

školní rok 2022/2023

Metody molekulární biologie - cvičení

Petr Beneš
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Přírodovědecká fakulta MU

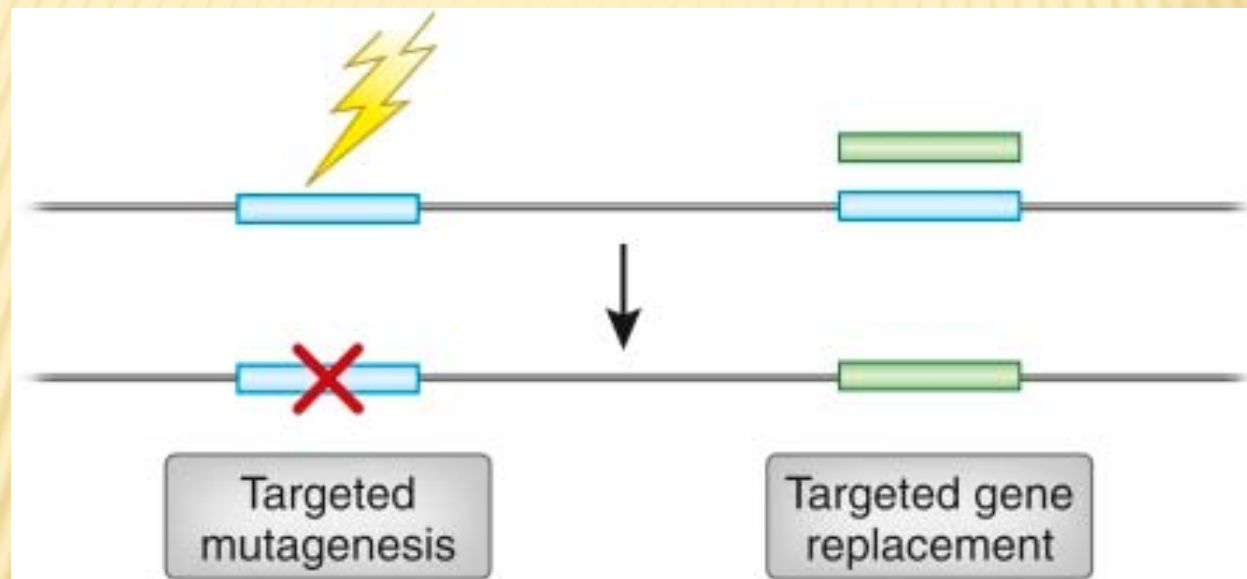
Cíl cvičení

Potlačení/umlčení exprese vybraného genu

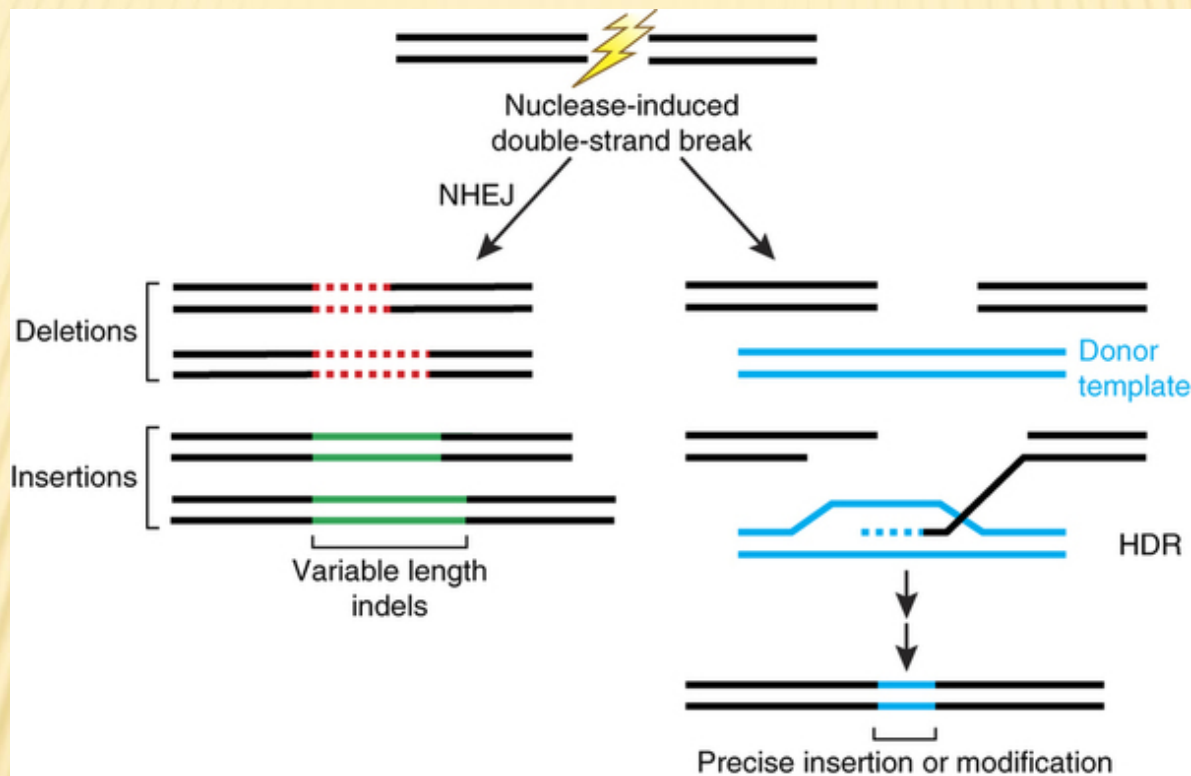
- a) mutageneze v genomu pomocí metody CRISPR/Cas9
- b) post-transkripčním umlčením exprese pomocí shRNA

→ funkce genu ...

Cílená mutageneze přímo v genomech



Cílená mutagenese přímo v genomech



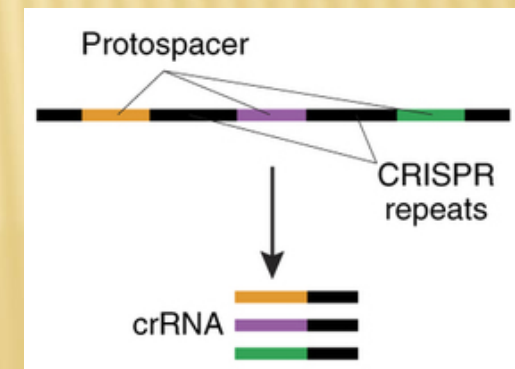
- od 80. let - četnost homologické rekombinace je zvýšena indukci dsDNA zlomů
- snaha o vývoj systému pro sekvenčně specifickou tvorbu dsDNA zlomů

CRISPR/Cas9

- ✗ CRISPR-associated protein 9 - nukleáza ze *Streptococcus pyogenes*
- ✗ adaptivní imunita bakterií proti virům (obecně cizorodé DNA)
- ✗ RGN - RNA-guided nuclease
- ✗ sekvenční specifita je dána **interakcí DNA-RNA**

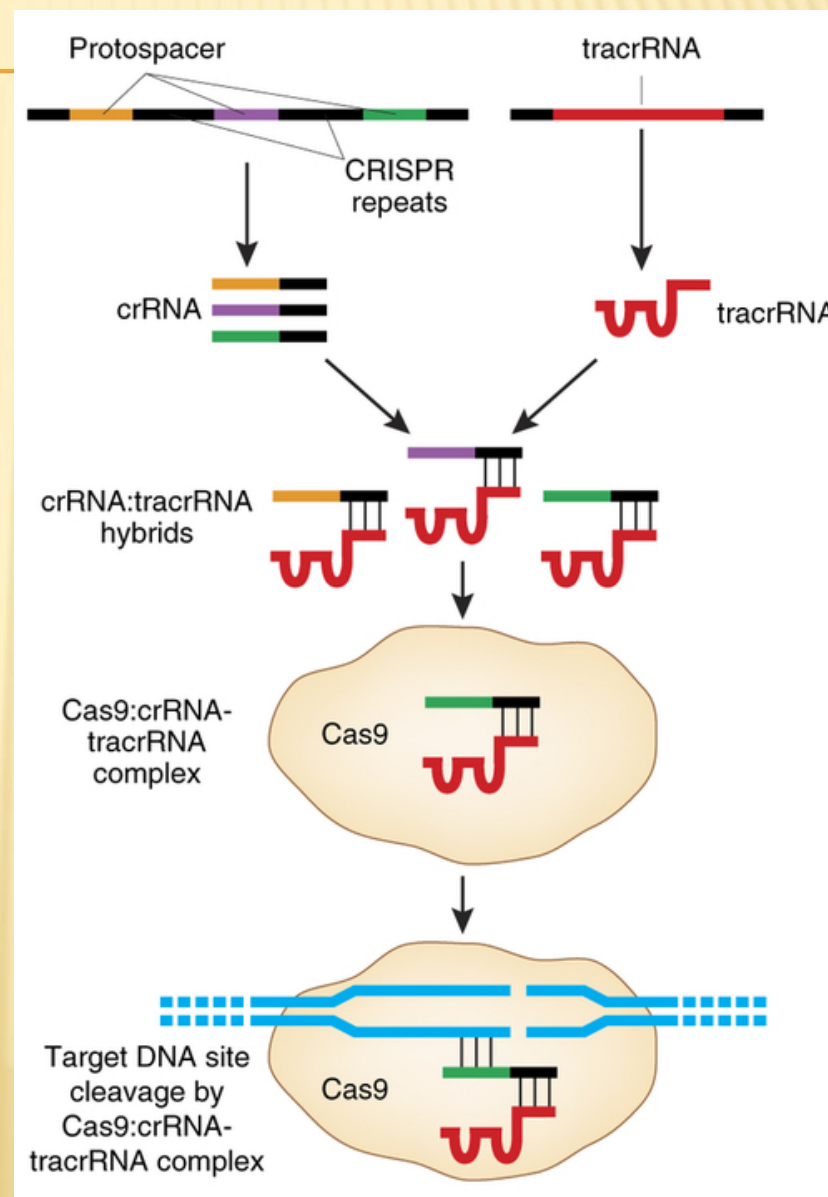
Bakterie - inkorporace cizorodé DNA do CRISPR repetice v genomu
- tyto následně přepsány do RNA (pre-crRNA → crRNA)

crRNA - protospacer - fragment cizorodé DNA
- CRISPR repetice



CRISPR/Cas9

- crRNA poté hybridizuje s transaktivující CRISPR RNA (tracrRNA)
- tento komplex RNA interaguje s Cas9 nukleázou
- protospacer RNA navede celý komplex k cizorodé DNA (komplementarita sekvencí)
- výsledný ribonukleoproteinový komplex štěpí cizorodou komplementární DNA



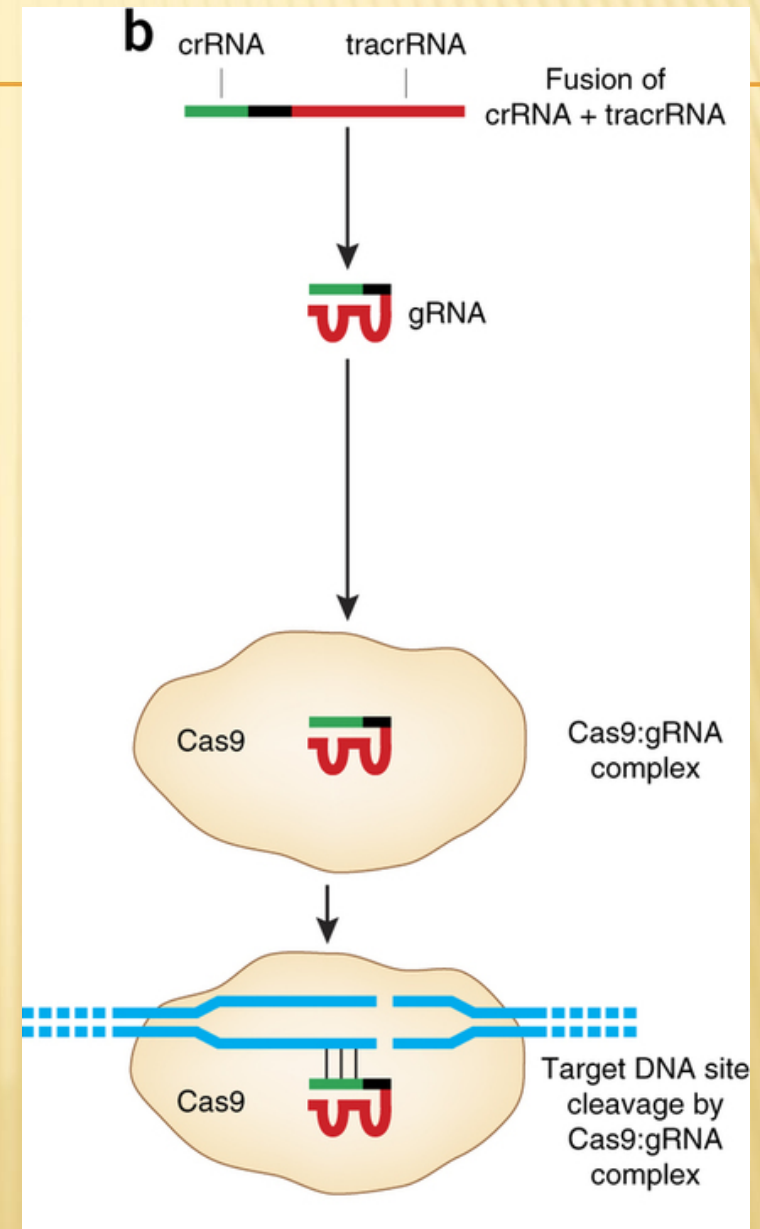
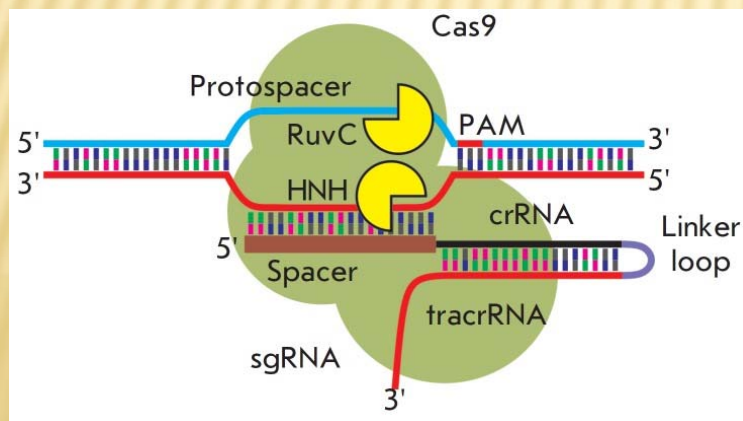
CRISPR/Cas9

- celý systém modifikován pro cílenou mutagenezi

Vektor:

- guideRNA (gRNA) = crRNA + tracrRNA
- součástí gRNA i 20nt komplementární úsek k cílovému místu v genomové DNA

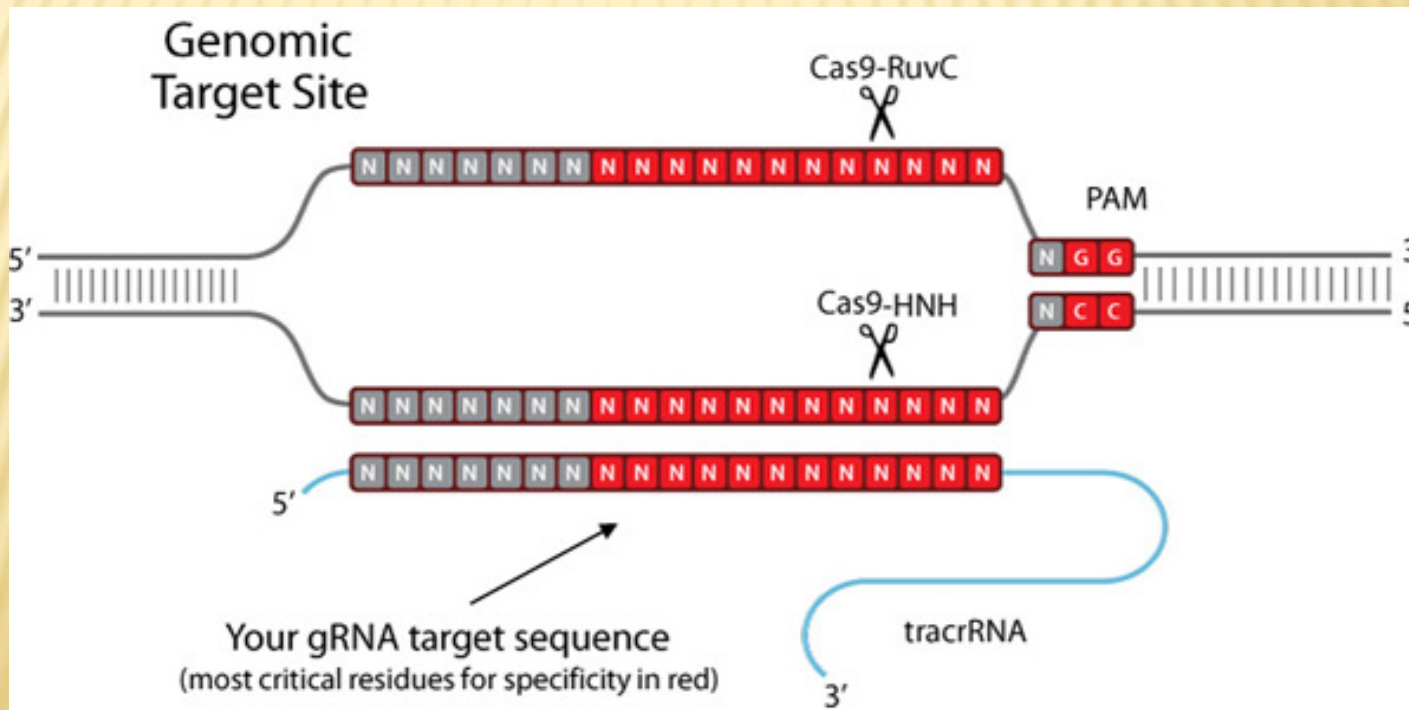
+ koexprese Cas9 nukleázy



CRISPR/Cas9

PAM - protospacer adjacent motif

- sekvence v těsném sousedství s gDNA
- nutná pro účinné štěpení Cas9 nukleázou
- původní systém „NGG“ (ale vývoj systémů s jinými sekvencemi)
- dle systému cílová sekvence musí být ve formátu $N_{20}GG$



