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## METHODS FOR NOMINATION OF NUCLEASE ON-/OFF-TARGET EDITING LOCATIONS, DESIGNATED "CTL-seq" (CRISPR Tag Linear-seq)

#### Abstract

Described herein are methods for identifying and nominating on- and off-target CRISPR editing sites with improved accuracy and sensitivity.

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## **Background/Summary**

CROSS REFERENCE TO RELATED APPLICATIONS [0001] This application is a continuation of U.S. patent application Ser. No. 17/382,945, filed on Jul. 22, 2021, which claims priority to U.S. Provisional Patent Application No. 63/055,460, filed on Jul. 23, 2020, which is incorporated by reference herein in its entirety.

## REFERENCE TO SEQUENCE LISTING

[0002] This application was filed with a Sequence Listing XML in ST.26 XML format in accordance with 37 C.F.R. § 1.831. The Sequence Listing XML file submitted in the USPTO Patent Center, "013670-9056-US03\_sequence\_listing\_xml\_1 May 2025.xml," was created on May 1, 2025, contains 273 sequences, has a file size of 248.0 kilobytes (253,952 bytes), and is incorporated by reference in its entirety into the specification.

#### TECHNICAL FIELD

[0003] Described herein are methods for identifying and nominating on- and off-target CRISPR editing sites with improved accuracy and sensitivity.

#### BACKGROUND

[0004] CRISPR (clustered regularly interspaced short palindromic repeats) has revolutionized genomics by permitting the simple introduction of changes to the genetic code. CRISPR systems, such as Cas9 and Cas12a proteins, are guided to their target by RNA oligonucleotide sequences bound by the Cas proteins (forming ribonucleoprotein protein; RNP), where the enzyme creates double stranded breaks (DSBs) in DNA sequences. Native cellular machinery repairs DSBs, generally using non-homologous end joining (NHEJ) or homology directed repair (HDR) molecular pathways. DNA repaired through NHEJ, which occurs at on- and off-target locations, often contains indels (insertions/deletions), which can lead to mutations and change the function of encoded genes. Thus, identifying these locations is critical to deconvoluting the impact of on- and off-target editing on biological phenotypes.

[0005] To date, no "gold standard" method exists to identify or nominate off-target editing locations for CRISPR or other nucleases. Many methods have been developed. These methods use a variety of strategies, including the detection of endogenous repair machinery assembled at DSBs (Discover-Seq [1]), the integration of a DNA tag sequence into the host cell genome (GUIDE-Seq; see U.S. Pat. No. 9,822,407), iGUIDE [2, 3]), or by cutting DNA in vitro (BLISS [4], CIRCLE-Seq [5], SiteSeq [6]).

[0006] Cellular or cell based (sometimes referred to as in vivo) and biochemical (sometimes referred to as in vitro) off-target assay nomination systems each have their advantages. Proteins bound to the DNA and epigenetic marks modify the function of nuclease activity, suggesting that cellular or cell based methods may better identify actual editing targets [7]. However, biochemical methods have nominated sites not identified through cellular or cell based methods, suggesting biochemical methods may be more comprehensive [5, 6]. Nevertheless, these current tools tend to have imperfect sensitivity [5, 6] (see FIG. 1).

[0007] What is needed is a method for detecting and nominating on- and off-target CRISPR editing sites with improved accuracy and sensitivity.

#### SUMMARY

[0008] One embodiment described herein is a method for identifying and nominating on- and offtarget CRISPR edited sites with improved accuracy and sensitivity, the process comprising the steps of: (a) co-delivering a guide sequence RNA (sgRNA) or a two-part CRISPR RNA: transactivating crRNA (crRNA: tracrRNA) duplex, one or more tag sequences, and an RNA-guided endonuclease to cells; (b) incubating the cells for a period of time sufficient for double strand breaks to occur; (c) isolating genomic DNA from the cells, fragmenting the genomic DNA, and ligating the fragmented genomic DNA to a unique molecular index containing a universal adapter sequence; (d) amplifying the ligated DNA fragments using primers targeting the tag and universal adapter sequences to produce a first set of amplified sequences; (e) amplifying the first set of amplified sequences using universal sequencing primers targeting the tails of Tag-pTOP or TagpBOT primers to produce a second set of amplified sequences; (f) sequencing the pooled sequences and obtaining sequencing data; and (g) identifying on-/off-target CRISPR editing loci. In one aspect, the universal sequencing primers target SP1 or SP2 sequence (SEQ ID NO: 7, 8) tails on the Tag-pTOP or Tag-pBOT primers to produce a second set of amplified sequences. In another aspect, the universal sequencing primers target predesigned non-homologous sequence (SEQ ID NO: 269-273) tails on the Tag-pTOP or Tag-pBot primers to produce a second set of amplified sequences. In another aspect, the universal sequencing primers target predesigned 13-mer tails on the Tag-pTOP or Tag-pBot primers to produce a second set of amplified sequences. In another aspect, step (g) comprises executing on a processor: (i) aligning the sequence data to a reference genome; (ii) identifying on-/off-target CRISPR editing loci; and (iii) outputting the alignment, analysis, and results data as custom-formatted files, tables or graphics. In another aspect, the method further comprises a step following step (e) comprising: (e1) normalizing the second set of amplified sequences to produce concentration normalized libraries, pooling the normalized libraries with other samples to produce pooled libraries; and continuing with steps (f)-(i). In another aspect, step (d) uses a supression PCR method. In another aspect, the RNA-guided endonuclease comprises an endogenously-expressed Cas enzyme, a Cas expression vector, a Cas protein, or a Cas RNP complex. In another aspect, the RNA-guided endonuclease comprises an endogenously-expressed Cas9 enzyme, a Cas9 expression vector, a Cas9 protein, or a Cas9 RNP complex. In another aspect, the cells comprise human or mouse cells. In another aspect, the period of time is about 24 hours to about 96 hours. In another aspect, multiple tag sequences are co-delivered. In another aspect, the tag sequences comprise double-stranded deoxyribooligonucleotides (dsDNA) comprising 52-base pairs. In another aspect, the tag sequences comprise a 5'-terminal phosphate, and phosphorothioate linkages between the 1.sup.st and 2.sup.nd, 2.sup.nd and 3.sup.rd, 50.sup.th and 51.sup.st, and 51.sup.st and 52.sup.nd nucleotides. In another aspect, the tag sequences comprise a double stranded DNA comprising the complementary top and bottom strand pairs of SEQ ID NO: 1-2 or 7-268.

[0009] Other embodiments described herein are on- and off-target CRISPR editing sites identified or nominated using the methods described herein.

[0010] Another embodiment described herein is a method for designing 52-base pair tag sequences, the method comprising, executing on a processor: (a) randomly generating 13-nucleotide sequences with 40-90% GC content, max homopolymer length A: 2, C: 3, G: 2, T: 2, weighted homopolymer rate <20, self-folding Tm<50° C., and self-dimer Tm<50° C.; (b) removing sequences that perfectly align to a particular genome or that are homopolymers or GG or CC dinucleotide motifs and obtaining a set of 13-mers; (c) selecting a subset of the 13-mer sequences that contain one or less CC or GG dinucleotide motifs; (d) concatenating four of the of 13-mer subset sequences to form random 52-mer sequences; (e) aligning the random 52-mer sequences to a genome; (f) removing the random 52-mer sequences that have similarity to the genome to produce a subset of 52-mer sequences; and (h) outputting the subset of 52-mer sequences and generating the complementary strands to produce double stranded 52-base pair tag sequences. In one aspect, the genome is human

or mouse. In another aspect, the 52-base pair tag sequences are-non complementary to the genome. In another aspect, the method further comprises designing primers for the 52-base pair tag sequences. In another aspect, the 52-base pair tag sequences comprise a 5'-terminal phosphate, and phosphorothioate linkages between the 1.sup.st and 2.sup.nd, 2.sup.nd and 3.sup.rd, 50.sup.th and 51.sup.st, and 51.sup.st and 52.sup.nd nucleotides of the 52-base pair tag sequences. In another aspect, the method further comprises synthesizing oligonucleotides comprising the 52-base pair tag sequences, the complement of the 52-base pair tag sequences, or primers for the 52-base pair tag sequences.

[0011] Other embodiments described herein are one or more 52-base pair tag sequences designed using the methods described herein. In one aspect, the 52-base pair tag sequence comprises a double stranded DNA comprising the top and bottom strand pairs of SEQ ID NO: 1-2 or 7-268. [0012] Another embodiment described herein is a method for designing primers partially complementary to the 52-base pair tag sequences of claim 23 and an adapter primer, the method comprising, executing on a processor: (a) designing tag primers that are partially complementary to the top and bottom strands of tag sequences; and (b) designing an adapter primer that is partially complementary to the top strand of the adapter sequence; wherein: the tag primers comprise a 5'universal tail sequence; and the adapter primer comprises a sequence complementary to the tails of Tag-pTOP or Tag-pBOT primers. In one aspect, the 5'-universal tail sequence is complementary to an SP1 or SP2 sequence (SEQ ID NO: 7, 8), a locus specific segment, a ribonucleotide (rN) 6nucleotides from the 3'-end, a 3'-end mismatch, a 3'-end block (3'-C.sub.3 spacer), a predesigned non-homologous sequence (SEQ ID NO: 269-273), or a predesigned 13-mer sequence. In another aspect, the primers partially complementary to top and bottom strands of the tag sequences comprise a tail sequence complementary to the SP1 sequence (SEQ ID NO: 7) and the adapter primer comprises a sequence complementary to the SP2 sequence (SEQ ID NO: 8) tail on the TagpTOP or Tag-pBOT primers; or the primers partially complementary to top and bottom strands of the tag sequences comprise a tail sequence complementary to the SP2 sequence (SEQ ID NO: 8) and the adapter primer comprises a sequence complementary to the SP1 sequence (SEQ ID NO: 7) tail on the Tag-pTOP or Tag-pBOT primers. In another aspect, the amplification of a nucleic acid molecule with the primers that are complementary to the top and bottom strands of tag sequences and primers that are complementary to the top strand of the adapter sequence produces a PCR product that comprises a portion of the tag sequence, a sgDNA sequence, and the adapter sequence. In another aspect, the method further comprises synthesizing oligonucleotides comprising the sequences of the forward and reverse tag primers and the adapter primer. In another aspect, the 52base pair tag sequences and primers partially complementary to the 52-base pair tag sequences are designed and selected using an algorithm predicting whether the primers are likely to be partially complementary and have a propensity to form primer-dimers.

[0013] Other embodiments described herein are one or more primers partially complementary to the 52-base pair tag sequences and one or more adapter primers designed using the methods described herein. In one aspect, the primers comprise the sequences of SEQ ID NO: 3, 4; and the adapter primer, wherein the adapter primer comprises the sequence of SEQ ID NO: 5. [0014] Another embodiment described herein is the use of one or more double-stranded 52-base pair tag sequences for identifying on- and off-target CRISPR editing sites.

## **Description**

#### **DESCRIPTION OF THE DRAWINGS**

[0015] FIG. **1** shows fraction of reads shared by three biological replicates are shown in white sectors; whereas reads shared by two replicates, or present in a single replicate, are shown in black sectors. Table 1 shows GUIDE-seq [3] based nomination for 4 different gRNAs in triplicate in a

96-well format. gRNA complexes were generated by mixing equimolar amounts of Alt-R crRNA-XT and Alt-R tracrRNA. HEK293 cells stably expressing Cas9 were transfected with 10 UM gRNA and 0.5 UM dsODN GUIDE-seq tag using the Nucleofector™ system (Lonza). After 72 hrs, genomic DNA (gDNA) was isolated. Genomic DNA was fragmented, and adapters were ligated using the Lotus DNA library preparation kit (IDT). Libraries were generated by amplification from the inserted tag to the ligated adapters [3]. Libraries were then sequenced in paired-end fashion on an Illumina® platform.

[0016] FIG. 2 shows that GUIDE-Seq finds more off-target locations than can be validated through rhAmpSeq targeted amplification. Presented results are an aggregate of 331 GUIDE-Seq nominated sites when delivering gRNA sequences (internally named: AR, CTNNB1, EMX1, GRHPR, HPRT38087, HPRT38285, VEGFA) into HEK293 cells stably expressing WT Cas9. GUIDE-seq nominated off-targets assigned >0.1% of the total reference genome aligned reads for each guide were designed and targeted by one rhAmpSeq panel all reference genome aligned. In subsequent experiments, gRNAs were again delivered to the same cells, and editing was assayed with rhAmpSeq. Targets were called "edited" if the treated condition had observed indels  $\geq$  the untreated control sample at  $\geq$ 1%.

[0017] FIG. **3** illustrates that GUIDE-Seq tag integration rate varies. The graph shows the percentage of Tag integration (normalized to % Editing) for 118 unique Cas9 on/off-target sites that had InDel editing in rhAmpSeq panels targeting GUIDE-Seq nominated on/off-target loci for guide sequences targeting the RAG1, RAG2, and EMX1 genes. Each guide was co-delivered with the 34-base pair GUIDE-Seq, dsODN tag into HEK293 cells stably expressing Cas9 by nucleofection. DNA was extracted 72 hrs later, amplified by rhAmpSeq multiplex PCR, sequenced on an Illumina® MiSeq, and analyzed through a custom pipeline. The normalized tag integration rate is calculated as the percentage of sequenced reads at each target containing the tag sequence divided by the total reads containing an allele divergent from the reference genome (indicating Cas9 editing).

[0018] FIG. **4** shows the design of rhAmpSeq primers against alien sequence tags. A cartoon diagram shows the steps of the design process using the rhAmpSeq design pipeline including design of forward primers against the top (1) and bottom (2) strands, discarding unneeded primers, and selecting tag-targeting primers that have 5'-overlapping, but not 3'-overlapping sequences, so that the top/bottom strand primer dimers would hairpin (3).

[0019] FIG. **5** shows an overview of the rhAmpSeq design pipeline used to construct the overlapping primer designs. In the pipeline, a known sequence is appended onto the 5'-end and 3'-end of each tag sequence, the inputs are quality-controlled and assays (shown in FIG. **4**A) are designed against the top and bottom strand of each tag. Primers targeting each tag strand are paired such that at least 4-nucelotides 3' of the RNA nucleotide do not overlap between primers targeting the same tag, and primer pairs are ranked and selected. Hg38 and mm38 acronyms represent versions of the human and mouse genomes, respectively.

[0020] FIG. **6** illustrates hairpin formation if overlapping primers generate PCR amplicons. The diagram shows a representative target sequence and hairpin PCR product of undesired short amplicons from overlapping primer regions with complementary 5' primer tail ends at the 3'- and 5'-end of the PCR product.

[0021] FIG. 7 shows the number of target sites (black bars) with integration of the specified single tag (SEQ ID NO: 9-40) or pools of tags described in Table 5 (SEQ ID NO: 9-40, 45-268). The striped bar (CTLmax) shows the maximum number of target sites that theoretically can be found if a combination of the single tags (SEQ ID NO: 9-40) is used (23 sites out of a maximum of 32 sites). Pool A1 contains all the single tags (SEQ ID NO: 9-40). Pools B1-6 contain 16 different tags each (SEQ ID NO: 45-268). Pool C1 contains all tags tested (SEQ ID NO: 9-40, 45-268). Integration events were determined using an in-house data analysis tool.

[0022] FIG. **8** shows the number of target sites (black bars) with integration of the specified single

tag (SEQ ID NO: 9-40) or pools of tags described in Table 5 (SEQ ID NO: 9-40, 45-268). The striped bar (CTLmax) shows the maximum number of target sites that theoretically can be found if a combination of the single tags (SEQ ID NO: 9-40) is used (47 sites out of a maximum of 53 sites). Pool A1 contains all the single tags (SEQ ID NO: 9-40). Pools B1-6 contain 16 different tags each (SEQ ID NO: 45-268). Pool C1 contains all tags tested (SEQ ID NO: 9-40, 45-268). Integration events were determined using an in-house data analysis tool.

