

1. gRNAs targeting Tns3 ATG (5') with efficiently cutting and Indel generation

Mus musculus sequence found at [chr11:8549019-8549117](#)

Found 12 possible guide sequences in input (98 bp). Click on a PAM NGG match to show its guide sequence.

Shown below are the PAM site and the nucleotide at position -3 5' of it.

Colors **green**, **yellow** and **red** indicate high, medium and low specificity of the PAM's guide sequence in the genome.

```

0      10      20      30      40      50      60      70      80      90
ttcctcccttgagGACCGTGGTTCAGTCCAGCCATGGAGGACGCCAGCACTGGACCTACGTATGTCACCGAGCGTATCATCGCAGTGTCTT
CCT...c      CCG...g      CCA...c      CCA...g      CCA...a      CCT...c      CCG...c
CCC...g
CCT...c
CCA...g
c...TGG
t...AGG
  
```

	Position/ Strand	Guide Sequence + PAM Restriction Enzymes	Specificity Score	Efficacy Score	Prox. GC	Out-of- Frame Score	Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to matches sort <input type="checkbox"/> exons only <input type="checkbox"/> chr11 only
gRNA#2	60 / rev	TACGCTCGGTGACATACGTA AGG PCR primers Restr. Enzymes: SnaBI	98	7	-	64 %	0-0-0-0-9 0-0-0-0-0 9 off-targets	4:intron:Deaf17 4:intron:Ror2 4:intergenic:Ccdc13-Gm17163 show all...
gRNA#1	74 / rev	GGACTGCGATGATACGCT CGG PCR primers	97	68	-	69 %	0-0-0-2-34 0-0-0-0-0 36 off-targets	4:intergenic:Gm11381-Mylk4 4:intergenic:Gm4487-Gm24073 4:intron:Diap1 show all...
	48 / rev	CATACGTAAGTCCAGTCC TGG PCR primers	95	19	-	68 %	0-0-0-1-24 0-0-0-0-0 25 off-targets	3:intergenic:Pk3r1-Gm25808 4:intergenic:Bche-Gm6098 4:intergenic:Gm22684-Anxa1 show all...
	56 / fw	CATGGAGGACAGCCAGAAC TGG PCR primers	80	12	-	55 %	0-0-3-10-135 0-0-0-0-1 148 off-targets	2:intron:Colec10 2:intron:Zfp7 3:exon:Zfp217 show all...
	3 / rev	GAACCAGGCTCTGCAAGGG AGG PCR primers	75	3	+	82 %	0-0-0-21-78 0-0-0-2-1 99 off-targets	3:intergenic:Dnm1-Ciz1 3:intergenic:Gm15336/Arhgap26-Gm15336 3:exon:Mmp25 show all...

gRNA #1

GGACTGCGATGATACGCT
AGCGTATCATCGCAGTGTCC

RECOGNIZED SEQUENCE 2 (PAM on the - strand)
forward sequence corresponding (+ strand)

55% GC

gRNA #2

TACGCTCGGTGACATACGTA
TACGTATGTCACCGAGCGTA

RECOGNIZED SEQUENCE 1 (PAM on the - strand)
forward sequence corresponding (+ strand)

50% GC

gRNA #1 (25+25pb)

Forward 5' - 3' CACCGGACTGCGATGATACGCT
Reverse 3' - 5' CCTGTGACGCTACTATGCGACAAA
Reverse 5' - 3' AAACAGCGTATCATCGCAGTGTCC

gRNA #2 (25+25pb)

Forward 5' - 3' CACCGTACGCTCGGTGACATACGTA
Reverse 3' - 5' CATGCGAGCCACTGTATGCATCAAA
Reverse 5' - 3' AAACAGCGTATCATCGCAGTGTCC

Genomic sequence and gRNA for Tns3

gacaggcgaggtggccttcagctcagctctacatgcagatcctttgggtggctgctgttcccagctttctccgaagccct
cattagtgagtggttcacatcctttttctgttatccttttcttgacctcctcttccctccttgagGACCGTGGTTCCTGA
GTCCAGCCATGGAGGACAGCCACGAACCTGGACCTTACGTATGTCACCGAGCGTATCATCGCAGTGTCCCTTCCCTGCCAG
TTGTTTACAGAGGAGTCTACTTTGCACAGCCTGCAGGAAGTCACACGCATGCTCAAGTGTAAAGCATGGAGACAACCTACCTG
gtgagtggggatgatagccacgggtgtgggtggatgggctggagggcaggagtcctcagaggactcgtgtgagggccagg
gtgtccctgtgcttgccttgcttacacctgaggaatggttgggggctggaatctgactcatgagcttaggggcatg
tcctctacccttctattgttagctttgctgtgtgtgtgacacgggctgtgacagttgactgtaggggtggctgctgct

