



(51) International Patent Classification:

C12N 15/00 (2006.01) C12N 9/22 (2006.01)
C12N 15/113 (2010.01) A61K 48/00 (2006.01)
C12N 15/90 (2006.01) A61P 27/02 (2006.01)

(21) International Application Number:

PCT/US2016/040962

(22) International Filing Date:

5 July 2016 (05.07.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/188,013 2 July 2015 (02.07.2015) US

(71) Applicant: THE JOHNS HOPKINS UNIVERSITY
[US/US]; 3400 North Charles Street, Baltimore, MD
21218 (US).

(72) Inventors: JUN, Albert, S.; 7906 Sherwood Avenue,
Towson, MD 21204 (US). RANGANATHAN, Vinod;
1201 W. Mt. Royal Avenue, Unit 549, Baltimore, MD
21217 (US). ZACK, Donald; 3 Ruby Field Court, Bal-
timore, MD 21209 (US).

(74) Agents: RUSSELL, Hathaway, P. et al.; Foley Hoag
LLP, Seaport West, 155 Seaport Boulevard, Boston, MA
02210-2600 (US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))

(54) Title: CRISPR/CAS9-BASED TREATMENTS

(57) Abstract: Described herein are methods for treating disorders affecting ocular and non-ocular tissue, such as corneal dys-
trophies and microsatellite expansion diseases. The methods use a nuclease system, such as Clustered Regularly Interspaced Short
Palindromic Repeats (CRISPR)/CRISPR associated (Cas) 9 (CRISPR-Cas9), to cut and/or repair genomic DNA. Such methods may
further comprise a DNA double-stranded break (DSB) repair system comprising a repair template in combination with a Non-Homo-
logous End- Joining (NHEJ) or Homology Directed Repair (HDR) targeted to the one or more CRISPR-Cas9 cleavage sites.



CRISPR/CAS9-BASED TREATMENTS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No: 62/188,013, filed July 2, 2015, the contents of which is incorporated herein by reference in its entirety.

5

BACKGROUND

Corneal dystrophies are a group of disorders that are generally inherited, bilateral, symmetric, slowly progressive, and not predominantly related to environmental or systemic factors (1,2). Corneal dystrophies can affect any anatomic layer, cell type, or tissue of the cornea and result in loss of corneal clarity and reduction in vision (1,3). Corneal dystrophies as a group affect >4% of the US population, and corneal transplantation is definitive treatment for corneal dystrophies of sufficient severity to cause significant vision loss. Fuchs endothelial corneal dystrophy (FECD) is the most common corneal dystrophy affecting approximately 4% of the US population. Approximately 70% of FECD cases are caused by a microsatellite trinucleotide repeat expansion in the transcription factor 4 (TCF4) gene (4). Additional microsatellite expansion diseases have been described (5).

15

Thus there is a great need for novel and improved therapies for treating disorders affecting ocular and non-ocular tissues, like corneal dystrophies and microsatellite expansion diseases affecting the eye and other tissues and organs throughout the body.

20

SUMMARY OF THE INVENTION

Described herein are methods for treating disorders affecting ocular and non-ocular tissues, such as corneal dystrophies and microsatellite expansion diseases. The methods use a nuclease system, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated (Cas) 9 (CRISPR-Cas9), to cut and/or repair genomic DNA. The CRISPR-Cas9-based gene editing can be used to inactivate or correct gene mutations causing corneal dystrophies and microsatellite expansion diseases, thereby providing a gene therapy approach for these groups of diseases.

25

One aspect of the invention relates to a method for treating a disorder affecting ocular tissue in a subject, the method comprising administering to the ocular area of the subject a therapeutically effective amount of a nuclease system comprising a genome targeted nuclease and a guide DNA comprising at least one targeted genomic sequence.

30

In certain embodiments, the nuclease can be provided as a protein, RNA, DNA, or an expression vector comprising a nucleic acid that encodes the nuclease.

In certain embodiments, the guide DNA can be provided as an RNA molecule (gRNA), DNA molecule, or an expression vector comprising a nucleic acid that encodes the gRNA.

In certain embodiments, the guide DNA may be provided as one, two, three, four, five, six, seven, eight, nine, or ten RNA molecules (gRNA), DNA molecules, or expression vectors comprising a nucleic acid that encodes the gRNA, or any combination thereof.

In certain embodiments, the nuclease system can be CRISPR-Cas9.

In certain embodiments, the nuclease system inactivates or excises gene mutations.

In certain embodiments, the system further comprises a DNA double-stranded break (DSB) repair system.

In certain embodiments, the DSB repair system comprises a repair template in combination with or without a Non-Homologous End-Joining (NHEJ) or Homology Directed Repair (HDR) targeted to the one or more CRISPR-Cas9 cleavage site, said site corrects or edits a genomic mutation.

In certain embodiments, the DSB repair system is provided by the host cell machinery.

In certain embodiments, the genome targeted nuclease can be Cas9.

In certain embodiments, the disorder can be a corneal dystrophy or microsatellite expansion disease.

In certain embodiments, the ocular area can be the cornea.

In certain embodiments, the guide DNA comprises at least one, two, three, four, five, six, seven, eight, nine, or ten targeted genomic sequences.

In certain embodiments, the target genomic sequences are selected from any one of the nucleotide sequences set forth in SEQ ID NOs: 1-172 and 174-342, or any combination thereof.

In certain embodiments, the nuclease system can be administered topically to the surface of the eye.

In certain embodiments, the nuclease system can be administered on or outside the cornea, sclera, to the intraocular, subconjunctival, sub-tenon, or retrobulbar space, or in or around the eyelids.

In certain embodiments, the nuclease system can be administered by implantation, injection, or virally.

Another aspect of the invention relates to a method for treating a disorder affecting non-ocular tissue in a subject, the method comprising administering to the non-ocular tissue
5 of the subject a therapeutically effective amount of a nuclease system comprising a genome targeted nuclease and a guide DNA comprising at least one targeted genomic sequence.

In certain embodiments, the nuclease can be provided as a protein, RNA, DNA, or an expression vector comprising a nucleic acid encoding the nuclease.

In certain embodiments, the guide DNA can be provided as an RNA molecule
10 (gRNA), DNA molecule, or an expression vector comprising a nucleic acid that encodes the gRNA.

In certain embodiments, the nuclease system can be CRISPR-Cas9.

In certain embodiments, the nuclease system inactivates or excises gene mutations.

In certain embodiments, the method further comprises a DNA double-stranded
15 break (DSB) repair system.

In certain embodiments, the DSB repair system comprises a repair template in combination with a Non-Homologous End-Joining (NHEJ) or Homology Directed Repair (HDR) targeted to the one or more CRISPR-Cas9 cleavage site, said site corrects or edits a genomic mutation.

20 In certain embodiments, the genome targeted nuclease can be Cas9.

In certain embodiments, the disorder can be microsatellite expansion disease.

In certain embodiments, the guide DNA comprises at least one, two, three, four, five, six, seven, eight, nine, or ten targeted genomic sequences.

In certain embodiments, the target genomic sequences are selected from any one of
25 the nucleotide sequences set forth in SEQ ID NOs: 1-172 and 174-342, or any combination thereof.

In certain embodiments, the nuclease system is administered topically, intravascularly, intradermally, transdermally, parenterally, intravenously, intramuscularly, intranasally, subcutaneously, regionally, percutaneously, intratracheally, intraperitoneally,
30 intraarterially, intravesically, intratumorally, inhalationonly, perfusiononly, lavagely, directly via injection, or orally via administration and formulation.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the

detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 contains four panels (A)-(D) describing two identified sites as targetable by Cas9 using the gRNA sequences that overlap with the respective mutations and their ability to disrupt dominant mutations in genes known to be causative in corneal dystrophies. **Panel (A)** depicts targeting of TGFBI exon 124 in HEK293 cells using the CRISPR-Cas9 system. The % gene modification by non-homologous end-joining (% indel) is indicated below. **Panel (B)** depicts an image trace of the gel indicating the peaks used for quantification. **Panel (C)** depicts targeting of TGFBI exon 555 in HEK293 cells using the CRISPR-Cas9 system. The % gene modification by non-homologous end-joining (% indel) is indicated below. **Panel (D)** depicts an image trace of the gel indicating the peaks used for quantification.

Figure 2 contains three panels (A)-(C) describing identified sites as targetable by Cas9 using the gRNA sequences that correspond to target sequences within the intron between exon 2 and exon 3 of the TCF4 gene. **Panel (A)** depicts in HEK293 cells using the CRISPR/Cas9 system 6 gRNAs targeting intronic sequences downstream (Table 4) of the trinucleotide repeat expansion which causes Fuchs corneal dystrophy. Molecular weight ladder is shown in the far left and far right lanes. Control lane indicates no gRNA and no Cas9 transfection. Cas9 lane indicates transfection with Cas9 but no gRNA. Arrows indicate major cleavage product produced by non-homologous end-joining, and % gene modification by non-homologous end-joining is indicated below. **Panel (B)** depicts image traces of the gel indicating the peaks used for quantification. **Panel (C)** depicts expected digest sizes for each gRNA.

Figure 3 contains three panels (A)-(C) describing identified sites as targetable by Cas9 using the gRNA sequences that correspond to target sequences within the intron between exon 2 and exon 3 of the TCF4 gene. **Panel (A)** depicts in HEK293 cells using the CRISPR/Cas9 system 6 gRNAs targeting intronic sequences upstream (Table 3) of the trinucleotide repeat expansion which causes Fuchs corneal dystrophy. Molecular weight ladder is shown in the far right lane. Control lane indicates no gRNA and no Cas9

transfection. Arrows indicate major cleavage products produced by non-homologous end-joining, and % gene modification by non-homologous end-joining is indicated below.

Panel (B) depicts image traces of the gel indicating the peaks used for quantification. **Panel (C)** depicts expected digest sizes for each gRNA.

5

DETAILED DESCRIPTION

Described herein are methods for treating eye disorders, such as corneal dystrophies and microsatellite expansion diseases. The methods use a nuclease system, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated (Cas) 9 (CRISPR-Cas9), to cut, nick, and/or repair genomic DNA.

As used herein, the term “eye disease” may encompass disorders of the eye including, but not limited to corneal dystrophies and microsatellite expansion diseases.

As used herein, the term “corneal dystrophy” or “corneal dystrophies” describes a group of disorders that are generally inherited, bilateral, symmetric, slowly progressive, and not predominantly related to environmental or systemic factors (1,2). Corneal dystrophies, include (but may not be limited to) the following : Epithelial Basement Membrane Dystrophy (aka Map-Dot-Fingerprint Dystrophy, Cogan Microcystic Epithelial Dystrophy, Anterior Basement Membrane Dystrophy); Epithelial Recurrent Erosion Dystrophies (aka Franceschetti Corneal Dystrophy, Dystrophia Smolandiensis, Dystrophia Helsinglandica); Subepithelial Mucinous Corneal Dystrophy; Meesmann Corneal Dystrophy (aka Juvenile Hereditary Epithelial Dystrophy, Stocker Holt Dystrophy); Lisch Epithelial Corneal Dystrophy (aka Band-Shaped and Whorled Microcystic Dystrophy); Gelatinous Drop-like Corneal Dystrophy (aka Subepithelial Amyloidosis, Primary Familial Amyloidosis (of Grayson)); Reis–Bucklers Corneal Dystrophy (aka Corneal Dystrophy of Bowman layer, type I (CDB I), Geographic Corneal Dystrophy (of Weidle), Atypical Granular Corneal Dystrophy, Granular Corneal Dystrophy, Type 3, Anterior Limiting Membrane Dystrophy, Type 1, Superficial Granular Corneal Dystrophy); Thiel–Behnke Corneal Dystrophy (aka Corneal Dystrophy of Bowman layer, Type II (CDB2), Honeycomb-Shaped Corneal Dystrophy, Anterior Limiting Membrane Dystrophy, Type II, Curly Fibers Corneal Dystrophy, Waardenburg–Jonkers Corneal Dystrophy); Lattice Corneal Dystrophy, Type 1 (Classic) (aka Biber-Haab-Dimmer Dystrophy); Lattice Corneal Dystrophy, Type 2 (aka Familial Amyloidosis (Finnish Type or Gelsolin Type), Meretoja Syndrome); Lattice Corneal Dystrophy, Type III; Lattice Corneal Dystrophy, Type IIIA; Lattice Corneal

Dystrophy, Type I/IIIA; Lattice Corneal Dystrophy, Type IV; Polymorphic (Corneal Amyloidosis; Granular Corneal Dystrophy, Type 1 (aka Corneal Dystrophy Groenouw Type I); Granular Corneal Dystrophy, Type 2 (aka Avellino Dystrophy, Combined Granular-Lattice Dystrophy); Macular Corneal Dystrophy (aka Groenouw Corneal Dystrophy Type II, Fehr Speckled Dystrophy); Schnyder Corneal Dystrophy (aka Schnyder Crystalline Corneal Dystrophy (SCCD), Schnyder Crystalline Dystrophy Sine Crystals, Hereditary Crystalline Stromal Dystrophy of Schnyder, Crystalline Stromal Dystrophy, Central Stromal Crystalline Corneal Dystrophy, Corneal Crystalline Dystrophy of Schnyder, Schnyder Corneal Crystalline Dystrophy); Congenital Stromal Corneal Dystrophy (aka Congenital Hereditary Stromal Dystrophy); Fleck Corneal Dystrophy (aka François-Neetens Speckled (Mouchetée) Corneal Dystrophy); Posterior Amorphous Corneal Dystrophy (aka Posterior Amorphous Stromal Dystrophy); Central Cloudy Dystrophy of Francois; Pre-Descemet Corneal Dystrophy; Fuchs Endothelial Corneal Dystrophy (aka Endoepithelial Corneal Dystrophy); Posterior Polymorphous Corneal Dystrophy (aka Posterior Polymorphous Dystrophy, Schlichting Dystrophy); Congenital Hereditary Endothelial Dystrophy (aka Maumenee Corneal Dystrophy); X-linked Endothelial Corneal Dystrophy.

All of the above disorders are caused by known or putative genetic mutations. Corneal dystrophies yet to be described will be caused by known or putative genetic mutations. Thus, all genetic corneal dystrophies can be amenable to the nuclease system, like CRISPR-Cas9, for gene therapy involving correction or inactivation of the mutant allele.

As used herein, “microsatellite sequences”, also called short tandem repeats, are short DNA sequences (usually 2-5 nucleotides) which are repeated, typically in the range of 5-50 times. These sequences are present throughout the human genome and can become mutated and/or increased in the number of repeats. Some microsatellite sequences, if they expand beyond a certain length, can result in microsatellite expansion diseases. All known or yet to be described microsatellite expansion diseases will be caused by expansions in known or putative genes. Thus, all microsatellite expansion diseases can be amenable to CRISPR-Cas9 gene therapy involving correction or inactivation of the mutant allele.

Microsatellite expansion diseases as used herein may encompass diseases that affect ocular and non-ocular tissues, including (but may not be limited to) the following

disorders: Blepharophimosis, ptosis and epicanthus inversus syndactyly; Cleidocranial dysplasia; Congenital central hypoventilation syndrome, Haddad syndrome DM (Myotonic dystrophy); FRAXA (Fragile X syndrome); FRAXE (Fragile XE mental retardation); FRDA (Friedreich's ataxia); Fuchs' Endothelial Corneal Dystrophy; FXTAS
 5 (Fragile X-associated tremor/ataxia syndrome); Hand-foot-genital syndrome; HD (Huntington's disease); Holoprosencephaly; Mental retardation with growth hormone deficiency; Mental retardation, epilepsy, West syndrome, Partington syndrome; Oculopharyngeal muscular dystrophy; SBMA (Spinal and bulbar muscular atrophy); SCA1 (Spinocerebellar ataxia Type 1); SCA12 (Spinocerebellar ataxia Type 12); SCA17
 10 (Spinocerebellar ataxia Type 17); SCA2 (Spinocerebellar ataxia Type 2); SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease); SCA6 (Spinocerebellar ataxia Type 6); SCA7 (Spinocerebellar ataxia Type 7); SCA8 (Spinocerebellar ataxia Type 8); Synpolydactyly.

As used herein, the term “eye”, “eye area” or “ocular area” of the subject
 15 encompasses the cornea, conjunctiva, sclera, fovea, macula, optic nerve, retina, lens, iris, pupil, to the intraocular, subconjunctival, sub-tenon, or retrobulbar space, or in or around the eyelids, and other anatomical features of the eye.

As used herein the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated (Cas) 9 nuclease are an extremely versatile and accurate
 20 approach to cut and/or repair genomic DNA (6). CRISPR-Cas9-based gene editing can be used to inactivate or correct gene mutations causing corneal dystrophies and microsatellite expansion diseases, thereby providing a gene therapy approach for these groups of diseases. The naturally occurring CRISPR system from *S. pyogenes* has been modified to utilize a
 25 single guide RNA (gRNA) consisting of a 20 nucleotide (nt) target sequence and an additional structural RNA portion which binds the Cas9 double strand nuclease (6,7). The CRISPR-Cas9 system from *S. pyogenes* has the potential to cut at any 20 nt sequence adjacent to a 5'-NGG-3' protospacer-adjacent motif (PAM), or alternate PAM sequences and bioinformatics provides tools to map target sites (8, 10). DNA cut by Cas9 is repaired
 30 by endogenous cellular mechanisms, including non-homologous end-joining (NHEJ), which produces insertion deletion mutations that can inactivate the original mutant allele. Thus, CRISPR-Cas9 can correct disease causing genetic mutations by cutting DNA in close enough proximity to a protein coding mutation to inactivate it through frameshifting. Alternatively, CRISPR-Cas9 can correct disease causing genetic mutations, either coding or

non-coding, by cutting DNA on both sides of a mutation to excise it, or nicking on different strands flanking the mutation or repeat, if the distance is under 200bp or so, or through the use of a repair template and homology directed repair (HDR) targeted to one or more CRISPR-Cas9 cleavage sites. Thus, specific mutant sequences can be gene edited and
5 repaired.