#### DETAILED DESCRIPTION

[0023] Described herein are methods for detecting and nominating on- and off-target CRISPR editing sites with improved accuracy and sensitivity. The intracellular context information is maintained by building upon prior in vivo nomination methods. The sensitivity is expanded by codelivering a set of unique, predefined sequence tags. In one aspect, the co-delivered set of predefined unique tags may range from 13-80 base pairs. In another aspect, the co-delivered set of predefined tags may be comprised of 13 base pair tag sequence tags, 26 base pair tag sequence tags, 39 base pair tag sequence tags, 52 base pair tag sequence tags, 65 base pair tag sequence tags, or 78 base pair tag sequence tags. In another aspect, the unique predefined tags are a set of 52-base pair tag sequence tags (the increased length of the sequence tags improves the ability to find good primer landing sites for rhPrimers). This limitation is believed to be mitigated by using a diversity of tag sequences that are distinct from human and mouse genomes. The specificity is improved by building upon Integrated DNA Technologies (IDT)'s rhAmp technology that uses RNAaseH2 (*Pyrococcus abyssi*) to unblock primers that have correctly annealed to their target; this yields lower rates of false priming. Specificity can be further enhanced by only nominating targets using reads that contain an expected tag sequence at the 5'-end. The incorporation of suppression PCR into this method permits ease of use. The prior in vivo methods (e.g., GUIDE-seq and iGUIDE) require parallel PCR reactions (2 pool amplification) to amplify by annealing to and extending from the top and bottom strand of the tags. Here, suppression PCR is used to allow both pools to be amplified simultaneously without causing problematic dimer sequences.

[0024] A GUIDE-Seq dsDNA tag was co-delivered with one guide RNA to HEK293 cells constitutively expressing Cas9 using nucleofection. See U.S. Pat. No. 9,822,407, which is incorporated by reference herein for such teachings. A total of four different guide RNAs were tested in this fashion. Ribonucleoprotein complexes (RNPs) between the expressed Cas9 and guide RNA form within the cells, introducing double stranded breaks. Repaired breaks can contain the co-delivered tags. After delivery, cells were incubated, and the resulting DNA was extracted. Target amplification was performed according to the GUIDE-Seq protocol and assayed with a modified version of the GUIDE-Seq analytical pipeline (github.com/aryeelab/guideseq). Nominated targets were compared between three biological replicates (unique guideRNA+Tag co-deliveries). Not all nominated targets were common to all biological replicates (commonly/total nominated targets: 7/31, 6/19, 2/4, 3/5 respectively; see Table 1). However, >90% of the total reads, attributed to any target, were attributed to common targets (on average; see FIG. 1).

