

mMYB

[Mus musculus myeloblastosis oncogene \(Myb\), transcript variant 2, mRNA](#)

1. 3,420 bp linear mRNA

Accession: NM_010848.3 GI: 110556653

[Protein](#) [PubMed](#) [Taxonomy](#)

[GenBank](#) [FASTA](#) [Graphics](#)

[Mus musculus myeloblastosis oncogene \(Myb\), transcript variant 1, mRNA](#)

2. 3,777 bp linear mRNA

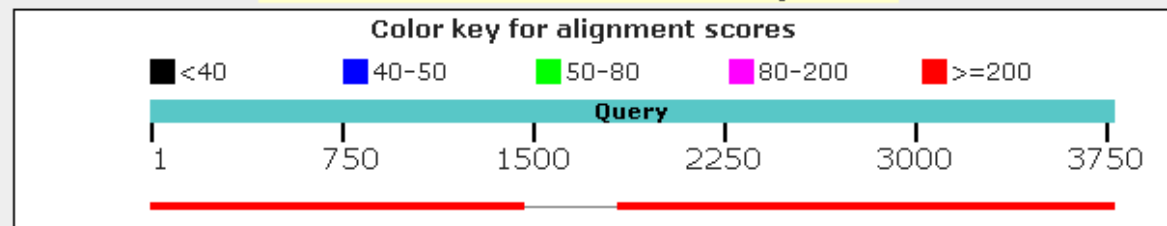
Accession: NM_001198914.1 GI: 312032463

[Protein](#) [PubMed](#) [Taxonomy](#)

[GenBank](#) [FASTA](#) [Graphics](#)

Distribution of the top 2 Blast Hits on 1 subject sequences ⓘ

Mouse over to see the title, click to show alignments



343 / fw	AGAAATATGGTCCGAAGCGT TGG Cloning / PCR primers	97	54	35	71	0-0-0-1-43 0-0-0-0-0 44 off-targets	4:intergenic:Scnn1g-Scnn1b 4:intron:App 4:intergenic:SNORA17-BC018507 show all...
334 / rev	GCAATAACAGACCAACGCTT CGG Enzymes: <i>Bme18I</i> , <i>Hpy188I</i> , <i>PspPI</i> Cloning / PCR primers	94	55	41	63	0-0-0-4-42 0-0-0-0-1 46 off-targets	4:intergenic:AC133509.1-Ostf1 4:intergenic:Psd3-Gm17236 4:intron:Appb2 show all...
82 / fw	TGTGTGACCATGACTACGAT GGG Enzymes: <i>XcmI</i> , <i>ApeKI</i> , <i>Fsp4HI</i> Cloning / PCR primers	91	69	48	77	0-0-0-5-66 0-0-0-0-0 71 off-targets	3:exon:Atp2c2 4:exon:6330439K17Rik 3:intergenic:Lect2-Tgfb1 show all...
211 / rev	CACTGCACATCTGTCCGGTT GGG Enzymes: <i>MspI</i> , <i>BsaWI</i> Cloning / PCR primers	91	43	79	72	0-0-2-5-56 0-0-0-0-0 63 off-targets	3:intron:1700019E19Rik 3:intergenic:Tgfb2-AC131777.1 4:intron:Kif16b show all...
234 / rev	GCACTTTCTGCCACCGGTGT TGG Enzymes: <i>AsiGI</i> , <i>BsaWI</i> , <i>Cfr10I</i> , <i>SgrAI</i> Cloning / PCR primers	91	45	54	47	0-0-1-3-89 0-0-0-0-0 93 off-targets	3:intron:Vav3 4:intergenic:Abca13-Eif3s6-ps1 4:intron:Usp48 show all...

T1 out of 65

<Previous [Next](#)>

Sequence: AGAAATATGGTCCGAAGCGTTGG

Efficacy score by CRISPRater: 0.48 LOW

Oligo pair with 5' extension fwd: CACCgAGAAATATGGTCCGAAGCGT rev: AAACACGCTTCGGACCATATTTCTC

Oligo pair with 5' substitution fwd: CACCgGAAATATGGTCCGAAGCGT rev: AAACACGCTTCGGACCATATTTCC

T3 out of 65

<Previous [Next](#)>

Sequence: GCAATAACAGACCAACGCTTCGG

Efficacy score by CRISPRater: 0.62 MEDIUM

Oligo pair fwd: CACCGCAATAACAGACCAACGCTT rev: AAACAAGCGTTGGTCTGTTATTGC

Name	Primer Sequence
guideRNA334rvU6senseMLM3636	ACACCgcaataacagaccaacgcttg
guideRNA334rvU6antisenseMLM3636	AAAACaagcgttggtctgttattgc

PCR to amplify the on-target site

Use these primers to amplify a genomic fragment around the on-target site:

