Supplementary Information for

**BEAR reveals that increased fidelity variants can successfully reduce the mismatch-tolerance of adenine but not cytosine base editors**

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**Contents:**

## Supplementary Figure 1 – Canonical and non-canonical 5’ splice sites examined in BEAR

## Supplementary Figure 2 – Splice site variants for identifying candidate BEAR sequences

## Supplementary Figure 3 – Versatility of BEAR allowing of its compatibility with different sequences

## Supplementary Figure 4 – Correlations between the editing efficiencies of ABE and CBE variants

## Supplementary Figure 5 – Correlations between base editor activities with 50 mismatching sgRNAs

## Supplementary Figure 6 – Off-target activities of different ABE variants on target 7 with 50 mismatching sgRNAs

## Supplementary Figure 7 – Off-target activities of different ABE variants on three additional targets with 31 mismatching sgRNAs

**Supplementary Methods** – Detailed plasmid construction

**Supplementary Table 1** – List of oligonucleotides

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## Figure S1 – Canonical and non-canonical 5’ splice sites examined in BEAR

Canonical (GT) or non-canonical (AT or GC) 5’ splice sites were constructed into the split GFP coding plasmid (**Fig. 1**) and were transformed either into HEK293T (blue) or N2a (grey) cells. Columns represent means +/- SD of three parallel transfections.

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## Figure S2 – Splice site variants for identifying candidate BEAR sequences in N2a cells

Flow cytometry measurements of GFP positive N2a cells transfected with ABE (**b**) or CBE (**c**) editing compatible reporter plasmids harboring systematically altered splice sites. The letters beneath the column charts represent the intended disrupted or pre-edited splice site sequences. Letters highlighted in blue indicate the bases that correspond to the canonical (**a**) 5’ - G GT AAGT - 3’ consensus splice site sequence, and sequence alterations are shown in black. Columns represent means, +/- SD of three parallel transfections.

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## Figure S3 – Versatility of BEAR allowing of its compatibility with different sequences

(**a)** The PAM motif was freely moved in context of the edited base in the entire span of the spacer, thus the editing window of ABE could be monitored. As a positive control for each edited position, a pre-edited plasmid was constructed. As negative controls, the disrupted 5’ss was co-transfected with dead nickase and nuclease (WT) SpCas9 and the corresponding sgRNAs. The heatmap shows the mean rates of GFP positive cells derived from three parallel transfections.

(**b)** Split mCherry (red) and mScarlet (yellow) coding sequences were separated by exactly the same intron and splice site that were used with GFP. Plasmids with disrupted splice sites were co-transfected with ABE (corrected), and splice site correction mediated fluorescence could be detected for both proteins. As positive controls, pre-edited plasmids were constructed to monitor the maximum theoretical extent of base editing. Columns represent means +/- SD of three parallel transfections.

GFP **(c)** and mScarlet **(d)** proteins were split at different amino acid positions within their sequence as indicated in the figure. All constructs expressed a high level of GFP and mScarlet, regardless of the protein or the amino acid site of splitting. Columns represent means +/- SD of three parallel transfections.

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## Figure S4 – Correlations between the editing efficiencies of ABE and CBE variants

Pearson’s correlation coefficients are shown for 34 on-target cleavage results (see **Figure 5**) compared for different ABE **(a)** and CBE **(b)** editors.

A total of 34 target sequences (from **Fig. 5**)were targeted by two codon optimized ABEs: ABERA (nABE) and ABEmax (**c**). These sequences were also targeted by three codon optimized CBEs: FNLS-CBE (CBE), BE4max and AncBE4max (**d**). The heatmaps show normalized on-target activity (measured/pre-edited) derived from three parallel transfections.

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## Figure S5 – Correlations between base editor activities with 50 mismatching sgRNAs

(**a**) Mean activity of different ABE variants with 1, 2, 3 or all mismatching sgRNAs shown in **Figure 6**.

(**b**) Pearson’s correlation coefficients characterizing the correlations between the editing activities of 7 ABE variants with 50 mismatching sgRNAs shown in **Figure 6**.

(**c**) Mean activity of different CBE variants with 1, 2, 3 or all mismatching sgRNAs shown in **Figure 6**.

(**d**) Pearson’s correlation coefficients characterizing the correlations between the editing activities of 8 CBE variants with 50 mismatching sgRNAs shown in **Figure 6**.

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## Figure S6 – Off-target activities of different ABE variants on target 7 with 50 mismatching sgRNAs

(**a**) Mismatch tolerance of dABE, nABE, ABEmax, x-ABE and 4 high fidelity ABE variants were compared utilizing exactly the same matching sgRNA (target 7 in **Fig. 5**) and 50 sgRNAs mismatching in one, two, three, four or five positions. Blue-yellow heatmaps show the mean normalized activity (off-target/on-target) derived from three parallel transfections. White-red heatmaps show the on-target activity (mean rates of GFP positive cells) derived from three parallel transfections.

(**b**) Pearson’s correlation coefficients characterizing the correlations between the editing activities of 8 ABE variants shown in **Figure S6a**.

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## Figure S7 – Off-target activities of different ABE variants on three additional targets with 31 mismatching sgRNAs

Mismatch tolerance of ABE and CBE and their high fidelity variants were compared utilizing exactly the same matching and 31 mismatching sgRNAs, the latter mismatching in either one or two positions, on targets 2 (**a**), 6 (**b**) and 17 (**c**). Blue-yellow heatmaps show the normalized activity (off-target/on-target) derived from three parallel transfections. White-red heatmaps show the on-target activity (mean rates of GFP positive cells) derived from three parallel transfections.

**Supplementary Methods – Detailed plasmid construction**

The sequences of oligos used to construct all plasmids are listed in Supplementary Table 1.

To construct BEAR-GFP plasmid candidates, a target cloning plasmid (pAT9624-BEAR-cloning) was cloned first, in which the target sequence is freely variable and can be cloned between the Esp3I sites via one-pot cloning (see below). The GFP halves were amplified via PCR from pEGFP-C1 (Clonthech) using primers *pAT9624-i1-for* and *pAT9624-i1-rev* and *pAT9624-i3-for* and *pAT9624-i3-rev*. The *Vim* intron sequence was amplified from the N2a genomic DNA with primers *pAT9624-i2-for* and *pAT9624-i2-rev*. The three inserts were cloned into an EcoRI and BshTI digested EGFP-C1 plasmid via Hi-Fi Assembly.

For splice site screens and for on-target experiments all BEAR targets were cloned to pAT9624-BEAR-cloning between the Esp3I sites via one-pot cloning. Briefly, 2 units of Esp3I enzyme, 1.5 units of DNA ligase, 1 mM DTT, 500 μM ATP, 50 ng vector and 5-5 μM of target-coding oligonucleotides were mixed in Tango buffer, and the mixture was incubated at 37 °C for 30 minutes before being transformed into NEB5-alpha competent cells.

To clone sgRNA targets used with BEAR-GFP, an sgRNA cloning plasmid pAT9658-sgRNA-mCherry was constructed from an EF1-mCherry coding plasmid and a pU6-pegRNA-GG-acceptor (Addgene #132777) via Hi-Fi Assembly. This plasmid expresses an mCherry protein in mammalian cells, which is applicable to monitor transfection efficiency, and an mRFP sequence in bacteria between the target cloning sites, which induces bacterial colonies to turn white instead of red upon successful cloning. The cloning site is BpiI, so all other enzyme recognition sites were mutated by introducing silent mutations into the mRFP1 plasmid. All sgRNA targets were cloned into this plasmid, to the BpiI sites, via one-pot cloning using the above described protocol with minute modifications (i.e. Green buffer and no DTT was used).

To clone the sgRNA targets used with BEAR-mScarlet and -mCherry, an sgRNA cloning plasmid pAT9679-BFP-sgRNA was constructed. mCherry in plasmid pAT9658-sgRNA-mCherry was replaced with BFP at the BamHI and BglII sites via amplifying BFP by PCR, using primers *pAT9679-for* and *-rev*, and assembling the fragments via Hi-Fi Assembly. This plasmid expresses a BFP protein in mammalian cells, which is applicable to monitor transfection efficiency, and can be used along with mScarlet and mCherry coding BEAR plasmids.

To construct the ABE coding pAT9676-ABE plasmid, the ABE coding sequence was amplified from pLenti-ABERA-P2A-Puro (Addgene #112675) using primers *pAT9676-ABE-for* and *-rev*, and it was cloned to the ZraI and PscI sites of a pUC19 plasmid via Hi-Fi Assembly. A BGH polyA sequence was amplified via PCR with primers *pAT9676-BGH-for* and *-rev*, and it was cloned to the PscI site of the previous construct via Hi-Fi Assembly.

To construct the dABE coding pAT9749-dABE plasmid from pAT9676-ABE, site-directed mutagenesis was applied to mutate the H840A amino acid, in order to gain a nuclease inactive ABE. pAT9676-ABE was digested with PscI and Eco32I. The respective discarded section of Cas9 was amplified from the same plasmid, in two fragments, using primers *pAT9749-F1-for* and *-rev* and *pAT9749-F2-for* and *-rev*,in which the overlapping parts coded the mutant amino acid. The vector and the two fragments were assembled via Hi-Fi Assembly.