TABLE-US-00001 TABLE 1 Identified off-target sites for four different gRNAs and relative level of editing at off-target sites compared to the on-target site Location C19orf84\_BR1 C19orf84\_BR2 C19orf84\_BR3 chr19\_51389306 100.00% 100.00% 100.00% chr9\_20224748 38.55% 16.43% 29.00% chr4\_28036434 16.33% 13.05% 14.36% chr15\_74256506 14.30% 18.18% 25.17% chr2\_171312919 11.40% 8.51% 7.93% chr8\_65742269 10.82% 1.17% 10.40% chr13\_96554656 8.70% 0.00% 0.00% chr4\_86807920 8.50% 9.21% 1.92% chr3 124485356 6.57% 0.00% 0.00% chr9\_20330398 5.60% 0.00% 0.00% chr11 71298123 5.12% 0.00% 0.00% chr7\_101729696 4.83% 0.00% 9.58% chr19\_10923882 3.67% 3.03% 0.00% chr10\_15548456 3.57% 15.38% 0.00% chr12 117097457 2.80% 0.00% 2.60% chr22 33493900 2.13% 0.00% 4.79% 0.00% 1.93% 0.55% chrX\_149763439 2.13% 3.83% chr17\_7435217 0.00% chr12\_26286721 1.74% 0.00% 5.06% chr16\_49704848 1.26% 5.01% 7.11%

```
chr12_51288216
                  1.06%
                           0.00%
                                    0.00% chr12_56010621
                                                             0.87%
                                                                      0.00%
                                                                               0.00%
                                                           0.29%
chr13 29717148
                  0.48%
                           0.00%
                                    0.00% chr1 3088065
                                                                    0.00%
                                                                             0.00%
                  0.19%
                           0.00%
                                                              0.19%
                                                                       0.00%
                                                                                0.00%
chr15 73442915
                                    0.55% chr10 118045968
chr14 102199972
                   0.00%
                            0.00%
                                     0.68% chr18 56334679
                                                               0.00%
                                                                        0.00%
                                                                                 2.33%
                  0.00%
                                                                      0.00%
chr21_36426137
                           0.00%
                                    2.19% chr5_139002763
                                                             0.00%
                                                                               3.83%
                  0.00%
                          0.00%
                                   3.83% Location C17orf99_BR1 C17orf99_BR2
chrX 58291642
C17orf99_BR3 chr17_78164110 100.00% 100.00% 100.00% chr22_24471716 15.00% 13.24%
                                    11.07%
                                              9.79% chr3 170476431
                                                                       5.86%
                                                                                3.97%
 10.86% chr10 101156881
                            6.22%
                                                                             4.63%
4.57% chr17 17692965
                         4.94%
                                  0.66%
                                                                    3.93%
                                           8.62% chr15 73400031
                                                                                      5.73%
chr19 15238775
                           0.00%
                                                                     0.00%
                                                                              1.59%
                  0.00%
                                    2.56% chr2 18362316
                                                            0.00%
chr2 171087784
                  0.00%
                           0.54%
                                    0.84% chr22 19959968
                                                             0.00%
                                                                               0.19%
                                                                      1.26%
chr22_32114104
                  0.00%
                           0.00%
                                    4.06% chr4_129034015
                                                             0.00%
                                                                      0.00%
                                                                               0.33%
chr5_61219030
                 0.00%
                          0.00%
                                                           0.00%
                                                                    0.00%
                                                                             1.86%
                                   0.33% chr5_66209615
                                                                     1.44%
chr7 69709389
                 0.00%
                          0.12%
                                   2.75% chr7_158662844
                                                            0.00%
                                                                              5.27%
chrX 9567397
                0.00%
                         0.00%
                                  0.23% chr19 55657073
                                                            0.00%
                                                                     0.66%
                                                                              0.00%
                  0.00%
                           2.47%
chr22 43788032
                                    0.00% Location C16orf90 BR1 C16orf90 BR2
C16orf90 BR3 chr16 3494817 100.00% 100.00% 100.00% chr2 109189307 75.32%
                                                                                    4.27%
52.05% chr22 24586001 45.45%
                                            0.00% chr10 104736568
                                   0.00%
                                                                      0.00%
8.22% Location ATAD3C_BR1 ATAD3C_BR2 ATAD3C_BR3 chr1_1450685 100.00% 100.00%
100.00% chr1_1503588 11.73% 10.07%
                                                                  2.47%
                                                                           1.86%
                                          9.27% chr1_1516015
                                                                                   5.14%
                           0.93%
chr19 32167960 26.34%
                                    0.00% chr2 111077960
                                                             0.00%
                                                                      1.12%
                                                                               0.00%
[0025] Additionally, nominated targets may not be replicable or detectable using orthogonal
methods. Using the GUIDE-Seq method, the GUIDE-Seq DNA tag was co-delivered with each of
6 guides (each tag is delivered with one guide RNA) to HEK293 cells constitutively expressing
Cas9 using nucleofection. rhAmpSeq multiplex amplicon panels were designed to amplify the
nominated targets, and we quantified editing in biological replicates. Of the 331 targets nominated
by GUIDE-Seq, only 41 (12%) could be verified with rhAmpSeq (see FIG. 2).
[0026] dsDNA tag sequences co-delivered with the guide RNAs into a stably expressing CRISPR
cell line, which are used in the NHEJ repair, are incorporated at varying rates. Here, the GUIDE-
Seq dsDNA tag was co-delivered with each of 6 guides into HEK293 cells constitutively
expressing Cas9. In another aspect, the dsDNA tag sequences co-delivered with CRISPR RNP,
which are used in the NHEJ repair, are incorporated at varying rates. Here, the GUIDE-Seq dsDNA
tag was co-delivered with each of 6 guides into HEK293 cells constitutively expressing Cas9.
rhAmpSeq panels were developed to amplify nominated targets, and in biological replicates, the
rates of tag integration were analyzed using a custom analytical pipeline. These results demonstrate
that tags are incorporated at 0-85% of edited genomic copies, varying by target (see FIG. 3).
Without being bound by any theory, it is hypothesized that the rate varies by sequence context.
[0027] Described herein are methods to improve the signal to noise ratio by combining Integrated
DNA Technology's rhAmpSeq<sup>TM</sup> technology, suppression PCR, and novel alien DNA sequence
designs to nominate nuclease off-target editing locations within a host genome.
[0028] In this method, Cas9, a sgRNA or a two-part CRISPR RNA: trans-activating crRNA
(crRNA: tracrRNA) duplex, and one or more double stranded DNA (dsDNA) tag sequences are
delivered to cells. Co-delivering multiple tags permits improved tag integration at off-target sites
(see below). The tag sequences have sequence content significantly different (i.e., alien) to the host
genome. After nuclease introduced DSBs, NHEJ repair will insert the tag sequence(s) into the
target site, forming known primer landing sites. After cells have time to repair the DSBs and
possibly further divide (such as after 72 hr), genomic DNA is isolated, fragmented (e.g., Covaris®
shearing, enzyme-based shearing, Tn5, etc.), ligated a unique molecular index (UMI)-containing
universal adapter sequence to the fragmented DNA, and the un-ligated material is removed. Next,
the DNA fragments are amplified by targeting primers to the tag and universal adapter sequences
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(Round 1 PCR). Using universal primers, a sample index (PCR2) is added, the amplified material is concentration normalized, pooled with other samples, and the pooled material is sequenced on an Illumina® (or similar) machine. The sequenced reads are aligned to a reference genome, and loci where large numbers of reads map may nominate on/off-target locations.

[0029] Alien sequences were designed by generating >1 M random 13-mer sequences with 40-90% GC content, max homopolymer length A: 2, C: 3, G: 2, T: 2, weighted homopolymer rate <20, self-folding Tm<50° C., and self-dimer Tm<50° C. From the list of sequences, sequences that aligned perfectly against human (GRCh38.p2; hg38) or mouse (GRCh38.p4; mm38) reference genomes or had troubling motif sequences (homopolymers, most G-G or C-C dinucleotide motifs) were removed, resulting in 479 sequences.

[0030] To design the 52-base pair tag sequences described herein, 49 13-mer oligo sequences were selected that contain≤1 C or G dinucleotide, and 10,000 unique combinations of four 13-mer sequences were generated. The length of each concatenated sequence (e.g., pasting four 13-mer sequences in a row using software) is 52-nucleotides. Next, each 52-nucleotide tag sequence was aligned against the human (GRCh38.p2) and mouse (GRChm38.p4) genomes using an internally modified version of bwa, called bwa-psm. Implementation of bwa-psm returns all possible secondary matches up to a defined threshold. A set of tag sequences (SEQ ID NO:1-2) were designed that were intended to work as a group, that had no similarity to the human or mouse genomes (max seed size: 7, seed edit distance: 2, max edit distance: 21, max gap open: 2, max gap extension: 3, mismatch penalty: 1, gap open penalty: 1, gap extension penalty: 1). [0031] Overlapping rhAmpSeq V1 primers (SEQ ID NO: 3-4) were designed complementary to the top and bottom strands of the tag and 5'-end of the adapter sequence (SEQ ID NO: 6) (FIG. 4). The tag-specific primers (SEQ ID NO: 3-4) contain a 5'-universal tail sequence matching the SP1 and SP2 primer sequences (SEQ ID NO: 7-8), a locus specific segment, a ribonucleotide (rN) 6nucleotides from the 3'-end, a 3'-end mismatch, and a 3'-end block (3'-C3 spacer). The adapterspecific primer (SEQ ID NO: 5) targets the 5'-end of the 5'-P5 adapter sequence (SEQ ID NO: 6), and the adapter sequence contains unique molecular index (UMI) sequence (Table 2). The primers were designed to target the plus and minus strands of the annealed tag such that, if these primers unexpectedly form a dimer, the formed product will hairpin, removing the oligo from the available reaction templates (e.g., supression PCR). (FIG. 6A-B). Primer sequences targeting the tags were chosen based on a proprietary design algorithm designed and implemented by IDT (internal copy of the algorithm with a public-facing UI: www.idtdna.com/site/account? ReturnURL=/site/order/designtool/index/RHAMPSEQ), which selects the most optimally performing primer pairs to amplify the intended template sequence. (FIG. 5). Primer sequences were assessed for non-specific binding to all other tag sequences and both human and mouse primary genome assemblies to verify they were unlikely to form off-target amplicons when combined with a universal adapter sequence and the presence of human or mouse genomic DNA. [0032] The primers were desired to work in pairs where one tag-specific primer (top or bottom strand) pairs with the adapter-specific primer (SEQ ID NO:5). This results in the amplification of a molecule that contains a portion of the tag, gDNA, and the adapter sequence when amplified using supression PCR methods (FIG. 4).

TABLE-US-00002 TABLE 2 Sequences Used for First Proof of Concept SEQ Sequence ID Type Name (5'.fwdarw.3') NO Tag 9022179029169042579 T\*C\*GTTCGTTC SEQ 04625907201907281 CGCTCTAACCGG ID CGAATCTACCGC NO: GCATATCTACGC 1 CGCA\*A\*T Tag 9022179029169042579 A\*T\*TGCGGCGT SEQ 04625907201907281\_r AGATATGCGCGG ID ev TAGATTCGCCGG NO: TTAGAGCGGAAC 2 GAAC\*G\*A Tag pFWD.ID\_Target1: acactctttccc SEQ Primers 9022179029169042579 tacacgacgctc ID 04625907201907281.12 ttccgatctTCT NO: 7.150.1.SP1 ACCGCGCATATC 3 TACrGCCGCT/ 3SpC3/ Tag pFWD.ID\_Target2: acactctttccc SEQ Primers 9022179029169042579 tacacgacgctc ID 04625907201907281.11 ttccgatctATA NO: 6.140.-1.SP1 TGCGCGGTAGAT 4

cttccgatctAA NO: TGATACGGCGAC 5 CACCGAGATCTA CArCAAGGC/ 3SpC3/ P5 Adapter Example Sequence AATGATACGGCG SEQ ACCACCGAGATC ID TACACTAGATCG NO: CNNWNNWNNACA 6 CTCTTTCCCTAC ACGACGCTCTTC CGATC\*T SP1 Sequencing Primer 1 acactetttccc SEQ tacacgacgctc ID ttccgatct NO: 7 cttccgatct NO: 8 "\*" SP2 Sequencing Primer 2 gtgactggagtt SEQ cagacgtgtgct ID indicates a phosphorothioate linkage; "rN" indicates a ribonucleotide, where N is the nucleotide preceded by the "r"; "/3SpC3/" indicates a 3'-C.sub.3 spacer. [0033] One embodiment described herein is a method for identifying and identifying and nominating on- and off-target CRISPR editing sites with improved accuracy and sensitivity, the process comprising the steps of: (a) co-delivering a guide sequence RNA (sgRNA) or a two-part CRISPR RNA: trans-activating crRNA (crRNA: tracrRNA) duplex and one or more tag sequences to cells; (b) incubating the cells for a period of time; (c) isolating genomic DNA from the cells, fragmenting the genomic DNA, and ligating the fragmented genomic DNA to a unique molecular index containing a universal adapter sequence; (d) amplifying the ligated DNA fragments using primers targeting the tag and universal adapter sequences to produce a first set of amplified sequences; (e) amplifying the first set of amplified sequences using universal sequencing primers targeting the tails of Tag-pTOP or Tag-pBOT primers to produce a second set of amplified sequences; (f) sequencing the pooled sequences and obtaining sequencing data; and (g) identifying on-/off-target CRISPR editing loci. In one embodiment, the universal sequencing primers target SP1 or SP2 sequence (SEQ ID NO: 7, 8) tails on the Tag-pTOP or Tag-pBOT primers to produce a second set of amplified sequences. In another embodiment, the universal sequencing primers target predesigned non-homologous sequence (Table 6; SEQ ID NO: 269-273) tails on the Tag-pTOP or Tag-pBot to produce a second set of amplified sequences. In yet another embodiment, the universal primers target predesigned 13-mer tails on the Tag-pTOP or Tag-pBOT primers to produce a second set of amplified sequences. In one embodiment, step (g) comprises executing on a processor: (i) aligning the sequence data to a reference genome; (ii) identifying on-/off-target CRISPR editing loci; and (iii) outputting the alignment, analysis, and results data as tables or graphics. In another embodiment, the method further comprises a step following step (e) comprising: (e1) normalizing the second set of amplified sequences to produce concentration normalized libraries, pooling the normalized libraries with other samples to produce pooled libraries; and continuing with steps (f)-(i). In one aspect, step (d) uses a supression PCR method. In another aspect, the cells constitutively express a Cas enzyme, are co-delivered with a Cas expression vector, are co-delivered with a Cas protein, or are co-delivered with a Cas RNP complex. In another aspect, the cells constitutively express a Cas9 enzyme, are co-delivered with a Cas9 expression vector, are co-delivered with a Cas9 protein, or are co-delivered with a Cas9 RNP complex. In another aspect, the cells comprise human or mouse cells. In another aspect, the period of time is about 24 hours to about 96 hours. In another aspect, multiple tag sequences are codelivered. In another aspect, the tag sequences comprise double-stranded deoxyribooligonucleotides (dsDNA) comprising 52-base pairs. In another aspect, the tag sequences comprise a 5'-terminal phosphate, and phosphorothioate linkages between the 1.sup.st and 2.sup.nd, 2.sup.nd and 3.sup.rd, 50.sup.th and 51.sup.st, and 51.sup.st and 52.sup.nd nucleotides. In another aspect, the tag sequences comprise a double stranded DNA comprising the top and bottom strand pairs of SEQ ID NO: 9-40 or 45-268. [0034] Another embodiment described herein is on- and off-target CRISPR editing sites identified or nominated using the methods described herein.

TCGCrCGGTTT/ 3SpC3/ Adapter Adapter Primer gtgactggagtt SEQ Primer cagacgtgtgct

[0035] Another embodiment described herein is a method for designing 52-base pair tag sequences, the method comprising, executing on a processor: (a) randomly generating 13-nucleotide sequences with 40-90% GC content, max homopolymer length A: 2, C: 3, G: 2, T: 2, weighted homopolymer rate <20, self-folding Tm<50° C., and self-dimer Tm<50° C.; (b) removing sequences that perfectly

align to a particular genome or that are homopolymers or GG or CC dinucleotide motifs and obtaining a set of 13-mers; (c) selecting a subset of the 13-mer sequences that contain one or less CC or GG dinucleotide motifs; (d) concatenating four of the of 13-mer subset sequences to form random 52-mer sequences; (e) aligning the random 52-mer sequences to a genome; (f) removing the random 52-mer sequences that have similarity to the genome to produce a subset of 52-mer sequences; and (h) outputting the subset of 52-mer sequences and generating the complementary strands to produce double stranded 52-base pair tag sequences. In one aspect, the genome is human or mouse. In one aspect, the 52-base pair tag sequences are not complementary to the genome. In another aspect, the method further comprises designing primers for the 52-base pair tag sequences. In another aspect, the 52-base pair tag sequences comprise a 5'-terminal phosphate, and phosphorothioate linkages between the 1.sup.st and 2.sup.nd, 2.sup.nd and 3.sup.rd, 50.sup.th and 51.sup.st, and 51.sup.st and 52.sup.nd nucleotides of the 52-base pair tag sequences. In another aspect, the method further comprises synthesising oligonucleotides comprising the 52-base pair tag sequences, the complement of the 52-base pair tag sequences, or primers for the 52-base pair tag sequences.

[0036] Another embodiment described herein is one or more 52-base pair tag sequences designed using the methods described herein. In one aspect, the 52-base pair tag sequence comprises a double stranded DNA comprising the complementary top and bottom strand pairs of SEQ ID NO: 9-40 or 45-268.

[0037] Another embodiment described herein is a method for designing primers partially complementary to the 52-base pair tag sequences described herein and an adapter primer, the method comprising, executing on a processor: (a) designing tag primers that are partially complementary to the top and bottom strands of tag sequences; and (b) designing an adapter primer that is partially complementary to the top strand of the adapter sequence; wherein: the tag primers comprise a 5'-universal tail sequence complementary to an SP1 or SP2 sequence (SEQ ID NO: 7, 8), a locus specific segment, a ribonucleotide (rN) 6-nucleotides from the 3'-end, a 3'-end mismatch, and a 3'-end block (3'-C3 spacer); and the adapter primer comprises a sequence complementary to the SP1 or SP2 sequence (SEQ ID NO: 7, 8). In one aspect, the primers partially complementary to top and bottom strands of the tag sequences comprise a sequence complementary to the SP1 sequence and the adapter primer comprises a sequence complementary to the SP2 sequence; or the primers partially complementary to top and bottom strands of the tag sequences comprise a sequence complementary to the SP2 sequence and the adapter primer comprises a sequence complementary to the SP1 sequence. In another aspect, amplification of a nucleic acid molecule with the primers that are complementary to the top and bottom strands of tag sequences and primers that are complementary to the top strand of the adapter sequence produces a PCR product that comprises a portion of the tag sequence, a sgDNA sequence, and the adapter sequence. In another aspect, the method further comprises synthesising oligonucleotides comprising the sequences of the forward and reverse tag primers and the adapter primer. [0038] In another embodiment described herein, the 52-base pair tag sequences and primers partially complementary to the 52-base pair tag sequences are designed and selected using an algorithm predicting whether the primers are likely to be partially complementary and have a propensity to form primer-dimers.

[0039] Another embodiment described herein is one or more primers partially complementary to the 52-base pair tag sequences and one or more adapter primers designed using the methods described herein. In one aspect, the primers partially complementary to the 52-base pair tag sequence comprise the sequences of SEQ ID NO: 3, 4; and the adapter primer comprises the sequence of SEQ ID NO:5.

[0040] Another embodiment described herein is the use of one or more double-stranded 52-base pair tag sequences for identifying on- and off-target CRISPR editing sites.

[0041] It will be apparent to one of ordinary skill in the relevant art that suitable modifications and

adaptations to the compositions, formulations, methods, processes, and applications described herein can be made without departing from the scope of any embodiments or aspects thereof. The compositions and methods provided are exemplary and are not intended to limit the scope of any of the specified embodiments. All the various embodiments, aspects, and options disclosed herein can be combined in any variations or iterations. The scope of the methods and processes described herein include all actual or potential combinations of embodiments, aspects, options, examples, and preferences herein described. The methods described herein may omit any component or step, substitute any component or step disclosed herein, or include any component or step disclosed elsewhere herein. It should also be understood that embodiments may include and otherwise be implemented by a combination of various hardware, software, and electronic components. For example, various microprocessors and application specific integrated circuits ("ASICs") can be utilized, as can software of a variety of languages. Also, servers and various computing devices can be used and can include one or more processing units, one or more computer-readable mediums, one or more input/output interfaces, and various connections (e.g., a system bus) connecting the components. Should the meaning of any terms in any of the patents or publications incorporated by reference conflict with the meaning of the terms used in this disclosure, the meanings of the terms or phrases in this disclosure are controlling. Furthermore, the specification discloses and describes merely exemplary embodiments. All patents and publications cited herein are incorporated by reference herein for the specific teachings thereof.

[0042] Various embodiments and aspects of the inventions described herein are summarized by the following clauses:

[0043] Clause 1. A method for identifying and nominating on- and off-target CRISPR edited sites with improved accuracy and sensitivity, the process comprising the steps of: [0044] (a) codelivering a guide sequence RNA (sgRNA) or a two-part CRISPR RNA: trans-activating crRNA (crRNA: tracrRNA) duplex, one or more tag sequences, and an RNA-guided endonuclease to cells; [0045] (b) incubating the cells for a period of time sufficient for double strand breaks to occur; [0046] (c) isolating genomic DNA from the cells, fragmenting the genomic DNA, and ligating the fragmented genomic DNA to a unique molecular index containing a universal adapter sequence; [0047] (d) amplifying the ligated DNA fragments using primers targeting the tag and universal adapter sequences to produce a first set of amplified sequences; [0048] (e) amplifying the first set of amplified sequences using universal sequencing primers targeting the tails of Tag-pTOP or Tag-pBOT primers to produce a second set of amplified sequences; [0049] (f) sequencing the pooled sequences and obtaining sequencing data; and [0050] (g) identifying on-/off-target CRISPR editing loci.

[0051] Clause 2. The method of clause 1, wherein the universal sequencing primers target SP1 or SP2 sequence (SEQ ID NO: 7, 8) tails on the Tag-pTOP or Tag-pBOT primers to produce a second set of amplified sequences.

[0052] Clause 3. The method of clause 1 or 2, wherein the universal sequencing primers target predesigned non-homologous sequence (SEQ ID NO: 269-273) tails on the Tag-pTOP or Tag-pBot primers to produce a second set of amplified sequences.

[0053] Clause 4. The method of any one of clauses 1-3, wherein the universal sequencing primers target predesigned 13-mer tails on the Tag-pTOP or Tag-pBot primers to produce a second set of amplified sequences.

[0054] Clause 5. The method of any one of clauses 1-4, wherein step (g) comprises executing on a processor:

[0055] Clause 6. aligning the sequence data to a reference genome; [0056] (a) (ii) identifying on-/off-target CRISPR editing loci; and [0057] (b) (iii) outputting the alignment, analysis, and results data as custom-formatted files, tables or graphics.

[0058] Clause 7. The method of any one of clauses 1-5, further comprising a step following step (e) comprising: [0059] (a) (e1) normalizing the second set of amplified sequences to produce

- concentration normalized libraries, pooling the normalized libraries with other samples to produce pooled libraries; and continuing with steps (f)-(i).
- [0060] Clause 8. The method of any one of clauses 1-6, wherein step (d) uses a supression PCR method.
- [0061] Clause 9. The method of any one of clauses 1-7, wherein the RNA-guided endonuclease comprises an endogenously-expressed Cas enzyme, a Cas expression vector, a Cas protein, or a Cas RNP complex.
- [0062] Clause 10. The method of any one of clauses 1-8, wherein the RNA-guided endonuclease comprises an endogenously-expressed Cas9 enzyme, a Cas9 expression vector, a Cas9 protein, or a Cas9 RNP complex.
- [0063] Clause 11. The method of any one of clauses 1-9, wherein the cells comprise human or mouse cells.
- [0064] Clause 12. The method of any one of clauses 1-10, wherein the period of time is about 24 hours to about 96 hours.
- [0065] Clause 13. The method of any one of clauses 1-11, wherein multiple tag sequences are codelivered.
- [0066] Clause 14. The method of any one of clauses 1-12, wherein the tag sequences comprise double-stranded deoxyribooligonucleotides (dsDNA) comprising 52-base pairs.
- [0067] Clause 15. The method of any one of clauses 1-13, wherein the tag sequences comprise a 5′-terminal phosphate, and phosphorothioate linkages between the 1.sup.st and 2.sup.nd, 2.sup.nd and 3.sup.rd, 50.sup.th and 51.sup.st, and 51.sup.st and 52.sup.nd nucleotides.
- [0068] Clause 16. The method of any one of clauses 1-14, wherein the tag sequences comprise a double stranded DNA comprising the complementary top and bottom strand pairs of SEQ ID NO: 1-2 or 7-268.
- [0069] Clause 17. On- and off-target CRISPR editing sites identified or nominated using the method of any one of clauses 1-15.
- [0070] Clause 18. A method for designing 52-base pair tag sequences, the method comprising, executing on a processor: [0071] (a) randomly generating 13-nucleotide sequences with 40-90% GC content, max homopolymer length A: 2, C: 3, G: 2, T: 2, weighted homopolymer rate <20, self-folding Tm<50° C., and self-dimer Tm<50° C.; [0072] (b) removing sequences that perfectly align to a particular genome or that are homopolymers or GG or CC dinucleotide motifs and obtaining a set of 13-mers; [0073] (c) selecting a subset of the 13-mer sequences that contain one or less CC or GG dinucleotide motifs; [0074] (d) concatenating four of the of 13-mer subset sequences to form random 52-mer sequences; [0075] (e) aligning the random 52-mer sequences to a genome; [0076] (f) removing the random 52-mer sequences that have similarity to the genome to produce a subset of 52-mer sequences; and [0077] (g) outputting the subset of 52-mer sequences and generating the complementary strands to produce double stranded 52-base pair tag sequences.
- [0078] Clause 19. The method of clause 17, wherein the genome is human or mouse.
- [0079] Clause 20. The method of clause 17 or 18, wherein the 52-base pair tag sequences are-non complementary to the genome.
- [0080] Clause 21. The method of any one of clauses 17-19, further comprising designing primers for the 52-base pair tag sequences.
- [0081] Clause 22. The method of any one of clauses 17-20, wherein the 52-base pair tag sequences comprise a 5'-terminal phosphate, and phosphorothioate linkages between the 1.sup.st and 2.sup.nd, 2.sup.nd and 3.sup.rd, 50.sup.th and 51.sup.st, and 51.sup.st and 52.sup.nd nucleotides of the 52-base pair tag sequences.
- [0082] Clause 23. The method of any one of clauses 17-21, further comprising synthesizing oligonucleotides comprising the 52-base pair tag sequences, the complement of the 52-base pair tag sequences, or primers for the 52-base pair tag sequences.
- [0083] Clause 24. One or more 52-base pair tag sequences designed using the methods of clauses

17-22.

[0084] Clause 25. The 52-base pair tag sequences of clause 23, wherein the 52-base pair tag sequence comprises a double stranded DNA comprising the top and bottom strand pairs of SEQ ID NO: 1-2 or 7-268.

[0085] Clause 26. A method for designing primers partially complementary to the 52-base pair tag sequences of clause 23 and an adapter primer, the method comprising, executing on a processor: [0086] (a) designing tag primers that are partially complementary to the top and bottom strands of tag sequences; and [0087] (b) designing an adapter primer that is partially complementary to the top strand of the adapter sequence; [0088] (c) wherein: [0089] (d) the tag primers comprise a 5′-universal tail sequence; and [0090] (e) the adapter primer comprises a sequence complementary to the tails of Tag-pTOP or Tag-pBOT primers.

[0091] Clause 27. The method of clause 25, wherein the 5′-universal tail sequence is complementary to an SP1 or SP2 sequence (SEQ ID NO: 7, 8), a locus specific segment, a ribonucleotide (rN) 6-nucleotides from the 3′-end, a 3′-end mismatch, a 3′-end block (3′-C3 spacer), a predesigned non-homologous sequence (SEQ ID NO: 269-273), or a predesigned 13-mer sequence.

[0092] Clause 28. The method of clause 25 or 26, wherein the primers partially complementary to top and bottom strands of the tag sequences comprise a tail sequence complementary to the SP1 sequence (SEQ ID NO: 7) and the adapter primer comprises a sequence complementary to the SP2 sequence (SEQ ID NO: 8) tail on the Tag-pTOP or Tag-pBOT primers; or the primers partially complementary to top and bottom strands of the tag sequences comprise a tail sequence complementary to the SP2 sequence (SEQ ID NO: 8) and the adapter primer comprises a sequence complementary to the SP1 sequence (SEQ ID NO: 7) tail on the Tag-pTOP or Tag-pBOT primers. [0093] Clause 29. The method of any one of clauses 25-27, wherein the amplification of a nucleic acid molecule with the primers that are complementary to the top and bottom strands of tag sequences and primers that are complementary to the top strand of the adapter sequence produces a PCR product that comprises a portion of the tag sequence, a sgDNA sequence, and the adapter sequence.

[0094] Clause 30. The method of any one of clauses 25-28, further comprising synthesizing oligonucleotides comprising the sequences of the forward and reverse tag primers and the adapter primer.

[0095] Clause 31. The method of any one of clauses 17-21 and 25-29, wherein the 52-base pair tag sequences and primers partially complementary to the 52-base pair tag sequences are designed and selected using an algorithm predicting whether the primers are likely to be partially complementary and have a propensity to form primer-dimers.

[0096] Clause 32. One or more primers partially complementary to the 52-base pair tag sequences and one or more adapter primers designed using the method of clauses 22-25.

[0097] Clause 33. The primers of clause 32, wherein the primers comprise the sequences of SEQ ID NO: 3, 4; and the adapter primer, wherein the adapter primer comprises the sequence of SEQ ID NO: 5.

[0098] Clause 34. Use of one or more double-stranded 52-base pair tag sequences for identifying on- and off-target CRISPR editing sites.

#### REFERENCES

[0099] 1. Wienert et al., "Unbiased detection of CRISPR off-targets in vivo using DISCOVER-seq," Science 364 (6437): 286-289 (2019). [0100] 2. Nobles et al., "IGUIDE: An improved pipeline for analyzing CRISPR cleavage specificity," Genome Biol. 20 (14): 4-9 (2019). [0101] 3. Tsai et al., "GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases," Nature Biotechnol. 33 (2): 187-197 (2015). [0102] 4. Yan et al., "BLISS is a versatile and quantitative method for genome-wide profiling of DNA double-strand breaks," Nature Commun. 8:15058 (2017). [0103] 5. Tsai et al., "CIRCLE-seq: a highly sensitive in vitro screen for genome-

wide CRISPR-Cas9 nuclease off-targets," Nature Methods 14 (6): 607-614 (2017). [0104] 6. Cameron et al., "Mapping the genomic landscape of CRISPR-Cas9 cleavage," Nature Methods 14 (6): 600-606 (2017). [0105] 7. Char and Moosburner, "Unraveling CRISPR-Cas9 genome engineering parameters via a library-on-library approach," Nature Methods 12 (9): 823-826 (2015). [0106] 8. Rand et al., "Headloop suppression PCR and its application to selective amplification of methylated DNA sequences," Nucleic Acids Res. 33 (14): e127 (2005).

#### **EXAMPLES**

Example 1

[0107] This experiment demonstrates the increased efficiency in tag integration when using doublestranded DNA tags with a length of 52-base pairs and varying genetic sequence. The sequences used are shown in Tables 3-5. Double-stranded tags were generated by hybridization of a top strand and a complementary bottom strand (Tables 3-4; SEQ ID NO: 9-40 or 45-268). Sixteen different tag designs were introduced separately into HEK293 cells constitutively expressing Cas9 together with a guideRNA which targets the EMX1 locus. Alternatively, either pools of 16 tags or one pool of 112 tags were introduced into HEK293 cells constitutively expressing Cas9 together with a guideRNA which targets the EMX1 locus. GuideRNAs were electroporated at a concentration of 10 UM, whereas the single Tag or pooled Tags were delivered at a final concentration of 0.5 μM. Tag integration levels were determined by targeted amplification using rhAmpSeq primers (SEQ ID NO: 3-4), enriching for known on- and off-target sites of the EMX1 guideRNA. The rhAmpSeq pool for EMX1 consists of 32 sites, which represent empirically determined ON and OFF target loci. Amplified products were sequenced on an Illumina® MiSeq, and tag integration levels were determined using custom software. This example shows that tag integration efficiency varies among single tag constructs individually with a range between 6 (CTL021) and 13 (CTL169, CTL079, CTL002) sites out of a maximum of 32 sites, and is therefore sequence dependent (Single Tags, FIG. 7). By taking the mathematical union of the single tag results, a hypothetical number of 23 sites was calculated (CTLmax, FIG. 7). The hypothesis that combining a pool of tags would increase the likelihood of tag integration was tested and was demonstrated (Pooled Tags, Table, FIG. 7). Pool A1 consists of the tags represented in the Single Tags (see Table 5) and demonstrated that 21 tag integration events were detected out of a maximum of 32 sites, which is higher than achieved with any of the single tags. Similarly, Pool B3 demonstrated integration of a tag at 21 sites out of a maximum of 32 sites. Again, variability between pools was shown (Pooled Tags, FIG. 7), indicating optimization of tag designs can potentially maximize tag integration. TABLE-US-00003 TABLE 3 Sequences Used for Second Proof of Concept SEQ ID Name Sequence (5'.fwdarw.3') NO CTL085\_/5Phos/A\*C\*GAGCGGTAGTCACCTA SEQ TOP\_tag GTCGTCGTACCAATTCGACGCACACTA ID CTCGC\*G\*C NO: 9 CTL085\_ /5Phos/G\*C\*GCGAGTAGTGTGCGTC SEQ BOT\_tag GAATTGGTACGACGACTAGGTGACTAC ID CGCTC\*G\*T NO: 10 CTL169 /5Phos/T\*A\*GCGCGAGTAGTCGGAC SEQ TOP tag GAGCGGTTACCAATACGCCGCACCTTA ID ATCCG\*C\*G NO: 11 CTL169 /5Phos/C\*G\*CGGATTAAGGTGCGGC SEQ BOT tag GTATTGGTAACCGCTCGTCCGACTACT ID CGCGC\*T\*A NO: 12 CTL137\_ /5Phos/T\*C\*GCGACAGTAGTCGTTC SEQ TOP\_tag GGCTAGGTACCTATTACCGCGTAGTTA ID GCGGC\*G\*T NO: 13 CTL137\_/5Phos/A\*C\*GCCGCTAACTACGCGG SEQ BOT\_tag TAATAGGTACCTAGCCGAACGACTACT ID GTCGC\*G\*A NO: 14 CTL042 /5Phos/C\*G\*CGCTACTAGGTGCGTC SEQ TOP\_tag GAATTGGTACCGATCCGCAATACACTA ID CTCGC\*G\*C NO: 15 CTL042 /5Phos/G\*C\*GCGAGTAGTGTATTGC SEQ BOT tag GGATCGGTACCAATTCGACGCACCTAG ID TAGCG\*C\*G NO: 16 CTL051\_

/5Phos/G\*G\*TAACGAGCGGTGCGTC SEQ TOP\_tag

GAATTGGTAACCGCTCGTCCGACCTTA ID ATCGC\*G\*C NO: 17 CTL051\_

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/5Phos/G*C*GCGATTAAGGTCGGAC SEQ BOT_tag
GAGCGGTTACCAATTCGACGCACCGCT ID CGTTA*C*C NO: 18 CTL167
/5Phos/T*T*CGGCGCTAGGTGCGGC SEQ TOP tag
GTATTGGTAACCGCTCGTCCGTTCGGC ID GCTAG*G*T NO: 19 CTL167
/5Phos/A*C*CTAGCGCCGAACGGAC SEQ BOT_tag
GAGCGGTTACCAATACGCCGCACCTAG ID CGCCG*A*A NO: 20 CTL026
/5Phos/T*A*CGCGACTAGGTGCGCG SEQ TOP_tag
ATTAAGGTACCTATTACCGCGCGACTA ID TGTGC*G*C NO: 21 CTL026
/5Phos/G*C*GCACATAGTCGCGCGG SEQ BOT tag
TAATAGGTACCTTAATCGCGCACCTAG ID TCGCG*T*A NO: 22 CTL068
/5Phos/G*T*CGCGCAGTGTAGCGCG SEQ TOP tag
ATTAAGGTACCTATTACCGCGTCGCGA ID CAGTA*G*T NO: 23 CTL068
/5Phos/A*C*TACTGTCGCGACGCGG SEQ BOT tag
TAATAGGTACCTTAATCGCGCTACACT ID GCGCG*A*C NO: 24 CTL138
/5Phos/A*A*CCGTCGATCCGCGCGT SEQ TOP tag
AGTATGGTACCGATCCGCAATACTAGC ID GCGAC*A*A NO: 25 CTL138
/5Phos/T*T*GTCGCGCTAGTATTGC SEQ BOT tag
GGATCGGTACCATACTACGCGCGGATC ID GACGG*T*T NO: 26 CTL079
/5Phos/T*C*GCTCGATTGGTTACGC SEQ TOP_tag GCACTACTTATGCGCTCGACTCGTTCG
ID GCTAG*G*T NO: 27 CTL079_/5Phos/A*C*CTAGCCGAACGAGTCG SEQ BOT_tag
AGCGCATAAGTAGTGCGCGTAACCAAT ID CGAGC*G*A NO: 28 CTL063
/5Phos/A*C*TGCGAGCGTACTTGTC SEQ TOP tag
GCGCTAGTACCAATTCGACGCAACCGC ID TCGTC*C*G NO: 29 CTL063
/5Phos/C*G*GACGAGCGGTTGCGTC SEQ BOT tag
GAATTGGTACTAGCGCGACAAGTACGC ID TCGCA*G*T NO: 30 CTL168
/5Phos/C*G*CATTAGTCGGTGCGGC SEQ TOP tag
GTATTGGTAACCGCTCGTCCGACGCGC ID TACCT*A*T NO: 31 CTL168
/5Phos/A*T*AGGTAGCGCGTCGGAC SEQ BOT_tag
GAGCGGTTACCAATACGCCGCACCGAC ID TAATG*C*G NO: 32 CTL021
/5Phos/A*T*TGCGGATCGGTGCGTC SEQ TOP tag
GAATTGGTAACCGCTCGTCCGTACGCG ID CACTA*C*T NO: 33 CTL021
/5Phos/A*G*TAGTGCGCGTACGGAC SEQ BOT tag
GAAGCGGTTACCAATTCGCGCACCGAT ID CCGCA*A*T NO: 34 CTL151
/5Phos/T*C*GGCGAGTAGTTGCGCG SEQ TOP_tag
GTTATGGTACCATAACCGCGCAGTAGT ID ACGCG*G*T NO: 35 CTL151
/5Phos/A*C*CGCGTACTACTGCGCG SEQ BOT tag
GTTATGGTACCATAACCGCGCAACTAC ID TCGCC*G*A NO: 36 CTL002
/5Phos/A*C*TAGCGATCGGTACCTA SEQ TOP tag
GCGCCGAAACCTATTACCGCGACCTAG ID CGTTG*C*G NO: 37 CTL002
/5Phos/C*G*CAACGCTAGGTCGCGG SEQ BOT tag
TAATAGGTTTCGGCGCTAGGTACCGAT ID CGCTA*G*T NO: 38 CTL134
/5Phos/T*A*GCGCGTCAAGAGCGCG SEQ TOP tag
GTTATGGTTTCGGCGCTAGGTTAACAG ID CGCGT*C*G NO: 39 CTL134
/5Phos/C*G*ACGCGCTGTTAACCTA SEQ BOT tag
GCGCCGAAACCATAACCGCGCTCTTGA ID CGCGC*T*A NO: 40 GuideSeg
/5Phos/G*T*TTAATTGAGTTGTCAT SEQ TOP tag ATGTTAATAACGGT*A*T ID NO: 41
GuideSeq /5Phos/A*T*ACCGTTATTAACATAT SEQ BOT tag GACAACTCAATTAA*A*C ID
NO: 42 EMX1 GAGTCCGAGCAGAAGAAGAA SEQ protospacer ID NO: 43 AR
GTTGGAGCATCTGAGTCCAG SEQ protospacer ID NO: 44 "/5Phos/" indicates a 5'-phosphate
moiety; "*" indicates a phosphorothioate linkage.
```

