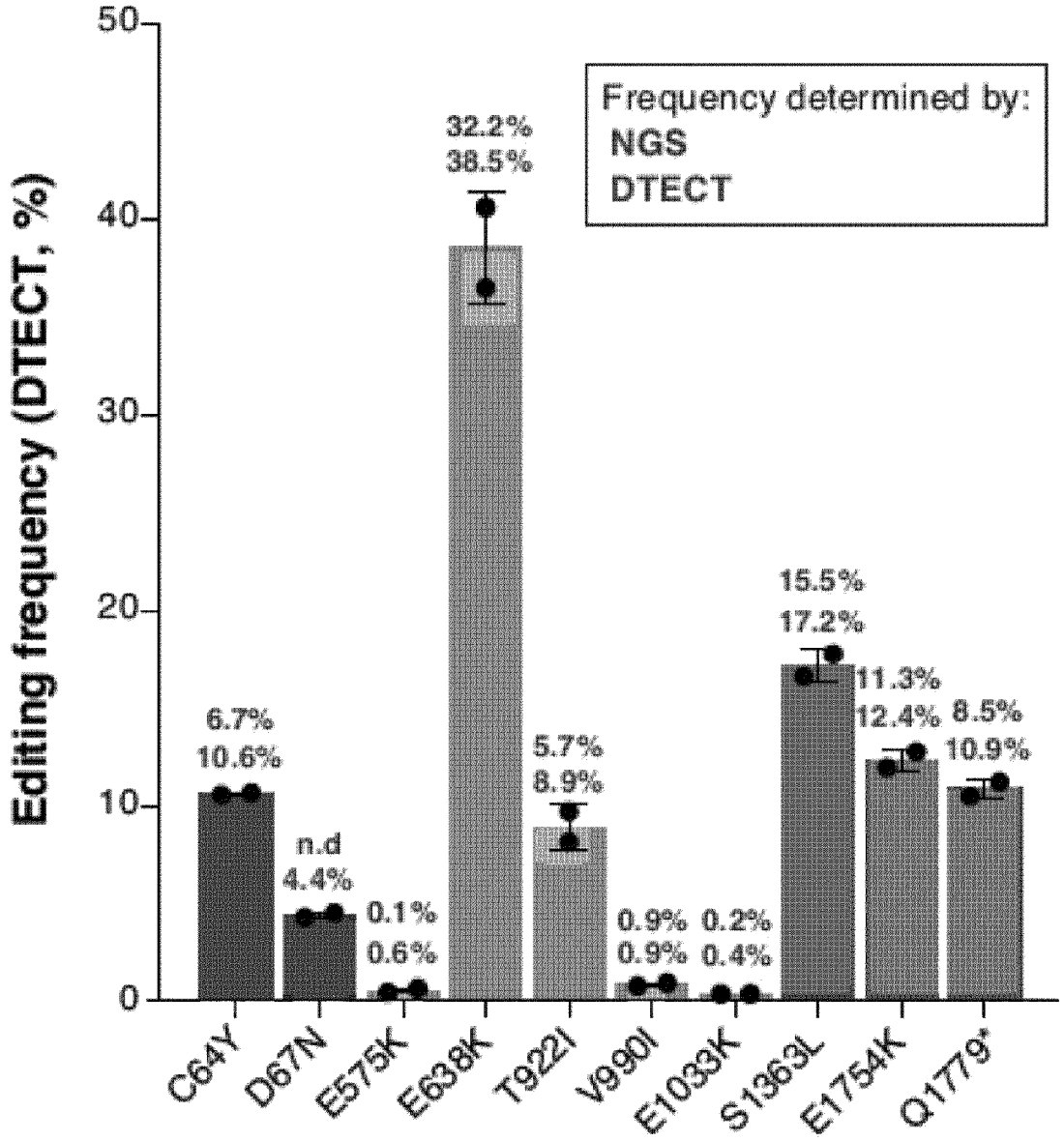
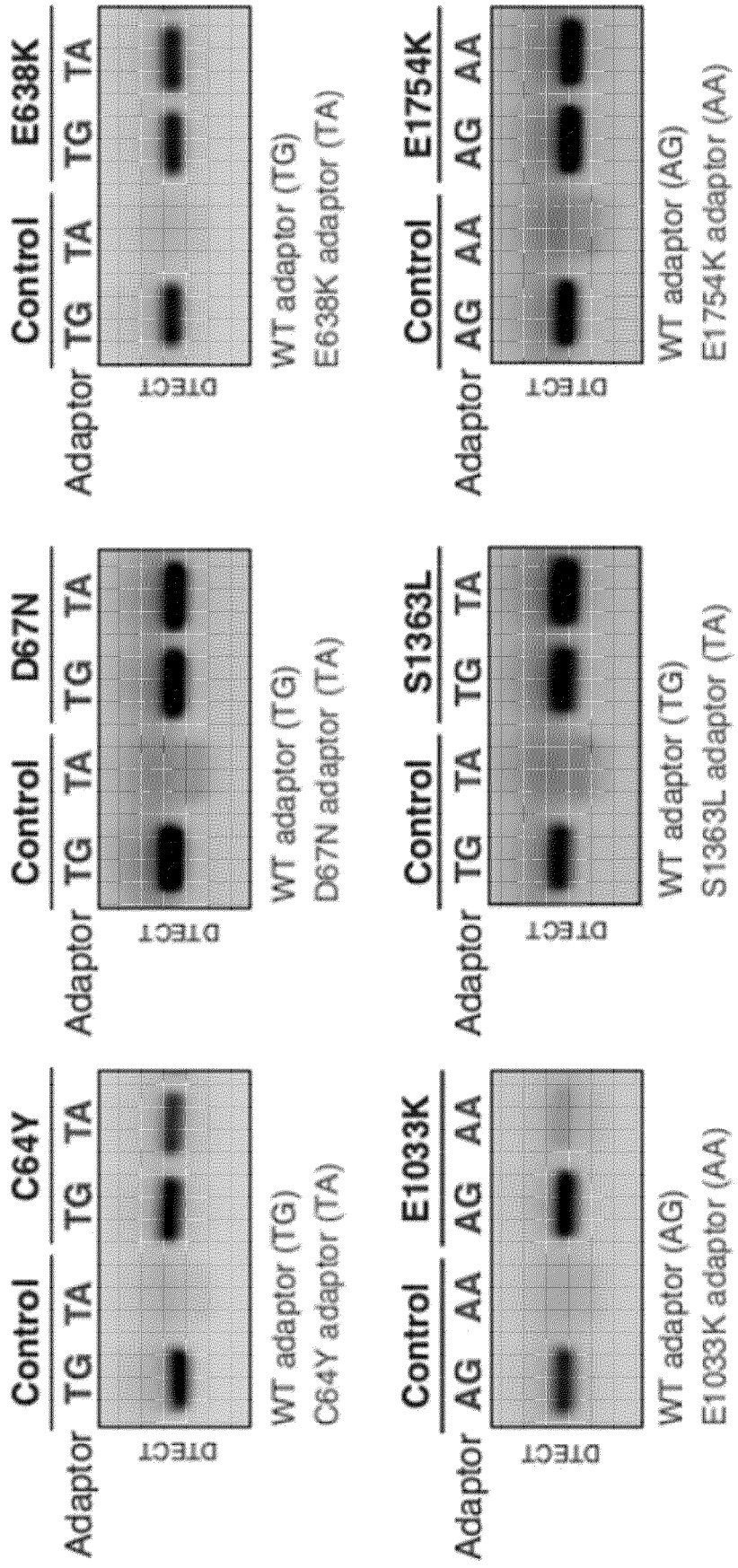

Editing frequency (DTECT, %)
50
40
30
20
10
0
Frequency determined by:
NGS
DTECT
6.7%
10.6%
n.d
4.4%
0.1%
0.6%
32.2%
38.5%
5.7%
8.9%
0.9%
0.9%
0.2%
0.4%
15.5%
17.2%
11.3%
12.4%
8.5%
10.9%
C64Y
D67N
E575K
E638K
T922I
V990I
E1033K
S1363L
E1754K
Q1779*

Figure 5C


Control C64Y
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
C64Y adaptor (TA)
Control D67N
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
D67N adaptor (TA)
Control E638K
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
E638K adaptor (TA)
Control E1033K
Adaptor AG AA AG AA
DTECT
WT adaptor (AG)
E1033K adaptor (AA)
Control S1363L
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
S1363L adaptor (TA)
Control E1754K
Adaptor AG AA AG AA
DTECT
WT adaptor (AG)
E1754K adaptor (AA)

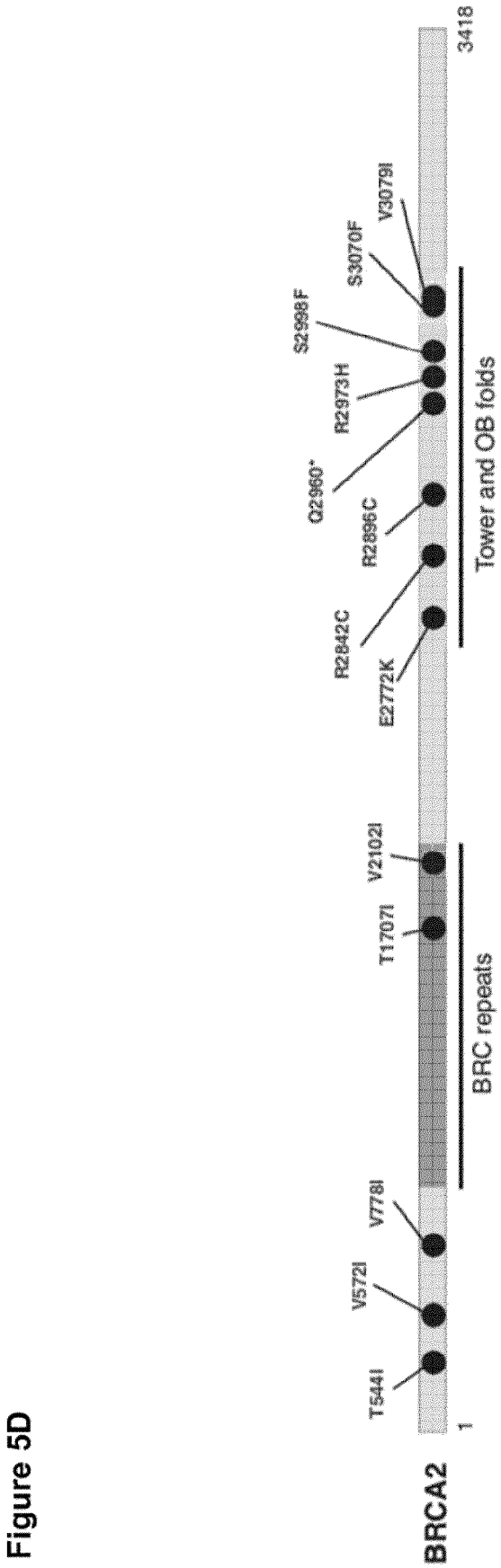

BRCA2
1
3418
T544I
V572I
V778I
T1707I
V2102I
R2842C
E2772K
R2896C
Q2960*
R2973H
S2998F
S3070F
V3079I
BRC repeats
Tower and OB folds

Figure 5D

Figure 5E

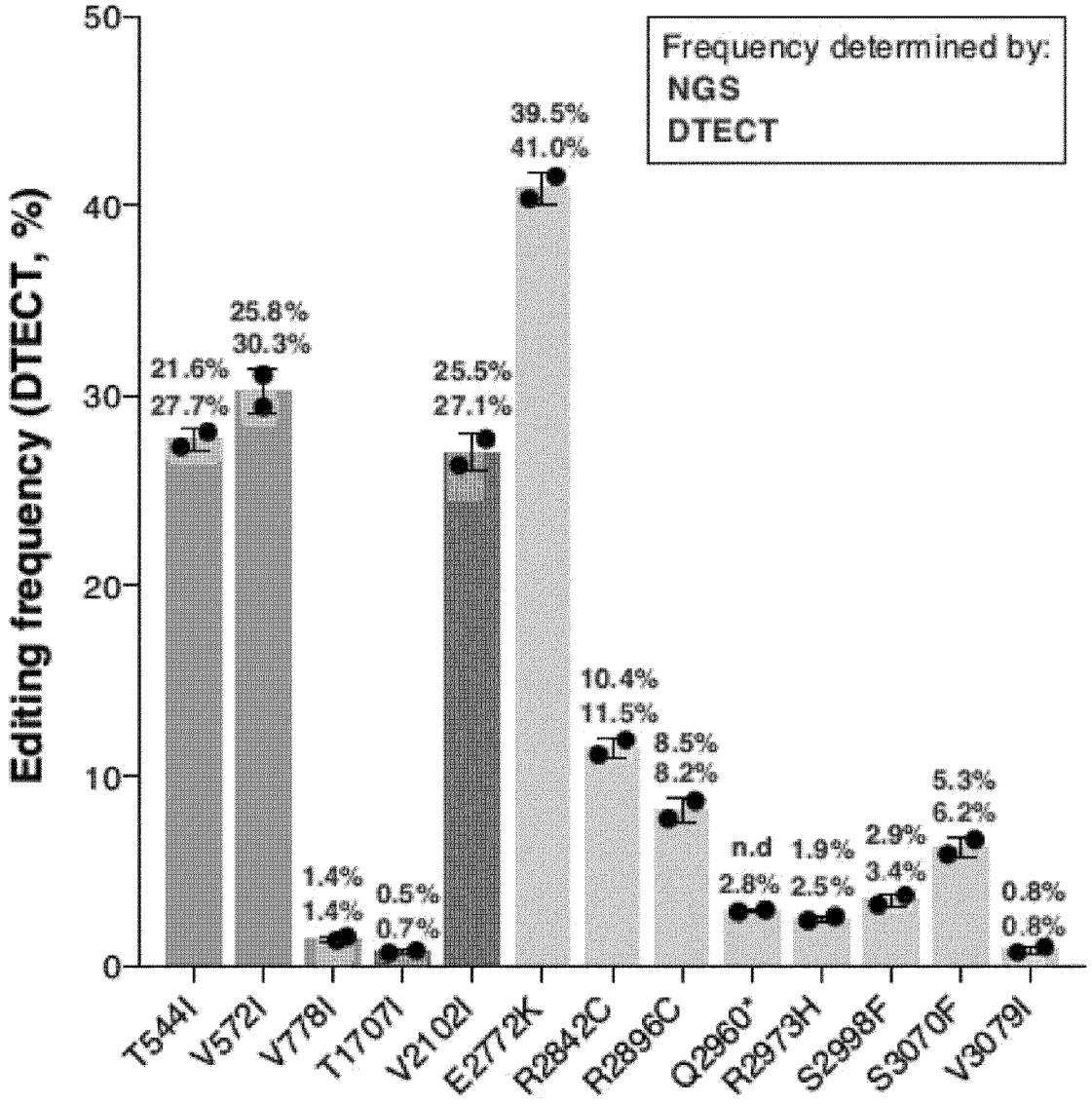

Frequency determined by:
NGS
DTECT
Editing frequency (DTECT, %)
50
40
30
20
10
0
21.6%
27.7%
25.8%
30.3%
1.4%
1.4%
0.5%
0.7%
25.5%
27.1%
39.5%
41.0%
10.4%
11.5%
8.5%
8.2%
n.d
2.8%
1.9%
2.5%
2.9%
3.4%
5.3%
6.2%
0.8%
0.8%
T544I
V572I
V778I
T1707I
V2102I
E2772K
R2842C
R2896C
Q2960*
R2973H
S2998F
S3070F
V3079I

Figure 5F

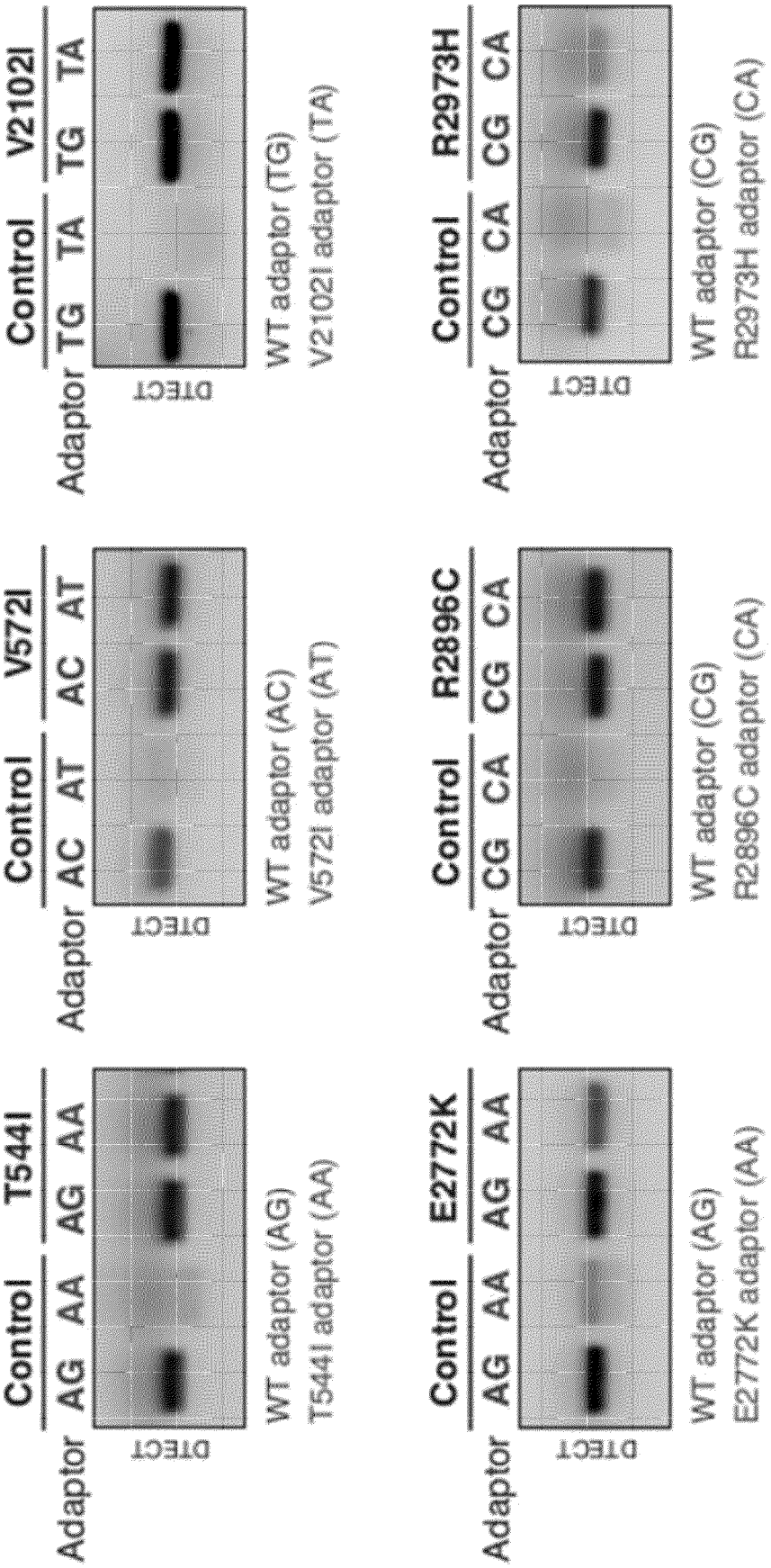
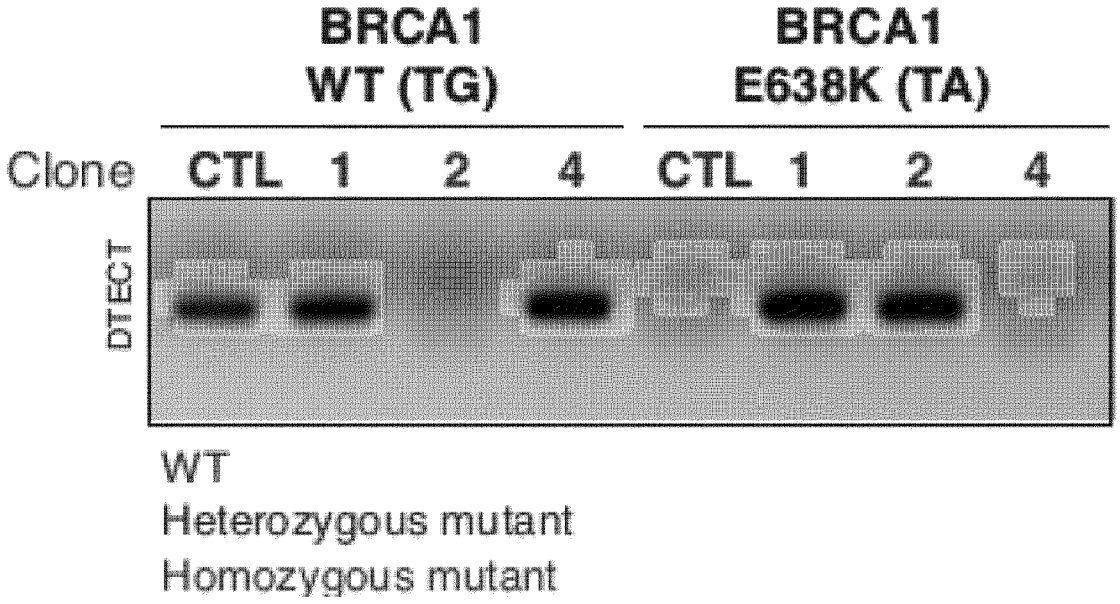

Control T544I
Adaptor AG AA AG AA
DTECT
WT adaptor (AG)
T544I adaptor (AA)
Control V572I
Adaptor AC AT AC AT
DTECT
WT adaptor (AC)
V572I adaptor (AT)
Control V2102I
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
V2102I adaptor (TA)
Control E2772K
Adaptor AG AA AG AA
DTECT
WT adaptor (AG)
E2772K adaptor (AA)
Control R2896C
Adaptor CG CA CG CA
DTECT
WT adaptor (CG)
R2896C adaptor (CA)
Control R2973H
Adaptor CG CA CG CA
DTECT
WT adaptor (CG)
R2973H adaptor (CA)

Figure 5G


BRCA1
WT (TG)
BRCA1
E638K (TA)
Clone
CTL 1 2 4
CTL 1 2 4
DTECT
WT
Heterozygous mutant
Homozygous mutant

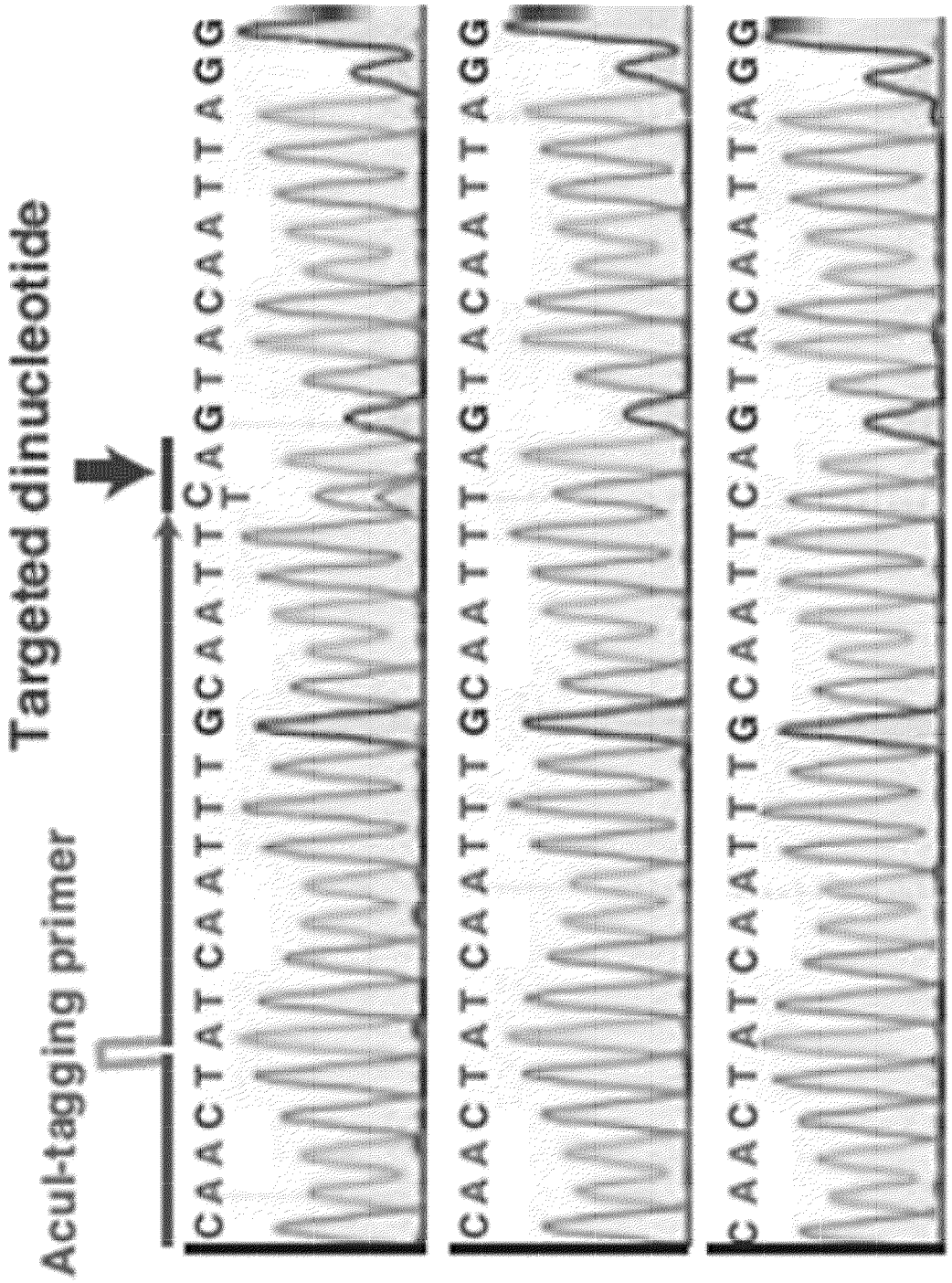
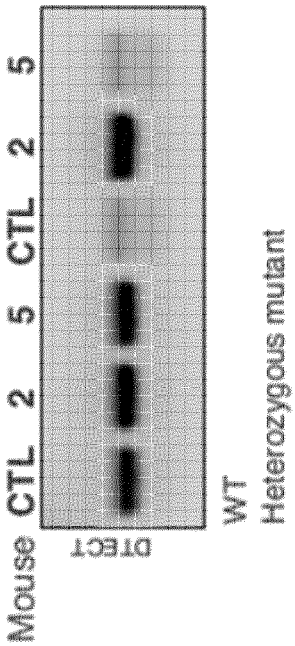

Targeted dinucleotide
AcuI-tagging primer
CAACTATCAATTTGCAATTCAGTACAATTAGG
Clone 1
heterozygous mutant
CAACTATCAATTTGCAATTTAGTACAATTAGG
Clone 2
homozygous mutant
CAACTATCAATTTGCAATTCAGTACAATTAGG
Clone 4
WT

Figure 5H


Brca1
WT (GA)
Brca1
S1598F (AA)
Mouse
CTL
2
5
CTL
2
5
DTECT
WT
Heterozygous mutant

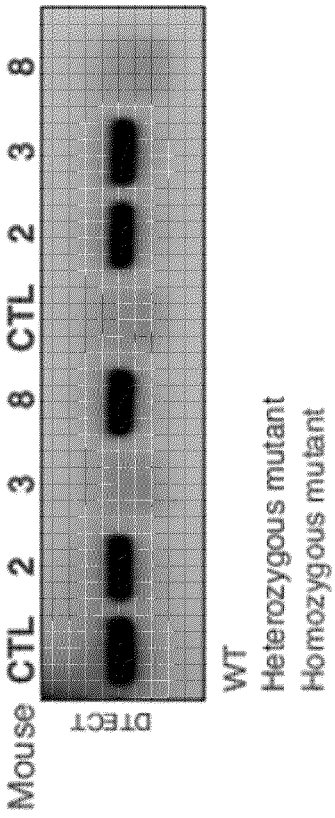

Bard1
WT (AG)
Bard1
S563F (TT)
Mouse
CTL
2
3
8
CTL
2
3
8
DTECT
WT
Heterozygous mutant
Homozygous mutant

Figure 5I

Figure 5J

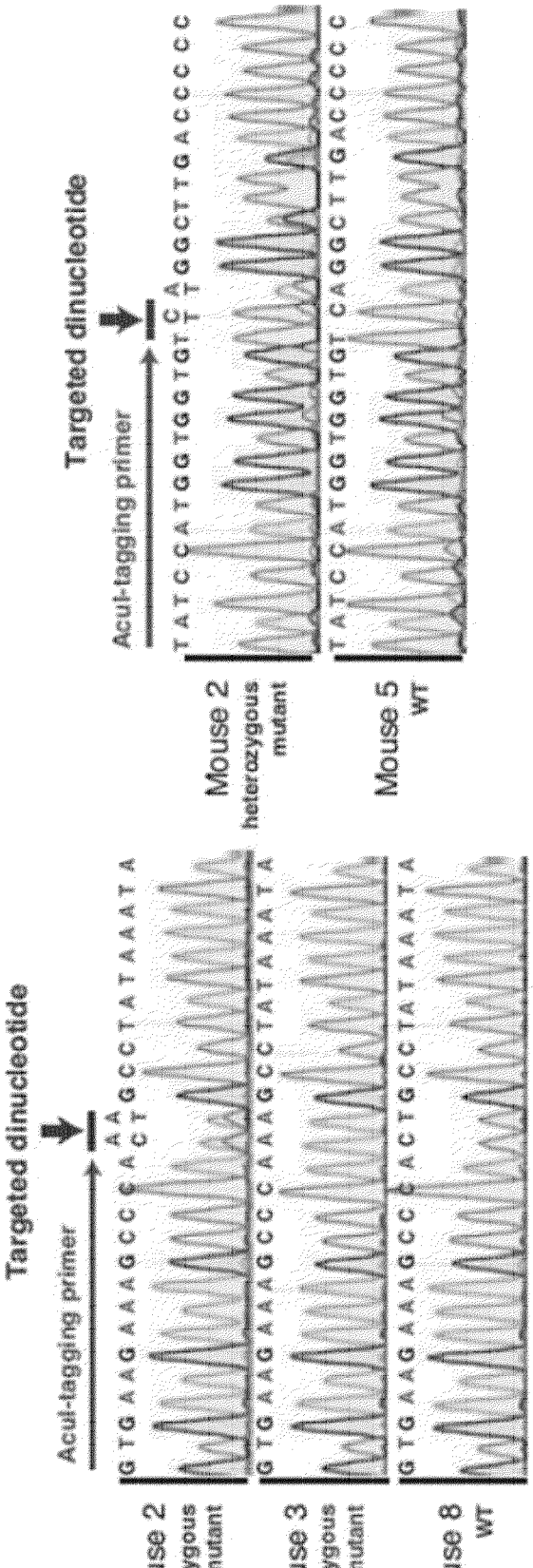

Targeted dinucleotide
Acul-tagging primer
Mouse 2
heterozygous
mutant
Mouse 5
WT
Mouse 2
heterozygous
mutant
Mouse 3
homozygous
mutant
Mouse 8
WT

Figure 6A

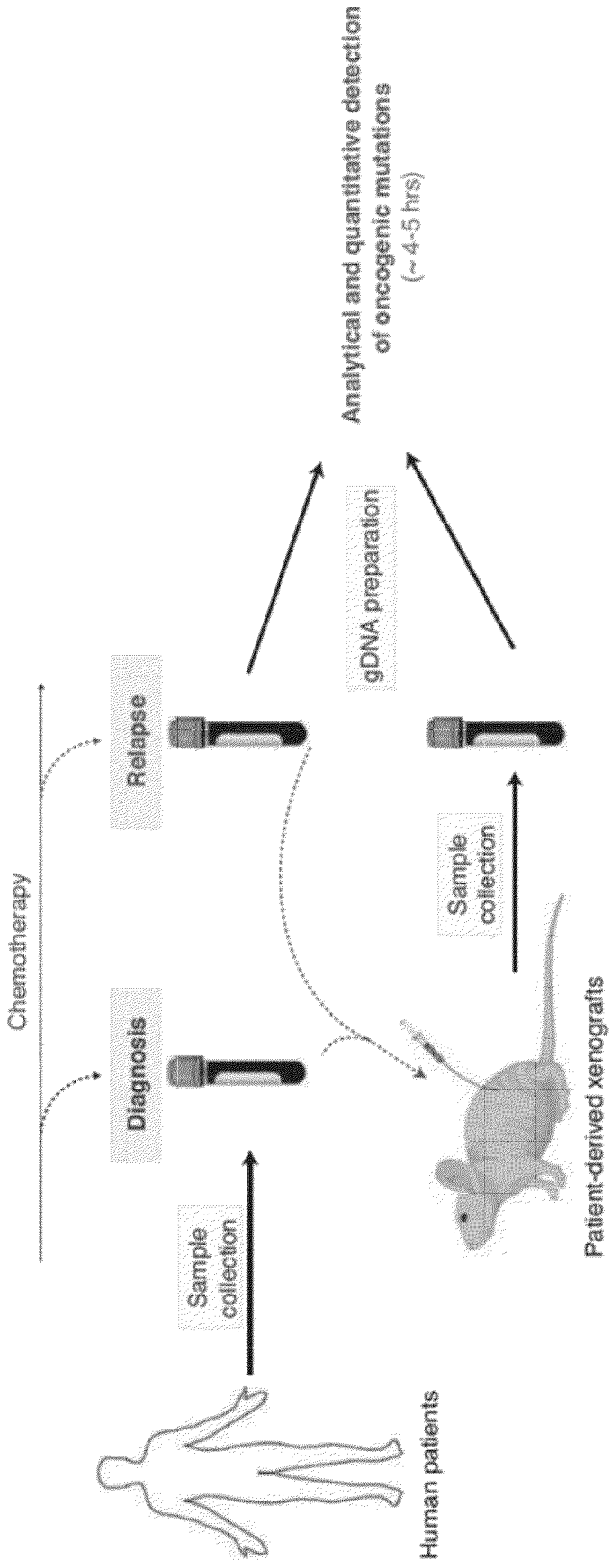

Human patients
Sample collection
Diagnosis
Chemotherapy
Relapse
Patient-derived xenografts
Sample collection
gDNA preparation
Analytical and quantitative detection of oncogenic mutations
(~ 4-5 hrs)

Figure 6B

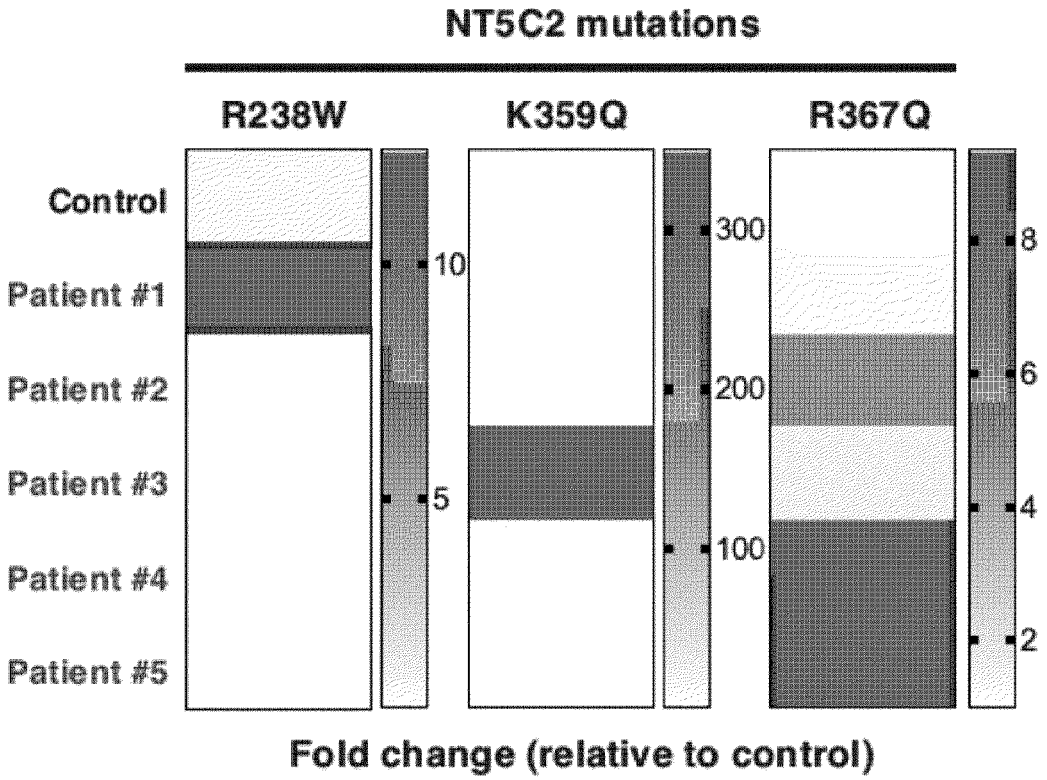


Figure 6C

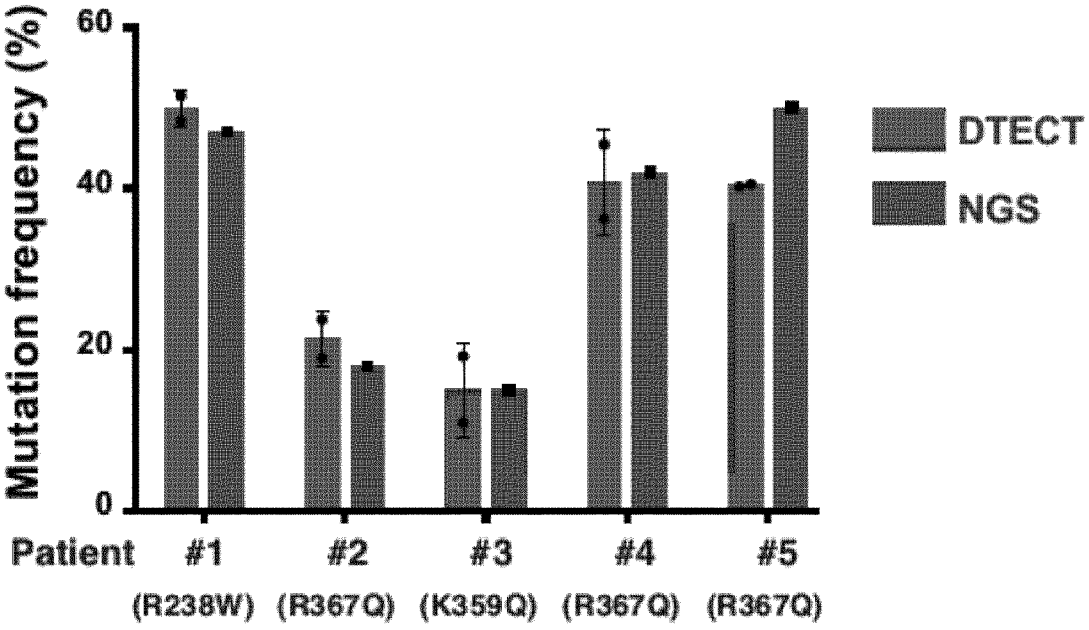


Figure 6D

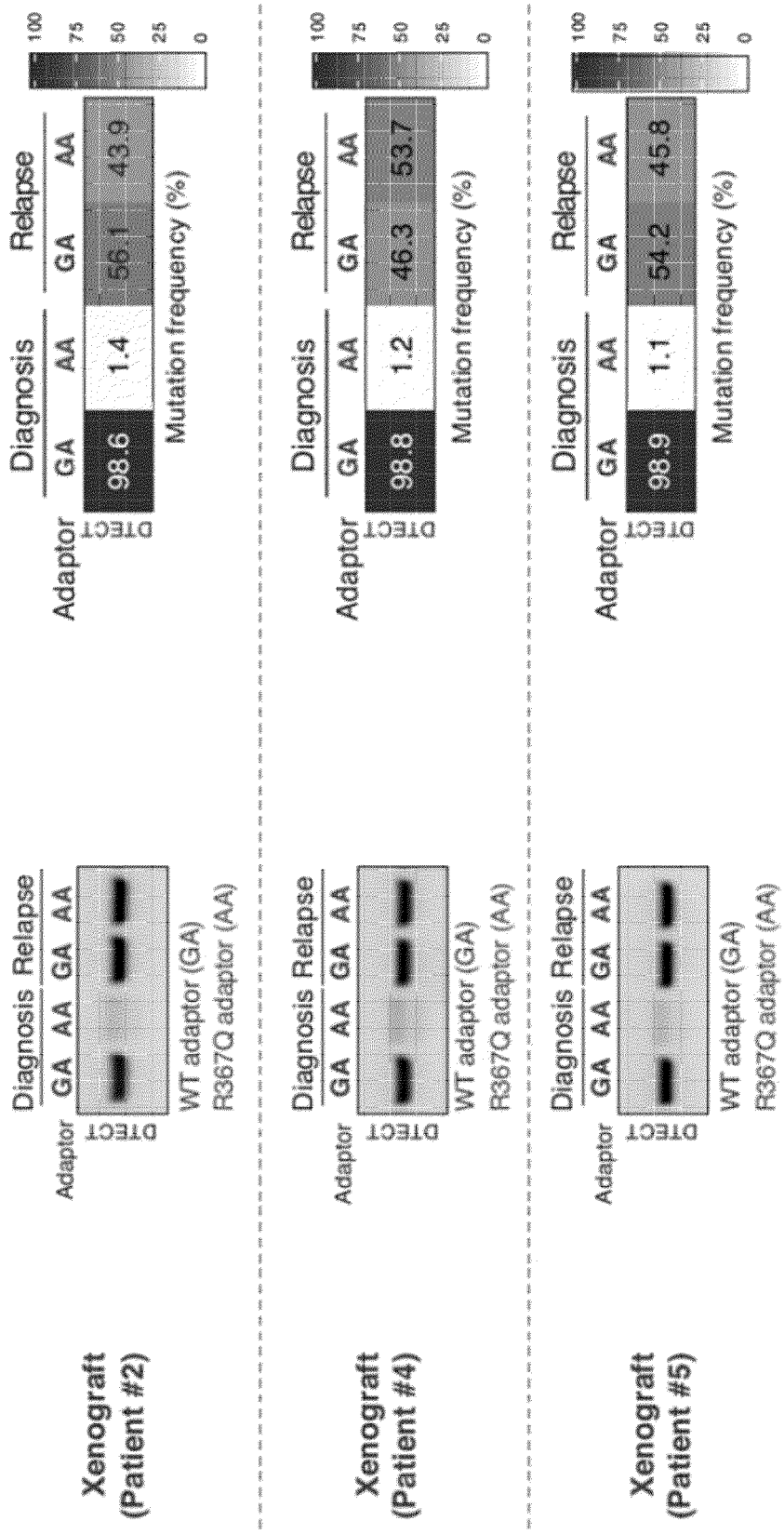

Xenograft (Patient #2)
Xenograft (Patient #4)
Xenograft (Patient #5)
Diagnosis
Relapse
GA
AA
GA
AA
Adaptor
DTECT
98.6
1.4
56.1
43.9
98.8
1.2
46.3
53.7
98.9
1.1
54.2
45.8
Mutation frequency (%)
100
75
50
25
0
WT adaptor (GA)
R367Q adaptor (AA)

Figure 7

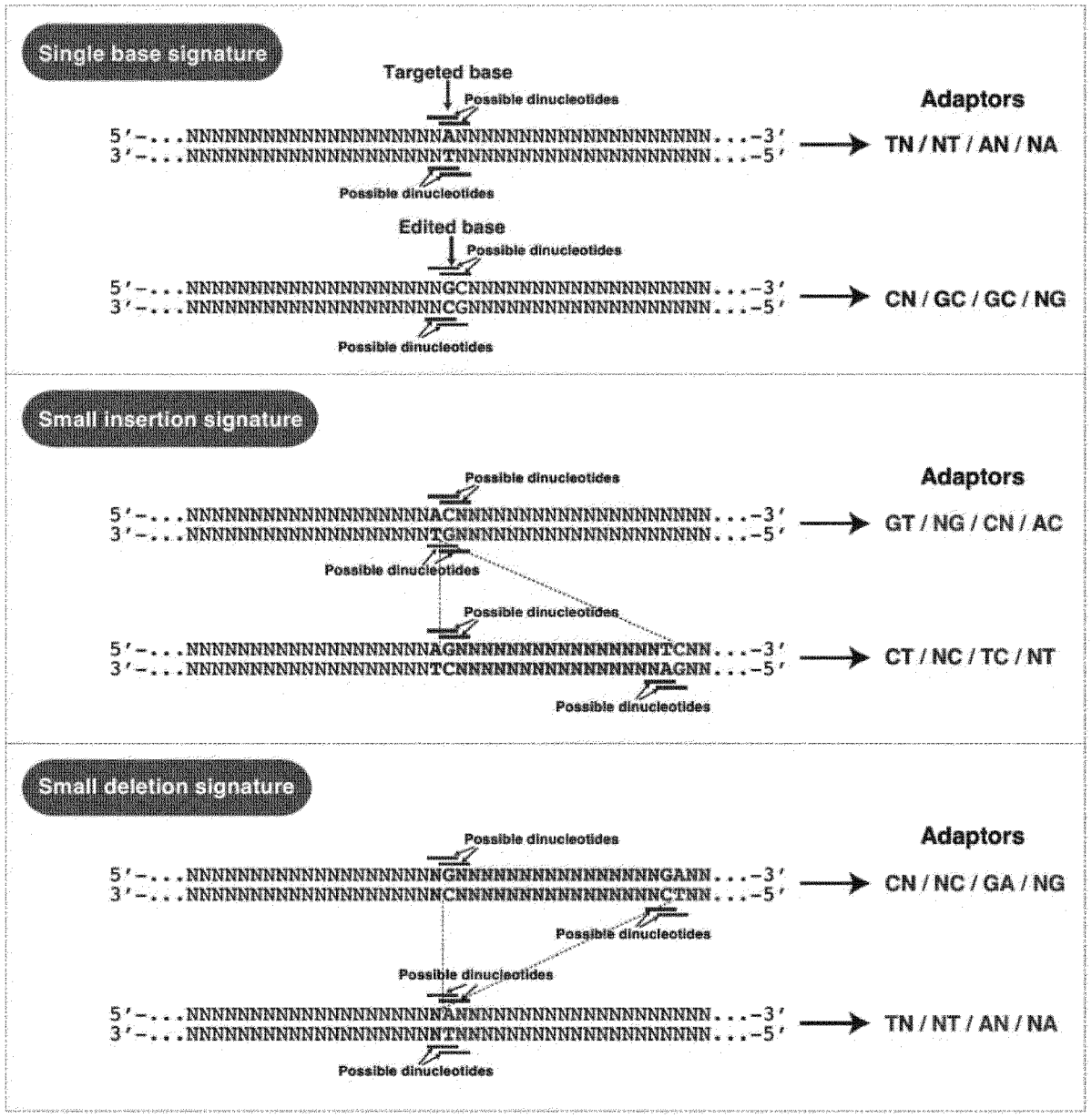

Single base signature
Targeted base
Possible dinucleotides
Adaptors
5′-...NNNNNNNNNNNNNNNNNNNNNANNNNNNNNNNNNNNNNNNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNNNTNNNNNNNNNNNNNNNNNNN...-5′
TN / NT / AN / NA
Possible dinucleotides
Edited base
Possible dinucleotides
5′-...NNNNNNNNNNNNNNNNNNNNNGCNNNNNNNNNNNNNNNNNNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNNNCGNNNNNNNNNNNNNNNNNNN...-5′
CN / GC / GC / NG
Possible dinucleotides
Small insertion signature
Possible dinucleotides
Adaptors
5′-...NNNNNNNNNNNNNNNNNNNNNACNNNNNNNNNNNNNNNNNNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNNNTGNNNNNNNNNNNNNNNNNNN...-5′
GT / NG / CN / AC
Possible dinucleotides
Possible dinucleotides
5′-...NNNNNNNNNNNNNNNNNNNNNAGNNNNNNNNNNNNNNNNNTCNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNNNTCNNNNNNNNNNNNNNNNNAGNN...-5′
CT / NC / TC / NT
Possible dinucleotides
Small deletion signature
Possible dinucleotides
Adaptors
5′-...NNNNNNNNNNNNNNNNNNNNNGNNNNNNNNNNNNNNNNNGANN...-3′
3′-...NNNNNNNNNNNNNNNNNNNNNCNNNNNNNNNNNNNNNNNCTNN...-5′
CN / NC / GA / NG
Possible dinucleotides
Possible dinucleotides
5′-...NNNNNNNNNNNNNNNNNNNNNANNNNNNNNNNNNNNNNNNNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNNNTNNNNNNNNNNNNNNNNNNNN...-5′
TN / NT / AN / NA
Possible dinucleotides

