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Contents:

GS :PCR primers for off-targets of TTTACAGTATGACCGAACAA *TGG*

In the table below, Illumina Nextera Handle sequences have been added and highlighted in bold. Primers for the on-target have been added for convenience. The table below is sorted by the CFD off-target score. Sites with very low CFD scores < 0.02 are unlikely to be cleaved, see our study Haeussler et al. 2016, Figure 2.

In the protocol by Matthew Canver, Harvard, two PCRs are run: one PCR to amplify the potential off-target, then a second PCR to extend the handles with Illumina barcodes. Please click here to download the protocol. Alternatively, you can have a look at Fu et al, 2014.

If a primer was not found, the reason is usually that the region around the off-target is too repetitive. To avoid unspecific primers, all repeats are masked for the primer design (not for off-target search). If you think that we should change the parameters here or should use different primer3 settings, please let us know.

Maximum amplicon length: 100 bp - for >= 75bp paired reads Primer Tm: 60 deg. Update

Name
GS_ontarget_mm0_exon_XM_027418074.1_NC_048598.1_37805770_F
GS_ontarget_mm0_exon_XM_027418074.1_NC_048598.1_37805770_R
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GS_mm4_intergenic_XM_027429551.1|LOC100774398/XM_027429559.2_NC_048601.1_65403254_R
GS_mm4_intron_XR_004768376.1_NC_048595.1_224812784_F
GS_mm4_intron_XR_004768376.1_NC_048595.1_224812784_R
GS_mm4_intergenic_XM_035450739.1/XM_027391808.2|LOC113832879_NW_023276806.1_75251958_F
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GS_mm4_intergenic_XM_035441132.1/XM_035441131.1/XM_027397566.2/XM_027397565.2/XM_027397564.2/XM_027397567.2|XM_035441133.1_NC_048595.1_4
GS_mm4_intergenic_XM_035441132.1/XM_035441131.1/XM_027397566.2/XM_027397565.2/XM_027397564.2/XM_027397567.2|XM_035441133.1_NC_048595.1_4
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GS_mm4_intergenic_XM_035447503.1/XM_035447502.1|XM_027427554.2_NC_048600.1_67354439_R
GS_mm4_intergenic_XM_027403130.2/XM_027403129.2|XM_027403132.2_NC_048595.1_96071776_F
GS_mm4_intergenic_XM_027403130.2/XM_027403129.2|XM_027403132.2_NC_048595.1_96071776_R
GS_mm4_intergenic_XM_035444505.1|XM_027415012.2_NC_048597.1_168189035_F
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GS_mm3_intergenic_XM_035446333.1|XM_027422235.2/XM_027422244.2/XM_027422243.2/XM_027422234.2/XM_027422238.2_NC_048599.1_62350732_R
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GS_mm3_intergenic_LOC100756423|XM_027392083.2_NW_023276806.1_121629563_R
GS_mm4_intergenic_XR_003481741.1|XR_003481508.2/XM_027397104.2_NC_048595.1_418919936_F
GS_mm4_intergenic_XR_003481741.1|XR_003481508.2/XM_027397104.2_NC_048595.1_418919936_R
GS_mm4_intergenic_XM_035441463.1|XM_035441699.1_NC_048596.1_192417044_F
GS_mm4_intergenic_XM_035441463.1|XM_035441699.1_NC_048596.1_192417044_R
GS_mm4_intergenic_XM_027409574.2|XM_027409248.1_NC_048596.1_248331943_F
GS_mm4_intergenic_XM_027409574.2|XM_027409248.1_NC_048596.1_248331943_R
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GS_mm4_intergenic_XM_027415224.2/XM_027415219.2/XM_027415218.2|XM_027415217.2_NC_048597.1_116978203_R
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GS_mm3_intron_XM_035444755.1_NC_048597.1_73237046_R
GS_mm4_intergenic_XR_003480591.1|XR_003480575.1_NW_023276806.1_105914801_F