## Example 2

[0108] This experiment demonstrates the increased efficiency in tag integration when using doublestranded DNA tags with a length of 52-base pairs and varying genetic sequence. The sequences used are shown in Tables 3-5. Double-stranded tags were generated by hybridization of a top strand and a complementary bottom strand (SEQ ID NO: 9-40 or 45-268). Sixteen different tag designs were introduced separately into HEK293 cells constitutively expressing Cas9 together with a guideRNA which targets the AR locus. Alternatively, either pools of 16 tags or one pool of 112 tags were introduced into HEK293 cells constitutively expressing Cas9 together with a guideRNA which targets the AR locus. GuideRNAs were electroporated at a concentration of 10 μM, whereas the single Tag or pooled Tags were delivered at a final concentration of 0.5 µM. Tag integration levels were determined by targeted amplification using rhAmpSeq primers (SEQ ID NO: 3-4), enriching for known on- and off-target sites of the AR guideRNA. The rhAmpSeq pool for AR consists of 53 sites which represent empirically determined ON and OFF target loci. Amplified products were sequenced on an Illumina® MiSeq, and tag integration levels were determined using custom software. This example shows that tag integration efficiency varies among single tag constructs individually with a range between 35 (CTL085, CTL134) and 41 sites (CTL002) out of a maximum of 53 sites, and is therefore sequence dependent (Single Tags, Table 5, FIG. 8). [0109] By taking the mathematical union of the single tag results, a hypothetical number of 47 sites was calculated (CTLmax, FIG. 8). The hypothesis that combining a pool of tags would increase the likelihood of tag integration was tested and was demonstrated (Pooled Tags, Table 5, FIG. 8). Pool B4 (see Table 5) demonstrated that 44 tag integration events were detected out of a maximum of 53 sites, which is higher than achieved with any of the single tags. Again, variability between pools was shown (Pooled Tags, Table 5, FIG. 8), indicating optimization of tag designs can potentially maximize tag integration.

```
TABLE-US-00004 TABLE
                    4 Tag
                          Sequences Name Sequence (5'.fwdarw.3') SEQ ID
CTL085 TOP tag /5Phos/A*C*GAGCGGTAGTCACCTAGTCGTACCAATTCGA SEQ
ID NO: 45 CGCACACTACTCGC*G*C CTL169_TOP_tag
/5Phos/T*A*GCGCGAGTAGTCGGACGAGCGGTTACCAATACGC SEQ
CGCACCTTAATCCG*C*G CTL137_TOP_tag
/5Phos/T*C*GCGACAGTAGTCGTTCGGCTAGGTACCTATTACC SEQ ID
                                                        NO:
GCGTAGTTAGCGGC*G*T CTL042_TOP_tag
/5Phos/C*G*CGCTACTAGGTGCGTCGAATTGGTACCGATCCGC SEQ
                                                         NO:
                                                             48
AATACACTACTCGC*G*C CTL051 TOP tag
/5Phos/G*G*TAACGAGCGGTGCGTCGAATTGGTAACCGCTCGT SEQ
                                                              49
                                                         NO:
CCGACCTTAATCGC*G*C CTL167_TOP_tag
/5Phos/T*T*CGGCGCTAGGTGCGGCGTATTGGTAACCGCTCGT SEQ
                                                         NO:
                                                             50
CCGTTCGGCGCTAG*G*T CTL026_TOP_tag
/5Phos/T*A*CGCGACTAGGTGCGCGATTAAGGTACCTATTACC SEQ
                                                         NO:
                                                             51
                                                     ID
GCGCGACTATGTGC*G*C CTL068 TOP tag
/5Phos/G*T*CGCGCAGTGTAGCGCGATTAAGGTACCTATTACC SEQ
                                                         NO:
                                                             52
                                                     ID
GCGTCGCGACAGTA*G*T CTL138_TOP_tag
/5Phos/A*A*CCGTCGATCCGCGCGTAGTATGGTACCGATCCGC SEQ
                                                     ID
                                                         NO:
                                                             53
AATACTAGCGCGAC*A*ACTL079_TOP_tag
/5Phos/T*C*GCTCGATTGGTTACGCGCACTACTTATGCGCTCG SEQ
                                                        NO:
                                                             54
                                                     ID
ACTCGTTCGGCTAG*G*T CTL063 TOP tag
/5Phos/A*C*TGCGAGCGTACTTGTCGCGCTAGTACCAATTCGA SEQ
                                                     ID
                                                         NO:
                                                             55
CGCAACCGCTCGTC*C*G CTL168 TOP tag
/5Phos/C*G*CATTAGTCGGTGCGGCGTATTGGTAACCGCTCGT SEQ
                                                     ID
                                                         NO:
                                                             56
CCGACGCGCTACCT*A*T CTL021_TOP_tag
```