Figure 8A

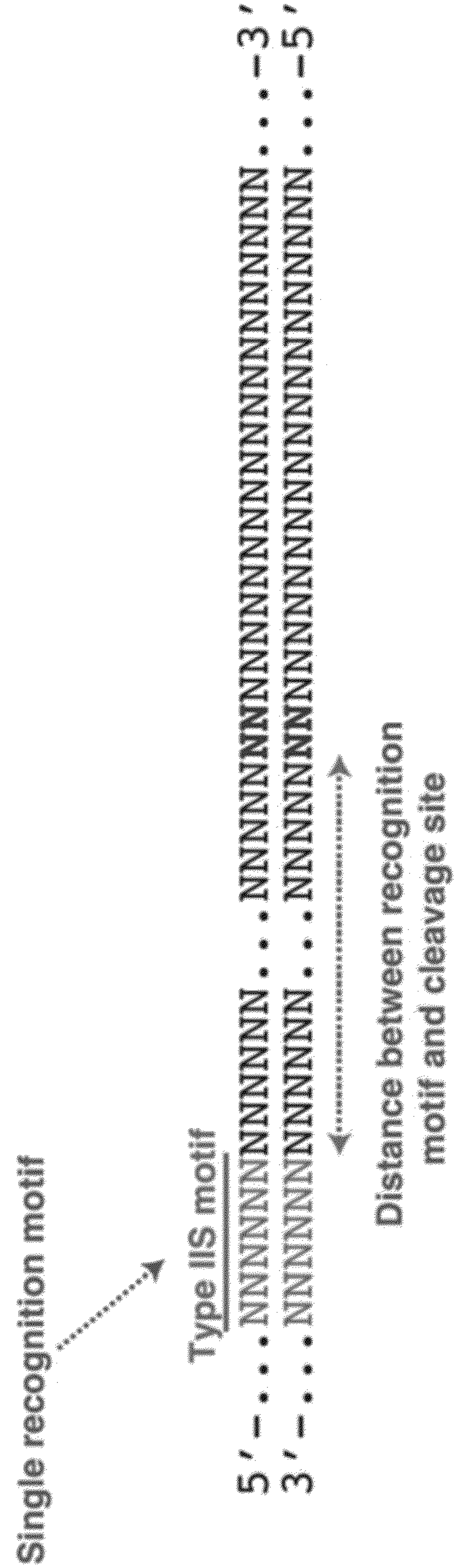
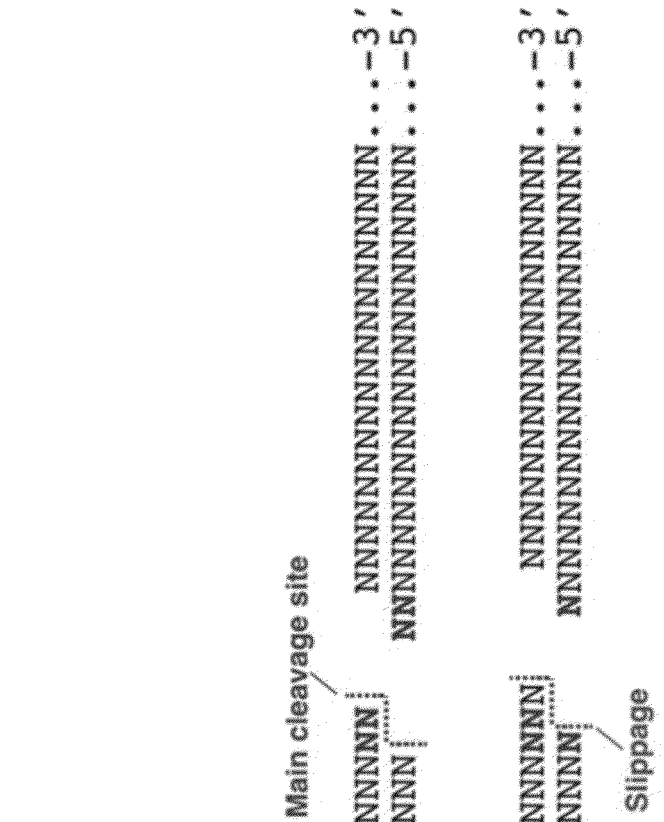

Single recognition motif
Type IIS motif
5′–. . .NNNNNNNNNNNN. . .NNNNNNNNNNNNNNNNNNNNNNNNNNN. . .–3′
3′–. . .NNNNNNNNNNNN. . .NNNNNNNNNNNNNNNNNNNNNNNNNNN. . .–5′
Distance between recognition motif and cleavage site

Figure 8B


Main cleavage site
Type IIS motif
Main digestion product
5′-....NNNNNNNNNNNNNNNNNNNN...-3′
3′-....NNNNNNNNNNNNNNNNNNNN...-5′
NNNNNNNNNNNNNNNNNNNNNNNNNN...-3′
NNNNNNNNNNNNNNNNNNNNNNNNNN...-5′
Type IIS motif
Digestion byproduct (slippage)
5′-....NNNNNNNNNNNNNNNNNNNNNN...
3′-....NNNNNNNNNNNNNNNNNNNN...
Slippage
NNNNNNNNNNNNNNNNNNNNNNNNNN...-3′
NNNNNNNNNNNNNNNNNNNNNNNNNN...-5′

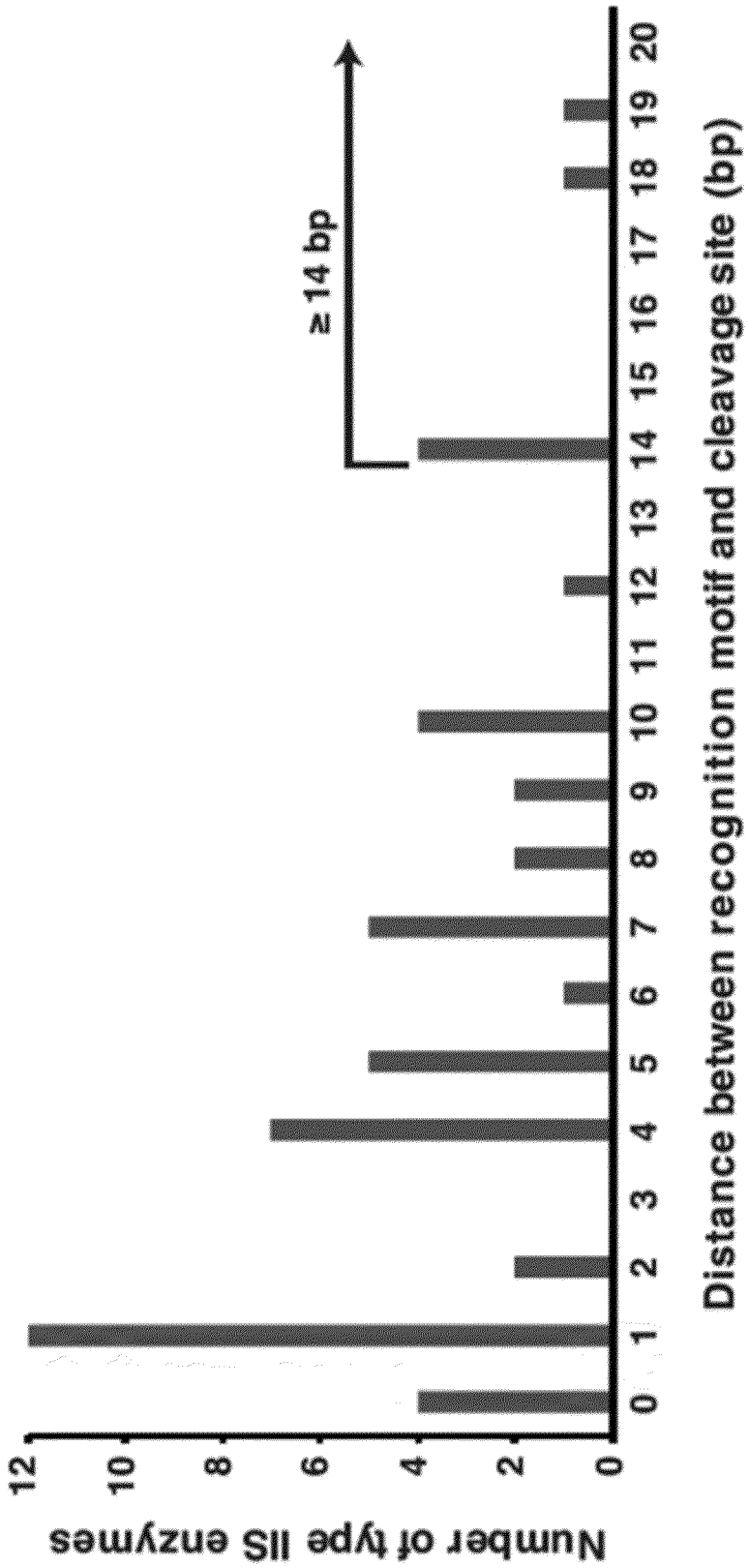

Number of type IIS enzymes
0
2
4
6
8
10
12
Distance between recognition motif and cleavage site (bp)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
≥ 14 bp

Figure 8C

Figure 8D

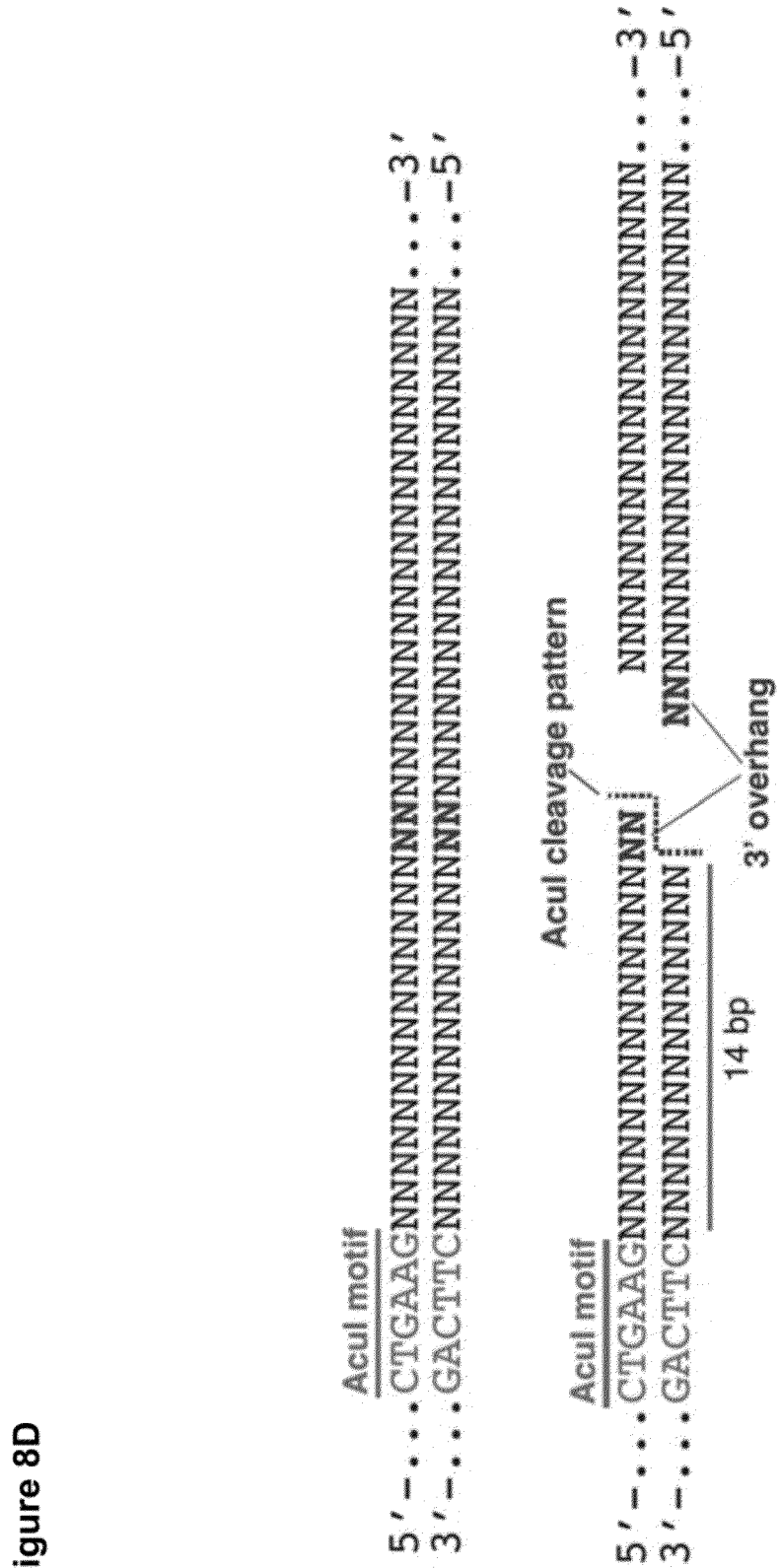

AcuI motif
5′-....CTGAAGNNNNNNNNNNNNNNNNNNNN....-3′
3′-....GACTTCNNNNNNNNNNNNNNNNNNNN....-5′
AcuI cleavage pattern
AcuI motif
5′-....CTGAAGNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNN....-3′
3′-....GACTTCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN....-5′
14 bp
3′ overhang

Figure 9A

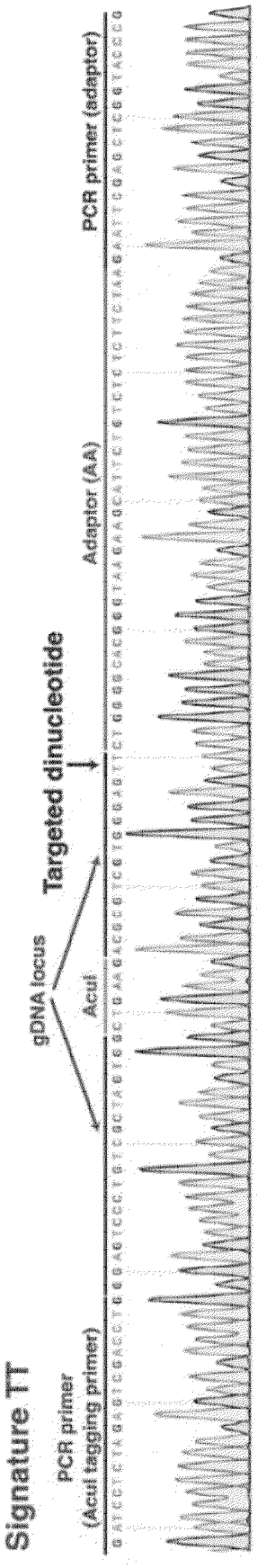

Signature TT
PCR primer
(AcuI tagging primer)
gDNA locus
AcuI
Targeted dinucleotide
Adaptor (AA)
PCR primer (adaptor)

Figure 9B

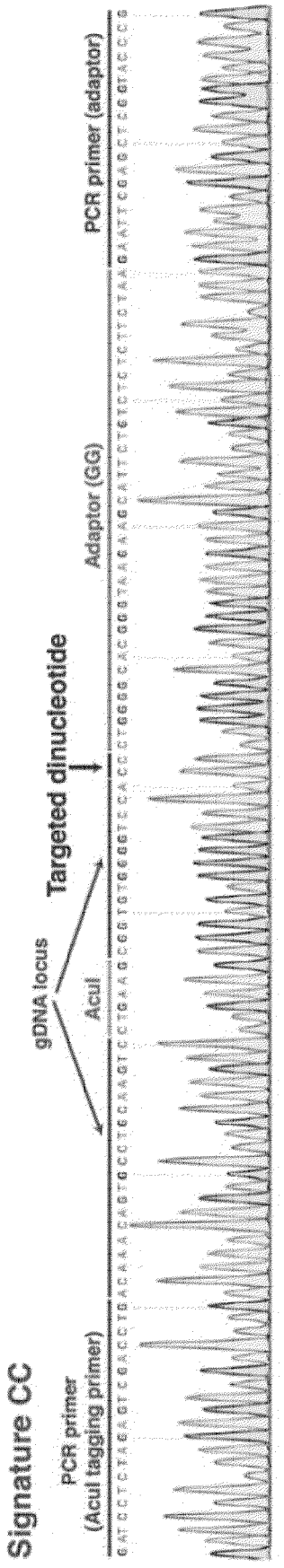
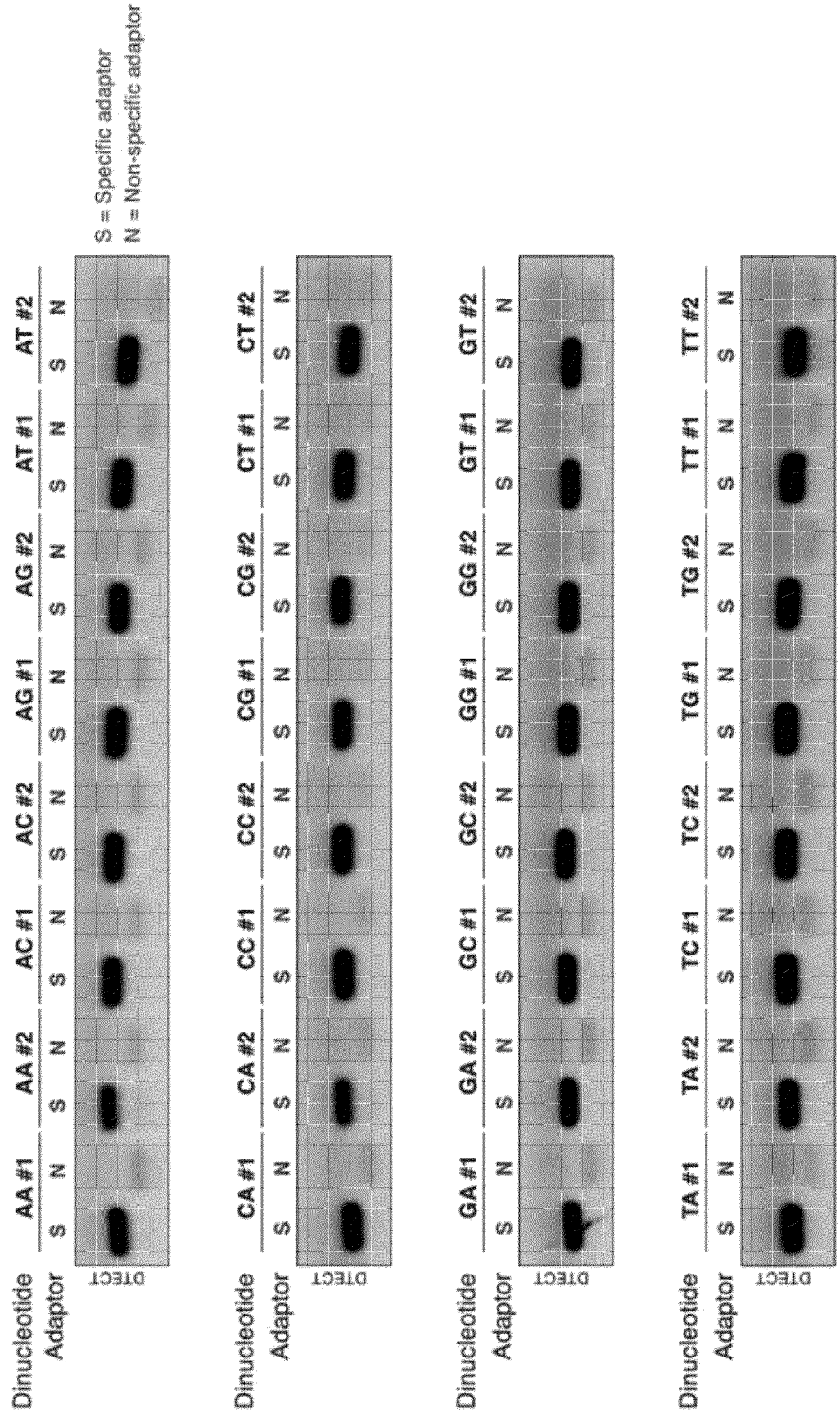

Signature CC
PCR primer
(AcuI tagging primer)
gDNA locus
AcuI
Targeted dinucleotide
Adaptor (GG)
PCR primer (adaptor)

Figure 9C


S = Specific adaptor
N = Non-specific adaptor
Dinucleotide
Adaptor
AA #1
AA #2
AC #1
AC #2
AG #1
AG #2
AT #1
AT #2
CA #1
CA #2
CC #1
CC #2
CG #1
CG #2
CT #1
CT #2
GA #1
GA #2
GC #1
GC #2
GG #1
GG #2
GT #1
GT #2
TA #1
TA #2
TC #1
TC #2
TG #1
TG #2
TT #1
TT #2
S
N
DTECT

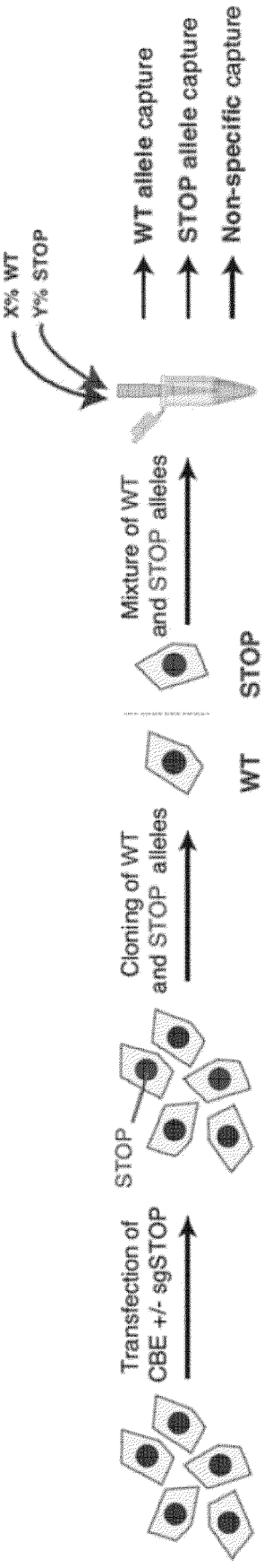

Transfection of
CBE +/- sgSTOP
STOP
Cloning of WT
and STOP alleles
WT
STOP
Mixture of WT
and STOP alleles
X% WT
Y% STOP
WT allele capture
STOP allele capture
Non-specific capture

Figure 10A

Figure 10B

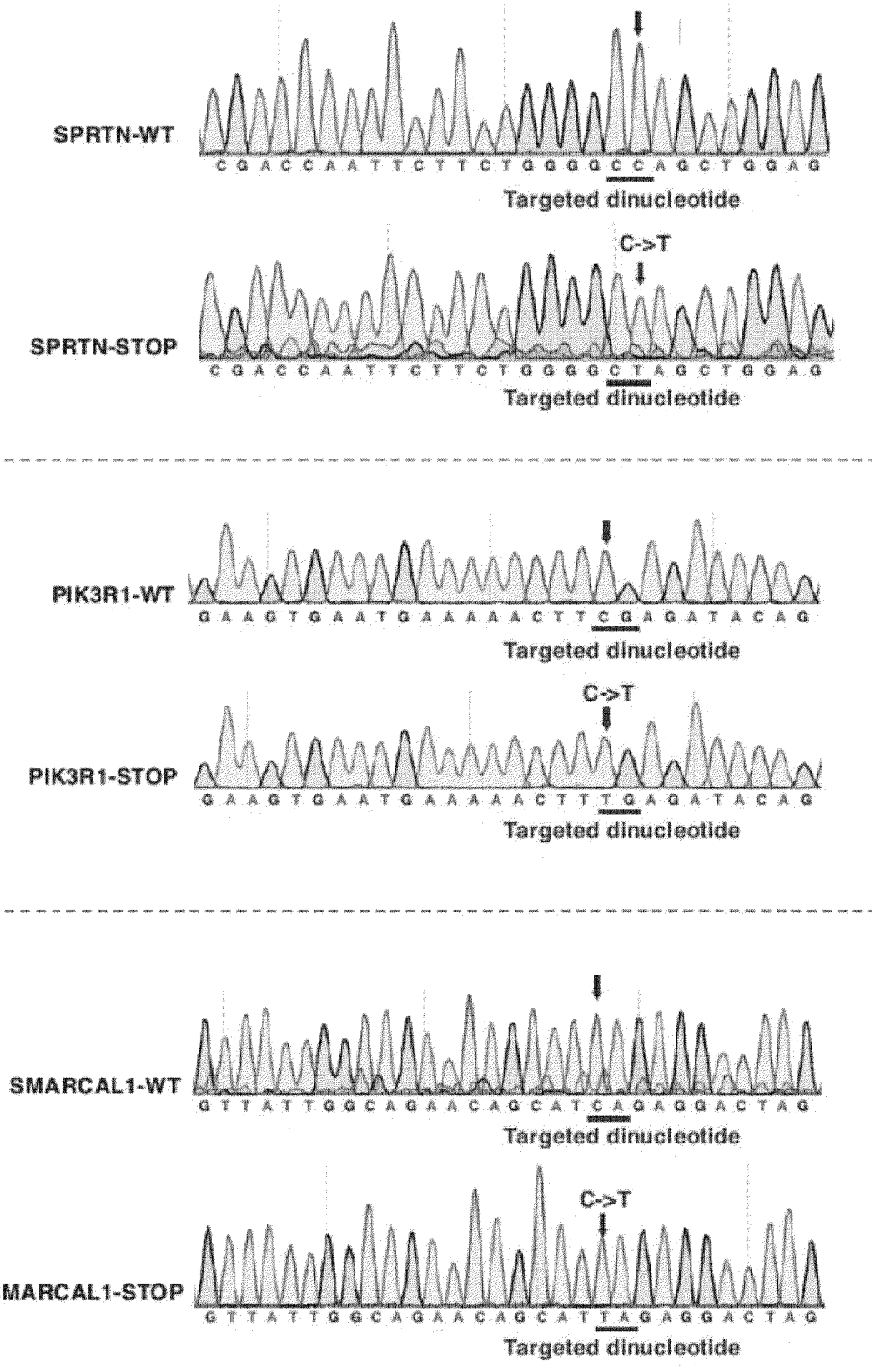

SPRTN-WT
C G A C C A A T T C T T C T G G G G C C A G C T G G A G
Targeted dinucleotide
C->T
SPRTN-STOP
C G A C C A A T T C T T C T G G G G C T A G C T G G A G
Targeted dinucleotide
PIK3R1-WT
G A A G T G A A T G A A A A A C T T C G A G A T A C A G
Targeted dinucleotide
C->T
PIK3R1-STOP
G A A G T G A A T G A A A A A C T T T G A G A T A C A G
Targeted dinucleotide
SMARCAL1-WT
G T T A T T G G C A G A A C A G C A T C A G A G G A C T A G
Targeted dinucleotide
C->T
SMARCAL1-STOP
G T T A T T G G C A G A A C A G C A T T A G A G G A C T A G
Targeted dinucleotide

Figure 10C

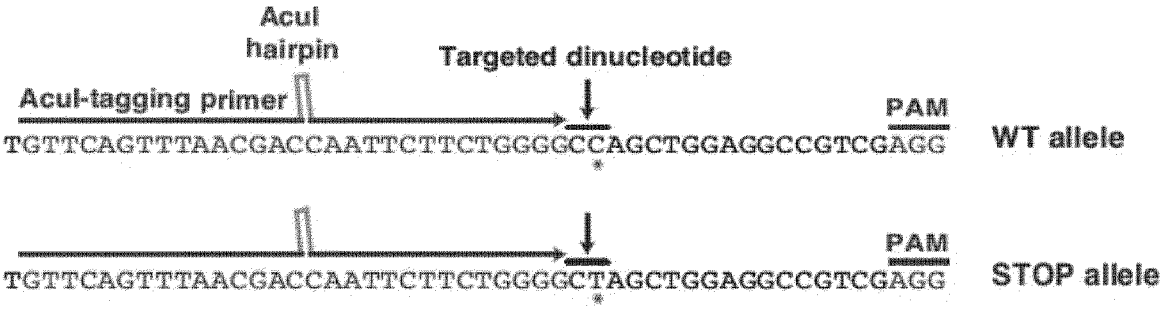
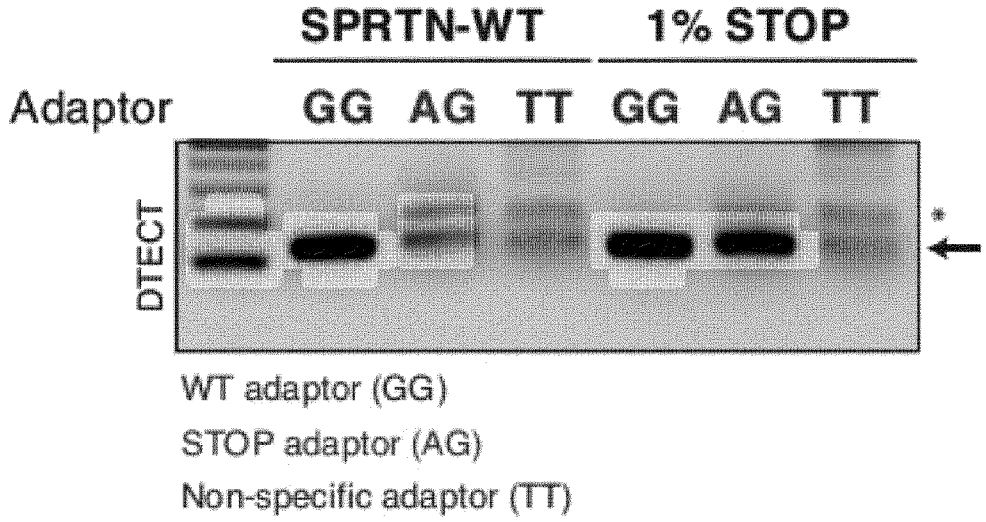


Figure 10D



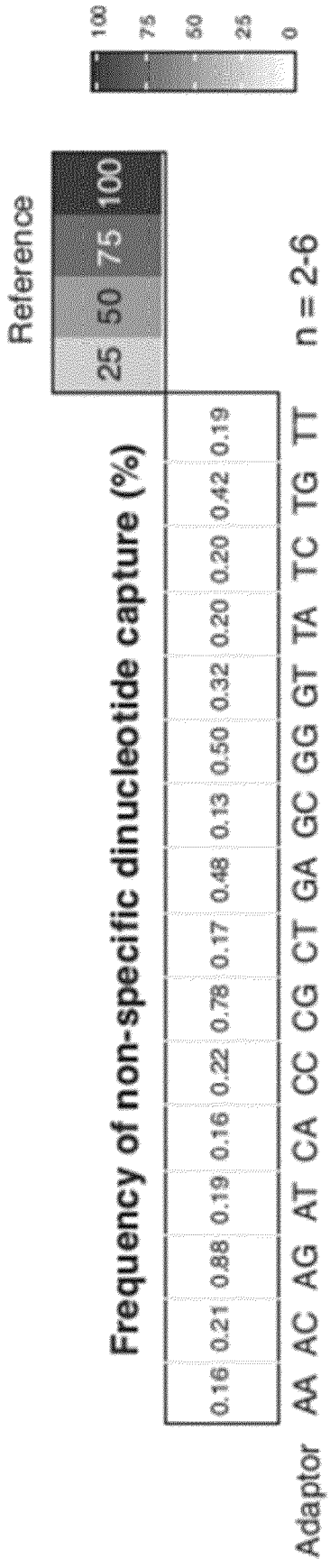

100
75
50
25
0
Reference
25 50 75 100
Frequency of non-specific dinucleotide capture (%)
0.16 0.21 0.88 0.19 0.16 0.22 0.78 0.17 0.48 0.13 0.50 0.32 0.20 0.20 0.42 0.19
Adaptor AA AC AG AT CA CC CG CT GA GC GG GT TA TC TG TT
n = 2-6

Figure 10E

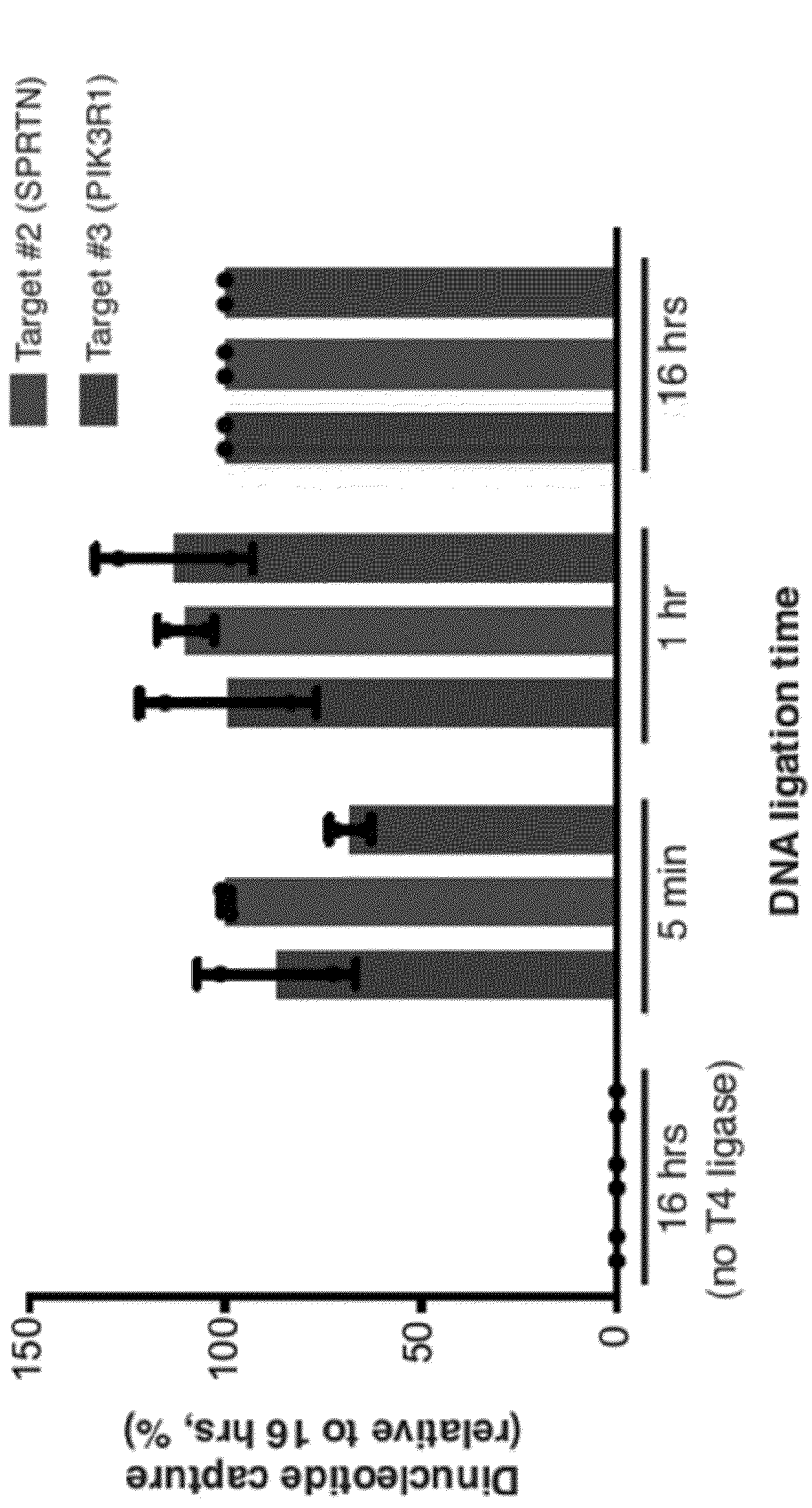

Target #1 (SMARCAL1)
Target #2 (SPRTN)
Target #3 (PIK3R1)
Dinucleotide capture
(relative to 16 hrs, %)
150
100
50
0
16 hrs
(no T4 ligase)
5 min
1 hr
16 hrs
DNA ligation time

Figure 10F

Figure 11A

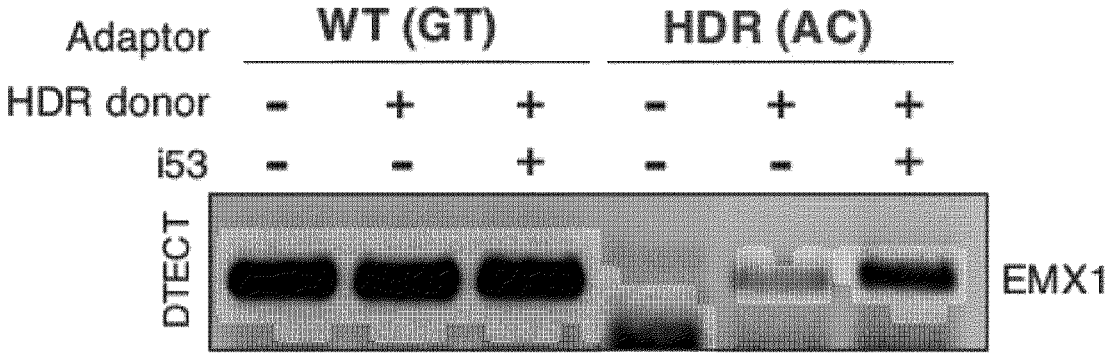


Figure 11B

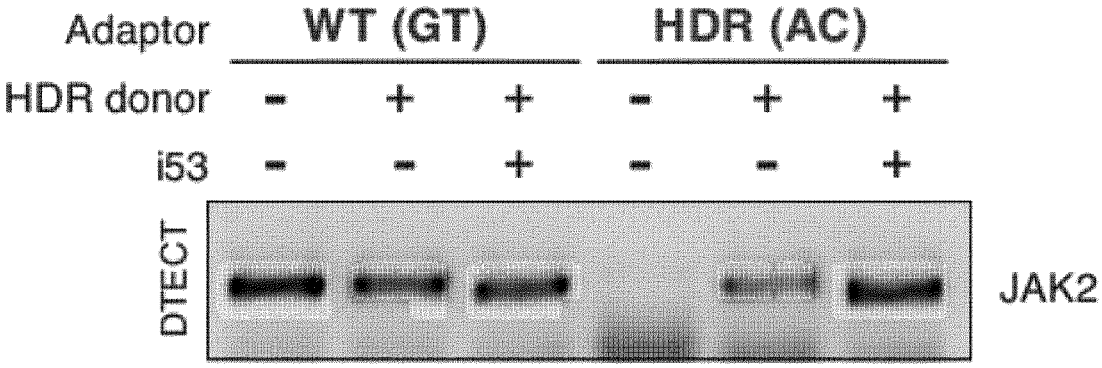


Figure 11C

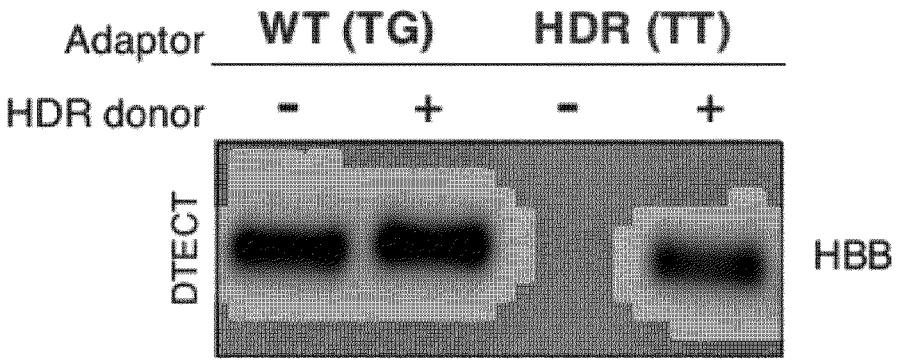


Figure 11D

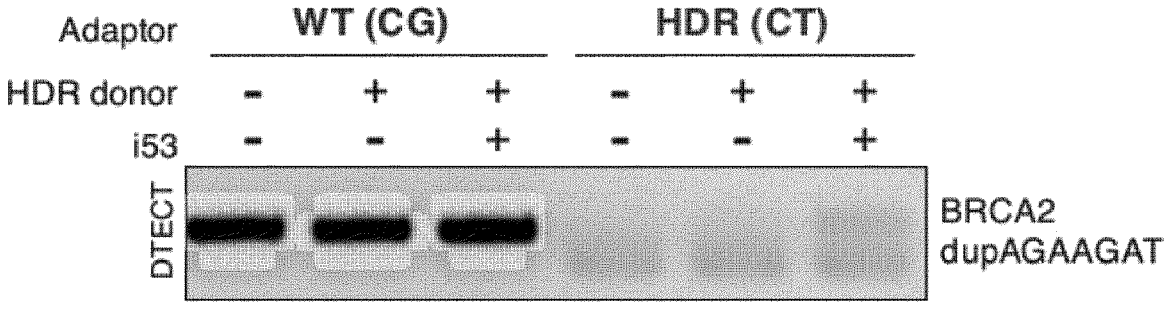


Figure 11E

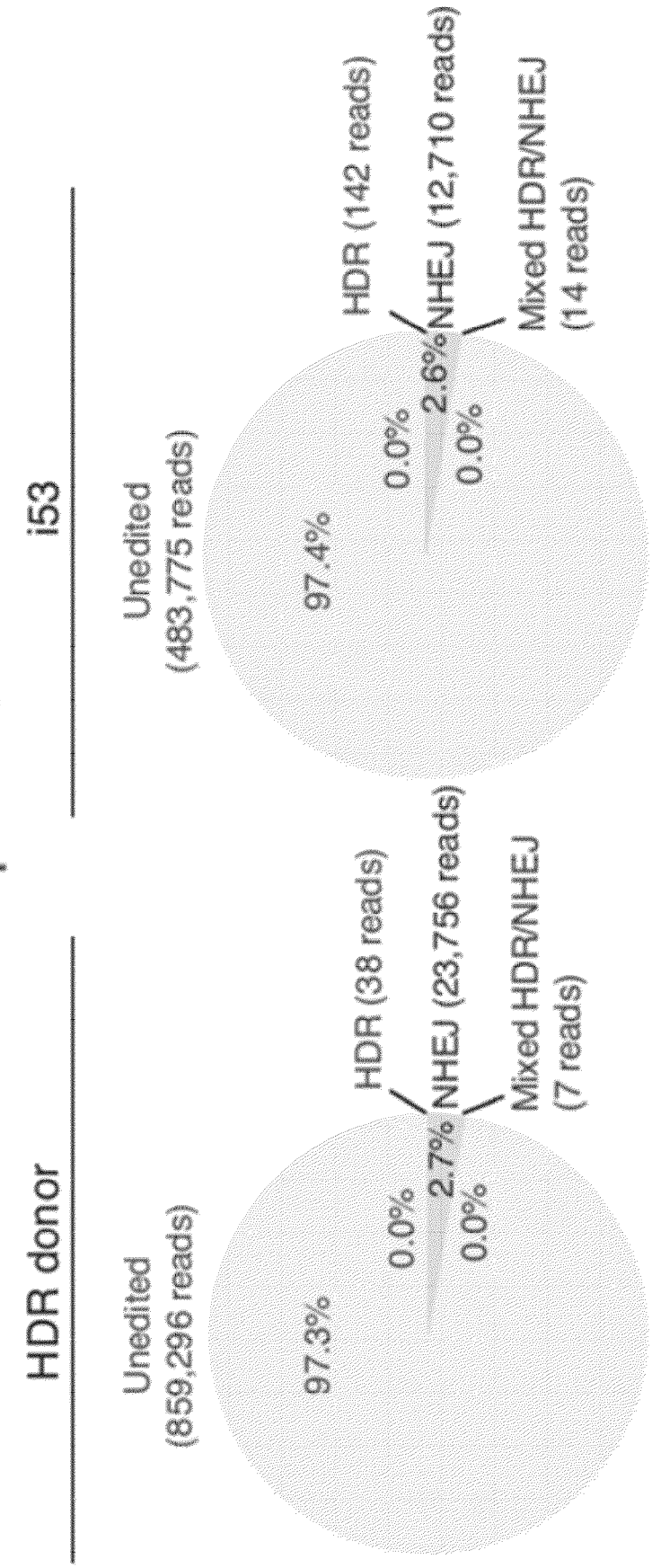

BRCA2 dupAGAAGAT
HDR donor
Unedited
(859,296 reads)
97.3%
HDR (38 reads)
0.0%
NHEJ (23,756 reads)
2.7%
Mixed HDR/NHEJ
(7 reads)
0.0%
i53
Unedited
(483,775 reads)
97.4%
HDR (142 reads)
0.0%
NHEJ (12,710 reads)
2.6%
Mixed HDR/NHEJ
(14 reads)
0.0%