To construct the CBE coding pAT9675-CBE plasmid, the CBE coding sequence was amplified from pLenti-FNLS-P2A-Puro (Addgene #110841) with primers *pAT9675-CBE-for* and *-rev*, and it was cloned to the ZraI and PscI sites of a pUC19 plasmid via Hi-Fi Assembly. A BGH polyA sequence was amplified by PCR, using primers *pAT9675-BGH-for* and *pAT9676-BGH-rev*, and it was cloned to the PscI site of the previous construct via Hi-Fi Assembly.

To construct the dCBE coding pAT9748-dCBE plasmid from pAT9675-CBE, site directed mutagenesis was applied to mutate the H840A amino acid, in order to gain a nuclease inactive CBE. pAT9675-CBE was digested with PscI and Eco32I. The respective discarded section of Cas9 was amplified from the same plasmid, in two fragments, with primers *pAT9749-F1-for* and *-rev* and *pAT9749-F2-for* and *-rev,* in whichthe overlapping parts coded the mutant amino acid. The vector and the two fragments were assembled via Hi-Fi Assembly.

The Cas9 coding plasmid used was pX330-Flag-wtSpCas9 (Addgene #92353). The dCas9 coding plasmid used was pX330-Flag-dSpCas9 (Addgene #92113). The nCas9 coding plasmid used was pX330-Flag-wtSpCas9-D10A (Addgene #80448).

To construct the pAT9750-BEAR-mCherry plasmid with disrupted 5’ss, the two mCherry halves were amplified from a pcDNA3.1-mCherry (Addgene #128744) plasmid via PCR using primers *pAT9750-i1-for* and *-rev* and *pAT9750-i3*-for and *-rev*. The *Vim* intron sequence was amplified from pAT9651-BEAR-GFP with primers *pAT9750-i2-for* and *pAT9750-i2-rev.* The three inserts were cloned into an EcoRI and BshTI digested EGFP-C1 plasmid via Hi-Fi Assembly.

To construct the pAT9751-BEAR-mCherry-preedited plasmid with pre-edited 5’ss, the two mCherry halves were amplified from a pcDNA3.1-mCherry (Addgene #128744) plasmid via PCR using primers *pAT9751-i1-for* and *pAT9750-i1-rev* and *pAT9750-i3*-for and *-rev*. The *Vim* intron sequence was amplified from pAT9651-BEAR-GFP with primers *pAT9750-i2-for* and *pAT9750-i2-rev.* The three inserts were cloned into an EcoRI and BshTI digested EGFP-C1 plasmid via Hi-Fi Assembly.

To construct the BEAR-mScarlet plasmids, mScarlet CDS from pCytERM\_mScarlet\_N1 (Addgene #85066) was cloned into pEGFP-C1 (pmScarlet-C1). To construct pAT9752-BEAR-mScarlet with disrupted 5’ss, the two pmScarlet halves were amplified from the mScarlet-C1 plasmid via PCR using primers *pAT9624-i1-for* and *pAT9752-i1-rev* and *pAT9752-i3*-for and *pAT9624-i3-rev*. The *Vim* intron sequence was amplified from pAT9651-BEAR-GFP with primers *pAT9750-i2-for* and *pAT9752-i2-rev.* The three inserts were cloned into an EcoRI and BshTI digested EGFP-C1 plasmid via Hi-Fi Assembly.

To construct pAT9753-BEAR-mScarlet with pre-edited 5’ss, the two mScarlet halves were amplified from the mScarlet-C1 plasmid via PCR using primers *pAT9624-i1-for* and *pAT9753-i1-rev* and *pAT9752-i3*-for and *pAT9624-i3-rev*. The *Vim* intron sequence was amplified from pAT9651-BEAR-GFP with primers *pAT9750-i2-for* and *pAT9752-i2-rev.* The three inserts were cloned into an EcoRI and BshTI digested EGFP-C1 plasmid via Hi-Fi Assembly.

To construct high fidelity ABE and CBE variants and xABE (pAT9991-eABE, pAT9992-HF-ABE, pAT9993-Hypa-ABE, pAT9994-HypaR661A-ABE, pAT9995-evoABE, pAT9996-HeF-ABE, pAT15064-eCBE, pAT15065-HF-CBE, pAT15066-Hypa-CBE, pAT15067-HypaR661A-CBE, pAT15068-evoCBE, pAT15069-HeF-CBE, pAT9784-xABE) increased fidelity Cas9 and xCas9 coding sequences (pX330-Flag-eSpCas9, -SpCas9-HF1, -HypaSpCas9, -Hypa-A-SpCas9, -evoSpCas9, -HeFSpCas9 – Addgene #126754-126459 and an in-house made xCas9 coding plasmid) were amplified by PCR using primers *pAT9991-for* and *pAT9749-F2-rev.* To construct the ABE variants, the amplified increased fidelity Cas9 fragments were cloned to a PScI and BglII digested pAT9676-ABE plasmid via Hi-Fi Assembly. To construct the CBE variants, the amplified increased fidelity Cas9 fragments were cloned to a PScI and BglII digested pAT9675-CBE plasmid via Hi-Fi Assembly. To append the UGI sequence to the CBE variants, high fidelity variant constructs and pAT9675-CBE were digested with NotI and Mva1269I, respectively and the UGI containing fragment was ligated with a T4 ligase.

To construct the editing window screen target plasmids with pre-edited or disrupted 5’ss, GFP halves were amplified from the EGFP-C1 plasmid with primers listed in the primer list below (insert 1 and insert 3), and the intron sequence (insert 2) was amplified from the pAT9651-BEAR-GFP plasmid with primers listed in the primer list below. The three inserts were cloned into an EcoRI and BshTI digested EGFP-C1 plasmid via Hi-Fi Assembly. All sgRNA targets were cloned to the plasmid pAT9658-sgRNA-mCherry plasmid using the protocol described above.

To construct BEAR-GFP and BEAR-mScarlet plasmids with different intron positions, GFP and mScarlet halves were amplified from pEGFP-C1 and mScarlet-C1 plasmids via PCR, using “insert 1 and insert 3” primers, as indicated in Supplementary Table 1 under the title “BEAR plasmids with different intron positions”. The *Vim* intron sequence was amplified from the pAT9651-BEAR-GFP plasmid via PCR, using “insert 2” primers.

To construct BEAR-GFP and BEAR-mScarlet expressing cell lines, the pSc1-puro (Addgene #80438) plasmid was modified to eliminate the sgRNA coding sequence, but not its target site. The GFP sequence was replaced by the coding sequences of BEAR-GFP and BEAR-mScarlet, which were cloned to the BshTI and EcoRI sites of this plasmid. The spacer that targets and linearizes these plasmids within the cells was cloned to pmCherry-gRNA (Addgene #80457) using the above described one-pot cloning method, utilizing oligos *U6-TL oligo-1* and *-2.* AAVS1 targeting spacers (AAVS1-a and -b) were cloned to pmCherry-gRNA (Addgene #80457) using the above described one-pot cloning method, utilizing oligos *AAVS1-a* and *-b, oligo-1 and -2.* U6-AAVS1-a was used to construct BEAR-GFP, and U6-AAVS1-b was used to create BEAR-mScarlet cell lines.

For the enrichment experiments genomic targets were cloned to pmCherry-gRNA (Addgene #80457) using the above described one-pot cloning method, utilizing oligos *sgRNA FANCF site 2 – oligo1, -2, sgRNA VEGFA site 3 – oligo1, -2, sgRNA HEK site 4 – oligo1, -2.* Genomic PCRs were conducted with primers *FANCFsite2-for* and *-rev*, *VEGFAsite3-for* and *-rev* and *HEKsite4-for* and *-rev,* respectively.

For the on-target experiments, sgRNA spacers (T1-T34) were cloned into the plasmid pAT9658-sgRNA-mCherry plasmid.

For the off-target experiments, mismatching sgRNA spacers (targets: T1, T2, T6, T7 and T17) were cloned into the plasmid pAT9658-sgRNA-mCherry plasmid.

To construct pAT15000-BE4max, pAT15001-AncBE4max and pAT15002-ABEmax, BE4max, AncBe4max and ABEmax, the corresponding coding sequences were amplified by PCR, using pCMV\_BE4max (Addgene # 112093), pCMV\_AncBE4max (Addgene # 112100) and pCMV\_ABEmax (Addgene # 112101), utilizing oligos *pAT15000-for* and *-rev*,and the products were cloned into an NcoI and PscI digestedpAT9675-CBE plasmid via HiFi Assembly.

