

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2025/0257352 A1 MCNEILL et al.

Aug. 14, 2025 (43) Pub. Date:

(54) METHODS FOR NOMINATION OF NUCLEASE ON-/OFF-TARGET EDITING LOCATIONS, DESIGNATED "CTL-SEQ" (CRISPR TAG LINEAR-SEQ)

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(21) Appl. No.: 19/067,623

(22) Filed: Feb. 28, 2025

Related U.S. Application Data

- (63) Continuation of application No. 17/382,945, filed on Jul. 22, 2021, now abandoned.
- (60) Provisional application No. 63/055,460, filed on Jul. 23, 2020.

Publication Classification

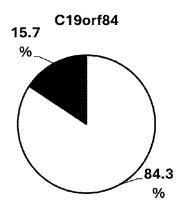
(51) Int. Cl. C12N 15/11 (2006.01)C12N 9/22 (2006.01)C12Q 1/6853 (2018.01)

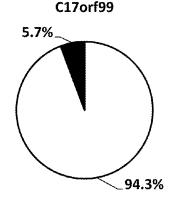
(52) U.S. Cl. CPC C12N 15/111 (2013.01); C12N 9/22 (2013.01); C12Q 1/6853 (2013.01); C12N 2310/20 (2017.05)

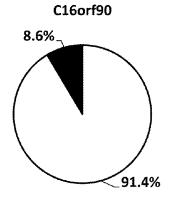
ABSTRACT (57)

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

Specification includes a Sequence Listing.







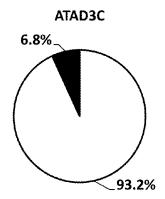
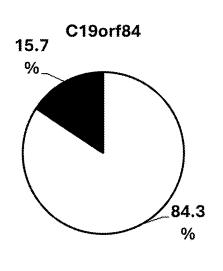
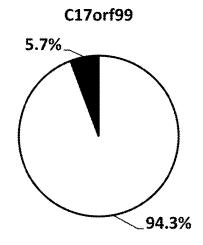
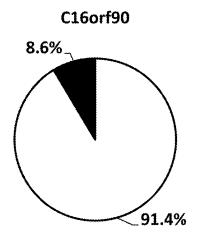


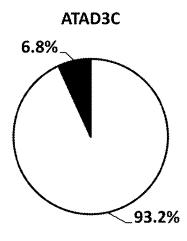


FIG. 1









Average Percentage Reads Present in 3 Biological Replicates Average Percentage Reads Present in 1 or 2 Biological Replicates

FIG. 2

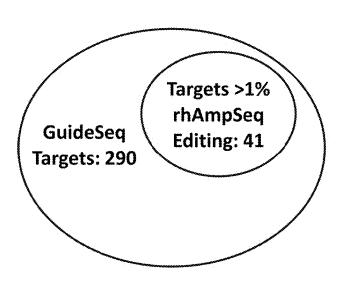
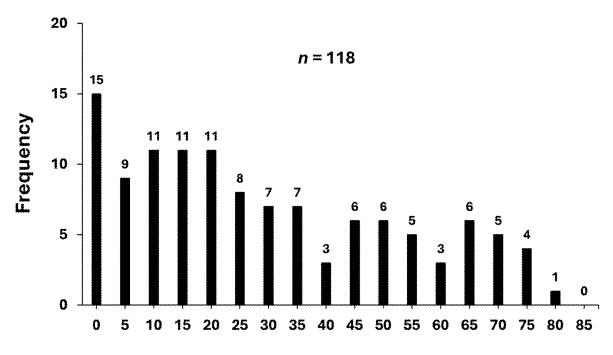


FIG. 3



Normalized Percentage Tag Integration Bins

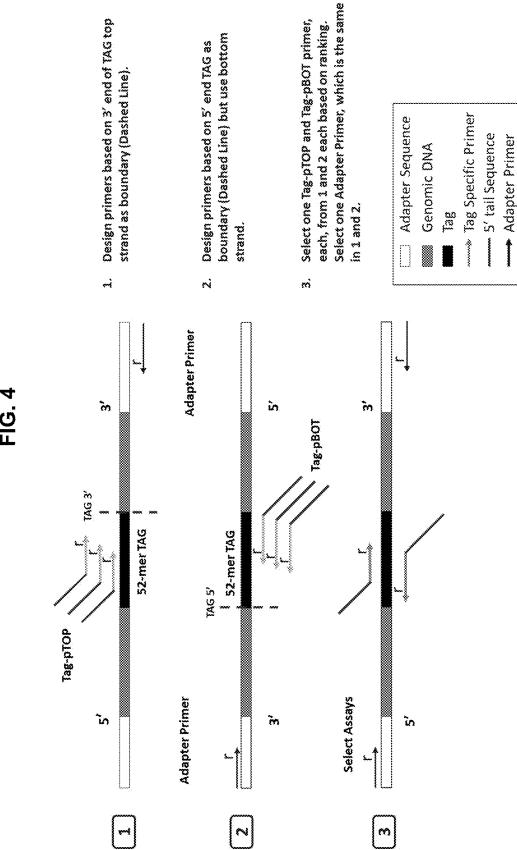
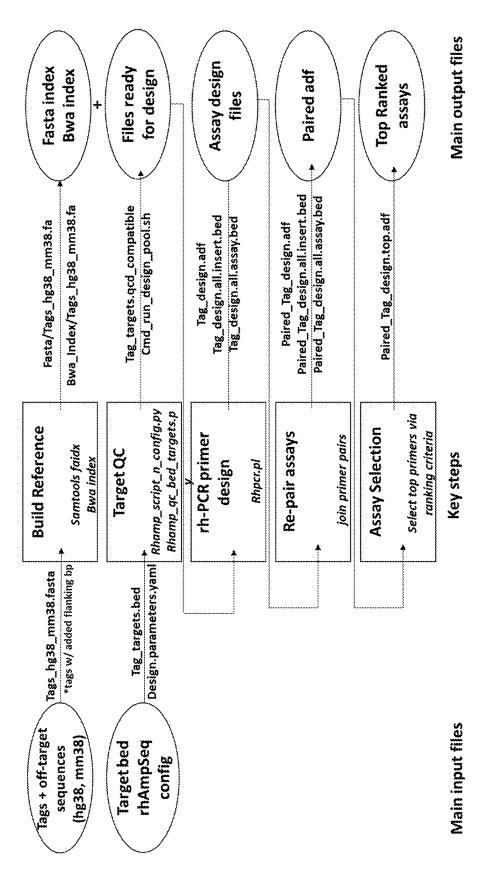


FIG. 5

Design pipeline overview



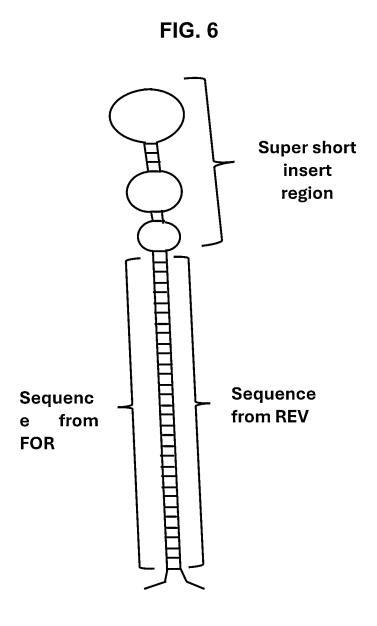


FIG. 7

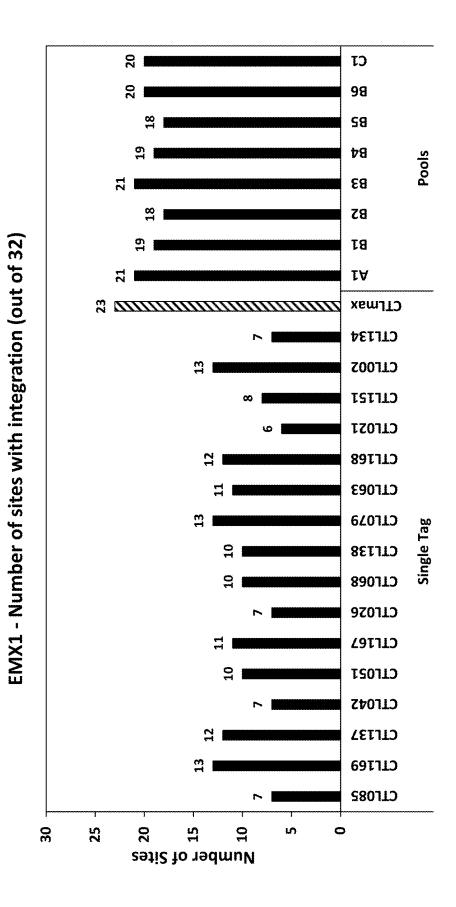
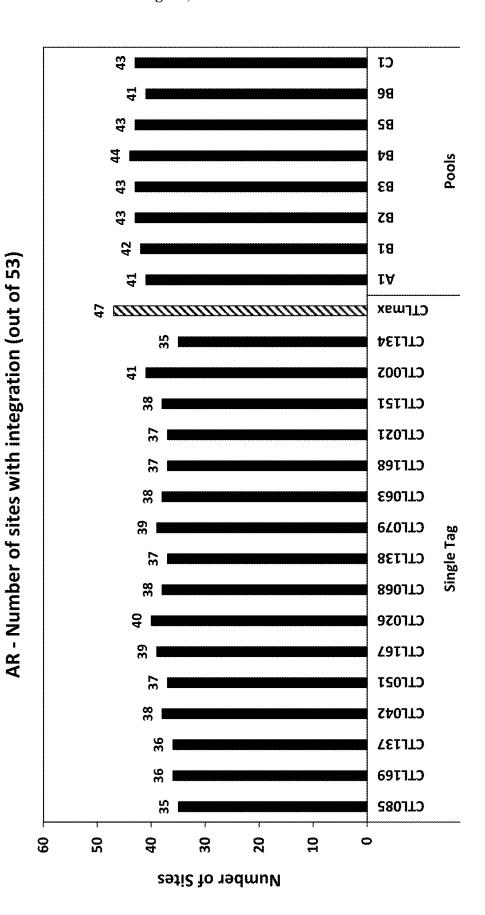


FIG. 8



METHODS FOR NOMINATION OF NUCLEASE ON-/OFF-TARGET EDITING LOCATIONS, DESIGNATED "CTL-SEQ" (CRISPR TAG LINEAR-SEQ)

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 off-target 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: trans-activating 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 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 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 endogenouslyexpressed 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 1st and 2^{nd} , 2^{nd} and 3^{rd} , 50^{th} and 51^{st} , and 51^{st} and 52^{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 selfdimer 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^{st} and 2^{nd} , 2^{nd} and 3^{rd} , 50^{th} and 51^{st} , and 51^{st} and 52^{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 TagpTOP 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) 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. 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 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. 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 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.

[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 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 GUIDEseq [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 NucleofectorTM 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 ≥ the untreated control sample at ≥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. 4A) 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 codelivered 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 (Pvrococcus 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 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%

TABLE 1-continued

	target sites for four g at off-target sites		
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%
chrX_149763439	2.13%	0.00%	3.83%
chr17_7435217	1.93%	0.00%	0.55%
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%
chr13_29717148	0.48%	0.00%	0.00%
chr1_3088065	0.29%	0.00%	0.00%
chr15_73442915	0.19%	0.00%	0.55%
chr10_118045968	0.19%	0.00%	0.00%
chr14_102199972	0.00%	0.00%	0.68%
chr18_56334679	0.00%	0.00%	2.33%
chr21_36426137	0.00%	0.00%	2.19%
chr5_139002763	0.00%	0.00%	3.83%
chrX_58291642	0.00%	0.00%	3.83%
Location	C17orf99_BR1	C17orf99_BR2	C17orf99_BR3
chr17_78164110	100.00%	100.00%	100.00%
chr22_24471716	15.00%	13.24%	10.86%
chr10_101156881	6.22%	11.07%	9.79%
chr3_170476431	5.86%	3.97%	4.57%
chr17_17692965	4.94%	0.66%	8.62%
chr15_73400031	3.93%	4.63%	5.73%
chr19_15238775	0.00%	0.00%	2.56%
chr2_18362316	0.00%	0.00%	1.59%
chr2_171087784	0.00%	0.54%	0.84%
chr22_19959968	0.00%	1.26%	0.19%
chr22_32114104	0.00%	0.00%	4.06%
chr4_129034015	0.00%	0.00%	0.33%
chr5_61219030	0.00%	0.00%	0.33%
chr5_66209615	0.00%	0.00%	1.86%
chr7_69709389	0.00%	0.12%	2.75%
chr7_158662844	0.00%	1.44%	5.27%
chrX_9567397	0.00%	0.00%	0.23%
chr19_55657073	0.00%	0.66%	0.00%
chr22_43788032	0.00%	2.47%	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%	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_1450685 chr1_1503588 chr1_1516015	100.00% 11.73% 2.47%	100.00% 10.07%	100.00% 9.27% 5.14%

[0025] Additionally, nominated targets may not be replicable or detectable using orthogonal methods. Using the GUIDE-Seq method, the GUIDE-Seq DNA tag was codelivered 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).

0.93%

1.12%

0.00%

0.00%

26.34%

0.00%

chr19 32167960

chr2_111077960

[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™ 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, enzymebased 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 (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) 6-nucleotides from the 3'-end, a 3'-end mismatch, and a 3'-end block (3'-C3 spacer). The adapter-specific 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 pres-

[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).

ence of human or mouse genomic DNA.

TABLE 2

Sequences Used for First Proof of Concept				
Туре	Name	Sequence (5'→3')	SEQ ID NO	
Tag	9022179029169042579 04625907201907281	T*C*GTTCGTTC CGCTCTAACCGG CGAATCTACCGC GCATATCTACGC CGCA*A*T	SEQ ID NO: 1	
Tag	9022179029169042579 04625907201907281_r ev	A*T*TGCGGCGT AGATATGCGCGG TAGATTCGCCGG TTAGAGCGGAAC GAAC*G*A	SEQ ID NO: 2	
Tag Primers	pFWD.ID_Target1: 9022179029169042579 04625907201907281.12 7.150.1.SP1	acactettteec tacacgacgete ttecgatetTCT ACCGCGCATATC TACCGCCGCT/ 3SpC3/	SEQ ID NO: 3	

TABLE 2-continued

Seque	nces Used for First Pr	oof of Concept	:
Туре	Name	Sequence (5'→3')	SEQ ID NO
Tag Primers	pFWD.ID_Target2: 9022179029169042579 04625907201907281.11 6.1401.SP1	acactetttece tacacgacgete ttecgatetATA TGCGCGGTAGAT TCGCrCGGTTT/ 3SpC3/	SEQ ID NO:
Adapter Primer	Adapter Primer	gtgactggagtt cagacgtgtgct cttccgatctAA TGATACGGCGAC CACCGAGATCTA CArCAAGGC/ 3SpC3/	SEQ ID NO:
P5 Adapter	Example Sequence	AATGATACGGCG ACCACCGAGATC TACACTAGATCG CNNWNNWNNACA CTCTTTCCCTAC ACGACGCTCTTC CGATC*T	SEQ ID NO:
SP1	Sequencing Primer 1	acactettteee tacacgacgete tteegatet	SEQ ID NO: 7
SP2	Sequencing Primer 2	gtgactggagtt cagacgtgtgct cttccgatct	SEQ ID NO:

"*" indicates a phosphorothioate linkage; "rN" indicates a ribonucleotide, where N is the nucleotide preceded by the "r"; "/3SpC3/" indicates a 3'-C $_3$ spacer.

[0033] One embodiment described herein is a method for identifying and identifying and nominating on- and offtarget CRISPR editing sites with improved accuracy and sensitivity, the process comprising the steps of: (a) codelivering 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 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^{st} and 2^{nd} , 2^{nd} and 3^{rd} , 50^{th} and 51^{st} , and 51^{st} and 52^{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.

[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 selfdimer 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^{st} and 2^{nd} , 2^{nd} and 3^{rd} , 50^{th} and 51^{st} , and 51^{st} and 52^{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 computerreadable 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) co-delivering 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

[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 co-delivered.

[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^{st} and 2^{nd} , 2^{nd} and 3^{rd} , 50^{th} and 51^{st} , and 51^{st} and 52^{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^{st} and 2^{nd} , 2^{nd} and 3^{rd} , 50^{th} and 51^{st} , and 51^{st} and 52^{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.