Figure 11F

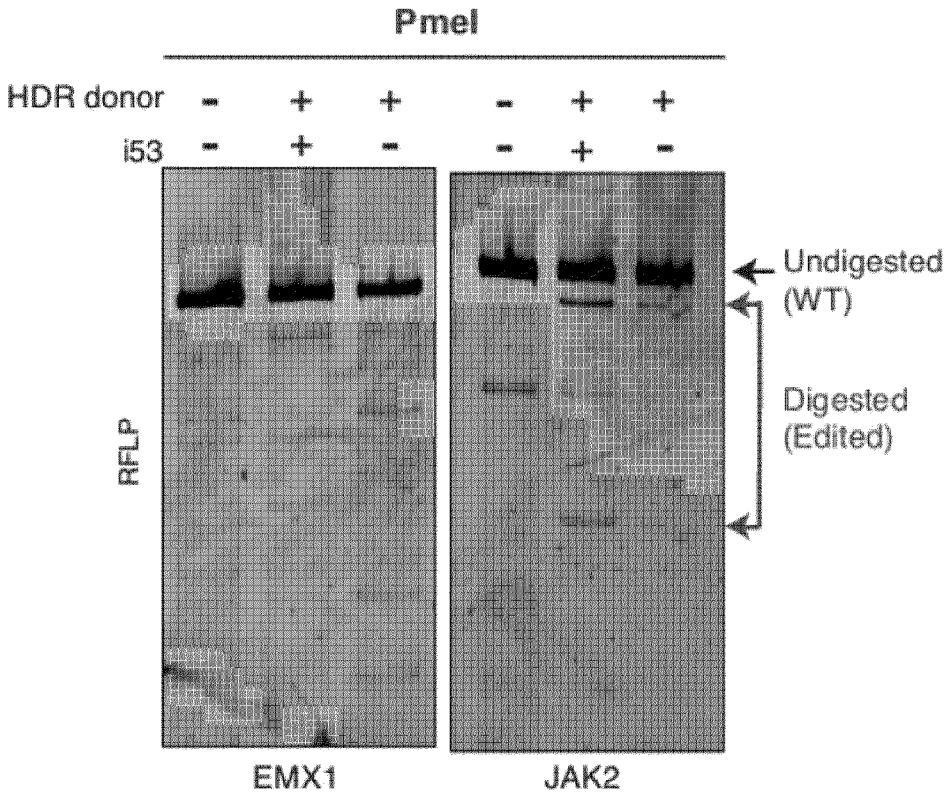


Figure 11G

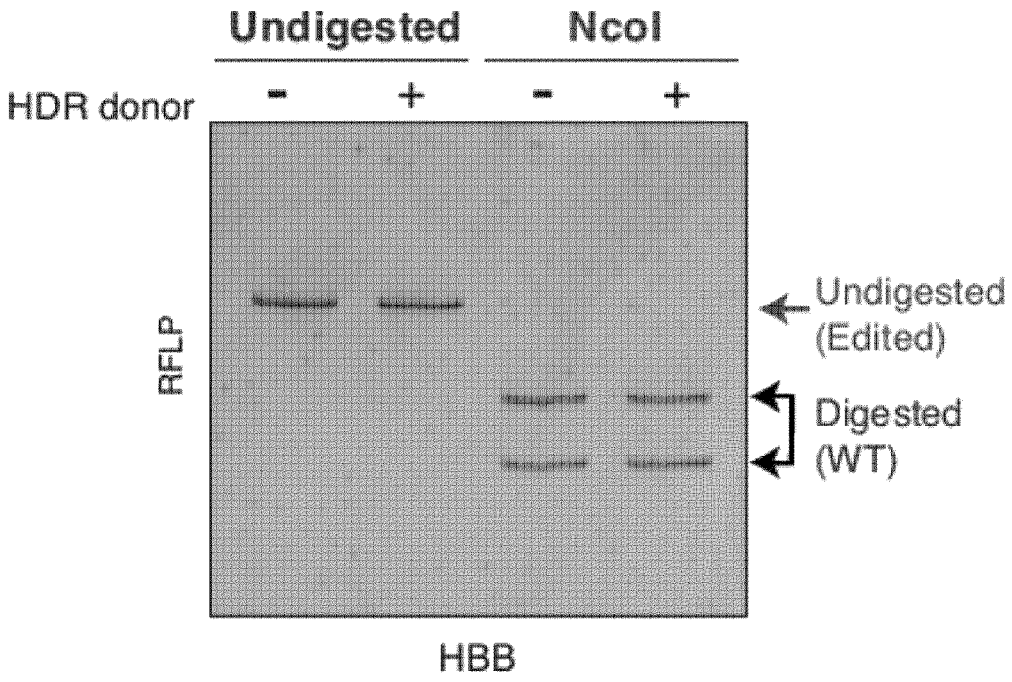


Figure 11H

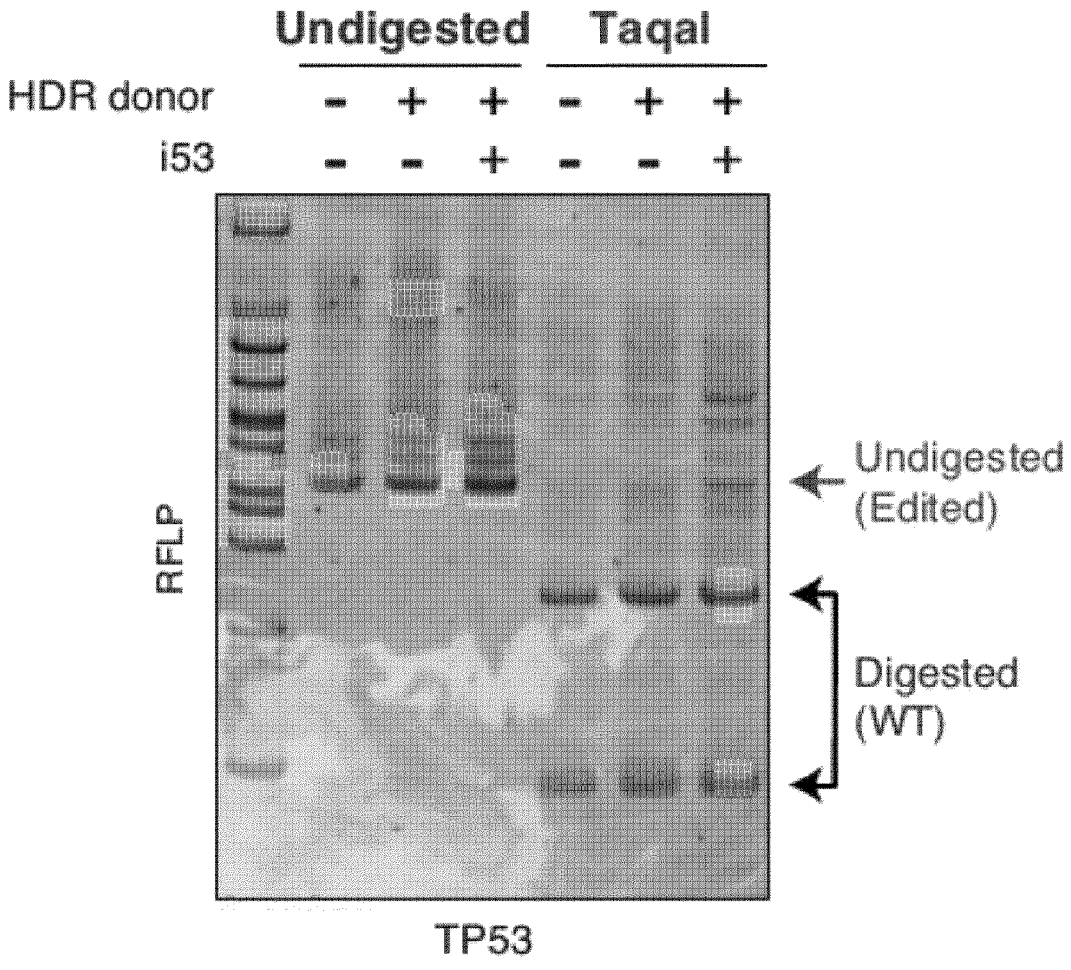

Undigested
TaqaI
HDR donor
- + + - + +
i53
- - + - - +
RFLP
Undigested
(Edited)
Digested
(WT)
TP53

Figure 11I

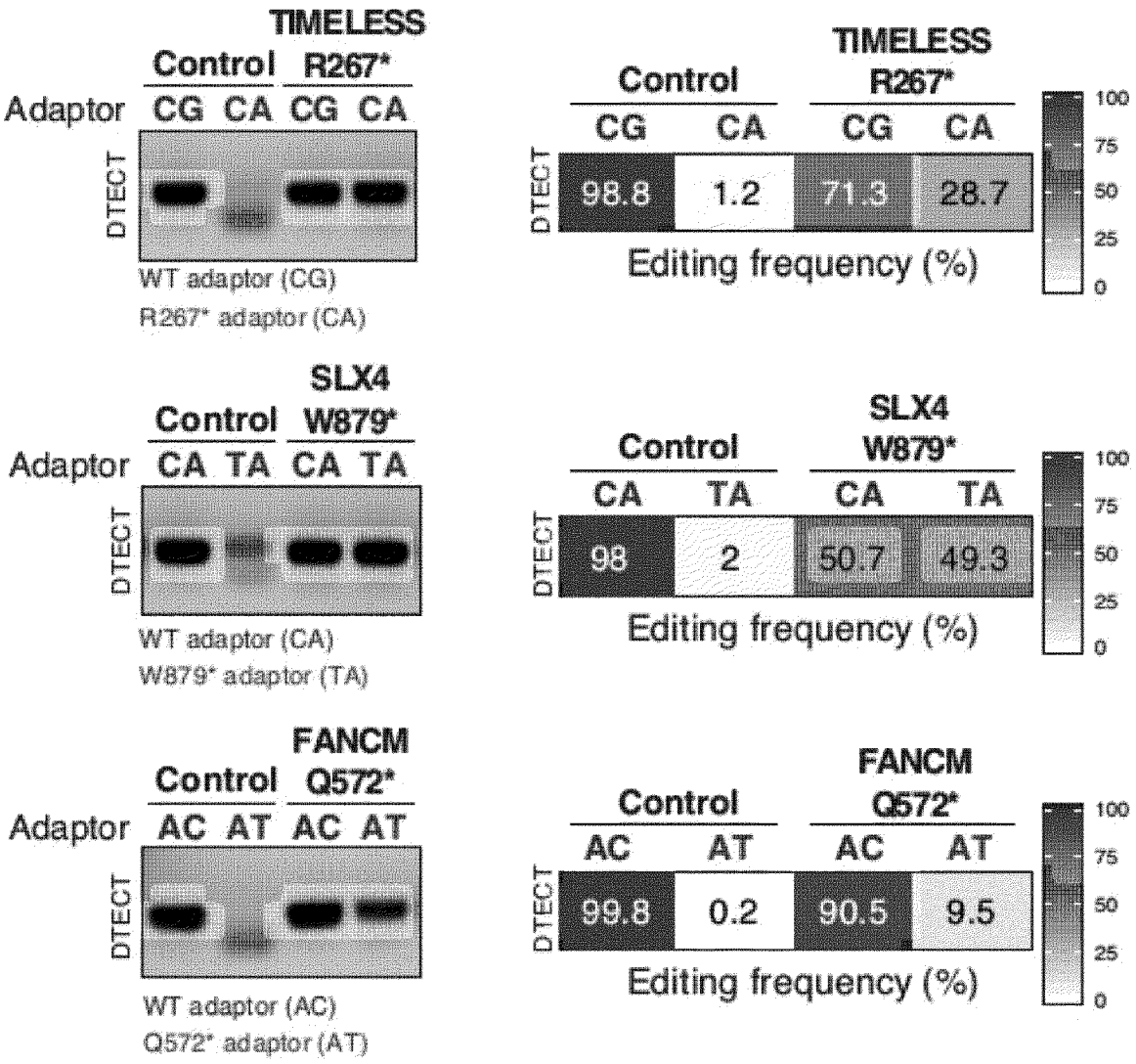

TIMELESS
Control
R267*
Adaptor
CG CA CG CA
DTECT
WT adaptor (CG)
R267* adaptor (CA)
TIMELESS
Control
R267*
CG CA CG CA
DTECT
98.8 1.2 71.3 28.7
Editing frequency (%)
100 75 50 25 0
SLX4
Control
W879*
Adaptor
CA TA CA TA
DTECT
WT adaptor (CA)
W879* adaptor (TA)
SLX4
Control
W879*
CA TA CA TA
DTECT
98 2 50.7 49.3
Editing frequency (%)
100 75 50 25 0
FANCM
Control
Q572*
Adaptor
AC AT AC AT
DTECT
WT adaptor (AC)
Q572* adaptor (AT)
FANCM
Control
Q572*
AC AT AC AT
DTECT
99.8 0.2 90.5 9.5
Editing frequency (%)
100 75 50 25 0

Figure 11J

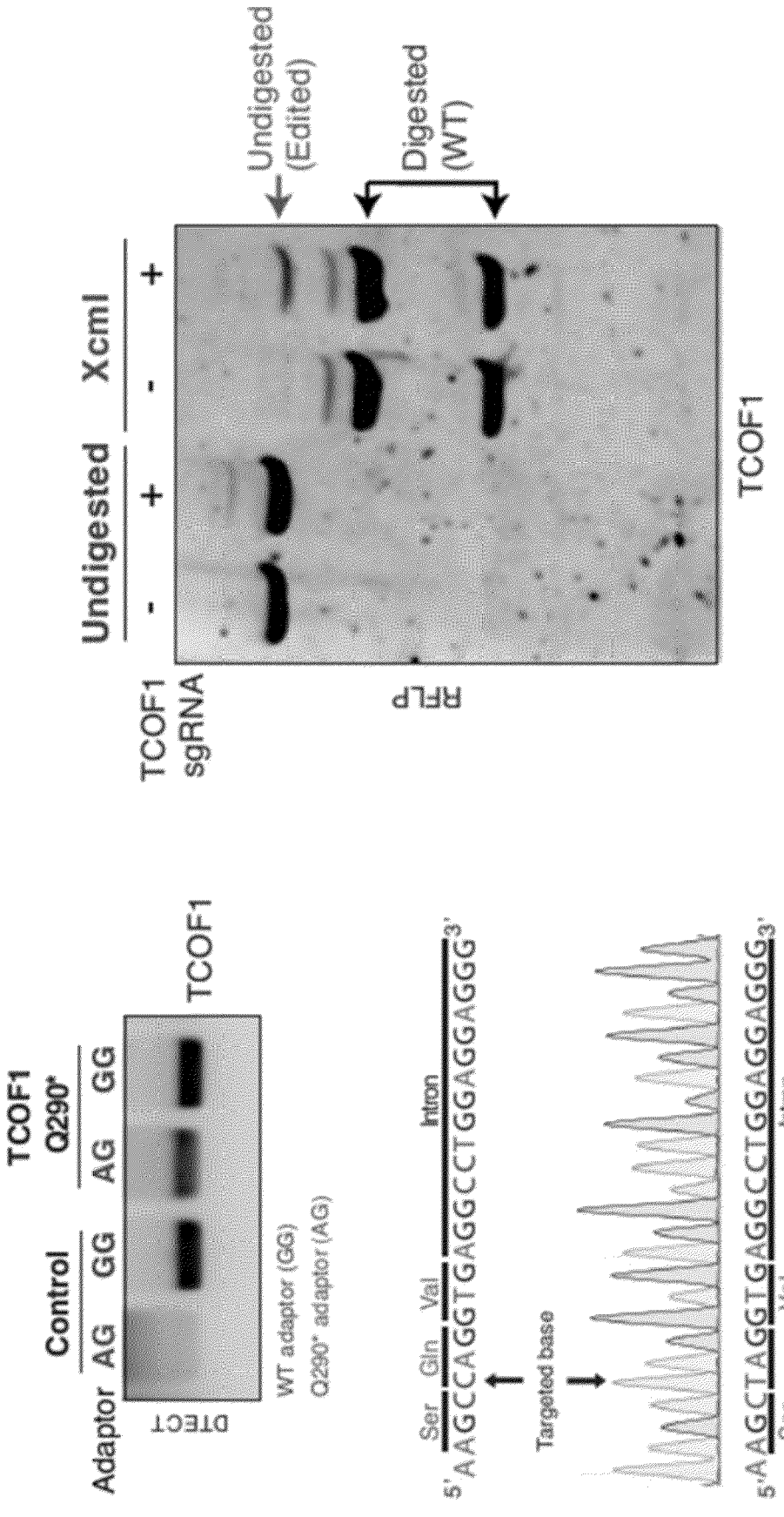

TCOF1
sgRNA
Undigested
-
+
XcmI
-
+
RFLP
Undigested
(Edited)
Digested
(WT)
TCOF1
TCOF1
Q290*
Control
Adaptor
AG
GG
AG
GG
DTECT
TCOF1
WT adaptor (GG)
Q290* adaptor (AG)
Ser
Gln
Val
Intron
5'AAGCCAGGTGAGGCCTGGAGGAGGG3'
Targeted base
5'AAGCTAGGTGAGGCCTGGAGGAGGG3'
Ser
*
Val
Intron

Figure 12A

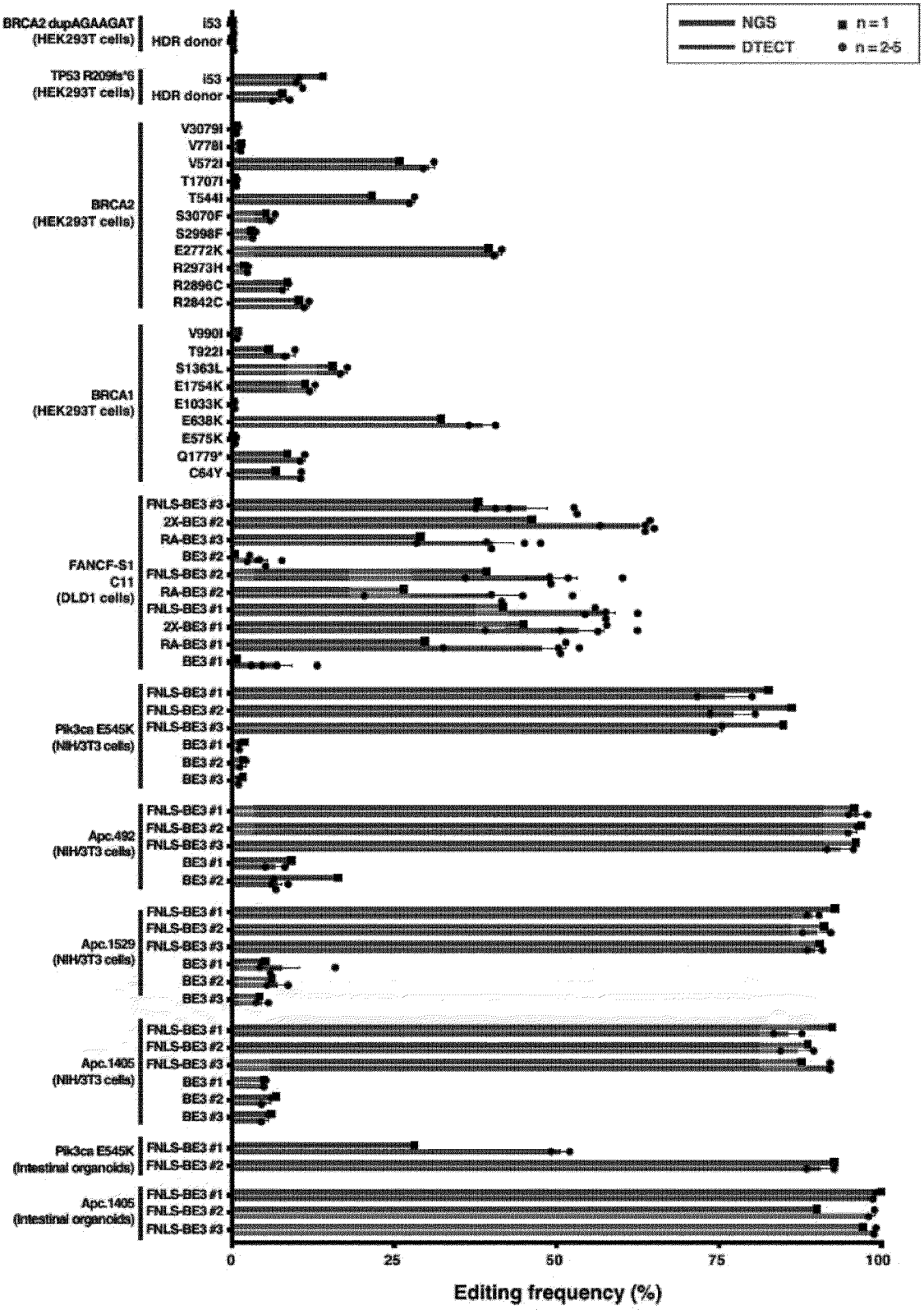

NGS
DTECT
n=1
n=2-5
BRCA2 dupAGAAGAT (HEK293T cells)
TP53 R209fs*6 (HEK293T cells)
BRCA2 (HEK293T cells)
BRCA1 (HEK293T cells)
FANCF-S1 C11 (DLD1 cells)
Pik3ca E545K (NIH/3T3 cells)
Apc.492 (NIH/3T3 cells)
Apc.1529 (NIH/3T3 cells)
Apc.1405 (NIH/3T3 cells)
Pik3ca E545K (Intestinal organoids)
Apc.1405 (Intestinal organoids)
i53
HDR donor
V3079I
V778I
V572I
T1707I
T544I
S3070F
S2998F
E2772K
R2973H
R2896C
R2842C
V990I
T922I
S1363L
E1754K
E1033K
E638K
E575K
Q1779*
C64Y
FNLS-BE3 #3
2X-BE3 #2
RA-BE3 #3
BE3 #2
FNLS-BE3 #2
RA-BE3 #2
FNLS-BE3 #1
2X-BE3 #1
RA-BE3 #1
BE3 #1
FNLS-BE3 #1
FNLS-BE3 #2
FNLS-BE3 #3
BE3 #1
BE3 #2
BE3 #3
0
25
50
75
100
Editing frequency (%)

Figure 12B

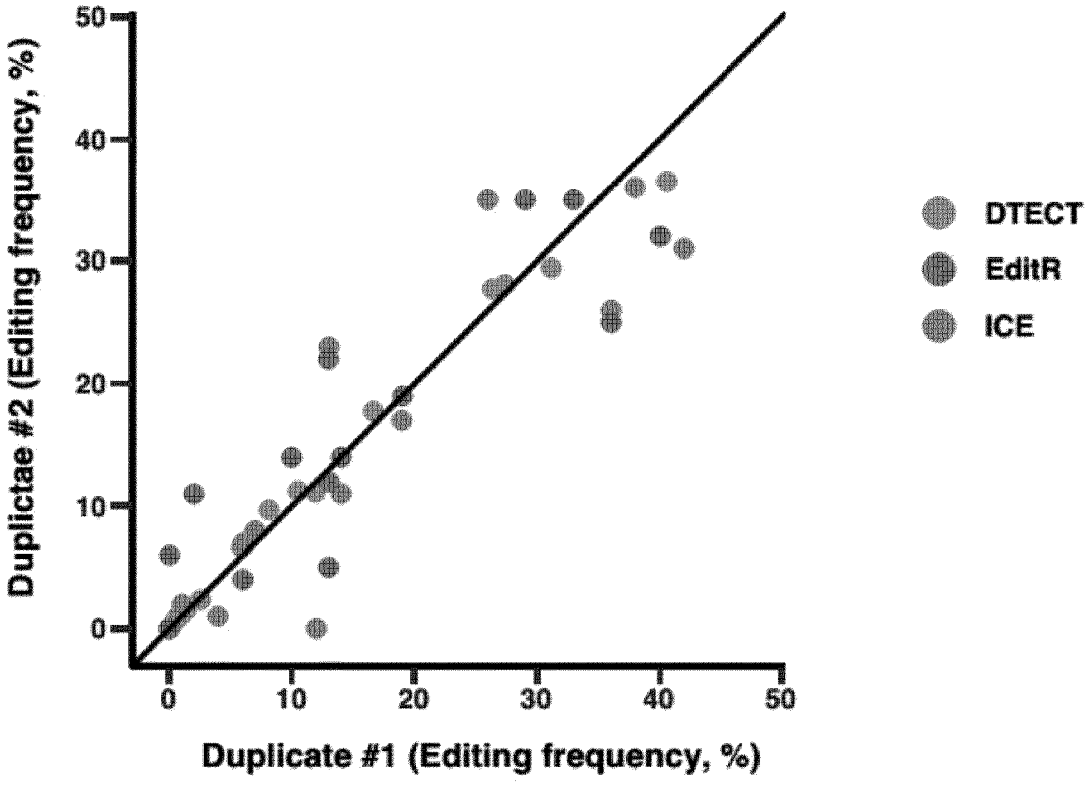
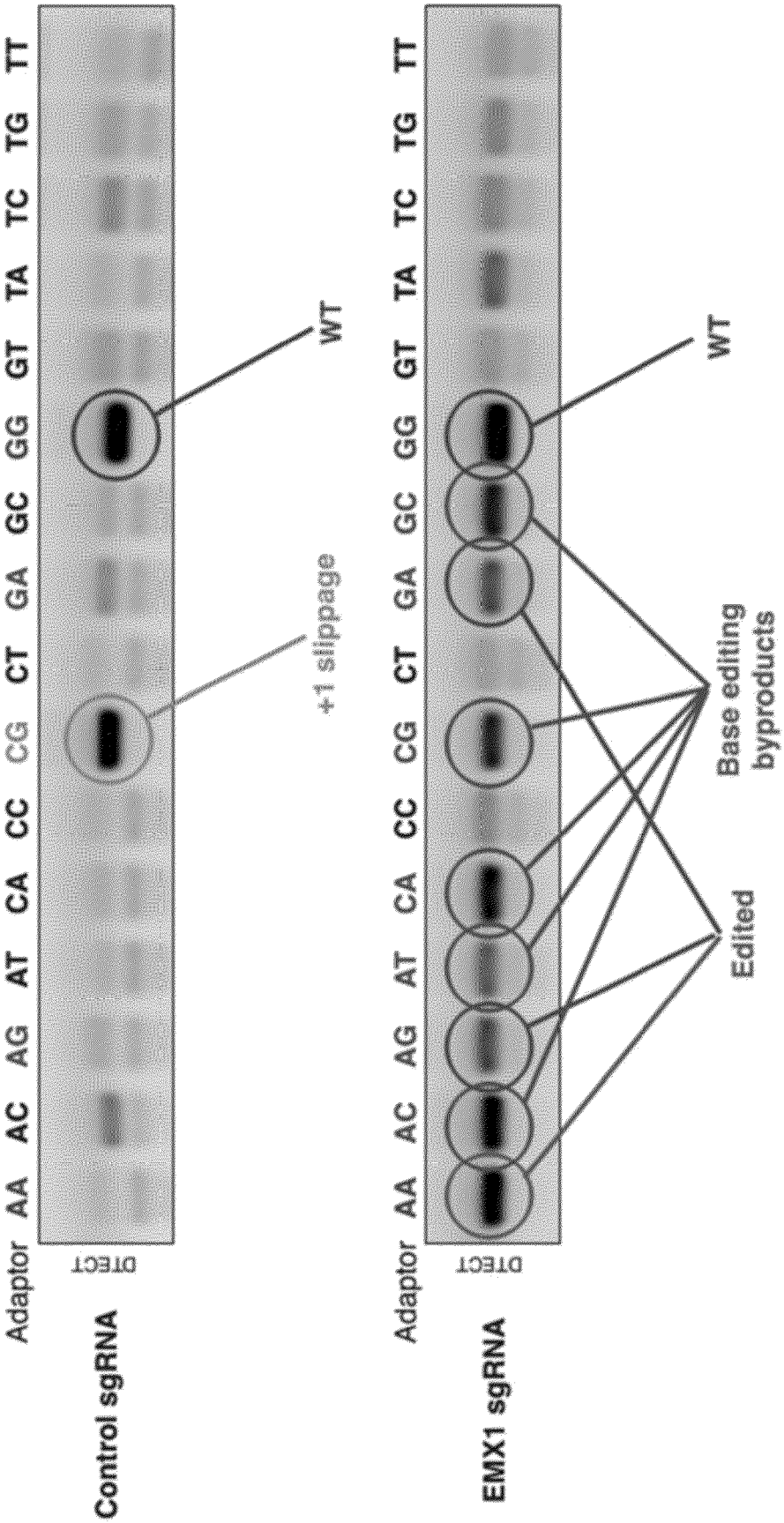
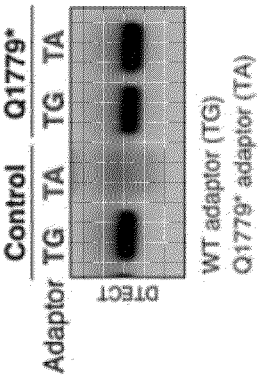
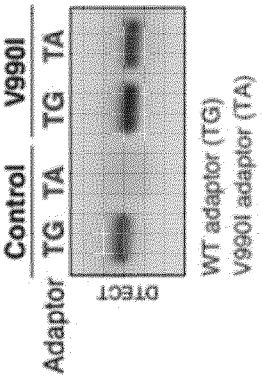
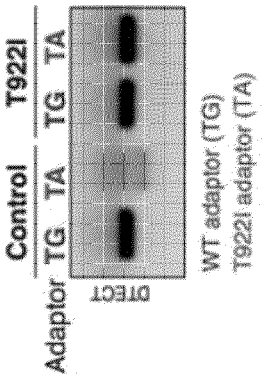

Duplictae #2 (Editing frequency, %)
Duplicate #1 (Editing frequency, %)
0
10
20
30
40
50
DTECT
EditR
ICE

Figure 13A


Adaptor
AA AC AG AT CA CC CG CT GA GC GG GT TA TC TG TT
DTECT
Control sgRNA
WT
+1 slippage
Adaptor
AA AC AG AT CA CC CG CT GA GC GG GT TA TC TG TT
DTECT
EMX1 sgRNA
WT
Base editing byproducts
Edited


Control Q1779*
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
Q1779* adaptor (TA)


Control V990I
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
V990I adaptor (TA)


Control T922I
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
T922I adaptor (TA)

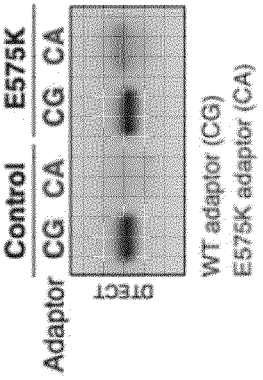

Control E575K
Adaptor CG CA CG CA
DTECT
WT adaptor (CG)
E575K adaptor (CA)

Figure 13B

Figure 13C

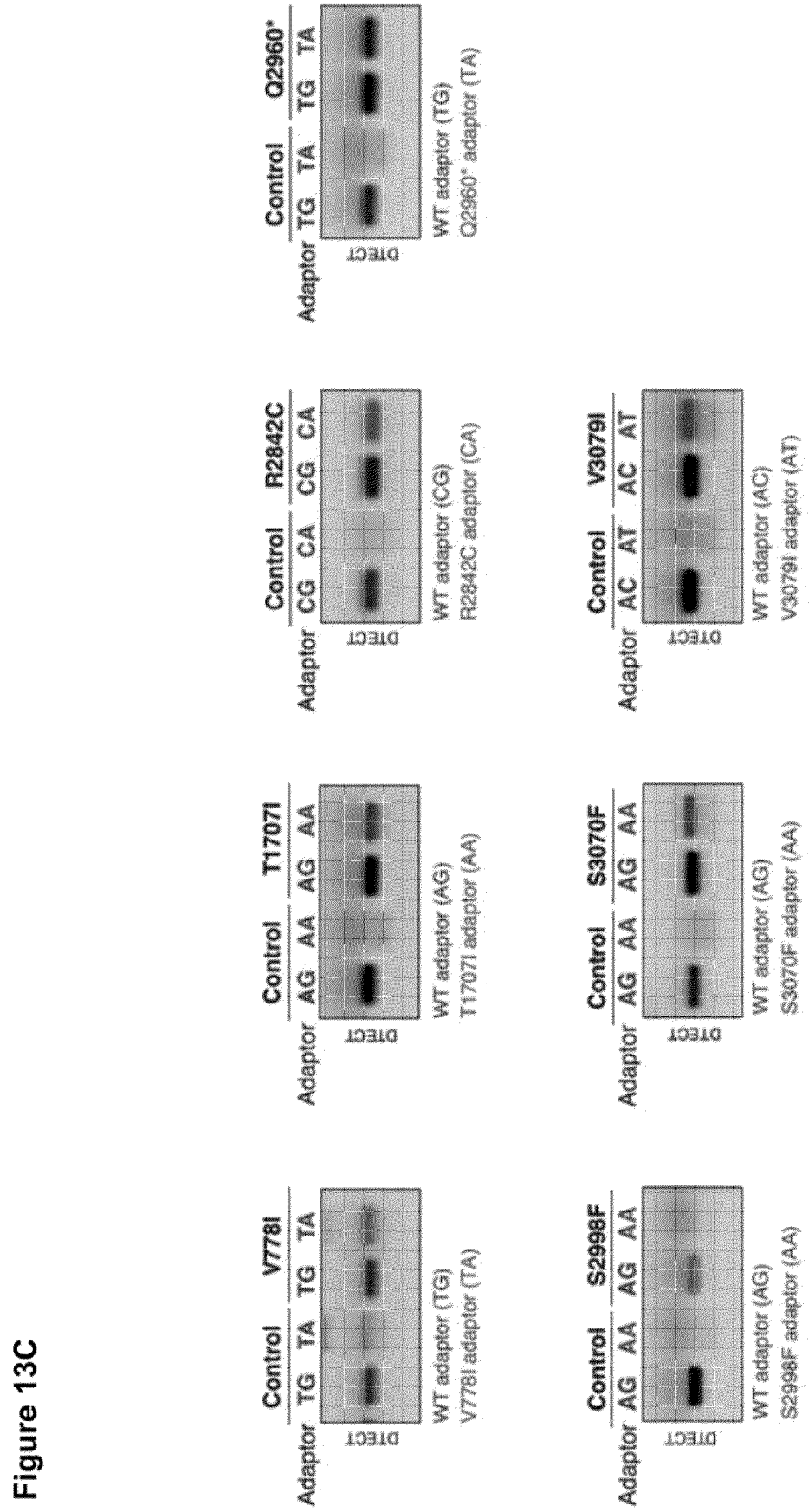

Control Q2960*
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
Q2960* adaptor (TA)
Control R2842C
Adaptor CG CA CG CA
DTECT
WT adaptor (CG)
R2842C adaptor (CA)
Control V3079I
Adaptor AC AT AC AT
DTECT
WT adaptor (AC)
V3079I adaptor (AT)
Control T1707I
Adaptor AG AA AG AA
DTECT
WT adaptor (AG)
T1707I adaptor (AA)
Control S3070F
Adaptor AG AA AG AA
DTECT
WT adaptor (AG)
S3070F adaptor (AA)
Control V778I
Adaptor TG TA TG TA
DTECT
WT adaptor (TG)
V778I adaptor (TA)
Control S2998F
Adaptor AG AA AG AA
DTECT
WT adaptor (AG)
S2998F adaptor (AA)

Figure 14A

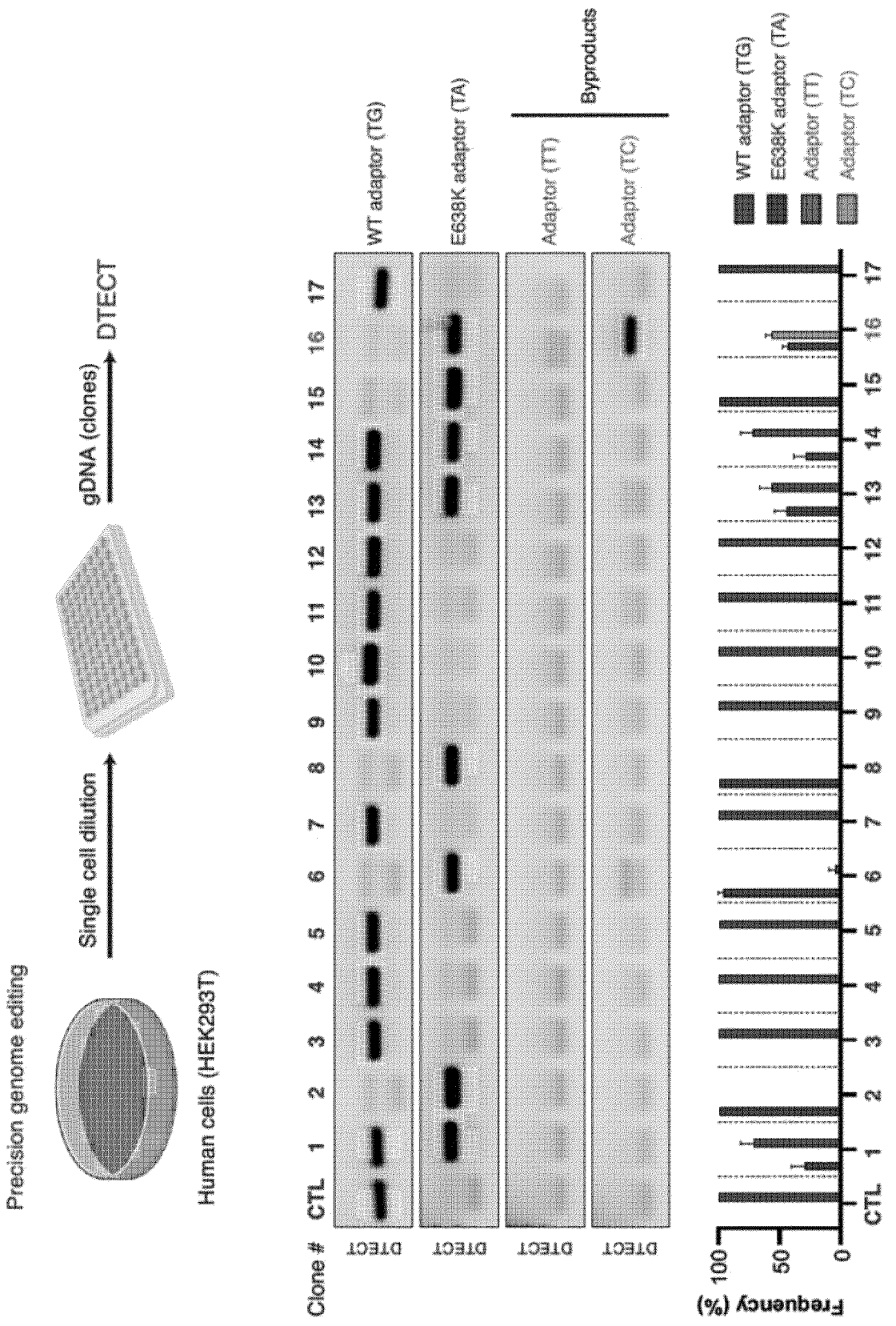

Precision genome editing
Human cells (HEK293T)
Single cell dilution
gDNA (clones)
DTECT
Clone # CTL 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
DTECT
WT adaptor (TG)
E638K adaptor (TA)
Byproducts
Adaptor (TT)
Adaptor (TC)
Frequency (%)
100
50
0

Figure 14B

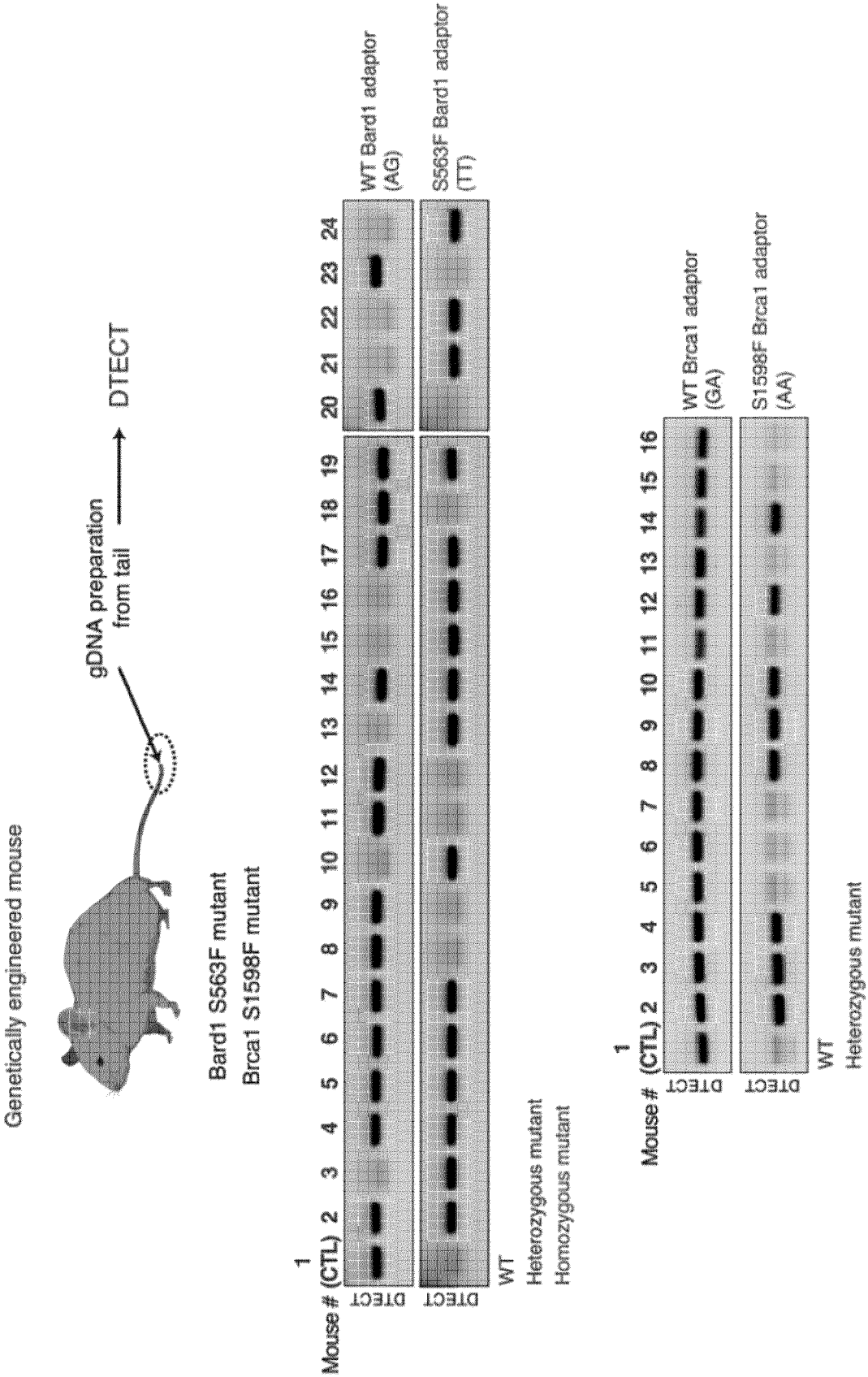

Genetically engineered mouse
gDNA preparation from tail
DTECT
Bard1 S563F mutant
Brca1 S1598F mutant
Mouse # 1 (CTL) 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
DTECT
DTECT
WT Bard1 adaptor (AG)
S563F Bard1 adaptor (TT)
WT
Heterozygous mutant
Homozygous mutant
Mouse # 1 (CTL) 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
DTECT
DTECT
WT Brca1 adaptor (GA)
S1598F Brca1 adaptor (AA)
WT
Heterozygous mutant

Figure 15A

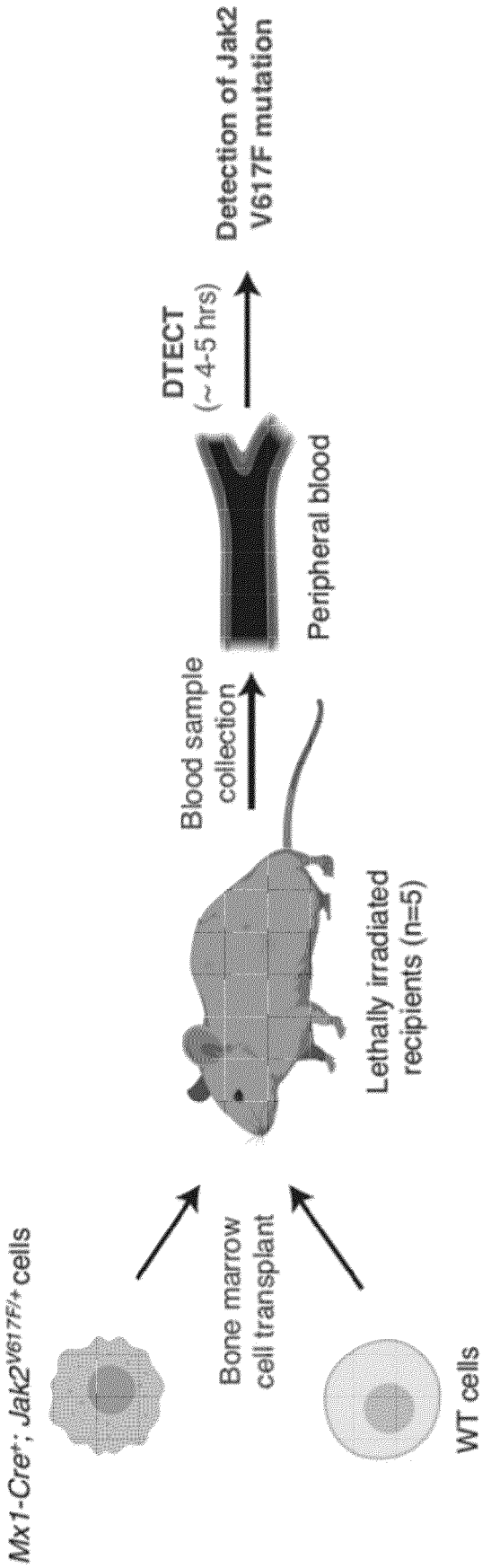

Mx1-Cre+; Jak2V617F/+ cells
WT cells
Bone marrow cell transplant
Lethally irradiated recipients (n=5)
Blood sample collection
Peripheral blood
DTECT (~ 4-5 hrs)
Detection of Jak2 V617F mutation