**Supplementary Table 1 – List of oligonucleotides**

|  |  |  |
| --- | --- | --- |
| **Oligonucleotides used for BEAR-GFP target cloning plasmid pAT9624** | |  |
|  |  |  |
| **oligo name** | **oligo sequence** |  |
| pAT9624-i1-for | GTGAACCGTCAGATCCGCTAG |  |
| pAT9624-i1-rev | TGAGACGTAAGATCTCCTCGTCTCGCACGTAGCCTTCGGGCATG |  |
| pAT9624-i2-for | GAGACGAGGAGATCTTACGTCTCAATTTTTTAGTTAAAATATGGGAAAG |  |
| pAT9624-i2-rev | CGTCCTTGAAGAAGATGGTGCGCTCCTGCTCAAAAAAGAAAC |  |
| pAT9624-i3-for | GAGCGCACCATCTTCTTCAAGGACG |  |
| pAT9624-i3-rev | CCCGCGGTACCGTCGAC |  |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning splice site screen target plasmids (Fig. 2, Supplementary Fig.2)** | | |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **P1 - pre-edited** | CGTGCAGGTTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCAACCTG |
| **P1 - disrupted** | CGTGCAGATTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCAATCTG |
| **P2 - pre-edited** | CGTGCAGGCTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCAGCCTG |
| **P2 - disrupted** | CGTGCAGACTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCAGTCTG |
| **P3 - pre-edited** | CGTGCAGGGTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCACCCTG |
| **P3 - disrupted** | CGTGCAGAGTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCACTCTG |
| **P4 - pre-edited** | CGTGCAGGATGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCATCCTG |
| **P4 - disrupted** | CGTGCAGAATGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCATTCTG |
| **P5 - pre-edited** | CGTGCAGAGTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCACTCTG |
| **P5 - disrupted** | CGTGCAGAATGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCATTCTG |
| **P6 - pre-edited** | CGTGCAGGGTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCACCCTG |
| **P6 - disrupted** | CGTGCAGAATGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCATTCTG |
| **P7 - pre-edited** | CGTGCAAGTTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCAACTTG |
| **P7 - disrupted** | CGTGCAAATTGAGGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCCTCAATTTG |
| **P8 - pre-edited** | CGTGCAGGTAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTACCTG |
| **P8 - disrupted** | CGTGCAGATAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTATCTG |
| **P9 - pre-edited** | CGTGCAGGCAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTGCCTG |
| **P9 - disrupted** | CGTGCAGACAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTGTCTG |
| **P10 - pre-edited** | CGTGCAGGCAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTGCCTG |
| **P10 - disrupted** | CGTGCAGACAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTGTCTG |
| **P11 - pre-edited** | CGTGCAGGGAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTCCCTG |
| **P11 - disrupted** | CGTGCAGAGAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTCTCTG |
| **P12 - pre-edited** | CGTGCAGAGAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTCTCTG |
| **P12 - disrupted** | CGTGCAGAAAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTTTCTG |
| **P13 - pre-edited** | CGTGCAGGGAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTCCCTG |
| **P13 - disrupted** | CGTGCAGAAAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTTTCTG |
| **P14 - pre-edited** | CGTGCAAGTAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTACTTG |
| **P14 - disrupted** | CGTGCAAATAAGTGCATAGACTGCGGGTTG | AAATCAACCCGCAGTCTATGCACTTATTTG |
| **P15 - pre-edited** | CGTGCAGGTTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAACCTG |
| **P15 - disrupted** | CGTGCAGGCTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAGCCTG |
| **P16 - pre-edited** | CGTGCAGATTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAATCTG |
| **P16 - disrupted** | CGTGCAGACTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAGTCTG |
| **P17 - pre-edited** | CGTGCAGTTTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAAACTG |
| **P17 - disrupted** | CGTGCAGTCTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAGACTG |
| **P18 - pre-edited** | CGTGCAGCTTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAAGCTG |
| **P18 - disrupted** | CGTGCAGCCTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAGGCTG |
| **P19 - pre-edited** | CGTGCAGTCTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAGACTG |
| **P19 - disrupted** | CGTGCAGCCTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAGGCTG |
| **P20 - pre-edited** | CGTGCAGTTTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAAACTG |
| **P20 - disrupted** | CGTGCAGCCTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAGGCTG |
| **P21 - pre-edited** | CGTGCAAGTTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAACTTG |
| **P21 - disrupted** | CGTGCAAGCTGCGGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCCGCAGCTTG |
| **P22 - pre-edited** | CGTGCAGGTAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTACCTG |
| **P22 - disrupted** | CGTGCAGGCAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTGCCTG |
| **P23 - pre-edited** | CGTGCAGATAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTATCTG |
| **P23 - disrupted** | CGTGCAGACAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTGTCTG |
| **P24 - pre-edited** | CGTGCAGTTAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTAACTG |
| **P24 - disrupted** | CGTGCAGTCAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTGACTG |
| **P25 - pre-edited** | CGTGCAGTTAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTAACTG |
| **P25 - disrupted** | CGTGCAGCCAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTGGCTG |
| **P26 - pre-edited** | CGTGCAGTCAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTGACTG |
| **P26 - disrupted** | CGTGCAGCCAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTGGCTG |
| **P27 - pre-edited** | CGTGCAGTTAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTAACTG |
| **P27 - disrupted** | CGTGCAGCCAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTGGCTG |
| **P28 - pre-edited** | CGTGCAAGTAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTACTTG |
| **P28 - disrupted** | CGTGCAAGCAAGTGCTGGAGGTGGGGGTTG | AAATCAACCCCCACCTCCAGCACTTGCTTG |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning sgRNA targets on Fig. 2c, d** | | |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **P1-gRNA** | CACCGCAGATTGAGGCATAGACTG | AAACCAGTCTATGCCTCAATCTGC |
| **P9-gRNA** | CACCGCAGACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTCTGC |
| **P14-gRNA** | CACCGCAAATAAGTGCATAGACTG | AAACCAGTCTATGCACTTATTTGC |
| **P15-gRNA** | CACCGCAGGCTGCGGCTGGAGGTG | AAACCACCTCCAGCCGCAGCCTGC |
| **P24-gRNA** | CACCGCAGTCAAGTGCTGGAGGTG | AAACCACCTCCAGCACTTGACTGC |
|  |  |  |
| **Oligonucleotides used for cloning ABE, -CBE, dABE, -dCBE and their increased fidelity variants, Fig. 2-6** | | |
|  |  |  |
| **oligo name** | **oligo sequence** |  |
| pAT9676-ABE-for | ATTTCCCCGAAAAGTGCCACCTGACGTCCAGCAGAGATCCACTTTGG |  |
| pAT9676-ABE-rev | TGGCCTTTTGCTGGCCTTTTGCTCACATGTCATTTCTTTTTCTTAGCTTGACCAG | |
| pAT9676-BGH-for | GCTAAGAAAAAGAAATGACATGTCCTAGAGCTCGCTGATCAGCCTCG |  |
| pAT9676-BGH-rev | TTTTGCTGGCCTTTTGCTCAGCGGCCGCTCCCCAG |  |
| pAT9749-F1-for | GAGGAAAACGAGGACATTCTGGAAGAT |  |
| pAT9749-F1-rev | GAAAGCTCTGAGGCACGATGGCGTCCACATCGTAGTCGG |  |
| pAT9749-F2-for | CCGACTACGATGTGGACGCCATCGTGCCTCAGAGCTTTC |  |
| pAT9749-F2-rev | GGCTGATCAGCGAGCTCTAGG |  |
| pAT9675-CBE-for | ATTTCCCCGAAAAGTGCCACCTGACGTCCAGCAGAGATCCACTTTGG |  |
| pAT9675-CBE-rev | TGGCCTTTTGCTGGCCTTTTGCTCACATGTCAGACTTTCCTCTTCTTCTTGG |  |
| pAT9675-BGH-for | AGAAGAAGAGGAAAGTCTGACATGTCCTAGAGCTCGCTGATCAGCCTCG |  |
| pAT9991-for | GAACCGGATCTGCTATCTGCAAGA |  |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning editing window BEAR target plasmids (Fig. 3a)** | | |
|  |  |  |
| **oligo name** | **insert1 - fwd oligo** |  |
| Window - disrupted - A20-1-i1-for | GTGAACCGTCAGATCCGCTAG |  |
| Window - disrupted - A20-19-i1-rev | CCGCAGTCTATGCCACACCCATCAGGGCACGGGCAG |  |
| Window - disrupted - A20-1-i2-rev | GGGTGGTCACGAGGGTGGGCCTGCTCAAAAAAGAAAC |  |
| Window - disrupted - A20-1-i3-for | GCCCACCCTCGTGACCAC |  |
| Window - disrupted - A20-1-i3-rev | CCCGCGGTACCGTCGAC |  |
| Window - disrupted - A20-19-i1-rev | CCGCAGTCTATGCCACACCCATCAGGGCACGGGCAG |  |
| Window - disrupted - A20-19-i2-for | GGGTGTGGCATAGACTGCGGG |  |
| Window - disrupted - A18-i2-for | CAAGCTGCCCGTGCCCTGATGGGTTGGCATAGACTGCGGG |  |
| Window - disrupted - A17-i2-for | CAAGCTGCCCGTGCCCTGATGGGTGGCATAGACTGCGGG |  |
| Window - disrupted - A16-i2-for | CAAGCTGCCCGTGCCCTGATGGGTGCATAGACTGCGGG |  |
| Window - disrupted - A15-i2-for | CAAGCTGCCCGTGCCCTGATGGGTCATAGACTGCGGGTTG |  |
| Window - disrupted - A14-i2-for | CAAGCTGCCCGTGCCCTGATGGGTATAGACTGCGGGTTG |  |
| Window - disrupted - A13-i2-for | CAAGCTGCCCGTGCCCTGATGGGTTAGACTGCGGGTTG |  |
| Window - disrupted - A12-i2-for | CAAGCTGCCCGTGCCCTGATGGGTAGACTGCGGGTTG |  |
| Window - disrupted - A11-i2-for | CAAGCTGCCCGTGCCCTGATGGGTGACTGCGGGTTGA |  |
| Window - disrupted - A10-i2-for | CAAGCTGCCCGTGCCCTGATGGGTACTGCGGGTTGATTTTTTAG |  |
| Window - disrupted - A9-i2-for | CAAGCTGCCCGTGCCCTGATGGGTCTGCGGGTTGATTTTTTAG |  |
| Window - disrupted - A8-i2-for | CAAGCTGCCCGTGCCCTGATGGGTGCGGGTTGATTTTTTAG |  |
| Window - disrupted - A7-i2-for | CAAGCTGCCCGTGCCCTGATGGGTCGGGTTGATTTTTTAG |  |
| Window - disrupted - A6-i2-for | CAAGCTGCCCGTGCCCTGATGGGTGGGTTGATTTTTTAGTTAAAATATG |  |
| Window - disrupted - A5-1-i2-for | CAAGCTGCCCGTGCCCTGATGGGGGGTTGATTTTTTAGTTAAAATATG |  |
|  |  |  |
| Window - preedited 20-1-i1-for | GTGAACCGTCAGATCCGCTAG |  |
| Window - preedited 20-1-i2-rev | GGGTGGTCACGAGGGTGGGCCTGCTCAAAAAAGAAAC |  |
| Window - preedited 20-1-i3-for | GCCCACCCTCGTGACCAC |  |
| Window - preedited 20-1-i3-rev | CCCGCGGTACCGTCGAC |  |
| Window - preedited 20-19-i1-rev | CCGCAGTCTATGCCACACCCACCAGGGCACGGGCAG |  |
| Window - preedited 18-1-i1-rev | ACCAGGGCACGGGCAG |  |
| Window - preedited 20-19-i2-rev | GGGTGTGGCATAGACTGCGGG |  |
| Window - preedited 18-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTTGGCATAGACTGCGGG |  |
| Window - preedited 17-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTGGCATAGACTGCGGG |  |
| Window - preedited 16-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTGCATAGACTGCGGG |  |
| Window - preedited 15-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTCATAGACTGCGGGTTG |  |
| Window - preedited 14-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTATAGACTGCGGGTTG |  |
| Window - preedited 13-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTTAGACTGCGGGTTG |  |
| Window - preedited 12-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTAGACTGCGGGTTG |  |
| Window - preedited 11-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTGACTGCGGGTTGA |  |
| Window - preedited 10-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTACTGCGGGTTGATTTTTTAG |  |
| Window - preedited 9-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTCTGCGGGTTGATTTTTTAG |  |
| Window - preedited 8-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTGCGGGTTGATTTTTTAG |  |
| Window - preedited 7-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTCGGGTTGATTTTTTAG |  |
| Window - preedited 6-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGTGGGTTGATTTTTTAGTTAAAATATG |  |
| Window - preedited 5-1-i2-rev | CAAGCTGCCCGTGCCCTGGTGGGGGGTTGATTTTTTAGTTAAAATATG |  |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning editing window sgRNA targets** | | |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **sgRNA - window - 20** | CACCATGGGTGTGGCATAGACTGC | AAACGCAGTCTATGCCACACCCAT |
| **sgRNA - window - 19** | CACCGATGGGTGTGGCATAGACTG | AAACCAGTCTATGCCACACCCATC |
| **sgRNA - window - 18** | CACCGTGATGGGTTGGCATAGACTG | AAACCAGTCTATGCCAACCCATCAC |
| **sgRNA - window - 17** | CACCGCTGATGGGTGGCATAGACTG | AAACCAGTCTATGCCACCCATCAGC |
| **sgRNA - window - 16** | CACCGCCTGATGGGTGCATAGACTG | AAACCAGTCTATGCACCCATCAGGC |
| **sgRNA - window - 15** | CACCGCCCTGATGGGTCATAGACTG | AAACCAGTCTATGACCCATCAGGGC |
| **sgRNA - window - 14** | CACCGCCCTGATGGGTATAGACTG | AAACCAGTCTATACCCATCAGGGC |
| **sgRNA - window - 13** | CACCGTGCCCTGATGGGTTAGACTG | AAACCAGTCTAACCCATCAGGGCAC |
| **sgRNA - window - 12** | CACCGGTGCCCTGATGGGTAGACTG | AAACCAGTCTACCCATCAGGGCACC |
| **sgRNA - window - 11** | CACCGCGTGCCCTGATGGGTGACTG | AAACCAGTCACCCATCAGGGCACGC |
| **sgRNA - window - 10** | CACCGCCGTGCCCTGATGGGTACTG | AAACCAGTACCCATCAGGGCACGGC |
| **sgRNA - window - 9** | CACCGCCCGTGCCCTGATGGGTCTG | AAACCAGACCCATCAGGGCACGGGC |
| **sgRNA - window - 8** | CACCGCCCGTGCCCTGATGGGTGC | AAACGCACCCATCAGGGCACGGGC |
| **sgRNA - window - 7** | CACCGTGCCCGTGCCCTGATGGGTC | AAACGACCCATCAGGGCACGGGCAC |
| **sgRNA - window - 6** | CACCGCTGCCCGTGCCCTGATGGGT | AAACACCCATCAGGGCACGGGCAGC |
| **sgRNA - window - 5** | CACCGCTGCCCGTGCCCTGATGGG | AAACCCCATCAGGGCACGGGCAGC |
| **sgRNA - window - 4** | CACCAGCTGCCCGTGCCCTGATGG | AAACCCATCAGGGCACGGGCAGCT |