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EXAMPLES

Example 1

[0107] This experiment demonstrates the increased efficiency in tag integration when using double-stranded 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

SEO

ID

NO

SEQ

ID

NO:

15

Name

CTL042

TOP_tag

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 3

Sequen	ces Used for Second Proof of Concept		CTL026
Name	Sequence (5'→3')	SEQ ID NO	BOT_ta
CTL085_ TOP_tag	/5Phos/A*C*GAGCGGTAGTCACCTA GTCGTCGTACCAATTCGACGCACACTA CTCGC*G*C	SEQ ID NO:	CTL068 TOP_ta
CTL085_ BOT_tag	/5Phos/G*C*GCGAGTAGTGTGCGTC GAATTGGTACGACGACTAGGTGACTAC CGCTC*G*T	SEQ ID NO: 10	CTL068 BOT_ta
CTL169_ TOP_tag	/5Phos/T*A*GCGCGAGTAGTCGGAC GAGCGGTTACCAATACGCCGCACCTTA ATCCG*C*G	SEQ ID NO: 11	CTL138 TOP_ta
CTL169_ BOT_tag	/5Phos/C*G*CGGATTAAGGTGCGGC GTATTGGTAACCGCTCGTCCGACTACT CGCGC*T*A	SEQ ID NO: 12	CTL138 BOT_ta
CTL137_ TOP_tag	/5Phos/T*C*GCGACAGTAGTCGTTC GGCTAGGTACCTATTACCGCGTAGTTA GCGGC*G*T	SEQ ID NO: 13	CTL079 TOP_ta
CTL137_ BOT_tag	/5Phos/A*C*GCCGCTAACTACGCGG TAATAGGTACCTAGCCGAACGACTACT GTCGC*G*A	SEQ ID NO: 14	CTL079 BOT_ta

TABLE 3-continued

Sequences Used for Second Proof of

Concept

Sequence (5'→3')

CTCGC*G*C

/5Phos/C*G*CGCTACTAGGTGCGTC

GAATTGGTACCGATCCGCAATACACTA

		15
CTL042_ BOT_tag	/5Phos/G*C*GCGAGTAGTGTATTGC GGATCGGTACCAATTCGACGCACCTAG TAGCG*C*G	SEQ ID NO: 16
CTL051_ TOP_tag	/5Phos/G*G*TAACGAGCGGTGCGTC GAATTGGTAACCGCTCGTCCGACCTTA ATCGC*G*C	SEQ ID NO: 17
CTL051_ BOT_tag	/5Phos/G*C*GCGATTAAGGTCGGAC GAGCGGTTACCAATTCGACGCACCGCT CGTTA*C*C	SEQ ID NO: 18
CTL167_ TOP_tag	/5Phos/T*T*CGGCGCTAGGTGCGGC GTATTGGTAACCGCTCGTCCGTTCGGC GCTAG*G*T	SEQ ID NO: 19
CTL167_ BOT_tag	/5Phos/A*C*CTAGCGCCGAACGGAC GAGCGGTTACCAATACGCCGCACCTAG CGCCG*A*A	SEQ ID NO: 20
CTL026_ TOP_tag	/5Phos/T*A*CGCGACTAGGTGCGCG ATTAAGGTACCTATTACCGCGCGACTA TGTGC*G*C	SEQ ID NO: 21
CTL026_ BOT_tag	/5Phos/G*C*GCACATAGTCGCGCGG TAATAGGTACCTTAATCGCGCACCTAG TCGCG*T*A	SEQ ID NO: 22
CTL068_ TOP_tag	/5Phos/G*T*CGCGCAGTGTAGCGCG ATTAAGGTACCTATTACCGCGTCGCGA CAGTA*G*T	SEQ ID NO: 23
CTL068_ BOT_tag	/5Phos/A*C*TACTGTCGCGACGCGG TAATAGGTACCTTAATCGCGCTACACT GCGCG*A*C	SEQ ID NO: 24
CTL138_ TOP_tag	/5Phos/A*A*CCGTCGATCCGCGCGT AGTATGGTACCGATCCGCAATACTAGC GCGAC*A*A	SEQ ID NO: 25
CTL138_ BOT_tag	/5Phos/T*T*GTCGCGCTAGTATTGC GGATCGGTACCATACTACGCGCGGATC GACGG*T*T	SEQ ID NO: 26
CTL079_ TOP_tag	/5Phos/T*C*GCTCGATTGGTTACGC GCACTACTTATGCGCTCGACTCGTTCG GCTAG*G*T	SEQ ID NO: 27
CTL079_ BOT_tag	/5Phos/A*C*CTAGCCGAACGAGTCG AGCGCATAAGTAGTGCGCGTAACCAAT CGAGC*G*A	SEQ ID NO: 28

TABLE 3-continued

Sequences Used for Second Proof of Concept			
Name	Sequence (5'→3')	SEQ ID NO	
CTL063_ TOP_tag	/5Phos/A*C*TGCGAGCGTACTTGTC GCGCTAGTACCAATTCGACGCAACCGC TCGTC*C*G	SEQ ID NO: 29	
CTL063_ BOT_tag	/5Phos/C*G*GACGAGCGGTTGCGTC GAATTGGTACTAGCGCGACAAGTACGC TCGCA*G*T	SEQ ID NO: 30	
CTL168_ TOP_tag	/5Phos/C*G*CATTAGTCGGTGCGGC GTATTGGTAACCGCTCGTCCGACGCGC TACCT*A*T	SEQ ID NO: 31	
CTL168_ BOT_tag	/5Phos/A*T*AGGTAGCGCGTCGGAC GAGCGGTTACCAATACGCCGCACCGAC TAATG*C*G	SEQ ID NO: 32	
CTL021_ TOP_tag	/5Phos/A*T*TGCGGATCGGTGCGTC GAATTGGTAACCGCTCGTCCGTACGCG CACTA*C*T	SEQ ID NO: 33	
CTL021_ BOT_tag	/5Phos/A*G*TAGTGCGCGTACGGAC GAAGCGGTTACCAATTCGCGCACCGAT CCGCA*A*T	SEQ ID NO: 34	
CTL151_ TOP_tag	/5Phos/T*C*GGCGAGTAGTTGCGCG GTTATGGTACCATAACCGCGCAGTAGT ACGCG*G*T	SEQ ID NO: 35	
CTL151_ BOT_tag	/5Phos/A*C*CGCGTACTACTGCGCG GTTATGGTACCATAACCGCGCAACTAC TCGCC*G*A	SEQ ID NO: 36	
CTL002_ TOP_tag	/5Phos/A*C*TAGCGATCGGTACCTA GCGCCGAAACCTATTACCGCGACCTAG CGTTG*C*G	SEQ ID NO: 37	
CTL002_ BOT_tag	/5Phos/C*G*CAACGCTAGGTCGCGG TAATAGGTTTCGGCGCTAGGTACCGAT CGCTA*G*T	SEQ ID NO: 38	
CTL134_ TOP_tag	/5Phos/T*A*GCGCGTCAAGAGCGCG GTTATGGTTTCGGCGCTAGGTTAACAG CGCGT*C*G	SEQ ID NO: 39	
CTL134_ BOT_tag	/5Phos/C*G*ACGCGCTGTTAACCTA GCGCCGAAACCATAACCGCGCTCTTGA CGCGC*T*A	SEQ ID NO: 40	
GuideSeq_ TOP_tag	/5Phos/G*T*TTAATTGAGTTGTCAT ATGTTAATAACGGT*A*T	SEQ ID NO: 41	
GuideSeq_ BOT_tag	/5Phos/A*T*ACCGTTATTAACATAT GACAACTCAATTAA*A*C	SEQ ID NO: 42	

TABLE 3-continued

Sequenc	es Used for Second Proof of Concept	
Name	Sequence (5'→3')	SEÇ ID NO
EMX1 protospacer	GAGTCCGAGCAGAAGAAGAA	SEQ ID NO:
AR protospacer	GTTGGAGCATCTGAGTCCAG	SEQ ID NO: 44

[&]quot;/5Phos/" indicates a 5'-phosphate moiety; "*" indicates a phosphorothioate linkage.

Example 2

[0108] This experiment demonstrates the increased efficiency in tag integration when using double-stranded 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 4

	Tag Sequences			
Name	Sequence (5'→3') SEQ	ID	NO	
CTL085_TOP_tag	/5Phos/A*C*GAGCGGTAGTCACCTAGTCGTCGTACCAATTCGASEQ CGCACACTACTCGC*G*C	ID	NO:	45
CTL169_TOP_tag	/5Phos/T*A*GCGCGAGTAGTCGGACGAGCGGTTACCAATACGCSEQCGCACCTTAATCCG*C*G	ID	NO:	46
CTL137_TOP_tag	/5Phos/T*C*GCGACAGTAGTCGTTCGGCTAGGTACCTATTACCSEQGCGTAGTTAGCGGC*G*T	ID	NO:	47
CTL042_TOP_tag	/5Phos/C*G*CGCTACTAGGTGCGTCGAATTGGTACCGATCCGCSEQ AATACACTACTCGC*G*C	ID	NO:	48
CTL051_TOP_tag	/ 5 Phos/G*G*TAACGAGCGGTGCGTCGAATTGGTAACCGCTCGTSEQCCGACCTTAATCGC*G*C	ID	NO:	49
CTL167_TOP_tag	/ 5Phos/T*T*CGGCGCTAGGTGCGGCGTATTGGTAACCGCTCGTSEQCGGTTCGGCGCTAG*G*T	ID	NO:	50
CTL026_TOP_tag	$/ 5 Phos/T \star A \star CGCGACTAGGTGCGCGATTAAGGTACCTATTACCSEQGCGCGACTATGTGC \star G \star C$	ID	NO:	51
CTL068_TOP_tag	/ 5Phos/G*T*CGCGCAGTGTAGCGCGATTAAGGTACCTATTACCSEQGCGTCGCGACAGTA*G*T	ID	NO:	52
CTL138_TOP_tag	/ 5Phos/A*A*CCGTCGATCCGCGCGTAGTATGGTACCGATCCGCSEQAATACTAGCGCGAC*A*A	ID	NO:	53
CTL079_TOP_tag	$/ \texttt{5Phos/T*C*GCTCGATTGGTTACGCGCACTACTTATGCGCTCGSEQ} \\ \texttt{ACTCGTTCGGCTAG*G*T} \\$	ID	NO:	54
CTL063_TOP_tag	/ 5Phos/A*C*TGCGAGCGTACTTGTCGCGCTAGTACCAATTCGASEQCGCAACCGCTCGTC*C*G	ID	NO:	55
CTL168_TOP_tag	/ 5Phos/C*G*CATTAGTCGGTGCGGCGTATTGGTAACCGCTCGTSEQCGCGACGCGCTACCT*A*T	ID	NO:	56
CTL021_TOP_tag	$/ \texttt{5Phos/A*T*TGCGGATCGGTGCGTCGAATTGGTAACCGCTCGTSEQ} \\ \texttt{CCGTACGCGCACTA*C*T} \\$	ID	NO:	57
CTL151_TOP_tag	$/ \texttt{5Phos/T*C*GGCGAGTAGTTGCGCGGTTATGGTACCATAACCGSEQ} \\ \texttt{CGCAGTAGTACGCG*G*T} \\$	ID	NO:	58
CTL002_TOP_tag	/5Phos/A*C*TAGCGATCGGTACCTAGCGCCGAAACCTATTACCSEQGCGACCTAGCGTTG*C*G	ID	NO:	59
CTL134_TOP_tag	/5Phos/T*A*GCGCGTCAAGAGCGCGGTTATGGTTTCGGCGCTASEQ GGTTAACAGCGCGT*C*G	ID	NO:	60
CTL085_BOT_tag	/5Phos/G*C*GCGAGTAGTGTGCGTCGAATTGGTACGACGACTASEQ GGTGACTACCGCTC*G*T	ID	NO:	61
CTL169_BOT_tag	/5Phos/C*G*CGGATTAAGGTGCGGCGTATTGGTAACCGCTCGTSEQ CCGACTACTCGCGC*T*A	ID	NO:	62
CTL137_BOT_tag	/5Phos/A*C*GCCGCTAACTACGCGGTAATAGGTACCTAGCCGASEQ ACGACTACTGTCGC*G*A	ID	NO:	63
CTL042_BOT_tag	/5Phos/G*C*GCGAGTAGTGTATTGCGGATCGGTACCAATTCGASEQCGCACCTAGTAGCG*C*G	ID	NO:	64
CTL051_BOT_tag	/5Phos/G*C*GCGATTAAGGTCGGACGAGCGGTTACCAATTCGASEQCGCACCGCTCGTTA*C*C	ID	NO:	65
CTL167_BOT_tag	/5Phos/A*C*CTAGCGCCGAACGGACGAGCGGTTACCAATACGCSEQCGCACCTAGCGCCG*A*A	ID	NO:	66
CTL026_BOT_tag	/5Phos/G*C*GCACATAGTCGCGCGGTAATAGGTACCTTAATCGSEQCGCACCTAGTCGCG*T*A	ID	NO:	67
CTL068_BOT_tag	/5Phos/A*C*TACTGTCGCGACGCGGTAATAGGTACCTTAATCGSEQCGCTACACTGCGCG*A*C	ID	NO:	68

TABLE 4-continued

	Tag Sequences			
Name	Sequence $(5'\rightarrow 3')$ SEQ	ID	NO	
CTL138_BOT_tag	/5Phos/T*T*GTCGCGCTAGTATTGCGGATCGGTACCATACTACSEQ GCGCGGATCGACGG*T*T	ID	NO:	69
CTL079_BOT_tag	/5Phos/A*C*CTAGCCGAACGAGTCGAGCGCATAAGTAGTGCGCSEQGTAACCAATCGAGC*G*A	ID	NO:	70
CTL063_BOT_tag	/ 5Phos/C*G*GACGAGCGGTTGCGTCGAATTGGTACTAGCGCGASEQCAAGTACGCTCGCA*G*T	ID	NO:	71
CTL168_BOT_tag	$/ {\tt 5Phos/A*T*AGGTAGCGCGTCGGACGAGCGGTTACCAATACGCSEQCGCACCGACTAATG*C*G}$	ID	NO:	72
CTL021_BOT_tag	/ 5Phos/A*G*TAGTGCGCGTACGGACGAGCGGTTACCAATTCGASEQCGCACCGATCCGCA*A*T	ID	NO:	73
CTL151_BOT_tag	$/ {\tt 5Phos/A*C*CGCGTACTACTGCGCGGTTATGGTACCATAACCGSEQCGCAACTACTCGCC*G*A}$	ID	NO:	74
CTL002_BOT_tag	$/ {\tt 5Phos/C*G*CAACGCTAGGTCGCGGTAATAGGTTTCGGCGCTASEQ} \\ {\tt GGTACCGATCGCTA*G*T} \\$	ID	NO:	75
CTL134_BOT_tag	$/ {\tt 5Phos/C*G*ACGCGCTGTTAACCTAGCGCCGAAACCATAACCGSEQCGCTCTTGACGCGC*T*A}$	ID	NO:	76
CTL161_TOP_tag	/ 5Phos/T*A*CACTGCGCGACACTGCGAGCGTACACCTTAATCGSEQCGCTAGTTAGCGGC*G*T	ID	NO:	77
CTL164_TOP_tag	$/ {\tt 5Phos/A*A*CCGTCGAGTGCACCGCGTACTACTAATGTCGAACSEQCGCTACGCGCACTA*C*T}$	ID	NO:	78
CTL030_TOP_tag	$/ \texttt{5Phos/C*G*CGGACTAAGGTGCGCGAGTAGTGTTACGCGCACTSEQ} \\ \texttt{ACTAATCTAGCCGC*G*A} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	ID	NO:	79
CTL088_TOP_tag	/ 5Phos/A*C*TAGTGCGACGAACTACTCGCGCTAACCAATTCGASEQCGCACCGATCGCTA*G*T	ID	NO:	80
CTL148_TOP_tag	/ 5Phos/A*A*TGTCGAACCGCGCGCGAGTAGTGTACCATAACCGSEQCGCACCTTAGTCCG*C*G	ID	NO:	81
CTL152_TOP_tag	/ 5Phos/G*C*GTCGAATTGGTACCGCCGACTTATACCAATACGCSEQCGCATAGGTAGCGC*G*T	ID	NO:	82
CTL007_TOP_tag	/ 5Phos/A*C*CTAGTAGCGCGGCGTCGAATTGGTACTAGCGCGASEQCAACGCGTAGTATG*G*T	ID	NO:	83
CTL141_TOP_tag	$/ {\tt 5Phos/A*C*CGCTGTTACCGCGCGATTAAGGTACGCCGCTAASEQCTACGGTACGGTCG*G*T}$	ID	NO:	84
CTL064_TOP_tag	/ 5Phos/A*C*CGCCGACTTATCGTTCGGCTAGGTACCAATTCGASEQCGCACTGCGAGCGT*A*C	ID	NO:	85
CTL158_TOP_tag	$/ \texttt{5Phos/A*C*CTTAATCCGCGACTGCGAGCGTACACCTATTACCSEQ} \\ \texttt{GCGCGACGCGCTGT*T*A} \\$	ID	NO:	86
CTL066_TOP_tag	/ 5Phos/A*C*GACGACTAGGTACCGCTCGTTACCTCTTGACGCGSEQCTAACCAATTCGAC*G*C	ID	NO:	87
CTL144_TOP_tag	/ 5Phos/A*C*CATACTACGCGGCGGTTCGACATTACCATAACCGSEQCGTAGTGCGAGCG*T*A	ID	NO:	88
CTL107_TOP_tag	$/ \texttt{5Phos/C*T*TGTACGGCGGTGCGGCGTATTGGTACCAATACGCSEQ} \\ \texttt{CGCTCGTCGCACTA*G*T} \\$	ID	NO:	89
CTL149_TOP_tag	$/ \texttt{5Phos/G*T*ACGCTCGCAGTACCGCCGACTTATACCTTAATCGSEQ} \\ \texttt{CGCACTAGCGCGAC*A*A} \\$	ID	NO:	90
CTL008_TOP_tag	$/ 5 \\ Phos/A*C*GACGACTAGGTTATGGTACGGCGTTAGCGCGAGTSEQ \\ AGTACCTTAGTCCG*C*G$	ID	NO:	91
CTL099_TOP_tag	/5Phos/A*C*GAGCGGTAGTCATAGGTAGCGCGTTCTTGACGCGSEQ CTAACCGATCGCTA*G*T	ID	NO:	92