CRISPR/Cas9 - zvýšení specifity - nikázy

Pouze 20 nt naváděcí sekvence

→ off-target efekty

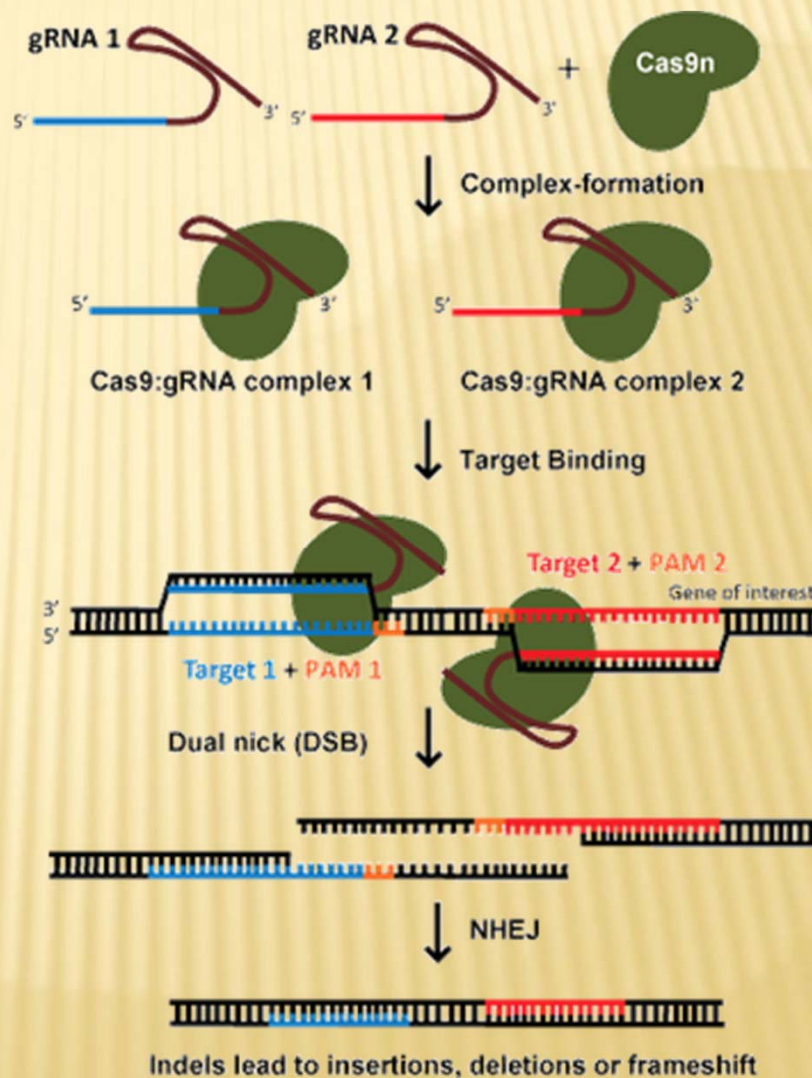
Cas9 - 2 nukleázové domény (RuvC, HNH)

Cas9 nikázy - mutanti v jedné doméně
- indukce cílených SSB

-design - 2x SSB blízko sebe
- a na opačných řetězcích

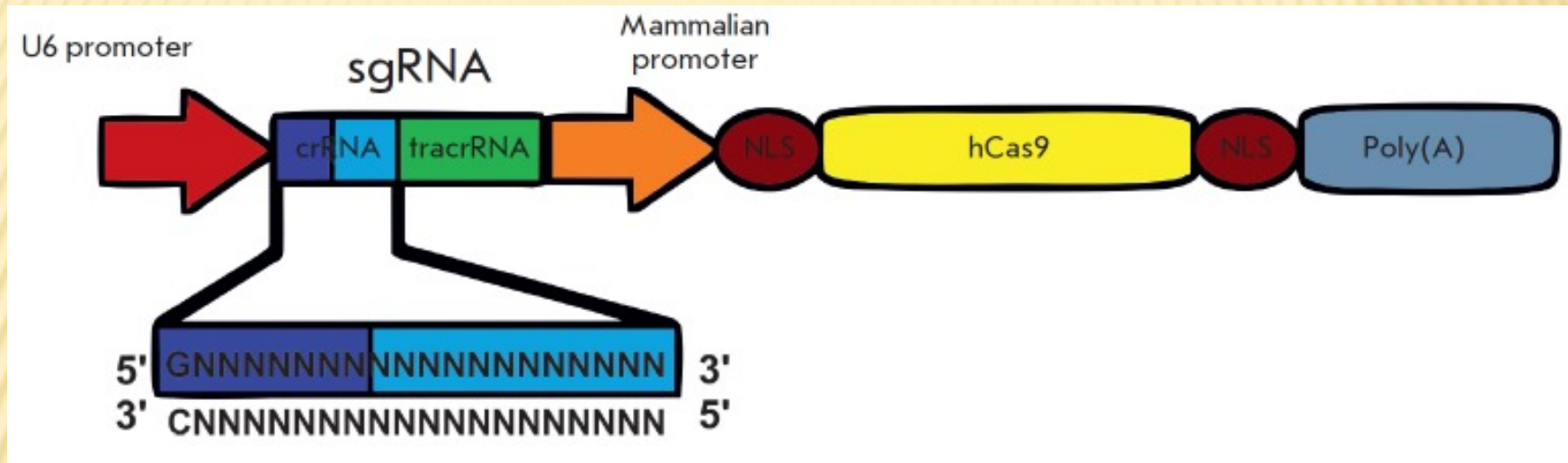
↓
DSB

vyšší specifita (?)



nebo využití upravených (více specifických forem Cas9) - SpCas9 - HF, eSpCas9

To KO gene - all you need is ...



Plasmid - gRNA, Cas9 promotor (konstitutivní nebo indukibilní)

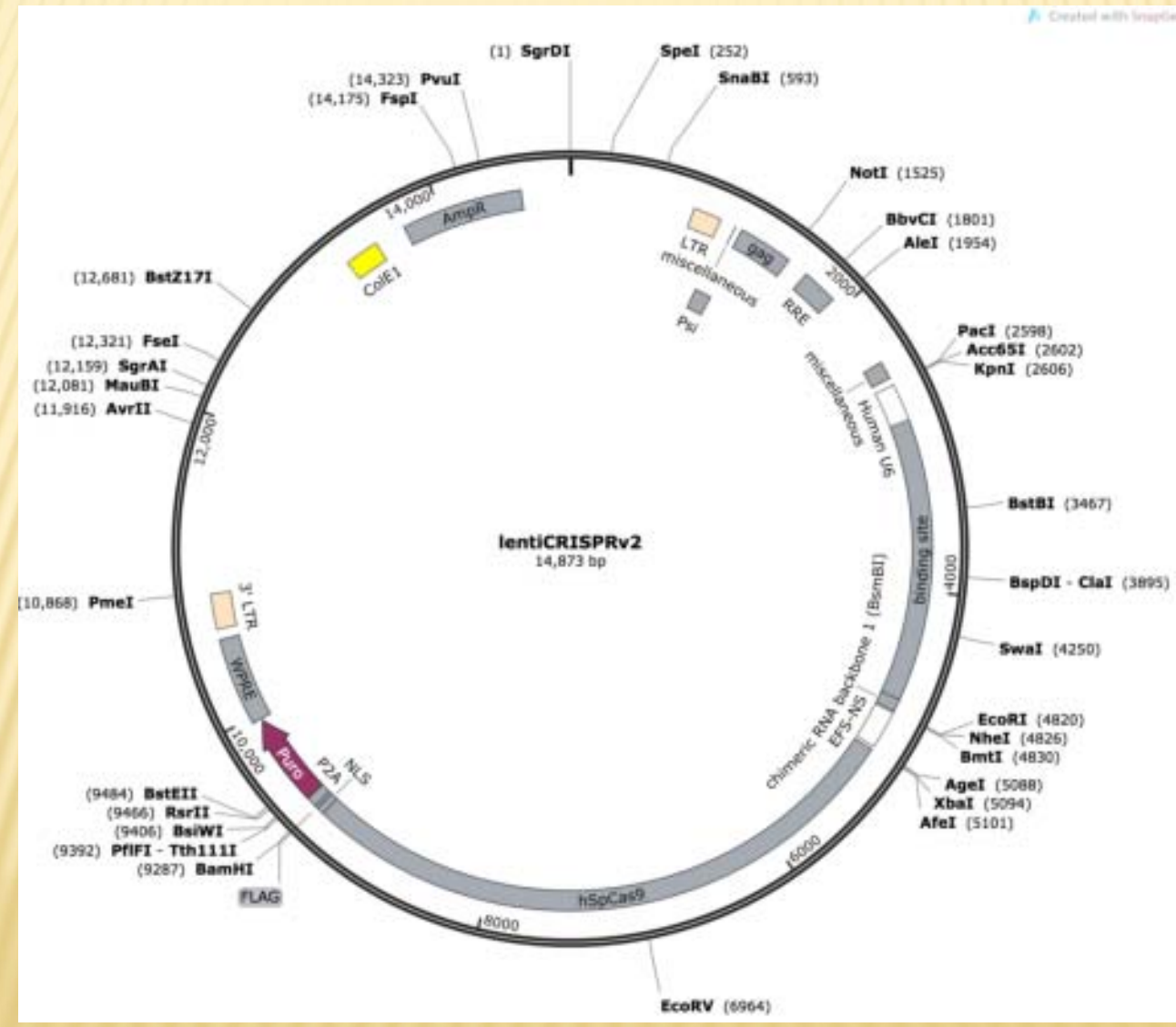
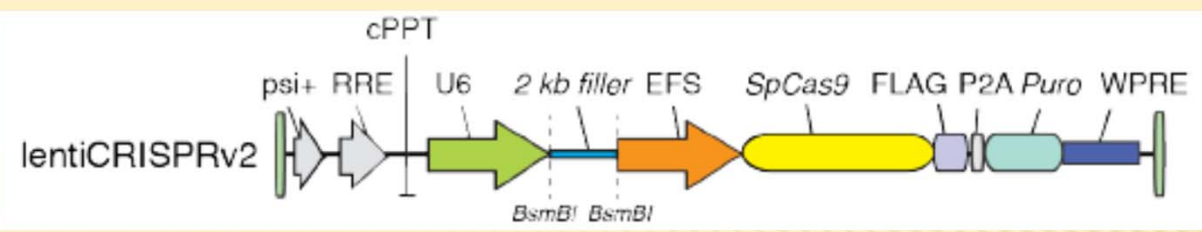
Virový vektor

Transfekce RNA po in vitro transcripci

Transfekce komplexu Cas9 + gRNA

<http://www.addgene.org/52961/>

lentiCRISPRv2



CRISPR/Cas9

<http://www.addgene.org/crispr/>

- dostupné vektory

Různé LifeTech firmy - různé platformy (modifikace původního systému)

- knihovny gDNA pro různé organismy
- validované gRNA

Pro design lze využít dostupný software (více než 30 online softwarů)

- https://en.wikipedia.org/wiki/CRISPR/Cas_Tools

- <http://crispor.tefor.net/>

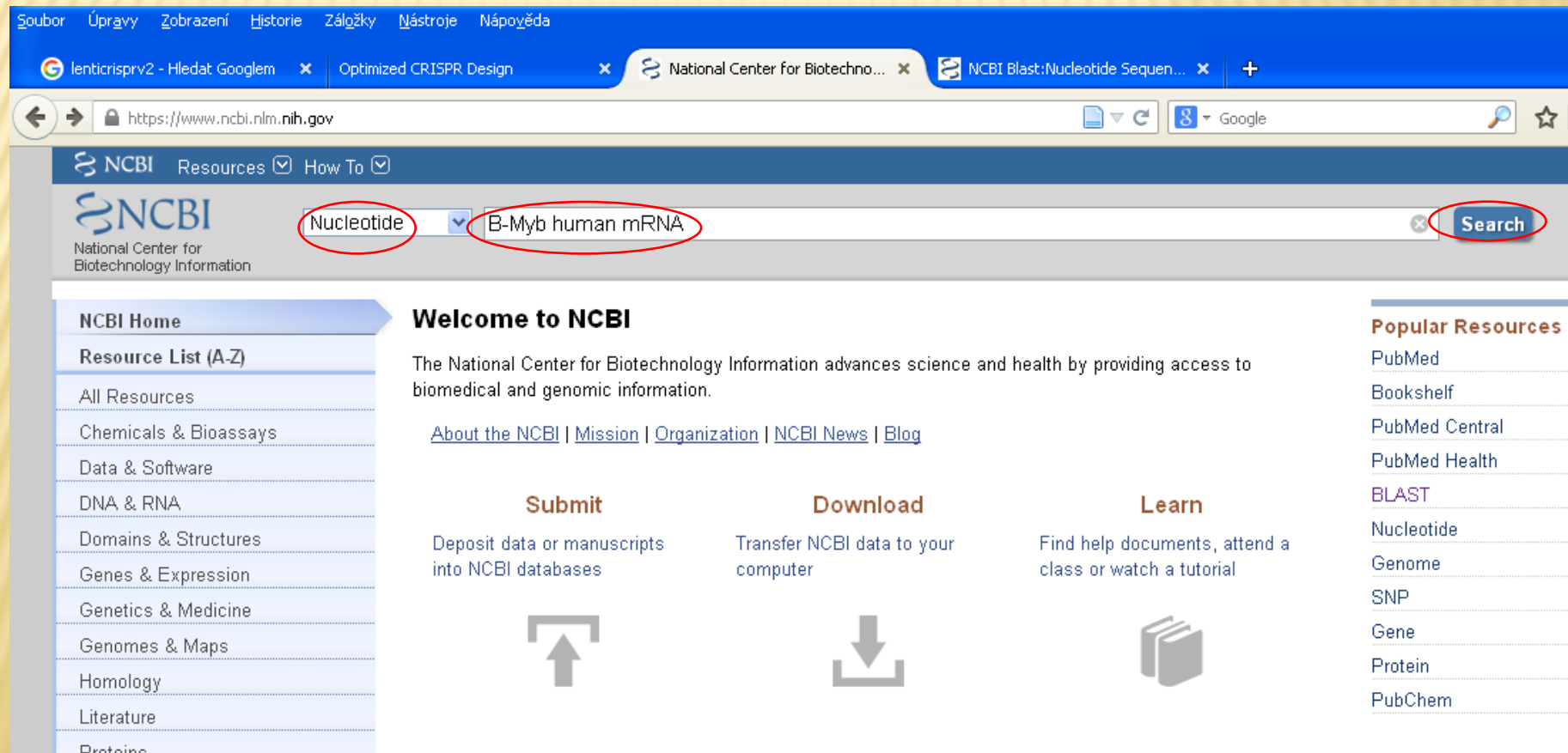
- <https://crispr.cos.uni-heidelberg.de/>

+ všechny větší i menší Lifetech firmy

design CRISPR/Cas9

Sekvence genu včetně znalosti kde začínají a končí exony

<https://www.ncbi.nlm.nih.gov/>



The screenshot shows the NCBI website interface. The search bar is located at the top right, with a dropdown menu set to 'Nucleotide' and the search term 'B-Myb human mRNA' entered. The search button is labeled 'Search'. The website header includes the NCBI logo and navigation links like 'Resources' and 'How To'. The main content area features a 'Welcome to NCBI' message and three columns of links: 'Submit', 'Download', and 'Learn'. A sidebar on the left lists various resources, and a 'Popular Resources' section is on the right.



Gene B-Myb human

Search

- NCBI Home
- Resource List (A-Z)
- All Resources
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- Data & Software
- DNA & RNA
- Domains & Structures
- Genes & Expression
- Genetics & Medicine
- Genomes & Maps
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- Literature
- Proteins
- Sequence Analysis
- Taxonomy
- Training & Tutorials
- Variation

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- Protein
- PubChem

NCBI News & Blog

Save and Share in PubMed Labs! 18 Jan 2019

We've recently added save and share options to PubMed Labs. From your PubMed Labs search results list you

Improved ClinVar search quickly connects you to information about variants

17 Jan 2019

If you've been searching in ClinVar you

February 6 Webinar: New Variation Services for Normalizing, Remapping, and Annotating Variants

16 Jan 2019

Join us on Wednesday, February 2019

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Nucleotide Nucleotide B-myb human mRNA Search
Create alert Advanced

The Nucleotide database will include EST and GSS sequences in early 2019. [Read more](#)

- Species
- Animals (63)
- Plants (7)
- Fungi (3)
- Bacteria (1)
- Customize ...

- Molecule types
- genomic DNA/RNA (16)
- mRNA (58)
- Customize ...

- Source databases
- INSDC (GenBank) (34)
- RefSeq (40)
- Customize ...

- Sequence Type
- Nucleotide (52)
- EST (22)

- Sequence length
- Custom range...

- Release date
- Custom range...

- Revision date
- Custom range...

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Summary 20 per page Sort by Default order

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GENE

Was this helpful?

MYBL2 – MYB proto-oncogene like 2

[Homo sapiens \(human\)](#)

Also known as: B-MYB, BMYB

GeneID: 4605

[RefSeq transcripts \(2\)](#) [RefSeq proteins \(2\)](#) [PubMed \(108\)](#)

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RefSeq transcripts +

RefSeq proteins +

Items: 1 to 20 of 74

Results by taxon

Top Organisms [Tree](#)

- Homo sapiens (42)
- Mus musculus (15)
- Oryza sativa Japonica Group (5)
- Aspergillus fumigatus Af293 (3)
- Xenopus laevis (2)
- All other taxa (7)
- More...

Find related data

Database:

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Search details

B-myb[All Fields] AND ("H sapiens"[Organism] OR hum Fields) AND mRNA[All Fields]

[Search](#)

Nucleotide

Nucleotide

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The Nucleotide database will include EST and GSS sequences in early 2019. Read more.

Species

Animals (2)

Customize ...

Molecule types

mRNA (2)

Customize ...

Source databases

RefSeq (2)

Customize ...

Sequence Type

Nucleotide (2)

Sequence length

Custom range...

Release date

Custom range...

Revision date

Custom range...