| **sgRNA - window - 3** | CACCAAGCTGCCCGTGCCCTGATG | AAACCATCAGGGCACGGGCAGCTT |
| **sgRNA - window - 2** | CACCGCAAGCTGCCCGTGCCCTGAT | AAACATCAGGGCACGGGCAGCTTGC |
| **sgRNA - window - 1** | CACCGCAAGCTGCCCGTGCCCTGA | AAACTCAGGGCACGGGCAGCTTGC |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning BEAR-mScarlet and BEAR-mCherry plasmids (Fig. 3b)** | | |
|  |  |  |
| **oligo name** | **oligo sequence** |  |
| pAT9750-i1-for | CGTCAGATCCGCTAGCGCTACCGGTCGCCACCATGGTGAGCAAG |  |
| pAT9750-i1-rev | ACCCGCAGTCTATGCACTTGTCTGCAGGGAGGAGTCC |  |
| pAT9750-i2-for | CAAGTGCATAGACTGC |  |
| pAT9750-i2-rev | ACTCGCCGTCCTGCTCAAAAAAGAAAC |  |
| pAT9750-i3-for | TTTTGAGCAGGACGGCGAGTTC |  |
| pAT9750-i3-rev | CGCGGTACCGTCGACTGCAGCCCTCTAGATGCATGCTCG |  |
| pAT9751-i1-rev | ACCCGCAGTCTATGCACTTGCCTGCAGGGAGGAGTCC |  |
| pAT9752-i1-rev | ACCCGCAGTCTATGCACTTGTCTGCGTCACGGTCACGGCG |  |
| pAT9752-i2-rev | AGGGAGGTGTCCTGCTCAAAAAAGAAAC |  |
| pAT9752-i3-for | TTTTGAGCAGGACACCTCCCTGGAGG |  |
| pAT9753-i1-rev | ACCCGCAGTCTATGCACTTGCCTGCGTCACGGTCACGGCG |  |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning pAT9679-BFP-sgRNA (Fig. 3b)** | | |
|  |  |  |
| **oligo name** | **oligo sequence** |  |
| pAT9679-for | ACACAGGTGTCGTGACGCGGGATCCGCCACCATGAGCG |  |
| pAT9679-rev | AGCGAGCTCTAGGACATGTAGATCTTAATTAAGCTTGTGCCCCAG |  |
|  |  |  |
|  |  |  |
| **BEAR plasmids with different intron positions (Fig. 3c, d)** | | |
|  |  |  |
| **oligo name** | **oligo sequence** |  |
| all intron constructs - i1-for | GTGAACCGTCAGATCCGCTAG |  |
| all intron constructs - i3-rev | CCCGCGGTACCGTCGAC |  |
| GFP 53-i1-rev | CTGTGGTGCAGATAAACTTCAGGGTCAGCTTGCC |  |
| GFP 53-i2-for | GAAGTTTATCTGCACCACAGGCAAGTGCATAGACTGCG |  |
| GFP 53-i2-rev | GGCAGCTTGCCTGCTCAAAAAAGAAAC |  |
| GFP 53-i3-for | TTTTGAGCAGGCAAGCTGCCCGTGCC |  |
| GFP 58-i1-rev | ATCAGGGCACGGGCAG |  |
| GFP 58-i2-for | CAAGCTGCCCGTGCCCTGATGGGTATAGACTGCGGGTTG |  |
| GFP 58-i2-rev | GGGTGGTCACGAGGGTGGGCCTGCTCAAAAAAGAAAC |  |
| GFP 58-i3-for | GCCCACCCTCGTGACCAC |  |
| GFP 87-i1-rev | CTGACTTGAAGAAATCGTGCTGCTTCATG |  |
| GFP 87-i2-for | GCACGATTTCTTCAAGTCAGGCAAGTGCATAGACTGCG |  |
| GFP 87-i2-rev | CCTTCGGGCATGGCTGCTCAAAAAAGAAAC |  |
| GFP 87-i3-for | TGAGCAGCCATGCCCGAAGGCTACG |  |
| GFP 117-i1-rev | CCGCAGTCTATGCCACACCCACCCTCAAACTTCACCTCGGCGCG |  |
| GFP 117-i2-for | GGGTGTGGCATAGACTGCGGG |  |
| GFP 117-i2-rev | CGGTTCACCAGGGTGTCGCCTGCTCAAAAAAGAAAC |  |
| GFP 117-i3-for | GCGACACCCTGGTGAACCG |  |
| mScarlet 65-i1-rev | CTGAGGGGAAAGGATGTCCCAGGAGAAGGG |  |
| mScarlet 65-i2-for | GGGACATCCTTTCCCCTCAGGCAAGTGCATAGACTGCG |  |
| mScarlet 65-i2-rev | CGTACATGAACTGCTCAAAAAAGAAAC |  |
| mScarlet 65-i3-for | TTTTGAGCAGTTCATGTACGGCTCCAGGG |  |
| mScarlet 71-i1-rev | CTGGAGCCGTACATAAACTGAGGGGACAGGATG |  |
| mScarlet 71-i2-for | CAGTTTATGTACGGCTCCAGGCAAGTGCATAGACTGCG |  |
| mScarlet 71-i2-rev | GGTGAAGGCCCTGCTCAAAAAAGAAAC |  |
| mScarlet 71-i3-for | TTTTGAGCAGGGCCTTCACCAAGCACC |  |
| mScarlet 78-i1-rev | CTGGGTGCTTGGTAAAGGCCCTGGAGCCGTA |  |
| mScarlet 78-i2-for | GGCCTTTACCAAGCACCCAGGCAAGTGCATAGACTGCG |  |
| mScarlet 78-i2-rev | GGGATGTCGGCTGCTCAAAAAAGAAAC |  |
| mScarlet 78-i3-for | TTTTGAGCAGCCGACATCCCCGACTACTATAAGCAG |  |
| mScarlet 110-i1-rev | ACCCGCAGTCTATGCACTTGCCTGCAGGGAGGAGTCC |  |
| mScarlet 110-i2-for | CAAGTGCATAGACTGC |  |
| mScarlet 110-i2-rev | ACTCGCCGTCCTGCTCAAAAAAGAAAC |  |
| mScarlet 110-i3-for | TTTTGAGCAGGACGGCGAGTTC |  |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for plasmids used in creating BEAR cell lines (Supplementary Fig.3, Fig. 4)** | | |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **U6-TL** | CACCGGCGCAACGCGATCGCGTAA | AAACTTACGCGATCGCGTTGCGCC |
| **U6-AAVS-1-a** | CACCACAGTGGGGCCACTAGGGAC | AAACGTCCCTAGTGGCCCCACTGT |
| **U6-AAVS-1-b** | CACCGGTCCCTAGTGGCCCCACTG | AAACCAGTGGGGCCACTAGGGACC |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning genomic sgRNA targets (Fig. 4)** | | |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **sgRNA FANCF site 2** | CACCGCTGCAGAAGGGATTCCATG | AAACCATGGAATCCCTTCTGCAGC |
| **sgRNA VEGFA site 3** | CACCGGTGAGTGAGTGTGTGCGTG | AAACCACGCACACACTCACTCACC |
| **sgRNA HEK site 4** | CACCGGCACTGCGGCTGGAGGTGG | AAACCCACCTCCAGCCGCAGTGCC |
|  |  |  |
|  |  |  |
|  |  |  |
| **Oligos for amplifying genomic targets (Fig. 4)** | |  |
|  |  |  |
| **oligo name** | **oligo sequence** |  |
| FANCFsite2-for | GGGCCGGGAAAGAGTTGCTG |  |
| FANCFsite2-rev | GCCCTACATCTGCTCTCCCTCC |  |
| VEGFAsite3-for | TCCAGATGGCACATTGTCAG |  |
| VEGFAsite3-rev | AGGGAGCAGGAAAGTGAGGT |  |
| HEKsite4-for | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAACCCAGGTAGCCAGAGAC | |
| HEKsite4-rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTTTCAACCCGAACGGAG | |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning on-target screen, target plasmids (Fig. 5)** | | |
|  |  |  |
| **On-target T1** | see "p9 not-edited" cloning, above |  |
| **On-target T2** | CGTGCAGACAAGTAGCTTGCCGGTGG | AAATCCACCGGCAAGCTACTTGTCTG |
| **On-target T3** | CGTGCAGACAAGTGCGACGTAAACGG | AAATCCGTTTACGTCGCACTTGTCTG |
| **On-target T4** | CGTGCAGACAAGTATGAACTTCAGGG | AAATCCCTGAAGTTCATACTTGTCTG |
| **On-target T5** | CGTGCAGACAAGTATCTTCTTCAAGG | AAATCCTTGAAGAAGATACTTGTCTG |
| **On-target T6** | CGTGCAGACAAGTAACTTCACCTCGG | AAATCCGAGGTGAAGTTACTTGTCTG |
| **On-target T7** | CGTGCAGACAAGTATGGTCCTGCTGG | AAATCCAGCAGGACCATACTTGTCTG |
| **On-target T8** | CGTGCAGACAAGTAGAGTGATCCCGG | AAATCCGGGATCACTCTACTTGTCTG |
| **On-target T9** | CGTGCAGACAAGTTTGAAGAAGATGG | AAATCCATCTTCTTCAAACTTGTCTG |
| **On-target T10** | CGTGCAGACAAGTGGCGACACCCTGG | AAATCCAGGGTGTCGCCACTTGTCTG |
| **On-target T11** | CGTGCAGACAAGTAGGGCGGACTGGG | AAATCCCAGTCCGCCCTACTTGTCTG |
| **On-target T12** | CGTGCAGACAAGTAGTGGTTGTCGGG | AAATCCCGACAACCACTACTTGTCTG |
| **On-target T13** | CGTGCAGACAAGTTCGCCCTCGCCGG | AAATCCGGCGAGGGCGAACTTGTCTG |
| **On-target T14** | CGTGCAGACAAGTATGCCACCTACGG | AAATCCGTAGGTGGCATACTTGTCTG |
| **On-target T15** | CGTGCAGACAAGTTGCACGCCGTAGG | AAATCCTACGGCGTGCAACTTGTCTG |
| **On-target T16** | CGTGCAGACAAGTTCGAGCTGAAGGG | AAATCCCTTCAGCTCGAACTTGTCTG |
| **On-target T17** | CGTGCAGACAAGTTCACGAGGGTGGG | AAATCCCACCCTCGTGAACTTGTCTG |
| **On-target T18** | CGTGCAGACAAGTTCGGGCATGGCGG | AAATCCGCCATGCCCGAACTTGTCTG |
| **On-target T19** | CGTGCAGACAAGTTCAAGGAGGACGG | AAATCCGTCCTCCTTGAACTTGTCTG |
| **On-target T20** | CGTGCAGACAAGTTGGTAGTGGTCGG | AAATCCGACCACTACCAACTTGTCTG |