TABLE 4-continued

	Tag Sequences			
Name	Sequence (5'→3') SEQ	ID	NO	
CTL089_TOP_tag	/5Phos/A*C*CGATCCGCAATGCGTCGAATTGGTACCATAACCGSEQCGCACCGCCGTACA*A*G	ID	NO:	93
CTL081_TOP_tag	/5Phos/A*C*TAGTGCGACGAACTACTGTCGCGAACCTATTACCSEQ GCGACCAATCGAGC*G*A	ID	NO:	94
CTL075_TOP_tag	/5Phos/A*C*CGCCGTACAAGTCGCGACAGTAGTAACCGCTCGTSEQCCGTTCGGCGCTAG*G*T	ID	NO:	95
CTL160_TOP_tag	$/ 5 \texttt{Phos} / \texttt{T*C*GTCGCACTAGTCGCATTAGTCGGTAGTAGTACGCSEQ} \\ \texttt{GGTATAGGTAGCGC*G*T} \\$	ID	NO:	96
CTL133_TOP_tag	$/ 5 \texttt{Phos/A*C*CAATTCGACGCTAGTTAGCGGCGTACACTACTCGSEQ} \\ \texttt{CGCGCACTCGACGG*T*T} \\$	ID	NO:	97
CTL076_TOP_tag	$/ 5 Phos/C \star G \star C G G TAATAGGTCGCGGTAATAGGTACGAGCGGTASEQ G T CACACTACT C G C \star G \star C$	ID	NO:	98
CTL024_TOP_tag	$/ \texttt{5Phos/T*C*} \\ \texttt{GGCGAGTAGTTTAGTGCGAGCGTAAGTAGTGCGCSEQ} \\ \texttt{GTAACCAATCGAGC*G*A} \\$	ID	NO:	99
CTL045_TOP_tag	/ 5 Phos/G*T*CGCGCAGTGTAGCGCGGTTATGGTACCATAACCGSEQCGCACTAGTGCGAC*G*A	ID	NO:	100
CTL009_TOP_tag	$/ \texttt{5Phos}/\texttt{T*A*TGCGCTCGACTGCGCGATTAAGGTAATGTCGAACSEQ} \\ \texttt{CGCAGTAGTACGCG*G*T} \\$	ID	NO:	101
CTL055_TOP_tag	/5Phos/A*C*TAGCGCGACAACGACTATGTGCGCACCAATTCGASEQCGCTACGCGCACTA*C*T	ID	NO:	102
CTL101_TOP_tag	$/ 5 \verb Phos/A*A*CTACTCGCCGACTTGTACGGCGGTACCAATTCGASEQCGCAACTAATCCGC*G*C$	ID	NO:	103
CTL135_TOP_tag	/5Phos/C*G*CGGATTAAGGTCTTGTACGGCGGTACCTAGCCGASEQ ACGTACGCGCACTA*C*T	ID	NO:	104
CTL155_TOP_tag	$/ \texttt{5Phos}/\texttt{T*A*GCGCGTCAAGACTTGTACGGCGGTACCGATCCGCSEQ} \\ \texttt{AATGCACTCGACGG*T*T} \\$	ID	NO:	105
CTL122_TOP_tag	/5Phos/C*G*CATTAGTCGGTGCGGCGTATTGGTACGACGACTASEQGGTACCAATACGCC*G*C	ID	NO:	106
CTL080_TOP_tag	/5Phos/A*C*CTAGTAGCGCGGCGCGGTTATGGTACCGACTAATSEQ GCGACTAGCGATCG*G*T	ID	NO:	107
CTL126_TOP_tag	/5Phos/A*C*TACTCGCGCTAACCTAGTCGTCGTAATCTAGCCGSEQCGTAACCTCGCAC*T*A	ID	NO:	108
CTL098_TOP_tag	/5Phos/A*C*CGCCGCTATACGCGCGATTAAGGTGTACGCTCGCSEQ AGTCGCGGACTAAG*G*T	ID	NO:	109
CTL038_TOP_tag	/5Phos/T*A*CGCGCACTACTAACCGTCGAGTGCGTACGCTCGCSEQ AGTACCGATCGCTA*G*T	ID	NO:	110
CTL139_TOP_tag	/5Phos/G*T*CGCGCAGTGTATAACAGCGCGTCGTTAGTGCGCGSEQ AGAACGACGACTAG*G*T	ID	NO:	111
CTL010_TOP_tag	/5Phos/G*C*GTCGAATTGGTCGCGTAGTATGGTACCGCCGCTASEQ TACACCAATACGCC*G*C	ID	NO:	112
CTL034_TOP_tag	/5Phos/T*A*CGCGCACTACTTACGCGACTAGGTACCGATCGCTSEQ AGTCGACGCGGTGT*T*A	ID	NO:	113
CTL117_TOP_tag	/5Phos/A*C*GCCGCTAACTATAGTTAGCGGCGTACCAATTCGASEQ	ID	NO:	114
CTL035_TOP_tag	/5Phos/C*G*CGGACTAAGGTTAGTTAGCGGCGTTACGCGCACTSEQ	ID	NO:	115
CTL121_TOP_tag	/5Phos/A*C*GACGACTAGGTACCGCCGACTTATACGCCGCTAASEQ	ID	NO:	116

TABLE 4-continued

Tag Sequences					
Name	Sequence (5'→3') SEQ	ID	NO		
CTL106_TOP_tag	/5Phos/C*G*GATCGACGGTTGCGCGAGTAGTGTAGTAGTACGCSEQ GGTTACACTGCGCG*A*C	ID	NO:	117	
CTL059_TOP_tag	/5Phos/A*T*TGCGGATCGGTACCGCCGACTTATACCGATCCGCSEQ AATTCGCTCGATTG*G*T	ID	NO:	118	
CTL157_TOP_tag	/5Phos/A*C*TGCGAGCGTACACTGCGAGCGTACACCTTAATCGSEQCGCACCGCTCGTTA*C*C	ID	NO:	119	
CTL015_TOP_tag	/ 5Phos/A*C*TACTGTCGCGATCGTCGCACTAGTTACGCTCGCASEQCTAATTGCGGATCG*G*T	ID	NO:	120	
CTL110_TOP_tag	$/ \texttt{5Phos}/\texttt{G} \star \texttt{G} \star \texttt{TAACGAGCGGTTCTCGCGCACTAATTAGTGCGCGSEQ} \\ \texttt{AGAACCATACTACG} \star \texttt{C} \star \texttt{G} \\$	ID	NO:	121	
CTL123_TOP_tag	/ 5Phos/A*C*TACTCGCGCTAGCGCGATTAAGGTACCTTAATCGSEQCCAACTACTCGCC*G*A	ID	NO:	122	
CTL014_TOP_tag	/ 5Phos/T*A*CGCGCACTACTCTTGTACGGCGGTACCAATTCGASEQCGCAACCGTCGAGT*G*C	ID	NO:	123	
CTL131_TOP_tag	/ 5Phos/A*A*CCGTCGATCCGATTGCGGATCGGTACCTTAATCGSEQCGCACTAGTGCGAC*G*A	ID	NO:	124	
CTL062_TOP_tag	/ 5Phos/A*G*TAGTGCGCGTATACACTGCGCGACACACTACTCGSEQCGCACCTTAATCCG*C*G	ID	NO:	125	
CTL044_TOP_tag	/ 5Phos/A*C*GCCGTACCATACGCGGTAATAGGTAGTAGTGCGCSEQGTATTCGGCGCTAG*G*T	ID	NO:	126	
CTL043_TOP_tag	$/ \texttt{5Phos}/\texttt{T*A*GCGCGTCAAGAACCTAGCGTTGCGATAAGTCGGCSEQ} \\ \texttt{GGTAGTACGCG*G*T} \\$	ID	NO:	127	
CTL118_TOP_tag	/ 5Phos/C*G*CATTAGTCGGTAATCTAGCCGCGAACCATAACCGSEQCGCACCGATCGCTA*G*T	ID	NO:	128	
CTL128_TOP_tag	/ 5Phos/T*A*TGGTACGCGTGCGGCGTATTGGTACGCCGCTAASEQCTAATAAGTCGGCG*G*T	ID	NO:	129	
CTL067_TOP_tag	/ 5Phos/G*C*GCGGTTATGGTGCGGCGTATTGGTACGAGCGGTASEQGTCAACCGCTCGTC*C*G	ID	NO:	130	
CTL020_TOP_tag	/ 5Phos/C*G*ACTATGTGCGCAACTACTCGCCGAACCATAACCGSEQCCTATGCGCTCGA*C*T	ID	NO:	131	
CTL006_TOP_tag	/ 5Phos/T*A*GTTAGCGGCGTACCGCTCGTTACCACCTTAATCGSEQCGCACCATACTACG*C*G	ID	NO:	132	
CTL017_TOP_tag	/ 5Phos/C*G*CATTAGTCGGTAGTAGTGCGCGTAAACCGCTCGTSEQCCGTTAGTGCGCGA*G*A	ID	NO:	133	
CTL057_TOP_tag	/ 5Phos/T*A*GCGCGAGTAGTACCGACTAATGCGTCTCGCGCACSEQTAAGACTACCGCTC*G*T	ID	NO:	134	
CTL078_TOP_tag	$/ \texttt{5Phos/T*A*CGCTCGCACTATCGCTCGATTGGTACCGCCGCTASEQ} \\ \texttt{TACACCATAACCGC*G*C} \\$	ID	NO:	135	
CTL031_TOP_tag	$/ {\tt 5Phos/A*C*CAATCGAGCGAAGTCGAGCGCATAACGCGCTACCSEQ} \\ {\tt TATACGCCGCTAAC*T*A} \\$	ID	NO:	136	
CTL136_TOP_tag	/ 5Phos/A*C*CTTAATCCGCGACTGCGAGCGTACACCGACTAATSEQGCGACTACTGTCGC*G*A	ID	NO:	137	
CTL165_TOP_tag	/ 5Phos/A*G*TAGTGCGCGTATCGCTCGATTGGTTCTTGACGCGSEQCTAGTATAGCGGCG*G*T	ID	NO:	138	
CTL039_TOP_tag	/ 5Phos/T*C*GTCGCACTAGTCGGTACGGTGCGCACATAGSEQTCGTATGGTACGGC*G*T	ID	NO:	139	
CTL036_TOP_tag	$/ {\tt 5Phos/C*G*CGGATTAAGGTAGTCGAGCGCATAACCGCGTACTSEQ} \\ {\tt ACTACGACGACTAG*G*T} \\$	ID	NO:	140	

TABLE 4-continued

	Tag Sequences			
Name	Sequence (5'→3') SEQ	ID	NO	
CTL048_TOP_tag	/5Phos/C*G*ACTATGTGCGCTACGCTCGCACTAACACTACTCGSEQ CGCACCTAGCGCCG*A*A	ID	NO:	141
CTL053_TOP_tag	/5Phos/A*C*CGCCGACTTATTCTCGCGCACTAATCGTCGCACTSEQ AGTAACCGTCGATC*C*G	ID	NO:	142
CTL072_TOP_tag	/5Phos/A*C*CTAGCGTTGCGACCGACTAATGCGGGTAACGAGCSEQGGTTATGGTACGGC*G*T	ID	NO:	143
CTL096_TOP_tag	/ 5 Phos/C*G*CGCTACTAGGTCGCGGTAATAGGTACCTAGCGTTSEQGCGACCTAGTCGCG*T*A	ID	NO:	144
CTL150_TOP_tag	$/ \texttt{5Phos/C*G*TTCGGCTAGGTACTACTCGCGCTACGCATTAGTCSEQ} \\ \texttt{GGTTCGCGACAGTA*G*T} \\$	ID	NO:	145
CTL084_TOP_tag	$/ \texttt{5Phos/C*G*GACGAGCGGTTCGCGGTAATAGGTACGACGACTASEQ} \\ \texttt{GGTTAGCTGACGGC*G*T} \\$	ID	NO:	146
CTL142_TOP_tag	$/ \texttt{5Phos}/\texttt{T*A*CGCTCGCACTAATTGCGGATCGGTACCGACTAATSEQ} \\ \texttt{GCGACCGCGTACTA*C*T} \\$	ID	NO:	147
CTL102_TOP_tag	/ 5Phos/A*C*CGACCGTACCGTATGGTACGGCGTTCTTGACGCGSEQCTAACCTAGCGCCG*A*A	ID	NO:	148
CTL154_TOP_tag	$/ {\tt 5Phos/G*C*GCGGATTAGTTAACCGTCGAGTGCACACTACTCGSEQCGCACTGCGAGCGT*A*C}$	ID	NO:	149
CTL112_TOP_tag	$/ \texttt{5Phos/A*C*CTTAATCCGCGACCGACTAATGCGTACGCGCACTSEQ} \\ \texttt{ACTATAAGTCGGCG*G*T} \\ \\ \texttt{T} \\$	ID	NO:	150
CTL145_TOP_tag	$/ {\tt 5Phos/A*C*CTTAATCCGCGGCGCGGTTATGGTACCGACTAATSEQGCGAACCGCTCGTC*C*G}$	ID	NO:	151
CTL060_TOP_tag	/ 5Phos/A*C*TGCGAGCGTACCTTGTACGGCGGTACCTAGTAGCSEQGCGATAAGTCGGCG*G*T	ID	NO:	152
CTL016_TOP_tag	$/ \texttt{5Phos/T*T*CGGCGCTAGGTACCTTAGTCCGCGTTCGGCGCTASEQ} \\ \texttt{GGTACCTAGCGTTG*C*G} \\$	ID	NO:	153
CTL159_TOP_tag	$/ \texttt{5Phos/A*C*CTAGTCGCGTACTTGTACGGCGGTACCTAGCCGASEQ} \\ \texttt{ACGAACCGTCGAGT*G*C} \\$	ID	NO:	154
CTL056_TOP_tag	/5Phos/A*C*CATAACCGCGCTACACTGCGCGACACCAATACGCSEQCGCTATGGTACGGC*G*T	ID	NO:	155
CTL162_TOP_tag	/5Phos/A*C*ACTACTCGCGCTACGCGACTAGGTAATGTCGAACSEQCGCACGCCGCTAAC*T*A	ID	NO:	156
CTL018_TOP_tag	/5Phos/A*C*CGACTAATGCGTAACAGCGCGTCGTTAGTGCGCGSEQ AGAACCTTAATCGC*G*C	ID	NO:	157
CTL115_TOP_tag	/5Phos/A*C*GCCGTACCATAACCGACTAATGCGATAAGTCGGCSEQ GGTACCAATACGCC*G*C	ID	NO:	158
CTL033_TOP_tag	/5Phos/G*T*ACGCTCGCAGTCGCGGTAATAGGTTCGGCGAGTASEQGTTACCATAACCGC*G*C	ID	NO:	159
CTL047_TOP_tag	/5Phos/C*G*GACGAGCGGTTGCGCGGTTATGGTACTAGTGCGASEQCGAGCGCACATAGT*C*G	ID	NO:	160
CTL108_TOP_tag	/5Phos/A*C*TACTCGCGCTAGCGCGATTAAGGTACGCCGCTAASEQ	ID	NO:	161
CTL041_TOP_tag	/5Phos/A*C*CAATTCGACGCAACTAATCCGCGCACCAATTCGASEQCGCAGTAGTGCGCG*T*A	ID	NO:	162
CTL061_TOP_tag	/5Phos/A*C*CGCCGCTATACACCTAGCGCCGAAGTACGCTCGCSEQ	ID	NO:	163
CTL166_TOP_tag	/5Phos/A*C*ACTACTCGCGCCGGACGAGCGGTTACCAATACGCSEQCGCTAGCGCGGATA*G*T	ID	NO:	164