GS_mm4_intergenic_XR_003480591.1|XR_003480575.1_NW_023276806.1_105914801_R
GS_mm4_intergenic_XM_027418322.2|XM_027418323.2_NC_048598.1_126687209_F
GS_mm4_intergenic_XM_027418322.2|XM_027418323.2_NC_048598.1_126687209_R
GS_mm4_intergenic_XM_027433529.1|XM_027433530.1_NC_048604.1_19648442_F
GS_mm4_intergenic_XM_027433529.1|XM_027433530.1_NC_048604.1_19648442_R
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GS_mm4_intron_XM_027409391.2/XM_027409390.2_NC_048596.1_239730517_R
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GS_mm4_intergenic_NR_162747.1|XR_003485533.2_NC_048598.1_324095_F
GS_mm4_intergenic_NR_162747.1|XR_003485533.2_NC_048598.1_324095_R
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GS_mm4_intergenic_XM_035449723.1|XM_035449807.1/XM_027432847.2/XM_027432846.2/XM_027432844.2/XM_027432841.2/XM_027432840.2/XM_027432839
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GS_mm4_intergenic_XM_035453013.1|XM_035452881.1/XM_027387841.2/XM_027387840.2/XM_027387842.2/XM_027387843.2_NW_023276807.1_67873342_R
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GS_mm4_intergenic_XM_027388908.2|XM_035451486.1/XM_035451485.1/XM_035451484.1/XM_035451483.1/XM_035451481.1/XM_027388906.2/XM_027388905
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GS_mm4_intergenic_XM_027415102.2|XM_035443223.1_NC_048597.1_164201437_F
GS_mm4_intergenic_XM_027415102.2|XM_035443223.1_NC_048597.1_164201437_R
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GS_mm4_intergenic_XM_027410037.1|XM_027409923.2_NC_048596.1_269705056_R
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GS_mm4_intergenic_XM_027412393.2/XM_027412392.2|XM_035443635.1_NC_048597.1_17062675_R
GS_mm4_intergenic_XM_027430858.1|XM_035449460.1_NC_048602.1_24593903_F
GS_mm4_intergenic_XM_027430858.1|XM_035449460.1_NC_048602.1_24593903_R
GS_mm4_intergenic_XM_027416582.2/XM_035445016.1|XM_027416530.2_NC_048598.1_3104829_F
GS_mm4_intergenic_XM_027416582.2/XM_035445016.1|XM_027416530.2_NC_048598.1_3104829_R
GS_mm4_intergenic_XM_027401349.2|XM_027401349.2/XM_027401348.2/XM_027401347.2_NC_048595.1_340449079_F
GS_mm4_intergenic_XM_027401349.2|XM_027401349.2/XM_027401348.2/XM_027401347.2_NC_048595.1_340449079_R
GS_mm4_intergenic_XM_035445044.1/XM_035445043.1/XM_035445042.1/XM_035445041.1/XM_035445040.1/XM_035445037.1|XM_035445035.1/XM_027419812
GS_mm4_intergenic_XM_035445044.1/XM_035445043.1/XM_035445042.1/XM_035445041.1/XM_035445040.1/XM_035445037.1|XM_035445035.1/XM_027419812
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GS_mm4_intergenic_LOC100766360|XR_003488641.2/XM_027434055.2/XM_027434054.2_NW_023276853.1_147710_R
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GS_mm4_intron_XM_027401056.2_NC_048595.1_284945160_R
GS_mm4_intergenic_XM_035439108.1|XM_027401086.2_NC_048595.1_287839766_F
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GS_mm4_intergenic_LOC113835376|XM_035444554.1/XM_027412661.2/XM_027412660.2/XM_027412659.2_NC_048597.1_47241613_F

GS_mm4_intergenic_LOC113835376|XM_035444554.1/XM_027412661.2/XM_027412660.2/XM_027412659.2_NC_048597.1_47241613_R
GS_mm4_intergenic_LOC107977897|XR_003485888.1_NC_048598.1_72026587_F
GS_mm4_intergenic_LOC107977897|XR_003485888.1_NC_048598.1_72026587_R

Off-target amplicon sequences with primers

These only list off-targets that have primers in the table above. Primers underlined, off-targets in bold.