CRISPR-Cas9 applied to corneal cells can correct the genetic defect causing corneal dystrophies and thus be used to treat these disorders. The CRISPR-Cas9 treatment could be administered topically to the surface of the eye, via implant, or via injection. The implant or injection could be administered to the cornea, sclera, to the intraocular, subconjunctival,
10 sub-tenon, or retrobulbar space, or in or around the eyelids. CRISPR-Cas9 can also be applied outside the cornea or eye to treat other microsatellite expansion diseases in addition to Fuchs endothelial corneal dystrophy. CRISPR-Cas9 approaches to treat corneal dystrophies and microsatellite expansion diseases could employ single or multiple guide RNAs to inactivate or excise gene mutations, or using a repair template to correct gene
15 mutations. In other embodiments, the CRISPR-Cas9 treatment may be applied to non-ocular tissue to correct the genetic defect causing microsatellite expansion diseases.

In certain embodiments, the routes of CRISPR-Cas9 treatment administration can vary with the location and nature of the cells or tissues to be contacted, and include, e.g., intravascular, intradermal, transdermal, parenteral, intravenous, intramuscular, intranasal,
20 subcutaneous, regional, percutaneous, intratracheal, intraperitoneal, intraarterial, intravesical, intratumoral, inhalation, perfusion, lavage, direct injection, and oral administration and formulation, or any of the following routes of administration. The term "systemic administration" refers to administration in a manner that results in the introduction of the composition into the subject's circulatory system or otherwise permits
25 its spread throughout the body. "Regional" administration refers to administration into a specific, and somewhat more limited, anatomical space, such as intraperitoneal, intrathecal, subdural, or to a specific organ. "Local administration" refers to administration of a composition or drug into a limited, or circumscribed, anatomic space, such as intratumoral injection into a tumor mass, subcutaneous injections, intradermal or intramuscular
30 injections. Those of skill in the art will understand that local administration or regional administration may also result in entry of a composition into the circulatory system i.e., rendering it systemic to one degree or another. For example, the term "intravascular" is understood to refer to delivery into the vasculature of a patient, meaning into, within, or in

a vessel or vessels of the patient, whether for systemic, regional, and/or local administration. In certain embodiments, the administration can be into a vessel considered to be a vein (intravenous), while in others administration can be into a vessel considered to be an artery. Veins include, but are not limited to, the internal jugular vein, a peripheral vein, a coronary vein, a hepatic vein, the portal vein, great saphenous vein, the pulmonary vein, superior vena cava, inferior vena cava, a gastric vein, a splenic vein, inferior mesenteric vein, superior mesenteric vein, cephalic vein, and/or femoral vein. Arteries include, but are not limited to, coronary artery, pulmonary artery, brachial artery, internal carotid artery, aortic arch, femoral artery, peripheral artery, and/or ciliary artery. It is contemplated that delivery may be through or to an arteriole or capillary.

The CRISPR-Cas system may be used facilitate targeted genome editing in eukaryotic cells, including mammalian cells, such as human cells. To facilitate genome editing, the cell to be modified is co-transfected with an expression vector encoding Cas9 or the Cas9 protein, DNA, or RNA itself, along with a guide-RNA molecule itself, or an expression vector comprising a nucleic acid molecule encoding the guide-RNA molecule. For example, in certain embodiments, the introduction of Cas9 can be done by transfecting in Cas9 as a protein, RNA, DNA, or expression vector comprising a nucleic acid that encodes Cas9. In certain embodiments, the guide DNA can itself be administered directly as an RNA molecule (gRNA), DNA molecule, or as expression vector comprising a nucleic acid that encodes the gRNA.

While many different CRISPR-Cas systems could be modified to facilitate targeted genome modification, the most commonly used CRISPR-Cas system in targeted genome modification is the CRISPR-Cas9 system *from S. pyogenes*. The CRISPR-Cas9 system requires only a single protein, Cas9, to catalyze double-stranded DNA breaks at sites targeted by a guide-RNA molecule.

Multiple guide RNA sequences can be encoded in a single CRISPR array to facilitate the simultaneous editing of multiple sites within a cell's genome. For example, a pair of guide RNAs can target proximally located sequences to facilitate the deletion of the intervening sequence. In some embodiments, Cas9 is encoded by a codon-optimized sequence. Plasmids encoding Cas9, including codon-optimized plasmids and plasmids encoding engineered Cas9 nickase are publicly available from Addgene (<http://www.addgene.org/CRISPR/>).

Additional information on the application of CRISPR-Cas systems to targeted genome engineering can be found in Jinek *et al.*, *Science* 337:816-821 (2012); Cho *et al.*, *Nature Biotechnology* 31:230-232 (2013); Cong *et al.*, *Science* 339:819-823 (2013); Jinek *et al.*, *eLife* 2:e00471 (2013); Mali *et al.*, *Science* 339:823-826 (2013); Qi *et al.*, *Cell* 152:1173-1183 (2013); Fu *et al.*, *Nature Biotechnology* 31:822-826 (2013); Fu *et al.*, *Nature Biotechnology* 31:822-826 (2013); Hsu *et al.*, *Nature Biotechnology* 31:827-832 (2013); Mali *et al.*, *Nature Biotechnology* 31:833-838 (2013); Pattanayak *et al.*, *Nature Biotechnology* 31:839-843 (2013) and WO/2013/142578, each of which is hereby incorporated by reference in its entirety.

In some embodiments of the methods provided herein, the target nucleic acid sequence is modified using a CRISPR/Cas system. In some embodiments, the CRISPR/Cas system is a CRISPR-Cas9 system. In some embodiments, the subject is administered a nucleic acid encoding Cas9 and a nucleic acid encoding a guide-RNA that is specific to a target nucleic acid sequence in the eye.

In some embodiments, the guide-RNA comprises a target-specific guide sequence (*e.g.*, a sequence that is complementary to a sequence of the target DNA sequence) and a guide-RNA scaffold sequence. In some embodiments, the target-specific guide sequence is a nucleic acid sequence selected from any one of SEQ ID NOs: 1-172 and 174-342, or any combination thereof. The target-specific guide sequence may comprise two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty nucleic acid sequences selected from the nucleotide sequences set forth in SEQ ID NOs: 1-172 and 174-342.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

The present description is further illustrated by the following examples, which should not be construed as limiting in any way.

EXAMPLES

CRISPR-Cas9 guide RNAs (gRNAs) targeting known mutations causing corneal dystrophies were identified (**Table 1a-1c**). Human genomic sequences corresponding to gRNA IDs in Table 1 are listed in **Table 2**.

Mutations in transforming growth factor beta-induced (TGFB1) gene are known to cause several forms of corneal dystrophies including Reis–Bücklers corneal dystrophy, Thiel-Behnke corneal dystrophy, Lattice corneal dystrophy, Granular corneal dystrophy, type 1, and Granular corneal dystrophy, type 2 (1). Missense mutations at two hotspots, R124 and R555, account for nearly 50% of the TGFB1-related corneal dystrophies (9).

In order to demonstrate the feasibility of CRISPR-based treatments for corneal dystrophies, two Cas9 targeting sites were identified that overlap with the genomic sequence encoding both R124 and R555 of TGFB1: 5'–

TCAGCTGTACACGGACCGCACGG –3' (SEQ ID NO: 145), and 5'–

AGAGAACGGAGCAGACTCTTGGG –3' (SEQ ID NO: 171), located in exons 4 and 12, respectively. Specific amino acid substitutions at these residues result in clinically distinct corneal dystrophies: R124C - Lattice corneal dystrophy, type I; R124H - Granular corneal dystrophy, type 2; R555W - Granular corneal dystrophy, type 1; and R555Q - Reis–Bücklers corneal dystrophy.

The two target sites were cloned in pH1v1 (Addgene 60244) as described (8), and HEK293 cells were co-transfected with Cas9 and guide RNA (gRNA) constructs. Forty-eight or sixty hours post transfection, genomic DNA was harvested and the sequence surrounding the target cut sites were amplified according to the primers listed in the Appendix A (see below). The PCR products were then purified and quantified before performing the T7 Endo I assay.

Briefly, 200ng of PCR product was denatured and then slowly re-annealed to allow for the formation of heteroduplexes, T7 Endonuclease I was added to the PCR products and incubated at 37°C for 25/30 minutes to cleave heteroduplexes. The reaction was stopped by putting PCR products on ice, purified and finally run on a 6% TBE PAGE gel to resolve the products. The gel was stained with SYBR-Gold/ Diamond Nucleic Acid dye from Promega, visualized, and quantified using ImageJ. Non-homologous end joining (NHEJ) frequencies were calculated using the binomial-derived equation: % gene modification = $1 - \sqrt{1 - \frac{(a+b)}{(a+b+c)}}$ X 100; where the values of “a” and “b” are equal to the integrated area of the cleaved fragments after background subtraction and “c” is equal to the integrated area of the un-cleaved PCR product after background subtraction.

The results (**Figure 1**) indicate that all identified sites were targetable by Cas9 using the gRNA sequences that either overlap with the respective mutations (TGFB1) or targets

5' or 3' of the repeat region (TCF4). These results demonstrate the ability to disrupt dominant mutations in genes known to be causative in corneal dystrophies.

CRISPR-Cas9 approaches to treat corneal dystrophies and microsatellite expansion diseases could employ single or multiple guide RNAs to inactivate or excise gene mutations, or using a repair template and homology directed repair to correct a gene mutation. In the case of the TCF4 microsatellite expansion causing FECD, one or more gRNAs targeting a region on one side of a microsatellite expansion or regions on both sides of a microsatellite expansion could be used. **Table 3** shows IDs and corresponding human genomic sequences for gRNA target sequences upstream of the TCF4 microsatellite expansion causing FECD. **Table 4** shows IDs and corresponding human genomic sequences for gRNA target sequences downstream of the same TCF4 microsatellite expansion. These gRNAs or others in the TCF4 gene could be used in any combination to correct the microsatellite expansion causing FECD. A similar approach using one or more gRNAs targeting a region on one side of a microsatellite expansion or regions on both sides of a microsatellite expansion could be used for other microsatellite expansion diseases, including but not limited to those listed in **Table 5**.

Appendix A

CRISPR Targets:

20 TGFBI (124)
 hs101533615:TCAGCTGTACACGGACCGCACGG (SEQ ID NO: 145)
 TGFBI (555)
 hs101534962:AGAGAACGGAGCAGACTCTTGGG (SEQ ID NO: 171)

25 TCF4 (downstream of trinucleotide repeat)

 hs056193532- AAGTGCAACAAGCAGAAAGGGGG (SEQ ID NO: 333)
 hs056193533- GGCTGCAAAGCTGCCTGCCTAGG (SEQ ID NO: 334)
 hs056193534- GCTGCAAAGCTGCCTGCCTAGGG (SEQ ID NO: 335)
 hs056193535- CTGCCTAGGGCTACGTTTCCTGG (SEQ ID NO: 336)
 30 hs056193536- CAGGAAACGTAGCCCTAGGCAGG (SEQ ID NO: 337)
 hs056193537- TTGCCAGGAAACGTAGCCCTAGG (SEQ ID NO: 338)

TCF4 (upstream of trinucleotide repeat)

 hs056193542- AAAGAGCCCCACTTGGAAGGCGG (SEQ ID NO: 195)
 35 hs056193543- GCCCACTTGGAAGGCGGTTTGG (SEQ ID NO: 196)
 hs056193545- TCCAAACCGCCTTCCAAGTGGG (SEQ ID NO: 198)
 hs056193546- ATCCAAACCGCCTTCCAAGTGGG (SEQ ID NO: 199)

gRNA Primers:

40 TGFBI(124)humanF;CTTATAAGTTCTGTATGAGACCACTTTTCCCTCAGCT
 GTACACGGACCGCAG (SEQ ID NO: 173)

TGFBI(124)humanR;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTGCG
GTCCGTGTACAGCTGAGG (SEQ ID NO: 343)

TGFBI(555)humanF;CTTATAAGTTCTGTATGAGACCACTTTTTCCAGAGA
ACGGAGCAGACTCTTG (SEQ ID NO: 344)

5 TGFBI(555)humanR;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACAAG
AGTCTGCTCCGTTCTCTGG (SEQ ID NO: 345)

hs537_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCTTGCCAGGAA
ACGTAGCCCTG (SEQ ID NO: 352)

10 hs537_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACAGGGCTAC
GTTTCCTGGCAAG (SEQ ID NO: 353)

hs536_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCAGGAAACGT
AGCCCTAGGCG (SEQ ID NO: 354)

15 hs536_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGCCTAGGG
CTACGTTTCCTGG; (SEQ ID NO: 355)

hs535_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCTGCCTAGGG
CTACGTTTCCG (SEQ ID NO: 356)

hs535_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGGAAACGT
AGCCCTAGGCAGG (SEQ ID NO: 357)

20 hs534_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCGCTGCAAAGC
TGCCTGCCTAG (SEQ ID NO: 358)

hs534_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCTAGGCAGG
CAGCTTTGCAGCG (SEQ ID NO: 359)

25 hs533_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCGGCTGCAAAG
CTGCCTGCCTG (SEQ ID NO: 360)

hs533_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACAGGCAGGC
AGCTTTGCAGCCG (SEQ ID NO: 361)

hs532_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCAAGTGCAACA
AGCAGAAAGGG (SEQ ID NO: 362)

30 hs532_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCCTTTCTGC
TTGTTGCACTTG (SEQ ID NO: 363)

hs542_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCAAAGAGCCCC
ACTTGGAAGGG (SEQ ID NO: 364)

35 hs542_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCCTTCCAA
GTGGGGCTCTTTG (SEQ ID NO: 365)

hs543_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCGCCCCACTTG
GAAGGCGGTTG (SEQ ID NO: 366)

hs543_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACAACCGCCT
TCCAAGTGGGGCG (SEQ ID NO: 367)

40 hs545_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCTCCAAACCGC
CTTCCAAGTGG (SEQ ID NO: 368)

hs545_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCACTTGGA
AGGCGGTTTGGAG (SEQ ID NO: 369)

45 hs546_H1for;CTTATAAGTTCTGTATGAGACCACTTTTTCCCATCCAAACCG
CCTTCCAAGTG (SEQ ID NO: 370)

hs546_H1rev;CCTTATTTTAACTTGCTATTTCTAGCTCTAAAACACTTGGA
GGCGGTTTGGATG (SEQ ID NO: 371)

T7Endo I Genomic Amplification Primers:

5 TGFB1124.1F;CCACCTGTAGATGTACCGTGCTCTC (SEQ ID NO: 346)
TGFB1124.1R;AGGGGCTGCAGACTCTGTGTTTAAG (SEQ ID NO: 347)
TGFB1555.1F;AAGGAAAATACCTCTCAGCGTGGTG (SEQ ID NO: 348)
TGFB1555.1R;AGGCCTAGGGGTAGTAAAGGCTTCC (SEQ ID NO: 349)
TCF4.3F;TGCTTTGGATTGGTAGGACCTGTTC (SEQ ID NO: 372)
TCF4.3R;GGATAATGCACACCTTCCCTGAGTC (SEQ ID NO: 373)

TGFBI exon 4 amplicon:

10 CCACCTGTAGATGTACCGTGCTCTCTGTGTCAGAGAAGGGAGGGTGTGGTTGGGCT
GGACCCCCAGAGGCCATCCCTCCTTCTGTCTTCTGCTCCTGCAGCCCTACCACTC
TCAAACCTTTACGAGACCCTGGGAGTCGTTGGATCCACCACCACTCAGCTGTAC
ACGGACCGCACGGAGAAGCTGAGGCCTGAGATGGAGGGGGCCCGGCAGCTTCAC
CATCTTCGCCCCTAGCAACGAGGCCTGGGCCTCCTTGCCAGCTGTGAGATGACC
15 TCCGTCTGCCCGGGGGA CTCTTATGGGGA ACTGCCTTACTTCCCCGAGGGGTGG
GCATGATGAATGGGAGTCTGCAGTCATTTCCCTACTGTTTCAGGAAGCTTTCTCCT
TAACCCCTTAGAAAAGGCTGTGGA ACTTGAGCTAAAATATGTCTTACCAGGTTG
CGTCTAATGCCCCCGTTCCCTACTGGGCAGAAAGACTTGGGTGCTTCCTGAGG
AGGGATCCTTGGCAGAAGAGAGGCCTGGGCTCACGAGGGCTGAGAACATGTTT
20 CCCAGAGTTGCAAGGACCCATCTCTTAAACACAGAGTCTGCAGCCCCT (SEQ ID
NO: 350)

TGFBI exon 12 amplicon:

25 AAGGAAAATACCTCTCAGCGTGGTGAGGTATTTAAGGAAAATACCTGTTGACA
GGTGACATTTTCTGTGTGTGTATCTACAGCATGCTGGTAGCTGCCATCCAGTCTG
CAGGACTGACGGAGACCCTCAACCGGGAAGGAGTCTACACAGTCTTTGCTCCC
ACAAATGAAGCCTTCCGAGCCCTGCCACCAAGAGAACGGAGCAGACTCTTGGG
TAAAGACCAACTTAAGTACACGTCTCCATTTTCTAAAGTAGTGATCCCTCAGG
30 GCCCCAGCAGCAAACAGTTGGCACATCAAGGATTGACTTGAAGGGATTTTATG
ACAAGACTATTAGTGAAAGAGTGGGCGGGACTAAAGGAACTAGCAAAGGATG
AGGCCAACCAGGGACTAGCAACCCTGGGAAGCCTTTACTACCCCTAGGCCT
(SEQ ID NO: 351)

TCF4 gene amplicon:

35 TGCTTTGGATTGGTAGGACCTGTTCCTTACATCTTACCTCCTAGTTACATCTTTT
CCTAGGATTCTTAAACTAGTATGGATATGCTGAGCATACATTCTTTAGAACCCT
TTTGGACTGTTTTGGTAAATTTTCGTAGTCGTAGGATCAGCACAAAGCGGAACCT
GACACACTTGTTGGAGTTTTACGGCTGTACTTGGTCCTTCTCCATCCCTTTGCTTC
CTTTTCCTAAACCAAGTCCCAGACATGTCAGGAGAATGAATTCATTTTTAATGC
40 CAGATGAGTTTGGTGTAAAGATGCATTTGTAAAGCAAATAAAAAGAATCCACA
AAACACACAAATAAAAATCCAAACCGCCTTCCAAGTGGGGCTCTTTCATGCTGCT
GCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
CTGCTGCTGCTGCTCCTCCTCCTCCTCCTCCTTCTCCTCCTCCTCCTCCTTCTA
GACCTTCTTTTGGAGAAATGGCTTTCGGAAGTTTTGCCAGGAAACGTAGCCCTA
45 GGCAGGCAGCTTTGCAGCCCCCTTTCTGCTTGTTGCACTTTCTCCATTCGTTCCCT
TTGCTTTTTTGCAGGCTCTGACTCAGGGAAGGTGTGCATTATCC (SEQ ID NO: 374)

Table 1a: Known gene mutations affecting the cornea and overlapping gRNA target sequences by ID and gene(s).