TABLE 4-continued

	Tag Sequences			
Name	Sequence (5'→3') SEQ	ID	NO	
CTL012_TOP_tag	/5Phos/T*C*GTCGCACTAGTACCTTAATCCGCGCGCAACGCTASEQ GGTACACTACTCGC*G*C	ID	NO:	165
CTL052_TOP_tag	$/ 5 Phos/C \star G \star CGCTACTAGGTACCGACTAATGCGCGCAACGCTASEQ \\ GGTAATGTCGAACC \star G \star C \\$	ID	NO:	166
CTL153_TOP_tag	$/ 5 \texttt{Phos} / \texttt{A*C*GAGCGGTAGTCACTACTGTCGCGACGCAACGCTASEQ} \\ \texttt{GGTTACACTGCGCG*A*C} \\$	ID	NO:	167
CTL094_TOP_tag	$/ 5 \texttt{Phos} / \texttt{A*C*CTAGTCGCGTACGCGTAGTATGGTACCGATCGCTSEQ} \\ \texttt{AGTGGTAACGAGCG*G*T} \\$	ID	NO:	168
CTL095_TOP_tag	$/ 5 Phos/G \star C \star GGTTCGACATTACCGACTAATGCGTATGCGCTCGSEQ \\ ACTACCTAGCGTTG \star C \star G \\$	ID	NO:	169
CTL105_TOP_tag	/5Phos/A*C*TGCGAGCGTACTCTCGCGCACTAAACGCCGCTAASEQ CTACGCGCTACTAG*G*T	ID	NO:	170
CTL109_TOP_tag	/5Phos/C*G*GTACGGTCGGTAATCTAGCCGCGAACCTTAGTCCSEQ GCGACCGCCGTACA*A*G	ID	NO:	171
CTL032_TOP_tag	/ 5Phos/T*C*GGCGAGTAGTTACGCGCTACCTATTCGCGGCTAGSEQATTACGCCGCTAAC*T*A	ID	NO:	172
CTL161_BOT_tag	/ 5Phos/A*C*GCCGCTAACTAGCGCGATTAAGGTGTACGCTCGCSEQAGTGCGCGCAGTG*T*A	ID	NO:	173
CTL164_BOT_tag	$/ \texttt{5Phos/A*G*TAGTGCGCGTAGCGGTTCGACATTAGTAGTACGCSEQ} \\ \texttt{GGTGCACTCGACGG*T*T} \\$	ID	NO:	174
CTL030_BOT_tag	/ 5Phos/T*C*GCGGCTAGATTAGTAGTGCGCGTAACACTACTCGSEQCGCACCTTAGTCCG*C*G	ID	NO:	175
CTL088_BOT_tag	$/ 5 Phos/A * C * TAGCGATCGGTGCGTCGAATTGGTTAGCGCGAGTSEQ \\ AGTTCGTCGCACTA * G * T$	ID	NO:	176
CTL148_BOT_tag	/ 5Phos/C*G*CGGACTAAGGTGCGCGGTTATGGTACACTACTCGSEQCGCGGGTTCGACA*T*T	ID	NO:	177
CTL152_BOT_tag	$/ \texttt{5Phos/A*C*GCGCTACCTATGCGGCGTATTGGTATAAGTCGGCSEQ} \\ \texttt{GGTACCAATTCGAC*G*C} \\$	ID	NO:	178
CTL007_BOT_tag	/ 5Phos/A*C*CATACTACGCGTTGTCGCGCTAGTACCAATTCGASEQCGCCGCGCTACTAG*G*T	ID	NO:	179
CTL141_BOT_tag	/ 5Phos/A*C*CGACCGTACCGTAGTTAGCGGCGTACCTTAATCGSEQCGCGGTAACGAGCG*G*T	ID	NO:	180
CTL064_BOT_tag	$/ \texttt{5Phos/G*T*ACGCTCGCAGTGCGTCGAATTGGTACCTAGCCGASEQ} \\ \texttt{ACGATAAGTCGGCG*G*T} \\$	ID	NO:	181
CTL158_BOT_tag	$/ 5 Phos/T *A *A CAGCGCGTCGCGCGGTAATAGGTGTACGCTCGCSEQ \\ AGTCGCGGATTAAG *G *T \\$	ID	NO:	182
CTL066_BOT_tag	$/ 5 Phos/G \star C \star GTCGAATTGGTTAGCGCGTCAAGAGGTAACGAGCSEQ \\ GGTACCTAGTCGTC \star G \star T$	ID	NO:	183
CTL144_BOT_tag	$/ 5 \texttt{Phos} / \texttt{T*A*CGCTCGCACTAGCGCGGTTATGGTAATGTCGAACSEQ} \\ \texttt{CGCCGCGTAGTATG*G*T} \\$	ID	NO:	184
CTL107_BOT_tag	/5Phos/A*C*TAGTGCGACGAGCGGCGTATTGGTACCAATACGCSEQCGCACCGCCGTACA*A*G	ID	NO:	185
CTL149_BOT_tag	$/ 5 \texttt{Phos} / \texttt{T*T*GTCGCGCTAGTGCGCGATTAAGGTATAAGTCGGCSEQ} \\ \texttt{GGTACTGCGAGCGT*A*C} \\$	ID	NO:	186
CTL008_BOT_tag	/5Phos/C*G*CGGACTAAGGTACTACTCGCGCTAACGCCGTACCSEQ ATAACCTAGTCGTC*G*T	ID	NO:	187
CTL099_BOT_tag	$/ 5 \texttt{Phos} / \texttt{A*C*TAGCGATCGGTTAGCGCGTCAAGAACGCGCTACCSEQ} \\ \texttt{TATGACTACCGCTC*G*T} \\$	ID	NO:	188

TABLE 4-continued

	Tag Sequences			
Name	Sequence (5'→3') SEQ	ID	NO	
CTL089_BOT_tag	/ 5Phos/C*T*TGTACGGCGGTGCGCGGTTATGGTACCAATTCGASEQCGCATTGCGGATCG*G*T	ID	NO:	189
CTL081_BOT_tag	/5Phos/T*C*GCTCGATTGGTCGCGGTAATAGGTTCGCGACAGTSEQAGTTCGTCGCACTA*G*T	ID	NO:	190
CTL075_BOT_tag	/5Phos/A*C*CTAGCGCCGAACGGACGAGCGGTTACTACTGTCGSEQCGACTTGTACGGCG*G*T	ID	NO:	191
CTL160_BOT_tag	$/ \texttt{5Phos/A*C*GCGCTACCTATACCGCGTACTACCGACTAATSEQ} \\ \texttt{GCGACTAGTGCGAC*G*A} \\$	ID	NO:	192
CTL133_BOT_tag	/ 5Phos/A*A*CCGTCGAGTGCGCGCGAGTAGTGTACGCCGCTAASEQCTAGCGTCGAATTG*G*T	ID	NO:	193
CTL076_BOT_tag	$/ 5 Phos/G \star C \star G C G A G T A G T G T A C C G C T C G T A C C T A T T A C C S E Q G C G A C C T A T T A C C S E Q G C G A C C T A T T A C C S E Q G C G A C C T A T T A C C S E Q G C G A C T A C C G C T A C C T A$	ID	NO:	194
CTL024_BOT_tag	/ 5 Phos/T*C*GCTCGATTGGTTACGCGCACTACTTACGCTCGCASEQCTAAACTACTCGCC*G*A	ID	NO:	195
CTL045_BOT_tag	$/ \texttt{5Phos/T*C*GTCGCACTAGTGCGCGGTTATGGTACCATAACCGSEQ} \\ \texttt{CGCTACACTGCGCG*A*C} \\$	ID	NO:	196
CTL009_BOT_tag	/ 5 Phos/A * C * CGCGTACTACTGCGGTTCGACATTACCTTAATCGSEQCGCAGTCGAGCGCA * T * A	ID	NO:	197
CTL055_BOT_tag	/ 5Phos/A*G*TAGTGCGCGTAGCGTCGAATTGGTGCGCACATAGSEQTCGTTGTCGCGCTA*G*T	ID	NO:	198
CTL101_BOT_tag	$/ 5 \\ Phos/G*C*GCGGATTAGTTGCGTCGAATTGGTACCGCCGTACSEQ \\ AAGTCGGCGAGTAG*T*T$	ID	NO:	199
CTL135_BOT_tag	$/ \texttt{5Phos/A*G*TAGTGCGCGTACGTTCGGCTAGGTACCGCCGTACSEQ} \\ \texttt{AAGACCTTAATCCG*C*G} \\$	ID	NO:	200
CTL155_BOT_tag	$/ \texttt{5Phos/A*A*CCGTCGAGTGCATTGCGGATCGGTACCGCCGTACSEQ} \\ \texttt{AAGTCTTGACGCGC*T*A} \\$	ID	NO:	201
CTL122_BOT_tag	/5Phos/G*C*GGCGTATTGGTACCTAGTCGTCGTACCAATACGCSEQCGCACCGACTAATG*C*G	ID	NO:	202
CTL080_BOT_tag	/5Phos/A*C*CGATCGCTAGTCGCATTAGTCGGTACCATAACCGSEQCGCCGCGCTACTAG*G*T	ID	NO:	203
CTL126_BOT_tag	/5Phos/T*A*GTGCGAGCGTATCGCGGCTAGATTACGACGACTASEQ GGTTAGCGCGAGTA*G*T	ID	NO:	204
CTL098_BOT_tag	/5Phos/A*C*CTTAGTCCGCGACTGCGAGCGTACACCTTAATCGSEQCGCGTATAGCGGCG*G*T	ID	NO:	205
CTL038_BOT_tag	/5Phos/A*C*TAGCGATCGGTACTGCGAGCGTACGCACTCGACGSEQGTTAGTAGTGCGCG*T*A	ID	NO:	206
CTL139_BOT_tag	/5Phos/A*C*CTAGTCGTCGTTCTCGCGCACTAACGACGCGCTGSEQ TTATACACTGCGCG*A*C	ID	NO:	207
CTL010_BOT_tag	/5Phos/G*C*GGCGTATTGGTGTATAGCGGCGGTACCATACTACSEQ GCGACCAATTCGAC*G*C	ID	NO:	208
CTL034_BOT_tag	/5Phos/T*A*ACAGCGCGTCGACTAGCGATCGGTACCTAGTCGCSEQ GTAAGTAGTGCGCG*T*A	ID	NO:	209
CTL117_BOT_tag	/5Phos/G*C*GCGGATTAGTTGCGTCGAATTGGTACGCCGCTAASEQ	ID	NO:	210
CTL035_BOT_tag	/5Phos/A*T*TGCGGATCGGTAGTGCGCGTAACGCCGCTAASEQ	ID	NO:	211
CTL121_BOT_tag	/5Phos/A*C*GCGCTACCTATTAGTTAGCGGCGTATAAGTCGGCSEQ GGTACCTAGTCGTC*G*T	ID	NO:	212

TABLE 4-continued

Tag Sequences				
Name	Sequence (5'→3') SEQ	ID	NO	
CTL106_BOT_tag	/5Phos/G*T*CGCGCAGTGTAACCGCGTACTACTACACTACTCGSEQ	ID	NO:	213
CTL059_BOT_tag	/5Phos/A*C*CAATCGAGCGAATTGCGGATCGGTATAAGTCGGCSEQ GGTACCGATCCGCA*A*T	ID	NO:	214
CTL157_BOT_tag	/5Phos/G*G*TAACGAGCGGTGCGCGATTAAGGTGTACGCTCGCSEQ AGTGTACGCTCGCA*G*T	ID	NO:	215
CTL015_BOT_tag	/ 5Phos/A*C*CGATCCGCAATTAGTGCGAGCGTAACTAGTGCGASEQCGATCGCGACAGTA*G*T	ID	NO:	216
CTL110_BOT_tag	$/ \texttt{5Phos/C*G*CGTAGTATGGTTCTCGCGCACTAATTAGTGCGCGSEQ} \\ \texttt{AGAACCGCTCGTTA*C*C} \\$	ID	NO:	217
CTL123_BOT_tag	$/ \texttt{5Phos/T*C*GGCGAGTAGTTGCGCGATTAAGGTACCTTAATCGSEQ} \\ \texttt{CGCTAGCGCGAGTA*G*T} \\$	ID	NO:	218
CTL014_BOT_tag	$/ {\tt 5Phos/G*C*ACTCGACGGTTGCGTCGAATTGGTACCGCCGTACSEQ} \\ {\tt AAGAGTAGTGCGCG*T*A} \\$	ID	NO:	219
CTL131_BOT_tag	$/ \texttt{5Phos}/\texttt{T*C*GTCGCACTAGTGCGCGATTAAGGTACCGATCCGCSEQ} \\ \texttt{AATCGGATCGACGG*T*T} \\$	ID	NO:	220
CTL062_BOT_tag	/ 5Phos/C*G*CGGATTAAGGTGCGCGAGTAGTGTGTCGCGCAGTSEQGTATACGCGCACTA*C*T	ID	NO:	221
CTL044_BOT_tag	/ 5Phos/A*C*CTAGCGCCGAATACGCGCACTACTACCTATTACCSEQGCGTATGGTACGGC*G*T	ID	NO:	222
CTL043_BOT_tag	$/ \texttt{5Phos/A*C*CGCGTACTACTACCGCCGACTTATCGCAACGCTASEQ} \\ \texttt{GGTTCTTGACGCGC*T*A} \\$	ID	NO:	223
CTL118_BOT_tag	/ 5Phos/A*C*TAGCGATCGGTGCGCGGTTATGGTTCGCGGCTAGSEQATTACCGACTAATG*C*G	ID	NO:	224
CTL128_BOT_tag	/ 5Phos/A*C*CGCCGACTTATTAGTTAGCGGCGTACCAATACGCSEQCGCACGCCGTACCA*T*A	ID	NO:	225
CTL067_BOT_tag	$/ {\tt 5Phos/C*G*GACGAGCGGTTGACTACCGCTCGTACCAATACGCSEQCGCACCATAACCGC*G*C}$	ID	NO:	226
CTL020_BOT_tag	/ 5Phos/A*G*TCGAGCGCATAGCGCGGTTATGGTTCGGCGAGTASEQGTTGCGCACATAGT*C*G	ID	NO:	227
CTL006_BOT_tag	$/ \texttt{5Phos/C*G*CGTAGTATGGTGCGCGATTAAGGTGGTAACGAGCSEQ} \\ \texttt{GGTACGCCGCTAAC*T*A} \\$	ID	NO:	228
CTL017_BOT_tag	/ 5Phos/T*C*TCGCGCACTAACGGACGAGCGGTTTACGCGCACTSEQACTACCGACTAATG*C*G	ID	NO:	229
CTL057_BOT_tag	$/ \texttt{5Phos/A*C*GAGCGGTAGTCTTAGTGCGCGAGACGCATTAGTCSEQ} \\ \texttt{GGTACTACTCGCGC*T*A} \\$	ID	NO:	230
CTL078_BOT_tag	$/ {\tt 5Phos/G*C*GCGGTTATGGTGTATAGCGGCGGTACCAATCGAGSEQCGATAGTGCGAGCG*T*A}$	ID	NO:	231
CTL031_BOT_tag	$/ \texttt{5Phos}/\texttt{T*A*GTTAGCGGCGTATAGGTAGCGCGTTATGCGCTCGSEQ} \\ \texttt{ACTTCGCTCGATTG*G*T} \\$	ID	NO:	232
CTL136_BOT_tag	$/ \texttt{5Phos}/\texttt{T*C*GCGACAGTAGTCGCATTAGTCGGTGTACGCTCGCSEQ} \\ \texttt{AGTCGCGGATTAAG*G*T} \\$	ID	NO:	233
CTL165_BOT_tag	/ 5Phos/A*C*CGCCGCTATACTAGCGCGTCAAGAACCAATCGAGSEQCGATACGCGCACTA*C*T	ID	NO:	234
CTL039_BOT_tag	$/ {\tt 5Phos/A*C*GCCGTACCATACGACTATGTGCGCACCGACCGTASEQCCGACTAGTGCGAC*G*A}$	ID	NO:	235
CTL036_BOT_tag	$/ {\tt 5Phos/A*C*CTAGTCGTCGTAGTAGTACGCGGTTATGCGCTCGSEQACTACCTTAATCCG*C*G}$	ID	NO:	236