Figure 15B

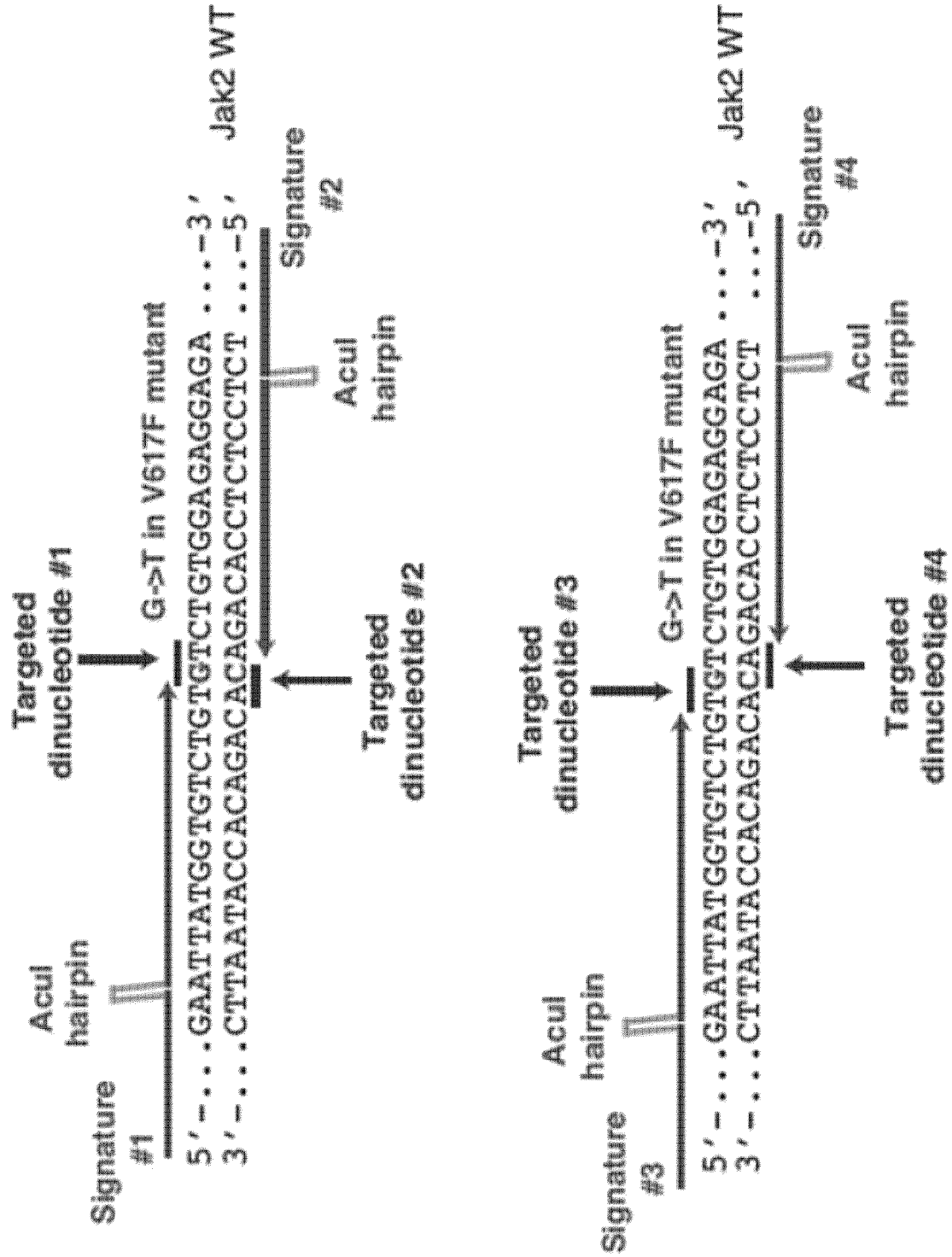
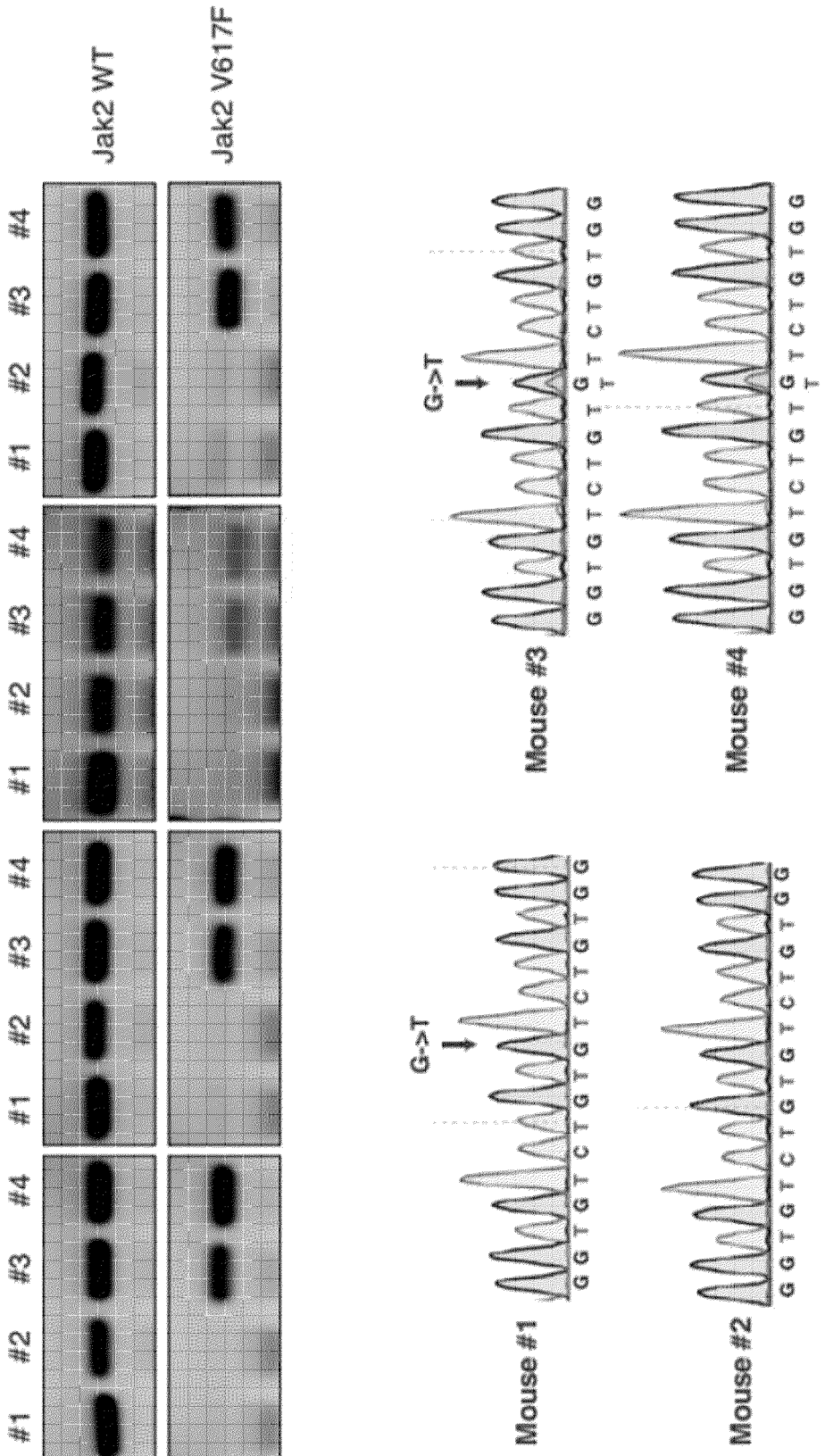

Signature #1
AcuI hairpin
Targeted dinucleotide #1
G->T in V617F mutant
Jak2 WT
Signature #2
AcuI hairpin
Targeted dinucleotide #2
Signature #3
AcuI hairpin
Targeted dinucleotide #3
G->T in V617F mutant
Jak2 WT
Signature #4
AcuI hairpin
Targeted dinucleotide #4

Figure 15C


Jak2 WT
Jak2 V617F
Signature #1
Signature #2
Signature #3
Signature #4
#1
#2
#3
#4
Mouse
DTECT
G->T
Mouse #1
Mouse #2
Mouse #3
Mouse #4

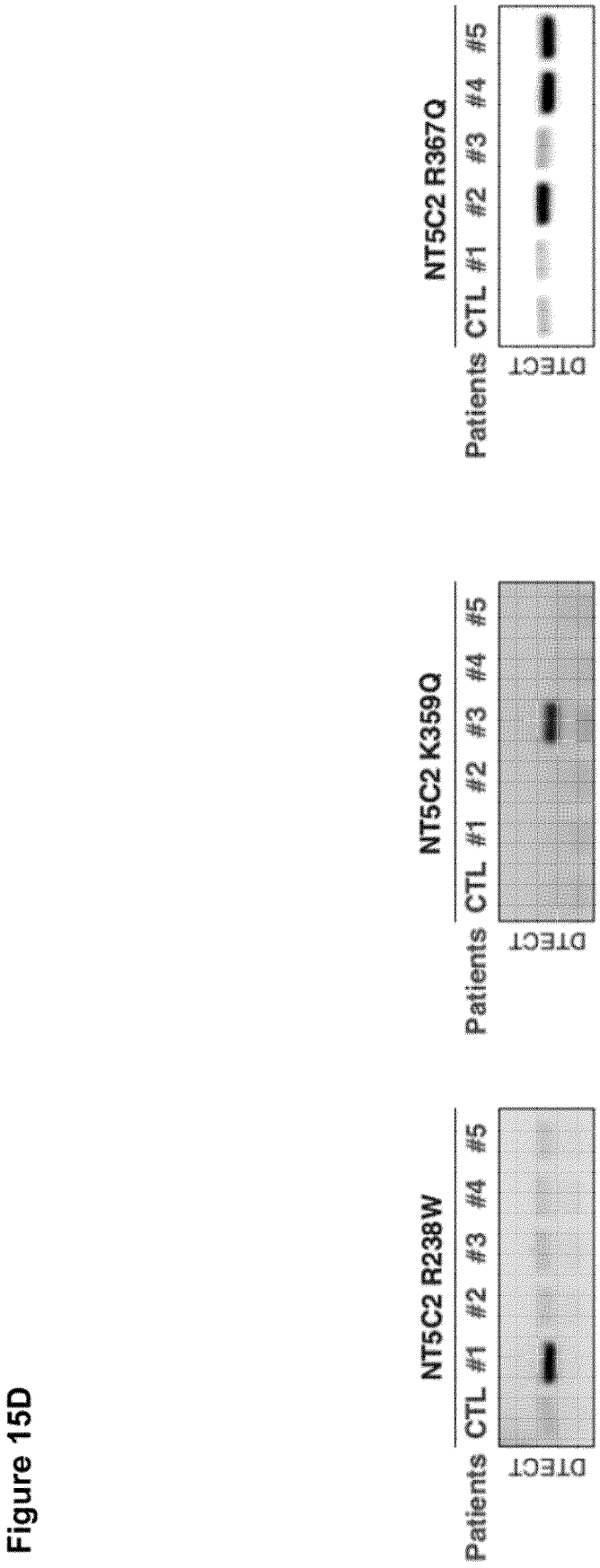

NT5C2 R238W
NT5C2 K359Q
NT5C2 R367Q
Patients
CTL
#1
#2
#3
#4
#5
DTECT

Figure 15D

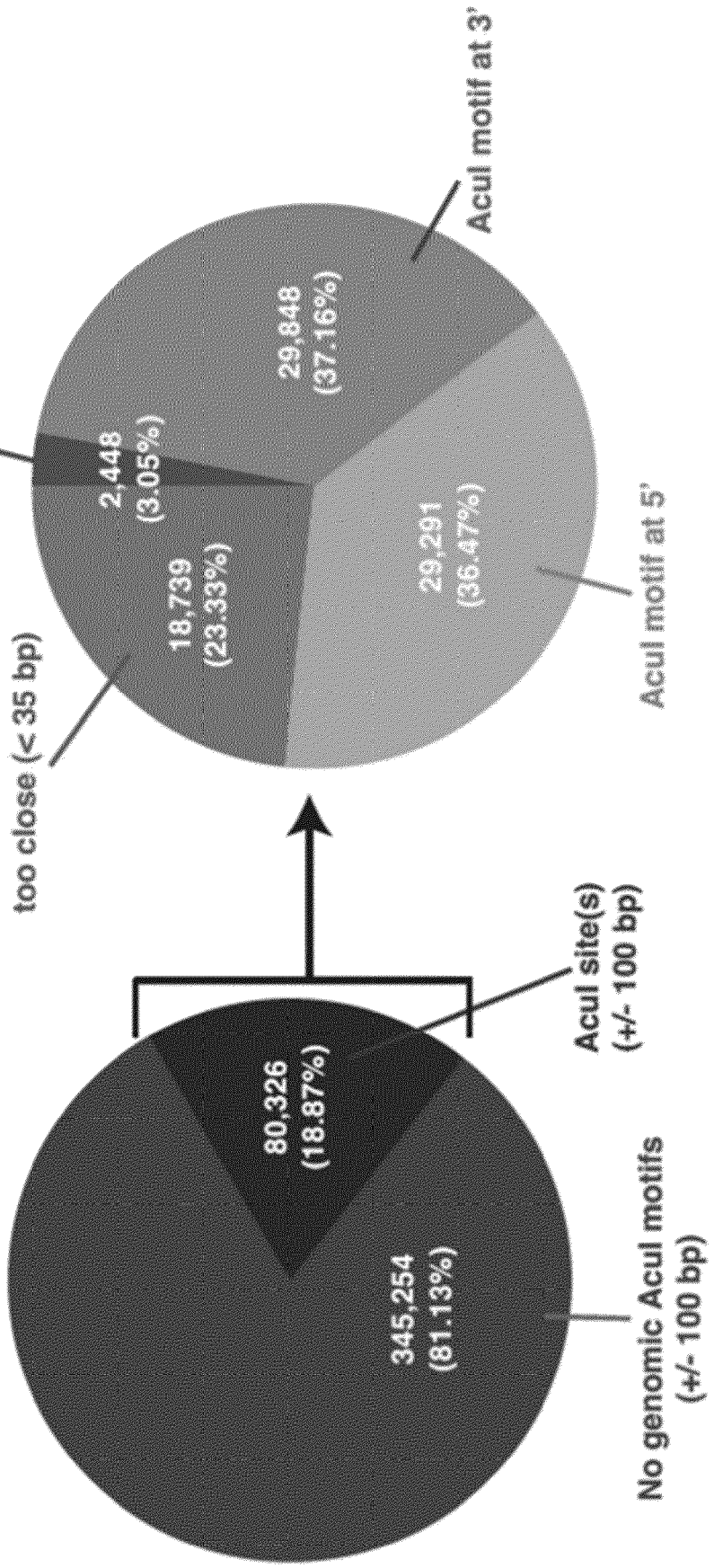

No genomic AcuI motifs
(+/- 100 bp)
345,254
(81.13%)
AcuI site(s)
(+/- 100 bp)
80,326
(18.87%)
AcuI motifs
at both 5' and 3'
2,448
(3.05%)
AcuI motif
too close (< 35 bp)
18,739
(23.33%)
AcuI motif at 3'
29,848
(37.16%)
AcuI motif at 5'
29,291
(36.47%)

Figure 16A

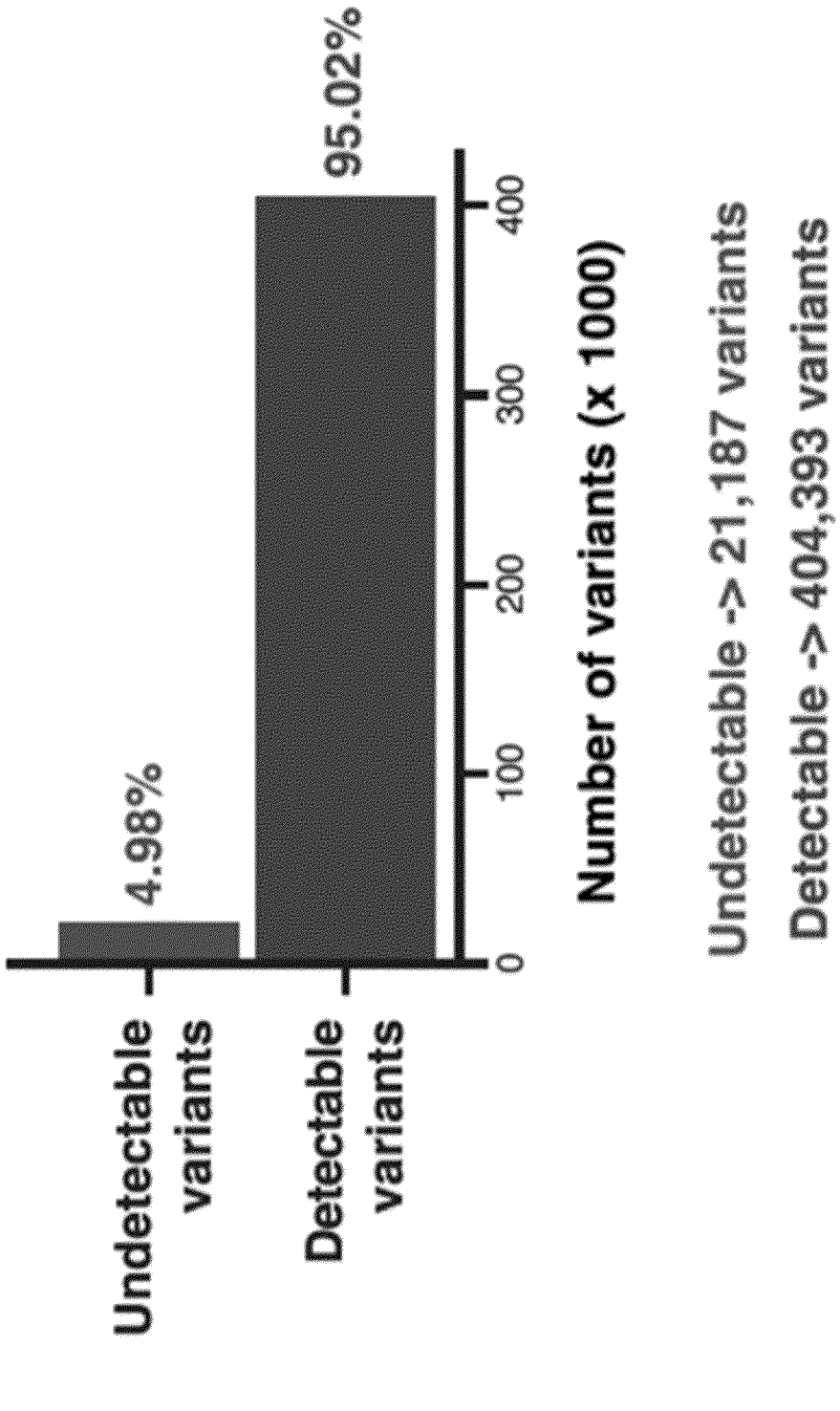

Undetectable variants
Detectable variants
4.98%
95.02%
0
100
200
300
400
Number of variants (x 1000)
Undetectable -> 21,187 variants
Detectable -> 404,393 variants

Figure 16B

Figure 16C

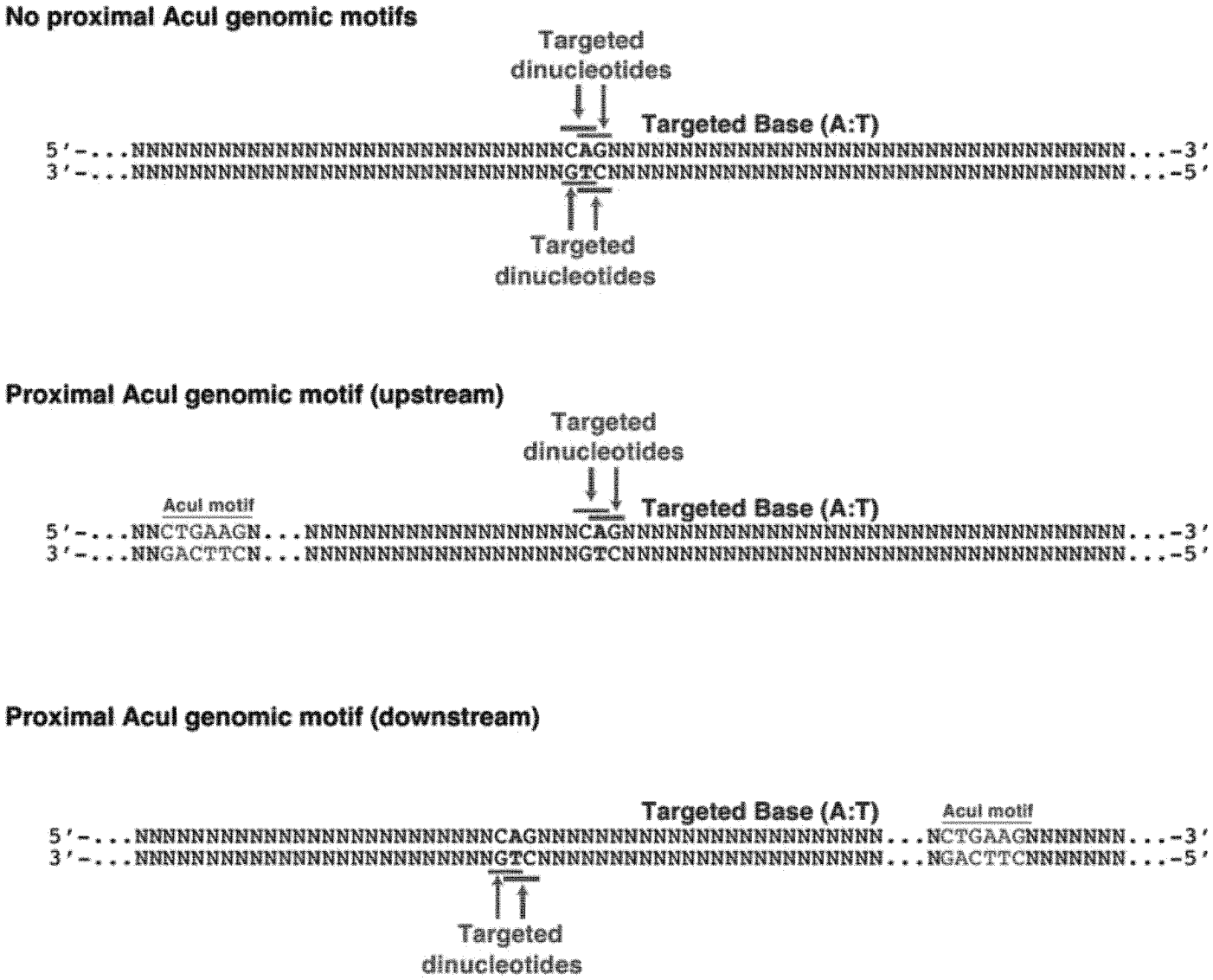
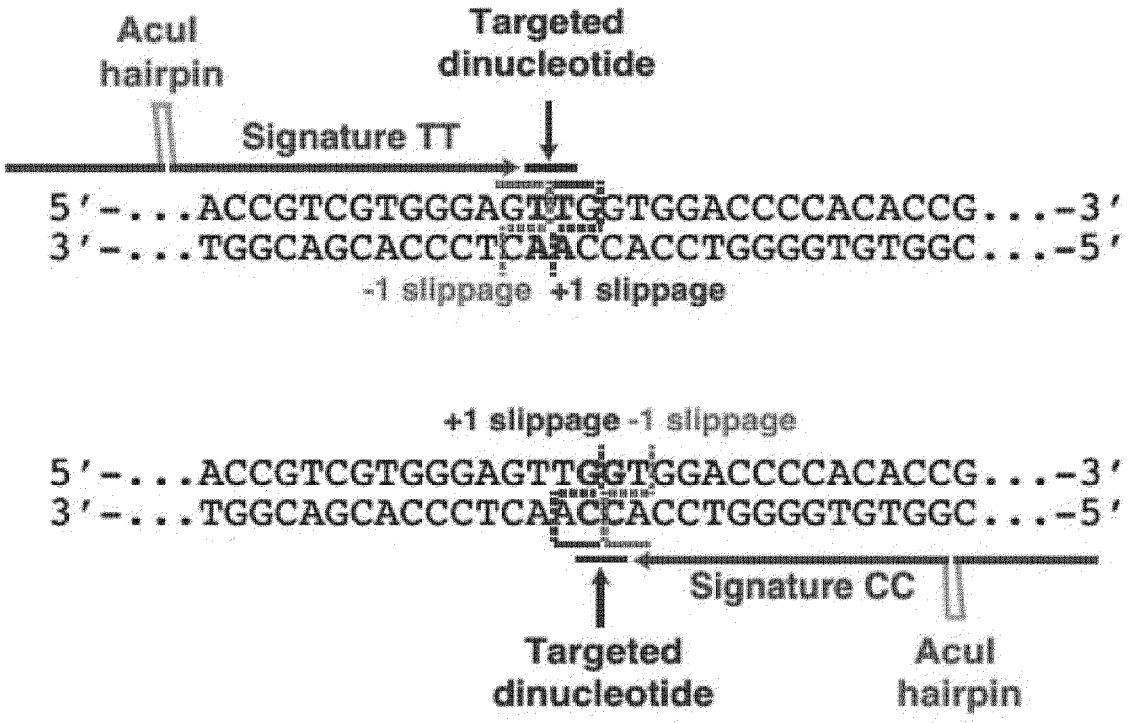

No proximal AcuI genomic motifs
Targeted dinucleotides
Targeted Base (A:T)
5′-...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...-5′
Targeted dinucleotides
Proximal AcuI genomic motif (upstream)
Targeted dinucleotides
AcuI motif
Targeted Base (A:T)
5′-...NNCTGAAGN...NNNNNNNNNNNNNNNNNNNNCAGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...-3′
3′-...NNGACTTCN...NNNNNNNNNNNNNNNNNNNNGTCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...-5′
Proximal AcuI genomic motif (downstream)
Targeted Base (A:T)
AcuI motif
5′-...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...NCTGAAGNNNNNNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...NGACTTCNNNNNNN...-5′
Targeted dinucleotides

Figure 17A


AcuI hairpin
Targeted dinucleotide
Signature TT
5′-...ACCGTCGTGGGAGTTGGTGGACCCCACACCG...-3′
3′-...TGGCAGCACCCTCAACCACCTGGGGTGTGGC...-5′
-1 slippage
+1 slippage
+1 slippage
-1 slippage
5′-...ACCGTCGTGGGAGTTGGTGGACCCCACACCG...-3′
3′-...TGGCAGCACCCTCAACCACCTGGGGTGTGGC...-5′
Signature CC
Targeted dinucleotide
AcuI hairpin

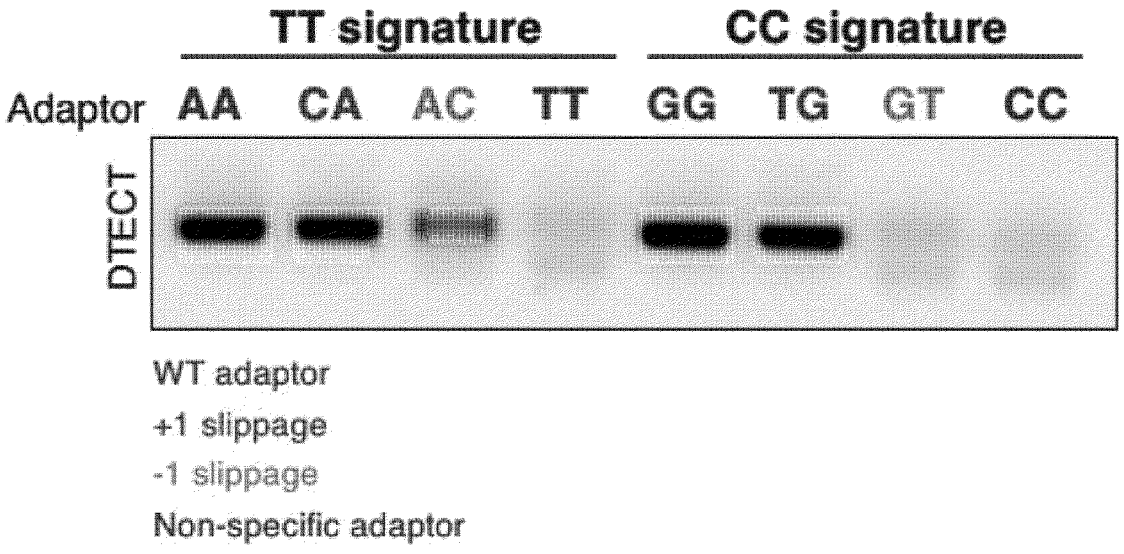

TT signature
CC signature
Adaptor
AA
CA
AC
TT
GG
TG
GT
CC
DTECT
WT adaptor
+1 slippage
-1 slippage
Non-specific adaptor

Figure 17B

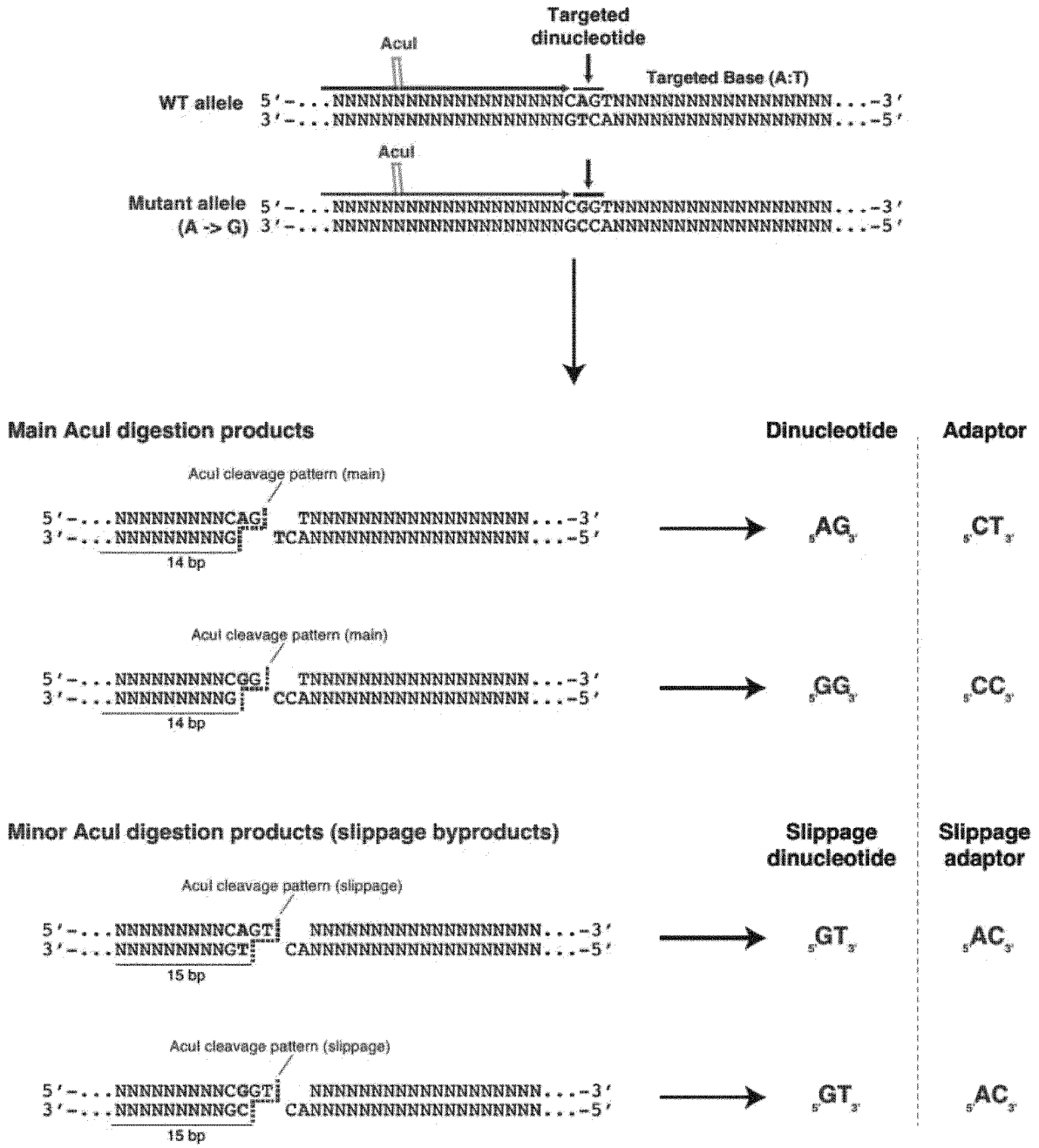

Targeted dinucleotide
AcuI
Targeted Base (A:T)
WT allele
5′-...NNNNNNNNNNNNNNNNNNNCAGTNNNNNNNNNNNNNNNNNNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNGTCANNNNNNNNNNNNNNNNNNN...-5′
AcuI
Mutant allele
(A -> G)
5′-...NNNNNNNNNNNNNNNNNNNCGGTNNNNNNNNNNNNNNNNNNN...-3′
3′-...NNNNNNNNNNNNNNNNNNNGCCANNNNNNNNNNNNNNNNNNN...-5′
Main AcuI digestion products
Dinucleotide
Adaptor
AcuI cleavage pattern (main)
5′-...NNNNNNNNNCAG
3′-...NNNNNNNNNG
TNNNNNNNNNNNNNNNNNNN...-3′
TCANNNNNNNNNNNNNNNNNN...-5′
14 bp
5′AG3′
3′CT5′
AcuI cleavage pattern (main)
5′-...NNNNNNNNNCGG
3′-...NNNNNNNNNG
TNNNNNNNNNNNNNNNNNNN...-3′
CCANNNNNNNNNNNNNNNNNN...-5′
14 bp
5′GG3′
3′CC5′
Minor AcuI digestion products (slippage byproducts)
Slippage dinucleotide
Slippage adaptor
AcuI cleavage pattern (slippage)
5′-...NNNNNNNNNCAGT
3′-...NNNNNNNNNGT
NNNNNNNNNNNNNNNNNNN...-3′
CANNNNNNNNNNNNNNNNNN...-5′
15 bp
5′GT3′
3′AC5′
AcuI cleavage pattern (slippage)
5′-...NNNNNNNNNCGGT
3′-...NNNNNNNNNGC
NNNNNNNNNNNNNNNNNNN...-3′
CANNNNNNNNNNNNNNNNNN...-5′
15 bp
5′GT3′
3′AC5′

Figure 18A

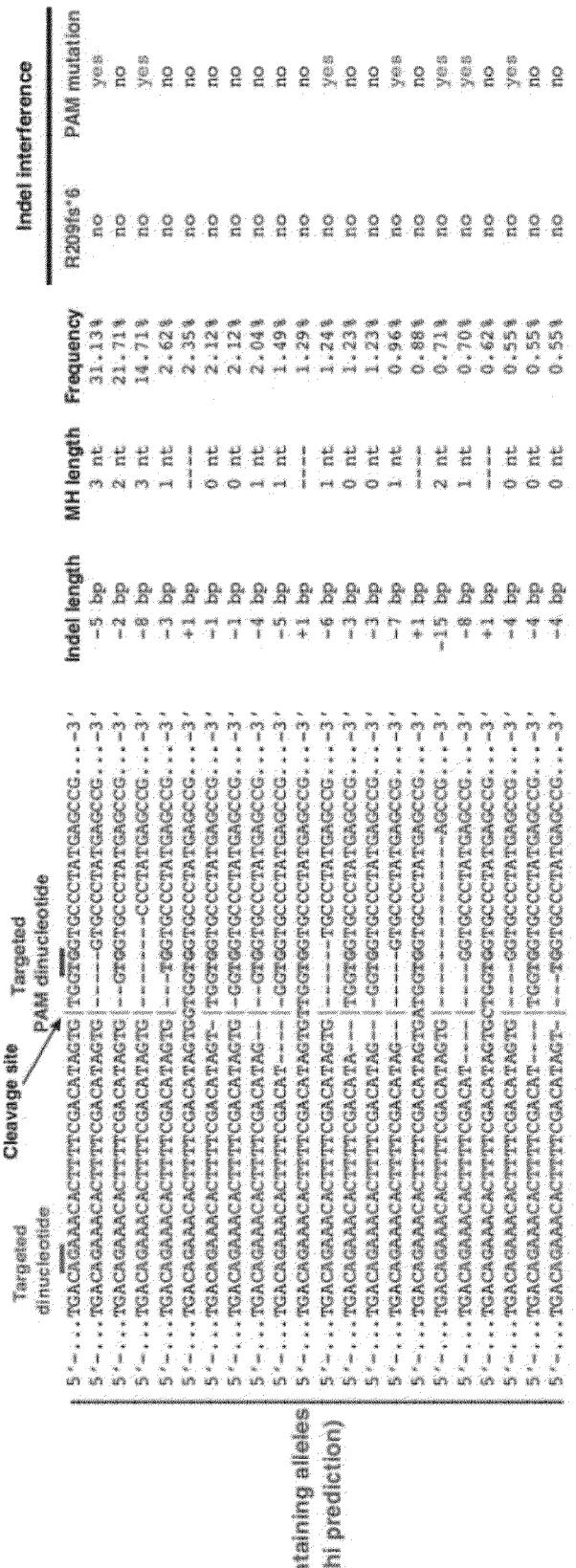
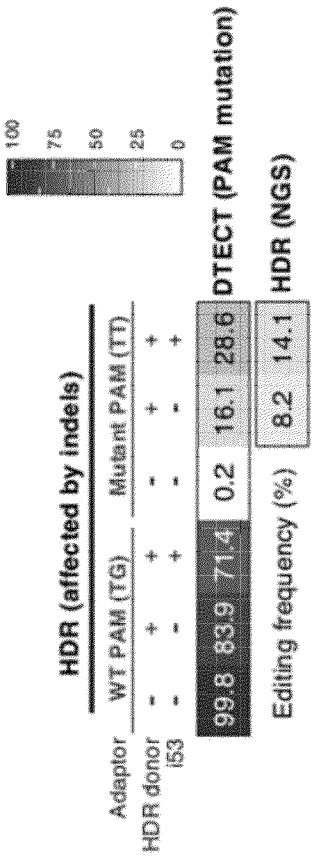

Indel interference
Indel-containing alleles (inDelphi prediction)
Targeted dinucleotide
Cleavage site
Targeted PAM dinucleotide
Indel length
MH length
Frequency
R209fs*6
PAM mutation
TP53 R209fs*6 (delAG)
PAM mutation
HDR-edited allele
R209fs*6 signature
Mutant PAM signature

Figure 18B


HDR (affected by indels)
Adaptor
WT PAM (TG)
Mutant PAM (TT)
HDR donor
i53
99.8 83.9 71.4 0.2 16.1 28.6
DTECT (PAM mutation)
Editing frequency (%)
8.2 14.1
HDR (NGS)
100
75
50
25
0

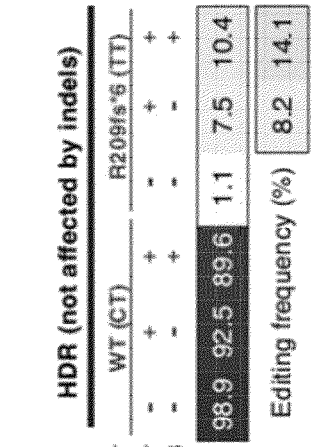

HDR (not affected by indels)
Adaptor
WT (CT)
R209fs*6 (TT)
HDR donor
i53
98.9 92.5 89.6 1.1 7.5 10.4
DTECT (R209fs*6)
Editing frequency (%)
8.2 14.1
HDR (NGS)

Figure 18C

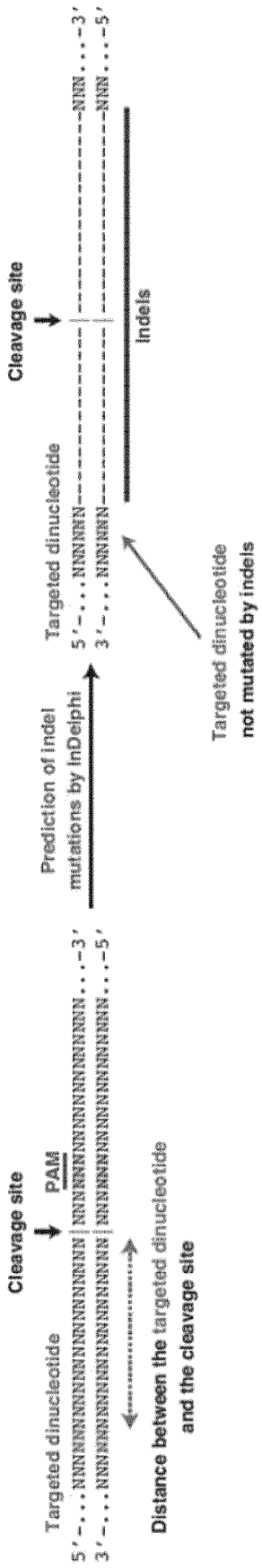
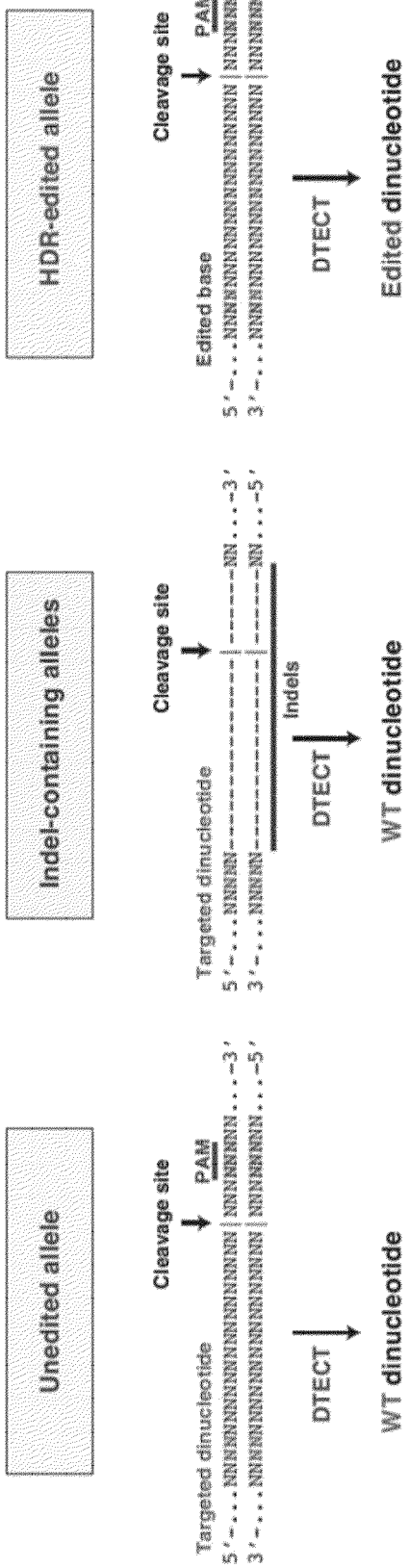

Cleavage site
PAM
Targeted dinucleotide
Distance between the targeted dinucleotide and the cleavage site
Prediction of indel mutations by inDelphi
Cleavage site
Targeted dinucleotide
indels
Targeted dinucleotide not mutated by indels

Figure 18D


HDR-edited allele
Cleavage site
PAM
Edited base
DTECT
Edited dinucleotide
Indel-containing alleles
Cleavage site
Targeted dinucleotide
Indels
DTECT
WT dinucleotide
Unedited allele
Cleavage site
PAM
Targeted dinucleotide
DTECT
WT dinucleotide

A VERSATILE METHOD FOR THE DETECTION OF MARKER-FREE PRECISION GENOME EDITING AND GENETIC VARIATION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit to and is a continuation of U.S. Non-provisional Pat. Application No. 17/192,836, filed Mar. 4, 2021, which claims benefit of U.S. Provisional Pat. Application Serial No. 62/985,746, filed on Mar. 5, 2020, which applications are incorporated by reference herein in their entireties.

FIELD OF DISCLOSURE

[0002] The present disclosure provides, inter alia, specially designed DNA adaptors and various methods and kits for carrying out and detecting marker-free precision genome editing and genetic variation using such adaptors.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] This application contains references to amino acids and/or nucleic acid sequences that have been filed as sequence listing text file "1035795-000704-seq.txt", file size of 63 KB, created on Apr. 8, 2021. The aforementioned sequence listing is hereby incorporated by reference in its entirety pursuant to 37 C.F.R. § 1.52(e)(5).

GOVERNMENT FUNDING

[0004] This invention was made with government support under grant no. GM117064, awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE DISCLOSURE

[0005] Precision genome editing allows the modeling and correction of desired genomic variants containing insertions or deletions of specific nucleotide sequences or changes in single DNA bases (Anzalone et al., 2019; Barbieri et al., 2017; Cong et al., 2013; Dow, 2015; Guo et al., 2018; Liu et al., 2018; Mali et al., 2013; Roy et al., 2018). Precision genome editing can be obtained by CRISPR-dependent homology-directed repair (HDR) of Cas9-induced DNA double-strand breaks (DSBs) (Jasin and Haber, 2016) or result from the use of alternative DSB-free methods, such as CRISPR-dependent base editing, which utilizes cytidine or adenosine deaminases fused to a nickase Cas9 (nCas9) mutant to generate base transitions (Gaudelli et al., 2017; Komor et al., 2016), and prime editing, which employs a reverse transcriptase-nCas9 fusion and a template prime editing guide RNA (pegRNA) to install into the genome a large variety of genomic changes, including transversions, transitions, small insertions and deletions (Anzalone et al., 2019).

[0006] Genome editing has been facilitated by the development of accessible and cost-effective methods for the detection of small insertions and deletions (indels) resulting from the repair of Cas9-induced DSBs, such as the T7E1 and Surveyor nuclease assays (Mashal et al., 1995; Qiu et al., 2004; Ran et al., 2013). However, since these methods do not determine the identity of DNA bases, they are ill-suited for the detection of genomic changes introduced by precision genome editing (Germini et al., 2018). Precision genome editing events can be detected by the addition of genomic markers by CRISPR-dependent HDR or prime editing, such as silent mutations that create or disrupt restriction sites, or selectable reporters encoding for antibiotic resistance or fluorescent proteins. However, the use of genomic markers entails an elaborate experimental design that is unique for each targeted site, thus complicating the insertion of the desired genetic modifications. In addition, genomic markers can cause unintended perturbations of coding or non-coding genomic elements. Moreover, marker-based detection methods are not compatible with CRISPR-dependent base editing strategies, which induce single DNA base changes (Rees and Liu, 2018). Alternatively, methods that employ Sanger sequencing or next-generation sequencing (NGS) enable the detection of precise genomic changes without the use of genomic markers (Brinkman et al., 2014; Pinello et al., 2016). However, Sanger sequencing-based approaches suffer from low sensitivity and precision due to variable quality of the sequencing reactions and background signals that often affect the sequencing reads (Brinkman et al., 2014; Brinkman et al., 2018). While NGS-based detection strategies are highly sensitive (Clement et al., 2019; Lindsay et al., 2016; Pinello et al., 2016), they remain expensive and time-consuming, which limits their value for the development of mutant cell lines and animal models and for applications that require a rapid turnaround time, such as the identification of pathogenic variants in certain clinical settings. Therefore, a simple, efficient, inexpensive and rapid method that enables quantitative detection of genetic variants in complex biological systems is needed. This disclosure is directed to meeting these and other needs.

SUMMARY OF THE DISCLOSURE

[0007] Genome editing technologies have transformed our ability to engineer desired genomic changes within living systems. However, detecting precise genomic modifications often requires sophisticated, expensive and time-consuming experimental approaches. The present disclosure provides DTECT (Dinucleotide signaTurE CapTure), a rapid and versatile detection method that relies on the capture of targeted dinucleotide signatures resulting from the digestion of genomic DNA amplicons by the type IIS restriction enzyme AcuI. DTECT enables the accurate quantification of marker-free precision genome editing events introduced by CRISPR-dependent homology-directed repair, base editing or prime editing in various biological systems, such as mammalian cell lines, organoids and tissues. Furthermore, DTECT allows the identification of oncogenic mutations in cancer mouse models, patient-derived xenografts and human cancer patient samples; it also allows the identification of genetic modifications incurred in various infectious diseases. Ultimately, DTECT enables the capture of signatures in nucleic acids from any organism including, e.g., viruses such as SARS-CoV-2. The ease, speed and cost efficiency by which DTECT identifies genomic signatures should facilitate the generation of marker-free cellular and animal models of human disease and expedite the detection of human pathogenic variants.

[0008] Accordingly, one embodiment of the present disclosure is a DNA adaptor comprising: (a) one strand with sequence of 5′-CTGGGGCACGGGTAAGAAG-CATTCTGTCTCTCTTCTAAGAATTCGAGCTCGG-TACC CG-3′ (SEQ ID NO: 230); and (b) one complementary strand with sequence of 5′-CGGGTACCGAGCTCGAATTCTTAGAAGAGAGACA-GAATGCTTCTTACCCGTGCCC CAGNN-3′ with "N" corresponding to A, T, G or C (SEQ ID NOs: 231-246).