Gene ID: Mutation	Gene(s)
NM_005202.3(COL8A2):c.1363_1364delCAinsGT	COL8A2
NM_005202.3(COL8A2):c.1363C>A (p.Gln455Lys)	COL8A2
NM_005202.3(COL8A2):c.1349T>G (p.Leu450Trp)	COL8A2
NM_001920.3(DCN):c.962delA (p.Lys321Argfs)	DCN
NM_001920.3(DCN):c.947delG (p.Gly316Aspfs)	DCN
NM_001920.3(DCN):c.967delT (p.Ser323Leufs)	DCN
NM_001920.3(DCN):c.941delC (p.Pro314Hisfs)	DCN
NM_000223.3(KRT12):c.55C>T (p.Arg19Trp)	KRT12
NM_000223.3(KRT12):c.43C>T (p.Pro15Ser)	KRT12
NM_000223.3(KRT12):c.427G>T (p.Val143Leu)	KRT12
NM_000223.3(KRT12):c.409G>C (p.Ala137Pro)	KRT12
NM_000223.3(KRT12):c.405A>C (p.Arg135Ser)	KRT12
NM_000223.3(KRT12):c.399T>G (p.Asn133Lys)	KRT12
NM_000223.3(KRT12):c.389A>C (p.Gln130Pro)	KRT12
NM_000223.3(KRT12):c.385A>G (p.Met129Val)	KRT12
NM_000223.3(KRT12):c.1298T>G (p.Leu433Arg)	KRT12
NM_000223.3(KRT12):c.1289G>C (p.Arg430Pro)	KRT12
NM_000223.3(KRT12):c.1286A>G (p.Tyr429Cys)	KRT12
NM_000223.3(KRT12):c.1277T>G (p.Ile426Ser)	KRT12
NM_000223.3(KRT12):c.1276A>G (p.Ile426Val)	KRT12
NM_000223.3(KRT12):c.*360A>C	KRT12
NM_000223.3(KRT12):c.386T>C (p.Met129Thr)	KRT12
NM_000223.3(KRT12):c.419T>G (p.Leu140Arg)	KRT12
NM_000223.3(KRT12):c.1285T>G (p.Tyr429Asp)	KRT12
NM_000223.3(KRT12):c.404G>T (p.Arg135Ile)	KRT12
NM_000223.3(KRT12):c.403A>G (p.Arg135Gly)	KRT12
NM_000223.3(KRT12):c.427G>C (p.Val143Leu)	KRT12
NM_057088.2(KRT3):c.109G>A (p.Gly37Arg)	KRT3
NM_057088.2(KRT3):c.1347C>A (p.Ala449=)	KRT3
NM_057088.2(KRT3):c.1508G>C (p.Arg503Pro)	KRT3
NM_057088.2(KRT3):c.1493A>T (p.Glu498Val)	KRT3
NM_057088.2(KRT3):c.1525G>A (p.Glu509Lys)	KRT3
NM_015040.3(PIKFYVE):c.1370C>T (p.Ser457Phe)	PIKFYVE
NM_015040.3(PIKFYVE):c.5018T>A (p.Phe1673Tyr)	PIKFYVE
NM_015040.3(PIKFYVE):c.4167_4170delAGTA (p.Glu1389Aspfs*16)	PIKFYVE
NM_015040.3(PIKFYVE):c.2962C>T (p.Gln988Ter)	PIKFYVE
NM_015040.3(PIKFYVE):c.3308A>G (p.Lys1103Arg)	PIKFYVE
NM_000351.4(STS):c.1331A>G (p.His444Arg)	STS
NM_000351.4(STS):c.1115G>C (p.Trp372Ser)	STS
NM_000358.2(TGFBI):c.593C>T (p.Ser198Phe)	TGFBI
NM_000358.2(TGFBI):c.1998G>C (p.Arg666Ser)	TGFBI
NM_000358.2(TGFBI):c.1526T>G (p.Leu509Arg)	TGFBI
NM_000358.2(TGFBI):c.1619T>C (p.Phe540Ser)	TGFBI
NM_000358.2(TGFBI):c.370C>A (p.Arg124Ser)	TGFBI

TGFBI, 3-BP DEL	TGFBI
NM_000358.2(TGFBI):c.1868G>A (p.Gly623Asp)	TGFBI
NM_000358.2(TGFBI):c.371G>A (p.Arg124His)	TGFBI
NM_000358.2(TGFBI):c.370C>T (p.Arg124Cys)	TGFBI
NM_000358.2(TGFBI):c.1663C>T (p.Arg555Trp)	TGFBI
NM_000358.2(TGFBI):c.[1637C>A;1652C>A]	TGFBI
NM_000358.2(TGFBI):c.371G>T (p.Arg124Leu)	TGFBI
NM_000358.2(TGFBI):c.1664G>A (p.Arg555Gln)	TGFBI
NM_000358.2(TGFBI):c.1501C>A (p.Pro501Thr)	TGFBI
NM_013319.2(UBIAD1):c.530G>A (p.Gly177Glu)	UBIAD1
NM_013319.2(UBIAD1):c.708C>G (p.Asp236Glu)	UBIAD1
NM_013319.2(UBIAD1):c.335A>G (p.Asp112Gly)	UBIAD1
NM_013319.2(UBIAD1):c.355A>G (p.Arg119Gly)	UBIAD1
NM_013319.2(UBIAD1):c.556G>A (p.Gly186Arg)	UBIAD1
NM_013319.2(UBIAD1):c.511T>C (p.Ser171Pro)	UBIAD1
NM_013319.2(UBIAD1):c.695A>G (p.Asn232Ser)	UBIAD1
NM_013319.2(UBIAD1):c.524C>T (p.Thr175Ile)	UBIAD1
NM_013319.2(UBIAD1):c.529G>C (p.Gly177Arg)	UBIAD1
NM_013319.2(UBIAD1):c.305A>G (p.Asn102Ser)	UBIAD1
NM_030751.5(ZEB1):c.2519A>C (p.Gln840Pro)	ZEB1
NM_030751.5(ZEB1):c.233A>C (p.Asn78Thr)	ZEB1

Table 1b. Known gene mutations affecting the cornea and overlapping gRNA target sequences by ID and condition(s).

<u>Gene ID: Mutation</u>	<u>Condition(s)</u>
NM_005202.3(COL8A2):c.1363_1364delCAinsGT	Corneal dystrophy Fuchs endothelial 1
NM_005202.3(COL8A2):c.1363C>A (p.Gln455Lys)	Corneal dystrophy Fuchs endothelial 1 Corneal dystrophy, posterior polymorphous, 2
NM_005202.3(COL8A2):c.1349T>G (p.Leu450Trp)	Corneal dystrophy Fuchs endothelial 1 Corneal dystrophy, posterior polymorphous, 2
NM_001920.3(DCN):c.962delA (p.Lys321Argfs)	Congenital Stromal Corneal Dystrophy
NM_001920.3(DCN):c.947delG (p.Gly316Aspfs)	Congenital Stromal Corneal Dystrophy
NM_001920.3(DCN):c.967delT (p.Ser323Leufs)	Congenital Stromal Corneal Dystrophy
NM_001920.3(DCN):c.941delC (p.Pro314Hisfs)	Congenital Stromal Corneal Dystrophy
NM_000223.3(KRT12):c.55C>T (p.Arg19Trp)	not provided
NM_000223.3(KRT12):c.43C>T (p.Pro15Ser)	not provided
NM_000223.3(KRT12):c.427G>T (p.Val143Leu)	not provided
NM_000223.3(KRT12):c.409G>C (p.Ala137Pro)	not provided
NM_000223.3(KRT12):c.405A>C (p.Arg135Ser)	not provided
NM_000223.3(KRT12):c.399T>G (p.Asn133Lys)	not provided
NM_000223.3(KRT12):c.389A>C (p.Gln130Pro)	not provided

NM_000223.3(KRT12):c.385A>G (p.Met129Val)	not provided
NM_000223.3(KRT12):c.1298T>G (p.Leu433Arg)	not provided
NM_000223.3(KRT12):c.1289G>C (p.Arg430Pro)	not provided
NM_000223.3(KRT12):c.1286A>G (p.Tyr429Cys)	not provided
NM_000223.3(KRT12):c.1277T>G (p.Ile426Ser)	not provided
NM_000223.3(KRT12):c.1276A>G (p.Ile426Val)	not provided
NM_000223.3(KRT12):c.*360A>C	not provided
NM_000223.3(KRT12):c.386T>C (p.Met129Thr)	Meesman's corneal dystrophy not provided
NM_000223.3(KRT12):c.419T>G (p.Leu140Arg)	Meesman's corneal dystrophy not provided
NM_000223.3(KRT12):c.1285T>G (p.Tyr429Asp)	Meesman's corneal dystrophy not provided
NM_000223.3(KRT12):c.404G>T (p.Arg135Ile)	Meesman's corneal dystrophy not provided
NM_000223.3(KRT12):c.403A>G (p.Arg135Gly)	Meesman's corneal dystrophy not provided
NM_000223.3(KRT12):c.427G>C (p.Val143Leu)	Meesman's corneal dystrophy not provided
NM_057088.2(KRT3):c.109G>A (p.Gly37Arg)	Malignant melanoma
NM_057088.2(KRT3):c.1347C>A (p.Ala449=)	Malignant melanoma
NM_057088.2(KRT3):c.1508G>C (p.Arg503Pro)	Meesman's corneal dystrophy not provided
NM_057088.2(KRT3):c.1493A>T (p.Glu498Val)	Meesman's corneal dystrophy not provided
NM_057088.2(KRT3):c.1525G>A (p.Glu509Lys)	Meesman's corneal dystrophy not provided
NM_015040.3(PIKFYVE):c.1370C>T (p.Ser457Phe)	Malignant melanoma
NM_015040.3(PIKFYVE):c.5018T>A (p.Phe1673Tyr)	Malignant melanoma
NM_015040.3(PIKFYVE):c.4167_4170delAGTA (p.Glu1389Aspfs*16)	Fleck corneal dystrophy
NM_015040.3(PIKFYVE):c.2962C>T (p.Gln988Ter)	Fleck corneal dystrophy
NM_015040.3(PIKFYVE):c.3308A>G (p.Lys1103Arg)	Fleck corneal dystrophy
NM_000351.4(STS):c.1331A>G (p.His444Arg)	X-linked ichthyosis with steryl-sulfatase deficiency
NM_000351.4(STS):c.1115G>C (p.Trp372Ser)	X-linked ichthyosis with steryl-sulfatase deficiency
NM_000358.2(TGFBI):c.593C>T (p.Ser198Phe)	Malignant melanoma
NM_000358.2(TGFBI):c.1998G>C (p.Arg666Ser)	Corneal epithelial dystrophy
NM_000358.2(TGFBI):c.1526T>G (p.Leu509Arg)	Corneal epithelial dystrophy
NM_000358.2(TGFBI):c.1619T>C (p.Phe540Ser)	Lattice corneal dystrophy type 3A
NM_000358.2(TGFBI):c.370C>A (p.Arg124Ser)	Groenouw corneal dystrophy type I
TGFBI, 3-BP DEL	Reis-Bucklers' corneal dystrophy
NM_000358.2(TGFBI):c.1868G>A (p.Gly623Asp)	Reis-Bucklers' corneal dystrophy
NM_000358.2(TGFBI):c.371G>A (p.Arg124His)	Avellino corneal dystrophy
NM_000358.2(TGFBI):c.370C>T (p.Arg124Cys)	Lattice corneal dystrophy Type I

NM_000358.2(TGFBI):c.1663C>T (p.Arg555Trp)	Groenouw corneal dystrophy type I
NM_000358.2(TGFBI):c.[1637C>A;1652C>A]	Lattice corneal dystrophy Type I
NM_000358.2(TGFBI):c.371G>T (p.Arg124Leu)	Reis-Bucklers' corneal dystrophy
NM_000358.2(TGFBI):c.1664G>A (p.Arg555Gln)	Thiel-Behnke corneal dystrophy
NM_000358.2(TGFBI):c.1501C>A (p.Pro501Thr)	Lattice corneal dystrophy type 3A
NM_013319.2(UBIAD1):c.530G>A (p.Gly177Glu)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.708C>G (p.Asp236Glu)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.335A>G (p.Asp112Gly)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.355A>G (p.Arg119Gly)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.556G>A (p.Gly186Arg)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.511T>C (p.Ser171Pro)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.695A>G (p.Asn232Ser)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.524C>T (p.Thr175Ile)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.529G>C (p.Gly177Arg)	Schnyder crystalline corneal dystrophy
NM_013319.2(UBIAD1):c.305A>G (p.Asn102Ser)	Schnyder crystalline corneal dystrophy
NM_030751.5(ZEB1):c.2519A>C (p.Gln840Pro)	Corneal dystrophy, fuchs endothelial, 6
NM_030751.5(ZEB1):c.233A>C (p.Asn78Thr)	Corneal dystrophy, fuchs endothelial, 6

Table 1c: Known gene mutations affecting the cornea and overlapping gRNA target sequences by ID and gRNA(s).