TABLE 4-continued

	Tag Sequences			
Name	Sequence (5'→3') SEQ	ID	ио	
CTL048_BOT_tag	/5Phos/T*T*CGGCGCTAGGTGCGCGAGTAGTGTTAGTGCGAGCSEQ GTAGCGCACATAGT*C*G	ID	NO:	237
CTL053_BOT_tag	/5Phos/C*G*GATCGACGGTTACTAGTGCGACGATTAGTGCGCGSEQAGAATAAGTCGGCG*G*T	ID	NO:	238
CTL072_BOT_tag	/5Phos/A*C*GCCGTACCATAACCGCTCGTTACCCGCATTAGTCSEQGGTCGCAACGCTAG*G*T	ID	NO:	239
CTL096_BOT_tag	/5Phos/T*A*CGCGACTAGGTCGCAACGCTAGGTACCTATTACCSEQGCGACCTAGTAGCG*C*G	ID	NO:	240
CTL150_BOT_tag	/5Phos/A*C*TACTGTCGCGAACCGACTAATGCGTAGCGCGAGTSEQ AGTACCTAGCCGAA*C*G	ID	NO:	241
CTL084_BOT_tag	$/ 5 Phos/A \star C \star GCCGCTAACTAACCTAGTCGTCGTACCTATTACCSEQ GCGAACCGCTCGTC \star C \star G$	ID	NO:	242
CTL142_BOT_tag	/ 5 Phos/A*G*TAGTACGCGGTCGCATTAGTCGGTACCGATCCGCSEQAATTAGTGCGAGCG*T*A	ID	NO:	243
CTL102_BOT_tag	$/ \texttt{5Phos}/\texttt{T*T*CGGCGCTAGGTTAGCGCGTCAAGAACGCCGTACCSEQ} \\ \texttt{ATACGGTACGGTCG*G*T} \\$	ID	NO:	244
CTL154_BOT_tag	$/ \texttt{5Phos}/\texttt{G} \star \texttt{T} \star \texttt{ACGCTCGCAGTGCGCGAGTAGTGTGCACTCGACGSEQ} \\ \texttt{GTTAACTAATCCGC} \star \texttt{G} \star \texttt{C}$	ID	NO:	245
CTL112_BOT_tag	$/ \texttt{5Phos/A*C*CGCCGACTTATAGTAGTGCGCGTACGCATTAGTCSEQ} \\ \texttt{GGTCGCGGATTAAG*G*T} \\$	ID	NO:	246
CTL145_BOT_tag	$/ {\tt 5Phos/C*G*GACGAGCGGTTCGCATTAGTCGGTACCATAACCGSEQ} \\ {\tt CGCCGCGGATTAAG*G*T} \\$	ID	NO:	247
CTL060_BOT_tag	$/ \texttt{5Phos/A*C*CGCCGACTTATCGCGCTACTAGGTACCGCCGTACSEQ} \\ \texttt{AAGGTACGCTCGCA*G*T} \\$	ID	NO:	248
CTL016_BOT_tag	$/ {\tt 5Phos/C*G*CAACGCTAGGTACCTAGCGCCGAACGCGGACTAASEQ} \\ {\tt GGTACCTAGCGCCG*A*A} \\$	ID	NO:	249
CTL159_BOT_tag	$/ 5 \texttt{Phos} / \texttt{G*C*ACTCGACGGTTCGTTCGGCTAGGTACCGCCGTACSEQ} \\ \texttt{AAGTACGCGACTAG*G*T} \\$	ID	NO:	250
CTL056_BOT_tag	/5Phos/A*C*GCCGTACCATAGCGGCGTATTGGTGTCGCGCAGTSEQGTAGCGCGGTTATG*G*T	ID	NO:	251
CTL162_BOT_tag	/5Phos/T*A*GTTAGCGGCGTGCGGTTCGACATTACCTAGTCGCSEQGTAGCGCGAGTAGT*G*T	ID	NO:	252
CTL018_BOT_tag	/5Phos/G*C*GCGATTAAGGTTCTCGCGCACTAACGACGCGCTGSEQ TTACGCATTAGTCG*G*T	ID	NO:	253
CTL115_BOT_tag	/5Phos/G*C*GGCGTATTGGTACCGCCGACTTATCGCATTAGTCSEQ GGTTATGGTACGGC*G*T	ID	NO:	254
CTL033_BOT_tag	/5Phos/G*C*GCGGTTATGGTAACTACTCGCCGAACCTATTACCSEQ GCGACTGCGAGCGT*A*C	ID	NO:	255
CTL047_BOT_tag	/5Phos/C*G*ACTATGTGCGCTCGTCGCACTAGTACCATAACCGSEQCGCAACCGCTCGTC*C*G	ID	NO:	256
CTL108_BOT_tag	/5Phos/A*A*TCTAGCCGCGATAGTTAGCGGCGTACCTTAATCGSEQCGCTAGCGCGAGTA*G*T	ID	NO:	257
CTL041_BOT_tag	/5Phos/T*A*CGCGCACTACTGCGTCGAATTGGTGCGCGGATTASEQGTTGCGTCGAATTG*G*T	ID	NO:	258
CTL061_BOT_tag	/5Phos/A*C*CGCCGCTATACACTGCGAGCGTACTTCGGCGCTASEQ GGTGTATAGCGGCG*G*T	ID	NO:	259
CTL166_BOT_tag	/5Phos/A*C*TACTCGCGCTAGCGGCGTATTGGTAACCGCTCGTSEQ CCGGCGCGAGTAGT*G*T	ID	NO:	260

TABLE 4-continued

	Tag Sequences					
Name	Sequence (5'→3') SEQ	ID	NO			
CTL012_BOT_tag	/5Phos/G*C*GCGAGTAGTGTACCTAGCGTTGCGCGCGGATTAASEQ GGTACTAGTGCGAC*G*A	ID	NO:	261		
CTL052_BOT_tag	/5Phos/G*C*GGTTCGACATTACCTAGCGTTGCGCGCATTAGTCSEQ GGTACCTAGTAGCG*C*G	ID	NO:	262		
CTL153_BOT_tag	/5Phos/G*T*CGCGCAGTGTAACCTAGCGTTGCGTCGCGACAGTSEQ AGTGACTACCGCTC*G*T	ID	NO:	263		
CTL094_BOT_tag	/5Phos/A*C*CGCTCGTTACCACTAGCGATCGGTACCATACTACSEQ GCGTACGCGACTAG*G*T	ID	NO:	264		
CTL095_BOT_tag	/5Phos/C*G*CAACGCTAGGTAGTCGAGCGCATACGCATTAGTCSEQ GGTAATGTCGAACC*G*C	ID	NO:	265		
CTL105_BOT_tag	/5Phos/A*C*CTAGTAGCGCGTAGTTAGCGGCGTTTAGTGCGCGSEQ AGAGTACGCT CGCA*G*T	ID	NO:	266		
CTL109_BOT_tag	/5Phos/C*T*TGTACGGCGGTCGCGGACTAAGGTTCGCGGCTAGSEQ ATTACCGACCGTAC*C*G	ID	NO:	267		
CTL032_BOT_tag	/5Phos/T*A*GTTAGCGGCGTAATCTAGCCGCGAATAGGTAGCGSEQ CGTAACTACTCGCC*G*A	ID	NO:	268		

[&]quot;/5Phos/" indicates a 5'-phosphate moiety; "*" indicates a phosphorothioate linkage.

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
	CTL169 CTL137 CTL042 CTL051 CTL167 CTL026 CTL068 CTL138 CTL079 CTL063	CTL164 CTL030 CTL088 CTL148 CTL152 CTL007 CTL141 CTL064 CTL158 CTL066	CTL081 CTL075 CTL160 CTL133 CTL076 CTL024 CTL045 CTL009 CTL055 CTL101	CTL139 CTL010 CTL034 CTL117 CTL035 CTL121 CTL106 CTL059 CTL157	CTL044 CTL043 CTL118 CTL128 CTL067 CTL020 CTL006 CTL017 CTL057 CTL057	CTL053 CTL072 CTL096 CTL150 CTL084 CTL142 CTL102 CTL154 0TL112 0TL1145	CTL115 CTL033 CTL047 CTL108 CTL041 CTL061 CTL166 CTL166 CTL012 CTL052 CTL153	Pool A1 Pool B1 Pool B2 Pool B3 Pool B4 Pool B5 Pool B6
	CTL021 CTL151 CTL002	CTL107 CTL149 CTL008	CTL155 CTL122 CTL080	CTL015 CTL110 CTL123 CTL014 CTL131	CTL136 CTL165 CTL039	CTL159 CTL056	CTL095 CTL105 CTL109	

TABLE 6

TABLE 6-continued

	Non-homologous tai	ls		Non-homologous t	ails
Jame	Sequence (5'→3')	SEQ ID NO:	Name	Sequence (5'→3')	SEQ ID NO:
1	ACGCGACTATACGCGCAATATGGT	SEQ ID NO: 269			
			H4	CGCGAGTACGTACGATTACCG	SEQ ID NO: 272
2	CTAGCGATACTACGCGATACGAGAT	SEQ ID NO: 270	Н5	ACGCGCGACTATACGCGCCTC	SEO ID NO: 273
[3	CATAGCGGTATTACGCGAGATTACGA	SEQ ID NO: 271	113	Acdededaciaiacdedecie	SEQ ID NO. 273
		SEQUENCE LISTING			

```
Sequence total quantity: 273
SEQ ID NO: 1 moltype = DNA length = 52
FEATURE Location/Qualifiers
source
                      1..52
                      mol type = other DNA
                      organism = synthetic construct
SEQUENCE: 1
tegttegtte egetetaace ggegaateta eegegeatat etaegeegea at
SEQ ID NO: 2
                       moltype = DNA length = 52
                       Location/Qualifiers
FEATURE
                       1..52
source
                      mol_type = other DNA
organism = synthetic construct
SEQUENCE: 2
                                                                   52
attgcggcgt agatatgcgc ggtagattcg ccggttagag cggaacgaac ga
                       moltype = DNA length = 57
SEQ ID NO: 3
FEATURE
                       Location/Qualifiers
                       1..57
source
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 3
                                                                   57
acactettte ectacaegae getetteega tettetaeeg egeatateta egeeget
SEQ ID NO: 4
                       moltype = DNA length = 58
FEATURE
                       Location/Qualifiers
source
                      1..58
                       mol_type = other DNA
                       organism = synthetic construct
SEOUENCE: 4
acactettte ectacaegae getetteega tetatatgeg eggtagatte geeggttt
                                                                   58
SEQ ID NO: 5
                      moltype = DNA length = 68
FEATURE
                       Location/Qualifiers
source
                      1..68
                       mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 5
gtgactggag ttcagacgtg tgctcttccg atctaatgat acggcgacca ccgagatcta 60
cacaaggc
SEQ ID NO: 6
                      moltype = DNA length = 78
FEATURE
                      Location/Qualifiers
                      1..78
source
                      mol type = other DNA
                      organism = synthetic construct
SEOUENCE: 6
aatgatacgg cgaccaccga gatctacact agatcgcnnw nnwnnacact ctttccctac 60
acgacgetet teegatet
SEQ ID NO: 7
                       moltype = DNA length = 33
FEATURE
                      Location/Qualifiers
source
                       1..33
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 7
acactettte ectacaegae getetteega tet
                                                                   33
SEQ ID NO: 8
                       moltype = DNA length = 34
```

Location/Qualifiers

FEATURE

		-continued	
source	134 mol_type = other DNA		
SEQUENCE: 8	organism = synthetic c	onstruct	
gtgactggag ttcagacgtg	tgctcttccg atct		34
SEQ ID NO: 9 FEATURE source	moltype = DNA length Location/Qualifiers 1.52 mol_type = other DNA organism = synthetic c		
SEQUENCE: 9 acgagcggta gtcacctagt	cgtcgtacca attcgacgca c		52
SEQ ID NO: 10 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic of		
SEQUENCE: 10 gcgcgagtag tgtgcgtcga	attggtacga cgactaggtg a	ctaccgctc gt	52
SEQ ID NO: 11 FEATURE source	<pre>moltype = DNA length Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 11	organism = synthetic c	onstruct	
	geggttaeca ataegeegea e	cttaatccg cg	52
SEQ ID NO: 12 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic c		
SEQUENCE: 12	attggtaacc gctcgtccga c		52
SEQ ID NO: 13 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA	= 52	5 <u>2</u>
SEQUENCE: 13	organism = synthetic c		52
SEQ ID NO: 14	ctaggtacct attaccgcgt a moltype = DNA length		52
FEATURE source	Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 14	organism = synthetic c		
	ataggtacet ageegaaega e		52
SEQ ID NO: 15 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic c		
SEQUENCE: 15 cgcgctacta ggtgcgtcga	attggtaccg atccgcaata c		52
SEQ ID NO: 16 FEATURE source	<pre>moltype = DNA length Location/Qualifiers 152</pre>	= 52	
GEOLDWAR 15	<pre>mol_type = other DNA organism = synthetic organism = synthetic</pre>	onstruct	
SEQUENCE: 16 gcgcgagtag tgtattgcgg	ateggtacea attegaegea e	ctagtagcg cg	52
SEQ ID NO: 17 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol type = other DNA	= 52	
SEQUENCE: 17	organism = synthetic c	onstruct	

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ggtaacgagc ggtgcgtcga	attggtaacc gctcgtccga ccttaatcgc gc	52
SEQ ID NO: 18 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 18	organism - synchecic consciuce	
	geggttacca attegaegea eegetegtta ee	52
SEQ ID NO: 19 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 19	organism = synthetic construct attggtaacc gctcgtccgt tcggcgctag gt	52
treggegera ggrgeggegr	accygeaace geregeeege reggegerag gr	32
SEQ ID NO: 20 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	
CECHENCE 20	organism = synthetic construct	
SEQUENCE: 20 acctagggc qaacqa	geggttacca atacgeegea cetagegeeg aa	52
SEQ ID NO: 21 FEATURE	moltype = DNA length = 52 Location/Qualifiers 152	
source	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 21 tacgcgacta ggtgcgcgat	taaggtacct attaccgcgc gactatgtgc gc	52
SEQ ID NO: 22 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 22 gcgcacatag tcgcgcggta	ataggtacet taategegea cetagtegeg ta	52
SEQ ID NO: 23 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
SEOUENCE: 23	<pre>mol_type = other DNA organism = synthetic construct</pre>	
~	taaggtacct attaccgcgt cgcgacagta gt	52
SEQ ID NO: 24 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>	
	organism = synthetic construct	
SEQUENCE: 24 actactgtcg cgacgcggta	ataggtacct taatcgcgct acactgcgcg ac	52
SEQ ID NO: 25 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 25 aaccqtcqat ccqcqcqtaq	organism = synthetic construct tatggtaccg atccgcaata ctagcgcgac aa	52
SEQ ID NO: 26	moltype = DNA length = 52	
FEATURE source	Location/Qualifiers 152 mol_type = other DNA	
GEOTIMAN C.	organism = synthetic construct	
SEQUENCE: 26 ttgtcgcgct agtattgcgg	ateggtacea tactaegege ggategaegg tt	52
SEQ ID NO: 27 FEATURE	moltype = DNA length = 52 Location/Qualifiers	