[0009] Another embodiment of the present disclosure is a method of preparing a DNA adaptor disclosed herein, comprising: (a) synthesizing one constant oligonucleotide with sequence of 5′-CTGGGGCACGGGTAAGAAG-CATTCTGTCTCTCTTCTAAGAATTCGAGCTCGG-TACC CG-3′ (SEQ ID NO: 230); (b) synthesizing one complementary oligonucleotide with sequence of 5′-CGGGTACCGAGCTCGAATTCTTAGAAGAGAGACA-GAATGCTTCTTACCCGTGCCC CAGNN-3′ with "N" corresponding to A, T, G or C (SEQ ID NOs: 231-246); (c) mixing the constant and complementary oligonucleotides; and (d) annealing the mixture to obtain the DNA adaptor.

[0010] Another embodiment of the present disclosure is a library of DNA adaptors prepared by methods disclosed herein, the library comprises 16 DNA adaptors, wherein each DNA adaptor has a different "NN".

[0011] Another embodiment of the present disclosure is a method for detecting a genetic modification, comprising the steps of: (a) amplifying a genomic locus of interest using a specially designed Type IIS restriction enzyme-tagging primer, comprising: (i) extracting genomic DNA from a biological sample of interest; (ii) synthesizing the Type IIS restriction enzyme-tagging primer based on the genomic locus of interest; (iii) amplifying the genomic locus of interest using the Type IIS restriction enzyme-tagging primer and a reverse primer; and (iv) purifying a Type IIS restriction enzyme-tagged genomic amplicon; (b) digesting the Type IIS restriction enzyme-tagged genomic amplicon with the Type IIS restriction enzyme; (c) isolating the smaller DNA fragment containing a genomic signature of interest exposed in a 3′ single-stranded overhang; (d) capturing the genomic signature of interest, comprising: (i) preparing the library of DNA adaptors disclosed herein; (ii) incubating the isolated smaller DNA fragment containing the 3′ overhang signature with the library of DNA adaptors and performing a ligation; and (iii) obtaining a ligated product; and (e) amplifying the ligated product to detect the presence of the genetic modification.

[0012] A further embodiment of the present disclosure is a kit for detecting a genetic modification of interest, comprising a specially designed Type IIS restriction enzyme-tagging primer disclosed herein, and a library of DNA adaptors disclosed herein, packaged together with instructions for its use.

[0013] Another embodiment of the present disclosure is a method for detecting a genetic modification, comprising the steps of: (a) amplifying a genomic locus of interest using a specially designed AcuI-tagging primer, comprising: (i) extracting DNA of interest; (ii) synthesizing the AcuI-tagging primer based on the genomic locus of interest; (iii) amplifying the genomic locus of interest using the AcuI-tagging primer and a reverse primer; and (iv) purifying an AcuI-tagged genomic amplicon; (b) digesting the AcuI-tagged genomic amplicon with restriction enzyme AcuI; (c) isolating the smaller DNA fragment containing a genomic signature of interest produced by AcuI-digestion; (d) capturing the genomic signature of interest, comprising: (i) preparing the library of DNA adaptors disclosed herein; (ii) incubating the isolated smaller DNA fragment with the library of DNA adaptors and performing a ligation; and (iii) obtaining a ligated product; and (e) amplifying the ligated product to detect the presence of the genetic modification.

[0014] An additional embodiment of the present disclosure is a kit for detecting a genetic modification, comprising a specially designed AcuI-tagging primer and a library of DNA adaptors disclosed herein, packaged together with instructions for its use.

[0015] Another embodiment of the present disclosure is a method for quantifying a genomic variant in a biological system, comprising the steps of: (a) obtaining a sample from the biological system; (b) amplifying a genomic locus of interest using a specially designed AcuI-tagging primer, comprising: (i) extracting DNA of interest; (ii) synthesizing the AcuI-tagging primer based on the genomic locus of interest; (iii) amplifying the genomic locus of interest using the AcuI-tagging primer and a reverse primer; and (iv) purifying an AcuI-tagged genomic amplicon; (c) digesting the AcuI-tagged genomic amplicon with restriction enzyme AcuI; (d) isolating the smaller DNA fragment containing a genomic signature of interest produced by the AcuI-digestion; (e) capturing the genomic signature of interest, comprising: (i) preparing the library of DNA adaptors disclosed herein; (ii) incubating the isolated smaller DNA fragment with the library of DNA adaptors and performing a ligation; and (iii) obtaining a ligated product; and (f) quantifying the genomic variant and determining its relative abundance.

[0016] Still another embodiment of the present disclosure is a method for identifying and quantifying an oncogenic mutation of interest in a biological sample, comprising the steps of: (a) obtaining a biological sample; (b) amplifying a genomic locus of interest using a specially designed AcuI-tagging primer, comprising: (i) extracting DNA of interest; (ii) synthesizing the AcuI-tagging primer based on the genomic locus of interest; (iii) amplifying the genomic locus of interest using the AcuI-tagging primer and a reverse primer; and (iv) purifying an AcuI-tagged genomic amplicon; (c) digesting the AcuI-tagged genomic amplicon with restriction enzyme AcuI; (d) isolating the smaller DNA fragment containing a genomic signature of interest produced by the AcuI-digestion; (e) capturing the genomic signature of interest, comprising: (i) preparing the library of DNA adaptors disclosed herein; (ii) incubating the isolated smaller DNA fragment with the library of DNA adaptors and performing a ligation; and (iii) obtaining a ligated product; (f) amplifying the ligated product to identify the presence of the oncogenic mutation of interest; and (g) quantifying the oncogenic mutation of interest, if present, and determining its frequency.

[0017] A further embodiment of the present disclosure is a process for marker-free detection of a precision genome editing event comprising carrying out Dinucleotide signaTurE CapTure (DTECT) on a nucleic acid sequence of interest.

[0018] Still another embodiment of the present disclosure is a method for detecting a virus variant of interest, comprising the steps of: (a) obtaining a nucleic acid of the virus

variant of interest from a biological sample; and (b) if the nucleic acid is DNA, carrying out Dinucleotide signaTurE CapTure (DTECT) to detect the variant of interest; or (c) if the nucleic acid is RNA, coverting it to DNA by reverse transcription PCR (RT-PCR) and then carrying out DTECT to detect the variant of interest.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIGS. **1**A-**1**C show the identification of targeted dinucleotide signatures using DTECT.

[0020] FIG. **1**A is a schematic representation of DTECT. The targeted genomic locus containing a hypothetical targeted dinucleotide (N= A, C, G or T; green) is PCR-amplified using a forward AcuI-tagging primer juxtaposed to the targeted dinucleotide and a locus-specific DNA primer (AcuI-tagging primer design and PCR, steps I and II). The AcuI-tagging primer (60 nt) is constituted of DNA sequences complementary to the genomic locus (purple) interrupted by a hairpin containing an AcuI recognition site (green), and a non-complementary DNA sequence (blue). The locus-specific reverse primer (red) is located at a distance >100 bp from the targeted dinucleotide. The obtained PCR product is subsequently cleaved by the AcuI restriction enzyme in a position adjacent to the targeted dinucleotide, resulting in the generation of two DNA fragments of 60 bp and >100 bp (AcuI digestion, step III). The 60 bp fragment containing the exposed signature of the targeted dinucleotide is then isolated using SPRI beads with higher affinity towards >100 bp DNA products (Small fragment isolation, step IV). The 60 bp fragment is then ligated to DNA adaptors containing 3′-overhangs of two bases complementary (specific) or not (non-specific) to the dinucleotide signature (Adaptor ligation, step V). The ligated product is then subjected to PCR amplification for analytical or quantitative detection (Detection PCR, step VI). The approximate time required for each step is indicated.

[0021] FIG. **1**B shows the schematics of the DTECT adaptor library. Control (green) and mutant (purple) dinucleotide signatures (left panel) are detected using a library of 16 unique adaptors (middle panel). The library contains adaptors with dinucleotides complementary to the control (green) or mutant (purple) signature, as well as non-specific adaptors (blue) (right panel).

[0022] FIG. **1**C shows the schematics of the positive and negative controls used in DTECT experiments to identify signatures of interest (e.g., mutant allele) in allele populations. In genomic DNA samples containing only the WT dinucleotide signature, the adaptor complementary to the WT dinucleotide signature (green) serves as a positive control, while the adaptor complementary to the mutant signature of interest (purple) and a non-specific adaptor (blue) are used as negative controls. In genomic DNA samples containing a mixture of the WT and the mutant dinucleotide signature, the adaptor complementary to the WT dinucleotide signature (green) is used as a positive control and a non-specific adaptor (blue) serves as a negative control. The adaptor complementary to the mutant dinucleotide signature (purple) is used to detect the presence of the variant of interest and quantify its frequency.

[0023] FIGS. **2**A-**2**K show the detection and quantification of dinucleotide signatures using DTECT.

[0024] FIG. **2**A shows the design of AcuI-tagging primers that allow the capture of two dinucleotide signatures (CC and TT; blue) on opposite DNA strands.

[0025] FIG. **2**B shows the PCR amplification (22 cycles) of the AcuI-digested DNA products containing the CC and TT signatures shown in FIG. **2**A, which have been captured using GG or AA adaptors.

[0026] FIG. **2**C shows the PCR amplification (22 cycles) of DNA fragments captured as in FIG. **2**B with or without dephosphorylation of the AcuI-digested products by the shrimp alkaline phosphatase (rSAP).

[0027] FIG. **2**D shows the PCR amplification (22 cycles) of DNA fragments captured as in FIG. **2**B in the absence or presence of AcuI, DNA adaptors (GG adaptor for signature CC; AA adaptor for signature TT) or T4 DNA ligase.

[0028] FIG. **2**E shows the schematic representation of the AcuI-tagging primer design for detecting four possible dinucleotide signatures (#1-4) containing the same targeted base (C:G, red) in the PIK3R1 gene.

[0029] FIG. **2**F shows the detection of the four dinucleotide signatures shown in FIG. **2**E by DTECT (18 PCR cycles) using specific (green) and non-specific (blue) adaptors.

[0030] FIG. **2**G shows the quantification by DTECT of the relative abundance of SMARCAL1, SPRTN and PIK3R1 WT (green) and STOP (purple) dinucleotide signatures in mixtures of WT and STOP alleles at predefined ratios. Graphs (left) represent the correlation between the frequency of WT and STOP variants determined by DTECT and the expected frequency of the same variants in the mixed populations for each of the above 3 genes. Error bars represent the s.d. of independent experiments (n = 2). Pearson correlation (r) was determined by comparing expected and DTECT-based frequency. Comparison of the mean frequency of STOP and WT signatures determined by DTECT and their expected frequency is shown in the right panel (n = 3 independent genes, SMARCAL1, SPRTN and PIK3R1).

[0031] FIG. **2**H shows the representation of the AcuI-tagging primers used to detect the WT and STOP alleles of the PIK3R1 gene. The targeted dinucleotides are shown in blue, the edited base is indicated with an asterisk and part of the AcuI-tagging primer sequence is shown in purple.

[0032] FIG. **2**I shows the PCR amplification (25 cycles) of WT and STOP PIK3R1 alleles (arrow) captured using DTECT from WT:STOP allele mixtures (i.e., 100:0 and 99:1). An adaptor (CG) specific for the WT allele is used as a positive control and a non-specific adaptor (TT) is used as a negative control. An adaptor that captures the STOP PIK3R1 allele (CA) serves as an additional negative control in the reaction containing only the WT allele. Background non-specific PCR products are indicated with an asterisk.

[0033] FIG. **2**J shows the fold change variation in the frequency of capture of each of the 16 dinucleotide signatures relative to the mean dinucleotide capture frequency. Oligonucleotides containing distinct dinucleotide signatures are captured using specific adaptors. The fraction of captured material is then quantified by qPCR and normalized to the mean value obtained from the capture of all 16 dinucleotide signatures. Error bars indicate the s.d. of 4 independent experiments. Dots represent individual data point.

[0034] FIG. **2**K shows the fold change variation in the frequency of capture of dinucleotide signatures with 1 A/T + 1

C/G, 2 A/T or 2 C/G bases relative to the mean dinucleotide capture frequency, determined as described in FIG. **2**J. Error bars represent the s.d. of 8 mean values for dinucleotides with 1 A/T + 1 C/G and 4 mean values for dinucleotides with 2 A/T and 2 C/G, as determined in FIG. **2**J.

[0035] FIGS. **3**A-**3**E show the detection and quantification of precision genome editing by CRISPR-mediated HDR, base editing and prime editing using DTECT.

[0036] FIG. **3**A shows the schematics of the protocol used to identify genomic changes introduced by CRISPR-dependent HDR, base editing or prime editing. In HDR experiments (blue), HEK293T cells were transfected with Cas9 and sgRNA targeting a gene of interest with or without donor DNA molecules. In base editing experiments (red), HEK293T cells were transfected with BE3 base editors with either control or base editing sgRNAs. Base editing experiments were also conducted in cells stably expressing FNLS-BE3. In prime editing experiments (grey), HEK293T cells were transfected with PE2 with or without pegRNA. Genomic DNA was then extracted from cell populations and subjected to DTECT using adaptors specific for WT (green) or edited (purple) variants.

[0037] FIG. **3**B shows the identification by DTECT of WT and HDR-edited (R209fs*6) TP53 alleles (top), WT and base-edited (Q223*) FANCD2 alleles (middle), and WT and prime-edited (CTT_ins) HEK3 alleles (bottom). Adaptors specific for the WT (CT, CA, CG; green) or edited (TT, TA; purple) signatures were utilized in DTECT experiments. Captured samples were subjected to analytical (left; 21 cycles) or quantitative PCR (right). In the HDR experiment, cells were transfected with Cas9, sgRNA and an ssODN specific for the TP53 locus with or without the HDR stimulatory factor i53. The ssODN was omitted in control reactions. In the base editing experiment, cells were transfected with BE3 and sgRNA to induce Q223* in FANCD2. In prime editing experiments, cells were transfected with PE2 and pegRNA to introduce a CTT insertion in the HEK3 locus.

[0038] FIG. **3**C provides the graphical representation of the correlation of DTECT- and NGS-based estimations of the frequency of genetic variants introduced by precision genome editing in human and mouse cells, and mouse intestinal organoids (n = 62). Data points in the dashed box (frequency <20%) of the left panel are shown enlarged on the right panel (n = 33). Error bars indicate the s.e.m. of 2-5 independent replicates. The source of the edited sample is indicated by distinct colors.

[0039] FIG. **3**D shows the schematic representation of the experiments conducted to measure the efficiency of precision genome editing in vivo using DTECT. Editing of the mouse liver was performed by hydrodynamic injection of the cytidine base editor (CBE) FNLS-BE3 and an sgRNA to introduce the Pik3ca E545K variant. DTECT (red) and NGS (green) were used to determine the efficiency of editing in the mouse liver sample.

[0040] FIG. **3**E shows the quantification by DTECT (red) and NGS (green) of the Pik3ca E545K variant introduced by CRISPR-mediated base editing in the mouse liver, as shown in FIG. **3**D. Error bars indicate the s.d. of 2 independent experiments. Dots represent individual data point.

[0041] FIGS. **4**A-**4**C show the identification of multiple genome editing events in a single locus or distinct loci by DTECT.

[0042] FIG. **4**A shows the detection by PCR (21 cycles) of allelic mixtures induced by CRISPR-mediated base editing events occurring at a CC sequence (green) in the EMX1 gene. The sequences of the EMX1 alleles resulting from four possible C->T base transitions (CC, CT, TC, TT) induced by CRISPR-mediated base editing and the adaptors to capture them (GG, AG, GA, AA) are shown. In these experiments HEK293T cells constitutively expressing the cytidine base editor (CBE) FNLS-BE3 were transfected with sgRNA targeting the EMX1 locus.

[0043] FIG. **4**B shows the schematics of the experiments conducted to detect multiple simultaneously induced variants using DTECT. HEK293T cells constitutively expressing the base editor FNLS-BE3 were transfected with two sgRNAs to introduce simultaneously the BRCA1 E638K and the BRCA2 E2772K mutations by CRISPR-mediated base editing.

[0044] FIG. **4**C shows the detection of multiple precision genome editing events introduced by CRISPR-mediated base editing in HEK293T cell populations, as illustrated in FIG. **4**B. WT and edited BRCA1 and BRCA2 alleles captured using adaptors specific for the WT (TG, AG; green) or edited (TA, AA; purple) alleles were subjected to analytical (left; 21 cycles) or quantitative PCR (right).

[0045] FIGS. **5**A-**5**J show the DTECT-mediated identification of clinically relevant BRCA½ mutations generated by precision genome editing and genotyping of cell lines and animal models carrying BRCA1 or BARD1 mutations.

[0046] FIG. **5**A shows the schematic representation of the human BRCA1 protein. BRCA1 domains and ClinVar BRCA1 mutations generated in this study are indicated.

[0047] FIG. **5**B shows the quantification using DTECT (red) and NGS (green) of the editing efficiency by which 10 BRCA1 mutations are introduced into HEK293T cells by CRISPR-mediated base editing. Experiments were conducted in cells expressing the base editor FNLS-BE3 upon transfection of sgRNAs to introduce the indicated mutations. Histograms show the mean frequency of the indicated variants estimated by DTECT and error bars represent the s.d. from 2 independent DTECT assays for the same Acultagged amplicon. n.d.: not determined, due to sequencing failure.

[0048] FIG. **5**C shows the analytical detection of the indicated BRCA1 mutations in HEK293T cell populations by DTECT (21 PCR cycles) using adaptors specific for WT (green) or mutant (purple) alleles.

[0049] FIG. **5**D shows the schematic representation of the human BRCA2 protein. BRCA2 domains and ClinVar BRCA2 mutations generated in this study are indicated.

[0050] FIG. **5**E shows the quantification using DTECT (red) and NGS (green) of the editing efficiency by which 13 BRCA2 mutations are introduced into HEK293T cells by CRISPR-mediated base editing, as described in FIG. **5**B.

[0051] FIG. **5**F shows the analytical detection of the indicated BRCA2 mutations in HEK293T cell populations by DTECT (21 PCR cycles) using adaptors specific for WT (green) or mutant (purple) alleles. Experiments were conducted as in FIG. **5**C.

[0052] FIG. **5**G shows the genotyping by DTECT-based analytical PCR (18 cycles) of single clones carrying WT and/or BRCA1 E638K mutant alleles derived from the BRCA1 E638K mutant cell population shown in FIG. **5**C. WT (#4, not edited), heterozygous (#1) and homozygous

(#2) BRCA1 mutant clones identified by DTECT are indicated.

[0053] FIG. **5**H shows the Sanger sequencing of WT, heterozygous and homozygous mutant amplicons shown in FIG. **5**G. The targeted dinucleotide is indicated in green and part of the sequence of the AcuI-tagging primer is indicated in purple.

[0054] FIG. **5**I shows the genotyping by DTECT-based analytical PCR of Bard1 S563F (left) and Brca1 S1598F (right) knock-in mutant mice (Bard1, 18 PCR cycles; Brca1, 20 PCR cycles). gDNA for DTECT analysis was obtained from mouse tail samples. WT (Bard1 #8 and Brca1 #5), heterozygous (Bard1 #2 and Brca1 #2) and homozygous (Bard1 #3) mutant mice identified by DTECT are indicated. No homozygous Brca1 S1598F mutant mice were identified in the analyzed mouse litters due to sub-Mendelian birth ratios (Billing et al., 2018).

[0055] FIG. **5**J shows the Sanger sequencing of WT, heterozygous and homozygous mutant amplicons shown in FIG. **5**I.

[0056] FIGS. **6**A-**6**D show the detection of oncogenic signatures in human clinical samples using DTECT.

[0057] FIG. **6**A shows the schematic representation of the experiments conducted on ALL patient-derived samples. Bone marrow samples from ALL patients were collected at diagnosis and after chemotherapy. PDXs were generated from the patient samples. The genomic DNA was recovered from the patient samples and PDX mouse models and subjected to analytical and quantitative detection of NT5C2 oncogenic mutations using DTECT.

[0058] FIG. **6**B provides the heat map showing the detection of NT5C2 oncogenic mutations in patient samples and a control sample using DTECT. Bone marrow samples from 5 patients were collected; genomic DNA was prepared and tested for the presence of 3 frequent NT5C2 mutations responsible for relapse to chemotherapy. A non-patient-derived gDNA sample was utilized as a control to estimate the levels of non-specific background in the DTECT assay. Data are shown as fold change in the frequency of mutant signatures in the patient samples relative to the control sample.

[0059] FIG. **6**C shows the graphical representation of the frequency of NT5C2 mutations determined by DTECT (red) and NGS (green) in the 5 human patient samples analyzed in FIG. **6**B. Error bars indicate the s.d. of 2 independent DTECT replicates.

[0060] FIG. **6**D shows the analytical and quantitative detection of the NT5C2 R367Q mutation in PDX models generated from ALL tumors of patients #2, #4 and #5 at diagnosis and after chemotherapy relapse. WT and mutant variants were captured using adaptors specific for the WT (GA, green) or mutant (AA, purple) allele and subjected to analytical (left; 18 PCR cycles) and quantitative PCR (right).

[0061] FIG. **7** shows the DTECT applications for the detection of precision genome editing and genetic variation. It shows the schematic representation of examples of targeted dinucleotide signatures generated by single base edits, small insertions and deletions that can be detected using DTECT. Examples of adaptors that can be used to detect the indicated genome editing events are shown on the right.

[0062] FIGS. **8**A-**8**D show the features of type IIS restriction enzymes compatible with DTECT and schematic representation of the AcuI digestion pattern.

[0063] FIG. **8**A shows the representation of two key features of type IIS restriction enzymes compatible with DTECT: 1) Binding of a single recognition motif (green); 2) Cleavage of a targeted DNA sequence (blue) far from the recognition motif.

[0064] FIG. **8**B shows the representation of the pattern of digestion of a type IIS enzyme, including the main digestion product and a cleavage byproduct due to slippage activity.

[0065] FIG. **8**C shows the graphical representation of the number of type IIS enzymes in function of the distance between their recognition motif and cleavage site.

[0066] FIG. **8**D shows the pattern of cleavage of the type IIS enzyme AcuI. AcuI cleaves DNA products 14/16 bp away from its recognition site (green), leaving a 3′-overhang of 2 DNA bases (blue).

[0067] FIGS. **9**A-**9**C show the Sanger sequencing reads of captured AcuI-digested DNA fragments and validation of the adaptor library.

[0068] FIGS. **9**A and **9**B show the Sanger sequencing reads of PCR amplicons of AcuI-digested DNA products containing the TT (FIG. **9**A) and CC (FIG. **9**B) signatures shown in FIG. **2**B, which have been captured using AA or GG adaptors. The DNA sequences of PCR primers (red), genomic locus (purple), targeted dinucleotides (blue), AcuI motif (green) and adaptors (brown) are shown.

[0069] FIG. **9**C shows the PCR amplification (18 cycles) of captured AcuI-digested DNA products by DTECT using specific (green) and non-specific (blue) DNA adaptors. Each of the 16 adaptors was tested for its ability to capture two independent dinucleotide signatures (#1 and #2).

[0070] FIGS. **10**A-**10**F show the identification of WT and STOP alleles in mixed solutions and quantification of non-specific dinucleotide capture and ligation efficiency in DTECT assays.

[0071] FIG. **10**A shows the schematics of the protocol used to identify and quantify WT and STOP alleles in mixed solutions, as shown in FIGS. **2**G-**2**I. Cells were transfected with the cytidine base editor (CBE) BE3 and an sgRNA to induce a STOP codon (sgSTOP) using iSTOP. WT and STOP alleles were then cloned and mixed at different WT:STOP ratios, as indicated in FIG. **2**G. DTECT was then used to capture WT and STOP signatures using adaptors specific for the WT (green) or STOP (purple) allele, as well as non-specific adaptors (blue). Captured material was then subjected to analytical or quantitative PCR.

[0072] FIG. **10**B shows the Sanger sequencing reads of WT and STOP alleles of SPRTN, SMARCAL1 and PIK3R1. The targeted dinucleotide signature is shown in green and the edited cytidine base (C-> T) is indicated by the blue arrow.

[0073] FIG. **10**C shows the representation of the AcuI-tagging primers used to detect the WT and STOP alleles of the SPRTN gene. The targeted dinucleotides are shown in blue, the edited base is indicated with an asterisk, the PAM sequence is show in red and part of the AcuI-tagging primer sequence is shown in purple.

[0074] FIG. **10**D shows the PCR amplification (25 cycles) of WT and STOP SPRTN alleles (arrow) captured using DTECT from WT:STOP allele mixtures (i.e., 100:0 and 99:1). An adaptor (AG) specific for the STOP SPRTN allele is utilized in the capture reaction, along with an adaptor spe-

cific for the WT allele (GG; positive control) and a non-specific adaptor (TT; negative control). Background non-specific PCR products are indicated with an asterisk.

[0075] FIG. **10**E shows the frequency of non-specific dinucleotide capture for each of the 16 adaptors used for DTECT. Adaptors containing the indicated dinucleotide sequences were utilized to capture AcuI-digested DNA fragments with non-complementary dinucleotides and the frequency of non-specific dinucleotide capture was quantified by qPCR. Mean frequency of non-specific dinucleotide capture is shown for 2-6 independent DNA ligation reactions using DNA fragments with distinct non-complementary dinucleotides. Adaptors complementary to +1 and -1 AcuI-dependent slippage events were excluded from the analysis.

[0076] FIG. **10**F shows the time course experiment to measure the efficiency of the ligation of AcuI-digested products to DNA adaptors. AcuI-digested products from 3 independent targets (SMARCAL1, SPRTN and PIK3R1), DNA adaptors and T4 ligase were incubated for 5 min, 1 hour or 16 hours, and the captured material was quantified by qPCR. A sample without T4 ligase was used as a negative control. The percentage of captured material at the different time points was obtained by normalization to the amount of captured material upon a 16-hour ligation reaction. Error-bars represent the s.d. of 2 independent experiments.

[0077] FIGS. **11**A-**11**J show the detection of CRISPR-mediated HDR and base editing events by DTECT, NGS and RFLP assays.

[0078] FIGS. **11**A-**11**D show the detection by analytical PCR (20 or 21 cycles) of WT and HDR-edited EMX1 (FIG. **11**A), JAK2 (FIG. **11**B), HBB (FIG. **11**C) and BRCA2 (FIG. **11**D) alleles captured using adaptors specific for the WT (green) or edited (purple) alleles. In these experiments HEK293T cells were transfected with Cas9, sgRNA and an HDR donor (ssODN) with or without the HDR stimulatory factor i53. The ssODN was omitted in control reactions. ssODNs introduce a PmeI site in EMX1 and JAK2, a sickle cell anemia mutation in HBB (i.e., G6V), and a breast cancer-associated small tandem duplication in BRCA2 (dupAGAAGAT).

[0079] FIG. **11**E shows the quantification of the efficiency of the insertion of the short tandem duplication dupAGAAGAT in the BRCA2 locus, as determined by NGS. The pie chart shows the distribution of NGS reads corresponding to HDR- and/or NHEJ-mediated repair events (HDR, red; NHEJ, blue; mixed HDR/NHEJ, green; unedited, brown) occurring at the BRCA2 locus in HEK293T cells transfected with Cas9/sgRNA and ssODN donor, with or without i53. In these experiments, the BRCA2 locus was amplified by PCR and subjected to NGS. The NGS reads were analyzed by CRISPResso.

[0080] FIG. **11**F shows the RFLP assay to monitor the gain of a PmeI restriction site introduced by ssODN-meditated HDR in the EMX1 and JAK2 loci under the same experimental conditions shown in FIG. **11**A and FIG. **11**B. Digested (edited) and undigested (WT) DNA products are indicated by arrows.

[0081] FIGS. **11**G-**11**H show the RFLP assays to monitor the loss of NcoI (FIG. **11**G) or TaqaI (FIG. **11**H) restriction sites in the HBB and TP53 loci, respectively, resulting from the insertion of the G6V and R209fs*6 mutations under the same experimental conditions shown in FIG. **11**C and FIG. **3**B. Digested (WT) and undigested (edited) DNA products are indicated by arrows.

[0082] FIG. **111** shows the detection of WT and nonsense mutant TIMELESS, SLX4 and FANCM alleles by DTECT using adaptors specific for the WT (green) or edited (purple) signatures. Experiments were performed in cells transfected with the cytidine base editor BE3 and sgRNA to induce the indicated nonsense mutations, which were detected by analytical (left; 21 cycles) or quantitative PCR (right).

[0083] FIG. **11**J shows the detection of WT and nonsense mutant TCOF1 alleles by DTECT (21 PCR cycles) using adaptors specific for the WT (GG, green) or edited (AG, purple) allele. Experiments were performed in cells transfected with BE3 and sgRNA to induce the indicated nonsense mutation in the TCOF1 gene. The introduction of the nonsense mutation was confirmed by Sanger sequencing (bottom) and by an RFLP assay that monitors the loss of an XcmI restriction site at the edited locus (right).

[0084] FIGS. **12**A-**12**B show the comparative analysis of DTECT-, Sanger- and NGS-based estimations of the frequency of genetic variants generated by precision genome editing.

[0085] FIG. **12**A shows the graphical representation of the frequency of mutations introduced by CRISPR-dependent HDR and base editing in human and mouse cells, and intestinal organoids. The FANCF, Pik3ca and Apc loci were edited in biological duplicate or triplicate using multiple base editors, and the resulting edited samples were previously described (Zafra et al., 2018). The BRCA½ loci were edited using BE3. The frequency values were determined by both DTECT (red) and NGS (green). NGS was conducted on standard PCR amplicons (FANCF, Pik3ca and Apc) or AcuI-tagged amplicons (BRCA½) of the edited loci. Error bars represent the s.e.m. of 2-5 independent DTECT assays per edited sample. The same frequency values are plotted in the graphs shown in FIG. **3**C.

[0086] FIG. **12**B shows the graphical representation of the correlation between technical duplicates obtained by DTECT (red), EditR (green) or ICE (blue). Each dot represents a distinct BRCA½ variant introduced in cells by precision genome editing. Technical duplicates of DTECT assays correspond to two independent ligation reactions for the same AcuI-digested amplicon and Sanger-based technical duplicates correspond to two independent sequencing reactions for the same PCR amplicon.

[0087] FIGS. **13**A-**13**C show the detection of base editing byproducts and clinically relevant BRCA½ mutations introduced by precision genome editing.

[0088] FIG. **13**A shows the detection by analytical PCR (21 cycles) of allelic mixtures induced by CRISPR-mediated base editing events occurring at a CC sequence in the EMX1 gene, as shown in FIG. **4**A. In these experiments HEK293T cells constitutively expressing the base editor FNLS-BE3 were transfected with a control sgRNA (top) or an sgRNA targeting the EMX1 locus (bottom). All possible 16 adaptors were used to capture EMX1 variants. Adaptors that capture the WT allele (GG) and +1 AcuI slippage event (CG) are shown in green and orange. Adaptors that capture C->T base editing events (AA, AG, GA) and C->A and C->G base editing byproducts (AC, AT, CA, CG, GC) are also shown.

[0089] FIGS. **13**B-**13**C show the analytical detection of the indicated BRCA1 (FIG. **13**A) and BRCA2 (FIG. **13**B) mutations in HEK293T cell populations by DTECT (21 PCR cycles) using adaptors specific for WT (green) or

mutant (purple) alleles. Experiments were conducted as in FIGS. **5**C and **5**F.

[0090] FIGS. **14**A-**14**B show the genotyping of mutant cellular clones and knock-in mice using DTECT.

[0091] FIG. **14**A shows the genotyping by DTECT-based analytical PCR (20 cycles) of HEK293T clones (17) carrying WT and/or BRCA1 E638K mutant alleles or base editing byproducts derived by single cell dilution from the BRCA1 E638K cell population shown in FIG. **5**C. Heterozygous and homozygous mutant clones are indicated in blue and purple, respectively. WT clones are indicated in green and a clone with a base editing byproduct is indicated in orange. Clones #1, #2, #4 and control (CTL) are also shown in FIG. **5**G. Quantification of each BRCA1 variant by qPCR is also shown (bottom). HEK293T cells have 4 BRCA1 alleles. Error bars correspond to two independent experiments.

[0092] FIG. **14**B shows the genotyping by DTECT-based analytical PCR of Bard1 S563F (top) and Brca1 S1598F (bottom) knock-in mutant mice (Bard1, 18 PCR cycles; Brca1, 20 PCR cycles). DTECT assays were conducted on gDNA isolated from mouse tail samples. Heterozygous and homozygous mutant mice are indicated in blue and purple, respectively, and WT mice are indicated in green. No homozygous Brca1 S1598F mutant mice were identified in the analyzed mouse litters due to sub-Mendelian birth ratios (Billing et al., 2018). Mice #1, #2, #3 and #8 (Bard1), and #1, #2, #5 (Brca1) are also shown in FIG. **5**I.

[0093] FIGS. **15**A-**15**D show the detection of oncogenic mutations in a mouse model of myeloproliferative neoplasm and in ALL patients using DTECT.

[0094] FIG. **15**A shows the schematics of the experiments conducted to detect the Jak2 V617F mutation in a mouse model of myeloproliferative neoplasm. Peripheral blood was collected from mice transplanted with a mixture of bone marrow cells either wild-type (WT) or carrying an inducible Jak2 V617F mutant allele (Mx1-Cre+;Jak2$^{V617F/+}$). DTECT was then utilized to determine the presence of the Jak2 V617F mutation in gDNA extracted from the collected blood samples.

[0095] FIG. **15**B shows the schematic representation of 4 AcuI-induced dinucleotide signatures that enable the identification of Jak2 WT and V617F alleles. The G in red is replaced by a T in the Jak2 V617F mutant allele.

[0096] FIG. **15**C shows the identification by DTECT-based analytical PCR (20 cycles) of the Jak2 V617F mutation in the blood of a mouse model of myeloproliferative neoplasm generated as described in FIG. **15**A. The Jak2 V617F mutation was identified using the 4 independent dinucleotide signatures shown in FIG. **15**B. gDNA samples from peripheral blood of WT mice were used as controls (#1 and #2) in this experiment. Sanger sequencing (bottom) was conducted to confirm the results obtained using DTECT.

[0097] FIG. **15**D shows the analytical detection of the indicated NT5C2 mutations in ALL patient samples by PCR (20 cycles). The frequency of the indicated mutations in the same patient samples is shown in FIG. **6**B.

[0098] FIGS. **16**A-**16**C show the analysis of ClinVar variants with proximal genomic AcuI motifs compatible with DTECT.

[0099] FIG. **16**A shows the Bioinformatic analysis of ClinVar database variants (425,580) with (80,326; blue) or without (345,254; green) genomic AcuI sites in close proximity (+/- 100 bp). Variants (green, right pie chart) with a single AcuI motif located 35 bp to 100 bp away on the 3′- (29,848) or 5′- (29,291) side can be detected using DTECT, as illustrated in FIG. **16**C. Variants (red, right pie chart) with an AcuI motif located <35 bp away (18,739) or with proximal AcuI motifs on both sides (2,448) cannot be detected using DTECT.

[0100] FIG. **16**B shows the percentage and number of ClinVar variants that can (95.02%, 404,393) or cannot (4.98%, 21,187) be detected using DTECT.

[0101] FIG. **16**C shows the schematic representation of genomic loci with or without an AcuI site in close proximity to the edited site. When a genomic AcuI site is located 35 bp to 100 bp away from the edited site, detection of the edited site can be obtained by designing 2 AcuI-tagging primers that anneal to the targeted locus between the genomic AcuI site and the edited base(s). This approach allows the capture of two independent dinucleotide signatures for each targeted site with one proximal AcuI site. Four independent dinucleotide signatures can be captured for targeted sites with no proximal AcuI sites.

[0102] FIGS. **17**A-**17**B show the detection of AcuI slippage events by DTECT.

[0103] FIG. **17**A shows the schematics of targeted dinucleotides (blue) and +1 (red) and -1 (orange) AcuI slippage events (left). Detection of AcuI slippage byproducts by DTECT (22 PCR cycles) using adaptors complementary to the targeted dinucleotide signatures (green) and to signatures generated by AcuI +1 (red) or -1 (orange) slippage (right). A non-specific adaptor (blue) is used as a control.

[0104] FIG. **17**B shows the schematic representation of DNA digestion products generated by precise AcuI cleavage (green) or +1 slippage (red) occurring at wild-type and mutant alleles. The dinucleotide signatures generated as a result of AcuI slippage byproducts and the complementary adaptors to capture them are indicated.

[0105] FIGS. **18**A-**18**D show the design of DTECT assays to avoid indel interference in CRISPR-mediated HDR experiments.

[0106] FIG. **18**A shows the InDelphi prediction (https://indelphi.giffordlab.mit.edu) of indel-containing alleles in the TP53 locus. The dinucleotides targeted to simultaneously introduce the TP53 R209fs*6 mutation and a G > T mutation in the PAM by CRISPR-dependent HDR are indicated in green and red, respectively. The Cas9 cleavage site is indicated in black. The dinucleotide signatures captured to detect the TP53 R209fs*6 and PAM mutations are shown in purple. The presence of indel interference in the distinct predicted alleles is indicated. MH, microhomology.

[0107] FIG. **18**B shows the DTECT-based quantification of the TP53 R209fs*6 and PAM mutations introduced by HDR using a single ssODN donor template, as shown in FIG. **18**A. Adaptors specific for the WT (CT and TG; green and red) or edited (TT; purple) signatures were used for quantification. HDR efficiency determined by NGS is also shown.

[0108] FIG. **18**C shows the schematic representation of the design of DTECT experiments to avoid interference of indels formed at DSBs during CRISPR-mediated HDR. Cas9-mediated DSBs are induced at a distance from a targeted dinucleotide (green) sufficient to avoid mutation of the targeted dinucleotide by indels (blue). The pattern of indel mutations is predicted using the InDelphi website.

[0109] FIG. **18**D shows the schematics of alleles generated by CRISPR-mediated HDR, including the unedited allele (green), indel-containing alleles (blue) and the HDR-

edited allele (purple). Using the experimental design shown in FIG. **18**C, DTECT captures both the unedited and the indel-containing alleles using an adaptor specific for the WT dinucleotide signature, while the HDR-edited allele is captured using an adaptor specific for the edited dinucleotide signature. The capture of indel-containing alleles with a WT adaptor ensures the accurate quantification of the frequency of the HDR-edited allele in the allele population.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0110] The present disclosure provides a versatile method that uses standard molecular biology techniques to detect variants introduced by precision genome editing or resulting from genetic variation. This detection method, designated Dinucleotide signaTurE CapTure (DTECT), enables accurate and sensitive quantification of marker-free precision genome editing events induced by CRISPR-dependent HDR, base editing and prime editing. In addition, we show that DTECT can readily identify oncogenic mutations in cancer mouse models, patient-derived xenograft models and cancer patient samples. These studies establish a cost-effective method for the rapid detection of genetic variants, which will aid the generation of marker-free cellular and animal models of human disease and expedite the detection of pathogenic variants for clinical applications.

[0111] Accordingly, one embodiment of the present disclosure is a DNA adaptor comprising: (a) one strand with sequence of 5′-CTGGGGCACGGGTAAGAAG-CATTCTGTCTCTCTTCTAAGAATTCGAGCTCGG-TACC CG-3′ (SEQ ID NO: 230); and (b) one complementary strand with sequence of 5′-CGGGTACCGAGCTCGAATTCTTAGAAGAGAGACA-GAATGCTTCTTACCCGTGCCC CAGNN-3′ with "N" corresponding to A, T, G or C (SEQ ID NOs: 231-246).

[0112] In some embodiments, the DNA adaptor is labeled with a detection molecule. Non-limiting examples of the detection molecule include a radiolabel, a fluorescent label, a biotinylated label, a non-fluorescent label, an enzyme, a hapten, a phosphorescent molecule, a chemiluminescent molecule, a chromophore, a luminescent molecule, a photoaffinity molecule, a color particle or a ligand.

[0113] Another embodiment of the present disclosure is a method of preparing a DNA adaptor disclosed herein, comprising: (a) synthesizing one constant oligonucleotide with sequence of 5′-CTGGGGCACGGGTAAGAAG-CATTCTGTCTCTCTTCTAAGAATTCGAGCTCGG-TACC CG-3′ (SEQ ID NO: 230); (b) synthesizing one complementary oligonucleotide with sequence of 5′-CGGGTACCGAGCTCGAATTCTTAGAAGAGAGACA-GAATGCTTCTTACCCGTGCCC CAGNN-3′ with "N" corresponding to A, T, G or C (SEQ ID NOs: 231-246); (c) mixing the constant and complementary oligonucleotides; and (d) annealing the mixture to obtain the DNA adaptor.

[0114] Another embodiment of the present disclosure is a library of DNA adaptors prepared by methods disclosed herein, the library comprises 16 DNA adaptors, wherein each DNA adaptor has a different "NN".

[0115] Another embodiment of the present disclosure is a method for detecting a genetic modification, comprising the steps of: (a) amplifying a genomic locus of interest using a specially designed Type IIS restriction enzyme-tagging primer, comprising: (i) extracting genomic DNA from a biological sample of interest; (ii) synthesizing the Type IIS restriction enzyme-tagging primer based on the genomic locus of interest; (iii) amplifying the genomic locus of interest using the Type IIS restriction enzyme-tagging primer and a reverse primer; and (iv) purifying a Type IIS restriction enzyme-tagged genomic amplicon; (b) digesting the Type IIS restriction enzyme-tagged genomic amplicon with the Type IIS restriction enzyme; (c) isolating the smaller DNA fragment containing a genomic signature of interest exposed in a 3′ single-stranded overhang; (d) capturing the genomic signature of interest, comprising: (i) preparing the library of DNA adaptors disclosed herein; (ii) incubating the isolated smaller DNA fragment containing the 3′ overhang signature with the library of DNA adaptors and performing a ligation; and (iii) obtaining a ligated product; and (e) amplifying the ligated product to detect the presence of the genetic modification.

[0116] In some embodiments, the genetic modification is selected from a base change, a deletion, or an insertion. In some embodiments, the genetic modification is selected from a single genomic change or multiple genomic changes. In some embodiments, the multiple genomic changes can occur within a single locus or distinct loci.

[0117] In some embodiments, the Type IIS restriction enzyme is selected from AcuI, BpmI, BpuEI, BsgI, MmeI and NmeAIII. In some embodiments, the Type IIS restriction enzyme is selected from AcuI and BpuEI. In some embodiments, the Type IIS restriction enzyme is AcuI.

[0118] In some embodiments, the Type IIS restriction enzyme-tagging primer is an oligonucleotide comprising: (a) a non-complementary handle sequence positioned on the 5′ side; (b) a complementary sequence of the genomic locus of interest on the 5′ side; (c) a recognition motif of the Type IIS restriction enzyme that is positioned at a predicted distance from its cleavage site to generate the genomic signature of interest; and (d) a complementary sequence of the genomic locus of interest on the 3′ side.

[0119] In some embodiments, the reverse primer is positioned at more than 100 bp downstream of the genomic locus of interest.

[0120] In some embodiments, the non-complementary handle sequence can have any suitable length. In some embodiments, the non-complementary handle sequence is 25 bp. In some embodiments, the non-complementary handle sequence can have any suitable sequence. In some embodiments, the non-complementary handle sequence is 5′-GCAATTCCTCACGAGACCCGTCCTG-3′ (SEQ ID NO: 3).

[0121] In some embodiments, the ligation in step (d)(ii) of the methods disclosed above is carried out by T4 DNA ligase.

[0122] A further embodiment of the present disclosure is a kit for detecting a genetic modification of interest, comprising a specially designed Type IIS restriction enzyme-tagging primer disclosed herein, and a library of DNA adaptors disclosed herein, packaged together with instructions for its use. In some embodiments, the Type IIS restriction enzyme is AcuI.

[0123] Another embodiment of the present disclosure is a method for detecting a genetic modification, comprising the steps of: (a) amplifying a genomic locus of interest using a specially designed AcuI-tagging primer, comprising: (i) extracting DNA of interest; (ii) synthesizing the AcuI-tag-

ging primer based on the genomic locus of interest; (iii) amplifying the genomic locus of interest using the AcuI-tagging primer and a reverse primer; and (iv) purifying an AcuI-tagged genomic amplicon; (b) digesting the AcuI-tagged genomic amplicon with restriction enzyme AcuI; (c) isolating the smaller DNA fragment containing a genomic signature of interest produced by AcuI-digestion; (d) capturing the genomic signature of interest, comprising: (i) preparing the library of DNA adaptors disclosed herein; (ii) incubating the isolated smaller DNA fragment with the library of DNA adaptors and performing a ligation; and (iii) obtaining a ligated product; and (e) amplifying the ligated product to detect the presence of the genetic modification.

[0124] In some embodiments, the AcuI-tagging primer is an oligonucleotide comprising: (a) a non-complementary handle sequence positioned on the 5′ side; and (b) a complementary sequence of the genomic locus of interest containing an AcuI motif (5′-CTGAAG-3′) positioned 14 bp upstream from the genomic locus of interest.

[0125] In some embodiments, the AcuI-tagging primer can have any suitable length. In some embodiments, the AcuI-tagging primer is 60 bp.

[0126] In some embodiments, the reverse primer is positioned at more than 100 bp downstream of the genomic locus of interest.

[0127] In some embodiments, the non-complementary handle sequence can have any suitable length. In some embodiments, the non-complementary handle sequence is 25 bp.

[0128] In some embodiments, the complementary sequence has the structure of: 5′-N(20)CTGAAGN(14)-3′ or 5′-N(15)CTGAAGN(14)-3′, with "N" corresponding to A, T, G or C, depending on the DNA sequence of the genomic locus of interest.

[0129] In some embodiments, the non-complementary handle sequence is 5′-GCAATTCCTCACGAGACCCGTCCTG-3′ (SEQ ID NO: 3) and the complementary sequence is 5′-N(15)CTGAAGN(14)-3′, with "N" corresponding to A, T, G or C.

[0130] In some embodiments, the ligation in step (d)(ii) of the methods disclosed above is carried out by T4 DNA ligase.

[0131] An additional embodiment of the present disclosure is a kit for detecting a genetic modification, comprising a specially designed AcuI-tagging primer and a library of DNA adaptors disclosed herein, packaged together with instructions for its use.

[0132] Another embodiment of the present disclosure is a method for quantifying a genomic variant in a biological system, comprising the steps of: (a) obtaining a sample from the biological system; (b) amplifying a genomic locus of interest using a specially designed AcuI-tagging primer, comprising: (i) extracting DNA of interest; (ii) synthesizing the AcuI-tagging primer based on the genomic locus of interest; (iii) amplifying the genomic locus of interest using the AcuI-tagging primer and a reverse primer; and (iv) purifying an AcuI-tagged genomic amplicon; (c) digesting the AcuI-tagged genomic amplicon with restriction enzyme AcuI; (d) isolating the smaller DNA fragment containing a genomic signature of interest produced by the AcuI-digestion; (e) capturing the genomic signature of interest, comprising: (i) preparing the library of DNA adaptors disclosed herein; (ii) incubating the isolated smaller DNA fragment with the library of DNA adaptors and performing a ligation; and (iii) obtaining a ligated product; and (f) quantifying the genomic variant and determining its relative abundance.

[0133] In some embodiments, the genomic variant is generated by precision genome editing. In some embodiments, the precision genome editing is CRISPER-dependent homology-directed repair, base editing or prime editing.