New Primers for PCR

CCT PAM in reverse strand

CCCTxxxX gRNA#1

TCACCGXX gRNA#2

2. gRNAs targeting Tns3 TGA (3') with efficiently cutting and Indel generation

Tns3 last part of the cDNA region containing the stop codon.

```
4301 CCCTGAGTAT GACCTTGGTT CAAGAACCTC CTCCAGTGTC TACAGTGGTA
4351 CACTTCAAGG TGTGAGCCCA GGGCATCACC CTGACTGACA ATCAGAGAAA
4401 GCTCTTCTTC CGGAGGCATT ACCCGGTGAG CAGTGTGATT TTCTGTGCTT
4451 TGGATCCACA AGACAGGAAG TGGATCAAAG ATGGCCCTTC TTCTAAAGTC
4501 TTTGGATTTG TGGCCCGGAA ACAGGGCAGT GCTACAGACA ACGTATGCCA
4551 CCTGTTTGCA GAGCATGACC CCGAGCAACC TGCCAGTGCC ATTGTCAACT
4601 TTGTGTCAAA GGTGATGATT GGCTCCCCTA AGAAGATCTG Agccttctct
4651 agccaaacct gccagatgcc tcatagggcc ctcaacatgg cagtgggggtg
4701 aggcagggcc cccattogta ccagattgac aatcttcaca tccagactc
4751 aaagagga gaagaatag acaactcttc aaaccaagaa caaacaatgt
4801 caccactgac tggccttaaa gaagctgctc ttccgacaca cctatatagt
4851 caggcgcat ttgagtggat ggatcttggc cgggaaatg cctcatcagg
4901 gccccaagga caagggtgtg acaaggaggg agcatggaag acaaggctgt
```

Query sequence for CRISPOR:

TTGTGTCAAAGGTCATGATTGGCTCCCCTAAGAAGATCTGAgccttctctagccaacctgccagatgcctcatagggcctcaacatggcagtgagggtga

TCATGATTGGCTCCCCT sequence also present in chr12:100150650-100150682> avoid to use it in the gRNA

1. Guide sequence: **GGCCCTATGAGGCATCTGGC** AGG
2. Guide sequence: **TATGAGGCATCTGGCAGGTT** TGG
3. Guide sequence: **TCTGGCAGGTTGGCTAGGA** AGG > more efficiently cutting gRNA

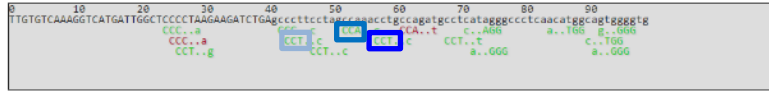
TTGTGTCAAAGGTCATGATTGGCTCCCCTAAGAAGATCTGAgccttctctagccaacctgccaacatggcagtgagggtgaggcag
ggcccccatctgtaccagattgacaatcttcacattccagactcaacaagaggagaagaatagacaactcttcaaccaagaacaacaatgtcaccactgactgg
ccttaaagaagctgctctccgacacacctatagtcaggcgcatcttgagtggatggatcttggcccgggaaatgcctcatcagggcccccaaggacaagggtgtg
acaaggaggagcatggaagacaaggctgt

Mus musculus (mm10) sequence found at chr11:8434434-8434534

Found 16 possible guide sequences in input (100 bp). Click on a PAM NGG match to show its guide sequence.

Shown below are the PAM site and the expected cleavage position located ~3bp 5' of the PAM site.

Colors **green**, **yellow** and **red** indicate high, medium and low specificity of the PAM's guide sequence in the genome.



Predicted guide sequences for PAMs

Ranked by default from highest to lowest specificity score (Hsu et al., Nat Biotechnol 2013) as on <http://crispr.mit.edu>.

Please cite the source when you use a score: Fusi, Chari, Xu, Doench, Wang, Moreno-Mateos, Housden, Prox, GC, -GG, Out-of-Frame

Our recommendation: Use Fusi for mammals, Moreno-Mateos for Zebrafish. Click on a column title to rank by its score.