57

NO:

/5Phos/A\*T\*TGCGGATCGGTGCGTCGAATTGGTAACCGCTCGT SEQ

```
CCGTACGCGCACTA*C*T CTL151_TOP_tag
/5Phos/T*C*GGCGAGTAGTTGCGCGGTTATGGTACCATAACCG SEQ ID
                                                            58
CGCAGTAGTACGCG*G*T CTL002 TOP tag
/5Phos/A*C*TAGCGATCGGTACCTAGCGCCGAAACCTATTACC SEQ
                                                       NO:
                                                            59
GCGACCTAGCGTTG*C*G CTL134_TOP_tag
/5Phos/T*A*GCGCGTCAAGAGCGCGGTTATGGTTTCGGCGCTA SEQ
                                                        NO:
                                                             60
GGTTAACAGCGCGT*C*G CTL085 BOT tag
/5Phos/G*C*GCGAGTAGTGTGCGTCGAATTGGTACGACGACTA SEQ
                                                        NO:
                                                             61
GGTGACTACCGCTC*G*T CTL169 BOT tag
/5Phos/C*G*CGGATTAAGGTGCGGCGTATTGGTAACCGCTCGT SEQ
                                                        NO:
                                                            62
CCGACTACTCGCGC*T*A CTL137 BOT tag
/5Phos/A*C*GCCGCTAACTACGCGGTAATAGGTACCTAGCCGA SEQ
                                                        NO:
                                                            63
ACGACTACTGTCGC*G*A CTL042 BOT tag
/5Phos/G*C*GCGAGTAGTGTATTGCGGATCGGTACCAATTCGA SEQ ID
                                                       NO:
                                                            64
CGCACCTAGTAGCG*C*G CTL051 BOT tag
/5Phos/G*C*GCGATTAAGGTCGGACGAGCGGTTACCAATTCGA SEQ
                                                             65
CGCACCGCTCGTTA*C*C CTL167 BOT tag
/5Phos/A*C*CTAGCGCCGAACGGACGAGCGGTTACCAATACGC SEQ ID
                                                             66
CGCACCTAGCGCCG*A*A CTL026_BOT_tag
/5Phos/G*C*GCACATAGTCGCGCGGTAATAGGTACCTTAATCG SEQ ID
CGCACCTAGTCGCG*T*A CTL068 BOT tag
/5Phos/A*C*TACTGTCGCGACGCGGTAATAGGTACCTTAATCG SEQ ID
                                                            68
CGCTACACTGCGCG*A*C CTL138 BOT tag
/5Phos/T*T*GTCGCGCTAGTATTGCGGATCGGTACCATACTAC SEQ ID NO:
GCGCGGATCGACGG*T*T CTL079 BOT tag
/5Phos/A*C*CTAGCCGAACGAGTCGAGCGCATAAGTAGTGCGC SEQ ID
                                                             70
GTAACCAATCGAGC*G*A CTL063_BOT_tag
/5Phos/C*G*GACGAGCGGTTGCGTCGAATTGGTACTAGCGCGA SEQ
                                                        NO:
                                                             71
CAAGTACGCTCGCA*G*T CTL168_BOT_tag
/5Phos/A*T*AGGTAGCGCGTCGGACGAGCGGTTACCAATACGC SEQ
                                                        NO:
                                                             72
CGCACCGACTAATG*C*G CTL021 BOT tag
/5Phos/A*G*TAGTGCGCGTACGGACGAGCGGTTACCAATTCGA SEQ
                                                             73
CGCACCGATCCGCA*A*T CTL151 BOT tag
/5Phos/A*C*CGCGTACTACTGCGCGGTTATGGTACCATAACCG SEQ ID
                                                       NO:
                                                            74
CGCAACTACTCGCC*G*A CTL002_BOT_tag
/5Phos/C*G*CAACGCTAGGTCGCGGTAATAGGTTTCGGCGCTA SEQ
                                                        NO:
                                                            75
GGTACCGATCGCTA*G*T CTL134 BOT tag
/5Phos/C*G*ACGCGCTGTTAACCTAGCGCCGAAACCATAACCG SEQ
                                                     ID
                                                        NO:
                                                             76
CGCTCTTGACGCGC*T*A CTL161 TOP tag
/5Phos/T*A*CACTGCGCGACACTGCGAGCGTACACCTTAATCG SEO
                                                     ID
                                                        NO:
                                                             77
CGCTAGTTAGCGGC*G*T CTL164_TOP_tag
/5Phos/A*A*CCGTCGAGTGCACCGCGTACTACTAATGTCGAAC SEQ
                                                     ID
                                                        NO:
                                                             78
CGCTACGCGCACTA*C*T CTL030_TOP_tag
/5Phos/C*G*CGGACTAAGGTGCGCGAGTAGTGTTACGCGCACT SEQ
                                                        NO:
                                                             79
                                                     ID
ACTAATCTAGCCGC*G*A CTL088 TOP tag
/5Phos/A*C*TAGTGCGACGAACTACTCGCGCTAACCAATTCGA SEQ
                                                        NO:
                                                             80
CGCACCGATCGCTA*G*T CTL148 TOP tag
/5Phos/A*A*TGTCGAACCGCGCGCGCGAGTAGTGTACCATAACCG SEQ
                                                        NO:
                                                             81
CGCACCTTAGTCCG*C*G CTL152_TOP_tag
/5Phos/G*C*GTCGAATTGGTACCGCCGACTTATACCAATACGC SEQ ID NO: 82
```