[0134] In some embodiments, the biological system is a mammalian cell line, an organoid, or a tissue.

[0135] In some embodiments, the quantification in step (f) of the methods disclosed above is carried out by quantitative PCR (qPCR).

[0136] Still another embodiment of the present disclosure is a method for identifying and quantifying an oncogenic mutation of interest in a biological sample, comprising the steps of: (a) obtaining a biological sample; (b) amplifying a genomic locus of interest using a specially designed AcuI-tagging primer, comprising: (i) extracting DNA of interest; (ii) synthesizing the AcuI-tagging primer based on the genomic locus of interest; (iii) amplifying the genomic locus of interest using the AcuI-tagging primer and a reverse primer; and (iv) purifying an AcuI-tagged genomic amplicon; (c) digesting the AcuI-tagged genomic amplicon with restriction enzyme AcuI; (d) isolating the smaller DNA fragment containing a genomic signature of interest produced by the AcuI-digestion; (e) capturing the genomic signature of interest, comprising: (i) preparing the library of DNA adaptors disclosed herein; (ii) incubating the isolated smaller DNA fragment with the library of DNA adaptors and performing a ligation; and (iii) obtaining a ligated product; (f) amplifying the ligated product to identify the presence of the oncogenic mutation of interest; and (g) quantifying the oncogenic mutation of interest, if present, and determining its frequency.

[0137] In some embodiments, the biological sample is obtained from a cancer animal model, a patient-derived xenograft (PDX), or a human cancer patient sample.

[0138] In some embodiments, the quantification in step (g) of the methods disclosed above is carried out by quantitative PCR (qPCR).

[0139] A further embodiment of the present disclosure is a process for marker-free detection of a precision genome editing event comprising carrying out Dinucleotide signaTurE CapTure (DTECT) on a nucleic acid sequence of interest.

[0140] DTECT can also be used to detect genetic signatures in any organism, for example, a virus. Thus, still another embodiment of the present disclosure is a method for detecting a virus variant of interest, comprising the steps of: (a) obtaining a nucleic acid of the virus variant of interest from a biological sample; and (b) if the nucleic acid is DNA, carrying out Dinucleotide signaTurE CapTure (DTECT) to detect the variant of interest; or (c) if the nucleic acid is RNA, coverting it to DNA by reverse transcription PCR (RT-PCR) and then carrying out DTECT to detect the variant of interest. This detection method is applicable to any type of virus including but not limited to a DNA virus, an RNA virus, a retrovirus, etc. In some embodiments, the virus is an RNA virus. In some embodiments, the virus is SARS-CoV-2.

[0141] The following examples are provided to further illustrate the methods of the present disclosure. These exam-

ples are illustrative only and are not intended to limit the scope of the disclosure in any way.

EXAMPLES

Example 1 Methods and Materials

Material Availability

[0142] Plasmids for DTECT quantification and expression of base editing sgRNAs targeting BRCA1, BRCA2 and FANCD2 have been deposited to Addgene (#139321-139333, and 139511).

Cell Line Generation and Single Clone Isolation

[0143] HEK293T and DLD1 cell lines were obtained from ATCC. Cells were cultured in DMEM (ThermoFisher Scientific) supplemented with 10% Fetalgro bovine growth serum (BGS, RMBIO) and 1% penicillin-streptomycin (ThermoFisher Scientific). Cells were grown at 37° C. with 5% CO_2 and tested regularly for mycoplasma. NIH/3T3 were maintained in DMEM supplemented with 10% bovine calf serum. Organoids were isolated and cultured as previously described (Zafra et al., 2018). To generate cells constitutively expressing FNLS-BE3-P2A-BlastR, HEK293T cells were infected with a lentivirus expressing the above construct. Viruses were produced in HEK293T in 6-well plates by transfecting 2 μg of FNLS-BE3-P2A-BlastR, 0.2 μg of Tat, 0.2 μg of Gag/Pol, 0.2 μg of Rev, 0.4 μg of VSV-G expressing plasmids in 250 μl of DMEM without serum. 9 μl of TransIT-293 (Mirus) were added to the DNA, mixed and incubated for 15 min at room temperature. The DNA transfection reagent mix was added dropwise to the cells and incubated at 37° C. with 5% CO_2. The next day the cell medium was replaced and cells were incubated for 48 hours. The medium containing lentiviruses was then collected and utilized to infect new HEK293T cells. 48 hours after infection, blasticidin was added to the medium until the uninfected control cells were killed. FNLS-BE3 expression was determined by western blot and the base editing activity of the construct was tested using previously validated sgRNAs. Single HEK293T clones were selected for high base editing efficiency. Clones were isolated by trypsinization of the initial cell population into individual cells. Cell density was evaluated by counting the cells with a hemocytometer and cells were diluted to approximately 0.13 cells/μl, equivalent to 20 cells per 150 μl. Serial dilutions were prepared and 150 μl of the diluted cell mixture were seeded into 96-well plates. Single clones were expanded and further examined for FNLS-BE3 expression and activity.

Editing of Cell Lines, Organoids and Mice

[0144] To induce CRISPR-mediated HDR editing, HEK293T cells were seeded at 50%-70% confluency into 24-well plates and reverse transfected with 0.25 μg of sgRNA and 0.25 μg of Cas9 expressing plasmid (Addgene #42230) with or without 0.5 μl of ssODN (40 μM) into 100 μl of DMEM without Fetalgro BGS and antibiotics. 3 μl of TransIT-293 (Mirus) were added to the DNA, mixed and incubated for 15 min at room temperature. Experiments involving i53 were done by adding 0.25 μg of i53 (Addgene #77939) to the transfection mixture. The gDNAs of cell populations and individual clones were recovered by resuspending the cell pellets in the Quick Extract DNA Extraction Solution (Epicentre), followed by incubation at 65° C. for 10 min and 95° C. for 5 min. The isolated gDNAs were diluted in H_2O, quantified using Nanodrop and stored at -20° C. or directly used in PCR reactions. In base editing experiments, we used cells constitutively expressing FNLS-BE3 or transfected with pCMV-BE3 (Addgene #73021) and sgRNAs, as described above. Empty plasmids (Addgene #100708) with no sgRNAs were used as controls. To determine the accuracy of the quantification of variant frequency by DTECT (FIG. **2**G), STOP codons were introduced into SPRTN, SMARCAL1 and PIK3R1 genes using iSTOP, as previously described (Billon et al., 2017). To isolate the WT alleles, the locus was amplified by PCR and cloned into the pCR-Blunt II-TOPO vector (ThermoFisher Scientific). The STOP alleles were isolated by PCR amplification using gDNA that was partially edited as template. The PCR product was subsequently digested using restriction enzymes that specifically cleave the WT PCR alleles (i.e., PvuII for SPRTN, SfaNI for SMARCAL1 and TaqaI for PIK3R1). The digestion reaction was loaded on a 2% agarose gel and the undigested PCR products were column purified (Zymoclean #D4008). The purified products were subsequently cloned into the pCR-Blunt II-TOPO vector (ThermoFisher Scientific). Cloned WT and STOP PCR fragments were confirmed by Sanger sequencing and are shown in FIG. **10**B. RFLP assays were conducted by digesting PCR amplicons of the edited genomic loci with enzymes that recognize restriction sites created or disrupted by editing of the targeted loci. Restriction digest products were run on 6% TBE polyacrylamide gels. Gels were run at 160 V in 1X TBE and stained for 5 min using SybrGold diluted in 1X TBE buffer. In prime editing experiments, 1 μg of pCMV-PE2 (Addgene #132775) was transfected into HEK293T cells along with 500 ng of control pegRNA (Addgene #132777) or pegRNA HEK3 insCTT (Addgene #132778). Three days after transfection, genomic DNA was recovered as above and the edited signature was identified with DTECT. Edited DLD1 (FANCF locus) and NIH/3T3 (Pik3ca and Apc loci) cell populations and mouse intestinal organoids (Pik3ca and Apc loci) were previously described (Zafra et al., 2018). Genomic DNA from the edited cell populations was used to quantify the editing efficiency by DTECT (FIG. **12**A).

[0145] In order to introduce multiple variants into the BRCA1 and BRCA2 genes, HEK293T cells expressing FNLS-BE3 were seeded at 50%-70% confluency into 24-well plates and reverse transfected with 1 μg of sgRNA into 100 μl of DMEM without Fetalgro BGS and antibiotics. 3 μl of TransIT-293 (Mirus) were added to the DNA, mixed and incubated for 15 min at room temperature. The DNA transfection mix was added dropwise to the cells and incubated at 37° C. with 5% CO_2 for 4 days. Single clones were generated and the gDNAs of cell populations and individual clones were recovered as describe above. Genomic loci were Sanger sequenced by Eton Bioscience or Genewiz. Sanger sequencing data were analyzed using Serial cloner and viewed by Snapgene Viewer. The sequencing profiles shown in this manuscript were generated by SnapGene Viewer. Quantitative detection of the editing level using the AcuI-tagged amplicon was done blindly.

[0146] In vivo mouse editing was performed as previously described (Zafra et al., 2018). Briefly, eight week-old

C57BL/6N mice (Charles River) were injected with 0.9% sterile sodium chloride solution containing 20 μg of pLenti-FNLS-P2A-Puro and 10 μg of sgRNA vector. The total injection volume corresponded to 20% of the individual mouse body weight and was injected into the lateral tail vein in 5-7 seconds. All animal experiments were authorized by the regional board of Karlsruhe, Germany.

Mouse Genotyping and Bone Marrow Transplantation

[0147] The generation of genetically engineered mice harboring the Brca1 S1598F and Bard1 S563F alleles was previously described (Billing et al., 2018; Shakya et al., 2011). Mouse genotyping was performed using DTECT on genomic DNA extracted from mouse tails. AcuI-tagging of the targeted loci was performed using 50 ng of gDNA (see DTECT protocol above). All primer sequences are listed in Table S1. Genotyping experiments were conducted blindly.

[0148] Competitive transplantation experiments were performed to assess chimerism of Jak2 V617F mutant cells in relation to wild-type support. Specifically, Mx1-Cre*;CD45.2 Jak2$^{V617F/+}$ and Mx1Cre^{+};CD45.1 wild-type mice were dosed with polyinosine-polycytosine (PIPC) 8 weeks prior to sacrifice to induce MPN in mutant mice. On day of sacrifice, dissected femurs and tibias were isolated and bone marrow flushed with a syringe into PBS. Red blood cells (RBCs) were lysed in ammonium chloride-potassium bicarbonate lysis buffer for 10 min on ice. 1.5×10^6 filtered whole donor Mx1-Cre^{+};Jak2$^{V617F/+}$ bone marrow cells (CD45.2) were then mixed with wild-type 1.5×10^6 competitor bone marrow cells (CD45.1) and transplanted via tail vein injection into lethally irradiated ($2 \times$ 550 Rad) CD45.1 host mice. Mice were then monitored serially for the development of MPN based on blood counts and donor chimerism by retroorbital bleed draws using heparinized microhematocrit capillary tubes (ThermoFisher Scientific). After 3 consecutive hematocrits of >65%, mice were then sacrificed for peripheral blood fluorescence-activated cell sorting (FACS) analysis and DNA extraction. All animal procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at Memorial Sloan Kettering Cancer Center. The conditional Mx1-Cre^{+};Jak2$^{V617F/+}$ mice are all C57BL/6 background and have been previously described (Mullally et al., 2010). Automated peripheral blood counts were obtained using a ProCyte Dx (IDEXX Laboratories) according to the manufacturer's protocol. For surface flow cytometry of mouse peripheral blood, bone marrow, and spleen, RBCs were lysed and stained with monoclonal antibodies in PBS plus 1% BSA for 1 hour on ice. For flow cytometry of erythroid lineage, bone marrow or splenic cells were stained without RBC lysis. DAPI was used for live/dead cell analysis. Cell populations were analyzed using an LSR Fortessa (Becton Dickinson), and data were analyzed with FlowJo software (Tree Star). DNA extraction was performed using the QIAamp DNA Micro Kit (Qiagen) per manufacturer's protocol.

Analysis of All Patient Samples and PDXs

[0149] DNA samples from leukemic ALL blasts obtained at diagnosis and after relapse were provided by multiple institutions, as previously described (Oshima et al., 2016). Informed consent was obtained at study entry and samples were collected under the supervision of local Institutional Review Boards for participating institutions and analyzed under the supervision of the Columbia University Irving Medical Center Institutional Review Board. Research was conducted in compliance with ethical regulations. ALL patients received standard combination chemotherapy at diagnosis. Diagnosis and relapse samples were harvested from bone marrow. High molecular weight genomic DNA from matched diagnosis and relapse samples of ALL patients was extracted from patient leukemic blasts or from xenografts using the DNeasy Blood & Tissue Kit (Qiagen) or the AllPrep DNA/RNA Mini Kit (Qiagen). Primary human xenograft ALL cells were passaged and harvested from the spleens of NRG (NOD.Cg-ag1tm1Moml12rgtm1Wjl/SzJ, The Jackson Laboratory) mice. Whole exome sequencing was performed and analyzed as previously described (Oshima et al., 2016).

Vector Construction and Cloning

[0150] sgRNAs were synthesized as complementary oligonucleotides (IDT) compatible with BbsI restriction sites located into the B52 plasmid (Addgene #100708). Oligonucleotides were designed as previously described (Billon et al., 2017). Cloned sgRNAs were verified by Sanger sequencing. Sequences of the sgRNAs are available in Table S1. ssODNs used in HDR experiments were synthesized as ultramer oligos (IDT) and their sequences are available in Table S1. To generate the FNLS-BE3-P2A-BlastR plasmid, the pLenti-FNLS-P2A-Puro plasmid (Addgene #110841) (Zafra et al., 2018) was modified by replacing the puromycin resistance gene with the blasticidin resistance gene. Briefly, the blasticidin resistance gene coding sequence was amplified by PCR and recombined using Gibson assembly into FNLS-BE3-P2A. The FNLS-BE3-P2A-BlastR sequence was verified by Sanger sequencing.

AcuI-Tagging Primer Design

[0151] The AcuI-tagging oligonucleotide enables the insertion of an AcuI motif (5′-CTGAAG-3′) 14 bp away from a targeted dinucleotide. This motif is inserted as a hairpin in the middle of a sequence complementary to the targeted genomic locus. The AcuI-tagging oligonucleotide is 60 bp-long and contains a non-complementary handle sequence of 20-25 bp. Common handle sequences used are PB547 (5′-GATCCTCTAGAGTCGACCTG-3′) (SEQ ID NO: 1) or PB1072 (5′-GCAATTCCTCACGA-GACCCGTCCTG-3′) (SEQ ID NO: 3) (Table S1). The oligonucleotide sequence complementary to the targeted genomic locus plus the AcuI motif has the following sequence: 5′-N(20)CTGAAGN(14)-3′ or 5′-N(15)CTGAAGN(14)-3′, with "N" corresponding to A, T, G or C bases complementary to the targeted locus. Reverse primers used in AcuI-tagging reactions were designed by Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) using the default parameters with the following changes: Mispriming library = "HUMAN" for amplifying from human genomic DNA or Mispriming library = "RODENT" for amplifying from mouse genomic DNA, Primer size "min = 25, Opt = 27, Max = 30", Primer Tm "Min = 57.0° C., Opt = 60.0° C., Max = 63.0° C.". Reverse primers are located >100 bp away from the targeted dinucleotides. All sequences of the primers used in this study are available in Table S1.

Adaptor Library Generation and Characterization

[0152] A set of 17 individual oligonucleotides constitutes the full adaptor library. This library contains: a) One constant oligonucleotide with the following sequence: 5′-CTGGGGCACGGGTAAGAAGCATTCTGTCTCTcttctaagaattcgagctcggtacccg-3′ (SEQ ID NO: 230). The lowercase nucleotide sequence located at the 3′-end of the constant oligonucleotide (5′-cttctaagaattcgagctcggtacccg-3′) (SEQ ID NO: 319) corresponds to the handle sequence used to detect the ligated products with either PB548 (5′-cgggtaccgagctcgaattc-3′) (SEQ ID NO: 2) or PB1073 (5′-cgggtaccgagctcgaattcttagaag-3′) (SEQ ID NO: 4); b) 16 variable oligonucleotides that contain a sequence complementary to the constant oligonucleotide plus one of 16 different dinucleotides at their 3′-end. The variable oligonucleotides have the following sequence: 5′-cgggtaccgagctcgaattcttagaagAGAGACAGAATGCTTCTTACCCGTGCCCCAGNN-3′. NN, with N = A, C, G or T (SEQ ID NOs: 231-246), corresponds to the dinucleotide that is different for each of the 16 oligos. The adaptor sequences are available in Table S1. The constant oligonucleotide and each variable oligonucleotide were resuspended at a concentration of 100 μM in H_2O. 2.5 μl of constant oligonucleotide and 2.5 μl of each variable oligonucleotide were mixed with 1X ligase buffer (ThermoFisher Scientific) and water in a 20 μl reaction. The reactions were placed in a thermocycler and oligonucleotides were annealed by incubating them for 5 min at 95° C., followed by a gradual temperature decrease from 95° C. to 15° C. After annealing was completed, 100 μl of water were added to dilute the adaptors in a 120 μl final volume. Adaptors were frozen and stored at -20° C.

[0153] The adaptor library was tested at two independent loci, as shown in FIG. **9**C. In this assay, AcuI-tagging oligonucleotides targeting the ampicillin resistance gene were designed following the rules detailed above (Table S1). First, we linearized the pUC19 plasmid as follows: 1.5 μg of pUC19, 1X CutSmart Buffer (NEB) and 0.75 μl of BamHI-HF were mixed in a 30 μl reaction and incubated for 2 hours at 37° C. The digested plasmid was subsequently purified on column (Zymoclean #D4008) and used as a template in PCR reactions with each AcuI-tagging primer and a constant reverse primer (5′-CCAATGCTTAATCAGTGAGG-3′) (SEQ ID NO: 320) located at the 3′-side of the ampicillin resistance gene. The PCRs were performed in a 25 μl reaction containing: 1 μM forward and reverse primers, 0.1 mM dNTP (NEB #N0447L), 1X Q5 buffer (NEB), 20 ng of digested pUC19, 1 unit of Q5 polymerase (NEB) and water. The PCR program used was the following: 95° C. for 1 min, 40 cycles of 95° C. for 10 s, 58° C. for 10 s, 72° C. for 45 s and a final amplification step of 1 min at 72° C. PCR reactions were loaded on a 2% agarose gel, extracted from gel and purified on column (Zymoclean #D4008). Finally, the DTECT protocol was applied as described below. Briefly, 0.5 pmol of AcuI-tagging PCR products were digested by AcuI for 30 min at 37° C. 10 μl of the digested products were purified with 18 μl of solid phase reversible immobilization magnetic beads (Beckman Coulter #A63881). 20 μl of supernatant (unbound fraction) were recovered and 0.5 μl of this supernatant were ligated using complementary and negative control adaptors for 1 hour at 25° C., followed by T4 ligase inactivation for 10 min at 65° C. The complementary and negative control adaptors used in FIG. **9**C are the following: AA #1 (Specific adaptor: TT, Non-specific adaptor: CC), AA #2 (TT, CC), AC #1 (GT, AC), AC #2 (GT, AA), AG #1 (CT, GA), AG #2 (CT, GA), AT #1 (AT, GG), AT #2 (AT, GG), CA #1 (TG, CA), CA #2 (TG, CA), CC #1 (GG, CC), CC #2 (GG, CC), CG #1 (CG, AA), CG #2 (CG, AA), CT #1 (AG, TT), CT #2 (AG, TT), GA #1 (TC, GA), GA #2 (TC, GA), GC #1 (GC, TT), GC #2 (GC, TT), GG #1 (CC, TT), GG #2 (CC, TT), GT #1 (AC, TG), GT #2 (AC, TG), TA #1 (TA, GG), TA #2 (TA, GG), TC #1 (GA, CT), TC #2 (GA, CT), TG #1 (CA, TG), TG #2 (CA, TG), TT #1 (AA, GG) and TT #2 (AA, GG). The ligated products were subsequently detected by PCR amplification using the primers PB547 (5′-gatcctctagagtcgacctg-3′) (SEQ ID NO: 1) and PB1073 (5′-cgggtaccgagctcgaattcttagaag-3′) (SEQ ID NO: 4). All primer sequences are listed in Table S1.

[0154] The measurement of the dinucleotide capture efficiency of each adaptor (FIGS. **2**J-**2**K) was determined by ligating the 16 different adaptors to annealed oligonucleotides containing complementary dinucleotides. To mimic the 5′ phosphorylation induced by AcuI in DTECT experiments, the reverse oligonucleotide (PB1449: 5′-gtagttcgccagttCTTCAGaatagtttgcgca CAGGACGGGTCTCGTGAGGAATTGC-3′) (SEQ ID NO: 91) was phosphorylated with PNK (NEB). The phosphorylation reaction was conducted as follows: 5 μl of PB1449 (100 μM), 4 μl of 5X ligase buffer, 0.5 μl of PNK in a 20 μl reaction. Phosphorylation was obtained upon incubation for 1 hour at 37° C., followed by heat inactivation of PNK for 20 min at 65° C. After incubation, the phosphorylated oligonucleotide PB1449 was annealed to 16 complementary oligonucleotides with the following sequence: 5′-GCAATTCCTCACGAGACCCGTCCTGTGCGCAAACTAT TCTGAAGAACTGGCGAACTACNN-3′ (SEQ ID NOs: 231-246). The two Ns indicate the dinucleotide that is different for each of the 16 oligos, with N = A, C, G or T. In the annealing reaction, 40 μl of 5X ligase buffer and 130 μl of H_2O were added to the phosphorylation reaction. 9.5 μl of this mix were used for annealing with 0.5 μl of each of the above 16 oligos (50 μM). Annealing, which was performed as described above for the library of adaptors, resulted in a 5′-phosphorylated double-stranded DNA with an overhang of 2 nucleotides, mimicking the product of AcuI digestion. The ligation between the adaptors and the phosphorylated products was performed as follows: 1 μl of annealed oligonucleotides, 2 μl of T4 ligase buffer, 0.5 μl of T4 ligase and 0.5 μl of adaptors in a 10 μl reaction. The ligation reaction was incubated for 1 hour at 25° C. and 10 min at 65° C. Detection was performed using qPCR as described below in the DTECT protocol.

[0155] The assay performed to measure the efficiency of DNA ligation (FIG. **10**F) was conducted in a master mix reaction equivalent to 5 μl per time point as follows: 0.5 μl of AcuI digested products, 1 μl of T4 ligase buffer and 0.5 μl of adaptors with or without 0.5 μl of T4 ligase. The reactions were incubated at 25° C. After 5 min, 5 μl were taken from the reaction and the T4 ligase was added for 10 min at 65° C. 1 hour after the start of the ligation reaction, 5 μl were additionally taken from the reaction and heat inactivated. The rest of the reaction was incubated overnight for 16 hours and heat inactivated. The amount of products captured was determined by qPCR as described below.

[0156] To calculate the frequency of non-specific dinucleotide capture shown in FIG. **10**E, AcuI-generated fragments of WT SMARCAL1, SPRTN and PIK3R1 amplicons

(obtained as described below) were ligated to each of the 16 library adaptors under the adaptor ligation conditions described above. The frequency of non-specific dinucleotide capture for all the adaptors non-complementary to the SMARCAL1, SPRTN and PIK3R1 dinucleotide signatures was calculated by qPCR analysis, as described below. Adaptors complementary to +1 and -1 AcuI-dependent slippage events were excluded from the analysis.

DTECT Protocol

[0157] The DTECT protocol consists of 6 steps (I-VI, FIG. **1**A). I) Design of the AcuI-tagging primer, as described above. II) Amplification of the genomic locus of interest using the AcuI-tagging primer. The genomic DNA (gDNA) is prepared using the Quick Extract Solution (Epicentre) by incubating the cells at 65° C. for 10 min and 95° C. for 5 min. The genomic DNA is quantified by Nanodrop, diluted to 200 ng/μl in H_2O and stored at -20° C. or immediately used in PCR reactions. PCRs were performed in a 25 μl or 50 μl solution containing: 1 μM forward and reverse primers, 0.1 mM dNTP (NEB #N0447L), 1X Q5 buffer (NEB), 10-200 ng of gDNA, 1 unit of Q5 polymerase (NEB) and water. PCR reactions were conducted as follows: 95° C. for 30 s; 40 cycles of 95° C. for 10 s, 58° C. for 10 s, 72° C. for 45 s; and final amplification at 72° C. for 1 min. When the AcuI-tagging PCR did not work on gDNA (<5% of the cases), a PCR using standard locus-specific primers was performed to amplify the targeted locus and the AcuI-tagging PCR was conducted using this amplicon as template DNA. PCR products were loaded on a 2% agarose gel and run in TAE buffer. PCR products were extracted from gel and column purified (Zymo Research #D4008) and the purified products were subsequently quantified using Nanodrop. III) Digestion of the AcuI-tagged genomic amplicon with AcuI. The purified PCR products were digested by 0.25 μl AcuI (NEB #0641L) in a 20 μl reaction containing 1X CutSmart Buffer (NEB) supplemented with 40 μM S-adenosylmethionine (SAM) and 100 ng of purified PCR product. The reaction was incubated for 1 hour at 37° C. with heat inactivation at 65° C. for 20 min. IV) Isolation of the AcuI-digested genomic amplicon by solid phase reversible immobilization (SPRI). 10 μl of the digestion reaction were subsequently mixed with 18 μl of Agencourt AMPure XP magnetic beads (Beckman Coulter #A63881) by pipetting up and down the beads 10 times (volume ratio of DNA:beads = 1:1.8) and then incubated at room temperature for 5 min. This procedure resulted in the binding of the larger digestion fragment (>100 bp) to the beads, while the smaller digested fragment (60 bp) remained in the supernatant. After incubation, the supernatant was isolated using a magnetic rack. 20 μl of the supernatant were recovered, diluted in 40 μl of H_2O and stored at -20° C. or immediately used for capture with DNA adaptors. V) Capture of the digested 60 bp-long products using DNA adaptors. The purified 60 bp-long DNA fragments were ligated to DNA adaptors generated as described above. The adaptors and the purified products were ligated in the following reaction: 6.5 μl of water, 2 μl of 5X ligase buffer (ThermoFisher Scientific), 0.5 μl of T4 ligase (ThermoFisher Scientific), 0.5 μl of adaptors and 0.5 μl of purified DNA product. The ligation reaction was performed for 1 hour at 25° C. in a thermocycler, followed by inactivation of the T4 ligase for 10 min at 65° C. The ligated products were stored at -20° C. or used directly for detection of the captured material. VI) Analytical or quantitative detection of the captured DNA products by PCR amplification. For analytical detection, the amplification of the captured material was performed by PCR in a 12.5 or 25 μl reaction volume containing 0.5 μM forward and reverse primers, 0.05 mM dNTP (NEB #N0447L), 1X Q5 buffer (NEB), 0.5-1 μl of ligated product, 0.1-0.2 μl of Q5 polymerase (NEB), 0.5-1 μl ligation reaction and water. PCR primers (PB1072 and PB1073) contained sequences complementary to the adaptor and handle (see above). The PCR program used was the following: 95° C. for 1 min, and different number of cycles (indicated in each figure legend) of 95° C. for 10 s, 65° C. for 5 s, 72° C. for 7 s. Detection of low abundant genomic variants (≤1% frequency) was generally obtained with 23-25 PCR cycles, while detection of greater amounts of edited products was achieved with 17-22 PCR cycles. 5 μl of the PCR reactions were incubated with SYBR Gold (Thermofisher Scientific #S-11494), loaded on a 2% agarose gel and run in 1X TAE buffer until the DNA was separated. Gels were developed using LI-COR Odyssey. qPCR was performed using QuantStudio 3 (Applied Biosystems). qPCR reactions were performed as follows: 5 μl of 2X SYBR Gold master mix (ThermoFisher Scientific #4367659), 0.1 μl of forward and reverse primers (PB1072 and PB1073, 100 μM) and 1 μl of ligated products (diluted 1:100 in H_2O) in a 10 μl reaction. The PCR program used in the qPCR reaction was the following: 95° C. for 10 s and 40 cycles of 60° C. 30 s, 95° C. 15 s. Quantification of the frequency of genomic variants was conducted as described below (Quantification and Statistical Analysis section).

Next-Generation Sequencing

[0158] Samples for NGS were prepared by amplifying the edited regions of interest by PCR. Samples were sequenced by the Genome Sciences Facility at The Pennsylvania State College of Medicine or by Genewiz and the results were analyzed by Genewiz, or by using an R-based script of the Ciccia laboratory or CRISPResso2 (Clement et al., 2019). To ensure that no biases were introduced during DTECT assays, the AcuI-tagging amplicons for the BRCA1 and BRCA2 mutant samples were sequenced by NGS and analyzed using an R-based script. In this analysis, 7 sequences with >6000 reads were filtered out from the analysis due to incorrect sequence. The editing frequency from the NGS results were determined using the formula: ((Number of reads for the edited dinucleotide) / (total number of reads)) x 100. Oligonucleotides used for PCR amplifications, Illumina sequencing adaptors and indexes are listed in Table S1.

Quantification and Statistical Analysis

[0159] Technical duplicates of each sample were performed in each qPCR reaction. A standard curve to determine the concentration of the captured material was generated using predefined concentrations of a DTECT ligation product (FIG. **1**A, step V) cloned into the pCR-Blunt II-TOPO vector (ThermoFisher Scientific; B650 plasmid, Addgene #139333) and oligos PB1072 and PB1073 (Table S1). The calculated standard curve corresponds to a linear curve with the following parameters: $y = -3.3245x + 7.5504$ and $R^2 = 0.99819$. Quantification of the frequency of genomic variants was determined by calculating the mean Ct score (Mean Ct) of the two technical duplicates for each

sample. The concentration of the captured material for each sample was determined using the following formula: Concentration = 10^((Mean Ct -7.5504)/-3.3245). The relative abundance between WT and mutant signatures was determined as follows: $Frequency^{Mutant}$ = ($Concentration^{Mutant}$ / ($Concentration^{Mutant}$ + $Concentration^{WT}$)) x 100 and $Frequency^{WT}$ = ($Concentration^{WT}$ / ($Concentration^{Mutant}$ + $Concentration^{WT}$)) x 100.

Data and Code Availability

[0160] R-based scripts of the Ciccia laboratory for analysis of NGS reads and ClinVar datasets are available upon request. Raw NGS reads of edited DLD1 and NIH/3T3 cells, organoids and liver samples are available under accession SRP151111 in the Sequence Read Archive. NGS reads have been deposited into the NCBI database and are and are accessible as BioProject # PRJNA603357. All uncropped gels, raw qPCR data and Sanger sequencing reads are available in Mendeley (https://data.mendeley.com/datasets/gtkk6sthtw/draft?a=ca72630e-56eb-4e29-bcdb-158b2c7d4123).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
Subcloning Efficiency DH5α	ThermoFisher Scientific	1 8265-017
Chemicals, Peptides, and Recombinant Proteins		
Q5 High-Fidelity DNA polymerase	NEB	M0491L
T4 DNA ligase	ThermoFisher Scientific	15224017
AcuI	NEB	R0641L
rSAP	NEB	M0371L
SybrGold (for gel staining)	ThermoFisher Scientific	S-11494
SybrGold (for qPCR)	ThermoFisher Scientific	4367659
BamHI—HF	NEB	R3136S
dNTPs	NEB	N0447L
T4 Polynucleotide Kinase	NEB	M0201S
Critical Commercial Assays		
Agencourt AMPure XP magnetic beads	Beckman Coulter	A63881
Zymoclean gel DNA recovery kit	Zymo Research	D4008
Quick Extract DNA Extraction Solution	Epicentre	QE09050
Zero BLUNT II TOPO PCR Cloning kit	ThermoFisher Scientific	450245
Deposited Data		
Unprocessed images of gels	This disclosure, Mendeley Data	Raw gel images
Raw Sanger sequencing files	This disclosure, Mendeley Data	Sequences of BRCA1-2 edited cells; Repeated sequences
Raw NGS sequencing files	This disclosure, NCBI	BioProject # PRJNA603357
Raw and processed qPCR data	This disclosure, Mendeley Data	Raw and processed qPCR data
Raw and processed DTECT, ICE, EditR and NGS data	This disclosure, Mendeley Data	Quantification of BRCA1-2 variants by DTECT, ICE, EditR and NGS
Human: HEK293T	ATCC	CRL-11268
Human: DLD1	ATCC	CCL-221
Mouse: NIH/3T3	ATCC	CRL-1658
Mouse: C57BL/6N	Charles River	C57BL/6NCrl
Mouse: $Brca1^{S1598F/+}$	Shakya et al, 2011	N/A
Mouse: $Bard1^{S563F/+}$	Billing et al, 2018	N/A
Mouse: $Mx1Cre^{+}$;CD45.1	Mullally et al, 2010	N/A
Mouse: $Mx1\text{-}Cre^{+}$;CD45.2 $Jak2^{V617F/+}$	Mullally et al, 2010	N/A
Mouse: NRG	The Jackson Laboratory	007799
Primers for PCR	This disclosure	Table S1
Oligonucleotides for sgRNA cloning	This disclosure	Table S1
ssODNs (for HDR)	This disclosure	Table S1
Oligonucleotides for adaptors	This disclosure	Table S1
Plasmid: B52 (containing 2 empty sgRNAs-expressing cassettes)	Addgene	100708
pCMV-PE2	Addgene	132775
pCMV-BE3	Addgene	73021
DTECT — Plasmid for standard curve	This disclosure, Addgene	139333
pTOPO-SPRTN WT	This disclosure	N/A
pTOPO-SPRTN STOP	This disclosure	N/A
pTOPO-SMARCAL1 WT	This disclosure	N/A
pTOPO-SMARCAL1 STOP	This disclosure	N/A
pTOPO-PIK3R1 WT	This disclosure	N/A
pTOPO-PIK3R1 STOP	This disclosure	N/A
pX330-U6-Chimeric_BB-CBh-hSpCas9	Addgene	42230
pCDNA3-Flag::UbvG08 I44A, deltaGG	Addgene	74939
pU6-Sp-pegRNA-HEK3-CTT_ins	Addgene	132778
Plasmids expressing sgRNAs for base editing of FANCD2, BRCA1 and BRCA2	This disclosure, Addgene	139321-139332, and 139511
R Studio Desktop IDE 1.0.143	RStudio	https://www.rstud io.com
Bioconductor R packages	Bioconductor	https://www.bioc onductor.org
R 3.4.1	The R project for statistical computing	https://www.r-project.org
ClinVar database	NCBI	https://www.ncbi. nlm.nih.gov/clinv ar/
Li-COR Odyssey	N/A	https://www.licor. com/bio/products /imaging_system s/odyssey
q-PCR QuantStudio 3	Applied Biosystems	N/A

Example 2 Design of DTECT, A Detection Method Based on the Capture of Dinucleotide Signatures

[0161] In our detection method, we take advantage of the property of type IIS restriction enzymes to generate single-stranded DNA overhangs at a specific distance from their recognition motif. Based on the above property, we hypothesized that single-stranded DNA overhangs generated by digestion of genomic DNA sequences with type IIS restriction enzymes could be captured and identified using DNA adaptors containing overhangs complementary to the exposed DNA signatures (FIG. 1A). To identify type IIS enzymes with efficient and accurate endonuclease activity, we analyzed the properties of known type IIS enzymes. Restriction enzymes optimal for our method exhibit the following characteristics: a) they cleave far from their recogni-

tion motif, thus enabling the incorporation of non-complementary type IIS recognition motifs into PCR primers without disrupting genomic DNA amplification (FIGS. **1**A and **8**A); b) they bind a single recognition motif (Bath et al., 2002) (FIG. **8**A); and c) they possess highly specific endonuclease activity, therefore generating a limited number of cleavage byproducts due to slippage activity (Lundin et al., 2015) (FIG. **8**B). Among the >40 known type IIS endonucleases, only 6 enzymes cleave at a distance ≥14 bp from their recognition motif (AcuI, BpmI, BpuEI, BsgI, MmeI and NmeAIII) (FIG. **8**C). Of those enzymes, only AcuI and BpuEI have a single recognition motif, and AcuI exhibits the lowest slippage activity of the two enzymes (slippage byproducts: AcuI, 1.1%; BpuEI, 41.4%) (Lundin et al., 2015). In particular, upon DNA cleavage AcuI exposes a dinucleotide signature located 15/16 nucleotides away from its recognition site (FIG. **8**D). Based on the above considerations, AcuI is the most suitable restriction enzyme for our detection method.

[0162] In our approach, the genomic locus of interest is PCR-amplified using a locus-specific DNA primer (red) and a DNA oligonucleotide (AcuI-tagging primer) containing two regions of complementarity to the genomic locus (purple) interrupted by an AcuI recognition site (AcuI hairpin, green) positioned 14 bp upstream of a dinucleotide of interest (FIG. **1**A, steps I and II). Tagging of the genomic amplicon with an AcuI motif allows AcuI-mediated digestion of the sequence of interest on the 3′-side of the targeted dinucleotide. Upon AcuI-mediated digestion, the signature of the targeted dinucleotide becomes exposed (FIG. **1**A, step III). To proceed with a single DNA fragment containing the targeted dinucleotide, the larger DNA fragment (>100 bp) resulting from AcuI-mediated digestion is removed using solid phase reversible immobilization (SPRI) beads (FIG. **1**A, step IV) and the smaller DNA fragment (60 bp) containing the targeted dinucleotide is ligated to an adaptor with a 3′-overhang complementary to the exposed signature (FIG. **1**A, step V). The ligated DNA products are subsequently detected by analytical or quantitative PCR (qPCR) (FIG. **1**A, step VI). This method, which we named DTECT (Dinucleotide signaTurE CapTure), can be completed within 4-5 hours (FIG. **1**A). A common set of DNA primers that anneal to constant regions in the AcuI-digested fragments (blue) and the ligated adaptors (brown) is utilized in all DTECT experiments (FIG. **1**A, step VI), avoiding locus-specific amplification bias and variability in qPCR efficiency among distinct sets of samples. Considering the total number of 16 unique dinucleotides (2^4), a library of 16 distinct adaptors is sufficient to capture all dinucleotide signatures that can be generated by AcuI (FIG. **1**B). Given the possible use of positive and negative controls to determine the efficiency and specificity of dinucleotide capture (FIG. **1**C), DTECT provides a highly controlled assessment of successful and specific capture of dinucleotide signatures

Example 3 DTECT Efficiently Captures Dinucleotide Signatures Generated by AcuI-Mediated Digestion

[0163] To demonstrate the feasibility of DTECT, we designed two AcuI-tagging DNA primers flanking four adjacent bases (5′-TTGG-3′) on opposite DNA strands (TT and CC signatures, blue) (FIG. **2**A). Upon PCR amplification using AcuI-tagging primers and locus-specific DNA primers, the PCR amplicons were digested and ligated to adaptors with either complementary or non-specific 3′-overhangs (GG or AA). Detection of the ligated products by PCR, as described above, revealed that the GG and AA adaptors specifically captured the DNA fragments containing the CC and TT dinucleotides, respectively (FIG. **2**B). Sanger sequencing confirmed that the amplicons of the ligated DNA products had the expected genomic sequence (purple) adjacent to the AcuI motif (green) and the GG or AA adaptors (brown) (FIGS. **9**A-**9**B). Importantly, robust amplification of captured DNA products was observed only upon 1) capture of the AcuI-digested products with complementary adaptors (FIG. **2**B), 2) AcuI-mediated cutting and generation of 5′-phosphorylated DNA fragments (FIGS. **2**C-**2**D), and 3) DNA ligation by the T4 DNA ligase (FIG. **2**D). We additionally showed that each individual DNA base can be identified by designing 4 independent AcuI-tagging primers (2 on each DNA strand), thus enabling the capture of 4 distinct signatures per genomic DNA base (FIGS. **2**E-**2**F). This DTECT feature allows flexible AcuI-mediated cleavage of genomic DNA amplicons containing targeted DNA sequences. In additional studies, we confirmed that each of the 16 possible dinucleotide signatures generated by AcuI at two independent target sites can be efficiently captured using DNA adaptors containing complementary DNA overhangs (FIG. **9**C). Together, these studies establish DTECT as a rapid and efficient method to identify DNA bases through the capture of AcuI-induced dinucleotide signatures using a common and unique set of adaptors.

Example 4 DTECT Enables Specific and Sensitive Quantification of DNA Variants

[0164] Next, we examined whether DTECT can determine the relative abundance of DNA variants with distinct DNA signatures, including low abundance DNA variants. To this end, we transfected HEK293T cells with sgRNAs that introduce nonsense mutations into the SPRTN, PIK3R1 and SMARCAL1 genes using iSTOP, a CRISPR-mediated base editing approach that creates STOP codons within genes of interest (Billon et al., 2017) (FIG. **10**A). We then cloned both WT and mutant alleles, which differ by a single base change (C -> T) (FIG. **10**B), and subjected them to PCR amplification using a locus-specific DNA primer and an AcuI-tagging primer flanking the iSTOP-targeted DNA base (FIG. **10**C). The WT and edited PCR products were then mixed at different ratios (WT - STOP allele = 100-0, 99-1, 90-10, 75-25, 50-50, 25-75 or 10-90) and digested with AcuI. The resulting DNA fragments were then captured using adaptors complementary to WT (green) and STOP (purple) dinucleotide signatures (FIG. **10**A). Remarkably, qPCR analysis of the captured DNA fragments accurately determined the relative abundance of the WT and STOP alleles at the three loci indicated above (FIG. **2**G), demonstrating that DTECT can estimate the frequency of dinucleotide signatures in a mixed population with high precision, including variants with low abundance (1%) (FIG. **2**G). Low abundance STOP variants in SPRTN and PIK3R1 were also detectable by analytical PCR (FIGS. **2**H-**2**I and **10**C-**10**D), confirming the high sensitivity and accuracy of DTECT. Importantly, direct comparison of the 16 DTECT adaptors revealed comparable efficiency in the capture of oligonucleotides containing complementary dinucleotide signatures (FIGS. **2**J-**2**K). In addition, all adaptors exhibited low levels of non-specific capture background (mean =

0.325%, ranging from 0.16% to 0.876%) (FIG. **10**E). The above observations indicate that the adaptor ligation is conducted under optimal conditions, as confirmed by kinetic analysis of the adaptor ligation reaction (FIG. **10**F). Together, these findings demonstrate that DTECT captures dinucleotide variants and quantifies their relative abundance with high specificity and sensitivity.

Example 5 DTECT Accurately Identifies Genomic Changes Introduced by CRISPR-Dependent HDR, Base Editing and Prime Editing in Mammalian Cells

[0165] To examine the ability of DTECT to identify precise genomic changes introduced into mammalian cell populations, we utilized CRISPR-mediated HDR for generating various types of disease-related mutations using single-stranded oligodeoxynucleotides (ssODNs), including a cancer-associated frameshift mutation in TP53 (i.e., R209fs*6), a missense mutation in HBB (i.e., G6V) that causes sickle cell anemia, a small tandem duplication in BRCA2 (dupAGAAGAT) identified in breast cancer, and small insertions into JAK2 and EMX1 (Paulsen et al., 2017), two genes associated with myeloproliferative disorders and Kallmann syndrome, respectively. Three days after co-transfection of Cas9 with site-specific sgRNAs and ssODNs into HEK293T cells, we harvested the cellular genomic DNA and utilized DTECT to determine by analytical and quantitative PCR whether the desired changes were incorporated into the targeted chromosomal loci (FIG. **3**A). For comparison, a restriction fragment length polymorphism (RFLP) assay that monitors restriction sites disrupted or created by the above mutations in the targeted genomic loci was conducted in parallel. In these experiments, DTECT readily captured the specific signature of the mutant variants (FIGS. **3**B and **11**A-**11**C), while the RFLP assay either failed to detect or weakly detected the same mutant variants (FIGS. **11**F-**11**H). In addition, DTECT was able to discern the HDR stimulatory effect induced by i53 (FIGS. **3**B and **11**A-**11**B), a genetically-encoded 53BP1 inhibitor that was previously shown to increase the frequency of HDR events (Canny et al., 2018), indicating that DTECT can be employed to compare the editing levels between distinct experimental conditions. Importantly, DTECT also clearly determined which mutations failed to be incorporated by the HDR machinery (e.g., BRCA2 dupAGAAGAT), as confirmed by NGS analysis (FIGS. **11**D-**11**E). Next, to determine whether DTECT can identify precise genomic changes introduced by CRISPR-mediated base editing in mammalian cell populations, we used a cytidine base editor to install nonsense mutations into the Fanconi anemia-associated genes FANCD2, FANCM and SLX4, the DNA replication and circadian clock gene TIMELESS and the Treacher Collins syndrome gene TCOF1. These experiments showed that DTECT was able to capture the signatures of the newly introduced variants in all of the above genes (FIGS. **3**B and **11**I-**11**J). Finally, to test whether DTECT is also able to identify genomic signatures generated by prime editing, we transiently transfected into HEK293T cells a prime editor and a pegRNA to introduce a 3-bp insertion (CTT_ins) in the HEK3 locus (Anzalone et al., 2019). As shown in FIG. **3**B, DTECT specifically identified the newly created signature and quantified its frequency in the transfected cell population, indicating that DTECT is also suitable to identify prime editing events. The specificity and accuracy of the above DTECT studies was confirmed by both positive and negative controls (e.g., CG and TT adaptors in the control unedited sample of FIG. **3**B).

[0166] To further confirm the accuracy of DTECT in quantifying precision genome editing, we compared the frequency of editing events determined by either DTECT or NGS across 62 samples derived from human cells, mouse cells and intestinal organoids, which were modified using CRISPR-mediated HDR or base editing (Zafra et al., 2018). As shown in FIG. **3**C (left panel) and 12A, the frequencies of editing events obtained by DTECT and NGS were comparable (mean frequency: DTECT, 35.43%; NGS, 33.47%; $r = 0.9857$, $n = 62$), indicating that the quantification of precision genome editing by DTECT is accurate. Similar to NGS, DTECT is also accurate in the detection of less abundant (< 20% frequency) variants (mean frequency: DTECT, 5.41%; NGS, 5.06%, $r = 0.843$, $n = 33$) (FIG. **3**C, right panel). Together, these experiments demonstrate that DTECT precisely identifies and quantifies genetic variants introduced by precision genome editing in various biological systems.

[0167] Recent studies led to the development of Sanger sequencing-based methods, such as ICE (Synthego; https://ice.synthego.com/#/) or EditR (Kluesner et al., 2018), that enable the detection of genomic variants based on the deconvolution of chromatogram peaks. To compare DTECT with the above methods, we subjected to Sanger sequencing the genomic amplicons of 23 samples edited by precision genome editing. In these experiments, we used two primers annealing to opposite DNA strands to obtain independent sequencing duplicates of the same amplicons, and analyzed the Sanger sequencing reads using either ICE or EditR. Notably, ~10% of the sequencing reactions failed to generate high quality reads required for ICE or EditR, despite using high quality amplicons for sequencing (Mendeley dataset, Data availability section). Independent repeats using new genomic amplicons did not improve the sequencing outcome (Mendeley dataset, Data availability section). In addition, we noted that technical duplicates of Sanger sequencing reactions analyzed by ICE or EditR displayed lower levels of consistency relative to technical replicates of DTECT assays (FIG. **12**B). These studies indicate that DTECT displays greater robustness and reliability compared to Sanger-based detection methods, which heavily rely on the quality of Sanger sequencing reactions.