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Items: 2

[Homo sapiens MYB proto-oncogene like 2 \(MYBL2\), transcript variant 1, mRNA](#)

1. 2,668 bp linear mRNA

Accession: NM_002466.4 GI: 1519243668

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[Homo sapiens MYB proto-oncogene like 2 \(MYBL2\), transcript variant 2, mRNA](#)

2. 2,713 bp linear mRNA

Accession: NM_001278610.1 GI: 519666782

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

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

Analyze these sequences

Run BLAST

Find in these sequences

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B-myb human mRNA (74)

Nucleotide

B myb human mRNA (12980)

Nucleotide

B myb human (13312)

Nucleotide

Homo sapiens mucin 1, cell surface associated (MUC1), transcript varia

Nucleotide

Homo sapiens mucin 1, cell surface associated (MUC1), transcript varia

Nucleotide

See more...

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Homo sapiens MYB proto-oncogene like 2 (MYBL2), transcript variant 1, mRNA

NCBI Reference Sequence: NM_002466.3

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

Go to

LOCUS NM_002466 2785 bp mRNA linear PRI 07-OCT-2016

DEFINITION Homo sapiens MYB proto-oncogene like 2 (MYBL2), transcript variant 1, mRNA.

ACCESSION NM_002466

VERSION NM_002466.3

KEYWORDS RefSeq.

SOURCE Homo sapiens (human)

ORGANISM [Homo sapiens](#)
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 2785)

AUTHORS Krakstad C, Tangen IL, Hoivik EA, Halle MK, Berg A, Werner HM, Raeder MB, Kusonmano K, Zou JX, Oyan AM, Stefansson I, Trovik J, Kalland KH, Chen HW and Salvesen HB.

TITLE ATAD2 overexpression links to enrichment of B-MYB-translational signatures and development of aggressive endometrial carcinoma

JOURNAL Oncotarget 6 (29), 28440-28452 (2015)

PUBMED [26308378](#)

REMARK GeneRIF: Data indicate that gene expression alterations in endometrial carcinoma samples with high ATAD2 expression showed upregulation of several cancer-related genes including B-MYB gene.

REFERENCE 2 (bases 1 to 2785)

AUTHORS Tao D, Pan Y, Jiang G, Lu H, Zheng S, Lin H and Cao F.

TITLE B-Myb regulates snail expression to promote epithelial-to-mesenchymal transition and invasion of breast cancer cell

JOURNAL Med. Oncol. 32 (1), 412 (2015)

PUBMED [25502082](#)

REMARK GeneRIF: We found that B-Myb upregulated expression of the key epithelial-to-mesenchymal transition regulator snail and that it mediated epithelial-to-mesenchymal transition activation and cell invasion by B-Myb.

REFERENCE 3 (bases 1 to 2785)

AUTHORS Tao D, Pan Y, Lu H, Zheng S, Lin H, Fang H and Cao F.

TITLE B-myb is a gene implicated in cell cycle and proliferation of breast cancer

Analyze this sequence

Run BLAST

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Highlight Sequence Features

Find in this Sequence

Articles about the MYBL2 gene

A human interactome in three quantitative dimensions organized by stoichiometry [Cell. 2015]

ATAD2 overexpression links to enrichment of B-MYB-translational signature [Oncotarget. 2015]

The BioPlex Network: A Systematic Exploration of the Human Interactome. [Cell. 2015]

See all...

Pathways for the MYBL2 gene

TFAP2A acts as a transcriptional repressor during retinoic acid induced cell differentiation

Transcriptional regulation by the AP-2 (TFAP2) family of transcription factors

Polo-like kinase mediated events

See all...

Reference sequence information

RefSeq alternative splicing

See the other reference mRNA sequence splice variant for the MYBL2 gene (NM_001278610.1).

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ORIGIN

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```

//

[Homo sapiens MYB proto-oncogene like 2 \(MYBL2\), transcript variant 1, mRNA](#)

2,668 bp linear mRNA

Accession: NM_002466.4 G: 1519243668

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

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

[Homo sapiens MYB proto-oncogene like 2 \(MYBL2\), transcript variant 2, mRNA](#)

2,713 bp linear mRNA

Accession: NM_001278610.1 G: 519666782

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

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Align two or more sequences

Enter Subject Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) Clear Subject subrange

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Program Selection

Optimize for

- Highly similar sequences (megablast)
- More dissimilar sequences (discontiguous megablast)
- Somewhat similar sequences (blastn)

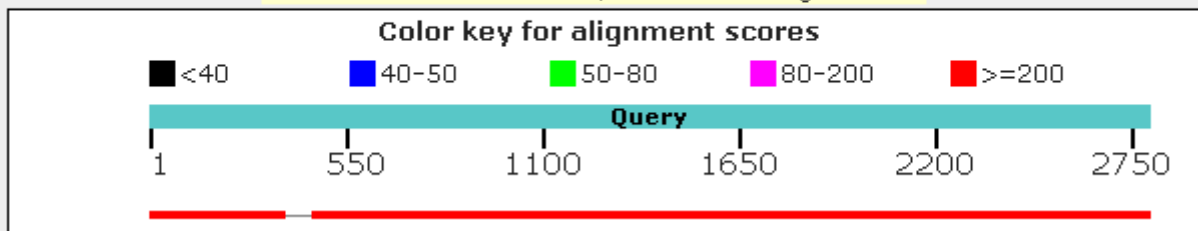
Choose a BLAST algorithm

BLAST Search nucleotide sequence using Megablast (Optimize for highly similar sequences)

Show results in a new window

Distribution of the top 2 Blast Hits on 1 subject sequences

Mouse over to see the title, click to show alignments



Homo sapiens MYB proto-oncogene like 2 (MYBL2), transcript variant 2, mRNA

Sequence ID: [NM_001278610.1](#) Length: 2713 Number of Matches: 2

Range 1: 380 to 2713 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
4311 bits(2334)	0.0	2334/2334(100%)	0/2334(0%)	Plus/Plus
Query 452	AACCGCACTGACCAGCAATGCCAGTACAGGTGGCTGAGAGTTTGAATCCAGACCTTGTG	511		
Sbjct 380	AACCGCACTGACCAGCAATGCCAGTACAGGTGGCTGAGAGTTTGAATCCAGACCTTGTG	439		
Query 512	AAGGGGCCATGGACCAAAAGAGGAAGACCAAAAAGTCATCGAGCTGGTTAAGAAGTATGCC	571		
Sbjct 440	AAGGGGCCATGGACCAAAAGAGGAAGACCAAAAAGTCATCGAGCTGGTTAAGAAGTATGCC	499		
Query 572	ACAAGCAGTGGACACTGATTGCCAAGCACCTGAAGGGCCGGCTGGGGAAGCAGTGCCTG	631		
Sbjct 500	ACAAGCAGTGGACACTGATTGCCAAGCACCTGAAGGGCCGGCTGGGGAAGCAGTGCCTG	559		
Query 632	GAACGCTGGCACAACCACCTCAACCCTGAGGTGAAGAAGTCTTGTGGACCGAGGAGGAG	691		
Sbjct 560	GAACGCTGGCACAACCACCTCAACCCTGAGGTGAAGAAGTCTTGTGGACCGAGGAGGAG	619		
Query 692	GACCGCATCATCTGCGAGGCCCAAGGTGCTGGGCAACCGCTGGGCCGAGATCGCCAAG	751		
Sbjct 620	GACCGCATCATCTGCGAGGCCCAAGGTGCTGGGCAACCGCTGGGCCGAGATCGCCAAG	679		
Query 752	ATGTTGCCAGGGAGGACAGACAATGCTGTGAAGAATCACTGGAATCTACCATCAAAGG	811		
Sbjct 680	ATGTTGCCAGGGAGGACAGACAATGCTGTGAAGAATCACTGGAATCTACCATCAAAGG	739		
Query 812	AAGGTGGACACAGGAGGCTTCTTGAGCGAGTCCAAAGACTGCAAGCCCCAGTGTACTTG	871		
Sbjct 740	AAGGTGGACACAGGAGGCTTCTTGAGCGAGTCCAAAGACTGCAAGCCCCAGTGTACTTG	799		
Query 872	CTGCTGGAGCTCGAGGACAAAGGACGGCTCCAGAGTGCACAGCCACGGAAGGCCAGGGA	931		
Sbjct 800	CTGCTGGAGCTCGAGGACAAAGGACGGCTCCAGAGTGCACAGCCACGGAAGGCCAGGGA	859		
Query 932	AGTCTTCTGACCAACTGGCCCTCCGTCCTCTACCATAAAGGAGGAGGAAAACAGTGG	991		
Sbjct 860	AGTCTTCTGACCAACTGGCCCTCCGTCCTCTACCATAAAGGAGGAGGAAAACAGTGG	919		
Query 992	GAGGAACCTGACAGCAGCCACCACATCGAAGGAACAGGACCCCATCGGTACAGATCTGG	1051		
Sbjct 920	GAGGAACCTGACAGCAGCCACCACATCGAAGGAACAGGACCCCATCGGTACAGATCTGG	979		

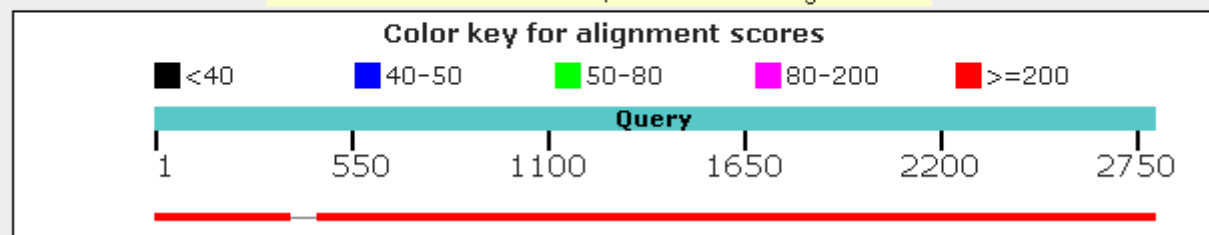
Query 2672	CCCCTGCTAGGATGGGGGATGTGGCCAGGGGTGCTCCTGTGCTCACCCCTCTCTTGGTGCA	2731
Sbjct 2600	CCCCTGCTAGGATGGGGGATGTGGCCAGGGGTGCTCCTGTGCTCACCCCTCTCTTGGTGCA	2659
Query 2732	tttttttGGAAGAATAAAAATGCCTCTCTTTTGTGCTGGTCTGGGAAAAAAAAA	2785
Sbjct 2660	TTTTTTTGAAGAATAAAAATGCCTCTCTTTTGTGCTGGTCTGGGAAAAAAAAA	2713

Range 2: 1 to 379 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
701 bits(379)	0.0	379/379(100%)	0/379(0%)	Plus/Plus
Query 1	ACTCAGGCCCCCTCCCCGGCGCCCGCGCCAGGACTGGGCGGCGCCGACGCGCTTGGCG	60		
Sbjct 1	ACTCAGGCCCCCTCCCCGGCGCCCGCGCCAGGACTGGGCGGCGCCGACGCGCTTGGCG	60		
Query 61	GGAGATAGAAAAGTGCTTCAACCCGCGCCGGCGGCGACTGCAGTTCCTGCGAGCGAGGAG	120		
Sbjct 61	GGAGATAGAAAAGTGCTTCAACCCGCGCCGGCGGCGACTGCAGTTCCTGCGAGCGAGGAG	120		
Query 121	CGCGGGACCTGCTGACACGCTGACGCCTTCGAGCGCGGCCCGGGGCCCGGAGCGGCCGGA	180		
Sbjct 121	CGCGGGACCTGCTGACACGCTGACGCCTTCGAGCGCGGCCCGGGGCCCGGAGCGGCCGGA	180		
Query 181	GCAGCCCCGGTCTGACccccggccccggctccccgctccgggctctgcccggggggggcga	240		
Sbjct 181	GCAGCCCCGGTCTGACccccggccccggctccccgctccgggctctgcccggggggggcga	240		
Query 241	gcgcgggcgcggtccgggccccgggggatgtctcggcggaacgctgcccaggatctggatga	300		
Sbjct 241	GCGCGGCGCGGTCCGGGCCGGGGGATGTCTCGCGGACGCGCTGCGAGGATCTGGATGA	300		
Query 301	GCTGCACTACCAGGACACAGATTGATGTCGGGAGCAGAGGGATAGCAAGTCAAGGT	360		
Sbjct 301	GCTGCACTACCAGGACACAGATTGATGTCGGGAGCAGAGGGATAGCAAGTCAAGGT	360		
Query 361	CAAATGGACCCATGAGGAG (379)			
Sbjct 361	CAAATGGACCCATGAGGAG (379)			

Distribution of the top 2 Blast Hits on 1 subject sequences

Mouse over to see the title, click to show alignments



266

```
atgtctcggcggacgcgctgcgaggatctggatgagctgcactaccaggacacagattcagatgtgccggagcag  
aggatagcaagtgcaaggtcaaatggacccatgaggaggacgagcagctgagggcctggtgaggcagtttggg  
cagcaggactggaagttcctggccagccacttcctaacgcactgaccagcaatgccagtacaggtggctgaga  
gttttgaaatccagaccttgtcaaggggcatggaccaaagaggaagaccaaaaagtcatcgagctggttaagaag
```

- u transkriptu 2 chybí 3. exon (zelený)

https://www.ncbi.nlm.nih.gov/nuccore/NM_002466.4

Homo sapiens MYB proto-oncogene like 2 (MYBL2), transcript variant 1, mRNA

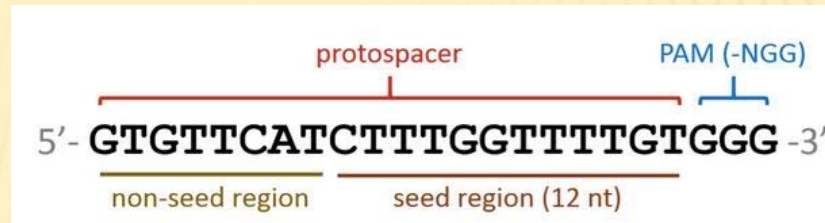
 (Ctrl) -

Chci-li začít
obě varianty
mRNA - cílím
na kratší
variantu

```
171 atgtctcggc
181 ggacgcgctg cgaggatctg gatgagctgc actaccagga cacagattca gatgtgccgg
241 agcagagggg tagcaagtgc aaggtcaaat ggaccatga ggaggacgag cagctgaggg
301 ccctgggtgag qcagtttgga caqcaggact ggaagtctct gccagaccac tcccctaacc
361 qcactgacca qcaatgccag tacaggtggc tgagagtttt gaatccagac cttgtcaagg
421 ggccatggac caaagaggaa gaccaaaaag tcatcagact ggttaagaag tatggcaca
481 agcagtggac actgattgcc aagcacctga agggccgctt ggggaagcag tgccgtgaa
541 gctggcaca ccacctcaac cctgaggtga agaagtcttg ctggaccgag gaggaggacc
601 gcatcatctg cgaggcccac aaggtgctgg gcaaccgctg gcccgagatc gccaatgt
661 tgccagggag qacagacaat gctgtgaaag atcactggaa ctctaccatc aaaaggaagg
721 tggacacagg aggcttcttg agcaggtcca aagactgcaa gccccagtg tacttgctgc
781 tggagctcga ggacaaggac gccctcaga gtgccagcc cacggaaggc cagggaaagc
841 ttctgaccaa ctggccctcc gtccctccta ccataaaggga ggaggaaac agtgaggagg
901 aacttgacgc agccaccaca tcgaaggaac agggaccat cgtacagat ctggacgcag
961 tgcgaacacc agagcccttg gaggaattcc cgaagcgtga ggaccaggaa ggctcccac
1021 cagaaacgag cctgccttac aagtgggtgg tggaggcagc taacctcctc atccctgctg
1081 tgggttctag cctctctgaa gccctggact tgatcgagtc ggaccctgat gcttggtgtg
1141 acctgagtaa atttgacctc cctgaggaac catctgcaga ggacagtatc aacaacagcc
1201 tagtgagct qcaagcgtca catcagcagc aagtcctgcc accccgcccag ccttcgccc
1261 tgggcccag tgtgaccgag taccgcctgg atggccacac catctcagac ctgagcccga
1321 qcagccgggg cgagctgatc cccatctccc ccagcactga agtcgggggc tctggcattg
1381 qcacaccgcc ctctgtgctc aagcggcaga ggaagaggcg tgtggctctg tcccctgtca
1441 ctgagaatag caccagtctg tccttctggt attcctgtaa cagcctcacg cccaagagca
1501 cacctgttaa gaccctgccc ttctcgccct cccagtttct gaacttctgg aacaacagg
1561 acacattgga gctggagagc ccctcgctga catccacccc agtgtgcagc cagaaggtgg
1621 tggtcaccac accactgcac cgggacaaga caccctgca ccagaaacat gctgcgtttg
1681 taaccccaga tcagaagtac tccatggaca aactcccca cacgccaacc ccgttcaaga
1741 agcccctgga gaagtacgga cccctgaagc ccctgccaca gaccccgcac ctggaggagg
1801 acttgaagga ggtgctgctg tctgaggctg qcatcgaact catcatcgag gacgacatca
1861 ggcccagaa gcagaagagg aagcctgggc tgccgagag ccccatcaag aaagtccgga
1921 agtctctgqc tcttgacatt gtggatgagg atgtgaaact gatgatgtcc acactgccc
1981 agtctctatc cttgccgaca actgcccctt caaactctc cagcctcacc ctgtcaggta
2041 tcaaagaaga caacagcttg ctcaaccagg gcttcttgca ggccaagccc gagaaggcag
2101 cagtggccca gaagcccga agccacttca cgacacctgc cctatgtcc agtgcctgga
2161 agacggtggc ctgcgggggg accagggacc agcttttcat gcaggagaaa gccggcagc
2221 tcctgggccc cctgaagccc agccacacat ctccgaccct catcttctcc tga
```


Online target prediction tools

Off-targets



Efficiency - „pravděpodobnost štěpení v cílovém místě“

Out-of-frame score - „pravděpodobnost vzniku posunové mutace“

- různé softwary různé metody/algoritmy scoringu

Chci-li mutovat gen → předčasný stop kodon, obvykle volím začátek genu
... cca do 500 bp od ATG

... ale pozor na alternativní iniciační kodóny
(tedy spíše ne první exon)

vs

non-sense mediated decay



Pravidlo 50 nt - neumíst'uji cílové místo štěpení blízko místa sestřihu

- aby mutace nezpůsobila vznik alternativního místa sestřihu

CDS

171..2201

/gene="MYBL2"

/gene_synonym="B-MYB; BMYB"

exon

191..284

/gene="MYBL2"

/gene_synonym="B-MYB; BMYB"

/inference="alignment:Splign:2.1.0"

exon

285..377

/gene="MYBL2"

/gene_synonym="B-MYB; BMYB"

/inference="alignment:Splign:2.1.0"

exon

378..598

/gene="MYBL2"

/gene_synonym="B-MYB; BMYB"

/inference="alignment:Splign:2.1.0"

exon

599..761

/gene="MYBL2"

/gene_synonym="B-MYB; BMYB"

/inference="alignment:Splign:2.1.0"

exon

762..1049

/gene="MYBL2"

/gene_synonym="B-MYB; BMYB"

/inference="alignment:Splign:2.1.0"

exon

1050..1463

/gene="MYBL2"

/gene_synonym="B-MYB; BMYB"

/inference="alignment:Splign:2.1.0"

http://crispor.tefor.net/

Step 1

Planning a lentiviral gene knockout screen? Use [CRISPOR Batch](#)

Sequence name (optional):

Enter a single genomic sequence, < 2000 bp, typically an exon

[Clear Box](#) - [Reset to default](#)

```
gtc atcgagctgg ttaagaagta tggcacaag cagtggacac
 421 tgattgccaa gcacctgaag gcccgctgg ggaagcagtg cctgaacgc
tggcacaacc
 481 acctcaacc tgagtgaaag aagcttctct ggaccgagga gaggaccgc
atcatctgcg
 541 aggccacaaa ggtgctgggc aaccgctggg ccgagatcgc caagatgttg ccaggag
```

Text case is preserved, e.g. you can mark ATGs with lowercase.
Instead of a sequence, you can paste a chromosome range, e.g. chr1:11,130,540-11,130,751

Step 2

Select a genome

Homo sapiens - Human - UCSC Feb. 2009 (GRCh37/hg19) + SNPs: 1000Genomes, ExaC

Note: pre-calculated exonic guides for this species are on the [UCSC Genome Browser](#).