| **On-target T21** | CGTGCAGACAAGTGGGTGGGCCAGGG | AAATCCCTGGCCCACCCACTTGTCTG |
| **On-target T22** | CGTGCAGACAAGTCTGTTCACCGGGG | AAATCCCCGGTGAACAGACTTGTCTG |
| **On-target T23** | CGTGCAGACAAGTGGCACCACCCCGG | AAATCCGGGGTGGTGCCACTTGTCTG |
| **On-target T24** | CGTGCAGACAAGTCTGGTCGAGCTGG | AAATCCAGCTCGACCAGACTTGTCTG |
| **On-target T25** | CGTGCAGACAAGTTCGACCAGGATGG | AAATCCATCCTGGTCGAACTTGTCTG |
| **On-target T26** | CGTGCAGACAAGTTCAGCGTGTCCGG | AAATCCGGACACGCTGAACTTGTCTG |
| **On-target T27** | CGTGCAGACAAGTAGGGTGGGCCAGG | AAATCCTGGCCCACCCTACTTGTCTG |
| **On-target T28** | CGTGCAGACAAGTGTCACGAGGGTGG | AAATCCACCCTCGTGACACTTGTCTG |
| **On-target T29** | CGTGCAGACAAGTGTGGTCACGAGGG | AAATCCCTCGTGACCACACTTGTCTG |
| **On-target T30** | CGTGCAGACAAGTCCGTAGGTCAGGG | AAATCCCTGACCTACGGACTTGTCTG |
| **On-target T31** | CGTGCAGACAAGTGTCGGGGTAGCGG | AAATCCGCTACCCCGACACTTGTCTG |
| **On-target T32** | CGTGCAGACAAGTATCGAGCTGAAGG | AAATCCTTCAGCTCGATACTTGTCTG |
| **On-target T33** | CGTGCAGACAAGTGACTTCAAGGAGG | AAATCCTCCTTGAAGTCACTTGTCTG |
| **On-target T34** | CGTGCAGACAAGTGTCGCCGATGGGG | AAATCCCCATCGGCGACACTTGTCTG |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning on-target screen sgRNA plasmids** | |  |
|  |  |  |
| **sgRNA - On-target T1** | see "P9-gRNA" cloning |  |
| **sgRNA - On-target T2** | CACCGCAGACAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGTCTGC |
| **sgRNA - On-target T3** | CACCGCAGACAAGTGCGACGTAAA | AAACTTTACGTCGCACTTGTCTGC |
| **sgRNA - On-target T4** | CACCGCAGACAAGTATGAACTTCA | AAACTGAAGTTCATACTTGTCTGC |
| **sgRNA - On-target T5** | CACCGCAGACAAGTATCTTCTTCA | AAACTGAAGAAGATACTTGTCTGC |
| **sgRNA - On-target T6** | CACCGCAGACAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGTCTGC |
| **sgRNA - On-target T7** | CACCGCAGACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTCTGC |
| **sgRNA - On-target T8** | CACCGCAGACAAGTAGAGTGATCC | AAACGGATCACTCTACTTGTCTGC |
| **sgRNA - On-target T9** | CACCGCAGACAAGTTTGAAGAAGA | AAACTCTTCTTCAAACTTGTCTGC |
| **sgRNA - On-target T10** | CACCGCAGACAAGTGGCGACACCC | AAACGGGTGTCGCCACTTGTCTGC |
| **sgRNA - On-target T11** | CACCGCAGACAAGTAGGGCGGACT | AAACAGTCCGCCCTACTTGTCTGC |
| **sgRNA - On-target T12** | CACCGCAGACAAGTAGTGGTTGTC | AAACGACAACCACTACTTGTCTGC |
| **sgRNA - On-target T13** | CACCGCAGACAAGTTCGCCCTCGC | AAACGCGAGGGCGAACTTGTCTGC |
| **sgRNA - On-target T14** | CACCGCAGACAAGTATGCCACCTA | AAACTAGGTGGCATACTTGTCTGC |
| **sgRNA - On-target T15** | CACCGCAGACAAGTTGCACGCCGT | AAACACGGCGTGCAACTTGTCTGC |
| **sgRNA - On-target T16** | CACCGCAGACAAGTTCGAGCTGAA | AAACTTCAGCTCGAACTTGTCTGC |
| **sgRNA - On-target T17** | CACCGCAGACAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGTCTGC |
| **sgRNA - On-target T18** | CACCGCAGACAAGTTCGGGCATGG | AAACCCATGCCCGAACTTGTCTGC |
| **sgRNA - On-target T19** | CACCGCAGACAAGTTCAAGGAGGA | AAACTCCTCCTTGAACTTGTCTGC |
| **sgRNA - On-target T20** | CACCGCAGACAAGTTGGTAGTGGT | AAACACCACTACCAACTTGTCTGC |
| **sgRNA - On-target T21** | CACCGCAGACAAGTGGGTGGGCCA | AAACTGGCCCACCCACTTGTCTGC |
| **sgRNA - On-target T22** | CACCGCAGACAAGTCTGTTCACCG | AAACCGGTGAACAGACTTGTCTGC |
| **sgRNA - On-target T23** | CACCGCAGACAAGTGGCACCACCC | AAACGGGTGGTGCCACTTGTCTGC |
| **sgRNA - On-target T24** | CACCGCAGACAAGTCTGGTCGAGC | AAACGCTCGACCAGACTTGTCTGC |
| **sgRNA - On-target T25** | CACCGCAGACAAGTTCGACCAGGA | AAACTCCTGGTCGAACTTGTCTGC |
| **sgRNA - On-target T26** | CACCGCAGACAAGTTCAGCGTGTC | AAACGACACGCTGAACTTGTCTGC |
| **sgRNA - On-target T27** | CACCGCAGACAAGTAGGGTGGGCC | AAACGGCCCACCCTACTTGTCTGC |
| **sgRNA - On-target T28** | CACCGCAGACAAGTGTCACGAGGG | AAACCCCTCGTGACACTTGTCTGC |
| **sgRNA - On-target T29** | CACCGCAGACAAGTGTGGTCACGA | AAACTCGTGACCACACTTGTCTGC |
| **sgRNA - On-target T30** | CACCGCAGACAAGTCCGTAGGTCA | AAACTGACCTACGGACTTGTCTGC |
| **sgRNA - On-target T31** | CACCGCAGACAAGTGTCGGGGTAG | AAACCTACCCCGACACTTGTCTGC |
| **sgRNA - On-target T32** | CACCGCAGACAAGTATCGAGCTGA | AAACTCAGCTCGATACTTGTCTGC |
| **sgRNA - On-target T33** | CACCGCAGACAAGTGACTTCAAGG | AAACCCTTGAAGTCACTTGTCTGC |
| **sgRNA - On-target T34** | CACCGCAGACAAGTGTCGCCGATG | AAACCATCGGCGACACTTGTCTGC |
|  |  |  |
|  |  |  |
| **Oligonucleotides used for cloning mismatching sgRNAs (Fig6, Supplementary Fig.6, Supplementary Fig.7)** | |  |
|  |  |  |
| **Target 1 mismatching sgRNAs (Fig. 6)** | |  |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **T1-1MM1** | CACCACAGACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTCTGT |
| **T1-1MM2** | CACCGTAGACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTCTAC |
| **T1-1MM3** | CACCGCGGACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTCCGC |
| **T1-1MM4** | CACCGCAAACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTTTGC |
| **T1-1MM5** | CACCGCAGGCAAGTGCATAGACTG | AAACCAGTCTATGCACTTGCCTGC |
| **T1-1MM6** | CACCGCAGATAAGTGCATAGACTG | AAACCAGTCTATGCACTTATCTGC |
| **T1-1MM7** | CACCGCAGACGAGTGCATAGACTG | AAACCAGTCTATGCACTCGTCTGC |
| **T1-1MM8** | CACCGCAGACAGGTGCATAGACTG | AAACCAGTCTATGCACCTGTCTGC |
| **T1-1MM9** | CACCGCAGACAAATGCATAGACTG | AAACCAGTCTATGCATTTGTCTGC |
| **T1-1MM10** | CACCGCAGACAAGAGCATAGACTG | AAACCAGTCTATGCTCTTGTCTGC |
| **T1-1MM11** | CACCGCAGACAAGTACATAGACTG | AAACCAGTCTATGTACTTGTCTGC |
| **T1-1MM12** | CACCGCAGACAAGTGTATAGACTG | AAACCAGTCTATACACTTGTCTGC |
| **T1-1MM13** | CACCGCAGACAAGTGCGTAGACTG | AAACCAGTCTACGCACTTGTCTGC |
| **T1-1MM14** | CACCGCAGACAAGTGCAAAGACTG | AAACCAGTCTTTGCACTTGTCTGC |
| **T1-1MM15** | CACCGCAGACAAGTGCATGGACTG | AAACCAGTCCATGCACTTGTCTGC |
| **T1-1MM16** | CACCGCAGACAAGTGCATAAACTG | AAACCAGTTTATGCACTTGTCTGC |
| **T1-1MM17** | CACCGCAGACAAGTGCATAGGCTG | AAACCAGCCTATGCACTTGTCTGC |
| **T1-1MM18** | CACCGCAGACAAGTGCATAGATTG | AAACCAATCTATGCACTTGTCTGC |
| **T1-1MM19** | CACCGCAGACAAGTGCATAGACAG | AAACCTGTCTATGCACTTGTCTGC |
| **T1-1MM20** | CACCGCAGACAAGTGCATAGACTA | AAACTAGTCTATGCACTTGTCTGC |
| **T1-2MM1** | CACCATAGACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTCTAT |
| **T1-2MM2** | CACCGTGGACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTCCAC |
| **T1-2MM3** | CACCGCGAACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTTCGC |
| **T1-2MM5** | CACCGCAGGTAAGTGCATAGACTG | AAACCAGTCTATGCACTTACCTGC |
| **T1-2MM7** | CACCGCAGACGGGTGCATAGACTG | AAACCAGTCTATGCACCCGTCTGC |
| **T1-2MM9** | CACCGCAGACAAAAGCATAGACTG | AAACCAGTCTATGCTTTTGTCTGC |
| **T1-2MM11** | CACCGCAGACAAGTATATAGACTG | AAACCAGTCTATATACTTGTCTGC |
| **T1-2MM13** | CACCGCAGACAAGTGCGAAGACTG | AAACCAGTCTTCGCACTTGTCTGC |