Gene ID: Mutation	gRNAs
NM_005202.3(COL8A2):c.1363_1364delCAinsGT	
NM_005202.3(COL8A2):c.1363C>A (p.Gln455Lys)	hs002677511, hs002677512, hs002677505, hs002677513, hs002677506, hs002677516, hs002677517, hs002677510, hs002677518
NM_005202.3(COL8A2):c.1349T>G (p.Leu450Trp)	hs002677507, hs002677508, hs002677509, hs002677511, hs002677512, hs002677505, hs002677513, hs002677506
NM_001920.3(DCN):c.962delA (p.Lys321Argfs)	hs028999998, hs029000002, hs029000003, hs028999999, hs029000004, hs029000000
NM_001920.3(DCN):c.947delG (p.Gly316Aspfs)	hs028999999, hs029000004, hs029000000, hs029000001,

	hs029000005
NM_001920.3(DCN):c.967delT (p.Ser323Leufs)	hs028999998, hs029000002, hs029000003
NM_001920.3(DCN):c.941delC (p.Pro314Hisfs)	hs029000000, hs029000001, hs029000005
NM_000223.3(KRT12):c.55C>T (p.Arg19Trp)	hs051021143, hs051021144, hs051021148, hs051021149, hs051021150, hs051021151, hs051021154, hs051021155
NM_000223.3(KRT12):c.43C>T (p.Pro15Ser)	hs051021148, hs051021149, hs051021150, hs051021151, hs051021154, hs051021155, hs051021157
NM_000223.3(KRT12):c.427G>T (p.Val143Leu)	hs051021071, hs051021069, hs051021070
NM_000223.3(KRT12):c.409G>C (p.Ala137Pro)	hs051021072, hs051021073
NM_000223.3(KRT12):c.405A>C (p.Arg135Ser)	hs051021073
NM_000223.3(KRT12):c.399T>G (p.Asn133Lys)	
NM_000223.3(KRT12):c.389A>C (p.Gln130Pro)	
NM_000223.3(KRT12):c.385A>G (p.Met129Val)	
NM_000223.3(KRT12):c.1298T>G (p.Leu433Arg)	hs051020754, hs051020755, hs051020748, hs051020750, hs051020752, hs051020756, hs051020758, hs051020753, hs051020759, hs051020760, hs051020763
NM_000223.3(KRT12):c.1289G>C (p.Arg430Pro)	hs051020752, hs051020756, hs051020758, hs051020753, hs051020759, hs051020760, hs051020763
NM_000223.3(KRT12):c.1286A>G (p.Tyr429Cys)	hs051020758, hs051020753, hs051020759, hs051020760, hs051020763
NM_000223.3(KRT12):c.1277T>G (p.Ile426Ser)	hs051020757, hs051020761, hs051020762
NM_000223.3(KRT12):c.1276A>G (p.Ile426Val)	hs051020757, hs051020761, hs051020762
NM_000223.3(KRT12):c.*360A>C	
NM_000223.3(KRT12):c.386T>C (p.Met129Thr)	
NM_000223.3(KRT12):c.419T>G (p.Leu140Arg)	hs051021069, hs051021070, hs051021072, hs051021073
NM_000223.3(KRT12):c.1285T>G (p.Tyr429Asp)	hs051020758, hs051020753, hs051020759, hs051020760, hs051020763
NM_000223.3(KRT12):c.404G>T (p.Arg135Ile)	hs051021073
NM_000223.3(KRT12):c.403A>G (p.Arg135Gly)	hs051021073
NM_000223.3(KRT12):c.427G>C (p.Val143Leu)	hs051021071, hs051021069, hs051021070

NM_057088.2(KRT3):c.109G>A (p.Gly37Arg)	hs027503914, hs027503915, hs027503916, hs027503917, hs027503911, hs027503912, hs027503918, hs027503919, hs027503921
NM_057088.2(KRT3):c.1347C>A (p.Ala449=)	hs027503292, hs027503297, hs027503293, hs027503299, hs027503294
NM_057088.2(KRT3):c.1508G>C (p.Arg503Pro)	
NM_057088.2(KRT3):c.1493A>T (p.Glu498Val)	
NM_057088.2(KRT3):c.1525G>A (p.Glu509Lys)	
NM_015040.3(PIKFYVE):c.1370C>T (p.Ser457Phe)	hs070544033, hs070544034, hs070544030, hs070544031
NM_015040.3(PIKFYVE):c.5018T>A (p.Phe1673Tyr)	hs070545604, hs070545605, hs070545601, hs070545606
NM_015040.3(PIKFYVE):c.4167_4170delAGTA (p.Glu1389Aspfs*16)	hs070544848, hs070544849, hs070544850, hs070544847, hs070544851
NM_015040.3(PIKFYVE):c.2962C>T (p.Gln988Ter)	hs070544887, hs070544888
NM_015040.3(PIKFYVE):c.3308A>G (p.Lys1103Arg)	hs131603026, hs131603028
NM_000351.4(STS):c.1331A>G (p.His444Arg)	hs131602458, hs131602459, hs131602461, hs131602462, hs131602463, hs131602464
NM_000351.4(STS):c.1115G>C (p.Trp372Ser)	
NM_000358.2(TGFBI):c.593C>T (p.Ser198Phe)	
NM_000358.2(TGFBI):c.1998G>C (p.Arg666Ser)	hs101535656, hs101535663, hs101535663, hs101535656
NM_000358.2(TGFBI):c.1526T>G (p.Leu509Arg)	hs101534814, hs101534815, hs101534824
NM_000358.2(TGFBI):c.1619T>C (p.Phe540Ser)	hs101534957
NM_000358.2(TGFBI):c.370C>A (p.Arg124Ser)	hs101533619, hs101533615, hs101533616, hs101533625
TGFBI, 3-BP DEL	
NM_000358.2(TGFBI):c.1868G>A (p.Gly623Asp)	hs101535371, hs101535375, hs101535372, hs101535376
NM_000358.2(TGFBI):c.371G>A (p.Arg124His)	hs101533619, hs101533615, hs101533616, hs101533625
NM_000358.2(TGFBI):c.370C>T (p.Arg124Cys)	hs101533619, hs101533615, hs101533616, hs101533625
NM_000358.2(TGFBI):c.1663C>T (p.Arg555Trp)	hs101534964, hs101534958, hs101534965, hs101534966, hs101534967, hs101534968, hs101534961, hs101534962
NM_000358.2(TGFBI):c.[1637C>A;1652C>A]	hs101534959, hs101534960
NM_000358.2(TGFBI):c.371G>T (p.Arg124Leu)	hs101533619, hs101533615, hs101533616, hs101533625
NM_000358.2(TGFBI):c.1664G>A (p.Arg555Gln)	hs101534964, hs101534958,

	hs101534965, hs101534966, hs101534967, hs101534968, hs101534961, hs101534962
NM_000358.2(TGFBI):c.1501C>A (p.Pro501Thr)	hs101534807, hs101534813, hs101534808, hs101534809, hs101534816, hs101534812, hs101534817, hs101534818, hs101534819, hs101534820, hs101534821
NM_013319.2(UBIAD1):c.530G>A (p.Gly177Glu)	hs001050141, hs001050144, hs001050142 & hs001050500, hs001050502, hs001050507, hs001050504
NM_013319.2(UBIAD1):c.708C>G (p.Asp236Glu)	hs001050535, hs001050534, hs001050538, hs001050546, hs001050540, hs001050547, hs001050542, hs001050543
NM_013319.2(UBIAD1):c.335A>G (p.Asp112Gly)	hs001050116
NM_013319.2(UBIAD1):c.355A>G (p.Arg119Gly)	hs001050115, hs001050119, hs001050117
NM_013319.2(UBIAD1):c.556G>A (p.Gly186Arg)	hs001050505, hs001050506
NM_013319.2(UBIAD1):c.511T>C (p.Ser171Pro)	hs001050143, hs001050140, hs001050141
NM_013319.2(UBIAD1):c.695A>G (p.Asn232Ser)	hs001050539, hs001050541, hs001050534, hs001050535, hs001050544, hs001050538, hs001050546
NM_013319.2(UBIAD1):c.524C>T (p.Thr175Ile)	hs001050143, hs001050140, hs001050141, hs001050144, hs001050142
NM_013319.2(UBIAD1):c.529G>C (p.Gly177Arg)	hs001050141, hs001050144, hs001050142 & hs001050500, hs001050502, hs001050507, hs001050504
NM_013319.2(UBIAD1):c.305A>G (p.Asn102Ser)	hs001050114
NM_030751.5(ZEB1):c.2519A>C (p.Gln840Pro)	hs013097041, hs013097042, hs013097045, hs013097046
NM_030751.5(ZEB1):c.233A>C (p.Asn78Thr)	hs013095774, hs013095775, hs013095776

Table 2: The gRNA target sequences by ID in Table 1 and corresponding human genomic sequence.

	#	ID	Genomic Sequence
5	1	hs001050117	GAGTGTGATGACAGGACACTTGTGG (SEQ ID NO:1)
	2	hs001050505	TGGATTCAAGTACGTGGCTCTGG (SEQ ID NO:2)
	3	hs001050119	TCCTGTTCATCACTCTTTTGTGG (SEQ ID NO:3)
	4	hs001050140	TCTGGCTCCTTTCTCTACACAGG (SEQ ID NO:4)
	5	hs001050141	GGCTCCTTTCTCTACACAGGAGG (SEQ ID NO:5)
10	6	hs001050141	GGCTCCTTTCTCTACACAGGAGG (SEQ ID NO:6)

	7	hs001050539	GTTGGAATGGAGAATGGCCTCGG (SEQ ID NO: 7)
	8	hs001050142	TCTACACAGGAGGTAAGATTTGG (SEQ ID NO: 8)
	9	hs001050142	TCTACACAGGAGGTAAGATTTGG (SEQ ID NO: 9)
	10	hs001050141	GGCTCCTTTCTCTACACAGGAGG (SEQ ID NO: 10)
5	11	hs001050144	CTTACCTCCTGTGTAGAGAAAGG (SEQ ID NO: 11)
	12	hs001050500	TCTCTGGATTTTCTGGCCGCAGG (SEQ ID NO: 12)
	13	hs001050502	GATTTTCTGGCCGCAGGAATTGG (SEQ ID NO: 13)
	14	hs001050504	AGGAATTGGATTCAAGTACGTGG (SEQ ID NO: 14)
	15	hs001050114	GTAAGTGTGACCAAATTACCGG (SEQ ID NO: 15)
10	16	hs028999998	ACCCGAATAAGAAGCCTTTTTTGG (SEQ ID NO: 16)
	17	hs001050504	AGGAATTGGATTCAAGTACGTGG (SEQ ID NO: 17)
	18	hs001050535	TCTCCATTCCAACAACACCAGGG (SEQ ID NO: 18)
	19	hs001050506	GGATTCAAGTACGTGGCTCTGGG (SEQ ID NO: 19)
	20	hs001050143	TGTAGAGAAAGGAGCCAGACAGG (SEQ ID NO: 20)
15	21	hs001050507	GTACTTGAATCCAATTCCTGCGG (SEQ ID NO: 21)
	22	hs001050534	TTCTCCATTCCAACAACACCAGG (SEQ ID NO: 22)
	23	hs001050535	TCTCCATTCCAACAACACCAGGG (SEQ ID NO: 23)
	24	hs001050538	TTCCAACAACACCAGGGACATGG (SEQ ID NO: 24)
	25	hs001050540	CCAGGGACATGGAGTCCGACCGG (SEQ ID NO: 25)
20	26	hs001050541	GGTGTGTGTGGAATGGAGAATGG (SEQ ID NO: 26)
	27	hs001050542	CAGGGACATGGAGTCCGACCGGG (SEQ ID NO: 27)
	28	hs001050543	GGACATGGAGTCCGACCGGGAGG (SEQ ID NO: 28)
	29	hs001050116	CTTTTTGTGGTCAATGCCCTTGG (SEQ ID NO: 29)
	30	hs001050115	ACCACAAAAGAGTGATGACAGG (SEQ ID NO: 30)
25	31	hs001050544	TGTCCCTGGTGTGTTGGAATGG (SEQ ID NO: 31)
	32	hs001050546	CTCCATGTCCCTGGTGTGTTGG (SEQ ID NO: 32)
	33	hs001050546	CTCCATGTCCCTGGTGTGTTGG (SEQ ID NO: 33)
	34	hs001050143	TGTAGAGAAAGGAGCCAGACAGG (SEQ ID NO: 34)
	35	hs001050547	CCGGTCGGACTCCATGTCCCTGG (SEQ ID NO: 35)
30	36	hs002677505	CCAAGTCACCTTTCTGCCCCAGG (SEQ ID NO: 36)
	37	hs002677506	CAAGTCACCTTTCTGCCCCAGGG (SEQ ID NO: 37)
	38	hs002677506	CAAGTCACCTTTCTGCCCCAGGG (SEQ ID NO: 38)
	39	hs013097041	GCAAACGATTCTGATTCCCCAGG (SEQ ID NO: 39)
	40	hs002677508	AAGGTGACTTGGGGCTCCCTGGG (SEQ ID NO: 40)
35	41	hs002677509	AAAGGTGACTTGGGGCTCCCTGG (SEQ ID NO: 41)
	42	hs002677510	CCCAGGGCTCCTGCCACCCCTGG (SEQ ID NO: 42)
	43	hs002677511	TGGGGCAGAAAGGTGACTTGGGG (SEQ ID NO: 43)
	44	hs002677512	CTGGGGCAGAAAGGTGACTTGGG (SEQ ID NO: 44)
	45	hs002677513	CCTGGGGCAGAAAGGTGACTTGG (SEQ ID NO: 45)
40	46	hs002677516	GCAGGAGCCCTGGGGCAGAAAGG (SEQ ID NO: 46)
	47	hs002677517	CAGGGGTGGCAGGAGCCCTGGGG (SEQ ID NO: 47)
	48	hs002677518	CCAGGGGTGGCAGGAGCCCTGGG (SEQ ID NO: 48)
	49	hs002677507	TTGGGGCTCCCTGGGCAGCCTGG (SEQ ID NO: 49)
	50	hs013095775	AGGGAATGCTAAGAACTGCTGGG (SEQ ID NO: 50)
45	51	hs013095776	GAATGCTAAGAACTGCTGGGAGG (SEQ ID NO: 51)
	52	hs013097042	AACGATTCTGATTCCCCAGGTGG (SEQ ID NO: 52)
	53	hs013097045	GAGTAGGTGTATGCCACCTGGGG (SEQ ID NO: 53)
	54	hs013097046	TGAGTAGGTGTATGCCACCTGGG (SEQ ID NO: 54)
	55	hs013095774	AAGGGAATGCTAAGAACTGCTGG (SEQ ID NO: 55)
50	56	hs027503293	CTCTCCATGCTGCTCGGCCTCGG (SEQ ID NO: 56)
	57	hs027503294	ATGCTGCTCGGCCTCGGCAATGG (SEQ ID NO: 57)
	58	hs027503297	GGCCGAGCAGCATGGAGAGATGG (SEQ ID NO: 58)
	59	hs027503299	ATTGCCGAGGCCGAGCAGCATGG (SEQ ID NO: 59)

	60	hs027503911	CTCCGCCAGCTCCCCCAGAGTGG (SEQ ID NO: 60)
	61	hs027503912	TCCGCCAGCTCCCCCAGAGTGGG (SEQ ID NO: 61)
	62	hs027503914	GGAGCTGGCGGAGGGGCCTATGG (SEQ ID NO: 62)
	63	hs027503915	CTCTGGGGGAGCTGGCGGAGGGG (SEQ ID NO: 63)
5	64	hs027503916	ACTCTGGGGGAGCTGGCGGAGGG (SEQ ID NO: 64)
	65	hs027503917	CACTCTGGGGGAGCTGGCGGAGG (SEQ ID NO: 65)
	66	hs027503918	GCCCACTCTGGGGGAGCTGGCGG (SEQ ID NO: 66)
	67	hs027503919	GTGGCCCACTCTGGGGGAGCTGG (SEQ ID NO: 67)
	68	hs027503921	AGCTGTGTGGCCCACTCTGGGGG (SEQ ID NO: 68)
10	69	hs027503292	GGCCATCTCTCCATGCTGCTCGG (SEQ ID NO: 69)
	70	hs028999999	GCCTTTTTTGGTGTGTGTCCAGG (SEQ ID NO: 70)
	71	hs029000000	TTTTTGGTGTGTGTGTCCAGGTGG (SEQ ID NO: 71)
	72	hs029000000	TTTTTGGTGTGTGTGTCCAGGTGG (SEQ ID NO: 72)
	73	hs028999999	GCCTTTTTTGGTGTGTGTCCAGG (SEQ ID NO: 73)
15	74	hs029000001	TTTTGGTGTGTGTGTCCAGGTGGG (SEQ ID NO: 74)
	75	hs029000002	ACCAAAAAGGCTTCTTATTCGGG (SEQ ID NO: 75)
	76	hs029000003	CACCAAAAAGGCTTCTTATTCGG (SEQ ID NO: 76)
	77	hs029000003	CACCAAAAAGGCTTCTTATTCGG (SEQ ID NO: 77)
	78	hs029000000	TTTTTGGTGTGTGTGTCCAGGTGG (SEQ ID NO: 78)
20	79	hs029000004	ACCTGGACACAACACCAAAAAGG (SEQ ID NO: 79)
	80	hs029000005	TCAAGTGACTTCTGCCCACCTGG (SEQ ID NO: 80)
	81	hs028999998	ACCCGAATAAGAAGCCTTTTTTGG (SEQ ID NO: 81)
	82	hs029000005	TCAAGTGACTTCTGCCCACCTGG (SEQ ID NO: 82)
	83	hs070544033	GAGTTCAGTACTGAGTCACTGTCTGGG (SEQ ID NO: 83)
25	84	hs051020748	CTTGGGCCTCCCCGTCCAGCAGG (SEQ ID NO: 84)
	85	hs051020750	GGGCCTCCCCGTCCAGCAGGCGG (SEQ ID NO: 85)
	86	hs051020752	CCTCCCCGTCCAGCAGGCGGCGG (SEQ ID NO: 86)
	87	hs051020753	CCCGTCCAGCAGGCGGCGGTAGG (SEQ ID NO: 87)
	88	hs051020755	CTGCTGGACGGGGAGGCCCAAGG (SEQ ID NO: 88)
30	89	hs051020756	CCGCCGCCTGCTGGACGGGGAGG (SEQ ID NO: 89)
	90	hs051020758	CTACCGCCGCCTGCTGGACGGGG (SEQ ID NO: 90)
	91	hs051020759	CCTACCGCCGCCTGCTGGACGGG (SEQ ID NO: 91)
	92	hs051020760	ACCTACCGCCGCCTGCTGGACGG (SEQ ID NO: 92)
	93	hs051020761	TCTCAATCTCCAGCTCCAGGCGG (SEQ ID NO: 93)
35	94	hs051020762	CTCAATCTCCAGCTCCAGGCGGG (SEQ ID NO: 94)
	95	hs051020757	AGGTCTCAATCTCCAGCTCCAGG (SEQ ID NO: 95)
	96	hs051020762	CTCAATCTCCAGCTCCAGGCGGG (SEQ ID NO: 96)
	97	hs051021069	CTAGAGCTCGCACCTTATCCAGG (SEQ ID NO: 97)
	98	hs051020763	TGAGACCTACCGCCGCCTGCTGG (SEQ ID NO: 98)
40	99	hs051020752	CCTCCCCGTCCAGCAGGCGGCGG (SEQ ID NO: 99)
	100	hs051020763	TGAGACCTACCGCCGCCTGCTGG (SEQ ID NO: 100)
	101	hs051020757	AGGTCTCAATCTCCAGCTCCAGG (SEQ ID NO: 101)
	102	hs051020763	TGAGACCTACCGCCGCCTGCTGG (SEQ ID NO: 102)
	103	hs051020758	CTACCGCCGCCTGCTGGACGGGG (SEQ ID NO: 103)
45	104	hs051020763	TGAGACCTACCGCCGCCTGCTGG (SEQ ID NO: 104)
	105	hs051021073	TGATAGATTAGCTTCCTACCTGG (SEQ ID NO: 105)
	106	hs051021073	TGATAGATTAGCTTCCTACCTGG (SEQ ID NO: 106)
	107	hs051021071	TAAGGTGCGAGCTCTAGAAGAGG (SEQ ID NO: 107)
	108	hs051021069	CTAGAGCTCGCACCTTATCCAGG (SEQ ID NO: 108)
50	109	hs051021070	AGCTCGCACCTTATCCAGGTAGG (SEQ ID NO: 109)
	110	hs051021070	AGCTCGCACCTTATCCAGGTAGG (SEQ ID NO: 110)
	111	hs051021072	ATTAGCTTCCTACCTGGATAAGG (SEQ ID NO: 111)
	112	hs051021072	ATTAGCTTCCTACCTGGATAAGG (SEQ ID NO: 112)