Position/ Strand	Guide Sequence + PAM Restriction Enzymes	Specificity Score	Predicted Efficiency							Out- of- Frame	Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to match exons only chr11 only	
			Fusi	Chari	Xu	Doench	Wang	Moreno-Mateos	Housden				Prox GC
88 / fw	CCCTCTAGGCCCTCATA AGG PCR primers	86	60	75	-0.3	19	61	36	3	-	65	0-0-2-5-77 0-0-0-0-0	2 exon Spbar1 2 intron Clf 2 intron Rims3 show all...
69 / rev	CCATTTAAGGCCCTATA AGG PCR primers	85	57	87	-0.1	17	51	51	5	-	70	0-0-0-4-79 0-0-0-1-1	2 exon Spbar1 2 intergenic Tcb17-Gm23926 4 intron Zfp19 show all...
76 / fw	AACCTGCGAATGCTCATA AGG PCR primers	79	54	30	-0.2	30	34	58	5	-	82	0-0-0-16-95 0-0-0-2-1	3 intergenic mmo4-79-Gm6214 3 intergenic Tl8-Gm16499-Gm22910 3 intergenic Ncam2-Gm23955 show all...
58 / rev	CCCTCTAAGCCCTCATA AGG PCR primers	77	51	75	-0.2	17	55	22	4	+	65	0-0-1-6-114 0-0-0-0-1	3 intergenic Gm25914-Epha4 2 intron Csk10 4 intergenic Trn-Mrps14 show all...
53 / rev	TATGAGCCTCTGCAAGTT AGG PCR primers	75	37	9	-0.4	4	42	67	5	-	63	0-0-0-10-107 0-0-0-1-2	3 intergenic Zfp11-Gm1101 3 intergenic Gm22529-Gpr37 3 intergenic Gm26465-Gm5283 show all...
94 / fw	AAGCCCTCAACAAGGAG AGG PCR primers	74	56	67	0.8	29	89	57	7	+	61	0-0-0-8-92 0-0-0-1-7	4 intergenic Nc102-16-18594 3 intergenic Cacuso1-Gm8984 2 exon Arhgap9/Gat1 show all...
44 / rev	TCTGCAGGTTGGCTAGGA AGG PCR primers	66	41	60	0.0	1	43	48	6	-	71	0-0-3-19-140 0-0-1-2-0	2 intergenic Ptx1-H2afy 2 intergenic Gm16026-Sub1 2 exon Ejab4-V5 show all...

3. Guide sequence: TCTGGCAGGTTGGCTAGGA AGG > best gRNA cutting efficiency

validation Primers

guideRna44revLeft	AGCCTTGCTTCCATGCTCC	Tm 60.035
guideRna44revRight	TGCTTGGCACATCGTAACT	Tm 59.964

Genomic fragment with validation primers and guide sequence

Genomic sequence chr11:8434468-8434491 including primers, forward strand:

AGCCTTGCTTCCATGCTCC CTCCTTGACAGCCCTTGTCTTGGGGCCCTGATGAGGCATTTCCCGGCCAAGATCCATCCACTCAAATGCGCCTGACTAT ATAGGTGTGTCGGAAGAGCAGCTTCTTAAGGCCAGTCAGTGGTGACATTGTTGTCTTGGTTGAAGAGTTGCATATCTTCTCCTCTGTTGAGTCTG GAATGTGAAGATTGCAATCTGGTACGAATGGGGCCCTGCCTCACCCACTGCCATGTTGAGGGCCCTATGAGGCATCTGGCAGGTTGGCTAGGAAGG GCTCAGATCTTCTAGGGGAGCCAATCATGACCTTTGACACAAAGTTGACAATGGCACTGGCAGGTTGCTCGGGTTCATGCTCTGCAAACAGGTGGCATA C GTTGTCTGTAGCACTGCCCTGTTCCGGGCCACAAATCCAAGACTCTGCAAGAGACAAGTCTAGTCAGAGGAGCCAGTACCGTGTGCAGCAAGCACCAT GCATGCATGCAGGCTTCTTCTCCTCATGATAGCAACCTGGGAACAGTACAGACTGCCCTTGC AGGTTACGATGTGCCAAGCA

Sequence length: 588

Method: Primer3.2 with default settings, target length 500-700 bp

Expression of guide RNA

Summary of all primers explained below

guideRna44revT7sense	TAGGTCTGGCAGGTTGGCTAGGA
guideRna44revT7antisense	AAACTCTAGCCAAACCTGCCAGA
guideRNA44revT7PromSense	TAATACGACTCACTATAGGTCTGGCAGGTTGGCTAGGAGTTTTAGAGCTAGAAATAGCAAG
guideRNAallT7PromAntisense	AAAAGCACGACTCGGTGCCACTTTTCAAGTTGATAACGGACTAGCCTATTTTAACTGCTATTTCTAGCTCTAAAAC
guideRNA44revU6sense	ACACCTCTGGCAGGTTGGCTAGGAG
guideRNA44revU6antisense	AAAACTCTAGCCAAACCTGCCAGACG