| **T1-2MM15** | CACCGCAGACAAGTGCATGAACTG | AAACCAGTTCATGCACTTGTCTGC |
| **T1-2MM17** | CACCGCAGACAAGTGCATAGGTTG | AAACCAACCTATGCACTTGTCTGC |
| **T1-2MM19** | CACCGCAGACAAGTGCATAGACAA | AAACTTGTCTATGCACTTGTCTGC |
| **T1-3MM1** | CACCATGGACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTCCAT |
| **T1-3MM2** | CACCGTGAACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTTCAC |
| **T1-3MM3** | CACCGCGAGCAAGTGCATAGACTG | AAACCAGTCTATGCACTTGCTCGC |
| **T1-3MM6** | CACCGCAGATGGGTGCATAGACTG | AAACCAGTCTATGCACCCATCTGC |
| **T1-3MM9** | CACCGCAGACAAAAACATAGACTG | AAACCAGTCTATGTTTTTGTCTGC |
| **T1-3MM12** | CACCGCAGACAAGTGTGAAGACTG | AAACCAGTCTTCACACTTGTCTGC |
| **T1-3MM15** | CACCGCAGACAAGTGCATGAGCTG | AAACCAGCTCATGCACTTGTCTGC |
| **T1-3MM18** | CACCGCAGACAAGTGCATAGATAA | AAACTTATCTATGCACTTGTCTGC |
| **T1-4MM1** | CACCATGAACAAGTGCATAGACTG | AAACCAGTCTATGCACTTGTTCAT |
| **T1-4MM2** | CACCGTGAGCAAGTGCATAGACTG | AAACCAGTCTATGCACTTGCTCAC |
| **T1-4MM5** | CACCGCAGGTGGGTGCATAGACTG | AAACCAGTCTATGCACCCACCTGC |
| **T1-4MM9** | CACCGCAGACAAAAATATAGACTG | AAACCAGTCTATATTTTTGTCTGC |
| **T1-4MM13** | CACCGCAGACAAGTGCGAGAACTG | AAACCAGTTCTCGCACTTGTCTGC |
| **T1-4MM17** | CACCGCAGACAAGTGCATAGGTAA | AAACTTACCTATGCACTTGTCTGC |
| **T1-5MM1** | CACCATGAGCAAGTGCATAGACTG | AAACCAGTCTATGCACTTGCTCAT |
| **T1-5MM2** | CACCGTGAGTAAGTGCATAGACTG | AAACCAGTCTATGCACTTACTCAC |
| **T1-5MM6** | CACCGCAGATGGAAGCATAGACTG | AAACCAGTCTATGCTTCCATCTGC |
| **T1-5MM11** | CACCGCAGACAAGTATGAGGACTG | AAACCAGTCCTCATACTTGTCTGC |
| **T1-5MM16** | CACCGCAGACAAGTGCATAAGTAA | AAACTTACTTATGCACTTGTCTGC |
|  |  |  |
|  |  |  |
| **Target 7 mismatching sgRNAs (Supplementary Fig.6)** | |  |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **T7-1MM1** | CACCACAGACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTCTGT |
| **T7-1MM2** | CACCGTAGACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTCTAC |
| **T7-1MM3** | CACCGCGGACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTCCGC |
| **T7-1MM4** | CACCGCAAACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTTTGC |
| **T7-1MM5** | CACCGCAGGCAAGTATGGTCCTGC | AAACGCAGGACCATACTTGCCTGC |
| **T7-1MM6** | CACCGCAGATAAGTATGGTCCTGC | AAACGCAGGACCATACTTATCTGC |
| **T7-1MM7** | CACCGCAGACGAGTATGGTCCTGC | AAACGCAGGACCATACTCGTCTGC |
| **T7-1MM8** | CACCGCAGACAGGTATGGTCCTGC | AAACGCAGGACCATACCTGTCTGC |
| **T7-1MM9** | CACCGCAGACAAATATGGTCCTGC | AAACGCAGGACCATATTTGTCTGC |
| **T7-1MM10** | CACCGCAGACAAGCATGGTCCTGC | AAACGCAGGACCATGCTTGTCTGC |
| **T7-1MM11** | CACCGCAGACAAGTGTGGTCCTGC | AAACGCAGGACCACACTTGTCTGC |
| **T7-1MM12** | CACCGCAGACAAGTACGGTCCTGC | AAACGCAGGACCGTACTTGTCTGC |
| **T7-1MM13** | CACCGCAGACAAGTATAGTCCTGC | AAACGCAGGACTATACTTGTCTGC |
| **T7-1MM14** | CACCGCAGACAAGTATGATCCTGC | AAACGCAGGATCATACTTGTCTGC |
| **T7-1MM15** | CACCGCAGACAAGTATGGCCCTGC | AAACGCAGGGCCATACTTGTCTGC |
| **T7-1MM16** | CACCGCAGACAAGTATGGTTCTGC | AAACGCAGAACCATACTTGTCTGC |
| **T7-1MM17** | CACCGCAGACAAGTATGGTCTTGC | AAACGCAAGACCATACTTGTCTGC |
| **T7-1MM18** | CACCGCAGACAAGTATGGTCCCGC | AAACGCGGGACCATACTTGTCTGC |
| **T7-1MM19** | CACCGCAGACAAGTATGGTCCTAC | AAACGTAGGACCATACTTGTCTGC |
| **T7-1MM20** | CACCGCAGACAAGTATGGTCCTGT | AAACACAGGACCATACTTGTCTGC |
| **T7-2MM1** | CACCATAGACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTCTAT |
| **T7-2MM2** | CACCGTGGACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTCCAC |
| **T7-2MM3** | CACCGCGAACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTTCGC |
| **T7-2MM5** | CACCGCAGGTAAGTATGGTCCTGC | AAACGCAGGACCATACTTACCTGC |
| **T7-2MM7** | CACCGCAGACGGGTATGGTCCTGC | AAACGCAGGACCATACCCGTCTGC |
| **T7-2MM9** | CACCGCAGACAAACATGGTCCTGC | AAACGCAGGACCATGTTTGTCTGC |
| **T7-2MM11** | CACCGCAGACAAGTGCGGTCCTGC | AAACGCAGGACCGCACTTGTCTGC |
| **T7-2MM13** | CACCGCAGACAAGTATAATCCTGC | AAACGCAGGATTATACTTGTCTGC |
| **T7-2MM15** | CACCGCAGACAAGTATGGCTCTGC | AAACGCAGAGCCATACTTGTCTGC |
| **T7-2MM17** | CACCGCAGACAAGTATGGTCTCGC | AAACGCGAGACCATACTTGTCTGC |
| **T7-2MM19** | CACCGCAGACAAGTATGGTCCTAT | AAACATAGGACCATACTTGTCTGC |
| **T7-3MM1** | CACCATGGACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTCCAT |
| **T7-3MM2** | CACCGTGAACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTTCAC |
| **T7-3MM3** | CACCGCGAGCAAGTATGGTCCTGC | AAACGCAGGACCATACTTGCTCGC |
| **T7-3MM6** | CACCGCAGATGGGTATGGTCCTGC | AAACGCAGGACCATACCCATCTGC |
| **T7-3MM9** | CACCGCAGACAAACGTGGTCCTGC | AAACGCAGGACCACGTTTGTCTGC |
| **T7-3MM12** | CACCGCAGACAAGTACAATCCTGC | AAACGCAGGATTGTACTTGTCTGC |
| **T7-3MM15** | CACCGCAGACAAGTATGGCTTTGC | AAACGCAAAGCCATACTTGTCTGC |
| **T7-3MM18** | CACCGCAGACAAGTATGGTCCCAT | AAACATGGGACCATACTTGTCTGC |
| **T7-4MM1** | CACCATGAACAAGTATGGTCCTGC | AAACGCAGGACCATACTTGTTCAT |
| **T7-4MM2** | CACCGTGAGCAAGTATGGTCCTGC | AAACGCAGGACCATACTTGCTCAC |
| **T7-4MM5** | CACCGCAGGTGGGTATGGTCCTGC | AAACGCAGGACCATACCCACCTGC |
| **T7-4MM9** | CACCGCAGACAAACGCGGTCCTGC | AAACGCAGGACCGCGTTTGTCTGC |
| **T7-4MM13** | CACCGCAGACAAGTATAACTCTGC | AAACGCAGAGTTATACTTGTCTGC |
| **T7-4MM17** | CACCGCAGACAAGTATGGTCTCAT | AAACATGAGACCATACTTGTCTGC |
| **T7-5MM1** | CACCATGAGCAAGTATGGTCCTGC | AAACGCAGGACCATACTTGCTCAT |
| **T7-5MM2** | CACCGTGAGTAAGTATGGTCCTGC | AAACGCAGGACCATACTTACTCAC |
| **T7-5MM6** | CACCGCAGATGGACATGGTCCTGC | AAACGCAGGACCATGTCCATCTGC |
| **T7-5MM11** | CACCGCAGACAAGTGCAACCCTGC | AAACGCAGGGTTGCACTTGTCTGC |
| **T7-5MM16** | CACCGCAGACAAGTATGGTTTCAT | AAACATGAAACCATACTTGTCTGC |
|  |  |  |
|  |  |  |
| **Target 2 mismatching sgRNAs (Supplementary Fig.7)** | |  |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **T2-1MM1** | CACCACAGACAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGTCTGT |
| **T2-1MM2** | CACCGTAGACAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGTCTAC |
| **T2-1MM3** | CACCGCGGACAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGTCCGC |
| **T2-1MM4** | CACCGCAAACAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGTTTGC |
| **T2-1MM5** | CACCGCAGGCAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGCCTGC |
| **T2-1MM6** | CACCGCAGATAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTATCTGC |
| **T2-1MM7** | CACCGCAGACGAGTAGCTTGCCGG | AAACCCGGCAAGCTACTCGTCTGC |
| **T2-1MM8** | CACCGCAGACAGGTAGCTTGCCGG | AAACCCGGCAAGCTACCTGTCTGC |
| **T2-1MM9** | CACCGCAGACAAATAGCTTGCCGG | AAACCCGGCAAGCTATTTGTCTGC |
| **T2-1MM10** | CACCGCAGACAAGCAGCTTGCCGG | AAACCCGGCAAGCTGCTTGTCTGC |
| **T2-1MM11** | CACCGCAGACAAGTGGCTTGCCGG | AAACCCGGCAAGCCACTTGTCTGC |