/SPhos/A*C*CTAGTAGCGCGGCGTTGAATTGGTACTAGCGCGA SEQ         ID         NO:         83           CAACGGTTAGTATG*G*T CTL141_TOP_tag         SPhos/A*C*CGCTCTTACCCGCGCGATTAAGGTACGCCGCTAA SEQ         ID         NO:         84           CTACGGTACGGTCG*G*T CTL064_TOP_tag         /SPhos/A*C*CGCCGACTGTACGTTCGGCTAGGTACACATTCGA SEQ         ID         NO:         85           /SPhos/A*C*CGACGCGACTGCGAGCGTACACCTATTACC SEQ         ID         NO:         86           /SPhos/A*C*CGACGACTGACGCACGCGACTGCGACACTTACCTCTTGACCG SEQ         ID         NO:         87           /SPhos/A*C*CACACTACGCGCGGTTCGACATTACCATAACCG SEQ         ID         NO:         87           /SPhos/A*C*CACACTCACCGCGGGTTCGACACTTACCATAACCG SEQ         ID         NO:         88           /SPhos/A*C*CACACTCTACCGCCGGGTTCGACATTACCATAACCG SEQ         ID         NO:         89           /SPhos/A*C*TACGGAGCGGTGCGGCGTATTGCACCATAACCG SEQ         ID         NO:         90           /SPhos/A*C*TACGCCCGACTACCGCCGACTTATACCTTAATCG SEQ         ID         NO:         91           /SPhos/A*C*CACACACTACGTTACGTAGGTACGCCGCTTAGCCGCAGT SEQ         ID         NO:         92           /SPhos/A*C*CACACACTACGCTACATCATCATAGCTACACTACCATAACCG SEQ         ID         NO:         93           /SPhos/A*C*CACACTACACACACTACTACTACTACCGCACACTACACCACTACCGCGACCACTACACCACCACTACCACCACACCACCACCACCACC	CGCATAGGTAGCGC*G*T CTL007_TOP_tag			
CAACGCCTACTATC*C*TCTIL141_TOP_tag  /FPhos/A*C*CGCTCGTTACCGCCGATTAAGGTACGCCGCTAA SEQ	0	ID	NO:	83
/SPhos/A*C**CGCTCGTTACCGCGCGATTAÄGGTACGCCGCTAA SEQ         ID         NO:         84           CTACGGTACGGTCG*G*T CTL064_TOP_tag         ID         NO:         85           CGCACTGCGAGCGT*A*C CTL158_TOP_tag         ID         NO:         86           GGCACTGCGAGCGT*A*C CTL158_TOP_tag         ID         NO:         86           GGCGACGGCGTCT**T*A CTL066_TOP_tag         SPhos/A*C*GACGACTAGGTACGCTCGTTACCTCTTGACGCG SEQ         ID         NO:         87           CTAACCAATTCGAC*G*C CTL144_TOP_tag         SPhos/A*C*ATACTACGCGCGGGTCGGGCGTATTGGTACCAATACG SEQ         ID         NO:         89           CGCTAGTGCGACAC**C*CTCGCAGTATTGGTACCAATACG SEQ         ID         NO:         89           CGCTAGTGCGACAC**C**CTL149_TOP_tag         SPhos/G*T*ACGCTCGCAGTACCGCCGACTTATACCATAACG         ID         NO:         90           CGCACTAGCGCGAC**A*ACTL008_TOP_tag         SPhos/A*C*GAGCACTAGGTTATGGTACGGCGAGT SEQ         ID         NO:         91           AGTACCTTAGTCCG**C**G**CTL099_TOP_tag         SPhos/A*C*GAGCACTACTAGTTAGGTACGACCTATACCGCGACCTACACCGCGACCTACACCGCACCGCGACCCGACCCGTACA**ACCTACTCGCACCCGACCCG	•			
CTACCGTACGGTCG*C*T CTL064_TOP_tag  SPhos/A*C*CGCCGACTTATCGTTCGGCTAGGTACCAATTCGA SEQ ID NO: 85  GCGCACTGCGAGCGT**AC CTL158_TOP_tag  SPhos/A*C*CTTAATCCGCGACTGCGAGCGTACACCTATTACC SEQ ID NO: 86  GCGCGACGCGCTGT*T*A CTL066_TOP_tag  SPhos/A*C*GACGACTAGGTACCGCTGGTTACCTCTTGACGGG SEQ ID NO: 87  CTAACCAATTCGAC*G*C CTL144_TOP_tag  SPhos/A*C*CATACTACGCGGGGTTCGACATTACCATAACCG SEQ ID NO: 88  GCGTACTGCGACGG*T**A CTL107_TOP_tag  SPhos/C*T*TGACGGGGGTGGGGCGTATTGGTACCAATACGC SEQ ID NO: 89  CGCTCGTCGCACCG*T**A CTL149_TOP_tag  SPhos/C*T*TGACGGGGGTGGGGCGTATTGGTACCAATACG SEQ ID NO: 90  CGCACTAGCGCGACTA*G*T CTL149_TOP_tag  SPhos/A*C*ACACACACAGCTTATACCATAACCG SEQ ID NO: 91  AGTACCTTACGCACCAC*C*CACACTTATACCATAACCG SEQ ID NO: 91  AGTACCTTACGCGCACTA*GGTTATGGTACGCCGTTAGCGCGAGT SEQ ID NO: 92  CGCACTAGCGCGAC**A*A CTL008_TOP_tag  SPhos/A*C*CACGCGTAGTATGGTACGCCGTTAGCGCGAGT SEQ ID NO: 92  CTAACCGATCGCTA*G*T CTL089_TOP_tag  SPhos/A*C*CGACTCGCAATTGGTACGCCGTTATGACCATAACCG SEQ ID NO: 93  GCCACCGCCGTACA**ACTCTDO*D_tag  SPhos/A*C*CGACCAATCGGTCGAATTGGTACCATAACCG SEQ ID NO: 94  GCGACCACGCCGTACA**ACTACTGTCGCAACTATTACC SEQ ID NO: 95  CGCACCGCCGTACA**ACTACTGTCGGAACTAATTACC SEQ ID NO: 95  CCGTTCGGCCACACACAAGTCGCGACAATGTATACCGCTGT SEQ ID NO: 96  GTATAGGTACGCC**C**CTL13*_TOP_tag  SPhos/A*C*CGCGTACAACTAGTTAGCGCGTAGTAGTACGCGCGACCGGGGGGGG	- $ 0$	ID	NO:	84
SPhos/A+C*CGCGACTITATCGTTCGGCTAGGTACCAATTCGA SEQ   ID   NO: 85				
CGCACTGCGAGCGT*A*C CTL158_TOP_tag /SPhos/A*C*CTTAATCCGCGACTGCAGCGTACACCTATTACC SEQ ID NO: 86 GGGCGACCGCGCTG*T*A CTL066_TOP_tag /SPhos/A*C*GACGACTAGGTACCGCTGTTACCTCTTGACGCG SEQ ID NO: 87 CTAACCAATTCGAC*G*C CTL144_TOP_tag /SPhos/A*C*CATACACGCGGCGGTTCGACATTACCATAACCG SEQ ID NO: 88 CGCTAGTGCGAGCG*T*A CTL107_TOP_tag /SPhos/C*T*TGTACGCGGGGGTGCGGGCGTATTGCTACACAATACCG SEQ ID NO: 89 CGCTCGTCGCACTA*C*T*CTL149_TOP_tag /SPhos/C*T*ACGCGGGGCGTATTGGTACCAATACG SEQ ID NO: 91 CGCACTAGCGCGACT*A*CTL149_TOP_tag /SPhos/A*C*GACCGACT*A*CTL008_TOP_tag /SPhos/A*C*GACCGACT*A*CTL008_TOP_tag /SPhos/A*C*GACCGAC*A*A*CTL008_TOP_tag /SPhos/A*C*GACCGAC*A*A*CTL008_TOP_tag /SPhos/A*C*GACCGAC*CACTTATGCTACCGCGACTACGCGCGACTAGCGCGACT*AGGTAGGAGGAGT SEQ ID NO: 92 CTAACCGATCGGTA*G*T*CTL089_TOP_tag /SPhos/A*C*CGACCGAC*GATTAGGTACGACGATTGGTACCATAACCG SEQ ID NO: 93 CGCACCGCCGTACA*A*G*CTL081_TOP_tag /SPhos/A*C*CGATCGCATAGGTCGAATTGGTACCATAACCG SEQ ID NO: 95 CGCACCACCGCCGTACA*A*G*CTL081_TOP_tag /SPhos/A*C*CGACCGAC*G*A*CTL075_TOP_tag /SPhos/A*C*CGACCGAC*G*A*CTL075_TOP_tag /SPhos/A*C*CGACCGAC*G*C*CAATTAGCTGCGAACATACCGCCGACCGCGAACCGACCGA	· · · · · · · · · · · · · · · · · · ·	ID	NO:	85
SPhos/A*C*CTTAATCCGCGACTGCGAGCĞTACACCTATTACC SEQ   ID   NO: 86	•		1.0.	
GCGCGACGCGTGT*T*A CTL066_TOP_tag /SPhos/A*C*GACGACTAGGTACCGCTCGTTACCTCTTGACGC SEQ ID NO: 87 CTAACCAATTCGAC*G*C*C*C*L144_TOP_tag /SPhos/A*C*CATACTACGCGGCGGTTCGACATTACCATAACCG SEQ ID NO: 88 CGCTAGTGCGAGCC*T*A CTL107_TOP_tag /SPhos/C*T*TGTACGCGGGTGCGGCGTATTGGTACCAATACGC SEQ ID NO: 89 CGCTCGTCGCACTA*C*T*CTL149_TOP_tag /SPhos/C*T*ACGCTGCAGTACGCCGCACTTATACCTTAATCG SEQ ID NO: 90 CGCACTAGCGCACTA*C*T*CTL149_TOP_tag /SPhos/C*T*ACGCTGCAGTACCGCCGACTTATACCTTAATCG SEQ ID NO: 91 AGTACCTTAGCGCGACA*A CTL008_TOP_tag /SPhos/A*C*GACGACTAGGTTATGGTACGACGCGAGT SEQ ID NO: 92 AGTACCTTAGTCCG*C*G*C*G*CTL099_TOP_tag /SPhos/A*C*GACGACTAGGTTATGGTACGGCGTTCTTGACGC SEQ ID NO: 92 CTAACCCGATCGCTA*G*T*CTL089_TOP_tag /SPhos/A*C*GACGACA*A*CTL078_TOP_tag /SPhos/A*C*CGATCCGCAATGCGTCGAATTGGTACCATAACCG SEQ ID NO: 93 CGCACCGCCGTACA*A*G*CTL081_TOP_tag /SPhos/A*C*CAATCGACGAACTACTGTCGCGAACCTATTACC SEQ ID NO: 94 CGCGACCGCGTACA*A*G*CTL078_TOP_tag /SPhos/A*C*GCGCTACAAGTCGCGACACTACTACGCTACCGC SEQ ID NO: 95 CCGTTCGGCGCTACAAGTCGCGACACTACTACGTACCGC SEQ ID NO: 95 CCGTTCGGCGCTACAAGTCGCAATACTGCGAACCTACTACGC SEQ ID NO: 95 CCGTTCGGCCGTACAAGTCGCAATTACCGGAACCTACTACTCG SEQ ID NO: 96 GGTATAGGTAGCGC*G*T*CTL133_TOP_tag /SPhos/A*C*CAATTCGACGCTAGTTAGCGGCCTACAACTACTCG SEQ ID NO: 97 CCGCGCACTCGACGG*T*T*CTL160_TOP_tag /SPhos/A*C*CAATTCGACGCTAGTTAGCGGCCTACACTACTCG SEQ ID NO: 98 GTCACACTCGACGG*T*T*CTL05_TOP_tag /SPhos/T*C*GCGCATATAGGTACGAGCGTAATAGGTACGACGGTA SEQ ID NO: 99 GTAACCAATCGACGC*G*A*CTL045_TOP_tag /SPhos/T*C*GGCGATATAGGTACGAACTATTCGAACCGCGCGCGCGCGC	<u> </u>	ID	NO.	86
/SPhos/A*C*GACGACTAGGTACCGCTCGTTACCTCTTGACGCG SEQ ID NO: 87 CTAACCAATTCGAC*G*C CTL144_TOP_tag SPhos/A*C*CATACTACGCGGCGGTTCGACATTACCATAACCG SEQ ID NO: 88 CGCTAGTGCGAGCG*T*A CTL107_TOP_tag /SPhos/C*T*TGTACGGCGGGTGCGGCGTATTGGTACCAATACCG SEQ ID NO: 89 CGCTCGTCGCACTA*G*T CTL149_TOP_tag /SPhos/C*T**GACGGCGGTGCGGCGTATTGGTACCAATACGC SEQ ID NO: 90 CGCACTAGCGCGACTA*CCGCCGACTTATACCTTAATCG SEQ ID NO: 91 ACTACCTTAGTCCGACTA*CTL149_TOP_tag /SPhos/A*C*GACGACTAGGTTATGGTACGGCGGAGT SEQ ID NO: 91 ACTACCTTAGTCCG*C*G CTL099_TOP_tag /SPhos/A*C*GACGACTAGGTTATGGTACGGCGTTACTGACGCG SEQ ID NO: 92 CTAACCGATCGCTA*C*T CTL089_TOP_tag /SPhos/A*C*GACGCGAATTGGTACGAATTGGTACCATAACCG SEQ ID NO: 93 CGCACCACGCCGCAATCGCTCGAATTGGTACCATAACCG SEQ ID NO: 94 CGCACCACTACGCTAC*A*C*T CTL089_TOP_tag /SPhos/A*C*TAGTGCGAATTGGTACCATAACCG SEQ ID NO: 94 CGCACCAATCGACAA*CG CTL091_TOP_tag /SPhos/A*C*GACCGCAACTCGCGAATCGGTAGTACATAACCG SEQ ID NO: 95 CCGTTCGGCGCTAC*A*A*C CTL081_TOP_tag /SPhos/A*C*CGCTCGAATCGCGACAGTAGTAGTACCGCCGCCCGT SEQ /SPhos/A*C*CGCTCGACAGTCGCGAATTACTCGGTAGTAGTACCGCCCCCGTT SEQ /SPhos/A*C*CGCTCGCATTAGTCGGAATTGGTACCATAACCG SEQ /SPhos/A*C*CAGTTCGACGATTAGTCGGTAGTAGTACCGCCCCCCCCCC	•	12	1,0,	
CTAACCAATTCGAC*G*C CTL144_TOP_tag /5Phos/A*C*CATACTACGCGCGCGTTCGACATTACCATAACCG SEQ ID NO: 88 CGCTAGTGCGAGCG*T*A CTL107_TOP_tag /5Phos/C*T*TGTACGGCGGTGCGGCGTATTGGTACCAATACGC SEQ ID NO: 89 CGCTCGTCGCACTA*C*T CTL149_TOP_tag /5Phos/G*T*ACGCTCGCAGTACCGCCGACTTATACCTTAATCG SEQ ID NO: 90 CGCACTAGCGCGAC*A*A CTL008_TOP_tag /5Phos/A*C*GACGACTAGGTTATGGTACCGCCGAGT SEQ ID NO: 91 AGTACCTTAGTCCC*C*G CTL099_TOP_tag /5Phos/A*C*GACGGCGTATTCATAGCTGTACCGCGAGT SEQ ID NO: 92 CTAACCGATCGCCGAT*G*T CTL089_TOP_tag /5Phos/A*C*CAGACGGTATCATAGGTACGCGCGTTCTTGACGCG SEQ ID NO: 93 CGCACCGCACTAGCTAGCTAGCTAGCTACGTACCATAACCG SEQ ID NO: 93 CGCACCGCACTACA*A*G CTL081_TOP_tag /5Phos/A*C*CAGTCCGCAATGCGTGGAATTGGTACCATAACCG SEQ ID NO: 94 GCGACCACGCCGTACA*A*G CTL081_TOP_tag /5Phos/A*C*CAGCGTACA*A*GTACTGCGCGAACCTATACC SEQ ID NO: 95 CCGTTCGGCGCTAGC*C*T CTL160_TOP_tag /5Phos/A*C*CAGCGTACA*G*T CTL160_TOP_tag /5Phos/A*C*CAGCGCGTACACTAGCTAGCGCGAACCTATACCG SEQ ID NO: 96 GGTATAGGTAGCG*G*T CTL133_TOP_tag /5Phos/A*C*CAATTCGACGTAGTTAGCGGCGTACACTACTCG SEQ ID NO: 97 CGGGCACTCGACGG*T*T CTL133_TOP_tag /5Phos/A*C*CAATTCGACGGT*TTAGCGGCGTACACTACTCG SEQ ID NO: 98 GTCACACTACTCGC*G*C*CTL024_TOP_tag /5Phos/C*G*CGGTAATAGGTCGCGGTAATAGGTACGC SEQ ID NO: 99 GTAACCAATCGACG*G*A CTL091_TOP_tag /5Phos/T*C*CGGCGAGTAGTTAGTGGGCGTAAGTAGTGCGC SEQ ID NO: 101 CGCACTACTGCGCG*G*A CTL091_TOP_tag /5Phos/C*T*CGGGCAGTGTAGCGGCGTAAGTAGTGCGC SEQ ID NO: 102 CGCACTAGTGCGCG*G*CTTAGCGGCGTAAGTAGTGCGC SEQ ID NO: 103 CGCACTAGTGCGCG*G*CTTAGCGGCGTAAGTAGTGCGC SEQ ID NO: 104 CGCACTAGTGCGAC*G*A CTL091_TOP_tag /5Phos/A*TGCGCGCGATTAAGGTGCGCGATAAGTGCGCACCAATTCGA SEQ ID NO: 103 CGCACTAGTGCGCG*C*C*CTL091_TOP_tag /5Phos/A*TGCGCGCGATTAAGGTGCGCGACCAATTCGA SEQ ID NO: 104 CGCAGTAGTACGCG*C*C*CTL1051_TOP_tag /5Phos/A*TGCGCGCGATTAAGGTGCGCGACCAATTCGA SEQ ID NO: 105 CGCAGTAGTACGCGCG*C*C*CTL135_TOP_tag /5Phos/C*G*CGGATTAAGGTCTTGACGCGGGTTACCATAGCGCACCAATTCGA SEQ ID NO: 104 ACGTACCGCACTA*C*C*C*C*C*CTL135_TOP_tag /5Phos/C*G*CGGATTAAGGTCTTGACGGCGGTACCTAGCCGA SEQ ID NO: 105 AATGCACTCGACGG*C*C*CTL108_TOP_tag /5Phos/C*G*CGGATTAAGGTCTT	- $ 0$	ID	NO:	87
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/SPhos/A*C*GACGACTAGGTTATGGTACGGCGTTAGCGCGAGT SEQ ID NO: 91 AGTACCTTAGTCCG*C*G CTL099_TOP_tag /SPhos/A*C*GAGCGGTAGTCATAGGTAGCGCGTTCTTGACGCG SEQ ID NO: 92 CTAACCGATCGCTA*G*T CTL089_TOP_tag /SPhos/A*C*CGATCGCAATGCGTCGAATTGGTACCATAACCG SEQ ID NO: 93 CGCACCGCCGTACA*A*G CTL081_TOP_tag /SPhos/A*C*TAGTGCGACGAACTACTGTCGCGAACCTATTACC SEQ ID NO: 94 GCGACCAATTCGACG*A*CTL075_TOP_tag /SPhos/A*C*CGCGTACA*G*CTL075_TOP_tag /SPhos/A*C*CGCCGTACA*G*CTCGCGAACTTAGTACCGCTCGT SEQ ID NO: 95 CCGTTCGGCGCTAG*G*T CTL160_TOP_tag /SPhos/T*C*GTCGCAACTCGCATTAGTCGCTAGTAGTACCGCSEQ ID NO: 96 GGTATAGGTAGCGCG*G*T*CTL133_TOP_tag /SPhos/A*C*CAATTCGACGTAGTAGCGGCGTACACTACTCG SEQ ID NO: 97 CGCGCACTCGACGG*T*T CTL076_TOP_tag /SPhos/C*G*CGGTAATAGGTGCGGTAACACTACTCG SEQ ID NO: 98 GTCACACTACTCGC*G*C CTL024_TOP_tag /SPhos/C*G*CGGTAATAGGTCGCGTAACTAGTACGGCGTA SEQ ID NO: 99 GTAACCAATCGACG*G*A CTL004_TOP_tag /SPhos/T*C*GGCGAGTAGTTAGTGCGAGCGTAACTAGTGCGC SEQ ID NO: 100 CGCACTAGTCGACG*A*CTL009_TOP_tag /SPhos/G*T*CGGCCACTGACGCGGTATAGGTACCATAACCG SEQ ID NO: 101 CGCACTAGTCGCACG*A*CTL009_TOP_tag /SPhos/A**TAGCGCGCACTAGCGCGGTTATAGTCGAAC SEQ ID NO: 102 CGCACTAGTCGACG*G*T*CTL010_TOP_tag /SPhos/A**TAGCGCGACAACGACTATGTGCGCACCAATTCGA SEQ ID NO: 103 CGCAACTAATCCGC*G*C*C*CTL1135_TOP_tag /SPhos/A*C*TAGCGCGACTACTGTACGGCGGTACCAATTCGA SEQ ID NO: 103 CGCAACTAATCCGC*G*C*C*C*C*CTL135_TOP_tag /SPhos/A*C*TAGCGCGACTACGGCGGTACCAATTCGA SEQ ID NO: 103 CGCAACTAATCCGC*G*C*C*C*C*C*C*C*C*C*C*C*C*C*C*C*C*		עו	NO:	90
AGTACCTTAGTCCG*C*G CTL099_TOP_tag /5Phos/A*C*GAGCGGTAGTCATAGGTAGCACGCGTTCTTGACGCG SEQ	5	ID	NO.	01
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SPhos/A*C*TAGTGCGACGAACTACTGTCGCGAACCTATTACC SEQ   ID   NO: 94	•	ID	NO:	93
SPhos/A*C*CGCGTACAAGTCGCGACAGTAGTAACCGCTCGT SEQ   ID   NO:   95   NO:   96   NO:   97   NO:   96   NO:   97   NO:   97   NO:   97   NO:   97   NO:   98   NO:   99	9	ID	NIO	0.4
No.   Sphos/A*C*CGCCGTACAAGTCGCGACAGTAGTAACCGCTCGT SEQ   No.   Specific control of the property of the prope	•	ID	NO:	94
CCGTTCGGCGCTAG*G*T CTL160_TOP_tag  /SPhos/T*C*GTCGCACTAGTCGCATTAGTCGGTAGTAGTACGC SEQ ID NO: 96 GGTATAGGTAGCGC*G*T CTL133_TOP_tag  /SPhos/A*C*CAATTCGACGCTAGTTAGCGGCGTACACTACTCG SEQ ID NO: 97 CGCGCACTCGACGG*T*T CTL076_TOP_tag  /SPhos/C*G*CGGTAATAGGTCGCGGTAATAGGTACGAGCGGTA SEQ ID NO: 98 GTCACACTACTCGC*G*C CTL024_TOP_tag  /SPhos/T*C*GGCGAGTAGTTAGTGCGAGCGTAAGTAGTGCGC SEQ ID NO: 99 GTAACCAATCGACG*C*G*A CTL045_TOP_tag  /SPhos/G*T*CGCGCAGTGTAGCGGGGTAATAGGTACCATAACCG SEQ ID NO: 100 CGCACTAGTGCGAC*G*A CTL045_TOP_tag  /SPhos/T*A*TGCGCTCGACTGCGGGATTAAGGTAATGTCGAAC SEQ ID NO: 101 CGCAGTAGTACGCG*G*T CTL055_TOP_tag  /SPhos/A*C*TAGCGCGACTACTGCGCGATTAAGGTAATGTCGAAC SEQ ID NO: 102 CGCACTAGCGCACTA*C*T CTL101_TOP_tag  /SPhos/A*A*CTACTCGCCGACTTGTACGGCGGTACCAATTCGA SEQ ID NO: 103 CGCAACTAATCCGC*G*C*CTL135_TOP_tag  /SPhos/C*G*CGGATTAAGGTCTTGTACGGCGGTACCAATTCGA SEQ ID NO: 104 ACGTACGCGCACTA*C*T CTL155_TOP_tag  /SPhos/C*G*CGGATTAAGGTCTTGTACGGCGGTACCTAGCCGA SEQ ID NO: 104 ACGTACGCGCACTA*C*T CTL155_TOP_tag  /SPhos/T*A*GCGCGTCAAGACTTGTACGGCGGTACCTAGCCGA SEQ ID NO: 105 AATGCACTCGACG*T*CTL122_TOP_tag  /SPhos/C*G*CATTAGTCGGTGCGGCGTACCGATCCGC SEQ ID NO: 106 GGTACCAATACCCC*G*C*CTL080_TOP_tag	9			0=
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SPhos/A*C*CAATTCGACGCTAGTTAGCGGCGTACACTACTCG SEQ   ID   NO: 97   NO: 108	·	ID	NO:	96
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                                                        NO:
                                                            156
CGCACGCCGCTAAC*T*A CTL018_TOP_tag
/5Phos/A*C*CGACTAATGCGTAACAGCGCGTCGTTAGTGCGCG SEQ
                                                    ID
                                                        NO:
                                                             157
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AGAACCTTAATCGC*G*C CTL115_TOP_tag			
/5Phos/A*C*GCCGTACCATAACCGACTAATGCGATAAGTCGGC SEQ	ID	NO:	158
GGTACCAATACGCC*G*C CTL033_TOP_tag			
/5Phos/G*T*ACGCTCGCAGTCGCGGTAATAGGTTCGGCGAGTA SEQ	ID	NO:	159
GTTACCATAACCGC*G*C CTL047_TOP_tag			
/5Phos/C*G*GACGAGCGGTTGCGCGGTTATGGTACTAGTGCGA SEQ	ID	NO:	160
CGAGCGCACATAGT*C*G CTL108_TOP_tag			
/5Phos/A*C*TACTCGCGCTAGCGCGATTAAGGTACGCCGCTAA SEQ	ID	NO:	161
CTATCGCGGCTAGA*T*T CTL041_TOP_tag			
/5Phos/A*C*CAATTCGACGCAACTAATCCGCGCACCAATTCGA SEQ	ID	NO:	162
CGCAGTAGTGCGCG*T*A CTL061_TOP_tag			-
/5Phos/A*C*CGCCGCTATACACCTAGCGCCGAAGTACGCTCGC SEQ	ID	NO:	163
AGTGTATAGCGGCG*G*T CTL166_TOP_tag			
/5Phos/A*C*ACTACTCGCGCCGGACGAGCGGTTACCAATACGC SEQ	ID	NO:	164
CGCTAGCGCGAGTA*G*T CTL012_TOP_tag		1.0,	10.
/5Phos/T*C*GTCGCACTAGTACCTTAATCCGCGCGCAACGCTA SEQ	ID	NO:	165
GGTACACTACTCGC*G*C CTL052_TOP_tag	12	1.0.	100
/5Phos/C*G*CGCTACTAGGTACCGACTAATGCGCGCAACGCTA SEQ	ID	NO:	166
GGTAATGTCGAACC*G*C CTL153_TOP_tag	12	110.	100
/5Phos/A*C*GAGCGGTAGTCACTACTGTCGCGACGCAACGCTA SEQ	ID	NO:	167
GGTTACACTGCGCG*A*C CTL094_TOP_tag	110	110.	107
/5Phos/A*C*CTAGTCGCGTACGCGTAGTATGGTACCGATCGCT SEQ	ID	NO:	168
AGTGGTAACGAGCG*G*T CTL095_TOP_tag	110	110.	100
/5Phos/G*C*GGTTCGACATTACCGACTAATGCGTATGCGCTCG SEQ	ID	NO:	169
ACTACCTAGCGTTG*C*G CTL105_TOP_tag	נו	110.	105
/5Phos/A*C*TGCGAGCGTACTCTCGCGCACTAAACGCCGCTAA SEQ	ID	NO:	170
CTACGCGCTACTAG*G*T CTL109_TOP_tag	10	110.	170
/5Phos/C*G*GTACGGTCGGTAATCTAGCCGCGAACCTTAGTCC SEQ	ID	NO:	171
GCGACCGCCGTACA*A*G CTL032_TOP_tag	ID	110.	1/1
/5Phos/T*C*GGCGAGTAGTTACGCGCTACCTATTCGCGGCTAG SEQ	ID	NO:	172
ATTACGCCGCTAAC*T*A CTL161_BOT_tag	ID	110.	1,2
/5Phos/A*C*GCCGCTAACTAGCGCGATTAAGGTGTACGCTCGC SEQ	ID	NO:	173
AGTGTCGCGCAGTG*T*A CTL164_BOT_tag	יוו	110.	1/5
/5Phos/A*G*TAGTGCGCGTAGCGGTTCGACATTAGTAGTACGC SEQ	ID	NO:	174
GGTGCACTCGACGG*T*T CTL030_BOT_tag	ינו	110.	1/ 4
/5Phos/T*C*GCGGCTAGATTAGTAGTGCGCGTAACACTACTCG SEQ	ID	NO:	175
CGCACCTTAGTCCG*C*G CTL088_BOT_tag	מו	110.	175
/5Phos/A*C*TAGCGATCGGTGCGTCGAATTGGTTAGCGCGAGT SEQ	ID	NO:	176
AGTTCGTCGCACTA*G*T CTL148_BOT_tag	עו	110.	170
/5Phos/C*G*CGGACTAAGGTGCGCGGTTATGGTACACTACTCG SEQ	ID	NΟ·	177
CGCGCGGTTCGACA*T*T CTL152_BOT_tag	עו	110.	1//
/5Phos/A*C*GCGCTACCTATGCGGCGTATTGGTATAAGTCGGC SEQ	ID	NO:	170
GGTACCAATTCGAC*G*C CTL007_BOT_tag	ID	110.	170
/5Phos/A*C*CATACTACGCGTTGTCGCGCTAGTACCAATTCGA SEQ	ID	NO:	179
•	11)		1/3
CCCCCCCTACTAC*C*TCTI141ROTtog	ID	1,0,	
CGCCGCGCTACTAG*G*T CTL141_BOT_tag /5Phos/A*C*CGACCGTACCGTAGTTAGCGGCGTACCTTAATCG SEO			180
/5Phos/A*C*CGACCGTACCGTAGTTAGCGGCGTACCTTAATCG SEQ			180
/5Phos/A*C*CGACCGTACCGTAGTTAGCGGCGTACCTTAATCG SEQ CGCGGTAACGAGCG*G*T CTL064_BOT_tag	ID	NO:	
/5Phos/A*C*CGACCGTACCGTAGTTAGCGGCGTACCTTAATCG SEQ CGCGGTAACGAGCG*G*T CTL064_BOT_tag /5Phos/G*T*ACGCTCGCAGTGCGTCGAATTGGTACCTAGCCGA SEQ	ID		180 181
/5Phos/A*C*CGACCGTACCGTAGTTAGCGGCGTACCTTAATCG SEQ CGCGGTAACGAGCG*G*T CTL064_BOT_tag	ID ID	NO:	