We have 637 genomes, but not yours? Search [NCBI assembly](#) and send a GCF_/GCA_ID to [CRISPOR support](#).

Step 3

Select a Protospacer Adjacent Motif (PAM)

20bp-NGG - Sp Cas9, SpCas9-HF1, eSpCas9 1.1

SUBMIT

Homo sapiens (hg19), chr20:42315492-42315712, forward genomic strand

Your input sequence is 221 bp long. It contains 36 possible guide sequences.

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

Click on a match for the PAM NGG below to show its 20 bp-long guide sequence. (Need help? Look at the [CRISPOR manual](#))

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

Gene Models:

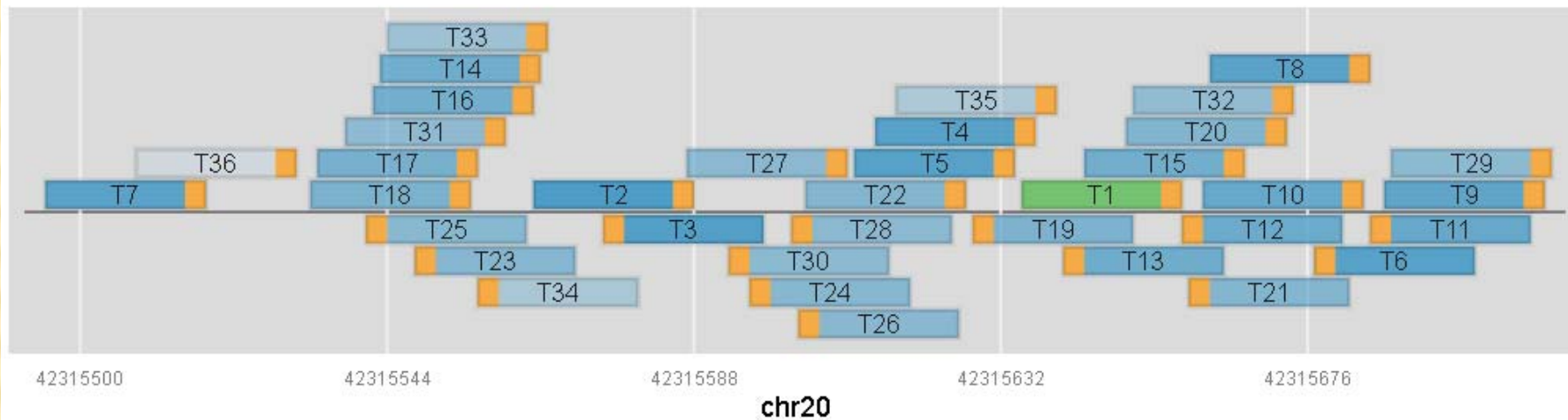
Variant database: Min. frequency: [Missing a variant database? We can add it.](#)

Position	0	10	20	30	40	50	60	70	80	90	100	110		
VariantsI.....													
Sequence	gtcatcgagctggttaagaagtatggcacaaagcagtggaactgattgccaagcacctgaagggccggctggggaagcagtgccgtgaacgctggcacaccacctcaacc													
			---TGG		---TGG		CCA---	---AGG	---TGG		CCG---	---TGG	CCA---	CC
							CCT---	---GGG	---GGG				CCT---	C
								---CGG						
								---GGG						

Download for: [SerialCloner \(free\)](#) - [ApE \(free\)](#) - [GenomeCompiler](#) - [Benchling](#) - [SnapGene](#) - [Geneious](#) - [Vector NTI](#) - [LaserGene](#) - [Genbank](#) - [FASTA](#)

Position/ Strand ⓘ	Guide Sequence + PAM + Restriction Enzymes ⓘ + Variants ⓘ <input type="checkbox"/> Only G- <input type="checkbox"/> Only GG- <input type="checkbox"/> Only A- ⓘ	MIT Specificity Score ⓘ	CFD Spec. score ⓘ	Predicted Efficiency ⓘ <small>Show all scores</small> Doench '16 Mor.-Mateos		Outcome Out-of-Frame Lindel		Off-targets for 0-1-2-3-4 mismatches + next to PAM ⓘ	Genome Browser links to matches sorted by score ⓘ <input type="checkbox"/> exons only <input type="checkbox"/> chr20 only
84 / rev	GGTGGTTGTGCCAGCGTTCA CGG ⚠ Inefficient Enzymes: <i>BceAI</i> , <i>Hpy168II</i> , <i>MwoI</i> Cloning / PCR primers	90	95	41	69	49	76	0 - 0 - 0 - 11 - 90 0 - 0 - 0 - 0 - 0 101 off-targets	3:intergenic:RN7SKP277-PNPT1P2 4:intergenic:PAR3-AS1-SS18L2P1 4:intergenic:CTD-2521M24.5-BST2 show all...
94 / fw	GGAAGCAGTGCCGTGAACGC TGG Enzymes: <i>Hpy168II</i> , <i>MwoI</i> , <i>BstC8I</i> Cloning / PCR primers	90	93	56	36	59	64	0 - 0 - 0 - 7 - 93 0 - 0 - 0 - 0 - 0 100 off-targets	4:intron:CDK11B 4:intergenic:CDK11A/RP1-283E3.8-RP1-283E3.8/CDK11 4:exon:AC104695.4 show all...
143 / fw	GAAGTCTTGCTGGACCGAGG AGGG...I.....I..... Enzymes: <i>BseRI</i> , <i>TaqII</i> , <i>BseDI</i> Cloning / PCR primers	90	91	66	64	40	79	0 - 0 - 0 - 5 - 95 0 - 0 - 0 - 0 - 1 100 off-targets	4:intron:RAPGEF4 4:intergenic:IGHVII-26-2-IGHV7-27 4:intergenic:AC024590.1-RP11-481E4.1 show all...
164 / fw	GGAGGACCGCATCATCTGCG AGG Enzymes: <i>BshFI</i> , <i>BstAPI</i> , <i>MwoI</i> , <i>PspPI</i> Cloning / PCR primers	89	93	65	43	48	83	0 - 0 - 0 - 3 - 47 0 - 0 - 0 - 0 - 0 50 off-targets	4:exon:DTX1 3:intron:DCP1A 4:exon:DTX2 show all...
186 / rev	TCTTGGCGATCTCGGCCAG CGGI.....I..... Enzymes: <i>BseYI</i> , <i>MspA1I</i> , <i>LpnPI</i> , <i>PspPI</i> Cloning / PCR primers	87	94	70	74	67	83	0 - 0 - 1 - 7 - 55 0 - 0 - 0 - 1 - 1	4:intergenic:RP11-380I10.4-RPL5P22 4:intergenic:RNU6-596P-RP11-756K15.2 4:intron:RP11-544L8__B.4 show all...

Pozor na možné mutace/polymorfismy



Legend for off-target site position: E = exonic; I = intronic; - = intergenic

Legend for the CRISPRater score: LOW efficacy (score<0.56); MEDIUM efficacy (0.56<=score<=0.74); HIGH efficacy (score>0.74)

T1 out of 36

<Previous [Next](#)>

Sequence: GGAGGACCGCATCATCTGCGAGG

Efficacy score by CRISPRater: 0.81 HIGH

Oligo pair fwd: CACCGGAGGACCGCATCATCTGCG rev: AAACCGCAGATGATGCGGTCCTCC

Top 20 offtarget sites out of 27 (including on target, for full list see xls file)

Coordinates	strand	MM	target_seq	PAM	distance	gene name	gene id
chr20:42315635-42315657	+	0	GGAGGACC [GCATCATCTGCG]	AGG	0	E MYBL2	ENSG00000101057
chr1:156457648-156457670	-	4	CAAGGGCC [GGATCATCTGCG]	AGG	2634	I MEF2D	ENSG00000116604

84 / rev	GGTGGTTGTGCCAGCGTTCA CGG ▲ Inefficient Enzymes: <i>BceAI</i> , <i>Hpy166II</i> , <i>MwoI</i> Cloning / PCR primers	90	95	41	69	49	76	0 - 0 - 0 - 11 - 90 0 - 0 - 0 - 0 - 0 101 off-targets
94 / fw	GGAAGCAGTGCCGTGAACGC TGG Enzymes: <i>Hpy166II</i> , <i>MwoI</i> , <i>BstC8I</i> Cloning / PCR primers	90	93	56	36	59	64	0 - 0 - 0 - 7 - 93 0 - 0 - 0 - 0 - 0 100 off-targets
143 / fw	GAAGTCTTGCTGGACCGAGG AGGG...I.....I.... Enzymes: <i>BseRI</i> , <i>TaqII</i> , <i>BseDI</i> Cloning / PCR primers	90	91	66	64	40	79	0 - 0 - 0 - 5 - 95 0 - 0 - 0 - 0 - 1 100 off-targets
164 / fw	GGAGGACCGCATCATCTGCG AGG Enzymes: <i>BshFI</i> , <i>BstAPI</i> , <i>MwoI</i> , <i>PspPI</i> Cloning / PCR primers	89	93	65	43	48	83	0 - 0 - 0 - 3 - 47 0 - 0 - 0 - 0 - 0 50 off-targets

T1 out of 36

<Previous [Next](#)>

Sequence:

GGAGGACCGCATCATCTGCGAGG

Efficacy score by CRISPRater: 0.81 HIGH

T3 out of 36

<Previous [Next](#)>

Sequence:

GGTGGTTGTGCCAGCGTTCACGG

Efficacy score by CRISPRater: 0.71 MEDIUM

T2 out of 36

<Previous [Next](#)>

Sequence:

GGAAGCAGTGCCGTGAACGCTGG

Efficacy score by CRISPRater: 0.74 HIGH

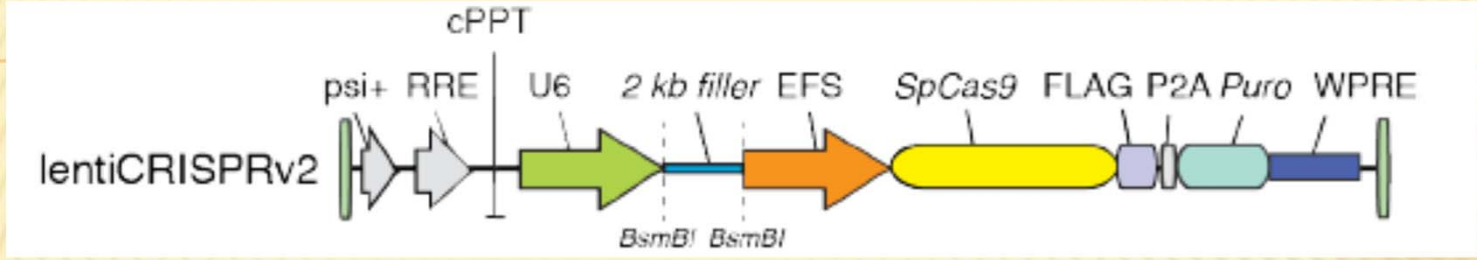
T4 out of 36

<Previous [Next](#)>

Sequence:

GAAGTCTTGCTGGACCGAGGAGG

Efficacy score by CRISPRater: 0.83 HIGH

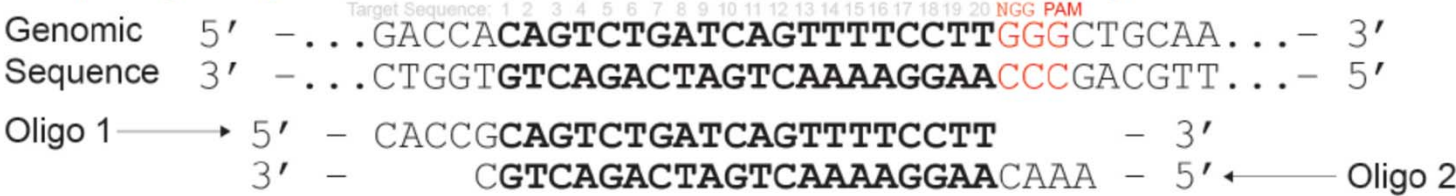


Target Guide Sequence Cloning Protocol

In order to clone the target sequence into the lentiCRISPRv2 or lentiGuide-Puro backbone, synthesize two oligos of the following form. **All plasmids have the same overhangs** after *BsmBI* digestion and the same oligos can be used for cloning into lentiCRISPRv2, lentiGuide-Puro or lentiCRISPRv1.



Example oligo design: Note that the NGG PAM is **not** included in the designed oligos.



84 / rev	GGTGGTTGTGCCAGCGTTCA CGG ⚠ Inefficient Enzymes: <i>BceAI</i> , <i>Hpy166II</i> , <i>MwoI</i> Cloning / PCR primers	90	95	41	69	49	76	0 - 0 - 0 - 11 - 90 0 - 0 - 0 - 0 - 0 101 off-targets
94 / fw	GGAAGCAGTGCCGTGAACGC TGG Enzymes: <i>Hpy166II</i> , <i>MwoI</i> , <i>BstC8I</i> Cloning / PCR primers	90	93	56	36	59	64	0 - 0 - 0 - 7 - 93 0 - 0 - 0 - 0 - 0 100 off-targets
143 / fw	GAAGTCTTGCTGGACCGAGG AGGG...I...I..... Enzymes: <i>BseRI</i> , <i>TaqII</i> , <i>BseDI</i> Cloning / PCR primers	90	91	66	64	40	79	0 - 0 - 0 - 5 - 95 0 - 0 - 0 - 0 - 1 100 off-targets
164 / fw	GGAGGACCGCATCATCTGCG AGG Enzymes: <i>BshFI</i> , <i>BstAPI</i> , <i>MwoI</i> , <i>PspPI</i> Cloning / PCR primers	89	93	65	43	48	83	0 - 0 - 0 - 3 - 47 0 - 0 - 0 - 0 - 0 50 off-targets

← return to the list of all guides

Guide sequence: GGAAGCAGTGCCGTGAACGC TGG

Contents:

- Cloning or expression of guide RNA
 - T7 *in vitro* expression from a plasmid
 - T7 *in vitro* expression from overlapping oligonucleotides
 - U6 expression from an Addgene plasmid
 - Direct PCR for *C. intestinalis*
 - Lentiviral vectors: Cloning with Gibson assembly
 - Summary of main cloning/expression primers
- PCR to amplify the on-target site
- Restriction sites for PCR validation
- PCR to amplify off-target sites
- Saturating mutagenesis using all guides

U6 expression from an Addgene plasmid

The guide sequence ggaggaccgcatcatctgcg does not contain the motif TTTT, which terminates RNA polymerase in mammalian cells.