Example 6 DTECT Enables the Identification of Precision Genome Editing Events In Vivo

[0168] The modeling and correction of pathogenic mutations in adult mice is critical for the development of novel approaches to therapeutic intervention against cancer and other diseases (Chadwick et al., 2017; Gao et al., 2018; Levy et al., 2020; Ryu et al., 2018; Song et al., 2020; Villiger et al., 2018; Yin et al., 2016; Yin et al., 2014). To determine whether DTECT can determine editing levels in adult mouse tissue, we hydrodynamically delivered into the mouse liver (Tschaharganeh et al., 2014) a cytidine base editor and an sgRNA introducing the oncogenic Pik3ca E545K mutation (Zafra et al., 2018) (FIG. **3**D). We then used both DTECT and NGS to quantify the oncogenic Pik3ca signature in DNA samples derived from the edited livers of two mice.

DTECT analysis identified base editing events in the mouse liver at a ~1-2% frequency, comparable to the editing rates obtained by NGS (FIG. **3**E). This study revealed that DTECT can accurately quantify low abundance genetic variants introduced by precision genome editing in vivo.

Example 7 DTECT is Capable of Identifying Multiple Genome Editing Events Occurring within A Single Locus or Distinct Loci

[0169] The above studies indicate that DTECT can determine the identity of individual genomic changes. To examine whether DTECT can also identify complex sets of mutations, we employed CRISPR-dependent base editing to target two adjacent cytosines in the EMX1 locus that had previously been converted into four distinct dinucleotide combinations (i.e., CC, CT, TC or TT) by base editing (Komor et al., 2016) (FIG. **4**A). As shown in FIG. **4**A, DTECT readily distinguished each of the four combinations in an sgRNA-dependent manner, demonstrating that DTECT can identify a complex mixture of allelic variants. Furthermore, we also detected base editing byproducts (FIG. **13**A), suggesting that DTECT could be used to optimize conditions that reduce the formation of these byproducts (Komor et al., 2017; Wang et al., 2017). Additionally, to determine whether DTECT can be employed to monitor genomic changes at multiple loci, we simultaneously introduced two clinically relevant point mutations into two distinct genes (i.e., BRCA1 and BRCA2) (FIG. **4**B). As shown in FIG. **4**C, DTECT correctly identified these genomic changes, indicating that it can readily detect complex genome editing events occurring within single or multiple genomic loci.

Example 8 DTECT Expedites the Derivation of Marker-Free Cell Lines Carrying Clinically Relevant Mutations and Facilitates the Genotyping of Cellular and Animal Disease Models

[0170] Precision genome editing allows the modeling of clinically relevant gene variants. Given that DTECT enables the identification of newly created DNA signatures without requiring the insertion of markers or elaborate experimental design specific for each edited site, we tested whether DTECT could facilitate the generation of multiple cell lines harboring clinically relevant mutations. In particular, we focused our attention on mutations in the BRCA 1 and BRCA2 genes, which in heterozygosity can predispose women to the development of breast and/or ovarian cancer (Apostolou and Fostira, 2013), whereas in homozygosity can cause Fanconi anemia (Ceccaldi et al., 2016). More than 7,000 clinically associated SNVs have been identified in BRCA½, according to the ClinVar database, but efforts to characterize their functional impact and pathogenic potential have been limited in part due to the challenge of generating cell lines that carry such a large number of individual homozygous and heterozygous variants. To determine whether DTECT can facilitate the production of cell lines harboring clinically relevant BRCA½ SNVs, we expressed a cytidine base editor in HEK293T cells along with individual sgRNAs to generate 23 different BRCA½ mutations identified in patients with ovarian and breast cancers, as reported in ClinVar (FIGS. **5**A and **5**D). We then used DTECT to determine by analytical PCR which variants were introduced in the transfected cell populations and quantify the editing efficiency for each variant by qPCR (FIGS. **5**B-**5**C, **5**E-**5**F and **13**B-**13**C). The accuracy of DTECT in the quantification of the editing events was confirmed by NGS (FIGS. **5**B and **5**E). The above approach proved effective for rapidly identifying cell populations with high levels of editing. Upon isolation of single clones from edited cell populations (e.g., BRCA1 E638K mutant cells), we tested whether DTECT could be used for clone genotyping. Importantly, DTECT allowed rapid genotyping of multiple clones (FIG. **14**A) and accurately determined the genotype of each clone, including WT, homozygous and heterozygous mutant clones (FIGS. **5**G-**5**H), thus expediting the production of marker-free isogenic heterozygous and homozygous mutant cells.

[0171] Given the ability of DTECT to correctly determine the genotype of cellular clones, we then tested whether DTECT could also be applied to mouse genotyping. To this end, we obtained tail DNA samples from genetically engineered mice carrying knock-in mutations in Brca1 (S1598F) and its partner protein Bard1 (S563F) (Billing et al., 2018). As shown in FIGS. **5**I-**5**J and **14**B, DTECT accurately determined the genotype of 24 Bard1 S563F mutant mice and 16 Brca1 S1598F mutant mice. These findings indicate that DTECT can be employed to rapidly determine the genotype of genetically engineered mice, thus facilitating the derivation, maintenance and analysis of marker-free animal models.

Example 9 DTECT Identifies the Presence of Oncogenic Mutations in Cancer Mouse Models and Human Cancer Patient Samples

[0172] Precise and rapid detection of pathogenic variants in patients is critical for accurate diagnosis and personalized therapy. Given the ability of DTECT to identify genetic variants rapidly and accurately, we tested whether DTECT could be utilized to expedite the identification of pathogenic variants in pre-clinical and clinical settings. In particular, we examined whether DTECT could identify the presence of oncogenic variants in various biological systems. In our studies we focused our attention on the JAK2 V617F variant, which is present in the majority of patients with myeloproliferative neoplasm (MPN) (Levine et al., 2005). Mice transplanted with Jak2 V617F mutant bone marrow cells develop MPN and recapitulate the human disease (Mullally et al., 2010). Therefore, we analyzed the Jak2 V617F variant in the peripheral blood of mice transplanted with a mixture of bone marrow cells that do or do not carry an inducible Jak2 V617F variant (Bhagwat et al., 2014) (FIG. **15**A). As shown in FIGS. **15**B-**15**C, DTECT readily distinguished wild-type from V617F mutant Jak2 in the examined mouse blood samples, as detected using any of the four distinct Acul-tagging primers specific for the targeted bases. These experiments show that DTECT can identify oncogenic signatures of interest in mouse tissues in a marker-free manner, thus enabling the tracking of genetic variants in mouse models without requiring complex selection markers.

[0173] We next examined whether DTECT can identify the presence of specific oncogenic mutations in human samples from patients diagnosed with acute lymphoblastic leukemia (ALL), the most common form of childhood cancer (Inaba et al., 2013). Although most ALL patients respond to chemotherapy, ~20% suffer a relapse as a result of resistance

to chemotherapy (Bhojwani and Pui, 2013). Moreover, secondary genetic alterations that promote chemoresistance, including mutations in the NT5C2 gene (Tzoneva et al., 2018; Tzoneva et al., 2013), are found in a large fraction of ALL relapse cases (Dieck and Ferrando, 2019; Oshima et al., 2016). To test whether DTECT can identify these relapse-specific oncogenic signatures, we obtained matched DNA samples from the bone marrow of ALL patients at diagnosis and relapse and analyzed them for the presence of three common NT5C2 mutations (R238W, K359Q and R367Q) (FIGS. **6**A-**6**B). Remarkably, DTECT unambiguously detected the presence of oncogenic NT5C2 variants in all five patient samples (patient #1, R238W; patients #2, #4 and #5, R367Q; patient #3, K359Q) and accurately quantified their frequency in a manner comparable to NGS (FIGS. **6**B-**6**C and **15**D). Moreover, DTECT also identified the presence of the above NT5C2 variants in the patient-derived xenograft (PDX) models generated from these relapsed ALL patients (FIGS. **6**A and **6**D). These studies demonstrate that DTECT can identify oncogenic mutations of interest in PDX models and cancer patient samples.

Example 10 Discussion

[0174] In this study, we established DTECT as a sensitive method for the identification of genomic DNA signatures. In particular, we show that DTECT readily identifies precision genome editing events induced by CRISPR-dependent HDR, base editing and prime editing, including low abundance and complex genomic changes. In addition, we show that DTECT can be employed to identify pathogenic lesions of interest, such as oncogenic mutations, in cancer mouse models, PDXs, and cancer patient specimens. DTECT is a rapid (~4-5 hours) and easy-to-perform detection method that relies on standard molecular biology techniques (PCR, DNA digestion and ligation) and common laboratory reagents. This methodology is also not labor-intensive, given that it entails short periods (5-10 min) of sample processing followed by hands-free incubations. Importantly, DTECT assays utilize a unique and common set of adaptors that includes positive and negative controls to ensure specificity and accuracy. The ease, speed and cost efficiency by which DTECT identifies genetic variants in a wide variety of cellular and animal systems (e.g., cell lines, organoids, animal models, patient samples) should facilitate the generation and study of biological models of human diseases and expedite the detection of pathogenic variants for both pre-clinical and clinical applications.

[0175] Although highly robust, DTECT has three potential limitations. First, AcuI-induced dinucleotide byproducts can be generated if a genomic AcuI restriction site located in close proximity to the targeted dinucleotide is incorporated into the amplicon of the targeted locus. However, an analysis of the ClinVar database revealed that genomic AcuI sites occur relatively infrequently and 95% of clinically relevant variants (404,393 variants) are compatible with DTECT (FIGS. **16**A-**16**B). Second, dinucleotide byproducts may also occur due to AcuI slippage activity, resulting in the cleavage of DNA molecules 13 (-1) or 15 (+1), instead of 14, bases away from the AcuI recognition site. Nonetheless, we found that DTECT is able to identify AcuI slippage events, which occur mostly at position +1 relative to the standard AcuI cleavage site (Lundin et al., 2015) (FIG. **17**A). It is reasonable to anticipate that future optimization of AcuI architecture and improvements in the AcuI digestion protocol will limit its slippage activity. It is also important to note that AcuI byproducts resulting from either genomic AcuI motifs or AcuI slippage activity are easily predictable based on the sequence of the nucleotides flanking the targeted dinucleotide and they can be completely avoided by optimal design of the AcuI-tagging primer and appropriate adaptor selection, as shown in FIGS. **16**C and **17**B. Third, indel mutations formed at DSB sites generated by Cas nucleases in CRISPR-mediated HDR experiments can result in defective PCR amplification of indel-containing loci that have not undergone HDR and therefore cause an overestimation of the frequency of HDR events by DTECT (FIGS. **18**A and **18**B). However, given that the mutagenic spectrum of indel mutations induced by any sgRNA is predictable (Allen et al., 2018; Leenay et al., 2019; Shen et al., 2018; van Overbeek et al., 2016) (inDelphi web portal; https://indelphi.giffordlab.mit.edu/), the negative impact of indel mutations on DTECT-based quantification of CRISPR-mediated HDR events can be avoided by introducing the desired genomic changes in indel-free regions adjacent to CRISPR-induced cut sites (FIGS. **18**C and **18**D). This limitation does not affect the detection of CRISPR-mediated base editing and prime editing events, and naturally occurring genetic variants, which are accompanied by either very low frequency (Anzalone et al., 2019; Gaudelli et al., 2017; Komor et al., 2017; Yeh et al., 2018) or complete absence of DSB-induced indel formation, respectively.

[0176] In addition to its ease of use, speed and cost efficiency, DTECT has several advantages compared to other detection methods. A major benefit of DTECT is its versatility, which allows the detection and quantification of nucleotide substitutions, precise base insertions and deletions using the same small set of 16 predefined adaptors (FIGS. **1**B and **7**). Each editing event can be identified using 4 distinct signatures resulting from AcuI-mediated digestion of genomic DNA amplicons, indicating that the design of DTECT studies is flexible (FIGS. **2**E-**2**F and **15**B-**15**C). These features distinguish DTECT from strategies that employ allele-specific DNA oligonucleotides or probes to identify SNVs, which work with variable efficiency due to the competition between WT and mutant alleles and the number of variant DNA bases, thus requiring unique experimental design for the detection of each individual genetic variant. Given that both wild-type and mutant DNA signatures are captured from the same AcuI-digested PCR amplicon and that a common set of PCR primers is utilized for both analytical and quantitative detection of all variants (FIG. **1**A, step VI), DTECT exhibits limited technical variability across distinct experimental conditions. This aspect differentiates DTECT from Sanger sequencing-based detection methods, such as ICE and EditR, in which efficiency depends on the quality of the sequencing reads, which can vary greatly between sequencing platforms, samples and reactions (FIG. **12**B). In addition, DTECT displays greater sensitivity and flexibility compared to RFLP-based assays (FIGS. **11**A-**11**J) and exhibits similar precision to NGS (FIG. **3**C) at a lower cost and with a faster turnaround time (hours vs. days/weeks). Finally, DTECT directly identifies genetic variants independently of genomic markers, therefore enabling the analysis of scarless and marker-free cellular and animal models generated by precision genome editing. Given its ability to identify multiple independent genetic variants simultaneously (FIGS. **4**A-**4**C), DTECT

could expedite the generation of complex genomic changes, especially for genetic interaction studies, synthetic biology applications and molecular recording (Fahim Farzadfard, 2018).

[0177] The ability to model clinically relevant mutations in a marker-free manner is critical for assessing their potential pathogenicity, especially in the case of genes, such as BRCA1 and BRCA2, which have thousands of clinically-associated SNVs. Recent studies have led to the development of high-throughput saturation genome editing (SGE) to examine en masse the pathogenicity of BRCA1 variants (Findlay et al., 2018). Although highly useful for classifying BRCA1 SNVs, SGE requires the use of haploid cells and is therefore not compatible with the study of the functional impact of BRCA1 mutations in heterozygosity, as observed in BRCA1 mutation carriers (Apostolou and Fostira, 2013). BRCA½ heterozygous mutations have been recently shown to cause genome instability induced by DNA replication stress (Billing et al., 2018; Pathania et al., 2014; Tan et al., 2017). By facilitating the derivation of both heterozygous and homozygous BRCA½ mutant cells and animal models (FIGS. **5**A-**5**J), DTECT could help elucidate the underlying mechanisms by which genome instability causes breast and ovarian cancer development in BRCA½ mutation carriers. Our work demonstrated that DTECT can expedite the generation of a large variety of human genetic variants in various complex biological systems.

[0178] In addition to facilitating precision genome editing, we showed that DTECT can also be used to detect pathogenic variants in pre-clinical and clinical settings. In particular, DTECT can rapidly identify the presence of oncogenic variants in cancer mouse models (FIGS. **15**A-**15**D), thus facilitating the study of cancer pathogenesis and the development of novel cancer therapies. Furthermore, DTECT can also identify oncogenic mutations in samples from cancer patients and PDX mouse models (FIGS. **6**A-**6**D). The speed by which DTECT accurately and unambiguously identifies pathogenic variants could accelerate cancer diagnosis and expedite the testing of cancer therapies in PDX models, thus leading to more effective cancer treatments. We envision that future developments and implementations of the DTECT protocol may further simplify the detection of desired genomic signatures and increase the sensitivity of DTECT, thus expanding the number of possible DTECT applications and enabling early diagnosis of cancer and hereditary disorders through the detection of pathogenic variants in circulating cell-free tumor and fetal DNA (Zhang et al., 2019).

[0179] Collectively, our work established DTECT as a facile, rapid and cost-effective method for identifying genomic variants in various biological systems, such as mammalian cell lines, organoids, mouse tissues, PDX models and human patient samples. Given the growing number of genetic variants identified in the human population (Lek et al., 2016) and in human genetic disorders (McClellan and King, 2010), this versatile method for the detection of genomic signatures should facilitate the study of human genetic variation and expedite the diagnosis and treatment of human disease.

TABLE S1

Primers, ssODNs, adaptors and other oligos used in this disclosure.

Detection primers	Sequence (5′- -> 3′)	Notes
PB547	gatcctctagagtcgacctg (SEQ ID NO: 1)	Oligos for detection (step VI)
PB548	cgggtaccgagctcgaattc (SEQ ID NO: 2)	Oligos for detection (step VI)
PB1072	gcaattcctcacgagacccgtcctg (SEQ ID NO: 3)	Oligos for detection (step VI) - Only these oligos were used for qPCR
PB1073	cgggtaccgagctcgaattcttagaag (SEQ ID NO: 4)	Oligos for detection (step VI) - Only these oligos were used for qPCR

Acultagging primers	Sequence (5′- -> 3′): Handle for detection-gDNA-AcuI hairpin-gDNA	Notes
PB1021	gatcctctagagtcgacctgG-GAGTCCCTGTCGCTAGTGGCT-GAAGACGCGTCGTGGGAG (SEQ ID NO: 5)	AcuI for signature TT
PB1022	gatcctctagagtcgacctgACAAA-CAGTGCCTGCAAGTCCT-GAAGCGGTGTGGGGTCCA (SEQ ID NO: 6)	AcuI for signature CC
PB1071	GCAATTCCTCACGA-GACCCGTCCTGATTTCAGG-GAAGAAGCTGAAGTGAAT-GAAAAACTT (SEQ ID NO: 7)	AcuI for PIK3R1-STOP
PB1153	GCAATTCCTCACGA-GACCCGTCCTGTGTAGTTT-TACTTACCTGAAGTCTCGTCTC-CACAG (SEQ ID NO: 8)	AcuI for JAK2 (HDR)
PB1151	GCAATTCCTCACGA-GACCCGTCCTGAGGACATC-GATGTCACTGAAGCCTCCAAT-GACTAG (SEQ ID NO: 9)	AcuI for EMX1 (HDR)
PB1019	gatcctctagagtcgacctgAAACGGCA-GAAGCTGGAGGACTGAAGG-GAAGGGCCTGAGT (SEQ ID NO: 10)	AcuI for EMX1 (Base editing)
PB1080	GCAATTCCTCACGA-GACCCGTCCTGGTTCAGTT-TAACGACCTGAAG-CAATTCTTCTGGGG (SEQ ID NO: 11)	AcuI for SPRTN-STOP
PB1149	GCAATTCCTCACGA-GACCCGTCCTGTGTGTTCAC-TAGCAACTGAAGCCTCAAACA-GACAC (SEQ ID NO: 12)	AcuI for HBB (HDR)
PB1211	GCAATTCCTCACGA-GACCCGTCCTGGAGGAG-GAGGCCCCTCTGAAGGCAGG-GACACGAAG (SEQ ID NO: 13)	AcuI for TCOF1 (Base editing)
oligo plate	GAT CCT CTA GAG TCG ACC TGC CAA ATT ATA TAC CTT TTG GCT GAA GTT ATA TCA TTC TTA (SEQ ID NO: 14)	BRCA1 C64Y AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGT CTT CAC TGC TAG AAC AAC TCT GAA GAT CAA TTT GCA ATT (SEQ ID NO: 15)	BRCA1 E638K AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGA TAT TGC TTG AGC TGG CTT CCT GAA GTT TAA AAA CAT TTT (SEQ ID NO: 16)	BRCA1 E1033K AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGG GTT CAG CTT TCG TTT TGA ACT GAA GAG CAG ATT CTT TTT (SEQ ID NO: 17)	BRCA1 E575K AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGT CCT CTA GCA GAT TTT TCT TCT GAA GAC ATT TAG TTT TAA (SEQ ID NO: 18)	BRCA1 V990I AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGG GAA AGA ATG AGT CTA	BRCA1 T922I AcuI

TABLE S1-continued

Acultag-ging primers	Sequence (5′- -> 3′): Handle for detection-gDNA-AcuI hairpin-gDNA	Notes
	ATA TCT GAA GCA AGC CTG TAC AGA (SEQ ID NO: 19)	
oligo plate	GAT CCT CTA GAG TCG ACC TGC ATC ATT ACC AAA TTA TAT ACT GAA GCC TTT TGG TTA TAT (SEQ ID NO: 20)	BRCA1 D67N AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGG AGG GAG GGA GCT TTA CCT TCT GAA GTC TGT CCT GGG ATT (SEQ ID NO: 21)	BRCA1 E1754K AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGG AAG AAA ATA ATC AAG AAG ACT GAA GGC AAA GCA TGG ATT (SEQ ID NO: 22)	BRCA1 S1363L AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGG CAG TGA TTT TAC ATC TAA ACT GAA GTG TCC ATT TTA GAT (SEQ ID NO: 23)	BRCA1 Q1779* AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGG ATG GAG AAG ACA TCA TCT GCT GAA GGA TTA TAC ATA TTT (SEQ ID NO: 24)	BRCA2 R2842C AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGT GAA TCT TTT TCT TTT TTT GCT GAA GAA TAG CTT ACA ATA (SEQ ID NO: 25)	BRCA2 R2973H AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGC TGA GTA TTT GGC GTC CAT CCT GAA GAT CAG ATT TAT ATT (SEQ ID NO: 26)	BRCA2 S2998F AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGC AAA TTT TTA GAT CCA GAC TCT GAA GTC AGC CAT CTT GTT (SEQ ID NO: 27)	BRCA2 S3070F AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGA GTG CAA ATT AAT TTA CCT TCT GAA GTA ACA TAA GAG ATT (SEQ ID NO: 28)	BRCA2 E2772K AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGG GAA TAT TTG ATG GTC AAC CCT GAA GAG AAA GAA TAA ATA (SEQ ID NO: 29)	BRCA2 T1707I AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGA TCT TGT TCT GAG GTG GAC CCT GAA GTA ATA GGA TTT GTC (SEQ ID NO: 30)	BRCA2 V3079I AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGT AGG AAG GCC ATG GAA TCT GCT GAA GCT GAA CAA AAG GAA (SEQ ID NO: 31)	BRCA2 Q2960* AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGA ACT GAA GCC TCT GAA AGT GCT GAA GAC TGG AAA TAC ATA (SEQ ID NO: 32)	BRCA2 T544I AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGT TTA CCA TCA CGT GCA CTA ACT GAA GCA AGA CAG CAA GTT (SEQ ID NO: 33)	BRCA2 R2896C AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGT GGA AGC TGG CCA GCC ACC ACT GAA GCC ACA CAG AAT TCT (SEQ ID NO: 34)	BRCA2 V572I AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGT TGC CTC TAG AAA TCA TGA CCT GAA GTA GGT TTG ACA GAA (SEQ ID NO: 35)	BRCA2 V778I AcuI
oligo plate	GAT CCT CTA GAG TCG ACC TGT TTC TCT TAT CAA CAC GAG GCT GAA GAA GTA TTT TTG ATA (SEQ ID NO: 36)	BRCA2 V2102I AcuI
AA1	GAT CCT CTA GAG TCG ACC TGC AAA CGA CGA GCG TGA CAC CCT GAA GAC GAT GCC TGT AGC (SEQ ID NO: 37)	For adaptor library testing
AA2	GAT CCT CTA GAG TCG ACC TGT CGT TGG GAA CCG GAG CTG ACT GAA GAT GAA GCC ATA CCA (SEQ ID NO: 38)	For adaptor library testing

TABLE S1-continued

Acultag-ging primers	Sequence (5′- -> 3′): Handle for detection-gDNA-AcuI hairpin-gDNA	Notes
AC1	GAT CCT CTA GAG TCG ACC TGG AGC TGA ATG AAG CCA TAC CCT GAA GAA ACG ACG AGC GTG (SEQ ID NO: 39)	For adaptor library testing
AC2	GAT CCT CTA GAG TCG ACC TGG CTG AAT GAA GCC ATA CCA ACT GAA GAC GAC GAG CGT GAC (SEQ ID NO: 40)	For adaptor library testing
AG1	GAT CCT CTA GAG TCG ACC TGG AAC CGG AGC TGA ATG AAG CCT GAA GCA TAC CAA ACG ACG (SEQ ID NO: 41)	For adaptor library testing
AG2	GAT CCT CTA GAG TCG ACC TGT ACC AAA CGA CGA GCG TGA CCT GAA GAC CAC GAT GCC TGT (SEQ ID NO: 42)	For adaptor library testing
AT1	GAT CCT CTA GAG TCG ACC TGT GAA GCC ATA CCA AAC GAC GCT GAA GAG CGT GAC ACC ACG (SEQ ID NO: 43)	For adaptor library testing
AT2	GAT CCT CTA GAG TCG ACC TGA AAC GAC GAG CGT GAC ACC ACT GAA GCG ATG CCT GTA GCA (SEQ ID NO: 44)	For adaptor library testing
CA1	GAT CCT CTA GAG TCG ACC TGG ATC GTT GGG AAC CGG AGC TCT GAA GGA ATG AAG CCA TAC (SEQ ID NO: 45)	For adaptor library testing
CA2	GAT CCT CTA GAG TCG ACC TGA GCT GAA TGA AGC CAT ACC ACT GAA GAA CGA CGA GCG TGA (SEQ ID NO: 46)	For adaptor library testing
CC1	GAT CCT CTA GAG TCG ACC TGC TGA ATG AAG CCA TAC CAA ACT GAA GCG ACG AGC GTG ACA (SEQ ID NO: 47)	For adaptor library testing
CC2	GAT CCT CTA GAG TCG ACC TGA GCC ATA CCA AAC GAC GAG CCT GAA GGT GAC ACC ACG ATG (SEQ ID NO: 48)	For adaptor library testing
CG1	GAT CCT CTA GAG TCG ACC TGA CCG GAG CTG AAT GAA GCC ACT GAA GTA CCA AAC GAC GAG (SEQ ID NO: 49)	For adaptor library testing
CG2	GAT CCT CTA GAG TCG ACC TGA ATG AAG CCA TAC CAA ACG ACT GAA GCG AGC GTG ACA CCA (SEQ ID NO: 50)	For adaptor library testing
CT1	GAT CCT CTA GAG TCG ACC TGG CCA TAC CAA ACG ACG AGC GCT GAA GTG ACA CCA CGA TGC (SEQ ID NO: 51)	For adaptor library testing
CT2	GAT CCT CTA GAG TCG ACC TGT CAT GTA ACT CGC CTT GAT CCT GAA GGT TGG GAA CCG GAG (SEQ ID NO: 52)	For adaptor library testing
GA1	GAT CCT CTA GAG TCG ACC TGG GAG CTG AAT GAA GCC ATA CCT GAA GCA AAC GAC GAG CGT (SEQ ID NO: 53)	For adaptor library testing
GA2	GAT CCT CTA GAG TCG ACC TGG GAA CCG GAG CTG AAT GAA GCT GAA GCC ATA CCA AAC GAC (SEQ ID NO: 54)	For adaptor library testing
GC1	GAT CCT CTA GAG TCG ACC TGA ACC GGA GCT GAA TGA AGC CCT GAA GAT ACC AAA CGA CGA (SEQ ID NO: 55)	For adaptor library testing
GC2	GAT CCT CTA GAG TCG ACC TGA AGC CAT ACC AAA CGA CGA GCT GAA GCG TGA CAC CAC GAT (SEQ ID NO: 56)	For adaptor library testing
GG1	GAT CCT CTA GAG TCG ACC TGA CGA CGA GCG TGA CAC CAC GCT GAA GAT GCC TGT AGC AAT (SEQ ID NO: 57)	For adaptor library testing
GG2	GAT CCT CTA GAG TCG ACC TGA GCA ATG GCA ACA ACG	For adaptor library testing

TABLE S1-continued

Acultagging primers	Sequence (5′- -> 3′): Handle for detection-gDNA-AcuI hairpin-gDNA	Notes
	TTG CCT GAA GGC AAA CTA TTA ACT (SEQ ID NO: 58)	
GT1	GAT CCT CTA GAG TCG ACC TGC CGG AGC TGA ATG AAG CCA TCT GAA GAC CAA ACG ACG AGC (SEQ ID NO: 59)	For adaptor library testing
GT2	GAT CCT CTA GAG TCG ACC TGC ATA CCA AAC GAC GAG CGT GCT GAA GAC ACC ACG ATG CCT (SEQ ID NO: 60)	For adaptor library testing
TA1	GAT CCT CTA GAG TCG ACC TGC TTG ATC GTT GGG AAC CGG ACT GAA GGC TGA ATG AAG CCA (SEQ ID NO: 61)	For adaptor library testing
TA2	GAT CCT CTA GAG TCG ACC TGA TAC CAA ACG ACG AGC GTG ACT GAA GCA CCA CGA TGC CTG (SEQ ID NO: 62)	For adaptor library testing
TC1 (P-B1040)	GAT CCT CTA GAG TCG ACC TGc cgc ttt ttt gca caa cat gCT GAA Ggg gga tca tgt aac (SEQ ID NO: 63)	For adaptor library testing
TC2	GAT CCT CTA GAG TCG ACC TGC GTT GCG CAA ACT ATT AAC TCT GAA GGG CGA ACT ACT TAC (SEQ ID NO: 64)	For adaptor library testing
TG1	GAT CCT CTA GAG TCG ACC TGC GGA GCT GAA TGA AGC CAT ACT GAA GCC AAA CGA CGA GCG (SEQ ID NO: 65)	For adaptor library testing
TG2 (P-B1070)	gat cct cta gag tcg acc tgc cat acc aaa cga cga gcg tCT GAA Gga cac cac gat gcc (SEQ ID NO: 66)	For adaptor library testing
TT1	GAT CCT CTA GAG TCG ACC TGT GAC ACC ACG ATG CCT GTA GCT GAA GCA ATG GCA ACA ACG (SEQ ID NO: 67)	For adaptor library testing
TT2	GAT CCT CTA GAG TCG ACC TGG CCT GTA GCA ATG GCA ACA ACT GAA GCG TTG CGC AAA CTA (SEQ ID NO: 68)	For adaptor library testing
PB1477	GCAATTCCTCACGA-GACCCGTCCTGACCT-GAGTTCTTTCCCTGAAGCCA-CATCAGCGTGC (SEQ ID NO: 69)	FANCD2 AcuI
PB1257	GATCCTCTAGAGTC-GACCTGCCGCAGAGCTGA-GAAGTTATCTGAAGTGGCA-GAACAGCAT (SEQ ID NO: 70)	SMARCAL1 AcuI
PB1264	gatcctctagagtcgacctgGTTTTCATTT-CAGGGAAGAACTGAAGGT-GAATGAAAAACT (SEQ ID NO: 71)	PIK3R1 signatures
PB1265	gatcctctagagtcgacctgTCTCGTAC-CAAAAAGGTCCCCT-GAAGGTCTGCTGTATCTC (SEQ ID NO: 72)	PIK3R1 signatures
PB1266	gatcctctagagtcgacctgATCTCGTAC-CAAAAAGGTCCCT-GAAGCGTCTGCTGTATCT (SEQ ID NO: 73)	PIK3R1 signatures
PB1010	gatcctctagagtcgacctgTTTTCATTT-CAGGGAAGAAGCTGAAGT-GAATGAAAAACTT (SEQ ID NO: 74)	PIK3R1 signatures
PB1433	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacAA (SEQ ID NO: 75)	AA-Oligo to test dinucleotide capture efficiency (DTECT)
PB1434	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacAC (SEQ ID NO: 76)	AC-Oligo to test dinucleotide capture efficiency (DTECT)
PB1435	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacAG (SEQ ID NO: 77)	AG-Oligo to test dinucleotide capture efficiency (DTECT)
PB1436	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacAT (SEQ ID NO: 78)	AT-Oligo to test dinucleotide capture efficiency (DTECT)
PB1437	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacCA (SEQ ID NO: 79)	CA-Oligo to test dinucleotide capture efficiency (DTECT)
PB1438	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacCC (SEQ ID NO: 80)	CC-Oligo to test dinucleotide capture efficiency (DTECT)
PB1439	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacCG (SEQ ID NO: 81)	CG-Oligo to test dinucleotide capture efficiency (DTECT)
PB1440	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacCT (SEQ ID NO: 82)	CT-Oligo to test dinucleotide capture efficiency (DTECT)
PB1441	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacGA (SEQ ID NO: 83)	GA-Oligo to test dinucleotide capture efficiency (DTECT)
PB1442	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacGC (SEQ ID NO: 84)	GC-Oligo to test dinucleotide capture efficiency (DTECT)
PB1443	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacGG (SEQ ID NO: 85)	GG-Oligo to test dinucleotide capture efficiency (DTECT)
PB1444	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacGT (SEQ ID NO: 86)	GT-Oligo to test dinucleotide capture efficiency (DTECT)
PB1445	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacTA (SEQ ID NO: 87)	TA-Oligo to test dinucleotide capture efficiency (DTECT)
PB1446	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacTC (SEQ ID NO: 88)	TC-Oligo to test dinucleotide capture efficiency (DTECT)
PB1447	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacTG (SEQ ID NO: 89)	TG-Oligo to test dinucleotide capture efficiency (DTECT)
PB1448	GCAATTCCTCACGA-GACCCGTCCTGtgcgcaaactattCT-GAAGaactggcgaactacTT (SEQ ID NO: 90)	TT-Oligo to test dinucleotide capture efficiency (DTECT)
PB1449	gtagttcgccagttCTTCAGaatagtttgcg-caCAGGACGGGTCTCGTGAG-GAATTGC (SEQ ID NO: 91)	Complementary 5′-phosphorylated oligo
PB1321	GCAATTCCTCACGA-GACCCGTCCTGGTGGCTCCA-TAGGAACT-GAAGGTCTTTCTCTTGTT (SEQ ID NO: 92)	mouse Pik3ca (545) AcuI
PB1380	GCAATTCCTCACGA-GACCCGTCCTGTTATA-TACCTTTTGGCTGAAGTTATAT-CATTCTTA (SEQ ID NO: 93)	BRCA1 Cys64Tyr AcuI
PB1381	GCAATTCCTCACGA-GACCCGTCCTGACTGCTAGAA-CAACTCTGAAGATCAATTTG-CAATT (SEQ ID NO: 94)	BRCA1 Glu638Lys AcuI
PB1382	GCAATTCCTCACGA-GACCCGTCCTGGCTT-GAGCTGGCTTCCTGAAGTT-TAAAAACATTTT (SEQ ID NO: 95)	BRCA1 Glu1033Lys AcuI
PB1383	GCAATTCCTCACGA-GACCCGTCCT-GAGCTTTCGTTTTGAACTGAA-GAGCAGATTCTTTTT (SEQ ID NO: 96)	BRCA1 Glu575Lys AcuI
PB1386	GCAATTCCTCACGA-GACCCGTCCTGTAGCA-	BRCA1 Val990Ile AcuI

TABLE S1-continued

Acultag-ging primers	Sequence (5′- -> 3′): Handle for detection-gDNA-AcuI hairpin-gDNA	Notes
	GATTTTTCTTCTGAAGACATT-TAGTTTTAA (SEQ ID NO: 97)	
PB1388	GCAATTCCTCACGA-GACCCGTCCTGGAATGAGTC-TAATATCTGAAGCAAGCCTGTA-CAGA (SEQ ID NO: 98)	BRCA1 Thr922Ile AcuI
PB1389	GCAATTCCTCACGA-GACCCGTCCTGTTACCAAATTA-TATACTGAAGCCTTTTGGTTA-TAT (SEQ ID NO: 99)	BRCA1 Asp67Asn AcuI
PB1390	GCAATTCCTCACGA-GACCCGTCCTGAGGGAGCTT-TACCTTCTGAAGTCTGTCCTGG-GATT (SEQ ID NO: 100)	BRCA1 Glu1754Lys AcuI
PB1393	GCAATTCCTCACGA-GACCCGTCCTGAAATAATCAA-GAAGACTGAAGGCAAAGCATG-GATT (SEQ ID NO: 101)	BRCA1 Ser1363Leu AcuI
PB1394	GCAATTCCTCACGA-GACCCGTCCTGGATTTTACATC-TAAACTGAAGTGTCCATTTTA-GAT (SEQ ID NO: 102)	BRCA1 Gln1779Ter AcuI
PB1396	GCAATTCCTCACGA-GACCCGTCCTGAGAAGACAT-CATCTGCTGAAGGATTATACA-TATTT (SEQ ID NO: 103)	BRCA2 Arg2842Cys AcuI
PB1397	GCAATTCCTCACGA-GACCCGTCCTGCTTTTTCTTTT-TTTGCTGAAGAATAGCTTACAA-TA (SEQ ID NO: 104)	BRCA2 Arg2973His AcuI
PB1398	GCAATTCCTCACGA-GACCCGTCCTGTATTTGGCGTC-CATCCTGAAGATCAGATTTA-TATT (SEQ ID NO: 105)	BRCA2 Ser2998Phe AcuI
PB1399	GCAATTCCTCACGA-GACCCGTCCTGTTTTAGATCCA-GACTCTGAAGTCAGC-CATCTTGTT (SEQ ID NO: 106)	BRCA2 Ser3070Phe AcuI
PB1400	GCAATTCCTCACGA-GACCCGTCCTGAAATTAATT-TACCTTCTGAAGTAACATAAGA-GATT (SEQ ID NO: 107)	BRCA2 Glu2772Lys AcuI
PB1401	GCAATTCCTCACGA-GACCCGTCCTGATTTGATGGT-CAACCCTGAAGAGAAAGAA-TAAATA (SEQ ID NO: 108)	BRCA2 Thr1707Ile AcuI
PB1402	GCAATTCCTCACGA-GACCCGTCCTGGTTCT-GAGGTGGACCCTGAAGTAA-TAGGATTTGTC (SEQ ID NO: 109)	BRCA2 Val3079Ile AcuI
PB1403	GCAATTCCTCACGA-GACCCGTCCTGAGGCCATG-GAATCTGCTGAAGCTGAA-CAAAAGGAA (SEQ ID NO: 110)	BRCA2 Gln2960Ter AcuI
PB1405	GCAATTCCTCACGA-GACCCGTCCTGAAGCCTCT-GAAAGTGCTGAAGACTGGAAA-TACATA (SEQ ID NO: 111)	BRCA2 Thr544Ile AcuI
PB1406	GCAATTCCTCACGA-GACCCGTCCTGCTTATCAACAC-GAGGCTGAAGAAGTATTTTT-GATA (SEQ ID NO: 112)	BRCA2 Val2102Ile AcuI
PB1407	GCAATTCCTCACGA-GACCCGTCCTGCATCACGTG-CACTAACTGAAGCAAGACAG-CAAGTT (SEQ ID NO: 113)	BRCA2 Arg2896Cys AcuI
PB1408	GCAATTCCTCACGA-GACCCGTCCTGGCTGGCCAGC-CACCACTGAAGCCACACA-GAATTCT (SEQ ID NO: 114)	BRCA2 Val572Ile AcuI
PB1409	GCAATTCCTCACGA-GACCCGTCCTGTCTAGAAAT-CATGACCTGAAGTAGGTTTGA-CAGAA (SEQ ID NO: 115)	BRCA2 Val778Ile AcuI
PB1509	GCAATTCCTCACGA-GACCCGTCCTGG-CATTTTCTGCTGCTCTGAAGGT-GAAGAAAGCCCA (SEQ ID NO:	Bard1 S563F AcuI

TABLE S1-continued

Acultag-ging primers	Sequence (5′- -> 3′): Handle for detection-gDNA-AcuI hairpin-gDNA	Notes
	116)	
PB1513	GCAATTCCTCACGA-GACCCGTCCTGgagcggatagaga-caCTGAAGtatccatggtggtg (SEQ ID NO: 117)	Brca1 S1598F AcuI
PB1483	GCAATTCCTCACGA-GACCCGTCCTGTGTGCGAGTT-CAGGACTGAAGATCAC-CAAAAAAGT (SEQ ID NO:118)	NT5C2 R367Q AcuI
PB1486	GCAATTCCTCACGA-GACCCGTCCTGTTGGAGATCA-CATTTCTGAAGTTGGGGA-CATTTTA (SEQ ID NO:119)	NT5C2 K359Q AcuI
PB1493	GCAATTCCTCACGA-GACCCGTCCTGTTTCAGG-GAAAACTGCT-GAAGCCTTTGCTTCTGAG (SEQ ID NO:120)	NT5C2 R238W AcuI
PB1296	GCAATTCCTCACGA-GACCCGTCCTGTGATACT-GAAATTGACTGAAGTAGAAG-CAGAAGAT (SEQ ID NO:121)	BRCA2 dupAGAAGAT AcuI
PB1473	GCAATTCCTCACGA-GACCCGTCCTGGCCAGCGAGA-GATGGCTGAAGCAGAAAAGAA-GACT (SEQ ID NO: 122)	TIMELESS AcuI
PB1476	GCAATTCCTCACGA-GACCCGTCCTGGGG-CAGCGGGTGCCGCTGAAGGC-GAGGACGCTGAC (SEQ ID NO: 123)	SLX4 AcuI
PB1472	GCAATTCCTCACGA-GACCCGTCCTGACGTT-TACGGCCAGTCTGAAGTC-TACCCATTCGTT (SEQ ID NO: 124)	FANCM AcuI
PB1427	GCAATTCCTCACGA-GACCCGTCCTGGAAGCTCG-GAAAAGCCTGAAGGATC-CAGGTGCTGC (SEQ ID NO: 125)	FANCF AcuI
PB1430	GCAATTCCTCACGA-GACCCGTCCTGATGTAGAAT-TAAGAACTGAAGTCATGCCTC-CAGTT (SEQ ID NO: 126)	AcuI Apc.1529
PB1431	GCAATTCCTCACGA-GACCCGTCCTGCCCGGGG-CATTTCATCTGAAGCCCAG-GAGCTAGGT (SEQ ID NO: 127)	AcuI Apc.492
PB1318	GCAATTCCTCACGA-GACCCGTCCTGTTGA-GAGTCGCTCCACTGAAGTTGC-CAGCTCTGTT (SEQ ID NO: 128)	AcuI Apc.1405
PB1332	GCAATTCCTCACGA-GACCCGTCCTGAG-CATTTGGTTTTGACTGAAGAT-TATGGTGTCTGT (SEQ ID NO: 129)	AcuI Jak2 #1
PB1333	GCAATTCCTCACGA-GACCCGTCCTGCTGGCTTTACT-TACTCTGAAGCTCCTCTCCACA-GA (SEQ ID NO: 130)	AcuI Jak2 #2
PB1460	GCAATTCCTCACGA-GACCCGTCCTGAAG-CATTTGGTTTTGCTGAAGAAT-TATGGTGTCTG (SEQ ID NO: 131)	AcuI Jak2 #3
PB1461	GCAATTCCTCACGA-GACCCGTCCTGGCTGGCTT-TACTTACCTGAAGTCTCCTCTC-CACAG (SEQ ID NO: 132)	AcuI Jak2 #4
PB1545	GCAATTCCTCACGA-GACCCGTCCTGGAAG-CAGGGCTTCCTCT-GAAGTTCCTCTGCCATCA (SEQ ID NO: 133)	AcuI HEK3
PB1301	GCAATTCCTCACGA-GACCCGTCCTG-GAAATTTGCGTGTGGCTGAA-GAGTATTTGGATGAC (SEQ ID	AcuI TP53 R209fs delGA

TABLE S1-continued

Acultag-ging primers	Sequence (5′- -> 3′): Handle for detection-gDNA-AcuI hairpin-gDNA	Notes
	NO: 134)	
PB1535	GCAATTCCTCACGA-GACCCGTCCTGAACCAGACCT-CAGGCCTGAAGGGCTCA-TAGGGCAC (SEQ ID NO: 135)	AcuI TP53 delAG (PAM)

Standard PCR primers	Sequence (5′- -> 3′)	Notes
Ampicillin reverse	CCA ATG CTT AAT CAG TGA GG (SEQ ID NO: 136)	For adaptor library testing
AcuI-tagging oligo reverse	AAT CGC TTG ATC ACA GAT GTA TGT A (SEQ ID NO: 137)	PCR BRCA1 C64Y and BRCA1 D67N
AcuI-tagging oligo reverse	GAA GAC AAA ATA TTT GGG AAA ACC T (SEQ ID NO: 138)	PCR BRCA1 E638K and BRCA1 E575K
AcuI-tagging oligo reverse	TCT CGT TAC TGG AAG TTA GCA CTC T (SEQ ID NO: 139)	PCR BRCA1 E1033K and BRCA1 V990I
AcuI-tagging oligo reverse	ATT TCA CCA TCA TCT AAC AGG TCA T (SEQ ID NO: 140)	PCR BRCA1 T922I
AcuI-tagging oligo reverse	CAC CTC CTG CAT TCA AAA GAT TC (SEQ ID NO: 141)	PCR BRCA1 E1754K
AcuI-tagging oligo reverse	GCT GCT TCA CCT TAA ATA ACA AAA A (SEQ ID NO: 142)	PCR BRCA1 S1363L
AcuI-tagging oligo reverse	AGG GAC ATA TGG GAA AAA GAG TTA G (SEQ ID NO: 143)	PCR BRCA1 Q1779*
AcuI-tagging oligo reverse	TTA GAC CTG ATA TTT CTG TCC CTT G (SEQ ID NO: 144)	PCR BRCA2 R2842C
AcuI-tagging oligo reverse	ACC TCT ACT ACC TAT GTG GCT TGT G (SEQ ID NO: 145)	PCR BRCA2 R2973H
AcuI-tagging oligo reverse	GGT TTG TAC CGG TAG TTG TTG ATA C (SEQ ID NO: 146)	PCR BRCA2 S2998F and BRCA2 Q2960*
AcuI-tagging oligo reverse	AAA TAG CCC TGT ACA ATG AAA AGT AGA (SEQ ID NO: 147)	PCR BRCA2 S3070F and BRCA2 V30791
AcuI-tagging oligo reverse	TCA TAT ACG GCA GTA TGG TTA AGG T (SEQ ID NO: 148)	PCR BRCA2 E2772K
AcuI-tagging oligo reverse	GTG GCC CTA CCT CAA AAT TAT TAC T (SEQ ID NO: 149)	PCR BRCA2 T17071
AcuI-tagging oligo reverse	TAT CTA CCA TGT TTG AGT GAC CTG A (SEQ ID NO: 150)	PCR BRCA2 T5441 and BRCA2 V572I
AcuI-tagging oligo reverse	CTT CAT AAG TCA GTC TCA TCT GCA A (SEQ ID NO: 151)	PCR BRCA2 V2102I
AcuI-tagging oligo reverse	GTA CAG GAG GGA CAA AAA TAA AAC A (SEQ ID NO: 152)	PCR BRCA2 R2896C

