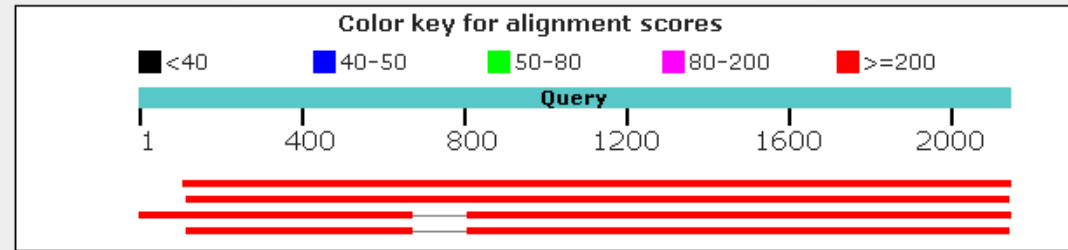


mTnfrsf9

Distribution of the top 6 Blast Hits on 4 subject sequences

Mouse over to see the title, click to show alignments



```
TTCCTGAAATTCAGGTGCTGCAGGCAGCCCTCAGCACAGAGAGCTGACAGGGAC
CCTGGGTCAGGGGTTCTGAGTTCAGCTGCCACTATTCTTTCACCTTTGGTGTC
CTGTGCATGTGACATTTGCCATGGGAAACAACGTGTTACAACGTGGTGGTCATTG
TGCTGCTGCTAGTGGGCTGTGAGAAGGTGGGAGCCGTGCAGAACTCCTGTGATAA
CTGTCAGCCTGTACTTTCTGCAGAAAATACAAATCCAGTCTGCAAGAGCTGCCCT
CCAAGTACCTTCTCCAGCATAGGTGGACAGCCGAAC TGTAACATCTGCAGAGGTG
GTGCAG
```

1 az 3 exon

Od ATG (pozice 132) po koniec tretho exonu je ta sekvence stejna ve vseh transkripnich variantach

Guide #1 89 GTTCGGCTGTCCACCTATGCTGG

Guide #2 79 AAACAACGTGTTACAACGTGGTGG

Oliga:

mTnfrsf9_CRISPR_A: CACCG GTTCGGCTGTCCACCTATGCTGG

mTnfrsf9_CRISPR_B: AAAC CCAGCATAGGTGGACAGCCGAAC C

10 siRNA Sequences (Up to 10 top scoring siRNA sequences are reported, sorted by the Start position and ranked as ★★★★★ to ★☆☆☆☆ to indicate knockdown probability). Select the sequence to order and click "Continue".

Select	No.	Start	Sequence(DNA)	Region	GC%	Tuschl's pattern match*	Rank ¹
<input type="checkbox"/>	1	203	GCAGAACTCCTGTGATAAC	ORF	47.37		★★★★☆
<input type="checkbox"/>	2	325	GAGTGTGTGCAGGCTATTT	ORF	47.37	BCD	★★★★☆
<input type="checkbox"/>	3	333	GCAGGCTATTCAGGTTCA	ORF	47.37		★★★★☆
<input type="checkbox"/>	4	380	GGAGTGTGAGTGCATTGAA	ORF	47.37		★★★★☆
<input type="checkbox"/>	5	484	CCTGTAGCTTGGGAACATT	ORF	47.37	BD	★★★★★
<input type="checkbox"/>	6	679	CCTGCAGGTCCTTACCTT	ORF	52.64		★★★★★
<input type="checkbox"/>	7	725	GCTGGCCCTGATCTTCATT	ORF	52.64		★★★★★
<input type="checkbox"/>	8	728	GGCCCTGATCTTCATACT	ORF	47.37		★★★★☆
<input type="checkbox"/>	9	749	CCTGTTCTCTGTGCTCAAA	ORF	47.37		★★★★★
<input type="checkbox"/>	10	828	GCTCAAGAGGAAGATGCTT	ORF	47.37	BCD	★★★★★