Select your Addgene plasmid:

To clone the guide into *lentiCRISPR v2 (Zhang lab)*, use these primers:

Name	Primer Sequence
guideRNA164fwU6senseentiCrispr	CACCggaggaccgcatcatctgcg
guideRNA164fwU6antisenseentiCrispr	AAACcgcagatgatgcggctctcc

Kontrola specifiity navržených oligonukleotidů k cílové sekvenci všech izoforem

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

GGAAGCAGTGCCGTGAACGCTGG

Query subrange [?](#)

From

To

Or, upload file Nevybrán žádný soubor [?](#)

Job Title

Enter a descriptive title for your BLAST search [?](#)

Align two or more sequences [?](#)

Choose Search Set

Database Standard databases (nr etc.): rRNA/ITS databases Genomic + transcript databases Betacoronavirus

Nucleotide collection (nr/nt) [?](#)

Organism **Optional** exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Exclude **Optional** Models (XM/XP) Uncultured/environmental sample sequences

Limit to **Optional** Sequences from type material

Entrez Query **Optional**

Enter an Entrez query to limit search [?](#) [YouTube](#) [Create custom database](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
<input checked="" type="checkbox"/>	Homo sapiens MYB proto-oncogene like 2 (MYBL2)_transcript variant 1_mRNA	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Homo sapiens isolate CHM13 chromosome 20	Homo sapiens	46.1	98.6	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Homo sapiens DNA_chromosome 20_nearly complete genome	Homo sapiens	46.1	98.6	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Homo sapiens MYB proto-oncogene like 2 (MYBL2)_transcript variant 2_mRNA	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Homo sapiens cDNA FLJ57697 complete cds_highly similar to Myb-related protein B	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Homo sapiens cDNA_FLJ95661_Homo sapiens v-myb myeloblastosis viral oncogene homolog (avian)-like 2 (...)	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Homo sapiens v-myb myeloblastosis viral oncogene homolog (avian)-like 2_mRNA (cDNA clone MGC:15600 ...	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Homo sapiens v-myb myeloblastosis viral oncogene homolog (avian)-like 2_mRNA (cDNA clone MGC:61523 ...	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Homo sapiens mRNA for MYB-related protein B variant_clone: FCC119C09	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Human DNA sequence from clone RP5-1028D15 on chromosome 20_complete sequence	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	Human mRNA for B-myb_gene	Homo sapiens	46.1	46.1	100%	6e-04	100.00%
<input checked="" type="checkbox"/>	V alpha 2.1-J alpha 6.1=T-cell receptor alpha chain variable region {C alpha region} [human_melanoma-specific...	Homo sapiens	32.2	32.2	69%	8.7	100.00%

Následně doporučuji manuální kontrolu, že cílová sekvence leží v exonu u všech izoforem (ne vždy musí mít izofomy stejně ohraničené exony)

Homo sapiens MYB proto-oncogene like 2 (MYBL2), transcript variant 1, mRNA

Sequence ID: [NM_002466.4](#) Length: 2668 Number of Matches: 1

Range 1: 523 to 545 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
46.1 bits(23)	6e-04	23/23(100%)	0/23(0%)	Plus/Plus

```
Query 1 GGAAGCAGTGCCGTGAACGCTGG 23
      |||
Sbjct 523 GGAAGCAGTGCCGTGAACGCTGG 545
```

```
exon 357..449
      /gene="MYBL2"
      /gene_synonym="B-MYB; BMYB"
      /inference="alignment:Splign:2.1.0"
exon 450..670
      /gene="MYBL2"
      /gene_synonym="B-MYB; BMYB"
      /inference="alignment:Splign:2.1.0"
exon 671..833
      /gene="MYBL2"
      /gene_synonym="B-MYB; BMYB"
      /inference="alignment:Splign:2.1.0"
```

Homo sapiens MYB proto-oncogene like 2 (MYBL2), transcript variant 2, mRNA

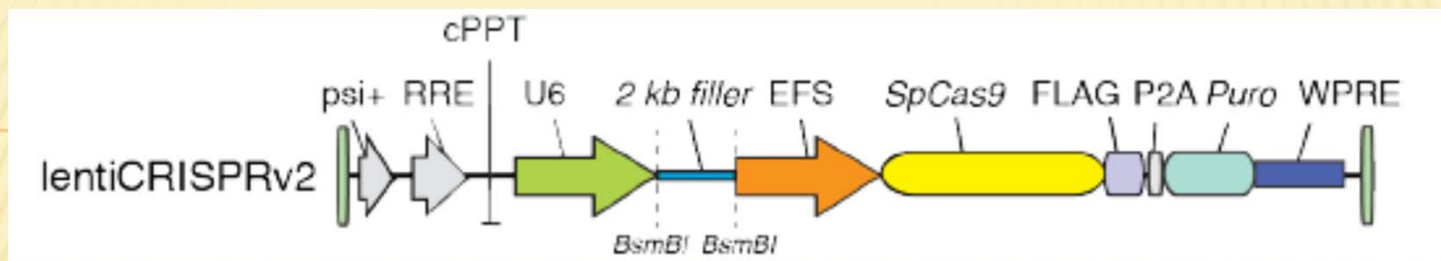
Sequence ID: [NM_001278610.2](#) Length: 2596 Number of Matches: 1

Range 1: 451 to 473 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
46.1 bits(23)	6e-04	23/23(100%)	0/23(0%)	Plus/Plus
Query 1	GGAAGCAGTGCCGTGAACGCTGG	23		
Sbjct 451	GGAAGCAGTGCCGTGAACGCTGG	473		

```
exon 285..377
      /gene="MYBL2"
      /gene_synonym="B-MYB; BMYB"
      /inference="alignment:Splign:2.1.0"
exon 378..598
      /gene="MYBL2"
      /gene_synonym="B-MYB; BMYB"
      /inference="alignment:Splign:2.1.0"
exon 599..761
      /gene="MYBL2"
      /gene_synonym="B-MYB; BMYB"
      /inference="alignment:Splign:2.1.0"
```

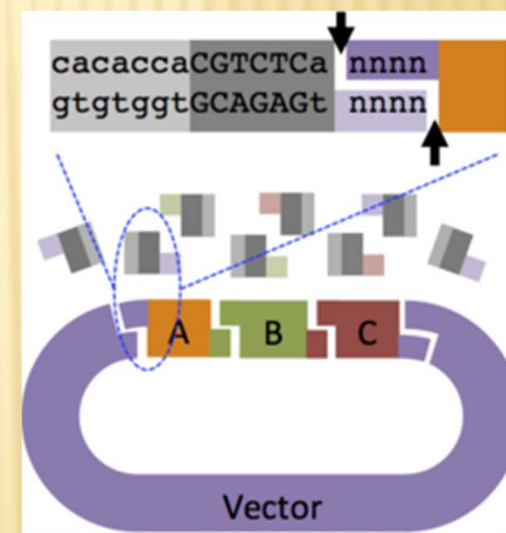



CACCggaagcagtgccgtgaacgc

AAACgcgttcacggcactgcttcc

CACCggaagcagtgccgtgaacgc
ccttcgtcacggcacttgcgCAAA

RE BsmBI (ESp3I)



ligace, transformace E. coli ligační směsí, expanze klonů, izolace plazmidové DNA, sekvenace

další postup ...

- přenos plazmidu do eukaryotických buněk (transfekce)
- selekce a expanze puromycin-rezistentních buněk
- příprava jednotlivých klonů metodou limitního ředění (ev. FACS)
- analýza exprese cílového genu na úrovni proteinu (westernův přenos)
- izolace genomové DNA klonu(ů) s nulovou expresí cílového proteinu
- amplifikace cíleného úseku genomové DNA + sekvenace

84 / rev	GGTGGTTGTGCCAGCGTTCA CGG ⚠ Inefficient Enzymes: <i>BceAI</i> , <i>Hpy166II</i> , <i>MwoI</i> Cloning / PCR primers	90	95	41	69	49	76	0 - 0 - 0 - 11 - 90 0 - 0 - 0 - 0 - 0 101 off-targets
94 / fw	GGAAGCAGTGCCGTGAACGC TGG Enzymes: <i>Hpy166II</i> , <i>MwoI</i> , <i>BstC8I</i> Cloning / PCR primers	90	93	56	36	59	64	0 - 0 - 0 - 7 - 93 0 - 0 - 0 - 0 - 0 100 off-targets
143 / fw	GAAGTCTTGCTGGACCGAGG AGGG...I.....I.... Enzymes: <i>BseRI</i> , <i>TaqII</i> , <i>BseDI</i> Cloning / PCR primers	90	91	66	64	40	79	0 - 0 - 0 - 5 - 95 0 - 0 - 0 - 0 - 1 100 off-targets
164 / fw	GGAGGACCGCATCATCTGCG AGG Enzymes: <i>BshFI</i> , <i>BstAPI</i> , <i>MwoI</i> , <i>PspPI</i> Cloning / PCR primers	89	93	65	43	48	83	0 - 0 - 0 - 3 - 47 0 - 0 - 0 - 0 - 0 50 off-targets

[← return to the list of all guides](#)

Guide sequence: GGAAGCAGTGCCGTGAACGC TGG

Contents:

- Cloning or expression of guide RNA
 - T7 *in vitro* expression from a plasmid
 - T7 *in vitro* expression from overlapping oligonucleotides
 - U6 expression from an Addgene plasmid
 - Direct PCR for *C. intestinalis*
 - Lentiviral vectors: Cloning with Gibson assembly
 - Summary of main cloning/expression primers
- PCR to amplify the on-target site
- Restriction sites for PCR validation
- PCR to amplify off-target sites
- Saturating mutagenesis using all guides

PCR to amplify the on-target site

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

OntargetGuideRna164fwLeft	ACTGATTGCCAAGCACCTGA	Tm 59.889
OntargetGuideRna164fwRight	GACAGAAGACAGGGCCTTCC	Tm 60.036

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

Maximum amplicon length: Primer Tm:

Genomic sequence chr20:42315634-42315657 including primers, genomic forward strand:

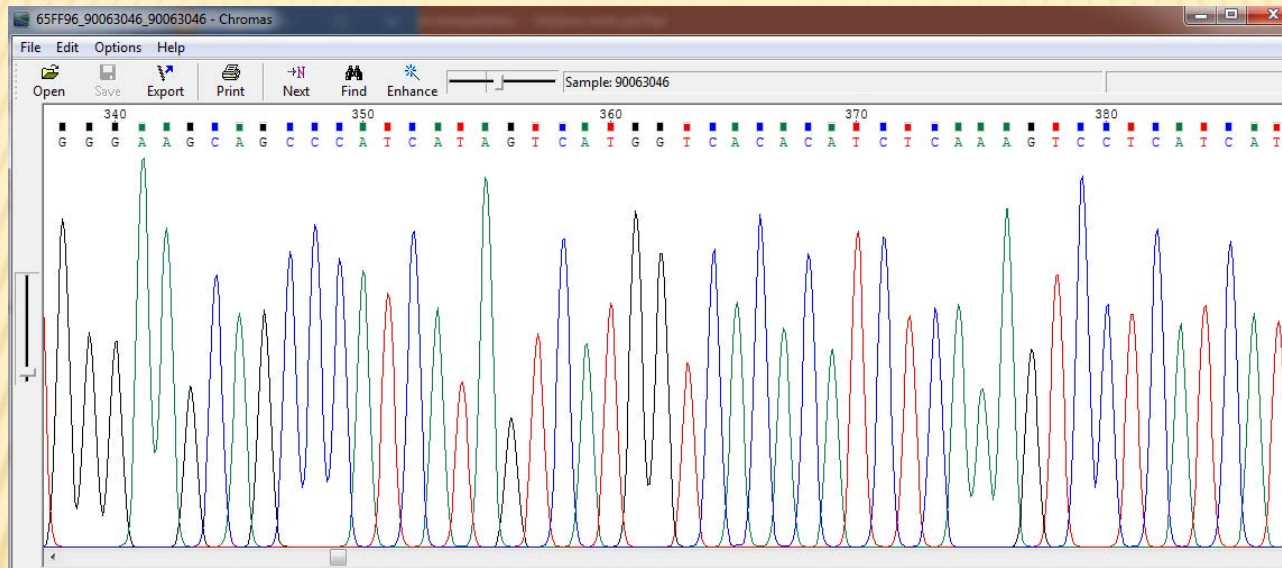
ACTGATTGCCAAGCACCTGAAAGGGCCGGCTGGGGAAGCAGTGCCGTGAACGCTGGCACAACCACCTCAACCCTGAGGTGA
AGAAGTCTTGCTGGACCGAGGAGGAGGACCGCATCATCTGCGAGGCCCACAAGGTGCTGGGCAACCGCTGGGCCGAGATC
GCCAAGATGTTGCCAGGGAGGTAAGCTGTCTTCTTGGGGTTGGGACAGGTTCCCGGGAGGCCAGGCCCGTGTCTTCTGAT
GGAGGAGGGTTCCTGGGTGGGTATTGTGGTCCTGTGCTCTTGTTCTGTAACACCCAGCCTCGGCTCCAGGGCTCCCAGAG
GCATGCATAGTCAGGAGGAAGGGTGGTTC~~CCCAGGGAAGGCCCTGTCTTCTGTC~~

ACTGATTGCCAAGCACCTGAAAGGGCCGGCTGGGGAAGCAGTGCCGTGAACGCTGGCACAACCACCTCAACCCTGAGGTGA
AGAAGTCTTGCTGGACCGAGGAGGAGGACCGCATCATCTGCGAGGCCCACAAGGTGCTGGGCAACCGCTGGGCCGAGATC
GCCAAGATGTTGCCAGGGAGGTAAGCTGTCTTCTTGGGGTTGGGACAGGTTCCCGGGAGGCCAGGCCCGTGTCTTCTGAT
GGAGGAGGGTTCCTGGGTGGGTATTGTGGTCCTGTGCTCTTGTTCTGTAACACCCAGCCTCGGCTCCAGGGCTCCCAGAG
GCATGCATAGTCAGGAGGAAGGGTGGTTC~~CCCAGGGAAGGCCCTGTCTTCTGTC~~

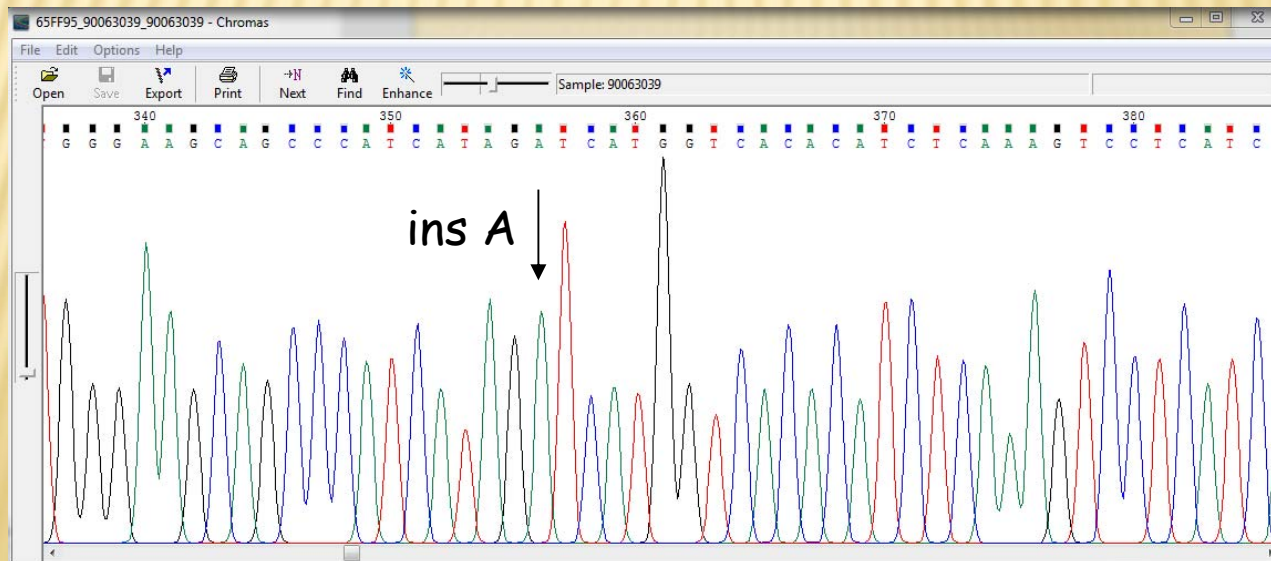
Sequence length: 373

Cílové místo štěpení alespoň 100 bp od primerů kvůli případným delším delecím

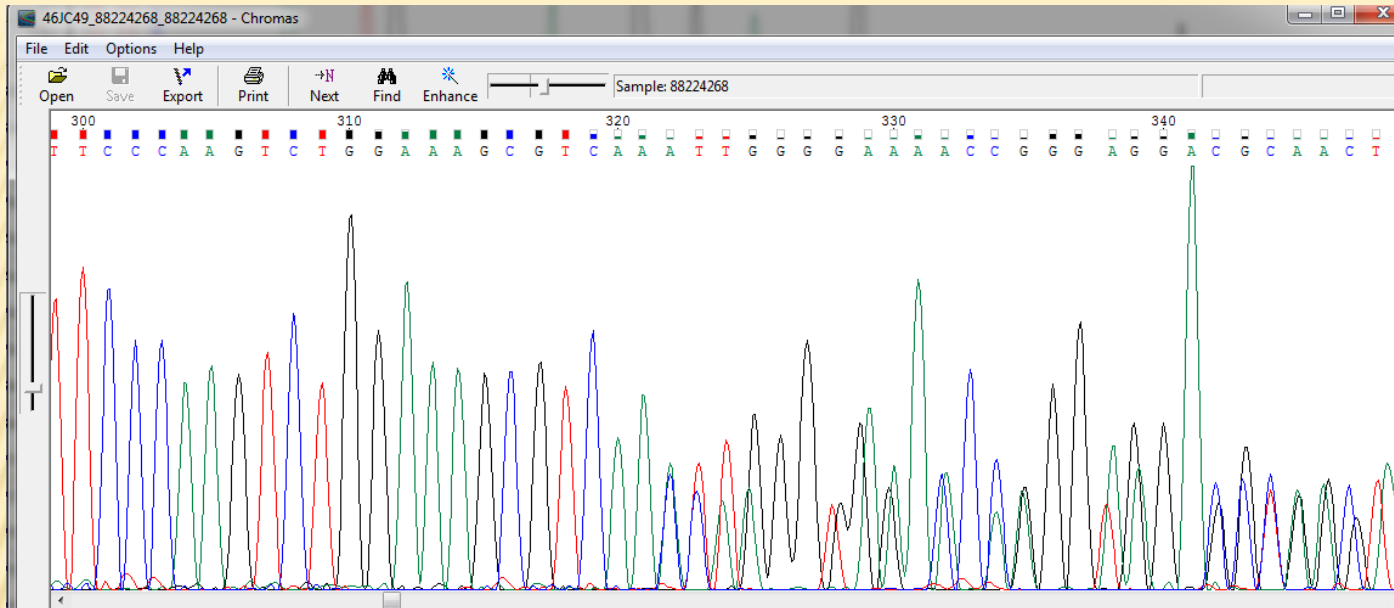
Sequencing results



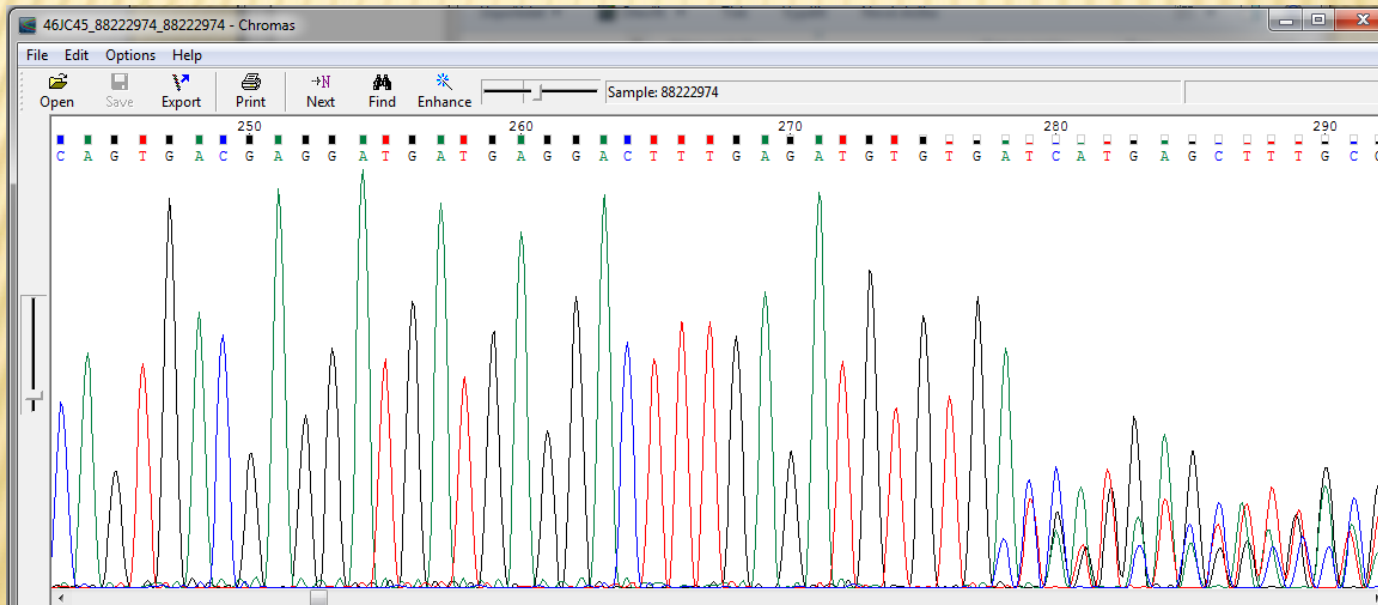
wt



Mutated
homozygot
X
Second allele
not detected



Heterozygot
2 alleles



Heterozygot
3 alleles
X
Not a single
cell clone

<http://crispid.gbiomed.kuleuven.be/>

Upload file



Please upload a trace file (ABI or SCF)