OntargetGuideRna334rvLeft	TTGCCCAAGTCTTACCGTCC	Tm 59.964
OntargetGuideRna334rvRight	ACAGGAAGTAAAGGGCTGGC	Tm 59.962

Genome fragment with validation primers (underlined) and guide sequence (yellow)

Maximum amplicon length: Primer Tm:

Genomic sequence chr10:20872412-20872435 including primers, genomic forward strand:

TTGCCCAAGTCTTACCGTCCGGGCAGCAGCTTTGCGATCTCTGCCATCTGTTCCCCAGACGCTTGTGTGCCTGGTAAAT
 GATTCTGTCCTCCTCTTCTGTCCAGGAGGTTTTCTTAACTTCTGGATTCAAATGGTTGTGCCACCTCTCCCGACACTGCT
 TTCCAATTCTCCCTTTTAAGTGCTTG **GCAATAACAGACCAACGCTTCGG** ACCATATTTCTGGACAAGCTCTATGACCTTC
 AGATGAACAGAAAAATAATCTGTGTTTTAATGTTACGGAAAGAGTCAGCGTCATCAAGCCTCAATGTTACAAGATCTTCA
GATCTTGAAATAATCTGCTTTCATCCACTGGACATGCAGGAGCCAGCCCTTTACTTCCTGT

TTGCCCAAGTCTTACCGTCCGGGCAGCAGCTTTGCGATCTCTGCCATCTGTTCCCCAGACGCTTGTGTGCCTGGTAAAT
 GATTCTGTCCTCCTCTTCTGTCCAGGAGGTTTTCTTAACTTCTGGATTCAAATGGTTGTGCCACCTCTCCCGACACTGCT
 TTCCAATTCTCCCTTTTAAGTGCTTG **GCAATAACAGACCAACGCTTCGG** ACCATATTTCTGGACAAGCTCTATGACCTTC
 AGATGAACAGAAAAATAATCTGTGTTTTAATGTTACGGAAAGAGTCAGCGTCATCAAGCCTCAATGTTACAAGATCTTCA
GATCTTGAAATAATCTGCTTTCATCCACTGGACATGCAGGAGCCAGCCCTTTACTTCCTGT