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Standard PCR primers	Sequence (5′- -> 3′)	Notes
AcuI-tagging oligo reverse	CCT TAA CTA GCT CTT TTG GGA CAA T (SEQ ID NO: 153)	PCR BRCA2 V778I
PB1150	GAAAATAGACCAATAGGCAGA-GAGAGTC (SEQ ID NO: 154)	HBB PCR rev
PB1152	TGTCATTAAGAGAGAGACTTT-TATTATTCC (SEQ ID NO: 155)	EMX1 PCR rev
PB1154	ATCCATCTACCTCAGTTTCCTA-TATCTATC (SEQ ID NO: 156)	JAK2 PCR rev
PB783	CCCTTTCCTGTAAAAACAATA-TAAAAA (SEQ ID NO: 157)	PIK3R1 PCR rev
PB764	TTCTGGAAAATGGATCTAAAGC-TAATA (SEQ ID NO: 158)	TCOF1 PCR RFLP for
PB765	TCACAATTCGTAGTCCTACTTC-TACCT(SEQ ID NO: 159)	TCOF1 PCR RFLP rev
TP226	ACGTTGATGGCAGTTGCAGGTC (SEQ ID NO: 160)	JAK2 (HDR) for
TP227	CTGACAGAGTTGCTAGA-CACTGGGTTG (SEQ ID NO: 161)	JAK2 (HDR) rev
PB969	AACGATCTTCAATATGCTTAC-CAAG (SEQ ID NO: 162)	HBB PCR RFLP for
PB970	CTTAACCATAGAAAAGAAGGG-GAAA (SEQ ID NO: 163)	HBB PCR RFLP rev
PB327	GCCATCCCCTTCTGTGAATGTTA-GAC(SEQ ID NO: 164)	EMX1 PCR for
PB328	GGAGATTGGAGACACGGAGAG-CAG (SEQ ID NO: 165)	EMX1 PCR rev
PB1302	AACTGTGCAATAGTTAAACC-CATTTAC (SEQ ID NO: 166)	PCR TP53 (HDR)
PB862	GTAGGTGTTCGGTAAATGT-TAATGG (SEQ ID NO: 167)	PCR FANCD2
PB863	AAGTCAAATCCCATACCCTACT-CAT(SEQID NO: 168)	PCR FANCD2
PB1334	TACTTGCTTTCAGTGTTGTGTTA-TAGG(SEQ ID NO: 169)	PCR Jak2 (mouse)
PB1335	ATTTGTTTACTGTAATCCTCATC-CATC(SEQ ID NO: 170)	PCR Jak2 (mouse)
PB1319	GGAAAAGTTTA-TAGGTGTCCCTTCTAC (SEQ ID NO: 171)	PCR Apc.1405
PB1320	AGCAGGTGTACTTCTGTCAGCTC (SEQ ID NO: 172)	PCR Apc.1405
PB1432	AATATTCTGCAGACTGA-TATTCTGGTT(SEQ ID NO: 173)	PCR Apc.492
PB1428	CGTTACTTAATTTTGAAAAACCT-CAAC (SEQ ID NO: 174)	PCR FANCF
PB1429	AGATTTGGGTTCTCTCTATAGC-CATT (SEQ ID NO: 175)	PCR FANCF
PB745	GACTCCAGTCAAAAATTCTCC-TAGTTA(SEQ ID NO: 176)	PCR FANCM
PB858	ATGTCTGCAGCTATAGTTAG-GAAGC (SEQ ID NO: 177)	PCR SLX4
PB859	ATCTCTCCCTGAGTTGATGA-GAAG(SEQID NO: 178)	PCR SLX4
PB764	TTCTGGAAAATGGATCTAAAGC-TAATA (SEQ ID NO: 179)	PCR TCOF1
PB765	TCACAATTCGTAGTCCTACTTC-TACCT(SEQ ID NO: 180)	PCR TCOF1
PB746	CTGTTTGTCCTAAACAAGATGT-GAAT (SEQ ID NO: 181)	PCR TIMELESS
PB747	CATTGGAGCAAGTTAAAACTA-CAAAAT (SEQ ID NO: 182)	PCR TIMELESS
PB1297	CCTTAACCTCTTGATGTATGA-GAAGAA (SEQ ID NO: 183)	PCR BRCA2 dupAGAAGAT
PB1298	AGTACATCTAAGAAATTGAG-CATCCTT(SEQ ID NO: 184)	PCR BRCA2 dupAGAAGAT
PB590	GTGTGTGTGCAATTATAAAA-GAAACTT (SEQ ID NO: 185)	PCR SMARCAL1
PB591	GTCAGCATTAGATGAGCTACTGA-GATT(SEQ ID NO: 186)	PCR SMARCAL1
PB1322	CTGTTCTACTTGTTGGTGGTGA-TAATA (SEQ ID NO: 187)	PCR mouse Pik3ca (545)
PB1323	ATGGTAAGAAATATGGTTAACAC-	PCR mouse

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Standard PCR primers	Sequence (5′- -> 3′)	Notes
	CAAG (SEQ ID NO: 188)	Pik3ca (545)
PB1510	CTATTTTAGGTTACTGGGAACA-GAATG (SEQ ID NO: 189)	Oligos for Bard1 S563F genotyping
PB1511	AAACTACATAACTACAACC-CAATGCTT(SEQ ID NO: 190)	Oligos for Bard1 S563F genotyping
PB1514	GAACCCCATACCTGGGATCT (SEQ ID NO: 191)	Oligos for Brca1 S1598F genotyping
PB1515	tcatacctcacaaggtgccta (SEQ ID NO: 192)	Oligos for Brca1 S1598F genotyping
PB1548	TTATCAGTTTTGGAGGATGTACA-TAAA (SEQ ID NO: 193)	PCR HEK3 rev
PB780	CTCCTTCCTCTTCCTACAG-TACTCC(SEQID NO: 194)	TP53 gDNA for (PAM)

Illumina primers (NGS)	Sequence (5′- -> 3′)	Notes
	Primers for amplifying AcuI-tagged amplicons	
SAM175	ACACTCTTTCCCTACAC-GACGCTCTTCCGATC TTTCCTCACGAGACCCGTCCTG (SEQ ID NO: 195)	Adaptor constant forward - Forward primer used with all amplicons - binds AcuI-tagging primer sequence
SAM176	AGACGTGTGCTCTTCCGATCTCTT-GATCACAG ATGTATGTA (SEQ ID NO: 196)	NGS BRCA1 C64Y AcuI
SAM177	AGACGTGTGCTCTTCCGATCT-CAAAATATTTG GGAAAACCT (SEQ ID NO: 197)	NGS BRCA1 E638K AcuI
SAM178	AGACGTGTGCTCTTCCGATCTT-TACTGGAAGT TAGCACTCT (SEQ ID NO: 198)	NGS BRCA1 E1033K AcuI
SAM179	AGACGTGTGCTCTTCCGATCT-CAAAATATTTG GGAAAACCT (SEQ ID NO: 199)	NGS BRCA1 E575K AcuI
SAM182	AGACGTGTGCTCTTCCGATCTT-TACTGGAAGT TAGCACTCT (SEQ ID NO: 200)	NGS BRCA1 V990I AcuI
SAM184	AGACGTGTGCTCTTCCGATCTAC-CATCATCTA ACAGGTCAT (SEQ ID NO: 201)	NGS BRCA1 T922I AcuI
SAM185	AGACGTGTGCTCTTCCGATCTCTT-GATCACAG ATGTATGTA (SEQ ID NO: 202)	NGS BRCA1 D67N AcuI
SAM186	AGACGTGTGCTCTTCC-GATCTCTCCTGCATTC AAAAGATTC (SEQ ID NO: 203)	NGS BRCA1 E1754K AcuI
SAM189	AGACGTGTGCTCTTCCGATCTTT-CACCTTAAA TAACAAAAA (SEQ ID NO: 204)	NGS BRCA1 S1363L AcuI
SAM190	AGACGTGTGCTCTTCCGATCTCA-TATGGGAAA AAGAGTTAG (SEQ ID NO: 205)	NGS BRCA1 Q1779* AcuI
SAM192	AGACGTGTGCTCTTCCGATCTCCT-GATATTTC TGTCCCTTG (SEQ ID NO: 206)	NGS BRCA2 R2842C AcuI
SAM193	AGACGTGTGCTCTTCCGATCTTAC-TACCTATG TGGCTTGTG (SEQ ID NO: 207)	NGS BRCA2 R2973H AcuI
SAM194	AGACGTGTGCTCTTCCGATCTG-TACCGGTAGT TGTTGATAC (SEQ ID NO: 208)	NGS BRCA2 S2998F AcuI
SAM195	AGACGTGTGCTCTTCC-GATCTCCTGTACAATG AAAAGTAGA (SEQ ID NO: 209)	NGS BRCA2 S3070F AcuI
SAM196	AGACGTGTGCTCTTCCGATCT-TACGGCAGTAT GGTTAAGGT (SEQ ID NO: 210)	NGS BRCA2 E2772K AcuI

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Illumina primers (NGS)	Sequence (5′- -> 3′)	Notes
	Primers for amplifying AcuI-tagged amplicons	
SAM197	AGACGTGTGCTCTTCCGATCTCC-TACCTCAAA ATTATTACT (SEQ ID NO: 211)	NGS BRCA2 T1707I AcuI
SAM198	AGACGTGTGCTCTTCC-GATCTCCTGTACAATG AAAAGTAGA (SEQ ID NO: 212)	NGS BRCA2 V3079I AcuI
SAM199	AGACGTGTGCTCTTCCGATCTG-TACCGGTAGT TGTTGATAC (SEQ ID NO: 213)	NGS BRCA2 Q2960* AcuI
SAM201	AGACGTGTGCTCTTCCGATCTAC-CATGTTTGA GTGACCTGA (SEQ ID NO: 214)	NGS BRCA2 T544I AcuI
SAM202	AGACGTGTGCTCTTCCGATCT-TAAGTCAGTCT CATCTGCAA (SEQ ID NO: 215)	NGS BRCA2 V2102I AcuI
SAM203	AGACGTGTGCTCTTCCGATCTG-GAGGGACAA AAATAAAACA (SEQ ID NO: 216)	NGS BRCA2 R2896C AcuI
SAM204	AGACGTGTGCTCTTCCGATCTAC-CATGTTTGA GTGACCTGA (SEQ ID NO: 217)	NGS BRCA2 V572I AcuI
SAM205	AGACGTGTGCTCTTCCGATCTAC-TAGCTCTTT TGGGACAAT (SEQ ID NO: 218)	NGS BRCA2 V778I AcuI
SAM113	caagcagaagacggcatacga-gatTGCCTCTTgtgactgga gttcagacgtgtgctcttccgatct (SEQ ID NO: 219)	N711
SAM64	aatgatacggcgaccaccgagatctacacACTG-CATAacact ctttccctacacgacg (SEQ ID NO: 220)	S506
TP370	acactctttccctacacgacgctcttccgatctGTT-TAAACAGT GGAATTCTAGAGTCA (SEQ ID NO: 221)	BRCA2_NGS_F
TP371	agacgtgtgctcttccgatctTTTTTG-CAGCTGTGTCATC C (SEQ ID NO: 222)	BRCA2 NGS R
TP372	acactctttccctacacgacgctcttcc-gatctGCCCCTCCTC AGCATCTTAT (SEQ ID NO: 223)	TP53 NGS F
TP373	agacgtgtgctcttccgatctCT-TAACCCCTCCTCCCAG AG (SEQ ID NO: 224)	TP53 NGS R

ssODNs:	
Sequence (5′- -> 3′)	Targeted gene
TTCCTTAGTCTTTCTTTGAAGCAGCAAGTATGATGAG-CAAGCTTTCTCA CAAGCATTTGGTTTTAAATTATGGAGTATGTGTgtt-taaacCTGTGGAGACG AGAGTAAGTAAAACTACAGGCTTTCTAATGCCTTTCT-CAGAGCATCTGT TTTTGTTTATATAGAAAATTCAGTTTCAGGATCA (SEQ ID NO: 225)	JAK2
AAGAAGGGCTCCCATCACATCAACCGGTGGCG-CATTGCCACGAAGCA GGCCAATGGGGAGGACATCGATGTCACCTCCAAT-GACTAgtttaaacGGG TGGGCAACCACAAACCCACGAGGGCA-GAGTGCTGCTTGCTGCTGGCC AGGCCCCTGCGTGGGCCCAAGCTGGACTCTGGC-CACTCCC(SEQID NO: 226)	EMX1
TACATTTGCTTCTGACACAACTGTGTTCACTAG-CAACCTCAAACAGACA CAATGGTGCATCTGACTCCTGTCGAGAAGTCTGCCGT-TACTGCCCTGT GGGGCAAGGTGAACGTGGATGAAGTTGGTGGT-GAGGCCCTGGG (SEQ ID NO: 227)	HBB
TCTTAGGTCTGGCCCCTCCTCAGCATCTTATCC-GAGTGGAAGGAAATT TGCGTGTGGAGTATTTGGATGACAAACACTTTTCGT-CATAGTGTGGTTG TGCCCTATGAGCCGCCTGAGGTCTGGTTTG-	TP53 R209f-s*6

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ssODNs:

Sequence (5′- -> 3′)	Targeted gene
CAACTGGGGTCTCTGGG AGGAGGGGTTAAGGGTGGTTGT (SEQ ID NO: 228)	
TTGTTTAAACAGTGGAATTCTAGAGTCACACTTCC-TAAAATATGCATTTT TGTTTTCACTTTTAGATATGATACTGAAATTGATA-GAAGCAGAAGATAG	BRCA2 dupA-GA
AAGATCGGCTATAAAAAAGATAATGGAAAGGGATGA-CACAGCTGCAAA AACACTTGTTCTCTGTGTTTCTGACATAAT (SEQ ID NO: 229)	AGAT

Libray of adaptors:

Oligo	Sequence (5′- -> 3′)	Notes
PB984	CTGGGGCACGGGTAAGAAG-CATTCTGTCTCTCT TCTAAgaattcgagctcggtacccg (SEQ ID NO: 230)	Oligo corresponds to the constant strand of the adaptor
PB985	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGGG (SEQ ID NO: 231)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ GG, expected to ligate to CC
PB986	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGAG (SEQ ID NO: 232)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ AG, expected to ligate to CT
PB987	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGAA (SEQ ID NO: 233)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ AA, expected to ligate to TT
PB988	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGTG (SEQ ID NO: 234)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ TG, expected to ligate to CA
PB989	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGTA (SEQ ID NO: 235)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ TA, expected to ligate to TA
PB990	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGCG (SEQ ID NO: 236)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ CG, expected to ligate to CG
PB991	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGCA (SEQ ID NO: 237)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ CA, expected to ligate to TG
PB992	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGCT (SEQ ID NO: 238)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ CT, expected to ligate to AG
PB993	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGGA (SEQ ID NO: 239)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ GA, expected to ligate to TC
P-B1000	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGAC (SEQ ID NO: 240)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ AC, expected to ligate to GT
P-B1001	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGAT (SEQ ID NO: 241)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ AT, expected to ligate to AT
P-B1002	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGCC (SEQ ID NO: 242)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ CC, expected to ligate to GG
P-B1003	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGGC (SEQ ID NO: 243)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ GC, expected to ligate to GC
P-B1004	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGGT (SEQ ID NO: 244)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ GT, expected to ligate to AC
P-B1005	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGTC (SEQ ID NO: 245)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ TC, expected to ligate to GA
P-B1006	cgggtaccgagctcgaattcTTAGAAGAGA-GACAGAATG CTTCTTACCCGTGCCCCAGTT (SEQ ID NO: 246)	Oligo corresponds to the variable strand of the adaptor. It contains a 3′ TT, expected to ligate to AA

Oligos (sgRNAs cloning):

Oligo	Sequence (5′- -> 3′)	Target/Notes
oligo plate	CAC CGT ACA TAA AGG ACA CTG TGA (SEQ ID NO: 247)	BRCA1 C64Y for
oligo plate	CAC CGC AAT TCA GTA CAA TTA GGT (SEQ ID NO: 248)	BRCA1 E638K for
oligo plate	CAC CGA TTT TCT CTA ATG TTA TTA (SEQ ID NO: 249)	BRCA1 E1033K for
oligo plate	CAC CGT TTT TCG AGT GAT TCT ATT (SEQ ID NO: 250)	BRCA1 E575K for
oligo plate	CAC CGT TTT AAC AAA TGA CTT GAT (SEQ ID NO: 251)	BRCA1 V9901 for
oligo plate	CAC CGA GAC AGT TAA TAT CAC TGC (SEQ ID NO: 252)	BRCA1 T922I for
oligo plate	CAC CGT TAT ATC ATT CTT ACA TAA (SEQ ID NO: 253)	BRCA1 D67N for
oligo plate	CAC CGG GGA TTC TCT TGC TCG CTT (SEQ ID NO: 254)	BRCA1 E1754K for
oligo plate	CAC CGT GGA TTC AAA CTT AGG TAT (SEQ ID NO: 255)	BRCA1 S1363L for
oligo plate	CAC CGT TAG ATC AAC TGG AAT GGA (SEQ ID NO: 256)	BRCA1 Q1779* for
oligo plate	CAC CGA TAT TTC GCA ATG AAA GAG (SEQ ID NO: 257)	BRCA2 R2842C for
oligo plate	CAC CGA CAA TAC GCA ACT TCC ACA (SEQ ID NO: 258)	BRCA2 R2973H for
oligo plate	CAC CGT ATA TTC TCT GTT AAC AGA (SEQ ID NO: 259)	BRCA2 S2998F for
oligo plate	CAC CGG TTC TGA GGT GGA CCT AAT (SEQ ID NO: 260)	BRCA2 S3070F for
oligo plate	CAC CGG AGA TTC TGG GGC TTC AAG (SEQ ID NO: 261)	BRCA2 E2772K for

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Oligos (sgRNAs cloning):

Oligo	Sequence (5′- -> 3′)	Target/Notes
oligo plate	CAC CGT AAA TAC TGC AGA TTA TGT (SEQ ID NO: 262)	BRCA2 T1707I for
oligo plate	CAC CGA GAA ACG ACA AAT CCT ATT (SEQ ID NO: 263)	BRCA2 V30791 for
oligo plate	CAC CGA AGG AAC AAG GTT TAT CAA (SEQ ID NO: 264)	BRCA2 Q2960* for
oligo plate	CAC CGC ATA CTG TTT GCT CAC AGA (SEQ ID NO: 265)	BRCA2 T5441 for
oligo plate	CAC CGG CTA CAG AAT TCT GTG TGG (SEQ ID NO: 266)	BRCA2 V5721 for
oligo plate	CAC CGA CAG AAC ATC CTT GGA AGT (SEQ ID NO: 267)	BRCA2 V778I for
oligo plate	AAA CTC ACA GTG TCC TTT ATG TAC (SEQ ID NO: 268)	BRCA1 C64Y rev
oligo plate	AAA CAC CTA ATT GTA CTG AAT TGC (SEQ ID NO: 269)	BRCA1 E638K rev
oligo plate	AAA CTA ATA ACA TTA GAG AAA ATC (SEQ ID NO: 270)	BRCA1 E1033K rev
oligo plate	AAA CAA TAG AAT CAC TCG AAA AAC (SEQ ID NO: 271)	BRCA1 E575K rev
oligo plate	AAA CAT CAA GTC ATT TGT TAA AAC (SEQ ID NO: 272)	BRCA1 V990I rev
oligo plate	AAA CGC AGT GAT ATT AAC TGT CTC (SEQ ID NO: 273)	BRCA1 T922I rev
oligo plate	AAA CTT ATG TAA GAA TGA TAT AAC (SEQ ID NO: 274)	BRCA1 D67N rev
oligo plate	AAA CAA GCG AGC AAG AGA ATC CCC (SEQ ID NO: 275)	BRCA1 E1754K rev
oligo plate	AAA CAT ACC TAA GTT TGA ATC CAC (SEQ ID NO: 276)	BRCA1 S1363L rev
oligo plate	AAA CTC CAT TCC AGT TGA TCT AAC (SEQ ID NO: 277)	BRCA1 Q1779* rev
oligo plate	AAA CCT CTT TCA TTG CGA AAT ATC (SEQ ID NO: 278)	BRCA2 R2842C rev
oligo plate	AAA CTG TGG AAG TTG CGT ATT GTC (SEQ ID NO: 279)	BRCA2 R2973H rev
oligo plate	AAA CTC TGT TAA CAG AGA ATA TAC (SEQ ID NO: 280)	BRCA2 S2998F rev
oligo plate	AAA CAT TAG GTC CAC CTC AGA ACC (SEQ ID NO: 281)	BRCA2 S3070F rev
oligo plate	AAA CCT TGA AGC CCC AGA ATC TCC (SEQ ID NO: 282)	BRCA2 E2772K rev
oligo plate	AAA CAC ATA ATC TGC AGT ATT TAC (SEQ ID NO: 283)	BRCA2 T17071 rev
oligo plate	AAA CAA TAG GAT TTG TCG TTT CTC (SEQ ID NO: 284)	BRCA2 V30791 rev
oligo plate	AAA CTT GAT AAA CCT TGT TCC TTC (SEQ ID NO: 285)	BRCA2 Q2960* rev
oligo plate	AAA CTC TGT GAG CAA ACA GTA TGC (SEQ ID NO: 286)	BRCA2 T5441 rev
oligo plate	AAA CCC ACA CAG AAT TCT GTA GCC (SEQ ID NO: 287)	BRCA2 V572I rev
oligo plate	AAA CAC TTC CAA GGA TGT TCT GTC (SEQ ID NO: 288)	BRCA2 V7781 rev
PB776	CACCGAACTTcGAGATACAG-CAGAC (SEQ ID NO: 289)	PIK3R1 R348* for
PB777	AAACGTCTGCTGTATCTC-gAAGTTC (SEQ ID NO: 290)	PIK3R1 R348* rev
PB551	CACCGGGCCAGCTG-GAGGCCGTCG (SEQ ID NO: 291)	SPRTN Q60* for
PB552	AAACCGACGGCCTC-CAGCTGGCCC(SEQ ID NO: 292)	SPRTN Q60* rev
PB756	CACCGAGCcAGGTGAGGCCTG-GAGG (SEQ ID NO: 293)	TCOF1 Q290* for
PB757	AAACCCTCCAGGCCT-CACCTgGCTC (SEQ ID NO: 294)	TCOF1 Q290* rev
TP212	CACCGAATTATGGAG-TATGTGTCTG (SEQ ID NO: 295)	JAK2 HDR for
TP213	AAACCAGACACATACTCCA-TAATTC (SEQ ID NO: 296)	JAK2 HDR rev
PB963	CACCGATGGTGCATCT-GACTCCTG(SEQ ID NO: 297)	HBB E6V HDR for

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Oligos (sgRNAs cloning):

Oligo	Sequence (5′- -> 3′)	Target/Notes
PB964	AAACCAGGAGTCAGATGCAC-CATC(SEQ ID NO: 298)	HBB E6V HDR rev
PB1017	CACCGAGTCCGAGCAGAA-GAAGAA (SEQ ID NO: 299)	EMX1 Base editing for
PB1018	AAACTTCTTCTTCTGCTCG-GACTC (SEQ ID NO: 300)	EMX1 Base editing rev
PB325	CACCGGTCACCTCCAATGAC-TAGGG (SEQ ID NO: 301)	EMX1 HDR for
PB326	AAACCCCTAGTCATTGGAGGT-GACC (SEQ ID NO: 302)	EMX1 HDR rev
PB1299	CACCGCACTTTTCGACA-TAGTGTGG (SEQ ID NO: 303)	TP53 R209fs*6
PB1300	AAACCCACACTATGTC-GAAAAGTGC (SEQ ID NO: 304)	TP53 R209fs*6
PB580	CACCGCAGCATCAGAGGAC-TAGCTC (SEQ ID NO: 305)	SMARCAL1 Q34*
PB581	AAACGAGCTAGTCCTCT-GATGCTGC (SEQ ID NO: 306)	SMARCAL1 Q34*
PB838	CACCGATTCCcAGCACGCT-GATGTG (SEQ ID NO: 307)	FANCD2 Q223* for
PB839	AAACCACATCAGCGTGCTgG-GAATC (SEQ ID NO: 308)	FANCD2 Q223* rev
E12	CAC CGA TAC ATT TTG TCT AGA CGT (SEQ ID NO: 309)	BRCA2 V2102I for
H06	AAA CAC GTC TAG ACA AAA TGT ATC (SEQ ID NO: 310)	BRCA2 V2102I rev
PB1294	CACCGTTTCACTTTTAGATAT-GATA(SEQ ID NO: 311)	BRCA2 dupAGAAGAT for
PB1295	AAACTATCATATCTAAAAGT-GAAAC (SEQ ID NO: 312)	BRCA2 dupAGAAGAT rev
PB738	CACCGAAGACTCGAGCCCTC-CAGCG (SEQ ID NO: 313)	TIMELESS R267* for
PB739	AAACCGCTGGAGGGCTC-GAGTCTTC (SEQ ID NO: 314)	TIMELESS R267* rev
PB834	CACCGCAGCcAGT-CAGCGTCCTCGC (SEQ ID NO: 315)	SLX4 W879* for
PB835	AAACGCGAGGACGCT-GACTgGCTGC (SEQ ID NO: 316)	SLX4 W879* rev
PB736	CACCGGTACAACGAATGGGTA-GAAC (SEQ ID NO: 317)	FANCM Q572* for
PB737	AAACGTTCTACCCATTCGTTG-TACC (SEQ ID NO: 318)	FANCM Q572* rev

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[0244] All documents cited in this application are hereby incorporated by reference as if recited in full herein.

[0245] Although illustrative embodiments of the present disclosure have been described herein, it should be understood that the disclosure is not limited to those described, and that various other changes or modifications may be made by one skilled in the art without departing from the scope or spirit of the disclosure.

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<400> SEQUENCE: 56

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<210> SEQ ID NO 57
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<212> TYPE: DNA
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<400> SEQUENCE: 57

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<210> SEQ ID NO 58
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<212> TYPE: DNA
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<400> SEQUENCE: 58

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<210> SEQ ID NO 59
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<400> SEQUENCE: 59

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<210> SEQ ID NO 60
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<212> TYPE: DNA
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<400> SEQUENCE: 60

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<210> SEQ ID NO 61
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<212> TYPE: DNA
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<400> SEQUENCE: 61

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<210> SEQ ID NO 62
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<212> TYPE: DNA
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<400> SEQUENCE: 62

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<210> SEQ ID NO 63
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 63

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<210> SEQ ID NO 64
<211> LENGTH: 60
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 64

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<210> SEQ ID NO 65
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 65

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<210> SEQ ID NO 66
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<400> SEQUENCE: 66

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<210> SEQ ID NO 67
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<400> SEQUENCE: 67

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<210> SEQ ID NO 68
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<400> SEQUENCE: 68

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<210> SEQ ID NO 69
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<400> SEQUENCE: 69

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<210> SEQ ID NO 70
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 70

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<210> SEQ ID NO 71
<211> LENGTH: 60
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 71

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<210> SEQ ID NO 72
<211> LENGTH: 60
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 72

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<210> SEQ ID NO 73
<211> LENGTH: 60
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 73

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<210> SEQ ID NO 74
<211> LENGTH: 60
<212> TYPE: DNA
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<400> SEQUENCE: 74

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<210> SEQ ID NO 75
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<400> SEQUENCE: 75

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<210> SEQ ID NO 76
<211> LENGTH: 60
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 76

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<210> SEQ ID NO 77
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 77

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<210> SEQ ID NO 78
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 78

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<210> SEQ ID NO 79
<211> LENGTH: 60

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 79

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<210> SEQ ID NO 80
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 80

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<210> SEQ ID NO 81
<211> LENGTH: 60
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 81

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<210> SEQ ID NO 82
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 82

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<210> SEQ ID NO 83
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 83

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<210> SEQ ID NO 84
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 84

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<210> SEQ ID NO 85
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 85

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<210> SEQ ID NO 86
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 86

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<210> SEQ ID NO 87
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 87

gcaattcctc acgagacccg tcctgtgcgc aaactattct gaagaactgg cgaactacta 60

<210> SEQ ID NO 88
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 88

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<210> SEQ ID NO 89
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 89

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<210> SEQ ID NO 90
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 90

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gcaattcctc acgagacccg tcctgtgcgc aaactattct gaagaactgg cgaactactt 60

<210> SEQ ID NO 91
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 91

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<210> SEQ ID NO 92
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 92

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<210> SEQ ID NO 93
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 93

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<210> SEQ ID NO 94
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 94

gcaattcctc acgagacccg tcctgactgc tagaacaact ctgaagatca atttgcaatt 60

<210> SEQ ID NO 95
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 95

gcaattcctc acgagacccg tcctggcttg agctggcttc ctgaagttta aaaacatttt 60

<210> SEQ ID NO 96
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

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<400> SEQUENCE: 96

gcaattcctc acgagacccg tcctgagctt tcgttttgaa ctgaagagca gattcttttt 60

<210> SEQ ID NO 97
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 97

gcaattcctc acgagacccg tcctgtagca gatttttctt ctgaagacat ttagttttaa 60

<210> SEQ ID NO 98
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 98

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<210> SEQ ID NO 99
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 99

gcaattcctc acgagacccg tcctgttacc aaattatata ctgaagcctt ttggttatat 60

<210> SEQ ID NO 100
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 100

gcaattcctc acgagacccg tcctgaggga gctttacctt ctgaagtctg tcctgggatt 60

<210> SEQ ID NO 101
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 101

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<210> SEQ ID NO 102
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 102

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<210> SEQ ID NO 103
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 103

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<210> SEQ ID NO 104
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 104

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<210> SEQ ID NO 105
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 105

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<210> SEQ ID NO 106
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 106

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<210> SEQ ID NO 107
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 107

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<210> SEQ ID NO 108
<211> LENGTH: 60

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 108

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<210> SEQ ID NO 109
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 109

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<210> SEQ ID NO 110
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 110

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<210> SEQ ID NO 111
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 111

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<210> SEQ ID NO 112
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 112

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<210> SEQ ID NO 113
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 113

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<210> SEQ ID NO 114
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 114

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<210> SEQ ID NO 115
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 115

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<210> SEQ ID NO 116
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 116

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<210> SEQ ID NO 117
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 117

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<210> SEQ ID NO 118
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 118

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<210> SEQ ID NO 119
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 119

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gcaattcctc acgagacccg tcctgttgga gatcacattt ctgaagttgg ggacatttta 60

<210> SEQ ID NO 120
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 120

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<210> SEQ ID NO 121
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 121

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<210> SEQ ID NO 122
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 122

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<210> SEQ ID NO 123
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 123

gcaattcctc acgagacccg tcctggggca gcgggtgccg ctgaaggcga ggacgctgac 60

<210> SEQ ID NO 124
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 124

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<210> SEQ ID NO 125
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

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<400> SEQUENCE: 125

gcaattcctc acgagacccg tcctggaagc tcggaaaagc ctgaaggatc caggtgctgc 60

<210> SEQ ID NO 126
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 126

gcaattcctc acgagacccg tcctgatgta gaattaagaa ctgaagtcat gcctccagtt 60

<210> SEQ ID NO 127
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 127

gcaattcctc acgagacccg tcctgcccgg ggcatttcat ctgaagccca ggagctaggt 60

<210> SEQ ID NO 128
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 128

gcaattcctc acgagacccg tcctgttgag agtcgctcca ctgaagttgc cagctctgtt 60

<210> SEQ ID NO 129
<211> LENGTH: 60
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 129

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<210> SEQ ID NO 130
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 130

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<210> SEQ ID NO 131
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 131

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<210> SEQ ID NO 132
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<212> TYPE: DNA
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gaagacaaaa tatttgggaa aacct 25

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gctgcttcac cttaaataac aaaaa 25

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gaaaatagac caataggcag agagagtc 28

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acgttgatgg cagttgcagg tc 22

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<400> SEQUENCE: 163

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<210> SEQ ID NO 164
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<400> SEQUENCE: 164

gccatcccct tctgtgaatg ttagac 26

<210> SEQ ID NO 165
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<212> TYPE: DNA
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<400> SEQUENCE: 165

ggagattgga gacacggaga gcag 24

<210> SEQ ID NO 166
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<212> TYPE: DNA
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aactgtgcaa tagttaaacc catttac 27

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<400> SEQUENCE: 167

gtaggtgttc ggtaaatgtt aatgg 25

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tacttgcttt cagtgttgtg ttatagg 27

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<400> SEQUENCE: 171

ggaaaagttt ataggtgtcc cttctac 27

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<400> SEQUENCE: 172

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<400> SEQUENCE: 173

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<400> SEQUENCE: 175

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<210> SEQ ID NO 176
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<400> SEQUENCE: 176

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<400> SEQUENCE: 177

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atgtctgcag ctatagttag gaagc 25

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<400> SEQUENCE: 178

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gtgtgtgtgc aattataaaa gaaactt 27

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<212> TYPE: DNA
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gtcagcatta gatgagctac tgagatt 27

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atggtaagaa atatggttaa caccaag 27

<210> SEQ ID NO 189
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 189

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aaactacata actacaaccc aatgctt 27

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<400> SEQUENCE: 191

gaaccccata cctgggatct 20

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<400> SEQUENCE: 192

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<212> TYPE: DNA
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<400> SEQUENCE: 193

ttatcagttt tggaggatgt acataaa 27

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<212> TYPE: DNA
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<400> SEQUENCE: 194

ctccttcctc ttcctacagt actcc 25

<210> SEQ ID NO 195
<211> LENGTH: 54

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 195

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 196

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<210> SEQ ID NO 197
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 197

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<210> SEQ ID NO 198
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<220> FEATURE:
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<400> SEQUENCE: 198

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<220> FEATURE:
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<400> SEQUENCE: 199

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 200

agacgtgtgc tcttccgatc tttactggaa gttagcactc t 41

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<210> SEQ ID NO 201
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 201

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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 202

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<210> SEQ ID NO 203
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 203

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<400> SEQUENCE: 204

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<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 205

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<210> SEQ ID NO 206
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 206

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agacgtgtgc tcttccgatc tcctgatatt tctgtccctt g 41

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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 207

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<400> SEQUENCE: 208

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<210> SEQ ID NO 209
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 209

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<211> LENGTH: 41
<212> TYPE: DNA
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 210

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<210> SEQ ID NO 211
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 211

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<210> SEQ ID NO 212
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<400> SEQUENCE: 212

agacgtgtgc tcttccgatc tcctgtacaa tgaaaagtag a 41

<210> SEQ ID NO 213
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 213

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<210> SEQ ID NO 214
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 214

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 215

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<220> FEATURE:
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<400> SEQUENCE: 216

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 217

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<210> SEQ ID NO 218
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 218

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<210> SEQ ID NO 219
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 219

caagcagaag acggcatacg agattgcctc ttgtgactgg agttcagacg tgtgctcttc 60

cgatct 66

<210> SEQ ID NO 220
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 221

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<210> SEQ ID NO 222
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<212> TYPE: DNA
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<400> SEQUENCE: 222

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<210> SEQ ID NO 224
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<400> SEQUENCE: 224

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<212> TYPE: DNA
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atca 184

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gacatcgatg tcacctccaa tgactagttt aaacgggtgg gcaaccacaa acccacgagg 120

gcagagtgct gcttgctgct ggccaggccc ctgcgtgggc ccaagctgga ctctggccac 180

tccc 184

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ctgactcctg tcgagaagtc tgccgttact gccctgtggg gcaaggtgaa cgtggatgaa 120

gttggtggtg aggccctggg 140

<210> SEQ ID NO 228
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<212> TYPE: DNA
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<220> FEATURE:
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atttggatga caaacacttt tcgtcatagt gtggttgtgc cctatgagcc gcctgaggtc 120

tggtttgcaa ctggggtctc tgggaggagg ggttaagggt ggttgt 166

<210> SEQ ID NO 229
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tttagatatg atactgaaat tgatagaagc agaagataga agatcggcta taaaaaagat 120

aatggaaagg gatgacacag ctgcaaaaac acttgttctc tgtgtttctg acataat 177

<210> SEQ ID NO 230
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<212> TYPE: DNA
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<210> SEQ ID NO 231
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<212> TYPE: DNA
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<400> SEQUENCE: 232

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<220> FEATURE:
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<210> SEQ ID NO 239
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<212> TYPE: DNA
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<220> FEATURE:
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<220> FEATURE:
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<400> SEQUENCE: 240

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<210> SEQ ID NO 241
<211> LENGTH: 60
<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 242

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<210> SEQ ID NO 243
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<220> FEATURE:
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<400> SEQUENCE: 243

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<400> SEQUENCE: 244

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<210> SEQ ID NO 246
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 246

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<210> SEQ ID NO 247
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 247

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<211> LENGTH: 24
<212> TYPE: DNA
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<400> SEQUENCE: 248

caccgcaatt cagtacaatt aggt 24

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<400> SEQUENCE: 250

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caccgttttt cgagtgattc tatt 24

<210> SEQ ID NO 251
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<400> SEQUENCE: 251

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<400> SEQUENCE: 253

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<212> TYPE: DNA
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<400> SEQUENCE: 254

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<220> FEATURE:
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<400> SEQUENCE: 255

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<210> SEQ ID NO 256
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<212> TYPE: DNA
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caccgttaga tcaactggaa tgga 24

<210> SEQ ID NO 257
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<212> TYPE: DNA
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<400> SEQUENCE: 257

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<210> SEQ ID NO 258
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 258

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<210> SEQ ID NO 260
<211> LENGTH: 24
<212> TYPE: DNA
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<400> SEQUENCE: 260

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 261

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<212> TYPE: DNA
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<220> FEATURE:
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caccgtaaat actgcagatt atgt 24

<210> SEQ ID NO 263
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<400> SEQUENCE: 263

caccgagaaa cgacaaatcc tatt 24

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<212> TYPE: DNA
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 264

caccgaagga acaaggttta tcaa 24

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<212> TYPE: DNA
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<400> SEQUENCE: 265

caccgcatac tgtttgctca caga 24

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<212> TYPE: DNA
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caccggctac agaattctgt gtgg 24

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<212> TYPE: DNA
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<220> FEATURE:
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caccgacaga acatccttgg aagt 24

<210> SEQ ID NO 268
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

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aaactcacag tgtcctttat gtac 24

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<212> TYPE: DNA
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<400> SEQUENCE: 269

aaacacctaa ttgtactgaa ttgc 24

<210> SEQ ID NO 270
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<212> TYPE: DNA
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aaactaataa cattagagaa aatc 24

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<212> TYPE: DNA
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<400> SEQUENCE: 271

aaacaataga atcactcgaa aaac 24

<210> SEQ ID NO 272
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aaacatcaag tcatttgtta aaac 24

<210> SEQ ID NO 273
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<400> SEQUENCE: 273

aaacgcagtg atattaactg tctc 24

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<210> SEQ ID NO 274
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<212> TYPE: DNA
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<400> SEQUENCE: 274

aaacttatgt aagaatgata taac 24

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aaacaagcga gcaagagaat cccc 24

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<212> TYPE: DNA
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<400> SEQUENCE: 276

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<210> SEQ ID NO 277
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 277

aaactccatt ccagttgatc taac 24

<210> SEQ ID NO 278
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 278

aaacctcttt cattgcgaaa tatc 24

<210> SEQ ID NO 279
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<212> TYPE: DNA
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<400> SEQUENCE: 279

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aaactgtgga agttgcgtat tgtc 24

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<400> SEQUENCE: 280

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<210> SEQ ID NO 281
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<212> TYPE: DNA
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<210> SEQ ID NO 283
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<210> SEQ ID NO 285
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aaacttgata aaccttgttc cttc 24

<210> SEQ ID NO 286
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 286

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<210> SEQ ID NO 287
<211> LENGTH: 24
<212> TYPE: DNA
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<210> SEQ ID NO 288
<211> LENGTH: 24
<212> TYPE: DNA
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aaacacttcc aaggatgttc tgtc 24

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 289

caccgaactt cgagatacag cagac 25

<210> SEQ ID NO 290
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<210> SEQ ID NO 291
<211> LENGTH: 24
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

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<210> SEQ ID NO 292
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 292

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<210> SEQ ID NO 293
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 293

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<210> SEQ ID NO 294
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 294

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<210> SEQ ID NO 295
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 295

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<210> SEQ ID NO 296
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 296

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<210> SEQ ID NO 297
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

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<400> SEQUENCE: 298

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<210> SEQ ID NO 299
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 299

caccgagtcc gagcagaaga agaa 24

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 300

aaacttcttc ttctgctcgg actc 24

<210> SEQ ID NO 301
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 301

caccggtcac ctccaatgac taggg 25

<210> SEQ ID NO 302
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 302

aaacccctag tcattggagg tgacc 25

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<210> SEQ ID NO 303
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Laboratory Synthesized

<400> SEQUENCE: 303

caccgcactt ttcgacatag tgtgg 25

<210> SEQ ID NO 304
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What is claimed is:

1. A method for detecting a genetic modification in a DNA sequence of interest, comprising the steps of:

(a) amplifying the DNA sequence of interest using a specially designed Type IIS restriction enzyme-tagging primer, comprising:

(i) obtaining the DNA sequence of interest from a biological sample;

(ii) synthesizing the Type IIS restriction enzyme-tagging primer based on the DNA sequence of interest;

(iii) amplifying the DNA sequence of interest using the Type IIS restriction enzyme-tagging primer and a reverse primer; and

(iv)purifying a Type IIS restriction enzyme-tagged amplicon;

(b) digesting the Type IIS restriction enzyme-tagged amplicon with the Type IIS restriction enzyme;

(c) isolating the smaller DNA fragment containing the genetic modification exposed in a 3′ single-stranded overhang;

(d) capturing the genetic modification, comprising:

(i) preparing a library of 16 DNA adaptors, wherein each DNA adaptor comprises one strand with sequence of 5′-CTGGGGCACGGGTAAGAAG-CATTCTGTCTCTCTTCTAAGAATTCGAG CTCGGTACCCG-3′ (SEQ ID NO: 230); and one complementary strand with sequence of 5′-CGGGTACCGAGCTCGAATTCTTAGAAGAGA-GACAGAATGCTTCTTAC CCGTGCCCCAGNN-3′ with "N" corresponding to A, T, G or C (SEQ ID NOs: 231-246), and wherein each DNA adaptor has a different "NN";

(ii) incubating the isolated smaller DNA fragment containing the 3′ overhang with the library of DNA adaptors and performing a ligation; and

(iii) obtaining a ligated product; and

(e) amplifying the ligated product to detect the presence of the genetic modification, wherein the DNA sequence of interest is a genomic locus or corresponds to a genomic locus of an RNA virus variant.

2. The method of claim **1**, wherein the DNA sequence of interest corresponds to a genomic locus of an RNA virus variant, and wherein obtaining the DNA sequence of interest comprises obtaining the RNA sequence from the RNA virus variant and converting it to the corresponding DNA sequence by reverse transcription PCR (RT-PCR).

3. The method of claim **2**, wherein the RNA virus is SARS-CoV-2.

4. The method of claim **1**, wherein the Type IIS restriction enzyme is selected from AcuI, BpmI, BpuEI, BsgI, MmeI and NmeAIII.

5. The method of claim **4**, wherein the Type IIS restriction enzyme is AcuI.

6. The method of claim **1**, wherein the Type IIS restriction enzyme-tagging primer is an oligonucleotide comprising:

(a) a non-complementary handle sequence positioned on the 5′ side;

(b) a complementary sequence of the genomic locus of interest on the 5′ side;

(c) a recognition motif of the Type IIS restriction enzyme that is positioned at a predicted distance from its cleavage site to generate the genomic signature of interest; and

(d) a complementary sequence of the genomic locus of interest on the 3′ side.

7. A kit for detecting a genetic modification of interest, comprising a specially designed Type IIS restriction enzyme-tagging primer according to claim **6**, and a library of DNA adaptors according to claim **1**, packaged together with instructions for its use.

8. The method of claim **5**, wherein the AcuI-tagging primer is an oligonucleotide comprising:

(a) a non-complementary handle sequence positioned on the 5′ side; and

(b) a complementary sequence of the genomic locus of interest containing an AcuI motif (5′-CTGAAG-3′) positioned 14 bp upstream from the genomic locus of interest.

9. The method of claim **8**, wherein the reverse primer is positioned at more than 100 bp downstream of the genomic locus of interest.

10. The method of claim **8**, wherein the non-complementary handle sequence is 25 bp.

11. The method of claim **8**, wherein the complementary sequence has the structure of: 5′-N(20)CTGAAGN(14)-3′ or 5′-N(15)CTGAAGN(14)-3′, with "N" corresponding to A, T, G or C, depending on the DNA sequence of the genomic locus of interest.

12. The method of claim **8**, wherein the non-complementary handle sequence is 5′-GCAATTCCTCACGA-GACCCGTCCTG-3′ (SEQ ID NO: 3) and the complementary sequence is 5′-N(15)CTGAAGN(14)-3′, with "N" corresponding to A, T, G or C.

13. A kit for detecting a genetic modification, comprising a specially designed AcuI-tagging primer and a library of DNA adaptors according to claim **1**, packaged together with instructions for its use.

14. A method for quantifying a genomic variant in a biological system, comprising the steps of:

(a) obtaining a sample from the biological system;

(b) amplifying a DNA sequence of interest using a specially designed AcuI-tagging primer, wherein the DNA sequence of interest is a genomic locus or corresponds to a genomic locus of an RNA virus variant, comprising:

(i) obtaining the DNA sequence of interest by (1) genomic extraction or (2) obtaining the RNA sequence from the RNA virus variant and converting it to the corresponding DNA sequence by reverse transcription PCR (RT-PCR);

(ii) synthesizing the AcuI-tagging primer based on the DNA sequence of interest;
(iii) amplifying the DNA sequence of interest using the AcuI-tagging primer and a reverse primer; and
(iv) purifying an AcuI-tagged amplicon;
(c) digesting the AcuI-tagged amplicon with restriction enzyme AcuI;
(d) isolating the smaller DNA fragment containing the genomic variant of interest produced by the AcuI-digestion;
(e) capturing the genomic variant of interest, comprising:
(i) preparing the library of DNA adaptors according to claim **1**;
(ii) incubating the isolated smaller DNA fragment with the library of DNA adaptors and performing a ligation; and
(iii) obtaining a ligated product; and
(f) quantifying the genomic variant and determining its relative abundance.

15. The method of claim **14**, wherein the genomic variant is generated by precision genome editing.

16. The method of claim **15**, wherein the precision genome editing is CRISPER-dependent homology-directed repair, base editing or prime editing.

17. The method of claim **14**, wherein the quantification in step (f) is carried out by quantitative PCR (qPCR).

18. A method for identifying and quantifying an oncogenic mutation of interest in a biological sample, comprising the steps of:
(a) obtaining a biological sample;
(b) amplifying a genomic locus of interest using a specially designed AcuI-tagging primer, comprising:
(i) extracting DNA of interest;
(ii) synthesizing the AcuI-tagging primer based on the genomic locus of interest;
(iii) amplifying the genomic locus of interest using the AcuI-tagging primer and a reverse primer; and
(iv) purifying an AcuI-tagged genomic amplicon;
(c) digesting the AcuI-tagged genomic amplicon with restriction enzyme AcuI;
(d) isolating the smaller DNA fragment containing the oncogenic mutation of interest produced by the AcuI-digestion;
(e) capturing the genomic signature of interest, comprising:
(i) preparing the library of DNA adaptors according to claim **1**;
(ii) incubating the isolated smaller DNA fragment with the library of DNA adaptors and performing a ligation; and
(iii) obtaining a ligated product;
(f) amplifying the ligated product to identify the presence of the oncogenic mutation of interest; and
(g) quantifying the oncogenic mutation of interest, if present, and determining its frequency.

19. The method of claim **18**, wherein the biological sample is obtained from a cancer animal model, a patient-derived xenograft (PDX), or a human cancer patient sample.

20. The method of claim **18**, wherein the quantification in step (g) is carried out by quantitative PCR (qPCR).

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