Start position

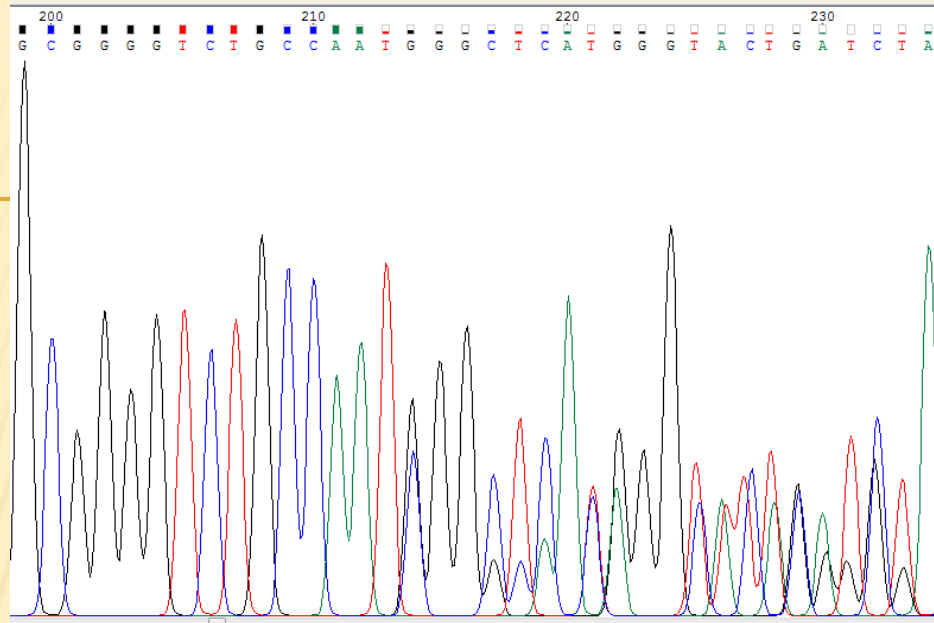
End position

Background cutoff (%)

Max insert size (bp)

Reference sequence

Analyze



```

1 -----ATGCGAGGGGCTTGGATCTAGCACCGCTGCTACTGCTACTGCTGCGATGGCGACCCGCTTTGCACGGCTCAGAGCAACT 100
1 ATTCTACTCCACCCACCATGGCGAGGGGCTTGGATCTAGCACCGCTGCTACTGCTACTGCTGCGATGGCGACCCGCTTTGCACGGCTCAGAGCAACT 100
1 ATTCTACTCCACCCACCATGGCGAGGGGCTTGGATCTAGCACCGCTGCTACTGCTACTGCTGCGATGGCGACCCGCTTTGCACGGCTCAGAGCAACT 100

101 -----GGCTCACAGGTATTGGTCGACTGCTC 200
83 GTACATGCCCCACCAACAAGATGACGGTCTGCGACACAAATGGCCAGGCGGGGTCTGCCAATGTCGGGCAATGGGCTCACAGGTATTGGTCGACTGCTC 182
101 GTACATGCCCCACCAACAAGATGACGGTCTGCGACACAAATGGCCAGGCGGGGTCTGCCAATG-----GGCTCACAGGTATTGGTCGACTGCTC 190
101 GTACATGCCCCACCAACAAGATGACGGTCTGCGACACAAATGGCCAGGCGGGGTCTGCCAAT--CGGGCAATGGGCTCACAGGTATTGGTCGACTGCTC 198

201 ----- 300
183 CACGCTAACTTCCAAGTGCCTGCTGCTCAAGGCGCGCATGAGCGCCCGGAAGAGCGGCCGAGCCTGGTGATGCCGAGCGAGCACGCGATACTGGACAAC 282
191 CACGCTAACTTCCAAGTGCCTGCTGCTCAAGGCGCGCATGAGCGCCCGGAAGAGCGGCCGAGCCTGGTGATGCCGAGCGAGCACGCGATACTGGACAAC 290
199 CACGCTAACTTCCAAGTGCCTGCTGCTCAAGGCGCGCATGAGCGCCCGGAAGAGCGGCCGAGCCTGGTGATGCCGAGCGAGCACGCGATACTGGACAAC 298

301 ----- 400
283 GATGGCCTCTACGACCCGGAGTGTGACGACAAGGGCCGCTTCAAGGCGCGCCAGTGCAACCAGACCTCGGTGTGCTGGTGCCTAACTCGGTGGGCGTGC 382
291 GATGGCCTCTACGACCCGGAGTGTGACGACGAGGGCCGCTTCAAGGCGCGCCAGTGCAACC----- 351
299 GATGGCCTCTACGACCCGGAGTGTGACGACCAGGGCCGCTTCAAGGCGCGCCA----- 351

```

Velmi dobrý je i - <https://ice.synthego.com/#/>

další postup ...

- eventuálně klonování zacíleného úseku genomové DNA do plazmidu vhodného pro sekvenaci
- sekvenace určitého počtu klonů (2 alely genu - min. 4 klony)
- analýza sekvenačních výstupů, identifikace mutací a jejich významu (ORF)

Jaké mutace lze očekávat?

- jakékoli (nejčastěji malé inserce/delece)
- Cas9 štěpí DNA 3-4 nukleotidy upstream od PAM sekvence
- ideální jsou malé posunové mutace, které mění čtecí rámeček
→ předčasný stop kodon

<https://www.ncbi.nlm.nih.gov/orffinder>

ORF finder searches for open reading frames (ORFs) in the DNA sequence you enter. The program returns the range of each ORF, along with its protein translation. Use ORF finder to search newly sequenced DNA for potential protein encoding segments, verify predicted protein using newly developed SMART BLAST or regular BLASTP.

This web version of the ORF finder is limited to the subrange of the query sequence up to 50 kb long. Stand-alone version, which doesn't have query sequence length limitation, is available for [Linux x64](#).

Examples (click to set values, then click Submit button) :

- NC_011604 Salmonella enterica plasmid pWES-1; genetic code: 11; 'ATG' and alternative initiation codons; minimal ORF length: 300 nt
- NM_000059; genetic code: 1; start codon: 'ATG only'; minimal ORF length: 150 nt



Enter Query Sequence

Enter accession number, gi, or nucleotide sequence in FASTA format:

[Empty text input field for query sequence]

From: [dropdown] To: [dropdown]

Choose Search Parameters

Minimal ORF length (nt): 75 [dropdown]
Genetic code: 1. Standard [dropdown]
ORF start codon to use:
 "ATG" only
 "ATG" and alternative initiation codons
 Any sense codon
Ignore nested ORFs:

Start Search / Clear

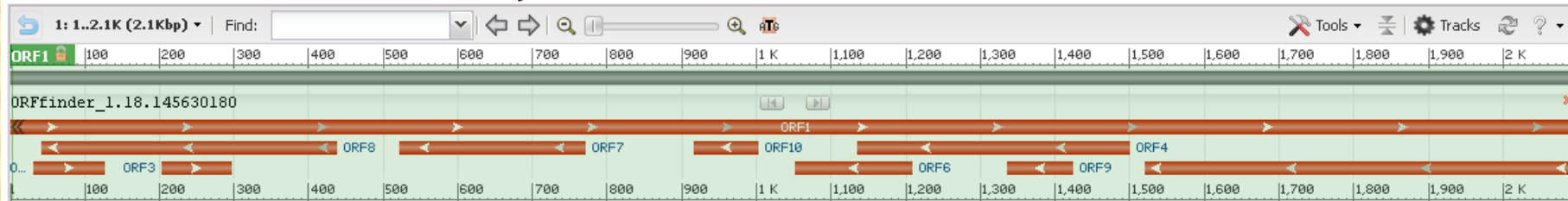
B-Myb

Vložíte-li sekvenci kódující sekvence genu (od ATG)

Open Reading Frame Viewer

Sequence

ORFs found: 10 Genetic code: 1 Start codon: 'ATG' only



ORF1 (700 aa)

Display ORF as...

Mark

```
>1c1|ORF1
MSRRTRCEDLDELHYQDTSADVPEQRDSKCKVKWTHREDE
QLRALVRFQGDQWKFLLASHFPNRTDQCCQYRWLRLVLPD
LVKCPWTKBEDQKVIELVKYGTQKQWTLIAKHLKGRLLKQ
CRERWVHNLNPEVKKSCWTREEDRIICEAHKVLGNRWAEI
AKMLPGRTDNAVKNHWNSTIKRVDTCGFLSEKDCPPV
YLLLELEDKDCGLQSAQPTGCGSLLTNWPSVPPTIKEEN
SEELAAATTSKEQEPICDLDLAVRTPEPLEEFPKREDQE
GSPPETSLPYKVVVEAANLLIPAVGSSLSEALDLIESDPD
AWCDLSKFDLPEEPSAEDSINNLSLVQLQASHQQVLPFRQ
PSALVPSVTEYRLDGHITISDLSRSSRGELIPISPSTEVCG
SGIGTPPSVLKRRQRKRRVALSPVTENSTLSFLDSCNSLT
PKSTPVKTLFPFSPSQFLNFWNRQDTLELESPLTSTPVCS
QKVVVITPLHRDRTPLHQKHAAFVTPDQKYSMDNTPHTPT
```

SmartBLAST ORF1

BLAST ORF1

BLAST marked set

BLAST Database:

UniProtKB/Swiss-Prot (swissprot)

Mark subset...

Marked: 0

Download marked set

as Protein FASTA

Label	Strand	Frame	Start	Stop	Length (nt aa)
ORF1	+	1	<1	2103	2103 700
ORF5	-	2	2093	1521	573 190
ORF8	-	2	437	42	396 131
ORF4	-	1	1500	1135	366 121
ORF7	-	2	770	522	249 82
ORF6	-	2	1208	1053	156 51
ORF2	+	2	32	127	96 31
ORF3	+	3	204	296	93 30
ORF9	-	3	1423	1337	87 28
ORF10	-	2	1002	917	87 28

Add six-frame translation track

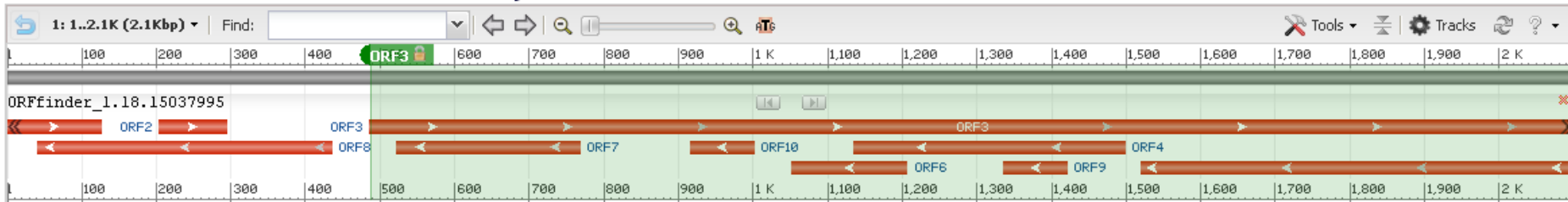
B-Myb - mutovaný

Open Reading Frame Viewer

Vložíte-li sekvenci kódující sekvence genu (od ATG)

Sequence

ORFs found: 10 Genetic code: 1 Start codon: 'ATG' only



Add six-frame translation track

ORF3 (538 aa)

Display ORF as...

Mark

```
>1c1|ORF3
MLPGRTDNAVKNHWNSTIKRKVDTCGFLSESKDCKPPVYL
LLELEDKDLQSAQPTREGQSLLTNWPSVPPTIKEEENSE
EELAAATTSKEQEPICGTDLDAVRTPEPLEEFPKREDQEGS
PPETSLPYRWVVEAANLLIPAVGSSLSREALDLIESDPDAW
CDLSKFDLPEEPSAEDSINNSLVQLQASHQQVLP RPQS
ALVPSVTFRYRLDGHITISDLRSRRCELIPIISPSTEVCSSC
IGTPPSVLKRQRKRRVALSPVTENSTSLSFLDSCNSLTPK
STPVKTLFPSPSQFLNFWNRQDTLELESPLSTSTPVCSQK
VVVTTPLHRDKTPLHQKHAAPVTPDQRYSDMNTPHPTPF
KNALEKYCPLKPLPQTPHLEEDLKEVLRSEAGIELIIEDD
IRPEKQRKPKGLRRSPIKKVKSLALDIVDEDVKLHMSTL
PKSLSLPTTAPSNSSSLTLCIKEDNSLLNQCFLQAKPEK
AAVAQKPRSHFTTTPAMSSAWKTVACGCTRDQLFMQEKAR
```

SmartBLAST ORF3

BLAST ORF3

BLAST marked set

BLAST Database:

UniProtKB/Swiss-Prot (swissprot)

Mark subset...

Marked: 0

Download marked set

as Protein FASTA

Label	Strand	Frame	Start	Stop	Length (nt aa)
ORF3	+	3	486	>2102	1617 538
ORF5	-	2	2092	1520	573 190
ORF8	-	2	436	41	396 131
ORF4	-	1	1499	1134	366 121
ORF7	-	2	769	521	249 82
ORF6	-	2	1207	1052	156 51
ORF1	+	1	<1	126	126 41
ORF2	+	2	203	295	93 30
ORF9	-	3	1422	1336	87 28
ORF10	-	2	1002	916	87 28

Organizace cvičení (CRISPR)

- skupiny po 12 lidech (2x 6 hod praktických cvičení)

Každá skupina

... před praktickou částí cvičení ...

- přidělen jeden cílový gen
- návrh strategie cílené mutagenese tohoto genu metodou CRISPR (miniprojekt)
- zaslání **nejpozději** týden před začátkem cvičení na **email vyučujícího dané skupinky**

... po praktické části cvičení ...

- výsledky získané na cvičeních budou zpracovány do tohoto miniprojektu
- včetně analýzy sekvenačních výstupů
- včetně vyhodnocení významu vzniklých mutací pro translaci cílového genu

Miniprojekt (.doc)

- stručný úvod + cíl
- detailní popis návrhu **gRNA sekvencí, oligonukleotidů a sekvencí primerů** pro vybraný gen

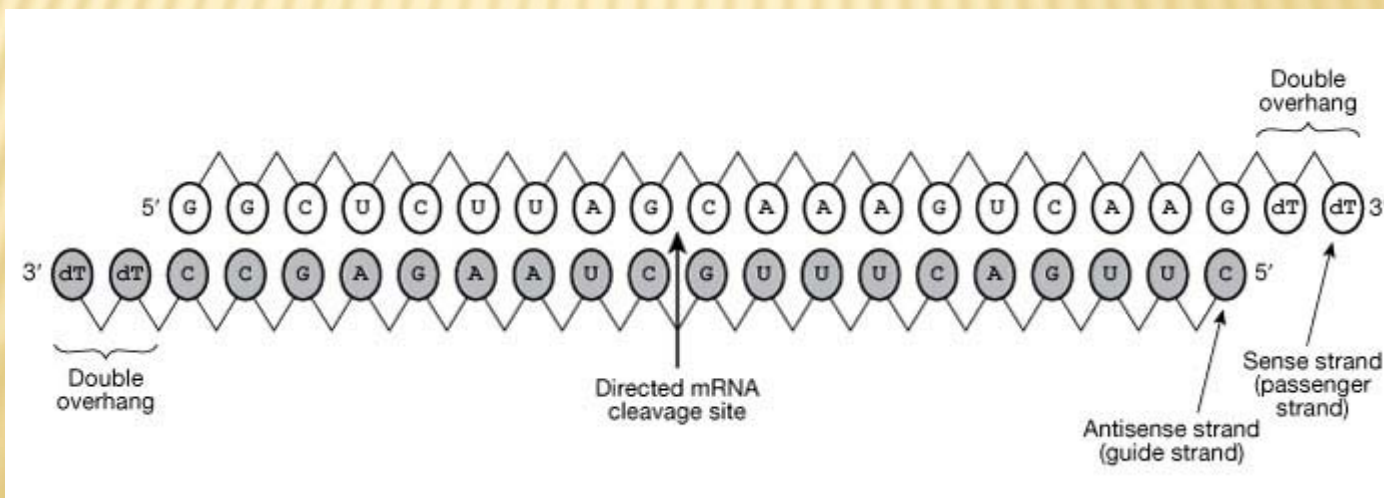
-
- stručný popis výsledků dílčích kroků cílené mutageneze z praktických cvičení
 - analýza výstupů ze sekvenace genomové DNA získaných klonů
 - závěr