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source	152 mol_type = other DNA		
SEQUENCE: 27	organism = synthetic c		
	actacttatg cgctcgactc g		52
SEQ ID NO: 28 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic c		
SEQUENCE: 28 acctagccga acgagtcgag	cgcataagta gtgcgcgtaa c		52
SEQ ID NO: 29 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic of		
SEQUENCE: 29 actgcgagcg tacttgtcgc	gctagtacca attcgacgca a	ccgctcgtc cg	52
SEQ ID NO: 30 FEATURE source	<pre>moltype = DNA length Location/Qualifiers 152 mol_type = other DNA</pre>	= 52	
SEQUENCE: 30	organism = synthetic c	onstruct	
	attggtacta gcgcgacaag t	acgctcgca gt	52
SEQ ID NO: 31 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic c		
SEQUENCE: 31	attggtaacc gctcgtccga c		52
SEQ ID NO: 32 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA	= 52	
SEQUENCE: 32 ataggtagcg cgtcggacga	organism = synthetic c geggttacca atacgccgca c		52
SEQ ID NO: 33 FEATURE	moltype = DNA length Location/Qualifiers		
source	152 mol_type = other DNA organism = synthetic c	onstruct	
SEQUENCE: 33 attgcggatc ggtgcgtcga	attggtaacc gctcgtccgt a	.cgcgcacta ct	52
SEQ ID NO: 34 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic of		
SEQUENCE: 34 agtagtgcgc gtacggacga	geggttacca attegacgea e		52
SEQ ID NO: 35 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol type = other DNA	= 52	
SEQUENCE: 35	organism = synthetic c		50
reggegagta gttgegeggt	tatggtacca taaccgcgca g		52
SEQ ID NO: 36 FEATURE source	<pre>moltype = DNA length Location/Qualifiers 152 mol_type = other DNA</pre>	= 52	
SEQUENCE: 36	organism = synthetic c	onstruct	

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accgcgtact actgcgcggt	tatggtacca taaccgcgca actactcgcc ga	52
SEQ ID NO: 37 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA</pre>	
SEQUENCE: 37	organism = synthetic construct	
	gccgaaacct attaccgcga cctagcgttg cg	52
SEQ ID NO: 38 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
GROVENGE 20	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 38 cgcaacgcta ggtcgcggta	ataggtttcg gcgctaggta ccgatcgcta gt	52
SEQ ID NO: 39 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
boarec	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 39	totaatttaa aaaataaatt oosooosa s	F2
	tatggtttcg gcgctaggtt aacagcgcgt cg	52
SEQ ID NO: 40 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 40 cgacgcgctg ttaacctagc	gccgaaacca taaccgcgct cttgacgcgc ta	52
SEQ ID NO: 41 FEATURE	moltype = DNA length = 34 Location/Qualifiers	
source	134 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 41 gtttaattga gttgtcatat	-	34
SEQ ID NO: 42 FEATURE	moltype = DNA length = 34 Location/Qualifiers	
source	134 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 42		
ataccgttat taacatatga	caactcaatt aaac	34
SEQ ID NO: 43 FEATURE source	<pre>moltype = DNA length = 20 Location/Qualifiers 120</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 43 gagtccgagc agaagaagaa		20
SEQ ID NO: 44 FEATURE source	<pre>moltype = DNA length = 20 Location/Qualifiers 120 mol_type = other DNA</pre>	
SEQUENCE: 44 gttggagcat ctgagtccag	organism = synthetic construct	20
		~~
SEQ ID NO: 45 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 45 acgagcggta gtcacctagt	cgtcgtacca attcgacgca cactactcgc gc	52
SEQ ID NO: 46 FEATURE	moltype = DNA length = 52 Location/Qualifiers	

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source	152 mol_type = other DNA		
SEQUENCE: 46	organism = synthetic co	onstruct	
tagegegagt agteggaega	geggttacca atacgeegea ee	cttaatccg cg	52
SEQ ID NO: 47 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 47 tegegacagt agtegttegg	organism = synthetic co		52
SEQ ID NO: 48 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA organism = synthetic co		
SEQUENCE: 48 cgcgctacta ggtgcgtcga	attggtaccg atccgcaata ca	actactcgc gc	52
SEQ ID NO: 49 FEATURE source	<pre>moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA</pre>	= 52	
SEQUENCE: 49	organism = synthetic co	onstruct	
	attggtaacc gctcgtccga cc	cttaatcgc gc	52
SEQ ID NO: 50 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA organism = synthetic co		
SEQUENCE: 50	-		
tteggegeta ggtgeggegt	attggtaacc gctcgtccgt tc	eggegetag gt	52
SEQ ID NO: 51 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 51	organism = synthetic co		52
SEQ ID NO: 52	moltype = DNA length =		52
FEATURE source	Location/Qualifiers 152	- 9 2	
SEOUENCE: 52	<pre>mol_type = other DNA organism = synthetic co</pre>	onstruct	
~	taaggtacct attaccgcgt cg	gegaeagta gt	52
SEQ ID NO: 53 FEATURE source	moltype = DNA length = Location/Qualifiers 1.52 mol_type = other DNA organism = synthetic co		
SEQUENCE: 53 aaccgtcgat ccgcgcgtag	tatggtaccg atccgcaata ct		52
SEQ ID NO: 54 FEATURE source	<pre>moltype = DNA length = Location/Qualifiers 152</pre>	= 52	
	<pre>mol_type = other DNA organism = synthetic co</pre>	onstruct	
SEQUENCE: 54 tegetegatt ggttaegege	actacttatg cgctcgactc gt	tcggctag gt	52
SEQ ID NO: 55 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol type = other DNA	- 52	
SEQUENCE: 55	organism = synthetic co	onstruct	

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actgcgagcg tacttgtcgc	gctagtacca attcgacgca accgctcgtc cg	52
SEQ ID NO: 56 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 56	3	
cgcattagtc ggtgcggcgt	attggtaacc getegteega egegetaeet at	52
SEQ ID NO: 57 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 57	organism = synthetic construct	
	attggtaacc getegteegt aegegeacta et	52
SEQ ID NO: 58 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEOUENCE: 58	organism = synthetic construct	
~	tatggtacca taaccgcgca gtagtacgcg gt	52
SEQ ID NO: 59 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	mol_type = other DNA	
SEQUENCE: 59	organism = synthetic construct	52
	gccgaaacct attaccgcga cctagcgttg cg	52
SEQ ID NO: 60 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 60 tagcgcgtca agagcgcggt	tatggtttcg gcgctaggtt aacagcgcgt cg	52
SEQ ID NO: 61 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 61 gcgcgagtag tgtgcgtcga	attggtacga cgactaggtg actaccgctc gt	52
SEQ ID NO: 62	moltype = DNA length = 52	
FEATURE source	Location/Qualifiers 152 mol type = other DNA	
	organism = synthetic construct	
SEQUENCE: 62 cgcggattaa ggtgcggcgt	attggtaacc gctcgtccga ctactcgcgc ta	52
SEQ ID NO: 63 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>	
CROHENCE . 63	organism = synthetic construct	
SEQUENCE: 63 acgccgctaa ctacgcggta	ataggtacct agccgaacga ctactgtcgc ga	52
SEQ ID NO: 64 FEATURE	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
source	mol_type = other DNA	
SEQUENCE: 64	organism = synthetic construct	
	atcggtacca attcgacgca cctagtagcg cg	52
SEQ ID NO: 65 FEATURE	moltype = DNA length = 52 Location/Qualifiers	

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source	152 mol type = other DNA		
SEQUENCE: 65	organism = synthetic construct		
	geggttacca attegaegea eegetegtta	cc	52
SEQ ID NO: 66 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
GROUPINGS 66	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 66 acctagegee gaaeggaega	geggttacca atacgeegea eetagegeeg	aa	52
SEQ ID NO: 67 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 67 gcgcacatag tcgcgcggta	ataggtacct taatcgcgca cctagtcgcg	ta	52
SEQ ID NO: 68 FEATURE	moltype = DNA length = 52 Location/Qualifiers 152		
source	mol_type = other DNA organism = synthetic construct		
SEQUENCE: 68 actactgtcg cgacgcggta	ataggtacet taategeget acaetgegeg	ac	52
SEQ ID NO: 69 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 69	organism = synthetic construct		
	ateggtacea tactaegege ggategaegg	tt	52
SEQ ID NO: 70 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 70	organism = synthetic construct		
acctagccga acgagtcgag	cgcataagta gtgcgcgtaa ccaatcgagc	ga	52
SEQ ID NO: 71 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152		
SEQUENCE: 71	<pre>mol_type = other DNA organism = synthetic construct</pre>		
~	attggtacta gcgcgacaag tacgctcgca	gt	52
SEQ ID NO: 72 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 72	organism = synthetic construct gcggttacca atacgccgca ccgactaatg	ca	52
SEQ ID NO: 73	moltype = DNA length = 52	3	
FEATURE source	Location/Qualifiers 152 mol type = other DNA		
SEQUENCE: 73	organism = synthetic construct		50
	geggttacca attegaegea eegateegea	at	52
SEQ ID NO: 74 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152		
SEQUENCE: 74	<pre>mol_type = other DNA organism = synthetic construct</pre>		
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accgcgtact actgcgcg	gt tatggtacca taaccgcgca actactcgcc ga	52	
SEQ ID NO: 75 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>		
	organism = synthetic construct		
SEQUENCE: 75			
cgcaacgcta ggtcgcgg	ta ataggtttcg gcgctaggta ccgatcgcta gt	52	
SEQ ID NO: 76 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 76	organism = synthetic construct		
cgacgcgctg ttaaccta	gc gccgaaacca taaccgcgct cttgacgcgc ta	52	
SEQ ID NO: 77 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 77	•		
tacactgcgc gacactgc	ga gegtacacet taategeget agttagegge gt	52	
SEQ ID NO: 78 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>		
	organism = synthetic construct		
SEQUENCE: 78 aaccgtcgag tgcaccgc	gt actactaatg togaacogot acgogoacta ct	52	
SEQ ID NO: 79 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 79 cgcggactaa ggtgcgcg	ag tagtgttacg cgcactacta atctageege ga	52	
SEQ ID NO: 80 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 80 actagtgcga cgaactac	te gegetaacca attegaegea eegategeta gt	52	
SEQ ID NO: 81 FEATURE	moltype = DNA length = 52 Location/Qualifiers		
source	152 mol_type = other DNA		
CECHENCE. 01	organism = synthetic construct		
SEQUENCE: 81 aatgtcgaac cgcgcgcg	ag tagtgtacca taaccgcgca ccttagtccg cg	52	
SEQ ID NO: 82 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 82	organism = synthetic construct		
	cg acttatacca atacgeegea taggtagege gt	52	
SEQ ID NO: 83 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 83	organism - synthetic constituet		
	ga attggtacta gcgcgacaac gcgtagtatg gt	52	
SEQ ID NO: 84 FEATURE	moltype = DNA length = 52 Location/Qualifiers		

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source	152 mol_type = other DNA		
SEQUENCE: 84	organism = synthetic c	onstruct	
accgctcgtt accgcgcgat	taaggtacgc cgctaactac g	gtacggtcg gt	52
SEQ ID NO: 85 FEATURE source	<pre>moltype = DNA length Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 85 accgccgact tatcgttcgg	organism = synthetic c ctaggtacca attcgacgca c		52
SEQ ID NO: 86 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic c		
SEQUENCE: 86 accttaatcc gcgactgcga	gcgtacacct attaccgcgc g		52
SEQ ID NO: 87 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 87	organism = synthetic c		
acgacgacta ggtaccgctc	gttacctctt gacgcgctaa c	caattegae ge	52
SEQ ID NO: 88 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic c		
SEQUENCE: 88	-		52
SEQ ID NO: 89 FEATURE source	<pre>gacattacca taaccgcgct a moltype = DNA length Location/Qualifiers 152 mol_type = other DNA</pre>	= 52	<i>3</i> 2
SEQUENCE: 89	organism = synthetic c	onstruct	
cttgtacggc ggtgcggcgt	attggtacca atacgccgct c	gtcgcacta gt	52
SEQ ID NO: 90 FEATURE source	<pre>moltype = DNA length Location/Qualifiers 152 mol type = other DNA</pre>	= 52	
SEQUENCE: 90	organism = synthetic c	onstruct	
	acttatacct taatcgcgca c	tagogogao aa	52
SEQ ID NO: 91 FEATURE source	moltype = DNA length Location/Qualifiers 152 mol_type = other DNA organism = synthetic c		
SEQUENCE: 91 acgacgacta ggttatggta	cggcgttagc gcgagtagta c	cttagteeg eg	52
SEQ ID NO: 92 FEATURE source	<pre>moltype = DNA length Location/Qualifiers 152 mol_type = other DNA</pre>	= 52	
SEQUENCE: 92	organism = synthetic c		
acgagcggta gtcataggta	gcgcgttctt gacgcgctaa c	cgatcgcta gt	52
SEQ ID NO: 93 FEATURE source	<pre>moltype = DNA length Location/Qualifiers 152 mol_type = other DNA</pre>	= 52	
SEQUENCE: 93	organism = synthetic c	onstruct	

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accgatccgc aatgcgtcga	attggtacca taaccgcgca ccgccgtaca ag	52
SEQ ID NO: 94 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
CROHENCE 04	organism = synthetic construct	
SEQUENCE: 94 actagtgcga cgaactactg	tegegaacet attacegega eeaategage ga	52
SEQ ID NO: 95 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA	
SEQUENCE: 95	organism = synthetic construct	
	agtagtaacc gctcgtccgt tcggcgctag gt	52
SEQ ID NO: 96 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 96		F2
	gteggtagta gtaegeggta taggtagege gt	52
SEQ ID NO: 97 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA	
	organism = synthetic construct	
SEQUENCE: 97 accaattcga cgctagttag	cggcgtacac tactcgcgcg cactcgacgg tt	52
SEQ ID NO: 98 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 98 cgcggtaata ggtcgcggta	ataggtacga gcggtagtca cactactcgc gc	52
SEQ ID NO: 99 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
SEQUENCE: 99	mol_type = other DNA organism = synthetic construct	
~	agcgtaagta gtgcgcgtaa ccaatcgagc ga	52
SEQ ID NO: 100 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 100 gtcgcgcagt gtagcgcggt	tatggtacca taaccgcgca ctagtgcgac ga	52
SEQ ID NO: 101 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
CHOURNON 101	organism = synthetic construct	
SEQUENCE: 101 tatgcgctcg actgcgcgat	taaggtaatg tcgaaccgca gtagtacgcg gt	52
SEQ ID NO: 102 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 102 actagegega caaegaetat	gtgcgcacca attcgacgct acgcgcacta ct	52
SEQ ID NO: 103	moltype = DNA length = 52	
FEATURE	Location/Qualifiers	

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source	152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 103	-		
aactactcgc cgacttgtac	ggcggtacca attcgacgca actaatccgc	: gc	52
SEQ ID NO: 104 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 104	organism = synthetic construct		
	ggcggtacct agccgaacgt acgcgcacta	ı ct	52
SEQ ID NO: 105 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 105	organism = synthetic construct		
	ggcggtaccg atccgcaatg cactcgacgg	; tt	52
SEQ ID NO: 106 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 106 cgcattagtc ggtgcggcgt	attggtacga cgactaggta ccaatacgc	: gc	52
SEQ ID NO: 107 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 107 acctagtagc gcggcgcggt	tatggtaccg actaatgcga ctagcgatcg	ı gt	52
SEQ ID NO: 108 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>		
	organism = synthetic construct		
SEQUENCE: 108 actactcgcg ctaacctagt	cgtcgtaatc tagccgcgat acgctcgcac	: ta	52
SEQ ID NO: 109 FEATURE	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
source	mol_type = other DNA organism = synthetic construct		
SEQUENCE: 109 accgccgcta tacgcgcgat	taaggtgtac gctcgcagtc gcggactaag	ı gt	52
SEQ ID NO: 110 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>		
SEQUENCE: 110	organism = synthetic construct		
	gagtgcgtac gctcgcagta ccgatcgcta	ı gt	52
SEQ ID NO: 111 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA		
SEQUENCE: 111	organism = synthetic construct		
	gegtegttag tgegegagaa egaegaetag	ı gt	52
SEQ ID NO: 112 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>		
SEQUENCE: 112	organism = synthetic construct		