| **T2-1MM12** | CACCGCAGACAAGTAACTTGCCGG | AAACCCGGCAAGTTACTTGTCTGC |
| **T2-1MM13** | CACCGCAGACAAGTAGTTTGCCGG | AAACCCGGCAAACTACTTGTCTGC |
| **T2-1MM14** | CACCGCAGACAAGTAGCCTGCCGG | AAACCCGGCAGGCTACTTGTCTGC |
| **T2-1MM15** | CACCGCAGACAAGTAGCTCGCCGG | AAACCCGGCGAGCTACTTGTCTGC |
| **T2-1MM16** | CACCGCAGACAAGTAGCTTACCGG | AAACCCGGTAAGCTACTTGTCTGC |
| **T2-1MM17** | CACCGCAGACAAGTAGCTTGTCGG | AAACCCGACAAGCTACTTGTCTGC |
| **T2-1MM18** | CACCGCAGACAAGTAGCTTGCTGG | AAACCCAGCAAGCTACTTGTCTGC |
| **T2-1MM19** | CACCGCAGACAAGTAGCTTGCCAG | AAACCTGGCAAGCTACTTGTCTGC |
| **T2-1MM20** | CACCGCAGACAAGTAGCTTGCCGA | AAACTCGGCAAGCTACTTGTCTGC |
| **T2-2MM1** | CACCATAGACAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGTCTAT |
| **T2-2MM2** | CACCGTGGACAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGTCCAC |
| **T2-2MM3** | CACCGCGAACAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTGTTCGC |
| **T2-2MM5** | CACCGCAGGTAAGTAGCTTGCCGG | AAACCCGGCAAGCTACTTACCTGC |
| **T2-2MM7** | CACCGCAGACGGGTAGCTTGCCGG | AAACCCGGCAAGCTACCCGTCTGC |
| **T2-2MM9** | CACCGCAGACAAACAGCTTGCCGG | AAACCCGGCAAGCTGTTTGTCTGC |
| **T2-2MM11** | CACCGCAGACAAGTGACTTGCCGG | AAACCCGGCAAGTCACTTGTCTGC |
| **T2-2MM13** | CACCGCAGACAAGTAGTCTGCCGG | AAACCCGGCAGACTACTTGTCTGC |
| **T2-2MM15** | CACCGCAGACAAGTAGCTCACCGG | AAACCCGGTGAGCTACTTGTCTGC |
| **T2-2MM17** | CACCGCAGACAAGTAGCTTGTTGG | AAACCCAACAAGCTACTTGTCTGC |
| **T2-2MM19** | CACCGCAGACAAGTAGCTTGCCAA | AAACTTGGCAAGCTACTTGTCTGC |
|  |  |  |
|  |  |  |
| **Target 6 mismatching sgRNAs (Supplementary Fig.7)** | |  |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **T6-1MM1** | CACCACAGACAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGTCTGT |
| **T6-1MM2** | CACCGTAGACAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGTCTAC |
| **T6-1MM3** | CACCGCGGACAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGTCCGC |
| **T6-1MM4** | CACCGCAAACAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGTTTGC |
| **T6-1MM5** | CACCGCAGGCAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGCCTGC |
| **T6-1MM6** | CACCGCAGATAAGTAACTTCACCT | AAACAGGTGAAGTTACTTATCTGC |
| **T6-1MM7** | CACCGCAGACGAGTAACTTCACCT | AAACAGGTGAAGTTACTCGTCTGC |
| **T6-1MM8** | CACCGCAGACAGGTAACTTCACCT | AAACAGGTGAAGTTACCTGTCTGC |
| **T6-1MM9** | CACCGCAGACAAATAACTTCACCT | AAACAGGTGAAGTTATTTGTCTGC |
| **T6-1MM10** | CACCGCAGACAAGCAACTTCACCT | AAACAGGTGAAGTTGCTTGTCTGC |
| **T6-1MM11** | CACCGCAGACAAGTGACTTCACCT | AAACAGGTGAAGTCACTTGTCTGC |
| **T6-1MM12** | CACCGCAGACAAGTAGCTTCACCT | AAACAGGTGAAGCTACTTGTCTGC |
| **T6-1MM13** | CACCGCAGACAAGTAATTTCACCT | AAACAGGTGAAATTACTTGTCTGC |
| **T6-1MM14** | CACCGCAGACAAGTAACCTCACCT | AAACAGGTGAGGTTACTTGTCTGC |
| **T6-1MM15** | CACCGCAGACAAGTAACTCCACCT | AAACAGGTGGAGTTACTTGTCTGC |
| **T6-1MM16** | CACCGCAGACAAGTAACTTTACCT | AAACAGGTAAAGTTACTTGTCTGC |
| **T6-1MM17** | CACCGCAGACAAGTAACTTCGCCT | AAACAGGCGAAGTTACTTGTCTGC |
| **T6-1MM18** | CACCGCAGACAAGTAACTTCATCT | AAACAGATGAAGTTACTTGTCTGC |
| **T6-1MM19** | CACCGCAGACAAGTAACTTCACTT | AAACAAGTGAAGTTACTTGTCTGC |
| **T6-1MM20** | CACCGCAGACAAGTAACTTCACCC | AAACGGGTGAAGTTACTTGTCTGC |
| **T6-2MM1** | CACCATAGACAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGTCTAT |
| **T6-2MM2** | CACCGTGGACAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGTCCAC |
| **T6-2MM3** | CACCGCGAACAAGTAACTTCACCT | AAACAGGTGAAGTTACTTGTTCGC |
| **T6-2MM5** | CACCGCAGGTAAGTAACTTCACCT | AAACAGGTGAAGTTACTTACCTGC |
| **T6-2MM7** | CACCGCAGACGGGTAACTTCACCT | AAACAGGTGAAGTTACCCGTCTGC |
| **T6-2MM9** | CACCGCAGACAAACAACTTCACCT | AAACAGGTGAAGTTGTTTGTCTGC |
| **T6-2MM11** | CACCGCAGACAAGTGGCTTCACCT | AAACAGGTGAAGCCACTTGTCTGC |
| **T6-2MM13** | CACCGCAGACAAGTAATCTCACCT | AAACAGGTGAGATTACTTGTCTGC |
| **T6-2MM15** | CACCGCAGACAAGTAACTCTACCT | AAACAGGTAGAGTTACTTGTCTGC |
| **T6-2MM17** | CACCGCAGACAAGTAACTTCGTCT | AAACAGACGAAGTTACTTGTCTGC |
| **T6-2MM19** | CACCGCAGACAAGTAACTTCACTC | AAACGAGTGAAGTTACTTGTCTGC |
|  |  |  |
|  |  |  |
| **Target 17 mismatching sgRNAs (Supplementary Fig.7)** | |  |
|  |  |  |
| **plasmid name** | **oligo1** | **oligo2** |
| **T17-1MM1** | CACCACAGACAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGTCTGT |
| **T17-1MM2** | CACCGTAGACAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGTCTAC |
| **T17-1MM3** | CACCGCGGACAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGTCCGC |
| **T17-1MM4** | CACCGCAAACAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGTTTGC |
| **T17-1MM5** | CACCGCAGGCAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGCCTGC |
| **T17-1MM6** | CACCGCAGATAAGTTCACGAGGGT | AAACACCCTCGTGAACTTATCTGC |
| **T17-1MM7** | CACCGCAGACGAGTTCACGAGGGT | AAACACCCTCGTGAACTCGTCTGC |
| **T17-1MM8** | CACCGCAGACAGGTTCACGAGGGT | AAACACCCTCGTGAACCTGTCTGC |
| **T17-1MM9** | CACCGCAGACAAATTCACGAGGGT | AAACACCCTCGTGAATTTGTCTGC |
| **T17-1MM10** | CACCGCAGACAAGCTCACGAGGGT | AAACACCCTCGTGAGCTTGTCTGC |
| **T17-1MM11** | CACCGCAGACAAGTCCACGAGGGT | AAACACCCTCGTGGACTTGTCTGC |
| **T17-1MM12** | CACCGCAGACAAGTTTACGAGGGT | AAACACCCTCGTAAACTTGTCTGC |
| **T17-1MM13** | CACCGCAGACAAGTTCGCGAGGGT | AAACACCCTCGCGAACTTGTCTGC |
| **T17-1MM14** | CACCGCAGACAAGTTCATGAGGGT | AAACACCCTCATGAACTTGTCTGC |
| **T17-1MM15** | CACCGCAGACAAGTTCACAAGGGT | AAACACCCTTGTGAACTTGTCTGC |
| **T17-1MM16** | CACCGCAGACAAGTTCACGGGGGT | AAACACCCCCGTGAACTTGTCTGC |
| **T17-1MM17** | CACCGCAGACAAGTTCACGAAGGT | AAACACCTTCGTGAACTTGTCTGC |
| **T17-1MM18** | CACCGCAGACAAGTTCACGAGAGT | AAACACTCTCGTGAACTTGTCTGC |
| **T17-1MM19** | CACCGCAGACAAGTTCACGAGGAT | AAACATCCTCGTGAACTTGTCTGC |
| **T17-1MM20** | CACCGCAGACAAGTTCACGAGGGC | AAACGCCCTCGTGAACTTGTCTGC |
| **T17-2MM1** | CACCATAGACAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGTCTAT |
| **T17-2MM2** | CACCGTGGACAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGTCCAC |
| **T17-2MM3** | CACCGCGAACAAGTTCACGAGGGT | AAACACCCTCGTGAACTTGTTCGC |
| **T17-2MM5** | CACCGCAGGTAAGTTCACGAGGGT | AAACACCCTCGTGAACTTACCTGC |
| **T17-2MM7** | CACCGCAGACGGGTTCACGAGGGT | AAACACCCTCGTGAACCCGTCTGC |
| **T17-2MM9** | CACCGCAGACAAACTCACGAGGGT | AAACACCCTCGTGAGTTTGTCTGC |
| **T17-2MM11** | CACCGCAGACAAGTCTACGAGGGT | AAACACCCTCGTAGACTTGTCTGC |
| **T17-2MM13** | CACCGCAGACAAGTTCGTGAGGGT | AAACACCCTCACGAACTTGTCTGC |
| **T17-2MM15** | CACCGCAGACAAGTTCACAGGGGT | AAACACCCCTGTGAACTTGTCTGC |
| **T17-2MM17** | CACCGCAGACAAGTTCACGAAAGT | AAACACTTTCGTGAACTTGTCTGC |
| **T17-2MM19** | CACCGCAGACAAGTTCACGAGGAC | AAACGTCCTCGTGAACTTGTCTGC |