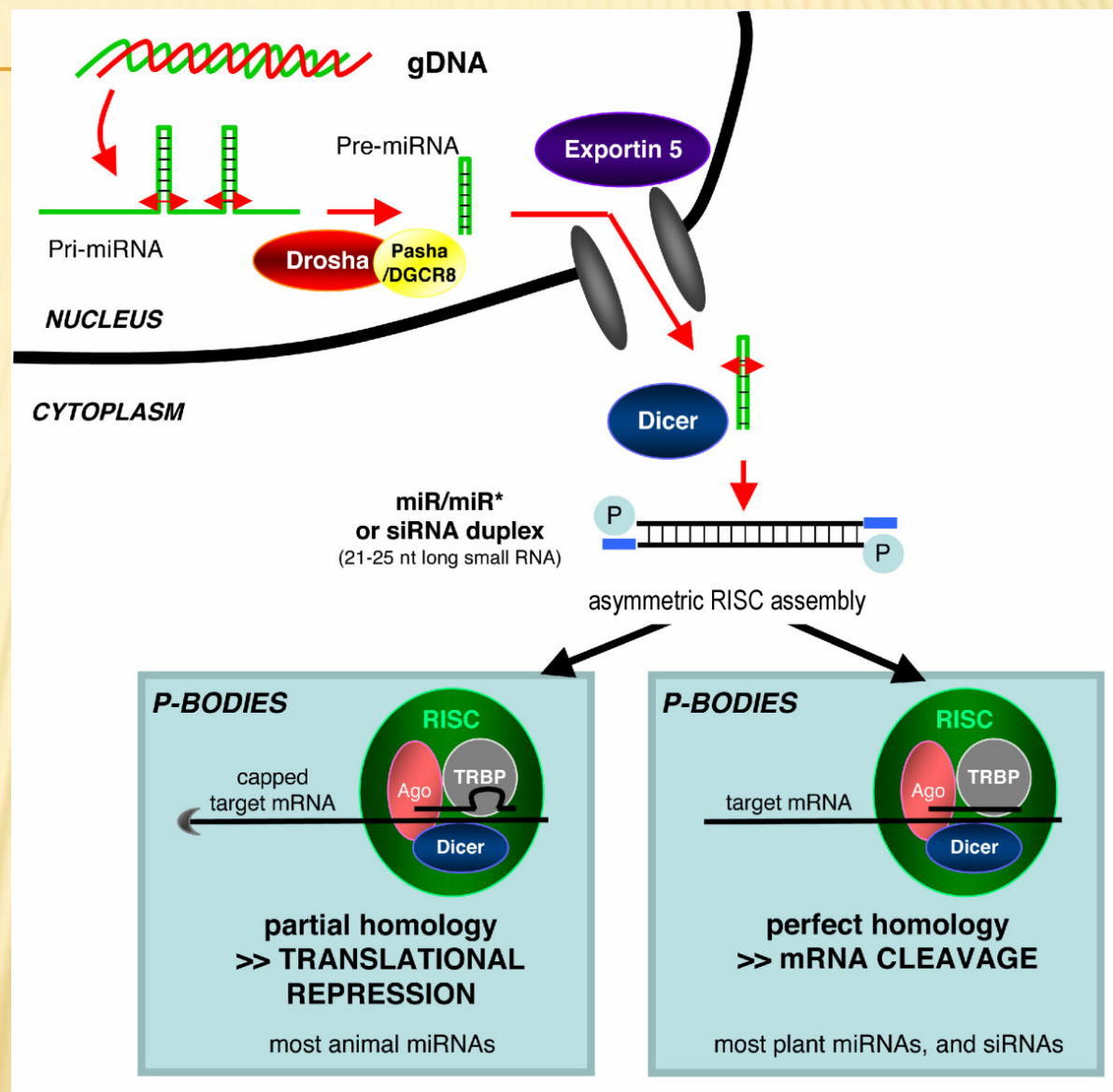
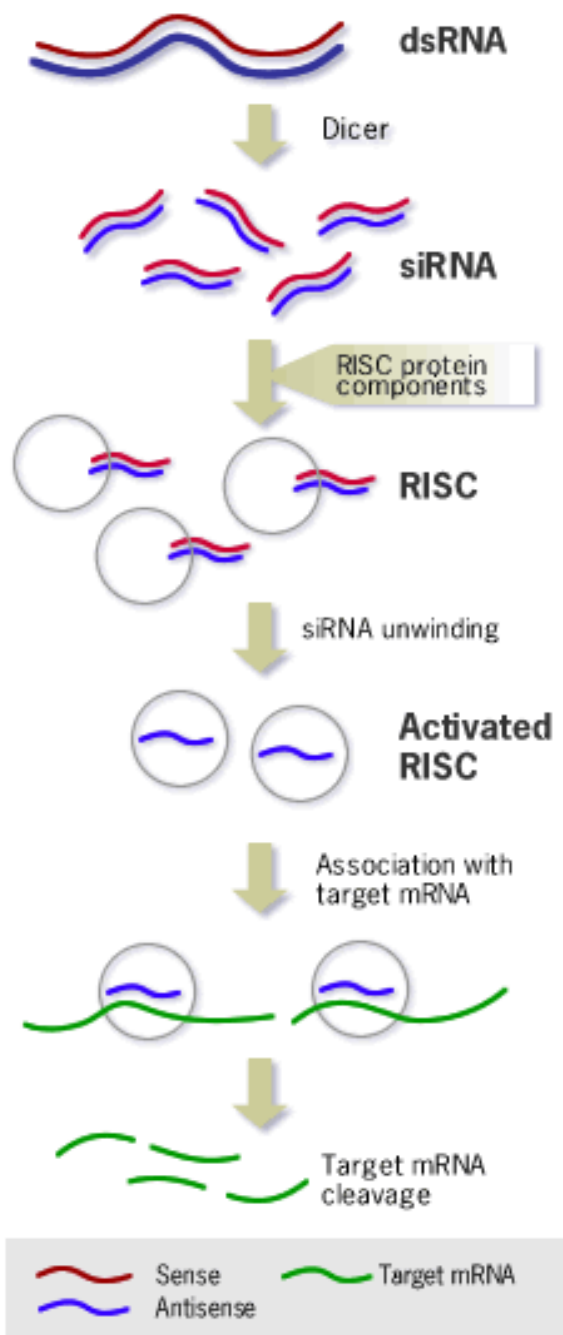
RNA interference

- mechanismus **post-transkripčního** umlčování genů
- využívá **dvouřetězcové RNA** pro interferenci s genovou expresí
(dsRNA efektivnější než ssRNA)
- podstatou je enzymová degradace nebo zastavení translace specifické mRNA
- miRNA vs siRNA

siRNA

- malé nekódující dsRNA o velikosti 21-25 nukleotidů
- negativní regulátory genové exprese, které obvykle zprostředkovávají degradaci cílové mRNA (úplná homologie)
- exogenní původ (viry, syntetické)
- v cytoplazmě procesovány proteinem Dicer
- siRNA se váže k RISC, jedno vlákno se degraduje a druhé zprostředkovává degradaci nebo inhibici translace příslušné mRNA





miRNA versus siRNA

- obě RNA negativně regulují translaci
- miRNA je endogenní, siRNA je exogenní
- miRNA může, ale obvykle není úplně komplementární určitému transkriptu, proto jedna miRNA může blokovat translaci několika/mnoha transkriptů (desítky až stovky)
- siRNA je obvykle zcela komplementární - obvykle štěpení jediné cílové mRNA

siRNA design

- **validované siRNA** dnes dodává řada firem (Dharmacon, Thermofisher, ...)
- různý algoritmus, délka
- chemické modifikace (zvýšení stability, redukce off-target efektů)...
- směsi siRNA
- **Kontroly !!!!!!!!!!!!!!!**

siRNA design

✘ 21-25 nt

✘ 30-60% GC

✘ ne cílené proti intronům, UTR a blízko ATG a terminačního kodónu

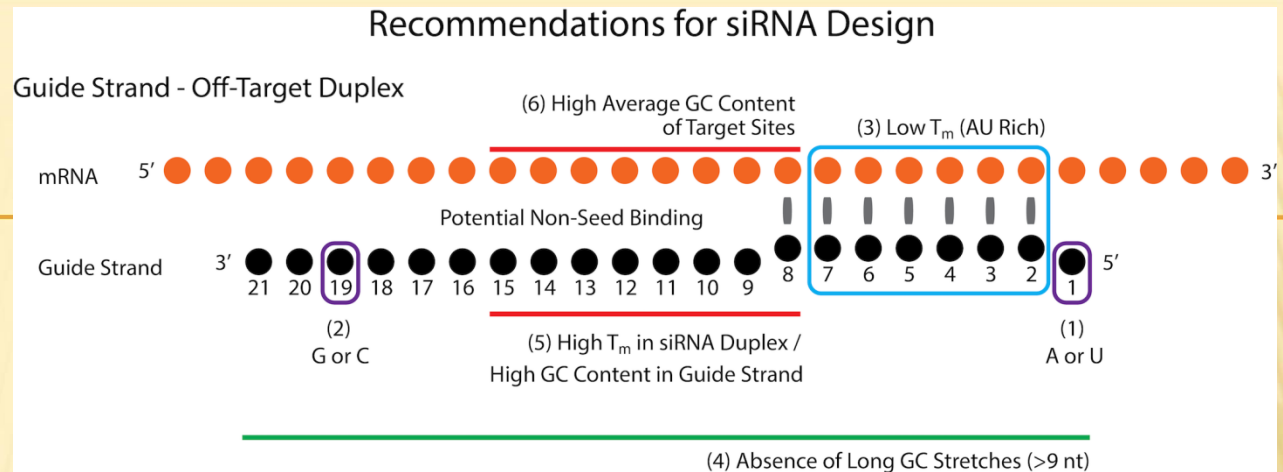
✘ homologie pouze k cílové mRNA (BLAST search)

<https://rnaidesigner.thermofisher.com/rnaiexpress/>

https://eu.idtdna.com/site/order/designtool/index/DSIRNA_CUSTO

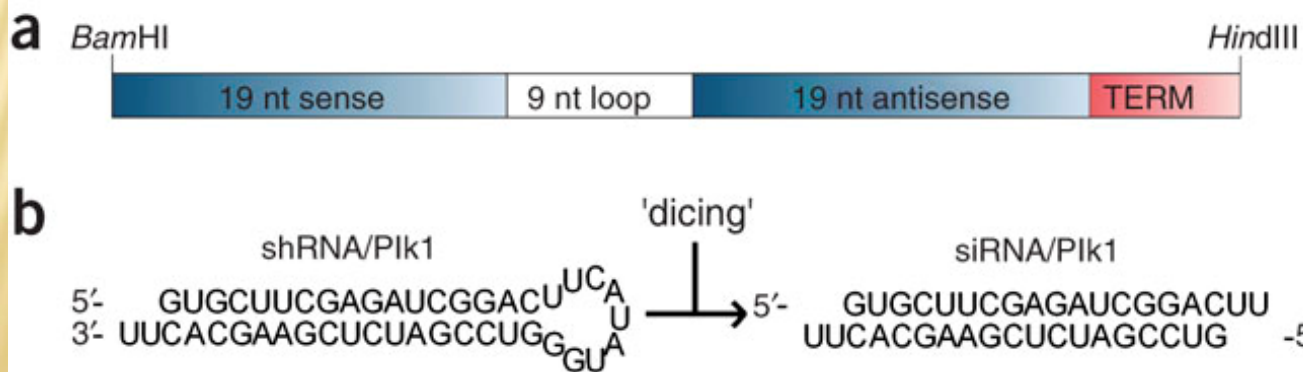
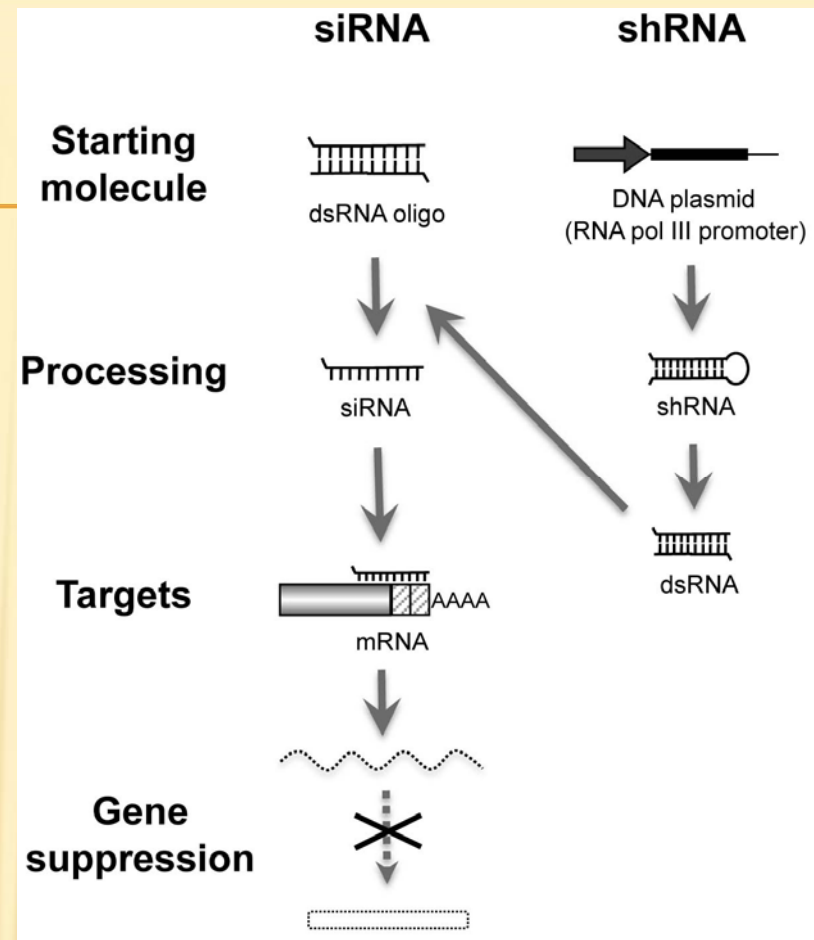
<https://www.invivogen.com/sirnazizard/>

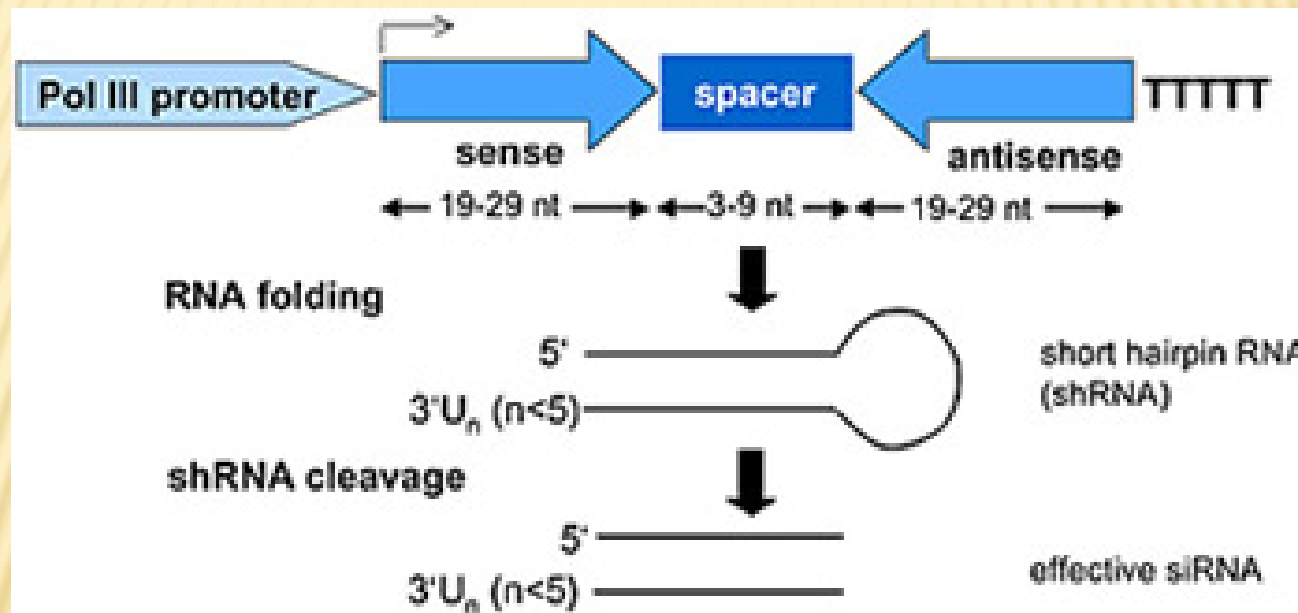
<http://sidirect2.rnai.jp/>



shRNA

- produkovány z exogenního vektoru
- promotor polymerázy III
- syntéza a zpracování v buňce obdobné jako i mikroRNA





atgtctcggcggacgcgctgcgaggatctggatgagctgcactaccagga cacagattcagatgtgc cggagcag
agggatag caagtg caaggt caaatggacccatgaggaggacgagcagctgaggg ccctggtgaggcagtttga
cagcagga ctggaagttcctggccagccacttccctaa ccgcactgaccagcaatgccagta caggtggctgaga
gttttgaatccaga ccttgt caaggggccatggaccaaagaggaagaccaaagtc atcgagctggttaagaag
tatggcacaagcagtggaactgat tggcaagcacctgaaggg ccggctgggaagcagtgccgtgaacgctgg
cacaacca cctcaa ccctgaggtgaagaagtcttgctggaccgaggaggaggaccgcatcatctgcgaggcccac
aaggtgctgggcaa ccgctgggcccagatcgccaagatg ttgcccaggaggagacagacaatgctgtgaagaatcac
tggaactctaccat caaaaggaaggtggacacaggaggcttcttgagcgagtccaagactgcaagcccccagtg
tacttgctgctggagctcgaggacaaggacggcctccagagtgc ccagcccacggaaggccagggaagtcttctg
accaactggccctc cgtccctcctaccataaaggaggaggaaaa cagtgaggaggaaacttg cagcagccacca ca
tcgaaggaacaggagcccatcggta cagatctggacgcagtgcaaacaccagagc ccttgagggaattcccgaag
cgtgagga ccaggaaggctcccaccagaaacgagcctgcctta caagtgggtggaggagcagctaacctcctc
atccctgctgtgggttctagcctctctgaagccctggacttgatcgagtcggaccctgatgcttgggtgtgacctg
agtaaatttgacctccctgaggaaccatctgcagagga cagtatcaacaa cagcctagtgcagctgcaagcgtca
catcagcagcaagt cctgccaccccgcagccttccgc cctggtgccagtggtgaccagtaaccgctggatggc
cacaccatctcagac cctgagccggagcagccggggcgagctgatccccatctccccagca ctgaagtcgggggc
tctggcat tggcacaccgcccctctgtgctcaagcggcagaggaagaggcgtgtggctctgtcccctgtcactgag
aatagcaccagctctgtcctt cctggattcctgtaacagcctcacgccc aagagcacacctgttaaga cctgccc
ttctcgccctcccagtttctgaacttctggaa caaacaggacacattggagctggagagcccctcgctgacatcc
accccagtggtgcagccagaaggtgggtggcaccacaccactgca ccggga caagacaccccctgcaccagaaacat
gctgcgtttgtaaccccagatcagaagta ctccatggacaacactccccacacgc caaccccgttcaagaacgcc
ctggagaagta cggaccccctgaagcccctgccacagaccccgcacctggaggaggacttgaaggagggtgctgcgt
tctgaggctggcatcgaactcatcatcgagga cgacatcaggcccgagaagcagaagaggaagcctgggctgcgg
cggagccc catcaagaaagtccggaagtctctggctcttgacattgtggatgaggatgtgaagctgatgatgtcc
aactgcccaagctctctatccttggccgacaactgccccttcaaa ctcttcagcctcacccctgtcaggtatcaaa
gaagacaa cagcttgctcaaccagggcttcttgaggcccaagcccgagaaggcagcagtggccagaagccccga
agccacttcacgacacctgcccctatgtccagtgccctggaagacgggtggcctgcgggggggaccaggaccagctt
ttcatgcaggagaaagcccggcagctcctggg ccgctgaagccagcca cacatctcgga cccctcatcttgtcc
tga

https://rnaidesigner.thermofisher.com/rnaiexpress/

The screenshot shows the ThermoFisher Scientific website for the BLOCK-iT™ RNAi Designer tool. The header includes the ThermoFisher logo, a search bar with 'All Categories' and a magnifying glass icon, and links for 'Contact Us', 'Sign In', and 'Quick Order'. A navigation bar below the header lists 'Applications & Techniques', 'Shop All Products', 'Services & Support', 'About Us', and 'Cloud'. A 'HELP' button is located in the top right corner of the main content area.