Sequence length: 380

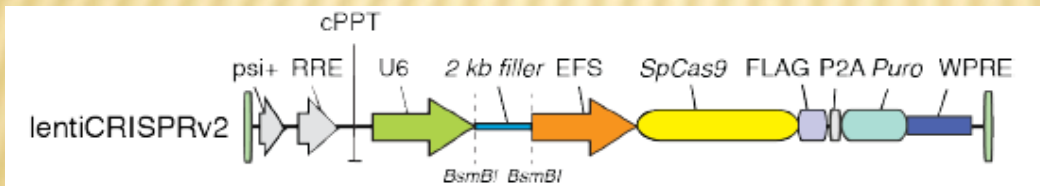
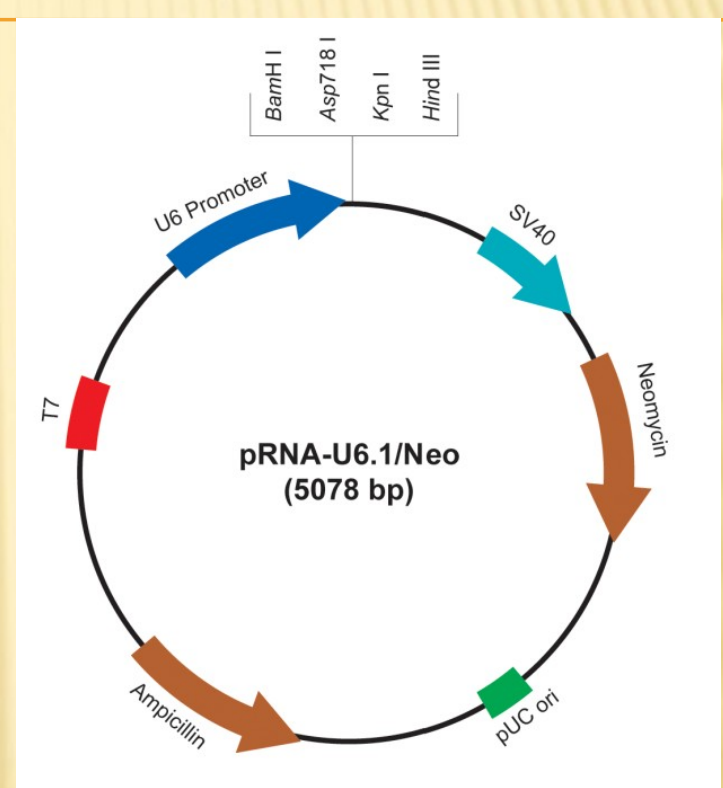
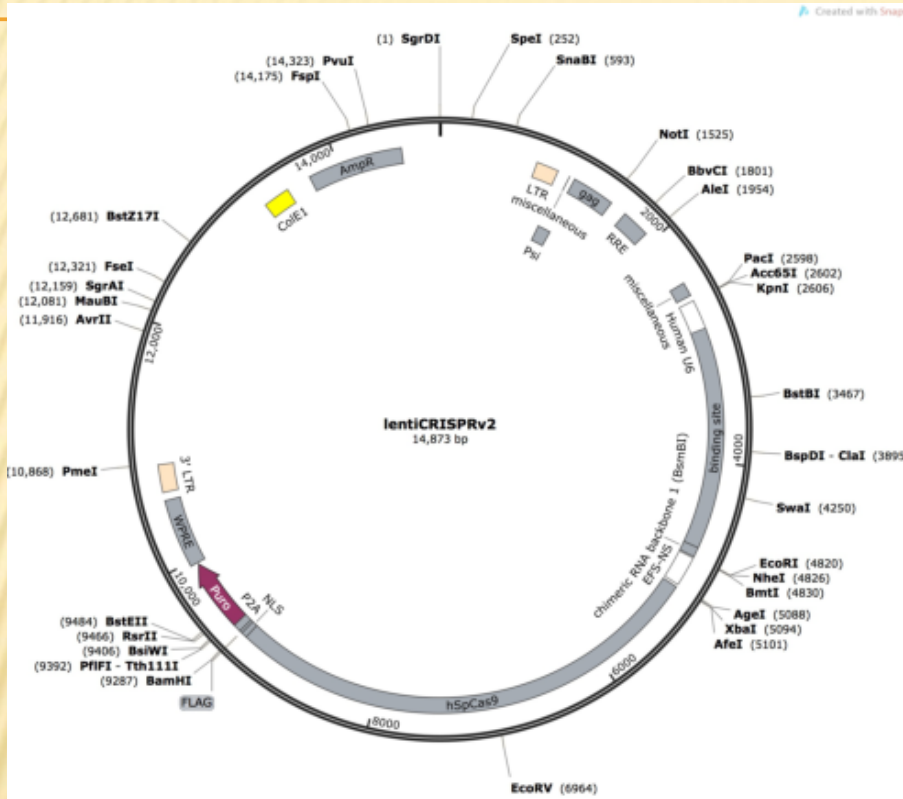
Select	No.	Start	Target sequence(DNA)	Region	GC%	Rank ¹
<input type="checkbox"/>	1	659	GGAGAGGTGGCACAACCATTT	ORF	52.39	★★★★★
<input type="checkbox"/>	2	793	GGACTGATAATGCTATCAAGA	ORF	38.1	★★★★★
<input type="checkbox"/>	3	1101	GCCATCCAGAGACACTATAAC	ORF	47.62	★★★★★
<input type="checkbox"/>	4	1504	GCTTAGATGCACCTACCTTAC	ORF	47.62	★★★★★
<input type="checkbox"/>	5	1542	GGTCACAAACTGACACCATGT	ORF	47.62	★★★★★
<input type="checkbox"/>	6	1671	GCCCTTGCAGCTCAAGAAATT	ORF	47.62	★★★★★
<input type="checkbox"/>	7	1844	GGAGTCGCCAACTGAGAAATC	ORF	52.39	★★★★★
<input type="checkbox"/>	8	1866	GGAAACTTCTTCTGCTCAAAC	ORF	42.86	★★★★★
<input type="checkbox"/>	9	2095	GGAAGACAGAGGACCAGATGA	ORF	52.39	★★★★★
<input type="checkbox"/>	10	2134	GGAAATACGTGAACGCGTTCT	ORF	47.62	★★★★★

BamH I

5' GATCCGACATTGAGATGTGTGACCATGACTACGAATAGTCA TGGTCACACATCTCAATG TTTTTTGGAAA 3'

3' GCGTA ACTCTACACCACTGGTACTGATGCTTATCAGTACCAGTGTGTAGAGTTACAAAAACCTTTTCGA 5'

Hind III



shRNA i gRNA

Spojení oligonukleotidů

Štěpení vektorů

Eluce fragmentů DNA z agarozového gelu

Ligace

Příprava kompetentních buněk E. coli

Transformace E. coli ligační směsí

Expanze klonů E. coli

Izolace plazmidové DNA

Ověření začlenění oligonukleotidu do vektoru (PCR, **sekvenace**)

Transfekce eukaryotických buněk

shRNA

SDS elektroforéza proteinů a imunobloting

gRNA

Selekce rezistentních buněk

Klonování rezistentních buněk metodou limitního ředění a expanze klonů

SDS elektroforéza proteinů a imunobloting

Izolace genomové DNA

PCR a klonování do sekvenačního vektoru

Sekvenace + analýza významu mutací