	113	hs051021073	TGATAGATTAGCTTCCTACCTGG (SEQ ID NO:113)
	114	hs051020758	CTACCGCCGCCTGCTGGACGGGG (SEQ ID NO:114)
	115	hs051021073	TGATAGATTAGCTTCCTACCTGG (SEQ ID NO:115)
	116	hs051021073	TGATAGATTAGCTTCCTACCTGG (SEQ ID NO:116)
5	117	hs051020754	TGCTGGACGGGGAGGCCCAAGGG (SEQ ID NO:117)
	118	hs051021143	CACTCTGCGAGGAGAGCCGCCGG (SEQ ID NO:118)
	119	hs051021144	ACTCTGCGAGGAGAGCCGCCGG (SEQ ID NO:119)
	120	hs051021148	GGAGAGCCGCCGGGACAGTCCGG (SEQ ID NO:120)
	121	hs051021149	GAGAGCCGCCGGGACAGTCCGG (SEQ ID NO:121)
10	122	hs051021150	AGAGCCGCCGGGACAGTCCGGGG (SEQ ID NO:122)
	123	hs051021151	GAGCCGCCGGGACAGTCCGGGG (SEQ ID NO:123)
	124	hs051021154	GCACCCCGGACTGTCCCGGCCGG (SEQ ID NO:124)
	125	hs051021155	TGCGCACCCCGGACTGTCCCGG (SEQ ID NO:125)
	126	hs051021155	TGCGCACCCCGGACTGTCCCGG (SEQ ID NO:126)
15	127	hs051021148	GGAGAGCCGCCGGGACAGTCCGG (SEQ ID NO:127)
	128	hs051021157	TCACTCTCAGTGCGCACCCCGG (SEQ ID NO:128)
	129	hs051021071	TAAGGTGCGAGCTCTAGAAGAGG (SEQ ID NO:129)
	130	hs070544030	CAGTGACTCAGTGAACCTCCGTGG (SEQ ID NO:130)
	131	hs070544031	GACTCAGTGAACCTCCGTGGAAGG (SEQ ID NO:131)
20	132	hs070545604	GCAAAAGCAATGATGGAGCTGGG (SEQ ID NO:132)
	133	hs070544034	GGAGTTCACTGAGTCACTGTCCG (SEQ ID NO:133)
	134	hs070544847	GATGACCAACAAGATGCTTTAGG (SEQ ID NO:134)
	135	hs070544849	CATCTTGTGTGGTCATCCACAGGG (SEQ ID NO:135)
	136	hs070544850	GCATCTTGTGTGGTCATCCACAGG (SEQ ID NO:136)
25	137	hs070544851	CGCTGCCTAAAGCATCTTGTTGG (SEQ ID NO:137)
	138	hs070544887	GGAGGAAGAAACAGCTGCTCAGG (SEQ ID NO:138)
	139	hs070544888	GAGGAAGAAACAGCTGCTCAGG (SEQ ID NO:139)
	140	hs131603026	ATGGAAGAGAAACTCATGATCGG (SEQ ID NO:140)
	141	hs070545601	CCATCATTGCTTTTGCTCTCAGG (SEQ ID NO:141)
30	142	hs070545605	AGCAAAAGCAATGATGGAGCTGG (SEQ ID NO:142)
	143	hs070545606	CCTGAGAGCAAAAGCAATGATGG (SEQ ID NO:143)
	144	hs070544848	TGTTGGTCATCCACAGGGAGTGG (SEQ ID NO:144)
	145	hs101533615	TCAGCTGTACACGGACCGCACGG (SEQ ID NO:145)
	146	hs101533616	CGGACCGCACGGAGAAGCTGAGG (SEQ ID NO:146)
35	147	hs101533625	CAGGCCTCAGCTTCTCCGTGCGG (SEQ ID NO:147)
	148	hs101533619	GCGGTCCGTGTACAGCTGAGTGG (SEQ ID NO:148)
	149	hs101533625	CAGGCCTCAGCTTCTCCGTGCGG (SEQ ID NO:149)
	150	hs101534964	GTTCTCTTGGTGGCAGGGCTCGG (SEQ ID NO:150)
	151	hs101533625	CAGGCCTCAGCTTCTCCGTGCGG (SEQ ID NO:151)
40	152	hs101535371	CCTGACATCATGGCCACAAATGG (SEQ ID NO:152)
	153	hs101534808	CGGGTGCTGACCCCCCAATGGG (SEQ ID NO:153)
	154	hs101534809	GGGTGCTGACCCCCCAATGGGG (SEQ ID NO:154)
	155	hs101534812	CCCCCAATGGGGACTGTCATGG (SEQ ID NO:155)
	156	hs101534813	CCATTGGGGGGGTCAGCACCCGG (SEQ ID NO:156)
45	157	hs101534815	ACTGTCATGGATGTCCTGAAGGG (SEQ ID NO:157)
	158	hs101534816	CATGACAGTCCCCATTGGGGGGG (SEQ ID NO:158)
	159	hs101534817	CCATGACAGTCCCCATTGGGGGG (SEQ ID NO:159)
	160	hs101534818	TCCATGACAGTCCCCATTGGGGG (SEQ ID NO:160)
	161	hs101534819	ATCCATGACAGTCCCCATTGGGG (SEQ ID NO:161)
50	162	hs101534820	CATCCATGACAGTCCCCATTGGG (SEQ ID NO:162)
	163	hs101534821	ACATCCATGACAGTCCCCATTGG (SEQ ID NO:163)
	164	hs101534824	TAAAGCGATTGTCTCCCTTCAGG (SEQ ID NO:164)
	165	hs101534957	AGACTGTGTAGACTCCTTCCCGG (SEQ ID NO:165)

	166	hs101533619	GCGGTCCGTGTACAGCTGAGTGG (SEQ ID NO:166)
	167	hs101534958	GAGCCCTGCCACCAAGAGAACGG (SEQ ID NO:167)
	168	hs101534960	GGCTCGGAAGGCTTCATTTGTGG (SEQ ID NO:168)
	169	hs101533619	GCGGTCCGTGTACAGCTGAGTGG (SEQ ID NO:169)
5	170	hs101534961	AAGAGAACGGAGCAGACTCTTGG (SEQ ID NO:170)
	171	hs101534962	AGAGAACGGAGCAGACTCTTGGG (SEQ ID NO:171)
	172	hs101534807	CCGGGTGCTGACCCCCCAATGG (SEQ ID NO:172)
	173	hs101534962	AGAGAACGGAGCAGACTCTTGGG (SEQ ID NO:171)
	174	hs101534959	GCTCGGAAGGCTTCATTTGTGGG (SEQ ID NO:174)
10	175	hs101534965	GCTCCGTTCTCTTGGTGGCAGGG (SEQ ID NO:175)
	176	hs101534966	TGCTCCGTTCTCTTGGTGGCAGG (SEQ ID NO:176)
	177	hs101534967	AGTCTGCTCCGTTCTCTTGGTGG (SEQ ID NO:177)
	178	hs101534968	AAGAGTCTGCTCCGTTCTCTTGG (SEQ ID NO:178)
	179	hs101535372	CATCATGGCCACAAATGGCGTGG (SEQ ID NO:179)
15	180	hs101535375	CCATTTGTGGCCATGATGTCAGG (SEQ ID NO:180)
	181	hs101535376	GACATGGACCACGCCATTTGTGG (SEQ ID NO:181)
	182	hs101533619	GCGGTCCGTGTACAGCTGAGTGG (SEQ ID NO:182)
	183	hs101535656	TTTTCTTTCAGGCTTCCCAGAGG (SEQ ID NO:183)
	184	hs101535656	TTTTCTTTCAGGCTTCCCAGAGG (SEQ ID NO:184)
20	185	hs101534814	GACTGTCATGGATGTCCTGAAGG (SEQ ID NO:185)
	186	hs101535663	ACCTAGTCGCACAGACCTCTGGG (SEQ ID NO:186)
	187	hs131602459	AGGAGGAAAAGCAAACAAGTGGG (SEQ ID NO:187)
	188	hs131602461	GGAAAAGCAAACAAGTGGGAAGG (SEQ ID NO:188)
	189	hs131602462	AAAGCAAACAAGTGGGAAGGAGG (SEQ ID NO:189)
25	190	hs131602463	ACAAGTGGGAAGGAGGTATCCGG (SEQ ID NO:190)
	191	hs131602464	CAAGTGGGAAGGAGGTATCCGGG (SEQ ID NO:191)
	192	hs002677511	TGGGGCAGAAAGGTGACTTGGGG (SEQ ID NO:192)
	193	hs131603028	TTAAGTAGGCGTTGCAGTAATGG (SEQ ID NO:193)
	194	hs131602458	TAGGAGGAAAAGCAAACAAGTGG (SEQ ID NO:194)

30 **Table 3:** The gRNA target sequences by ID and human genomic sequence in the TCF4 gene upstream of the microsatellite expansion causing Fuchs endothelial corneal dystrophy.

	<u>#</u>	<u>ID</u>	<u>Genomic Sequence</u>
	1	hs056193542	AAAGAGCCCCACTTGGAAGGCGG (SEQ ID NO:195)
	2	hs056193543	GCCCCACTTGGAAGGCGGTTTGG (SEQ ID NO:196)
35	3	hs056193544	GATTTTATTTGTGTGTTTTGTGG (SEQ ID NO:197)
	4	hs056193545	TCCAAACCGCCTTCCAAGTGGGG (SEQ ID NO:198)
	5	hs056193546	ATCCAAACCGCCTTCCAAGTGGG (SEQ ID NO:199)
	6	hs056193547	AATCCAAACCGCCTTCCAAGTGG (SEQ ID NO:200)
	7	hs056193548	CATCTTACACCAAACCTCATCTGG (SEQ ID NO:201)
40	8	hs056193549	TTTTTAATGCCAGATGAGTTTGG (SEQ ID NO:202)
	9	hs056193550	ATTCATTCTCCTGACATGTCTGG (SEQ ID NO:203)
	10	hs056193551	TTCATTCTCCTGACATGTCTGGG (SEQ ID NO:204)
	11	hs056193552	CTCCTGACATGTCTGGGACTTGG (SEQ ID NO:205)
	12	hs056193553	ACATGTCTGGGACTTGGTTTAGG (SEQ ID NO:206)
45	13	hs056193554	CTGGGACTTGGTTTAGGAAAAGG (SEQ ID NO:207)
	14	hs056193555	GGTTTAGGAAAAGGAAGCAAAGG (SEQ ID NO:208)
	15	hs056193556	GTTTAGGAAAAGGAAGCAAAGGG (SEQ ID NO:209)
	16	hs056193557	AACCAAGTCCCAGACATGTCAGG (SEQ ID NO:210)
	17	hs056193558	AGGAAAAGGAAGCAAAGGGATGG (SEQ ID NO:211)
50	18	hs056193559	AGGAAGCAAAGGGATGGAGAAGG (SEQ ID NO:212)
	19	hs056193560	TGGAGTTTACGGCTGTACTTGG (SEQ ID NO:213)

	20	hs056193561	GACACACTTGTGGAGTTTTACGG (SEQ ID NO:214)
	21	hs056193562	AGCGGAACTTGACACACTTGTGG (SEQ ID NO:215)
	22	hs056193563	GTCGTAGGATCAGCACAAAGCGG (SEQ ID NO:216)
	23	hs056193564	ATTTACCAAACAGTCCAAAGG (SEQ ID NO:217)
5	24	hs056193565	TTGGTAAATTTTCGTAGTCGTAGG (SEQ ID NO:218)
	25	hs056193566	TAGAACCCTTTTGGACTGTTTTGG (SEQ ID NO:219)
	26	hs056193567	ATACATTCTTTAGAACCCTTTTGG (SEQ ID NO:220)
	27	hs056193568	ATACTAGTTTTAAGAATCCTAGG (SEQ ID NO:221)
	28	hs056193569	TCCTAGGAAAAGATGTAAGTAGG (SEQ ID NO:222)
10	29	hs056193570	TAGGAAAAGATGTAAGTAGGAGG (SEQ ID NO:223)
	30	hs056193571	TAGGATTCTTAAAGTAGTATGG (SEQ ID NO:224)
	31	hs056193572	TAACTAGGAGGTAAGATGTAAGG (SEQ ID NO:225)
	32	hs056193573	GGAGGTAAGATGTAAGGAACAGG (SEQ ID NO:226)
	33	hs056193574	TCCTAGTTACATCTTTTCCTAGG (SEQ ID NO:227)
15	34	hs056193575	TAATGATGCTTTGGATTGGTAGG (SEQ ID NO:228)
	35	hs056193576	AAGCTAATGATGCTTTGGATTGG (SEQ ID NO:229)
	36	hs056193577	TAAACTTTAAAGAGACAACTGG (SEQ ID NO:230)
	37	hs056193578	AAAACCTTTAAAGAGACAACTGGG (SEQ ID NO:231)
	38	hs056193579	GTTTTAAGCTAATGATGCTTTGG (SEQ ID NO:232)
20	39	hs056193580	GGAAATGGAAAATAGAAAATAGG (SEQ ID NO:233)
	40	hs056193581	TTATTTATTGTTTTTGGAAATGG (SEQ ID NO:234)
	41	hs056193582	TCGTTTTATTATTGTTTTTGG (SEQ ID NO:235)
	42	hs056193583	GTAGTCTCAGTGTTTCAGACATGG (SEQ ID NO:236)
	43	hs056193584	TTCAGACATGGCCAAGTTTTAGG (SEQ ID NO:237)
	44	hs056193585	TCAGACATGGCCAAGTTTTAGGG (SEQ ID NO:238)
	45	hs056193586	CAGACATGGCCAAGTTTTAGGGG (SEQ ID NO:239)
	46	hs056193587	ACATGGCCAAGTTTTAGGGGTGG (SEQ ID NO:240)
	47	hs056193588	TTTAGGGGTGGTTTAGTTTTAGG (SEQ ID NO:241)
	48	hs056193589	TTAGGGGTGGTTTAGTTTTAGGG (SEQ ID NO:242)
	49	hs056193590	TAGGGGTGGTTTAGTTTTAGGGG (SEQ ID NO:243)
	50	hs056193591	ACTAAACCACCCCTAAACTTGG (SEQ ID NO:244)
	51	hs056193592	TGTCTATTTTTGCTTTCCACTGG (SEQ ID NO:245)
	52	hs056193593	GTCTATTTTTGCTTTCCACTGGG (SEQ ID NO:246)
	53	hs056193594	TCTATTTTTGCTTTCCACTGGGG (SEQ ID NO:247)
	54	hs056193595	TGGGGTGAGATTCCATTATTTGG (SEQ ID NO:248)
	55	hs056193596	GGGGTGAGATTCCATTATTTGGG (SEQ ID NO:249)
	56	hs056193597	GGGTGAGATTCCATTATTTGGGG (SEQ ID NO:250)
	57	hs056193598	CCATTATTTGGGGTAATCAGTGG (SEQ ID NO:251)
	58	hs056193599	CATTATTTGGGGTAATCAGTGGG (SEQ ID NO:252)
	59	hs056193600	ATTTGGGGTAATCAGTGGGTAGG (SEQ ID NO:253)
	60	hs056193601	ATAATGGAATCTCACCCAGTGG (SEQ ID NO:254)
	61	hs056193602	TTTGGGGTAATCAGTGGGTAGGG (SEQ ID NO:255)
	62	hs056193603	ATCAGTGGGTAGGGAATTGAAGG (SEQ ID NO:256)
	63	hs056193604	CCACTGATTACCCCAAATAATGG (SEQ ID NO:257)
	64	hs056193605	TTTTTTTTGAGTTTTATTACTGG (SEQ ID NO:258)
	65	hs056193606	TGTGGTGTGATGGAAGATTCAGG (SEQ ID NO:259)
	66	hs056193607	ACTATAATTTTGTGGTGTGATGG (SEQ ID NO:260)

67	hs056193608	AGTTTTTAACTATAATTTTGTGG (SEQ ID NO:261)
68	hs056193609	AAAGACCTTCATATTTACCAAGG (SEQ ID NO:262)
69	hs056193610	TGAATCCTTGGTAAATATGAAGG (SEQ ID NO:263)
70	hs056193611	TTTTTAATTGGCTGAATCCTTGG (SEQ ID NO:264)
71	hs056193612	ACTGTCCTTTAGATTCCTACTGG (SEQ ID NO:265)
72	hs056193613	GGACAGTAATAATTTTAAATTGG (SEQ ID NO:266)
73	hs056193614	TGGTTTCTAGCTGAAGTGTTTGG (SEQ ID NO:267)
74	hs056193615	GGTTTCTAGCTGAAGTGTTTGGG (SEQ ID NO:268)
75	hs056193616	AGAAACCAGTAGGAATCTAAAGG (SEQ ID NO:269)
76	hs056193617	CACTTCAGCTAGAAACCAGTAGG (SEQ ID NO:270)
77	hs056193618	AGTGCGGTAAGAAAGAACGGTGG (SEQ ID NO:271)
78	hs056193619	TTCAGTGCGGTAAGAAAGAACGG (SEQ ID NO:272)
79	hs056193620	TGATTTACTGGATTTTCAGTGCGG (SEQ ID NO:273)

Table 4: The gRNA target sequences by ID and human genomic sequence in the TCF4 gene downstream of the microsatellite expansion causing Fuchs endothelial corneal dystrophy.