BamH I

5' ... GATCCG **CCTGTAGCTTGGGAACATT** TTCAAGAGA **AATGTTCCCAAGCTACAGG** TTTTTTGAAA ... 3'

Sense loop Antisense Termination signal

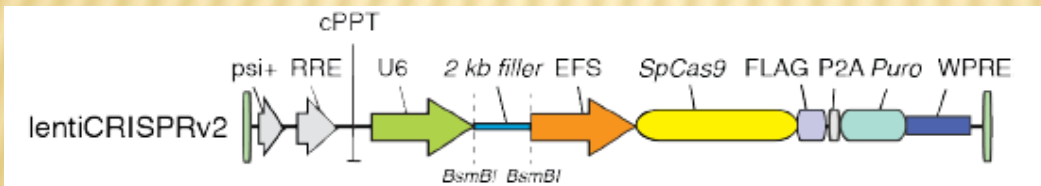
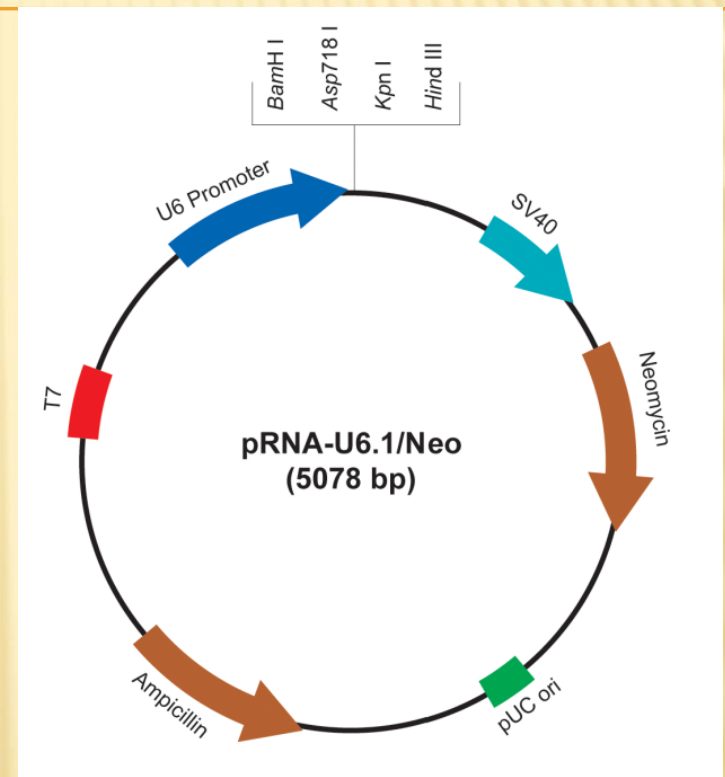
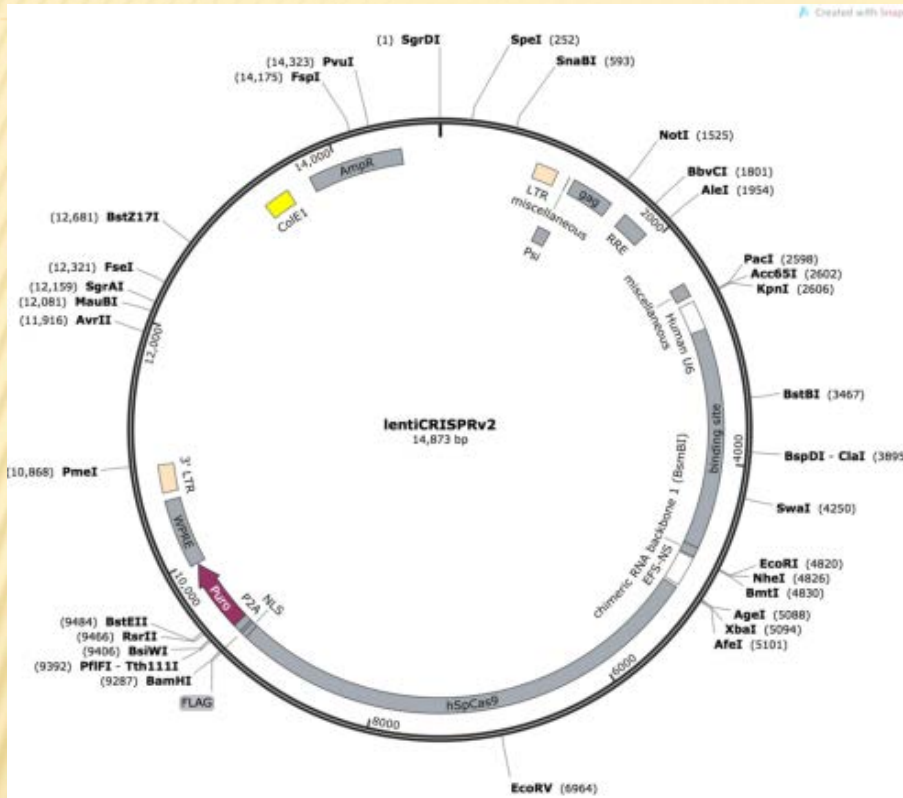
Hind III