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/5Phos/G*C*GTCGAATTGGTTAGCGCGTCAAGAGGTAACGAGC SEQ ID
GGTACCTAGTCGTC*G*T CTL144 BOT tag
/5Phos/T*A*CGCTCGCACTAGCGCGGTTATGGTAATGTCGAAC SEQ ID
                                                       NO:
                                                            184
CGCCGCGTAGTATG*G*T CTL107_BOT_tag
/5Phos/A*C*TAGTGCGACGAGCGGCGTATTGGTACCAATACGC SEQ
                                                        NO:
                                                             185
CGCACCGCCGTACA*A*G CTL149 BOT tag
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                                                       NO:
                                                            186
GGTACTGCGAGCGT*A*C CTL008 BOT tag
/5Phos/C*G*CGGACTAAGGTACTACTCGCGCTAACGCCGTACC SEQ
                                                        NO:
                                                             187
ATAACCTAGTCGTC*G*T CTL099 BOT tag
/5Phos/A*C*TAGCGATCGGTTAGCGCGTCAAGAACGCGCTACC SEQ
                                                             188
TATGACTACCGCTC*G*T CTL089 BOT tag
/5Phos/C*T*TGTACGGCGGTGCGCGGTTATGGTACCAATTCGA SEQ ID
                                                            189
CGCATTGCGGATCG*G*T CTL081 BOT tag
/5Phos/T*C*GCTCGATTGGTCGCGGTAATAGGTTCGCGACAGT SEQ ID
                                                            190
AGTTCGTCGCACTA*G*T CTL075 BOT tag
/5Phos/A*C*CTAGCGCCGAACGGACGAGCGGTTACTACTGTCG SEQ ID NO: 191
CGACTTGTACGGCG*G*T CTL160_BOT_tag
/5Phos/A*C*GCGCTACCTATACCGCGTACTACTACCGACTAAT SEQ ID NO: 192
GCGACTAGTGCGAC*G*A CTL133_BOT_tag
/5Phos/A*A*CCGTCGAGTGCGCGCGAGTAGTGTACGCCGCTAA SEQ ID NO: 193
CTAGCGTCGAATTG*G*T CTL076 BOT tag
/5Phos/G*C*GCGAGTAGTGTGACTACCGCTCGTACCTATTACC SEQ ID
                                                            194
GCGACCTATTACCG*C*G CTL024 BOT tag
/5Phos/T*C*GCTCGATTGGTTACGCGCACTACTTACGCTCGCA SEQ ID
                                                            195
CTAAACTACTCGCC*G*A CTL045_BOT_tag
/5Phos/T*C*GTCGCACTAGTGCGCGGTTATGGTACCATAACCG SEQ
                                                       NO:
                                                            196
CGCTACACTGCGCG*A*C CTL009 BOT tag
/5Phos/A*C*CGCGTACTACTGCGGTTCGACATTACCTTAATCG SEQ ID
                                                       NO:
                                                            197
CGCAGTCGAGCGCA*T*A CTL055_BOT_tag
/5Phos/A*G*TAGTGCGCGTAGCGTCGAATTGGTGCGCACATAG SEQ
                                                        NO:
                                                             198
TCGTTGTCGCGCTA*G*T CTL101 BOT tag
/5Phos/G*C*GCGGATTAGTTGCGTCGAATTGGTACCGCCGTAC SEQ
                                                        NO:
                                                            199
AAGTCGGCGAGTAG*T*T CTL135_BOT_tag
/5Phos/A*G*TAGTGCGCGTACGTTCGGCTAGGTACCGCCGTAC SEQ
                                                        NO:
                                                            200
AAGACCTTAATCCG*C*G CTL155 BOT tag
/5Phos/A*A*CCGTCGAGTGCATTGCGGATCGGTACCGCCGTAC SEQ
                                                    ID
                                                        NO:
                                                             201
AAGTCTTGACGCGC*T*A CTL122 BOT tag
/5Phos/G*C*GGCGTATTGGTACCTAGTCGTCGTACCAATACGC SEQ
                                                       NO:
                                                            202
CGCACCGACTAATG*C*G CTL080_BOT_tag
/5Phos/A*C*CGATCGCTAGTCGCATTAGTCGGTACCATAACCG SEQ
                                                    ID
                                                       NO:
                                                            203
CGCCGCGCTACTAG*G*T CTL126_BOT_tag
/5Phos/T*A*GTGCGAGCGTATCGCGGCTAGATTACGACGACTA SEQ
                                                        NO:
                                                            204
GGTTAGCGCGAGTA*G*T CTL098 BOT tag
/5Phos/A*C*CTTAGTCCGCGACTGCGAGCGTACACCTTAATCG SEQ
                                                        NO:
                                                            205
CGCGTATAGCGGCG*G*T CTL038 BOT tag
/5Phos/A*C*TAGCGATCGGTACTGCGAGCGTACGCACTCGACG SEQ
                                                             206
                                                        NO:
GTTAGTAGTGCGCG*T*A CTL139_BOT_tag
/5Phos/A*C*CTAGTCGTCGTTCTCGCGCACTAACGACGCGCTG SEQ
                                                             207
                                                    ID
                                                        NO:
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TTATACACTGCGCG*A*C CTL010_BOT_tag			
/5Phos/G*C*GGCGTATTGGTGTATAGCGGCGGTACCATACTAC SEQ	ID	NO:	208
GCGACCAATTCGAC*G*C CTL034_BOT_tag	110	1,0.	200
/5Phos/T*A*ACAGCGCGTCGACTAGCGATCGGTACCTAGTCGC SEQ	ID	NO:	209
GTAAGTAGTGCGCG*T*A CTL117_BOT_tag	12	1.0.	_00
/5Phos/G*C*GCGGATTAGTTGCGTCGAATTGGTACGCCGCTAA SEQ	ID	NO:	210
CTATAGTTAGCGGC*G*T CTL035_BOT_tag	12	1.0.	
/5Phos/A*T*TGCGGATCGGTAGTAGTGCGCGTAACGCCGCTAA SEQ	ID	NO:	211
CTAACCTTAGTCCG*C*G CTL121_BOT_tag		1.00	
/5Phos/A*C*GCGCTACCTATTAGTTAGCGGCGTATAAGTCGGC SEQ	ID	NO:	212
GGTACCTAGTCGTC*G*T CTL106_BOT_tag	12	1.0.	
/5Phos/G*T*CGCGCAGTGTAACCGCGTACTACACTACTCG SEQ	ID	NO:	213
CGCAACCGTCGATC*C*G CTL059_BOT_tag	12	1.0.	_10
/5Phos/A*C*CAATCGAGCGAATTGCGGATCGGTATAAGTCGGC SEQ	ID	NO:	214
GGTACCGATCCGCA*A*T CTL157_BOT_tag	12	110.	
/5Phos/G*G*TAACGAGCGGTGCGCGATTAAGGTGTACGCTCGC SEQ	ID	NO.	215
AGTGTACGCTCGCA*G*T CTL015_BOT_tag	12	110.	215
/5Phos/A*C*CGATCCGCAATTAGTGCGAGCGTAACTAGTGCGA SEQ	ID	NO:	216
CGATCGCGACAGTA*G*T CTL110_BOT_tag	10	110.	210
/5Phos/C*G*CGTAGTATGGTTCTCGCGCACTAATTAGTGCGCG SEQ	ID	NO:	217
AGAACCGCTCGTTA*C*C CTL123_BOT_tag	10	110.	217
/5Phos/T*C*GGCGAGTAGTTGCGCGATTAAGGTACCTTAATCG SEQ	ID	NO:	218
CGCTAGCGCGAGTA*G*T CTL014_BOT_tag	ינו	110.	210
/5Phos/G*C*ACTCGACGGTTGCGTCGAATTGGTACCGCCGTAC SEQ	ID	NO:	219
AAGAGTAGTGCGCG*T*A CTL131_BOT_tag	יוו	110.	213
/5Phos/T*C*GTCGCACTAGTGCGCGATTAAGGTACCGATCCGC SEQ	ID	NO:	220
AATCGGATCGACGG*T*T CTL062_BOT_tag	יוו	110.	220
/5Phos/C*G*CGGATTAAGGTGCGCGAGTAGTGTCGCGCAGT SEQ	ID	NO:	221
GTATACGCGCACTA*C*T CTL044_BOT_tag	ינו	110.	221
/5Phos/A*C*CTAGCGCCGAATACGCGCACTACTACCTATTACC SEQ	ID	NO·	222
GCGTATGGTACGGC*G*T CTL043_BOT_tag	ונו	110.	<b></b>
/5Phos/A*C*CGCGTACTACTACCGCCGACTTATCGCAACGCTA SEQ	ID	NΟ·	223
GGTTCTTGACGCGC*T*A CTL118_BOT_tag	110	110.	225
/5Phos/A*C*TAGCGATCGGTGCGCGGTTATGGTTCGCGGCTAG SEQ	ID	NO:	224
ATTACCGACTAATG*C*G CTL128_BOT_tag	ינו	110.	227
/5Phos/A*C*CGCCGACTTATTAGTTAGCGGCGTACCAATACGC SEQ	ID	NO:	225
CGCACGCCGTACCA*T*A CTL067_BOT_tag	110	110.	225
/5Phos/C*G*GACGAGCGGTTGACTACCGCTCGTACCAATACGC SEQ	ID	NO:	226
CGCACCATAACCGC*G*C CTL020_BOT_tag	מו	110.	220
/5Phos/A*G*TCGAGCGCATAGCGCGGTTATGGTTCGGCGAGTA SEQ	ID	NO:	227
GTTGCGCACATAGT*C*G CTL006_BOT_tag	ינו	110.	221
/5Phos/C*G*CGTAGTATGGTGCGCGATTAAGGTGGTAACGAGC SEQ	ID	NΟ·	228
GGTACGCCGCTAAC*T*A CTL017_BOT_tag	עוו	110.	220
/5Phos/T*C*TCGCGCACTAACGGACGAGCGGTTTACGCGCACT SEQ	ID	NO:	229
ACTACCGACTAATG*C*G CTL057_BOT_tag	110	110.	223
/5Phos/A*C*GAGCGGTAGTCTTAGTGCGCGAGACGCATTAGTC SEQ	ID	NO:	230
GGTACTACTCGCGC*T*A CTL078_BOT_tag	ענ	110.	200
/5Phos/G*C*GCGGTTATGGTGTATAGCGGCGGTACCAATCGAG SEQ	ID	NΟ·	231
CGATAGTGCGAGCG*T*A CTL031_BOT_tag	ינו	110.	<b>-</b> 01
/5Phos/T*A*GTTAGCGCGTATAGGTAGCGCGTTATGCGCTCG SEQ	ID	NΟ·	232
PER POLITION I TI OTTROCOCOCITITIOON TO THE FINANCIAL PROPERTY OF THE PROPERTY	ענ	110.	202