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mm4_intergenic_XM_035440452.1/XM_035440451.1/XM_035440450.1/XM_035440449.1/XM_027401158.2/XM_027401157.2/XM_027401156.2/XM_027401155.2/X

mm4_intergenic_XM_027429551.1|LOC100774398/XM_027429559.2_NC_048601.1_65403254

mm4_intergenic_XM_035450739.1/XM_027391808.2|LOC113832879_NW_023276806.1_75251958

mm4_intergenic_XM_035441132.1/XM_035441131.1/XM_027397566.2/XM_027397565.2/XM_027397564.2/XM_027397567.2|XM_035441133.1_NC_048595.1_4426

mm4_intergenic_XM_035447503.1/XM_035447502.1|XM_027427554.2_NC_048600.1_67354439

mm4_intergenic_XM_027403130.2/XM_027403129.2|XM_027403132.2_NC_048595.1_96071776

mm4_intergenic_XM_035444505.1|XM_027415012.2_NC_048597.1_168189035

mm4_intergenic_XR_003481741.1|XR_003481508.2/XM_027397104.2_NC_048595.1_418919936

mm4_intergenic_XM_035441463.1|XM_035441699.1_NC_048596.1_192417044

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mm4_intergenic_XM_027415224.2/XM_027415219.2/XM_027415218.2|XM_027415217.2_NC_048597.1_116978203

mm4_intergenic_XR_003480591.1|XR_003480575.1_NW_023276806.1_105914801

mm4_intergenic_XM_027433529.1|XM_027433530.1_NC_048604.1_19648442

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mm4_intergenic_XM_027412381.2|XM_027413320.2/XM_027413319.2/XM_027413318.2_NC_048597.1_16282132

mm4_intron_XM_035451716.1/XM_035451715.1_NW_023276806.1_86877996

mm4_intron_XM_027394572.2_NW_023276806.1_250313196

mm4_intergenic_XM_035449723.1|XM_035449807.1/XM_027432847.2/XM_027432846.2/XM_027432844.2/XM_027432841.2/XM_027432840.2/XM_027432839.2_N

mm4_intergenic_LOC100760297|XM_035441539.1_NC_048596.1_270343256

mm4_intron_XM_035449724.1_NC_048604.1_58433799

mm4_intron_XM_027406837.1_NC_048596.1_49172230

mm4_intergenic_XM_027388908.2|XM_035451486.1/XM_035451485.1/XM_035451484.1/XM_035451483.1/XM_035451481.1/XM_027388906.2/XM_027388905.2/X

mm4_intergenic_LOC100764068|LOC100758688_NW_023276807.1_17528491

mm4_intergenic_XM_035447174.1/XM_035447173.1/XM_027423732.2/XM_027423731.2/XM_027423729.2/XM_027423728.2|XM_035447174.1/XM_035447173.1/X

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mm4_exon_XM_027431898.2/XR_004771471.1/XR_004771472.1/XM_035450180.1/XM_035450179.1/XM_035450181.1_NC_048604.1_73068964

mm4_intron_XM_027416292.2/XM_027416291.2/XM_027416290.2_NC_048598.1_151749180

mm4_intergenic_XM_027410037.1|XM_027409923.2_NC_048596.1_269705056

mm4_intergenic_XM_027430858.1|XM_035449460.1_NC_048602.1_24593903

mm4_intergenic_XM_027401349.2|XM_027401349.2/XM_027401348.2/XM_027401347.2_NC_048595.1_340449079

mm4_intergenic_XM_035445044.1/XM_035445043.1/XM_035445042.1/XM_035445041.1/XM_035445040.1/XM_035445037.1|XM_035445035.1/XM_027419812.2/X

mm4_intron_XM_027401056.2_NC_048595.1_284945160
mm4_intergenic_XM_035439108.1|XM_027401086.2_NC_048595.1_287839766

Input file for Crispresso

Crispresso, written by Luca Pinello, is a software package to quantify the Cas9-induced mutations on off- or on-targets.

Click here to download an amplicon input file for Crispresso. For each off-target, it includes the off-target name, its PCR amplicon and the guide sequence. Keep a copy of this file.

After sequencing, run CRISPRessoPooled. The tool will map the reads to the amplicons and analyse the mutations:

```
CRISPRessoPooled -r1 Reads1.fastq.gz -r2 Reads2.fastq.gz -f crisporAmplicons_GS_aMGdfbP8brJISzWAaMfe.txt --name MY_EXPERIMENT
```