	<u>#</u>	<u>ID</u>	<u>Genomic Sequence</u>
5	1	hs056193473	AGATCTTTGAGGAGCTCTGAAGG (SEQ ID NO:274)
	2	hs056193474	AACAGTATGAAAGATCTTTGAGG (SEQ ID NO:275)
	3	hs056193475	ACAGCTTAGAGTTTATGCTAAGG (SEQ ID NO:276)
	4	hs056193476	CAGCTTAGAGTTTATGCTAAGGG (SEQ ID NO:277)
10	5	hs056193477	AGCATAAACTCTAAGCTGTTTGG (SEQ ID NO:278)
	6	hs056193478	TCTTTTAGTTTTAAGTTGGATGG (SEQ ID NO:279)
	7	hs056193479	TTTCTCTTTTAGTTTTAAGTTGG (SEQ ID NO:280)
	8	hs056193480	GTGATAATGGGGGCTGGGGTGGG (SEQ ID NO:281)
	9	hs056193481	AGTGATAATGGGGGCTGGGGTGG (SEQ ID NO:282)
15	10	hs056193482	TCTGTTCTTTCTTTTTCCTCAGG (SEQ ID NO:283)
	11	hs056193483	CAGAGTGATAATGGGGGCTGGGG (SEQ ID NO:284)
	12	hs056193484	ACAGAGTGATAATGGGGGCTGGG (SEQ ID NO:285)
	13	hs056193485	AACAGAGTGATAATGGGGGCTGG (SEQ ID NO:286)
	14	hs056193486	AAAGAACAGAGTGATAATGGGGG (SEQ ID NO:287)
20	15	hs056193487	GAAAGAACAGAGTGATAATGGGG (SEQ ID NO:288)
	16	hs056193488	AGAAAGAACAGAGTGATAATGGG (SEQ ID NO:289)
	17	hs056193489	AAGAAAGAACAGAGTGATAATGG (SEQ ID NO:290)
	18	hs056193490	TTTTCCTCAGGTTTCATTAGATGG (SEQ ID NO:291)
	19	hs056193491	TTGGCCATCTAATGAACCTGAGG (SEQ ID NO:292)
25	20	hs056193492	AGCAGTACTACTGCTACATTTGG (SEQ ID NO:293)
	21	hs056193493	AATGTAGCAGTAGTACTGCTTGG (SEQ ID NO:294)
	22	hs056193494	CCATAATGTTATCAAGATTCAGG (SEQ ID NO:295)
	23	hs056193495	AATGTTATCAAGATTCAGGTTGG (SEQ ID NO:296)
	24	hs056193496	GTTATCAAGATTCAGGTTGGAGG (SEQ ID NO:297)
30	25	hs056193497	TGAATCTTGATAACATTATGGGG (SEQ ID NO:298)
	26	hs056193498	CTGAATCTTGATAACATTATGGG (SEQ ID NO:299)
	27	hs056193499	CCTGAATCTTGATAACATTATGG (SEQ ID NO:300)

28	hs056193500	GAAAAACACTAGTTTTACCAAGG (SEQ ID NO:301)
29	hs056193501	TGTTTTTCTAGAGAGGCTGCTGG (SEQ ID NO:302)
30	hs056193502	AAACTAGTGTTTTTCTAGAGAGG (SEQ ID NO:303)
31	hs056193503	AACAACTTTTTTCTTCTCCTTGG (SEQ ID NO:304)
32	hs056193504	TTGTTTTATATTGAAAACCTTGG (SEQ ID NO:305)
33	hs056193505	GAAAACCTTGGCCATAAACGTGG (SEQ ID NO:306)
34	hs056193506	TGTCCATTTCCATCTCGTATAGG (SEQ ID NO:307)
35	hs056193507	CATTGCCACGTTTATGGCCAAGG (SEQ ID NO:308)
36	hs056193508	AATGGACATTGCCACGTTTATGG (SEQ ID NO:309)
37	hs056193509	AATCCTATACGAGATGGAAATGG (SEQ ID NO:310)
38	hs056193510	CAGGCAAATCCTATACGAGATGG (SEQ ID NO:311)
39	hs056193511	TATTTGGGTTCACATATGACAGG (SEQ ID NO:312)
40	hs056193512	TGGCACTTTTATTTTTATTTGGG (SEQ ID NO:313)
41	hs056193513	GTGGCACTTTTATTTTTATTTGG (SEQ ID NO:314)
42	hs056193514	ATTCTCATTTCTGCTCTAACAGG (SEQ ID NO:315)
43	hs056193515	AAATGAGAATTTAGTGCAGGTGG (SEQ ID NO:316)
44	hs056193516	ACGAAATGAGAATTTAGTGCAGG (SEQ ID NO:317)
45	hs056193517	GCATTTATTTTCGACCCTAATTGG (SEQ ID NO:318)
46	hs056193518	CTCTTCTTCGACGTATCTAGTGG (SEQ ID NO:319)
47	hs056193519	AAGAAGAGGGAAACCAATTAGGG (SEQ ID NO:320)
48	hs056193520	GAAGAAGAGGGAAACCAATTAGG (SEQ ID NO:321)
49	hs056193521	ACTAGATACGTCTGAAGAAGAGGG (SEQ ID NO:322)
50	hs056193522	CACTAGATACGTCTGAAGAAGAGG (SEQ ID NO:323)
51	hs056193523	TCAGAGCCTGCAAAAAGCAAAGG (SEQ ID NO:324)
52	hs056193524	GCAAAAAGCAAAGGAACGAATGG (SEQ ID NO:325)
53	hs056193525	TGCAGGCTCTGACTCAGGGAAGG (SEQ ID NO:326)
54	hs056193526	TTTTTGCAGGCTCTGACTCAGGG (SEQ ID NO:327)
55	hs056193527	CTTTTTGCAGGCTCTGACTCAGG (SEQ ID NO:328)
56	hs056193528	TTCGTTCCTTTGCTTTTTTGCAGG (SEQ ID NO:329)
57	hs056193529	AGAAAGTGCAACAAGCAGAAAGG (SEQ ID NO:330)
58	hs056193530	GAAAGTGCAACAAGCAGAAAGGG (SEQ ID NO:331)
59	hs056193531	AAAGTGCAACAAGCAGAAAGGGG (SEQ ID NO:332)
60	hs056193532	AAGTGCAACAAGCAGAAAGGGGG (SEQ ID NO:333)
61	hs056193533	GGCTGCAAAGCTGCCTGCCTAGG (SEQ ID NO:334)
62	hs056193534	GCTGCAAAGCTGCCTGCCTAGGG (SEQ ID NO:335)
63	hs056193535	CTGCCTAGGGCTACGTTTCTTGG (SEQ ID NO:336)
64	hs056193536	CAGGAAACGTAGCCCTAGGCAGG (SEQ ID NO:337)
65	hs056193537	TTGCCAGGAAACGTAGCCCTAGG (SEQ ID NO:338)
66	hs056193538	AAAGCCATTTCTCCAAAAGAAGG (SEQ ID NO:339)
67	hs056193539	TGGCTTTCGGAAGTTTGGCCAGG (SEQ ID NO:340)
68	hs056193540	TCTTTTGGAGAAATGGCTTTCGG (SEQ ID NO:341)
69	hs056193541	TAGACCTTCTTTTGGAGAAATGG (SEQ ID NO:342)

Table 5: Microsatellite expansion diseases/conditions with affected gene(s) in brackets.

	Blepharophimosis, ptosis and epicanthus inversus syndactyly [FOXL2]
	Cleidocranial dysplasia [RUNX2 CBFA1]
	Congenital central hypoventilation syndrome, Haddad syndrome [PHOX2B]
5	DM (Myotonic dystrophy) [DMPK]
	DRPLA (Dentatorubropallidolusian atrophy) [ATN1 or DRPLA]
	FRAXA (Fragile X syndrome) [FMR1]
	FRAXE (Fragile XE mental retardation) [AFF2 or FMR2]
	FRDA (Friedreich's ataxia) [FXN or X25]
10	Fuchs' Endothelial Corneal Dystrophy [TCF4]
	FXTAS (Fragile X-associated tremor/ataxia syndrome) [FMR1]
	Hand-foot-genital syndrome [HOXA13]
	HD (Huntington's disease) [HTT (Huntingtin)]
	Holoprosencephaly (HPE5) [ZIC2]
15	Mental retardation with growth hormone deficiency [SOX3]
	Mental retardation, epilepsy, West syndrome, Partington syndrome [ARX]
	Oculopharyngeal muscular dystrophy [PABPN1]
	SBMA (Spinal and bulbar muscular atrophy) [AR]
	SCA1 (Spinocerebellar ataxia Type 1) [ATXN1]
20	SCA12 (Spinocerebellar ataxia Type 12) [PPP2R2B or SCA12]
	SCA17 (Spinocerebellar ataxia Type 17) [TBP]
	SCA2 (Spinocerebellar ataxia Type 2) [ATXN2]
	SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease) [ATXN3]
	SCA6 (Spinocerebellar ataxia Type 6) [CACNA1A]
25	SCA7 (Spinocerebellar ataxia Type 7) [ATXN7]
	SCA8 (Spinocerebellar ataxia Type 8) [OSCA or SCA8]
	Synpolydactyly [HOXD13]

REFERENCES

- 30 1. Weiss JS, Moller HU, Aldave AJ, et al. IC3D classification of corneal dystrophies – edition 2. *Cornea* 2015;34:117-159.
2. Afshari NA, Bouchard CS, Colby KA, et al. Corneal dystrophies and ectasias. In: Weisenthal RW, ed. 2014–2015 Basic and Clinical Science Course, Section 8: External Disease and Cornea. San Francisco; American Academy of Ophthalmology; 2014:253–287.
- 35 3. Weiss JS, Moller HU, Lisch W et al. The IC3D classification of the corneal dystrophies. *Cornea* 2008;27(suppl2):S1-S83.
4. Wieben ED, Aleff RA, Eckloff BW, Atkinson EJ, Baheti S, Middha S, et al. Comprehensive Assessment of Genetic Variants Within TCF4 in Fuchs' Endothelial Corneal Dystrophy. *Investigative ophthalmology & visual science*. 2014;55(9):6101-7.
- 40 5. Nelson DL, Orr HT, Warren ST. The unstable repeats--three evolving faces of

neurological disease. *Neuron*. 2013 Mar 6;77(5):825-43.

6. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*. 2014;157(6):1262-78.
7. Doudna JA, Charpentier E. Genome editing. The new frontier of genome
5 engineering with CRISPR-Cas9. *Science*. 2014;346(6213):1258096.
8. Ranganathan V, Wahlin K, Maruotti J, Zack DJ. Expansion of the CRISPR-Cas9 genome targeting space through the use of H1 promoter-expressed guide RNAs. *Nature Communications*. 2014;5:4516.
9. Munier FL, Frueh BE, Othenin-Girard P, Uffer S, Cousin P, Wang MX, Heon E,
10 Black GC M, Blasi MA, Balestrazzi E, Lorenz B, Escoto R, Barraquer R, Hoeltzenbein M, Gloor B, Fossarello M, Singh AD, Arsenijevic Y, Zografos L, Schorderet DF. BIGH3 mutation spectrum in corneal dystrophies. *Invest. Ophthalmol. Vis. Sci*. 43: 949-954, 2002.
10. Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales AP, Li Z, Peterson RT, Yeh JJ, Aryee MJ, Joung JK. Engineered CRISPR-Cas9 nucleases
15 with altered PAM specificities. *Nature*. 2015 Jun 22. doi: 10.1038/nature14592.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention
20 described herein. While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention may become apparent to those skilled in the art upon review of this specification. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations. Such
25 equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A method for treating a disorder affecting ocular tissue in a subject, the method comprising administering to the ocular area of the subject a therapeutically effective amount of a nuclease system comprising a genome targeted nuclease and a guide DNA
5 comprising at least one targeted genomic sequence.
2. The method of claim 1, wherein the nuclease is provided as a protein, RNA, DNA, or an expression vector comprising a nucleic acid that encodes the nuclease.
3. The method of claim 1, wherein the guide DNA is provided as an RNA molecule (gRNA), DNA molecule, or an expression vector comprising a nucleic acid that encodes the
10 gRNA.
4. The method of claim 1, wherein the nuclease system is CRISPR-Cas9.
5. The method of claim 1, wherein the nuclease system inactivates or excises gene mutations.
6. The method of claim 1, further comprising a DNA double-stranded break (DSB)
15 repair system.
7. The method of claim 6, wherein the DSB repair system comprises a repair template in combination with or without a Non-Homologous End-Joining (NHEJ) or Homology Directed Repair (HDR) targeted to the one or more CRISPR-Cas9 cleavage site, said site corrects or edits a genomic mutation.
- 20 8. The method of claim 1, wherein the genome targeted nuclease is Cas9.
9. The method of claim 1, wherein the disorder is a corneal dystrophy or microsatellite expansion disease.
10. The method of claim 1, wherein the ocular area is the cornea.
11. The method of claim 1, wherein the guide DNA comprises at least one, two, three,
25 four, five, six, seven, eight, nine, or ten targeted genomic sequences.
12. The method of claim 11, wherein the target genomic sequences are selected from any one of the nucleotide sequences set forth in SEQ ID NOs: 1-172 and 174-342, or any combination thereof.
13. The method of claim 9, wherein the corneal dystrophy is selected from the group
30 consisting of Epithelial Basement Membrane Dystrophy, Epithelial Recurrent Erosion Dystrophies, Subepithelial Mucinous Corneal Dystrophy, Meesmann Corneal Dystrophy, Lisch Epithelial Corneal Dystrophy, Gelatinous Drop-like Corneal Dystrophy, Reis–Bucklers Corneal Dystrophy, Thiel–Behnke Corneal Dystrophy, Lattice Corneal

Dystrophy, Type 1 (Classic), Lattice Corneal Dystrophy, Type 2, Lattice Corneal Dystrophy, Type III, Lattice Corneal Dystrophy, Type IIIA, Lattice Corneal Dystrophy, Type I/IIIA, Lattice Corneal Dystrophy, Type IV, Polymorphic (Corneal) Amyloidosis, Granular Corneal Dystrophy, Type 1, Granular Corneal Dystrophy, Type 2, Macular
 5 Corneal Dystrophy, Schnyder Corneal Dystrophy, Congenital Stromal Corneal Dystrophy, Fleck Corneal Dystrophy, Posterior Amorphous Corneal Dystrophy, Central Cloudy Dystrophy of Francois, Pre-Descemet Corneal Dystrophy, Fuchs Endothelial Corneal Dystrophy, Posterior Polymorphous Corneal Dystrophy, Congenital Hereditary Endothelial Dystrophy, and X-linked Endothelial Corneal Dystrophy.

- 10 14. The method of claim 9, wherein the microsatellite expansion diseases is selected from the group consisting of Blepharophimosis, ptosis and epicanthus inversus syndactyly, Cleidocranial dysplasia, Congenital central hypoventilation syndrome, Haddad syndrome DM (Myotonic dystrophy), FRAXA (Fragile X syndrome), FRAXE (Fragile XE mental retardation), FRDA (Friedreich's ataxia), Fuchs' Endothelial Corneal Dystrophy, FXTAS
 15 (Fragile X-associated tremor/ataxia syndrome), Hand-foot-genital syndrome, HD (Huntington's disease), Holoprosencephaly, Mental retardation with growth hormone deficiency, Mental retardation, epilepsy, West syndrome, Partington syndrome, Oculopharyngeal muscular dystrophy, SBMA (Spinal and bulbar muscular atrophy), SCA1 (Spinocerebellar ataxia Type 1), SCA12 (Spinocerebellar ataxia Type 12), SCA17
 20 (Spinocerebellar ataxia Type 17), SCA2 (Spinocerebellar ataxia Type 2), SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease), SCA6 (Spinocerebellar ataxia Type 6), SCA7 (Spinocerebellar ataxia Type 7), SCA8 (Spinocerebellar ataxia Type 8), and Synpolydactyly.

15. The method of claim 1, wherein the nuclease system is administered topically to the
 25 surface of the eye.

16. The method of claim 1, wherein the nuclease system is administered on or outside the cornea, sclera, to the intraocular, subconjunctival, sub-tenon, or retrobulbar space, or in or around the eyelids.