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gcgtcgaatt ggtcgcgtag	tatggtaccg ccgctataca ccaatacgcc c	c 52	
SEQ ID NO: 113 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 113 tacgcgcact acttacgcga	ctaggtaccg atcgctagtc gacgcgctgt t	a 52	
SEQ ID NO: 114	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 114	organism = synthetic construct		
acgccgctaa ctatagttag	eggegtacea attegaegea aetaateege g	c 52	
SEQ ID NO: 115 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 115 cgcggactaa ggttagttag	cggcgttacg cgcactacta ccgatccgca a	t 52	
SEQ ID NO: 116 FEATURE	moltype = DNA length = 52 Location/Qualifiers		
source	<pre>152 mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 116 acgacgacta ggtaccgccg	acttatacgc cgctaactaa taggtagcgc g	t 52	
SEQ ID NO: 117 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	mol_type = other DNA organism = synthetic construct		
SEQUENCE: 117 cggatcgacg gttgcgcgag	tagtgtagta gtacgcggtt acactgcgcg a	c 52	
SEQ ID NO: 118 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
SEQUENCE: 118	<pre>mol_type = other DNA organism = synthetic construct</pre>		
	acttataccg atccgcaatt cgctcgattg g	t 52	
SEQ ID NO: 119 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 119 actgcgagcg tacactgcga	gegtacacet taategegea eegetegtta e	c 52	
SEQ ID NO: 120 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	mol_type = other DNA organism = synthetic construct		
SEQUENCE: 120 actactgtcg cgatcgtcgc	actagttacg ctcgcactaa ttgcggatcg g	t 52	
SEQ ID NO: 121 FEATURE	moltype = DNA length = 52 Location/Qualifiers		
source	152 mol_type = other DNA organism = synthetic construct		
SEQUENCE: 121 ggtaacgagc ggttctcqcg	cactaattag tgcgcgagaa ccatactacg c	g 52	
SEQ ID NO: 122	moltype = DNA length = 52	- -	
FEATURE	Location/Qualifiers		

		ntinuea	
source	152 mol_type = other DNA		
SEQUENCE: 122	organism = synthetic constr		
	taaggtacct taatcgcgca actact	egee ga	52
SEQ ID NO: 123 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic constr	at	
SEQUENCE: 123 tacgcgcact actcttgtac	ggcggtacca attcgacgca accgtc		52
SEQ ID NO: 124 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 124 aaccgtcgat ccgattgcgg	organism = synthetic constrateggtacct taatcgcgca ctagtg		52
SEQ ID NO: 125 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA		
SEQUENCE: 125	organism = synthetic constr	uct	
	cgcgacacac tactcgcgca ccttaa	tccg cg	52
SEQ ID NO: 126 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic constr	ugt	
SEQUENCE: 126	ataggtagta gtgcgcgtat tcggcg		52
SEQ ID NO: 127 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA		<i>32</i>
SEQUENCE: 127	organism = synthetic constr		
tagegegtea agaacetage SEQ ID NO: 128	gttgcgataa gtcggcggta gtagta moltype = DNA length = 52	cgcg gt	52
FEATURE source	Location/Qualifiers 152 mol_type = other DNA organism = synthetic constr	uct	
SEQUENCE: 128 cgcattagtc ggtaatctag	ccgcgaacca taaccgcgca ccgatc	gcta gt	52
SEQ ID NO: 129 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic constr	uct.	
SEQUENCE: 129 tatggtacgg cgtgcggcgt	attggtacgc cgctaactaa taagtc		52
SEQ ID NO: 130 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 130	organism = synthetic constrattggtacga gcggtagtca accgct		52
		-5-0 09	
SEQ ID NO: 131 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 131	organism = synthetic constr	uct	

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cgactatgtg cgcaactact	cgccgaacca taaccgcgct atgcgctcga ct	52	
SEQ ID NO: 132 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 132 tagttagegg egtacegete	gttaccacct taatcgcgca ccatactacg cg	52	
SEQ ID NO: 133 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 133	organism = synthetic construct	F0	
cgcattagtc ggtagtagtg	egegtaaace getegteegt tagtgegega ga	52	
SEQ ID NO: 134 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 134	organism = synthetic construct		
-	aatgegtete gegeactaag actaeegete gt	52	
SEQ ID NO: 135 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
GROUPINGE 125	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 135 tacgetegea ctategeteg	attggtaccg ccgctataca ccataaccgc gc	52	
SEQ ID NO: 136 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 136	organism = synthetic construct		
accaatcgag cgaagtcgag	cgcataacgc gctacctata cgccgctaac ta	52	
SEQ ID NO: 137 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152		
SEQUENCE: 137	<pre>mol_type = other DNA organism = synthetic construct</pre>		
~	gegtacaceg actaatgega etaetgtege ga	52	
SEQ ID NO: 138 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 138 agtagtgcgc gtatcgctcg	attggttctt gacgcgctag tatagcggcg gt	52	
SEQ ID NO: 139 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>		
SEQUENCE: 139	organism = synthetic construct		
	gtcggtgcgc acatagtcgt atggtacggc gt	52	
SEQ ID NO: 140 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	mol_type = other DNA organism = synthetic construct		
SEQUENCE: 140 cgcqgattaa qqtaqtcqaq	cgcataaccg cgtactacta cgacgactag gt	52	
SEQ ID NO: 141	moltype = DNA length = 52		
FEATURE	Location/Qualifiers		

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source	152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 141	organism - synthetic construct	
cgactatgtg cgctacgctc	gcactaacac tactegegea cetagegeeg aa	a 52
SEQ ID NO: 142 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 142	organism = synthetic construct	
_	cactaategt egeactagta acegtegate eg	g 52
SEQ ID NO: 143 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 143	organism = synthetic construct	
acctagcgtt gcgaccgact	aatgegggta aegageggtt atggtaegge gt	52
SEQ ID NO: 144 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 144 cgcgctacta ggtcgcggta	ataggtacct agcgttgcga cctagtcgcg ta	a 52
SEQ ID NO: 145 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>	
anaumuan 445	organism = synthetic construct	
SEQUENCE: 145 cgttcggcta ggtactactc	gcgctacgca ttagtcggtt cgcgacagta gt	52
SEQ ID NO: 146 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>	
SEQUENCE: 146	organism = synthetic construct	
cggacgagcg gttcgcggta	ataggtacga cgactaggtt agttagcggc gt	52
SEQ ID NO: 147 FEATURE	moltype = DNA length = 52 Location/Qualifiers 152	
source	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 147	atoggtacog actaatgoga cogogtacta ct	52
SEQ ID NO: 148	moltype = DNA length = 52	. 52
FEATURE source	Location/Qualifiers 152 mol type = other DNA	
SEQUENCE: 148	organism = synthetic construct	
	cggcgttctt gacgcgctaa cctagcgccg aa	a 52
SEQ ID NO: 149 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 149 gcgcggatta gttaaccgtc	gagtgcacac tactcgcgca ctgcgagcgt ac	52
SEQ ID NO: 150 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 150	J	

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accttaatcc	gcgaccgact	aatgegtaeg egeaetaeta taag	teggeg gt	52
SEQ ID NO: FEATURE source	151	moltype = DNA length = 5 Location/Qualifiers 152	2	
		<pre>mol_type = other DNA organism = synthetic cons</pre>	truct	
SEQUENCE: 1	.51	organism - synthetic cons	crucc	
accttaatcc	gcggcgcggt	tatggtaccg actaatgcga accg	ctcgtc cg	52
SEQ ID NO: FEATURE source	152	<pre>moltype = DNA length = 5 Location/Qualifiers 152 mol_type = other DNA</pre>	2	
SEQUENCE: 1	.52	organism = synthetic cons	truct	
actgcgagcg	taccttgtac	ggeggtaeet agtagegega taag	teggeg gt	52
SEQ ID NO: FEATURE source	153	<pre>moltype = DNA length = 5 Location/Qualifiers 152</pre>	2	
		<pre>mol_type = other DNA organism = synthetic cons</pre>	truct	
SEQUENCE: 1				
ttcggcgcta	ggtaccttag	teegegtteg gegetaggta eeta	gcgttg cg	52
SEQ ID NO: FEATURE source	154	moltype = DNA length = 5 Location/Qualifiers 152	2	
		<pre>mol_type = other DNA organism = synthetic cons</pre>	truct	
SEQUENCE: 1 acctagtcgc		ggcggtacct agccgaacga accg	tcgagt gc	52
SEQ ID NO: FEATURE	155	moltype = DNA length = 5 Location/Qualifiers	2	
source		152 mol_type = other DNA organism = synthetic cons	truct	
SEQUENCE: 1 accataaccg		cgcgacacca atacgccgct atgg	tacggc gt	52
SEQ ID NO: FEATURE source	156	<pre>moltype = DNA length = 5 Location/Qualifiers 152</pre>	2	
SEQUENCE: 1	E.c.	<pre>mol_type = other DNA organism = synthetic cons</pre>	truct	
~		ctaggtaatg tcgaaccgca cgcc	gctaac ta	52
SEQ ID NO: FEATURE source	157	<pre>moltype = DNA length = 5 Location/Qualifiers 152</pre>	2	
		<pre>mol_type = other DNA organism = synthetic cons</pre>	truct	
SEQUENCE: 1 accgactaat		gcgtcgttag tgcgcgagaa cctt	aatcgc gc	52
SEQ ID NO: FEATURE source	158	moltype = DNA length = 5 Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 1		organism = synthetic cons aatgcgataa gtcggcggta ccaa		52
SEQ ID NO: FEATURE source	159	moltype = DNA length = 5 Location/Qualifiers 152	2	
		<pre>mol_type = other DNA organism = synthetic cons</pre>	truct	
SEQUENCE: 1				
gtacgctcgc	agtcgcggta	ataggttcgg cgagtagtta ccat	aaccgc gc	52
SEQ ID NO: FEATURE	160	moltype = DNA length = 5 Location/Qualifiers	2	

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source	152 mol type = other DNA	
GROUPING 160	organism = synthetic construct	
SEQUENCE: 160 cggacgagcg gttgcgcggt	tatggtacta gtgcgacgag cgcacatagt cg 52	
SEQ ID NO: 161 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 161 actactegeg ctagegegat	organism = synthetic construct taaggtacgc cgctaactat cgcggctaga tt 52	
SEQ ID NO: 162 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 162 accaattoga ogcaactaat	ccgcgcacca attcgacgca gtagtgcgcg ta 52	
SEQ ID NO: 163 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 163	organism - synthetic constitut	
accgccgcta tacacctagc	gccgaagtac gctcgcagtg tatagcggcg gt 52	
SEQ ID NO: 164 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 164		
acactactcg cgccggacga	gcggttacca atacgccgct agcgcgagta gt 52	
SEQ ID NO: 165 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 165	organism = synthetic construct teegegegea aegetaggta cactactege ge 52	
SEQ ID NO: 166	moltype = DNA length = 52	
FEATURE source	Location/Qualifiers 152 mol type = other DNA	
SEQUENCE: 166	organism = synthetic construct	
~	aatgegegea aegetaggta atgtegaace ge 52	
SEQ ID NO: 167 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 167 acgagcggta gtcactactg	organism = synthetic construct tcgcgacgca acgctaggtt acactgcgcg ac 52	
SEQ ID NO: 168	moltype = DNA length = 52	
FEATURE source	Location/Qualifiers 152 mol_type = other DNA	
SEQUENCE: 168	organism = synthetic construct tatqqtaccq atcqctaqtq qtaacqaqcq qt 52	
SEQ ID NO: 169 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 169	organism = synthetic construct	

gcggttcgac attaccgact	aatgogtatg ogotogacta octagogttg og	52
SEQ ID NO: 170 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 170	organism - synthetic construct	
	cactaaacgc cgctaactac gcgctactag gt	52
SEQ ID NO: 171 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	
SEQUENCE: 171	organism = synthetic construct ccgcgaacct tagtccgcga ccgccgtaca ag	52
SEQ ID NO: 172 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 172 teggegagta gttaegeget	acctattcgc ggctagatta cgccgctaac ta	52
SEQ ID NO: 173 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 173	organism = synthetic construct taaggtgtac gctcgcagtg tcgcgcagtg ta	52
SEQ ID NO: 174	moltype = DNA length = 52	
FEATURE source	Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 174 agtagtgcgc gtagcggttc	gacattagta gtacgcggtg cactcgacgg tt	52
SEQ ID NO: 175 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
CROUDIAN 155	organism = synthetic construct	
SEQUENCE: 175 tcgcggctag attagtagtg	egegtaacac tactegegea cettagteeg eg	52
SEQ ID NO: 176 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	
SEQUENCE: 176 actagcgatc ggtgcgtcga	organism = synthetic construct attggttagc gcgagtagtt cgtcgcacta gt	52
SEQ ID NO: 177 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	
SEQUENCE: 177 cgcggactaa ggtgcgcggt	organism = synthetic construct tatggtacac tactcgcgcg cggttcgaca tt	52
SEQ ID NO: 178 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 178	organism = synthetic construct	
	attggtataa gtcggcggta ccaattcgac gc	52
SEQ ID NO: 179 FEATURE	moltype = DNA length = 52 Location/Qualifiers	

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source	152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 179	gctagtacca attcgacgcc gcgctactag gt	52
		52
SEQ ID NO: 180 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA	
SEQUENCE: 180	organism = synthetic construct	
	eggegtaeet taategegeg gtaacgageg gt	52
SEQ ID NO: 181 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	
SEQUENCE: 181	organism = synthetic construct	
	attggtacct agccgaacga taagtcggcg gt	52
SEQ ID NO: 182 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 182 taacagegeg tegegegeta	ataggtgtac gctcgcagtc gcggattaag gt	52
SEQ ID NO: 183	moltype = DNA length = 52	
FEATURE	Location/Qualifiers 152	
source	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 183	-	E2
	tcaagaggta acgagcggta cctagtcgtc gt	52
SEQ ID NO: 184 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 184 tacgctcgca ctagcgcggt	tatggtaatg tcgaaccgcc gcgtagtatg gt	52
SEQ ID NO: 185 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 185	-	52
	attggtacca atacgccgca ccgccgtaca ag	52
SEQ ID NO: 186 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 186 ttgtcgcgct agtgcgcgat	taaggtataa gteggeggta etgegagegt ac	52
SEQ ID NO: 187 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA	
CPOHENCE. 107	organism = synthetic construct	
SEQUENCE: 187 cgcggactaa ggtactactc	gegetaaege egtaceataa eetagtegte gt	52
SEQ ID NO: 188 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA	
SEQUENCE: 188	organism = synthetic construct	

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actagegate ggttagegeg	tcaagaacgc gctacctatg actaccgctc gt	52
SEQ ID NO: 189 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 189		50
erigiaegge ggigegeggi	tatggtacca attcgacgca ttgcggatcg gt	52
SEQ ID NO: 190 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 190	-	
tegetegatt ggtegeggta	ataggttege gacagtagtt egtegeaeta gt	52
SEQ ID NO: 191 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 191	organism = synthetic construct	
acctagegee gaaeggaega	geggttaeta etgtegegae ttgtaeggeg gt	52
SEQ ID NO: 192 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 192 acgcgctacc tataccgcgt	actactaccg actaatgcga ctagtgcgac ga	52
SEQ ID NO: 193 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	<pre>152 mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 193 aaccgtcgag tgcgcgcgag	tagtgtacgc cgctaactag cgtcgaattg gt	52
SEQ ID NO: 194 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 194 gcgcgagtag tgtgactacc	getegtacet attacegega cetattaceg eg	52
SEQ ID NO: 195 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	<pre>152 mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 195 tegetegatt ggttaegege	actacttacg ctcgcactaa actactcgcc ga	52
SEQ ID NO: 196 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>	
	organism = synthetic construct	
SEQUENCE: 196 tcgtcgcact agtgcgcggt	tatggtacca taaccgcgct acactgcgcg ac	52
SEQ ID NO: 197 FEATURE	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
source	nol_type = other DNA organism = synthetic construct	
SEQUENCE: 197 accgcgtact actgcggttc	gacattacet taategegea gtegagegea ta	52
SEQ ID NO: 198 FEATURE	moltype = DNA length = 52 Location/Qualifiers	