5' ... AGCTTTTCAAAAAA **CCTGTAGCTTGGGAACATT** TCTCTTGAA **AATGTTCCCAAGCTACAGG** CG ... 3'

BamH I

GATCCG**CCTGTAGCTTGGGAACATT**TTCAAGAGAA**AATGTTCCCAAGCTACAGG**TTTTTTTGAAA
GCGGACATCGAACCTTGTAAAAGTTCTCTT**TACAAGGGTTCGATGTCCAAAAACCTTTTCGA**

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

Klonování pro sekvenaci

Genom DNA:

CATGTGTGCTGGGGTTACAGCATCCACTACCACTCCGGGTATCTGCACACTGGTT
CCTGTTTAGCAAGCATGCTATCAGTCAAGCAACAGCAGCAGCCAGAGGACAACT
CATCTGACTGAGACACTTTCGGAATCTCCTTTGCTAGTGTCTGTGCATGTGACAT
TTCGCCATGGGAAACAACTGTTACAACGTGGTGGTCATTGTGCTGCTGCTAGTGG
GCTGTGAGAAGGTGGGAGCCGTGCAGAACTCCTGTGATAACTGTCAGCCTGGTAA
GTGCCAAAGTGACATGACTGTTGAAGACTCAGTTCAGTTAGCCTGGTGTCTTAGT
TAGGGTTCGTTGCTGTGAAGAGACACCACGGCCAAGGCAGCTCTTATAAAGAAC
AGCATTTAATTGGGGCTGGCTTACAGGCTCAGAGG

AAACATGTGCAGTCCTAAAATGCCAGTCATTGTGATGCCAGGAGGACAGACAGA
ATATTTATTTTGATATCCTCGAAAATGTCACAATGCTGAGAGAACTGGTCATTTGT
TGTCAGACACAAATGCCTGTGACAATTCTTGCAGGTACTTTCTGCAGAAAATACA
ATCCAGTCTGCAAGAGCTGCCCTCCAAGTACCTTCTCCAGCATAGGTGGACAGCC
GAACTGTAACATCTGCAGAGTGTGTGCAGGTAGGTCAGTCTGTCTGTCTGTCTGT
CTGTCTGGAAAGGAGAGCTTGCTGTTGCCAGGCTAGACTGGAACCTGTGACTCT
TGTTCTCAGCCTCCAAGTCTGGAGGTTTGCTTTAGAGGGGAGACATCTTCATCT
TTAAGACCATTGGGCAGAGTTAAGCTTTGATCCG