17. The method of claim 1, wherein the nuclease system is administered by
 30 implantation, injection, or virally.

18. A method for treating a disorder affecting non-ocular tissue in a subject, the method comprising administering to the non-ocular tissue of the subject a therapeutically effective

amount of a nuclease system comprising a genome targeted nuclease and a guide DNA comprising at least one targeted genomic sequence.

19. The method of claim 18, wherein the nuclease is provided as a protein, RNA, DNA, or an expression vector comprising a nucleic acid encoding the nuclease.

5 20. The method of claim 18, wherein the guide DNA is provided as an RNA molecule (gRNA), DNA molecule, or an expression vector comprising a nucleic acid that encodes the gRNA.

21. The method of claim 18, wherein the nuclease system is CRISPR-Cas9.

22. The method of claim 18, wherein the nuclease system inactivates or excises gene
10 mutations.

23. The method of claim 18, further comprising a DNA double-stranded break (DSB) repair system.

24. The method of claim 23, wherein the DSB repair system comprises a repair template in combination with a Non-Homologous End-Joining (NHEJ) or Homology Directed
15 Repair (HDR) targeted to the one or more CRISPR-Cas9 cleavage site, said site corrects or edits a genomic mutation.

25. The method of claim 18, wherein the genome targeted nuclease is Cas9.

26. The method of claim 18, wherein the disorder is microsatellite expansion disease.

27. The method of claim 18, wherein the guide DNA comprises at least one, two, three,
20 four, five, six, seven, eight, nine, or ten targeted genomic sequences.

28. The method of claim 27, wherein the target genomic sequences are selected from any one of the nucleotide sequences set forth in SEQ ID NOs: 1-172 and 174-342, or any combination thereof.

29. The method of claim 26, wherein the microsatellite expansion diseases is selected
25 from the group consisting of Blepharophimosis, ptosis and epicanthus inversus syndactyly, Cleidocranial dysplasia, Congenital central hypoventilation syndrome, Haddad syndrome DM (Myotonic dystrophy), FRAXA (Fragile X syndrome), FRAXE (Fragile XE mental retardation), FRDA (Friedreich's ataxia), Fuchs' Endothelial Corneal Dystrophy, FXTAS (Fragile X-associated tremor/ataxia syndrome), Hand-foot-genital syndrome, HD
30 (Huntington's disease), Holoprosencephaly, Mental retardation with growth hormone deficiency, Mental retardation, epilepsy, West syndrome, Partington syndrome, Oculopharyngeal muscular dystrophy, SBMA (Spinal and bulbar muscular atrophy), SCA1 (Spinocerebellar ataxia Type 1), SCA12 (Spinocerebellar ataxia Type 12), SCA17

(Spinocerebellar ataxia Type 17), SCA2 (Spinocerebellar ataxia Type 2), SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease), SCA6 (Spinocerebellar ataxia Type 6), SCA7 (Spinocerebellar ataxia Type 7), SCA8 (Spinocerebellar ataxia Type 8), and Synpolydactyly.

- 5 30. The method of claim 18, wherein the nuclease system is administered topically, intravascularly, intradermally, transdermally, parenterally, intravenously, intramuscularly, intranasally, subcutaneously, regionally, percutaneously, intratracheally, intraperitoneally, intraarterially, intravesically, intratumorally, inhalation only, perfusion only, lavage, directly via injection, or orally via administration and formulation.

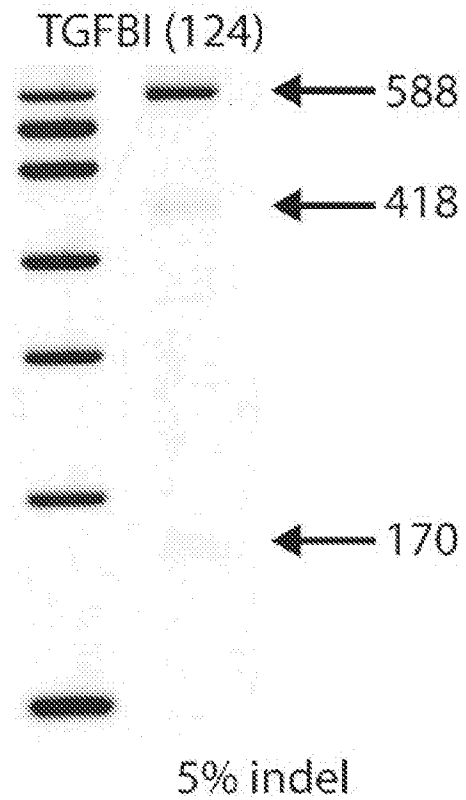
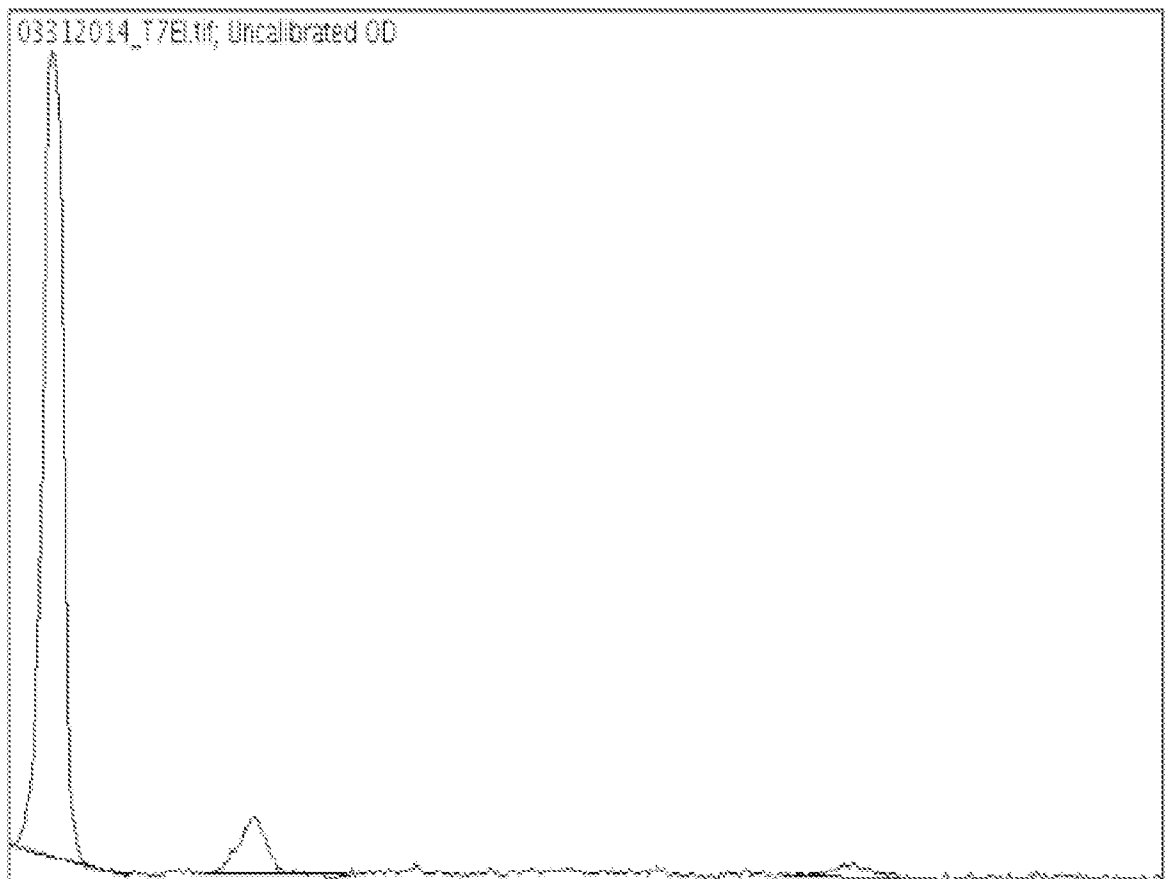
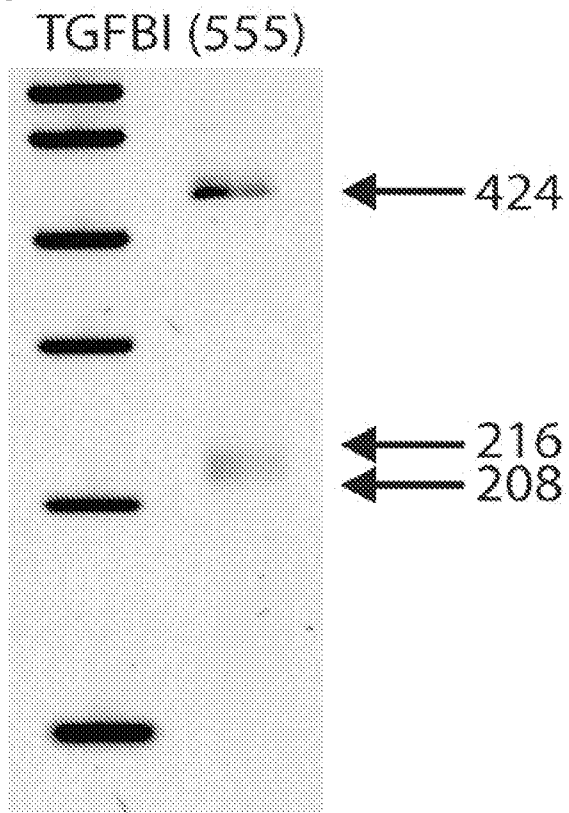
Figure 1**A****B**

Figure 1 (cont.)

C



17% indel

D

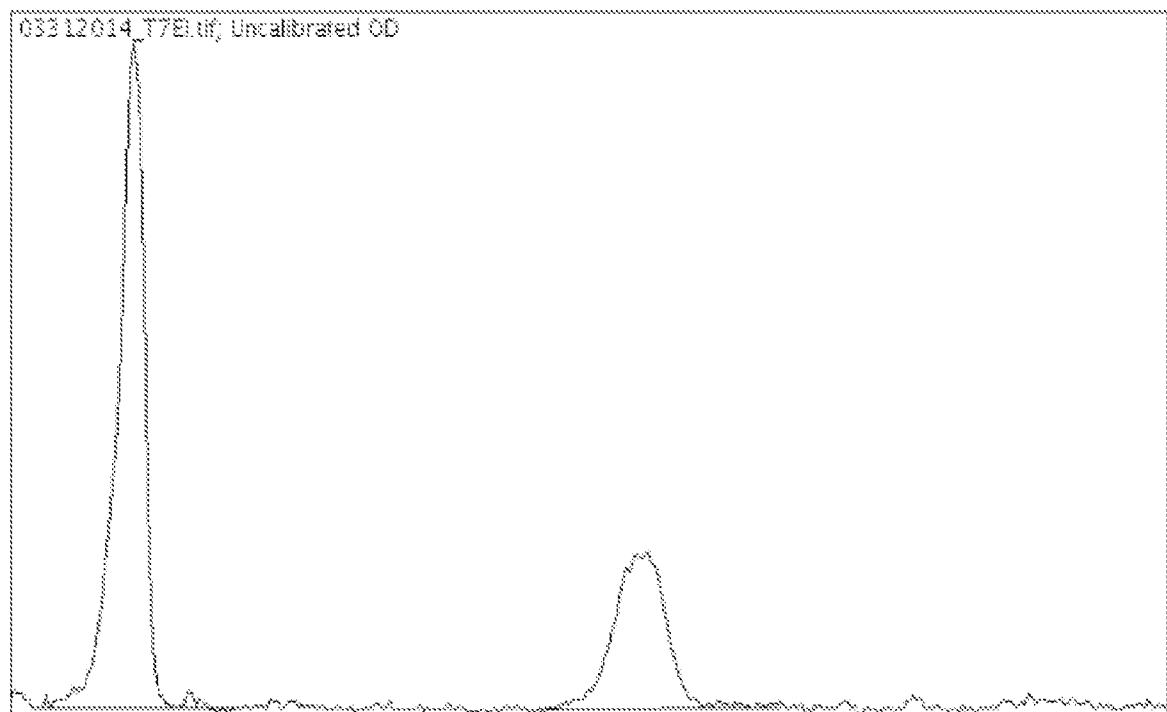


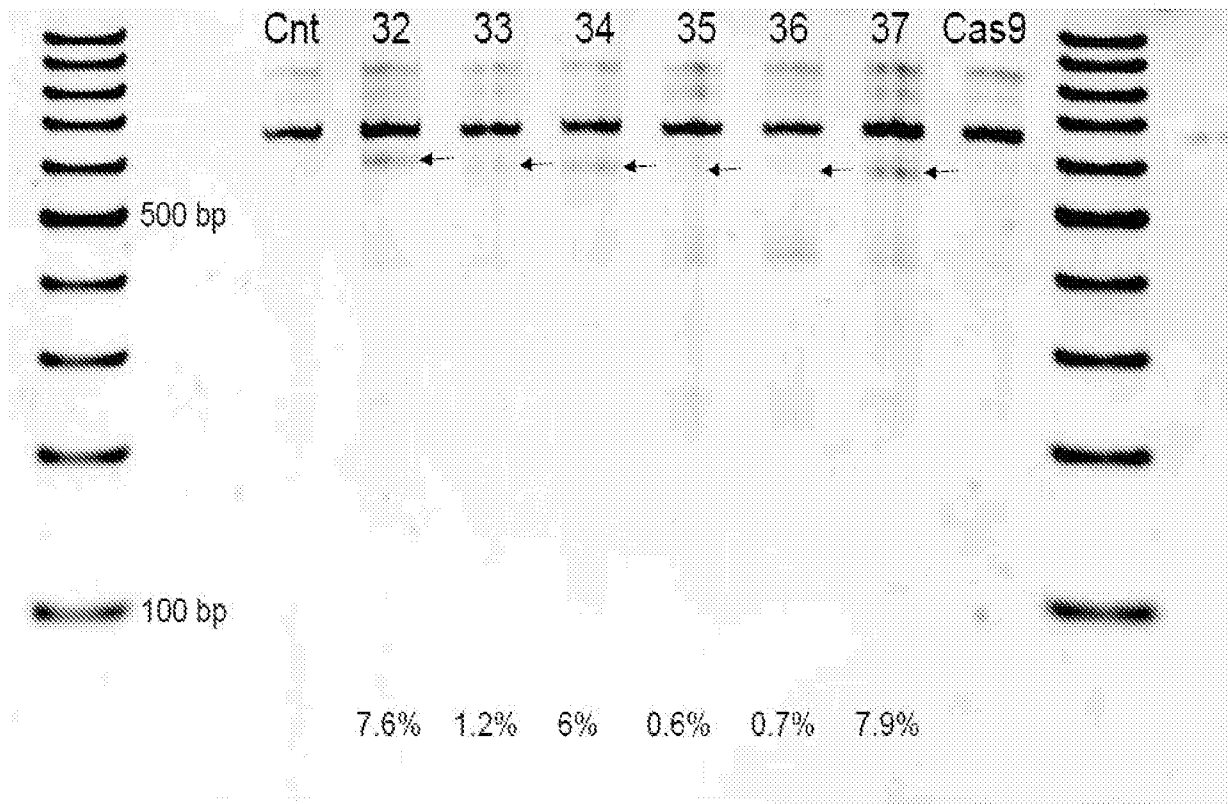
Figure 2**A**

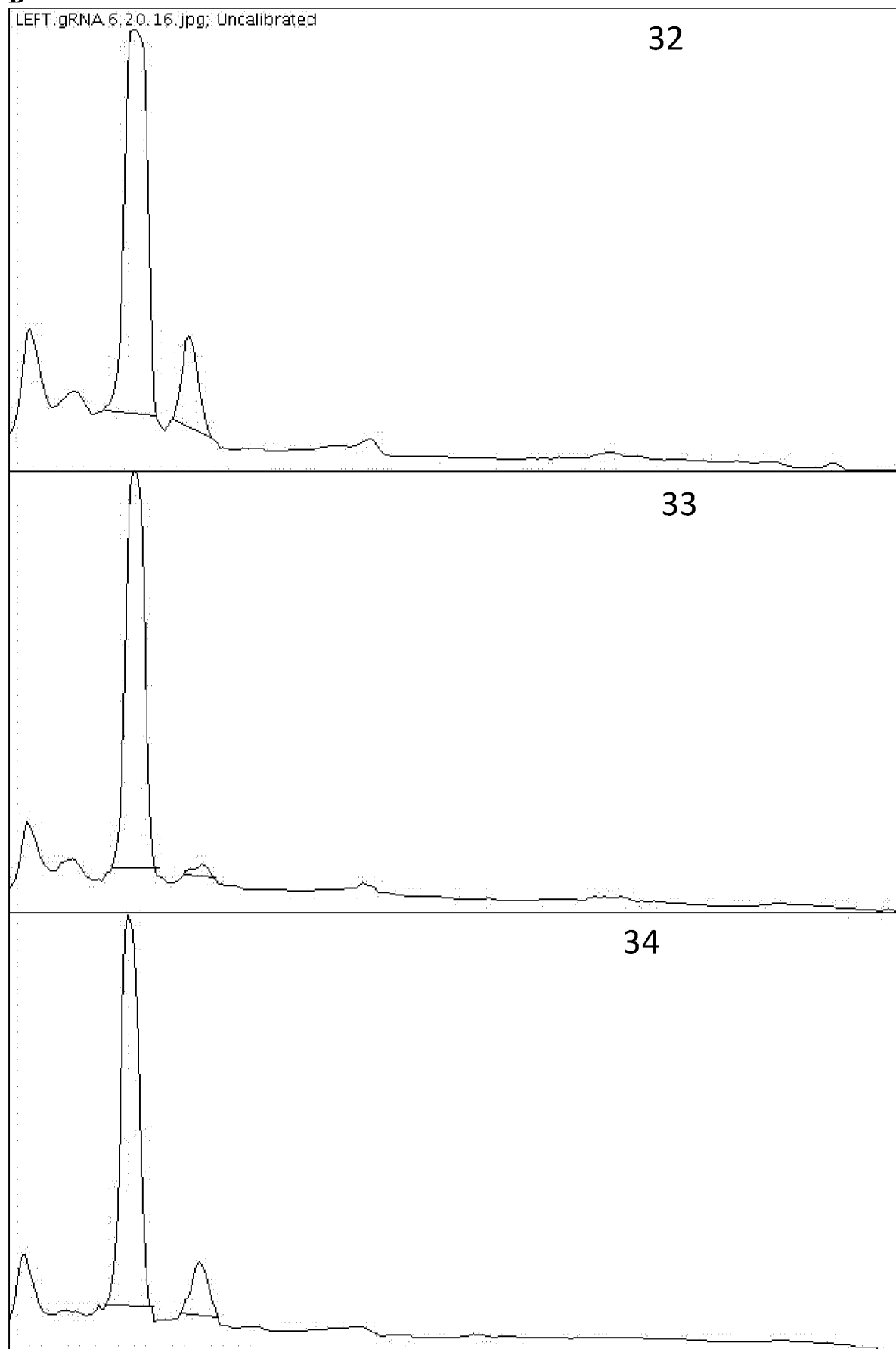
Figure 2 (cont.)**B**

Figure 2 (Cont.)