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source	152		
	<pre>mol_type = other DNA</pre>		
CEOUENCE 100	organism = synthetic construct		
SEQUENCE: 198 agtagtggg gtagggtgga	attggtgcgc acatagtcgt tgtcgcgcta gt	52	
-33-3-3- 33-3-3-			
SEQ ID NO: 199	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152		
Source	mol type = other DNA		
	organism = synthetic construct		
SEQUENCE: 199		F0	
gegeggatta gttgegtega	attggtaccg ccgtacaagt cggcgagtag tt	52	
SEQ ID NO: 200	moltype = DNA length = 52		
FEATURE	Location/Qualifiers		
source	152 mol type = other DNA		
	organism = synthetic construct		
SEQUENCE: 200			
agtagtgege gtaegttegg	ctaggtaccg ccgtacaaga ccttaatccg cg	52	
SEQ ID NO: 201	moltype = DNA length = 52		
FEATURE	Location/Qualifiers		
source	152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 201	3		
aaccgtcgag tgcattgcgg	ateggtaceg eegtacaagt ettgaegege ta	52	
SEQ ID NO: 202	moltype = DNA length = 52		
FEATURE	Location/Qualifiers		
source	152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 202			
gcggcgtatt ggtacctagt	cgtcgtacca atacgccgca ccgactaatg cg	52	
SEQ ID NO: 203	moltype = DNA length = 52		
FEATURE	Location/Qualifiers		
source	152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 203	organism = symmetry democrate		
accgatcgct agtcgcatta	gtcggtacca taaccgcgcc gcgctactag gt	52	
SEQ ID NO: 204	moltype = DNA length = 52		
FEATURE	Location/Qualifiers		
source	152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 204	organism - synthetic construct		
tagtgcgagc gtatcgcggc	tagattacga cgactaggtt agcgcgagta gt	52	
SEO ID NO: 205	moltype = DNA length = 52		
FEATURE	Location/Qualifiers		
source	152		
	mol_type = other DNA		
SEQUENCE: 205	organism = synthetic construct		
accttagtcc gcgactgcga	gcgtacacct taatcgcgcg tatagcggcg gt	52	
CEO ID NO. 200	moltano - DNA longth 50		
SEQ ID NO: 206 FEATURE	<pre>moltype = DNA length = 52 Location/Qualifiers</pre>		
source	152		
	mol_type = other DNA		
CECHENCE. 200	organism = synthetic construct		
SEQUENCE: 206 actagcgatc ggtactgcga	gcgtacgcac tcgacggtta gtagtgcgcg ta	52	
5 5 55 5 2 5 4	5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6		
SEQ ID NO: 207	moltype = DNA length = 52		
FEATURE	Location/Qualifiers 152		
source	mol type = other DNA		
	organism = synthetic construct		
SEQUENCE: 207			

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acctagtcgt	cgttctcgcg	cactaacgac gcgctgttat acact	gegeg ac	52
SEQ ID NO: FEATURE source	208	moltype = DNA length = 52 Location/Qualifiers 1.52 mol type = other DNA	2	
		organism = synthetic const	ruct	
SEQUENCE: 2 gcggcgtatt		ggcggtacca tactacgcga ccaat	tegae ge	52
SEQ ID NO: FEATURE source	209	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	2	
SEQUENCE: 2	09	organism = synthetic const	truct	
taacagcgcg	tcgactagcg	atcggtacct agtcgcgtaa gtagt	gegeg ta	52
SEQ ID NO: FEATURE source	210	moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA	2	
SEQUENCE: 2	10	organism = synthetic const	truct	
		attggtacgc cgctaactat agtta	agegge gt	52
SEQ ID NO: FEATURE source	211	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 2		organism = synthetic const		52
SEO ID NO:		moltype = DNA length = 52	3 3 3	52
FEATURE source	212	Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 2 acgcgctacc		organism = synthetic const		52
SEQ ID NO: FEATURE source	213	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 2	13	organism = synthetic const	ruct	
gtcgcgcagt	gtaaccgcgt	actactacac tactcgcgca accgt	tegate eg	52
SEQ ID NO: FEATURE source	214	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic const		
SEQUENCE: 2 accaatcgag		atoggtataa gtoggoggta cogat		52
SEQ ID NO: FEATURE source		moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	2	
SEQUENCE: 2 ggtaacgagc		organism = synthetic const taaggtgtac gctcgcagtg tacgc		52
SEQ ID NO: FEATURE source	216	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 2		organism = synthetic const		
accgatccgc	aattagtgcg	agcgtaacta gtgcgacgat cgcga	acagta gt	52
SEQ ID NO: FEATURE	217	moltype = DNA length = 52 Location/Qualifiers	2	

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source	152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 217	organism - synchetic construct		
cgcgtagtat ggttctcgcg	cactaattag tgcgcgagaa ccgctcgtta cc	52	
SEQ ID NO: 218	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152		
boarec	mol_type = other DNA		
SEQUENCE: 218	organism = synthetic construct		
-	taaggtacct taatcgcgct agcgcgagta gt	52	
SEQ ID NO: 219	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 219	attggtaccg ccgtacaaga gtagtgcgcg ta	52	
geaceegaeg geegeega	accegeacceg cogeacaaga geagegegeg ca	32	
SEQ ID NO: 220 FEATURE	moltype = DNA length = 52 Location/Qualifiers		
source	152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 220	taaggtaccg atccgcaatc ggatcgacgg tt	52	
cegeegeace agegegegat	taaggtaceg accegeaace ggategaegg te	32	
SEQ ID NO: 221 FEATURE	moltype = DNA length = 52 Location/Qualifiers		
source	152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 221	-		
cgcggattaa ggtgcgcgag	tagtgtgtcg cgcagtgtat acgcgcacta ct	52	
SEQ ID NO: 222	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 222	organism - synthetic constituct		
acctagegee gaataegege	actactacct attaccgcgt atggtacggc gt	52	
SEQ ID NO: 223	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152		
	mol_type = other DNA		
SEQUENCE: 223	organism = synthetic construct		
	acttatcgca acgctaggtt cttgacgcgc ta	52	
SEQ ID NO: 224	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152		
Bource	mol_type = other DNA		
SEQUENCE: 224	organism = synthetic construct		
	tatggttege ggetagatta eegactaatg eg	52	
SEQ ID NO: 225	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152		
	mol_type = other DNA		
SEQUENCE: 225	organism = synthetic construct		
	cggcgtacca atacgccgca cgccgtacca ta	52	
SEQ ID NO: 226	moltype = DNA length = 52		
FEATURE	Location/Qualifiers		
source	152 mol type = other DNA		
	organism = synthetic construct		
SEQUENCE: 226			

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cggacgagcg gttgactacc	gctcgtacca atacgccgca ccataaccgc g	c 52
SEQ ID NO: 227 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA	
	organism = synthetic construct	
SEQUENCE: 227 agtcgagcgc atagcgcggt	tatggttcgg cgagtagttg cgcacatagt c	g 52
SEQ ID NO: 228 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 228	-	50
cgcgtagtat ggtgcgcgat	taaggtggta acgagcggta cgccgctaac t	a 52
SEQ ID NO: 229 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	
SEQUENCE: 229	organism = synthetic construct	
tetegegeae taaeggaega	geggtttacg egeactacta eegactaatg eg	g 52
SEQ ID NO: 230 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 230 acgagoggta gtcttagtgc	gcgagacgca ttagtcggta ctactcgcgc ta	a 52
SEQ ID NO: 231 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 231 gcgcggttat ggtgtatagc	ggcggtacca atcgagcgat agtgcgagcg ta	a 52
SEQ ID NO: 232 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 232 tagttagcgg cgtataggta	gegegttatg egetegaett egetegattg g	t 52
SEQ ID NO: 233 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 233 tegegacagt agtegeatta	gtcggtgtac gctcgcagtc gcggattaag g	t 52
SEQ ID NO: 234 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 234 accgccgcta tactagcgcg	organism = synthetic construct tcaagaacca atcgagcgat acgcgcacta ct	t 52
SEQ ID NO: 235 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
CECHENCE	organism = synthetic construct	
SEQUENCE: 235 acgccgtacc atacgactat	gtgcgcaccg accgtaccga ctagtgcgac g	a 52
SEQ ID NO: 236 FEATURE	moltype = DNA length = 52 Location/Qualifiers	

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source	152		
	mol_type = other DNA		
SEQUENCE: 236	organism = synthetic construct		
	cgcggttatg cgctcgacta ccttaatccg cg	52	
SEQ ID NO: 237 FEATURE	<pre>moltype = DNA length = 52 Location/Qualifiers</pre>		
source	152 mol_type = other DNA		
SEQUENCE: 237	organism = synthetic construct		
-	tagtgttagt gegagegtag egeacatagt eg	52	
SEQ ID NO: 238 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152		
CECHENCE . 220	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 238 cggatcgacg gttactagtg	cgacgattag tgcgcgagaa taagtcggcg gt	52	
SEQ ID NO: 239 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
	mol_type = other DNA organism = synthetic construct		
SEQUENCE: 239 acgccgtacc ataaccgctc	gttaccegca ttagteggte geaacgetag gt	52	
SEQ ID NO: 240	moltype = DNA length = 52		
FEATURE source	Location/Qualifiers 152		
	<pre>mol_type = other DNA organism = synthetic construct</pre>		
SEQUENCE: 240 tacgcgacta ggtcgcaacg	ctaggtacct attaccgcga cctagtagcg cg	52	
SEQ ID NO: 241 FEATURE	moltype = DNA length = 52 Location/Qualifiers		
source	152 mol_type = other DNA organism = synthetic construct		
SEQUENCE: 241	-		
actactgtcg cgaaccgact	aatgegtage gegagtagta cetageegaa eg	52	
SEQ ID NO: 242 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
204200	mol_type = other DNA organism = synthetic construct		
SEQUENCE: 242 acgccgctaa ctaacctagt	cgtcgtacct attaccgcga accgctcgtc cg	52	
SEQ ID NO: 243 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>		
Source	mol_type = other DNA organism = synthetic construct		
SEQUENCE: 243 agtagtacgc ggtcgcatta	gtcggtaccg atccgcaatt agtgcgagcg ta	52	
SEQ ID NO: 244 FEATURE	moltype = DNA length = 52 Location/Qualifiers		
source	152 mol_type = other DNA		
SEQUENCE: 244	organism = synthetic construct tcaagaacgc cgtaccatac ggtacggtcg gt	52	
		52	
SEQ ID NO: 245 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152		
	<pre>mol_type = other DNA</pre>		
SEQUENCE: 245	organism = synthetic construct		

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gtacgctcgc agtgcgcgag	tagtgtgcac tcgacggtta actaatccgc gc	52
SEQ ID NO: 246 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
GROUPING OAS	organism = synthetic construct	
SEQUENCE: 246	agast agas thost sasts agast took at	E2
accgccgact tatagragig	cgcgtacgca ttagtcggtc gcggattaag gt	52
SEQ ID NO: 247 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA</pre>	
	organism = synthetic construct	
SEQUENCE: 247 cggacgagcg gttcgcatta	gteggtacea taacegegee geggattaag gt	52
SEQ ID NO: 248 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
boaree	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 248	3	
accgccgact tatcgcgcta	ctaggtaccg ccgtacaagg tacgctcgca gt	52
SEQ ID NO: 249 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 moltype = other DNA	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 249 cgcaacgcta ggtacctagc	gccgaacgcg gactaaggta cctagcgccg aa	52
SEQ ID NO: 250 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 250 gcactcgacg gttcgttcgg	ctaggtaccg ccgtacaagt acgcgactag gt	52
SEQ ID NO: 251 FEATURE	moltype = DNA length = 52 Location/Qualifiers	
source	152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 251 acgccgtacc atagcggcgt	attggtgtcg cgcagtgtag cgcggttatg gt	52
SEQ ID NO: 252 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 252 tagttagegg egtgeggtte	gacattacct agtcgcgtag cgcgagtagt gt	52
SEQ ID NO: 253 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA</pre>	
SEQUENCE: 253 gcgcgattaa ggttctcgcg	organism = synthetic construct cactaacgac gcgctgttac gcattagtcg gt	52
SEQ ID NO: 254 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152	
	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 254		
	acttatcgca ttagtcggtt atggtacggc gt	52
SEQ ID NO: 255 FEATURE	moltype = DNA length = 52 Location/Qualifiers	

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source	152 mol_type = other DNA		
SEQUENCE: 255 gcgcggttat ggtaactact	organism = synthetic cor cgccgaacct attaccgcga ct		52
SEQ ID NO: 256 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA organism = synthetic co		
SEQUENCE: 256 cgactatgtg cgctcgtcgc	actagtacca taaccgcgca ac		52
SEQ ID NO: 257 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA organism = synthetic co.		
SEQUENCE: 257 aatctagecg egatagttag	cggcgtacct taatcgcgct ag	cgcgagta gt	52
SEQ ID NO: 258 FEATURE source	moltype = DNA length = Location/Qualifiers 1.52 mol_type = other DNA organism = synthetic co		
SEQUENCE: 258			52
SEQ ID NO: 259 FEATURE source	<pre>attggtgcgc ggattagttg cg moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA</pre>	52	52
SEQUENCE: 259	organism = synthetic co	nstruct	
accgccgcta tacactgcga	gcgtacttcg gcgctaggtg ta	tagcggcg gt	52
SEQ ID NO: 260 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA		
SEQUENCE: 260 actactcgcg ctagcggcgt	organism = synthetic co		52
SEQ ID NO: 261 FEATURE source	<pre>moltype = DNA length = Location/Qualifiers 152 mol type = other DNA</pre>	52	
SEOUENCE: 261	organism = synthetic co	nstruct	
~	gttgcgcgcg gattaaggta ct	agtgcgac ga	52
SEQ ID NO: 262 FEATURE source	moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA organism = synthetic co.		
SEQUENCE: 262 geggttegae attacetage	gttgcgcgca ttagtcggta cc		52
SEQ ID NO: 263 FEATURE source	<pre>moltype = DNA length = Location/Qualifiers 152 mol type = other DNA</pre>	52	
SEQUENCE: 263	organism = synthetic co		
gtcgcgcagt gtaacctagc	gttgcgtcgc gacagtagtg ac	taccgctc gt	52
SEQ ID NO: 264 FEATURE source	<pre>moltype = DNA length = Location/Qualifiers 152 mol_type = other DNA</pre>	52	
SEQUENCE: 264	organism = synthetic co	nstruct	

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accgctcgtt accactagcg	ateggtacea tactaegegt aegegaetag gt	52
SEQ ID NO: 265 FEATURE source	<pre>moltype = DNA length = 52 Location/Qualifiers 152</pre>	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 265		
cgcaacgcta ggtagtcgag	cgcatacgca ttagtcggta atgtcgaacc gc	52
SEQ ID NO: 266 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 266	-	52
acctagtage gegtagttag	eggegtttag tgegegagag taegetegea gt	52
SEQ ID NO: 267 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol_type = other DNA	
SEQUENCE: 267	organism = synthetic construct	
	taaggttege ggetagatta eegaeegtae eg	52
SEQ ID NO: 268 FEATURE source	moltype = DNA length = 52 Location/Qualifiers 152 mol type = other DNA	
	organism = synthetic construct	
SEQUENCE: 268 tagttagcgg cgtaatctag	ccgcgaatag gtagcgcgta actactcgcc ga	52
SEO ID NO: 269	malterna DNA lamete 04	
FEATURE source	moltype = DNA length = 24 Location/Qualifiers 124	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 269		
acgcgactat acgcgcaata	tggt	24
SEQ ID NO: 270 FEATURE	<pre>moltype = DNA length = 25 Location/Qualifiers</pre>	
source	125 mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 270 ctagcgatac tacgcgatac	gagat	25
cragegarae raegegarae	gagac	23
SEQ ID NO: 271 FEATURE source	<pre>moltype = DNA length = 26 Location/Qualifiers 126</pre>	
204200	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 271	organism = symmetric competation	
catagoggta ttacgogaga	ttacga	26
SEQ ID NO: 272	moltype = DNA length = 21	
FEATURE source	Location/Qualifiers 121	
	<pre>mol_type = other DNA organism = synthetic construct</pre>	
SEQUENCE: 272 cgcgagtacg tacgattacc	a	21
SEQ ID NO: 273 FEATURE	moltype = DNA length = 21 Location/Qualifiers	
source	121 mol type = other DNA	
	organism = synthetic construct	
SEQUENCE: 273		21
acgcgcgact atacgcgcct	C	21

- 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 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 adapter-specific 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^{st} and 2^{nd} , 2^{nd} and 3^{rd} , 50^{th} and 51^{st} , and 51^{st} and 52^{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.

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