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ACTTCGCTCGATTG*G*T CTL136_BOT_tag
/5Phos/T*C*GCGACAGTAGTCGCATTAGTCGGTGTACGCTCGC SEQ ID
                                                            233
AGTCGCGGATTAAG*G*T CTL165 BOT tag
/5Phos/A*C*CGCCGCTATACTAGCGCGTCAAGAACCAATCGAG SEQ
                                                            234
                                                   ID
                                                       NO:
CGATACGCGCACTA*C*T CTL039 BOT tag
/5Phos/A*C*GCCGTACCATACGACTATGTGCGCACCGACCGTA SEQ ID
                                                       NO:
                                                            235
CCGACTAGTGCGAC*G*A CTL036 BOT tag
/5Phos/A*C*CTAGTCGTCGTAGTAGTACGCGGTTATGCGCTCG SEQ ID
                                                       NO:
                                                            236
ACTACCTTAATCCG*C*G CTL048 BOT tag
/5Phos/T*T*CGGCGCTAGGTGCGCGAGTAGTGTTAGTGCGAGC SEQ ID
                                                            237
GTAGCGCACATAGT*C*G CTL053_BOT_tag
/5Phos/C*G*GATCGACGGTTACTAGTGCGACGATTAGTGCGCG SEQ ID
                                                            238
AGAATAAGTCGGCG*G*T CTL072_BOT_tag
/5Phos/A*C*GCCGTACCATAACCGCTCGTTACCCGCATTAGTC SEQ ID
                                                           239
GGTCGCAACGCTAG*G*T CTL096 BOT tag
/5Phos/T*A*CGCGACTAGGTCGCAACGCTAGGTACCTATTACC SEQ ID
                                                            240
GCGACCTAGTAGCG*C*G CTL150 BOT tag
/5Phos/A*C*TACTGTCGCGAACCGACTAATGCGTAGCGCGAGT SEQ ID NO: 241
AGTACCTAGCCGAA*C*G CTL084_BOT_tag
/5Phos/A*C*GCCGCTAACTAACCTAGTCGTCGTACCTATTACC SEQ ID NO:
GCGAACCGCTCGTC*C*G CTL142 BOT tag
/5Phos/A*G*TAGTACGCGGTCGCATTAGTCGGTACCGATCCGC SEQ ID
AATTAGTGCGAGCG*T*A CTL102 BOT tag
/5Phos/T*T*CGGCGCTAGGTTAGCGCGTCAAGAACGCCGTACC SEQ ID
ATACGGTACGGTCG*G*T CTL154 BOT tag
/5Phos/G*T*ACGCTCGCAGTGCGCGAGTAGTGTGCACTCGACG SEQ ID NO: 245
GTTAACTAATCCGC*G*C CTL112_BOT_tag
/5Phos/A*C*CGCCGACTTATAGTAGTGCGCGTACGCATTAGTC SEQ ID NO: 246
GGTCGCGGATTAAG*G*T CTL145_BOT_tag
/5Phos/C*G*GACGAGCGGTTCGCATTAGTCGGTACCATAACCG SEQ ID
                                                            247
CGCCGCGATTAAG*G*T CTL060 BOT tag
/5Phos/A*C*CGCCGACTTATCGCGCTACTAGGTACCGCCGTAC SEQ ID
                                                            248
AAGGTACGCTCGCA*G*T CTL016 BOT tag
/5Phos/C*G*CAACGCTAGGTACCTAGCGCCGAACGCGGACTAA SEQ ID
                                                        NO:
                                                            249
GGTACCTAGCGCCG*A*A CTL159_BOT_tag
/5Phos/G*C*ACTCGACGGTTCGTTCGGCTAGGTACCGCCGTAC SEQ
                                                       NO:
                                                            250
AAGTACGCGACTAG*G*T CTL056_BOT_tag
/5Phos/A*C*GCCGTACCATAGCGGCGTATTGGTGTCGCGCAGT SEQ
                                                       NO:
                                                            251
GTAGCGCGGTTATG*G*T CTL162 BOT tag
/5Phos/T*A*GTTAGCGGCGTGCGGTTCGACATTACCTAGTCGC SEQ
                                                       NO:
                                                            252
GTAGCGCGAGTAGT*G*T CTL018_BOT_tag
/5Phos/G*C*GCGATTAAGGTTCTCGCGCACTAACGACGCGCTG SEQ ID
                                                       NO: 253
TTACGCATTAGTCG*G*T CTL115 BOT tag
/5Phos/G*C*GGCGTATTGGTACCGCCGACTTATCGCATTAGTC SEQ
                                                       NO:
                                                           254
                                                   ID
GGTTATGGTACGGC*G*T CTL033 BOT tag
/5Phos/G*C*GCGGTTATGGTAACTACTCGCCGAACCTATTACC SEQ
                                                   ID
                                                       NO:
                                                           255
GCGACTGCGAGCGT*A*C CTL047 BOT tag
/5Phos/C*G*ACTATGTGCGCTCGTCGCACTAGTACCATAACCG SEQ
                                                   ID
                                                       NO:
                                                            256
CGCAACCGCTCGTC*C*G CTL108_BOT_tag
/5Phos/A*A*TCTAGCCGCGATAGTTAGCGGCGTACCTTAATCG SEQ
                                                            257
                                                   ID
                                                       NO:
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CGCTAGCGCGAGTA*G*T CTL041_BOT_tag
/5Phos/T*A*CGCGCACTACTGCGTCGAATTGGTGCGCGGATTA SEQ
                                                                 258
GTTGCGTCGAATTG*G*T CTL061 BOT tag
/5Phos/A*C*CGCCGCTATACACTGCGAGCGTACTTCGGCGCTA SEQ
                                                         ID
                                                            NO:
                                                                 259
GGTGTATAGCGGCG*G*T CTL166_BOT_tag
/5Phos/A*C*TACTCGCGCTAGCGGCGTATTGGTAACCGCTCGT SEQ
                                                            NO:
                                                                 260
CCGGCGCGAGTAGT*G*T CTL012 BOT tag
/5Phos/G*C*GCGAGTAGTGTACCTAGCGTTGCGCGCGGATTAA SEQ
                                                             NO:
                                                                  261
                                                         ID
GGTACTAGTGCGAC*G*A CTL052 BOT tag
/5Phos/G*C*GGTTCGACATTACCTAGCGTTGCGCGCATTAGTC SEQ ID
                                                            NO:
                                                                 262
GGTACCTAGTAGCG*C*G CTL153 BOT tag
/5Phos/G*T*CGCGCAGTGTAACCTAGCGTTGCGTCGCGACAGT SEQ
                                                        ID
                                                                  263
AGTGACTACCGCTC*G*T CTL094_BOT_tag
/5Phos/A*C*CGCTCGTTACCACTAGCGATCGGTACCATACTAC SEQ ID
                                                            NO:
                                                                 264
GCGTACGCGACTAG*G*T CTL095 BOT tag
/5Phos/C*G*CAACGCTAGGTAGTCGAGCGCATACGCATTAGTC SEQ
                                                             NO:
                                                         ID
                                                                  265
GGTAATGTCGAACC*G*C CTL105 BOT tag
/5Phos/A*C*CTAGTAGCGCGTAGTTAGCGGCGTTTAGTGCGCG SEQ
                                                             NO:
                                                                  266
                                                         ID
AGAGTACGCT CGCA*G*T CTL109_BOT_tag
/5Phos/C*T*TGTACGGCGGTCGCGGACTAAGGTTCGCGGCTAG SEQ
                                                                  267
ATTACCGACCGTAC*C*G CTL032_BOT_tag
/5Phos/T*A*GTTAGCGGCGTAATCTAGCCGCGAATAGGTAGCG SEQ ID
CGTAACTCGCC*G*A "/5Phos/" indicates a 5'-phosphate moiety; "*"
phosphorothioate linkage.
TABLE-US-00005 TABLE 5 Pools of Tag Sequences Pools Tags Present in Pools Pool A1 Pool B1
Pool B2 Pool B3 Pool B4 Pool B5 Pool B6 Pool C1 CTL085 CTL161 CTL089 CTL098 CTL062
CTL048 CTL018 Pool A1 CTL169 CTL164 CTL081 CTL038 CTL044 CTL053 CTL115 Pool B1
CTL137 CTL030 CTL075 CTL139 CTL043 CTL072 CTL033 Pool B2 CTL042 CTL088 CTL160
CTL010 CTL118 CTL096 CTL047 Pool B3 CTL051 CTL148 CTL133 CTL034 CTL128 CTL150
CTL108 Pool B4 CTL167 CTL152 CTL076 CTL117 CTL067 CTL084 CTL041 Pool B5 CTL026
CTL007 CTL024 CTL035 CTL020 CTL142 CTL061 Pool B6 CTL068 CTL141 CTL045 CTL121
CTL006 CTL102 CTL166 CTL138 CTL064 CTL009 CTL106 CTL017 CTL154 CTL012 CTL079
CTL158 CTL055 CTL059 CTL057 0TL112 CTL052 CTL063 CTL066 CTL101 CTL157 CTL078
OTL145 CTL153 CTL168 CTL144 CTL135 CTL015 CTL031 CTL060 CTL094 CTL021 CTL107
CTL155 CTL110 CTL136 CTL016 CTL095 CTL151 CTL149 CTL122 CTL123 CTL165 CTL159
CTL105 CTL002 CTL008 CTL080 CTL014 CTL039 CTL056 CTL109 CTL134 CTL099 CTL126
CTL131 CTL036 CTL162 CTL032
TABLE-US-00006 TABLE 6 Non-homologous tails Name Sequence (5'.fwdarw.3') SEQ
ID NO: H1 ACGCGACTATACGCGCAATATGGT SEQ ID NO:
CTAGCGATACTACGCGATACGAGAT SEQ ID NO:
                                            270 H3
```

#### **Claims**

SEQ ID NO: 273

1. A method for identifying and nominating on- and off-target CRISPR edited sites with improved accuracy and sensitivity, the process comprising the steps of: (a) isolating genomic DNA from a cell having one or more tag sequences incorporated into a target site within a genome of the cell; (b) integrating a universal adapter sequence comprising a unique molecular index (UMI) into the

CGCGAGTACGTACGATTACCG SEQ ID NO: 272 H5 ACGCGCGACTATACGCGCCTC

CATAGCGGTATTACGCGAGATTACGA SEQ ID NO: 271 H4

isolated genomic DNA; (c) providing a multiplex PCR reaction mixture comprising: (i) one or more on-target oligonucleotide primers, each having a cleavage region comprising a ribonucleotide (rN) positioned 5' of a blocking group and a complementary region flanking the on-target genome edited locus, wherein the blocking group prevents primer extension and/or inhibits the oligonucleotide primer from serving as a template for DNA synthesis; (ii) one or more adapterspecific oligonucleotide primers, each having a cleavage region comprising a ribonucleotide (rN) positioned 5' of a blocking group and a complementary region flanking the 5' of the universal adapter sequence; and (iii) a cleaving enzyme, wherein the cleaving enzyme is an RNase H2 enzyme; (d) hybridizing the on-target oligonucleotide primer to the on-target genome edited locus to form an on-target double stranded substrate and hybridizing the one or more adapter-specific oligonucleotide primers to the 5' of the universal adapter sequence; (e) cleaving at a point within or adjacent to the cleavage region to remove the blocking group from the one or more on-target oligonucleotide primers and the one or more adapter-specific oligonucleotide primers; and (f) simultaneously amplifying a portion of the isolated genomic DNA comprising the one or more tag sequences and the universal adapter sequence; and (g) sequencing the amplified portion of the isolated genomic DNA, thereby identifying on- and off-target CRISPR edited sites.

- **2**. The method of claim 1, wherein the one or more adapter-specific oligonucleotide primers target SP1 or SP2 sequence (SEQ ID NO: 7, 8) tails on a top strand of the one or more on-target oligonucleotide primers or a bottom strand of the one or more on-target oligonucleotide primers.
- **3.** The method of claim 1, wherein the one or more adapter-specific oligonucleotide primers target predesigned non-homologous sequence (SEQ ID NO: 269-273) tails on a top strand of the one or more on-target oligonucleotide primers or a bottom strand of the one or more on-target oligonucleotide primers.
- **4.** The method of claim 1, wherein the one or more adapter-specific oligonucleotide primers target predesigned 13-mer tails on a top strand of the one or more on-target oligonucleotide primers or a bottom strand of the one or more on-target oligonucleotide primers.
- **5**. The method of claim 1, wherein the sequencing of step (g) further comprises executing on a processor: (i) aligning the sequence data to a reference genome; and (ii) outputting the alignment, analysis, and results data as custom-formatted files, tables or graphics.
- **6**. (canceled)
- 7. The method of claim 1, wherein step (d) uses a suppression PCR method.
- **8.** The method of claim 1, wherein the one or more on-target oligonucleotide primers comprise a first on-target oligonucleotide primer targeting a top strand of the isolated genomic DNA and a second on-target oligonucleotide primer targeting a bottom strand of the isolated genomic DNA.
- **9.** The method of claim 1, wherein the one or more adapter-specific oligonucleotide primers comprise a first adapter-specific oligonucleotide primer targeting a top strand of the isolated genomic DNA and a second adapter-specific oligonucleotide primer targeting a bottom strand of the isolated genomic DNA.
- **10**. The method of claim 1, wherein the cells comprise human or mouse cells.
- **11-12**. (canceled)
- **13.** The method of claim 1, wherein the one or more tag sequences comprise double-stranded deoxyribooligonucleotides (dsDNA) comprising 52-base pairs.
- **14**. The method of claim 1, wherein the one or more tag sequences comprise a 5'-terminal phosphate, and phosphorothioate linkages between the 1.sup.st and 2.sup.nd, 2.sup.nd and 3.sup.rd, 50.sup.th and 51.sup.st, and 51.sup.st and 52.sup.nd nucleotides.
- **15**. The method of claim 1, wherein the one or more tag sequences comprise a double stranded DNA comprising the complementary top and bottom strand pairs of SEQ ID NO: 1-2 or 7-268.
- **16**. On- and off-target CRISPR editing sites identified or nominated using the method of claim 1.
- **17-33**. (canceled)
- **34**. The method of claim 1, wherein the one or more tag sequences alien sequence content

containing no sequence identity to a mouse or human genome. **35**. The method of claim 1, wherein the cleavage region comprises a ribonucleotide (rN) that is positioned 6-nucleotides from the 3'-end.