B (cont.)

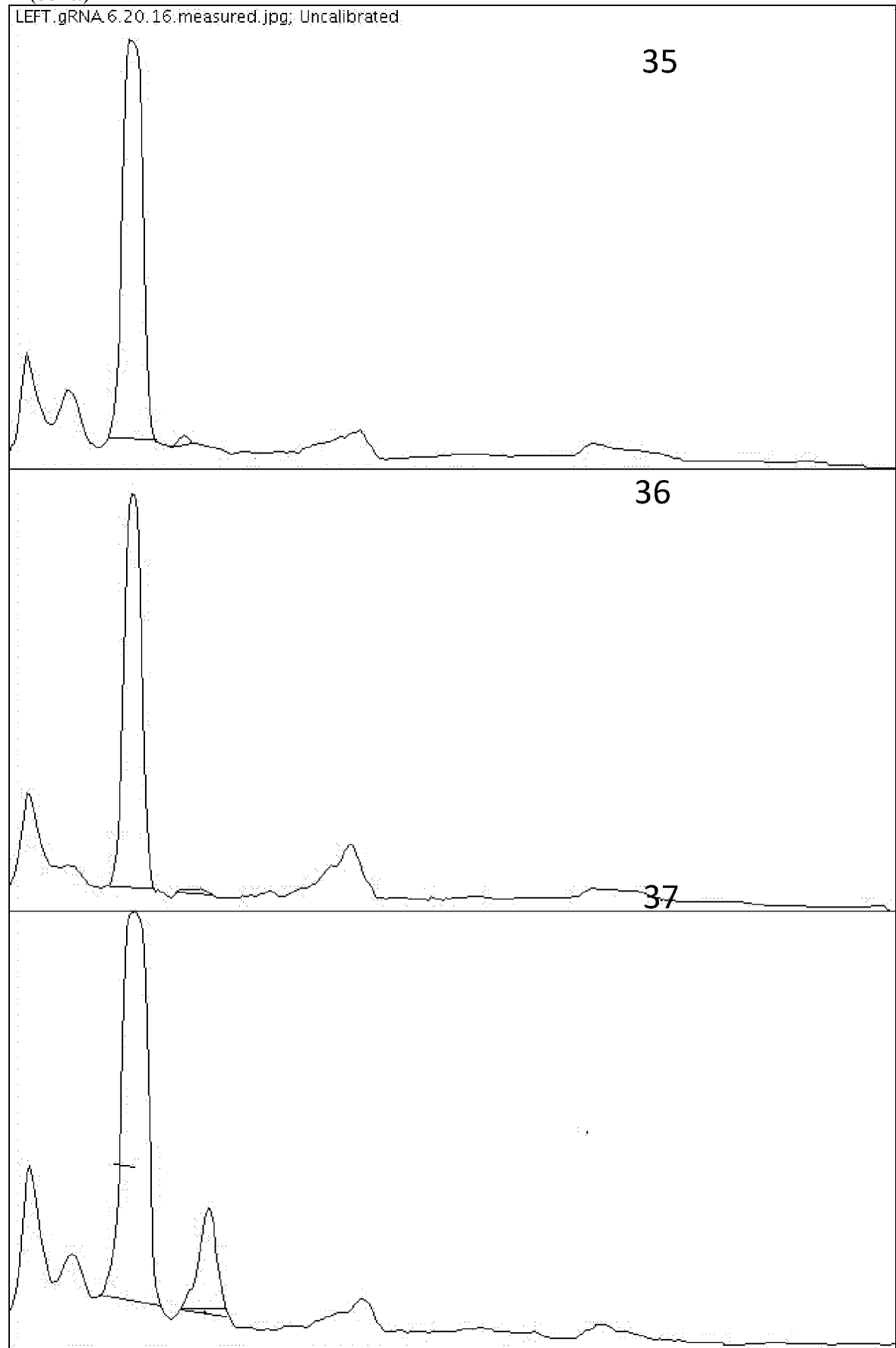


Figure 2 (cont.)

C

gRNA	expected digest 1	expected digest 2
hs056193532(32)	584	58
hs056193533(33)	563	79
hs056193534(34)	562	80
hs056193535(35)	549	93
hs056193536(36)	550	92
hs056193537(37)	546	96

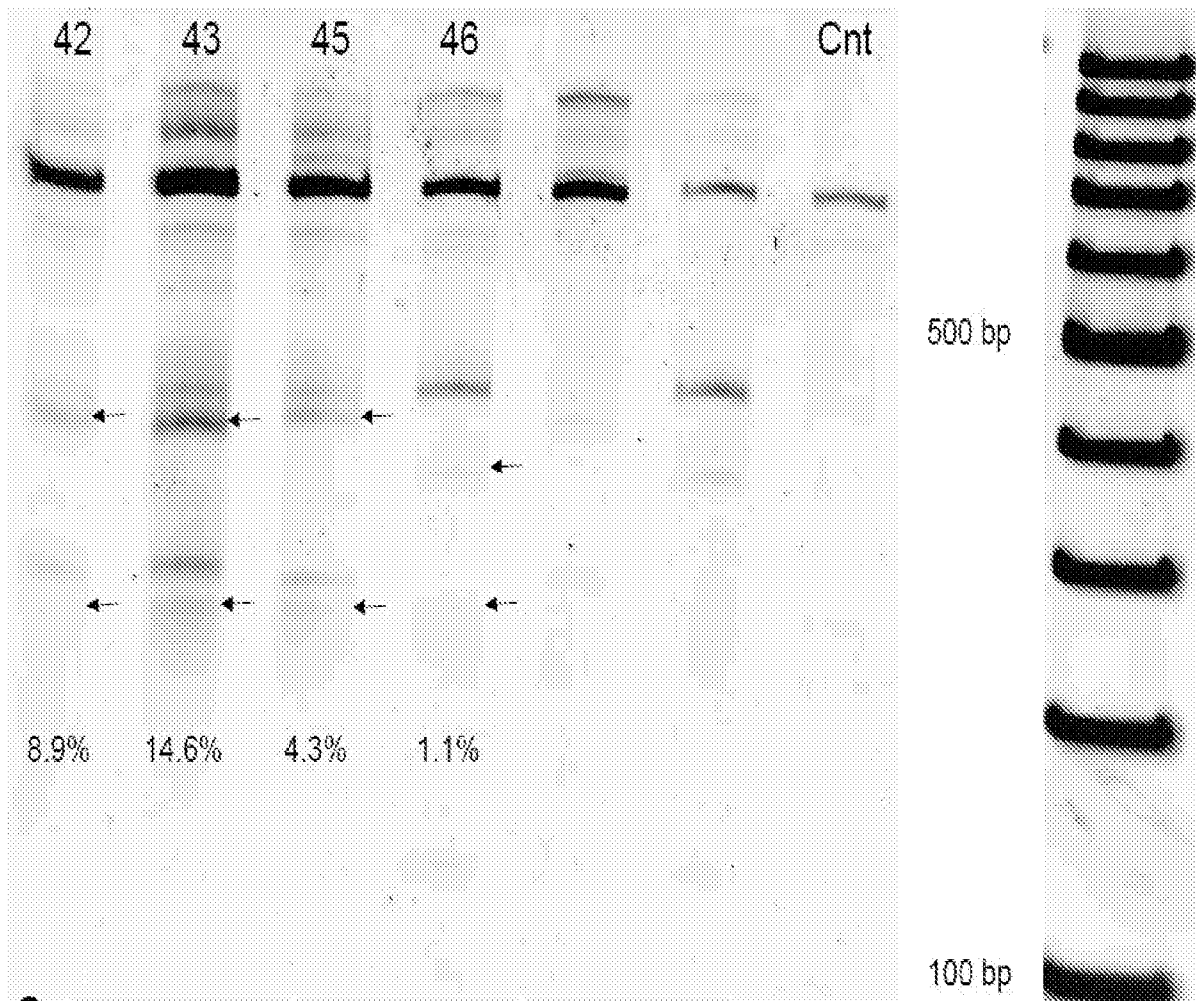
Figure 3**A**

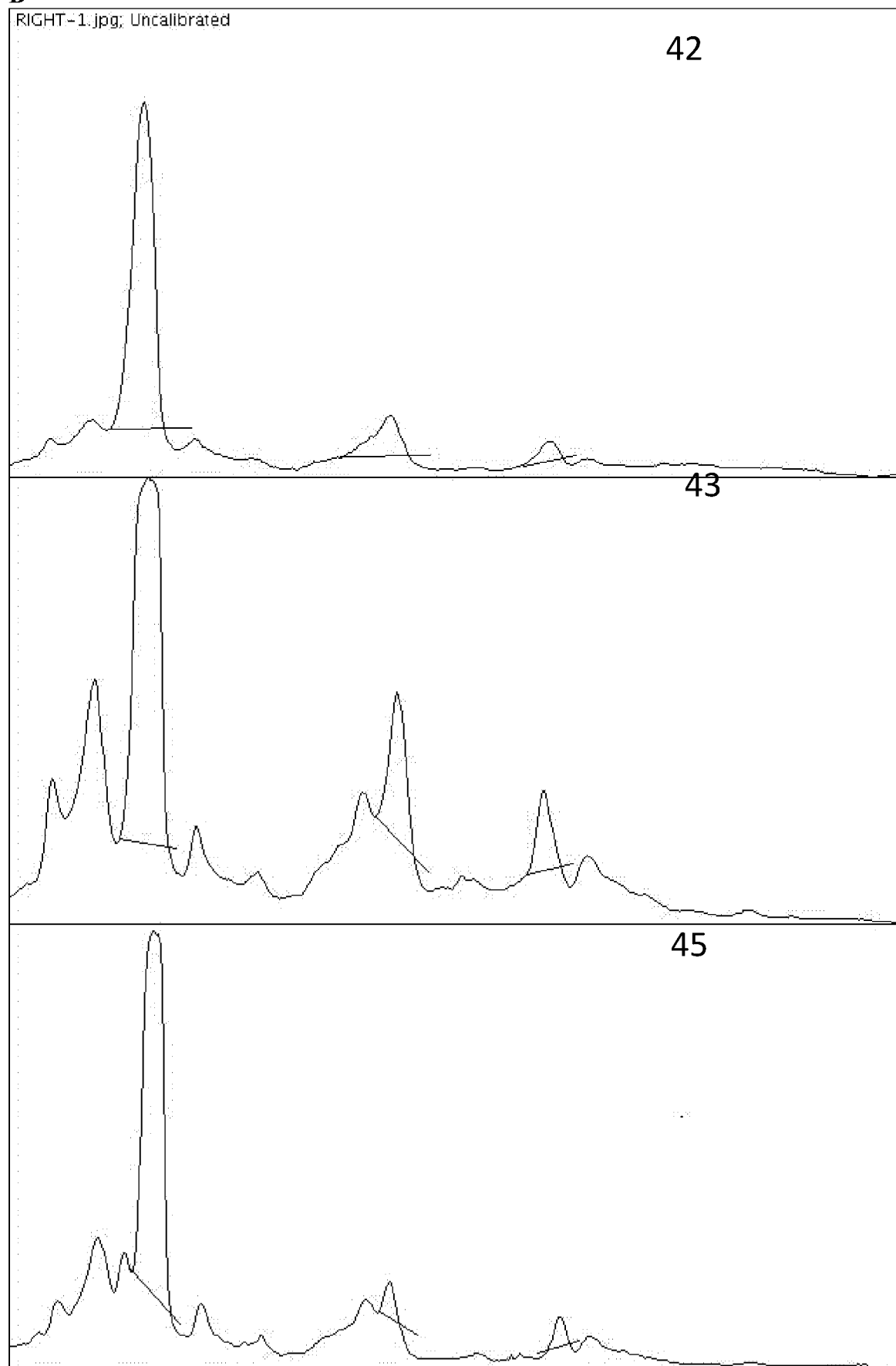
Figure 3 (cont.)**B**

Figure 3 (cont.)

B (cont.)

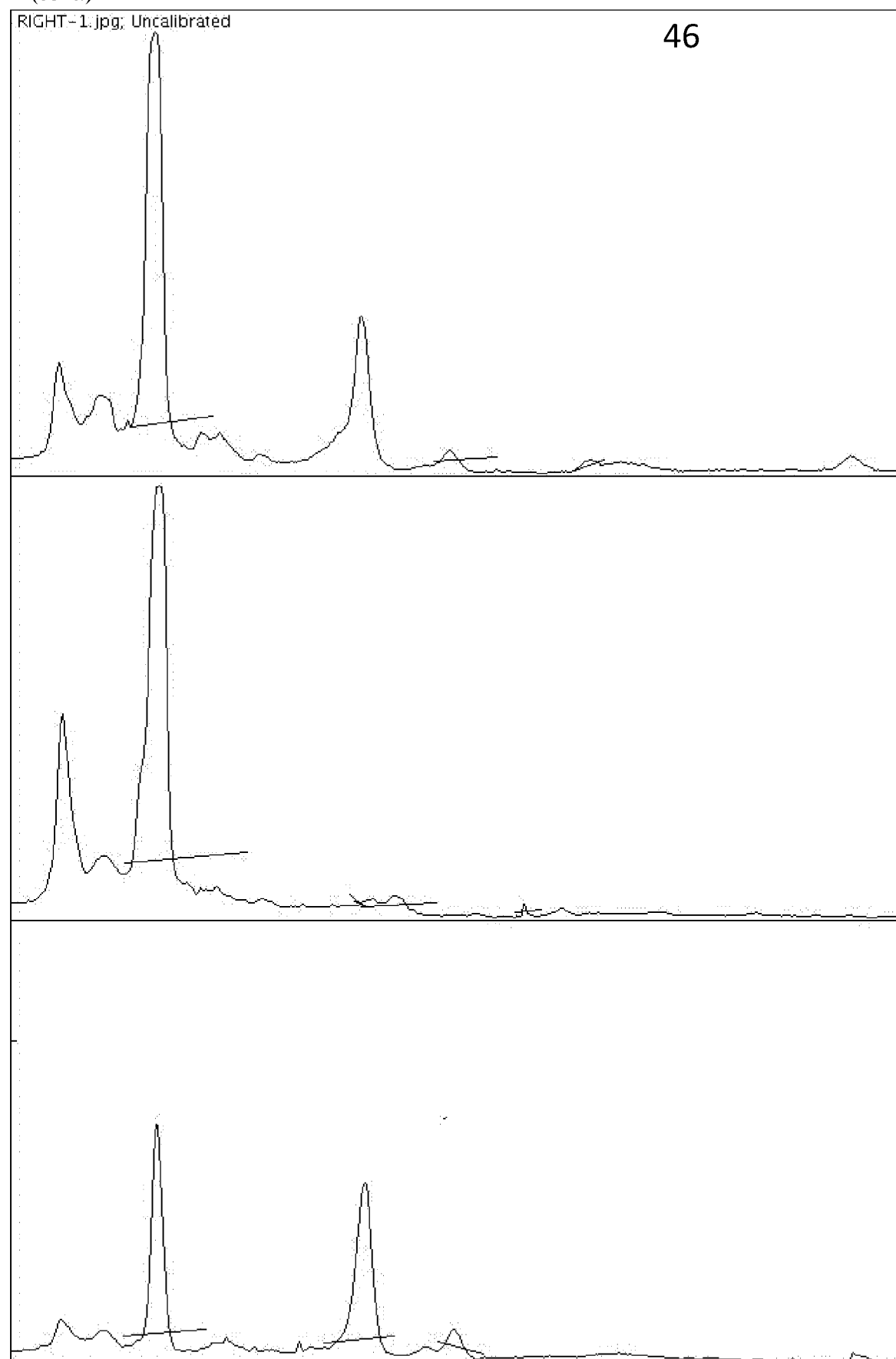


Figure 3 (cont.)

C

gRNA	expected digest 1	expected digest 2
hs056193542(42)	378	264
hs056193543(43)	365	277
hs056193545(45)	364	278
hs056193546(46)	363	279