BLOCK-iT™ RNAi Designer

The easiest way to design effective RNAi molecules for great results

See also:
[BLOCK-iT™ RNAi Express](#): Simplified online ordering of pre-designed and validated Stealth Select RNAi™ siRNA.

[Synthetics for in vivo RNAi](#)

[PlateSelect™](#): Order made-on-demand RNAi in customizable plate format at 1nmole scale!

Target Design Options:

- [Stealth RNAi™ siRNA](#)
- [siRNA](#)
- [miR RNAi](#)
- [shRNA](#)
- [siRNA to Stealth RNAi™ siRNA](#)
- [siRNA to shRNA](#)

Find out more about [Stealth RNAi™ siRNA](#), the next generation RNAi molecule or read about the benefits of [BLOCK-iT™ siRNA](#). [How to Order](#)

Step 1: Enter an accession number or provide a nucleotide sequence

Accession number:

OR

Nucleotide sequence: Enter only A, C, G, T, and U. See the online Help for additional information

```
atgtctcggcgacgcgctcggagatctggatgagctgcactaccaggacacagattcagatgtccggagcagagggatagcaagtgcaagtgcaaatggaccatgaggaggacgagcagctgaggccctgtgaggcagtttggacagcaggactggaagtctcggccagccactccctaacgcaactgaccagcaatgccagtagcaggtggctgagagtttgaatccagacctgtcaagggccatggaccaagaggaagaccaaaaagctatcagctggttaagaagtaggcacaaagcagtgacactgattgccaagcacctgaaggccggctgggaaagcagtgccctggaacgctggcacaaccacctcaacctgaggtgagaagctctgctgaccgagggaggaccgcacatctcgcaggcccaaggtgctgggcaaccgctggccgagatcgaagatgtccaggaggacagacaatgctgtgaagatcactggaactctaccataaaaaggaaggtggacacaggagctcttgagcagctccaagactgcaagccccagtgacctgctgagctcggagcaaggacggcctccagagtgcccagcccaggaaggccagggaaagctctgaccactggccctcctcctaccataaaggaggaggaacagtgaggaggaactgcagcagccaccatcgaaggaaacaggagcccacgtgacagatctggacgagtgcaacaccagagccctggaggaattcccgaagctgaggaccaggaagctcccaccagaaacgagcctgcttacaagtggtggaggcagtaacctctcatccctgctgggtctagcctctgaagccctggactgatcagctggaccctgatctgtgtgacctgagtaaatggacctcctgaggaaacctcgcagaggacagatcaacaacagcctagtcagctgcaagctcacatcagcaagctcctgcccaccccgccagcctccgcccctggtgcccagtgaccagtagccgctggatggccaccacctcagactgagccggagcagccggggcagctgacccatccccagcactgaagctggggcctggcaltggcaccgcccctctgctcaagcggcagaggaagggcgtgtggcctgtccctgctcactgagaatagcaccagctgtcctcctggtattcgtaacagcctcagcccagaagcaccctgtccctctcgcc
```


BLOCK-iT™ RNAi Designer

The easiest way to design effective RNAi molecules for great results

See also:

[BLOCK-iT™ RNAi Express](#): Simplified online ordering of pre-designed and validated Stealth Select RNAi™ siRNA.

[Synthetics for in vivo RNAi](#)

[PlateSelect™](#): Order made-on-demand RNAi in customizable plate format at 1nmole scale!

Target Design
Options:

[Stealth RNAi™ siRNA](#)

[siRNA](#)

[miR RNAi](#)

[shRNA](#)

[siRNA to Stealth RNAi™ siRNA](#)

[siRNA to shRNA](#)

Find out more about [Stealth RNAi™ siRNA](#), the next generation RNAi molecule. [How to Order](#)

Step 1: Enter an accession number or provide a nucleotide sequence

Accession number:

NM_002466.3

OR

Nucleotide sequence: Enter only A, C, G, T, and U. See the online Help for additional information

Step 2: If you entered an accession number in Step 1, select regions for target design

Open reading frame (ORF) 5' UTR 3' UTR

Step 3: Choose database for Blast

Human - Homo sapiens

NOTE: BLAST is used to compare input sequence with sequences in the database to find unique regions against which to design RNAi targets. The databases contain representative gene sequences for that species. Blast databases were updated on May 01, 2008 and the design output reflects the most up-to-date designs.

Step 4: Choose minimum and maximum G/C percentage

Minimum G/C percentage:

35%

Maximum G/C percentage:

55%

Step 5: Select siRNA design options and click "RNAi Design" to design siRNA.

[Default motif pattern:](#)

[Tuschl's motif pattern:](#)

P: Proprietary (Recommended)

A: AA(N19)TT

B: NA(N19)NN

C: NA(RN17Y)NN

D: NA(N18Y)NN

[RNAi Design](#)

Reset Form

Guarantee: The BLOCK-iT™ RNAi Designer is such an effective tool for the design of siRNAs that if you order the three best siRNA sequences designed by the BLOCK-iT™ RNAi Designer, we guarantee that two of them will give greater than 70% knockdown of mRNA, given that the transfection efficiency in your experiment is at least 80%. If two or more fail to knock down your target RNA by at least 70% under these conditions, Invitrogen will design and ship a fourth siRNA to your target for free*.

*Please contact Invitrogen Technical Services to take advantage of this offer (800-955-6288 ext 2). Please be prepared to fax or email your order reference number, oligo sequences and data showing your transfection efficiency and knockdown. This offer is good for one free duplex per target.

How to Order: You must have an account with Invitrogen to order custom oligos. After your oligos have been designed and added to the order form, you must enter a valid customer number that is keyed to your account. If you do not already have an account, contact your [local representative](#) to set up an account.

If you already know your RNAi oligo sequence: Order oligos online at [Order Custom Primers](#) or by [e-mail/Fax](#).

siRNA - default

Select	No.	Start	Sequence(DNA)	Region	GC%	Tuschl's pattern match*	Rank ¹
<input type="checkbox"/>	1	517	GCCATGGACCAAAGAGGAA	ORF	52.64		★★★★★
<input type="checkbox"/>	2	656	CCTGAGGTGAAGAAGTCTT	ORF	47.37		★★★★★
<input type="checkbox"/>	3	765	GGACAGACAATGCTGTGAA	ORF	47.37	B	★★★★★
<input type="checkbox"/>	4	954	CCGTCCTCCTACCATAAA	ORF	52.64		★★★★★
<input type="checkbox"/>	5	1177	GGGTTCTAGCCTCTCTGAA	ORF	52.64		★★★★★
<input type="checkbox"/>	6	1557	CCTTCCTGGATTCCTGTAA	ORF	47.37		★★★★★
<input type="checkbox"/>	7	1587	CCAAGAGCACACCTGTAA	ORF	47.37		★★★★★
<input type="checkbox"/>	8	1623	CCTCCCAGTTTCTGAACTT	ORF	47.37		★★★★★
<input type="checkbox"/>	9	1644	GGAACAAACAGGACACATT	ORF	42.11		★★★★★
<input type="checkbox"/>	10	1756	CCAGAAACATGCTGCGTTT	ORF	47.37	BD	★★★★★

siRNA - Tuschl

Select	No.	Start	Target sequence(DNA)	Region	GC%	Rank ¹
<input type="checkbox"/>	1	517	GCCATGGACCAAAGAGGAAGA	ORF	52.39	★★★★★
<input type="checkbox"/>	2	570	GCACAAAGCAGTGGACACTGA	ORF	52.39	★★★★☆
<input type="checkbox"/>	3	765	GGACAGACAATGCTGTGAAGA	ORF	47.62	★★★★★
<input type="checkbox"/>	4	1230	GGTGTGACCTGAGTAAATTTG	ORF	42.86	★★★★☆
<input type="checkbox"/>	5	1271	GCAGAGGACAGTATCAACAAC	ORF	47.62	★★★★☆
<input type="checkbox"/>	6	1585	GCCCAAGAGCACACCTGTAA	ORF	52.39	★★★★★
<input type="checkbox"/>	7	1644	GGAACAAACAGGACACATTGG	ORF	47.62	★★★★☆
<input type="checkbox"/>	8	1922	GCTGGCATCGAACTCATCATC	ORF	52.39	★★★★☆
<input type="checkbox"/>	9	2023	GGCTCTTGACATTGTGGATGA	ORF	47.62	★★★★☆
<input type="checkbox"/>	10	2024	GCTCTTGACATTGTGGATGAG	ORF	47.62	★★★★☆

shRNA

Select	No.	Start	Target sequence(DNA)	Region	GC%	Rank ¹
<input type="checkbox"/>	1	517	GCCATGGACCAAAGAGGAAGA	ORF	52.39	★★★★★
<input type="checkbox"/>	2	570	GCACAAAGCAGTGGACACTGA	ORF	52.39	★★★★☆
<input type="checkbox"/>	3	765	GGACAGACAATGCTGTGAAGA	ORF	47.62	★★★★★
<input type="checkbox"/>	4	1230	GGTGTGACCTGAGTAAATTTG	ORF	42.86	★★★★☆
<input type="checkbox"/>	5	1271	GCAGAGGACAGTATCAACAAC	ORF	47.62	★★★★☆
<input type="checkbox"/>	6	1585	GCCCAAGAGCACACCTGTAA	ORF	52.39	★★★★★
<input type="checkbox"/>	7	1644	GGAACAAACAGGACACATTGG	ORF	47.62	★★★★☆
<input type="checkbox"/>	8	1922	GCTGGCATCGAACTCATCATC	ORF	52.39	★★★★☆
<input type="checkbox"/>	9	2023	GGCTCTTGACATTGTGGATGA	ORF	47.62	★★★★☆
<input type="checkbox"/>	10	2024	GCTCTTGACATTGTGGATGAG	ORF	47.62	★★★★☆

http://sidirect2.rnai.jp/

... pro design siRNA

siDirect version 2.0 highly effective, target specific siRNA online design site. [Help](#)

Enter an accession number and retrieve sequence:
NM_002466

or Paste in a nucleotide sequence:

```
>sample sequence  
ggctgccaag aacctgcagg aggcagaaga atggtacaaa tccaagtttg ctgacctctc  
tgaggctgcc aaccggaaca atgacgccect ggcgccaggca aagcaggagt ccaactgagta  
ccggagacag gtgcagtccc tcacctgtga agtggatgcc cttaaaggaa ccaatgagtc  
cctggaacgc cagatgcgtg aaatggaaga gaactttgcc gttgaagctg ctaactacca  
agacactatt ggccgcctgc aggatgagat tcagaatatg aaggaggaaa tggctcgtca  
ccttcgtgaa taccaagacc tgctcaatgt taagatggcc cttgacattg agattgccac  
ctacaggaag ctgctggaag gcgaggagag caggatttct ctgctcttcc caaacttttc  
ctccctgaac ctgagggaaa ctaatctgga ttcactccct ctggttgata cccactcaaa  
aaggacactt ctgattaaga cggttgaaac tagagatgga caggttatca acgaaacttc  
tcagcatcac gatgaccttg aataaaaaat gcacacactc agtgcagcaa tatattacca
```

Options: [click here](#)

siDirect v.2.0 | Last modified on Dec 3, 2009.

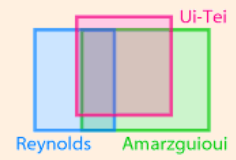
Nejnavštěvovanější Jak začít
tcagcatcac gatgaccttg aataaaaatt gcacacactc agtgcagcaa tatattacca

design siRNA

Options: [click here](#)

Functional siRNA selection algorithm by: ?

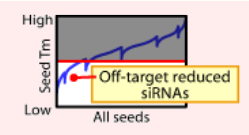
- Ui-Tei et al., *Nucleic Acids Res* **32**, 936-948 (2004) [Link](#) [\[hide options\]](#)
- Reynolds et al., *Nat Biotechnol* **22**, 326-330 (2004) [Link](#)
- Amarzguioui et al., *BBRC* **316**, 1050-1058 (2004) [Link](#)



- use combined rule:
- Ui-Tei + Reynolds + Amarzguioui
 - Ui-Tei + Reynolds × Amarzguioui
 - Ui-Tei × Reynolds × Amarzguioui

Minimization of seed-dependent off-target effects ?

- Seed-duplex stability:** Max Tm °C **new!**
(for reducing seed-dependent off-target effect)
Ui-Tei et al., *Nucleic Acids Res* **36**, 7100-7109 (2008) [Link](#)



Specificity check: Homo sapiens (human) non-redundant database ?

- Hide less-specific siRNAs [\[hide options\]](#)
- Show number of off-target hits within three mismatches

Other options

- Target range: from to
- Avoid contiguous G's or C's nt or more (for chemically synthesized siRNA)
- Avoid contiguous A's or T's nt or more (for shRNA vectors with pol III promoter)
- GC content: from % to %
- Custom pattern: ?
- Exclude pattern:
- Only show siRNAs that match all checked criteria

siDirect version 2.0 highly effective, target specific siRNA online design site. [Help](#)

Enter an accession number and retrieve sequence:



siDirect version 2.0 highly effective, target specific siRNA online design site. [Help](#)

Enter an accession number and retrieve sequence:

or Paste in a nucleotide sequence:

```
>NM_002466.4 Homo sapiens MYB proto-oncogene like 2 (MYBL2), transcript variant 1, mRNA
GACTGCAGTTCCTGCGAGCGAGGAGCGCGGGACCTGCTGACACGCTGACGCCTTCGAGCGCGGGCCCGGG
CCCCGAGCGGGCCGGAGCAGCCCCGGTCTGACCCCGGGCCCGGCTCCCGCTCCGGGCTCTGCCGGCGGGCG
GGCGAGCGCGCGCGGTCCGGGCCGGGGGATGTCTCGGCGGACCGCTGCGAGGATCTGGATGAGCTGC
ACTACCAGGACACAGATTTCAGATGTGCCGGAGCAGAGGGATAGCAAGTGCAAGGTCAAATGGACCCATGA
GGAGGACGAGCAGCTGAGGGCCCTGGTGGAGCAGTTTGGACAGCAGGACTGGAAGTTCCTGGCCAGCCAC
TTCCCTAACCGCACTGACCAGCAATGCCAGTACAGGTGGCTGAGAGTTTTGAATCCAGACCTTGTCAAGG
GGCCATGGACCAAAGAGGAAGACCAAAAAGTCATCGAGCTGGTTAAGAAGTATGGCACAAAGCAGTGGAC
ACTGATTGCCAAGCACCTGAAGGGCCGGCTGGGGAAGCAGTCCCGTGAACGCTGGCACAACCACCTCAAC
CCTGAGGTGAAGAAGTCTTGCTGGACCGAGGAGGAGGACCGCATCATCTGCGAGGCCACAAAGGTGCTGG
GCAACCGCTGGGCCGAGATCGCCAAGATGTTGCCAGGGAGGACAGACAATGCTGTGAAGAATCACTGGAA
CTCTACCATCAAAGGAAGGTGGACACAGGAGGCTTCTTGAGCGAGTCAAAGACTGCAAGCCCCAGTG
TACTTGCTGCTGGAGCTCGAGGACAAGGACGGCCTCCAGAGTGGCCAGCCACGGAAGGCCAGGGAAGTC
TTCTGACCAACTGGCCCTCCGTCCCTCCTACCATAAAGGAGGAGGAAAACAGTGAAGGAGAACTTGCAGC
ACCCACCACATCGAAGGAACAGGAGCCCATCGGTACAGATCTGGACGCAGTGCGAACACCAGAGCCCTTG
GACCAATTCGGCAAGCTGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGAC
```

siDirect version 2.0 result page. [Help](#)

2020-02-04 18:55:53, siDirect v.2.0

Query

Query name: NM_002466.4 Homo sapiens MYB proto-oncogene like 2 (MYBL2), transcript variant 1, mRNA
Query sequence: 2668 bp
Functional siRNA selection: Ui-Tei + Reynolds + Amarzguioui
Seed-duplex stability - Max Tm: 21.5°C
Specificity check: Homo sapiens non-redundant database
Avoid contiguous A's or T's: 4 nt

Effective siRNA candidates

target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5'→3') 21nt passenger (5'→3')	functional siRNA selection: Ui-Tei Reynolds Amarzguioui	seed-duplex stability (Tm);		specificity check: minimum number of mismatches against any off-targets;		contiguous A's or T's constraint
				guide	passenger	guide	passenger	
1662-1684	CAGAAACATGCTGCGTTTGTAAAC	UACAAACGCAGCAUGUUUCUG GAAACAUGCUGCGUUUGUAAC	URA	19.8 °C	12.1 °C	2 [detail]	3 [detail]	ok

... ale shRNA ???

www.invivogen.com/sirnawizard/



[FIND SIRNA SEQUENCES](#) [ADVANCED SEARCH](#) [DESIGN HAIRPIN INSERT](#) [SCRAMBLE SIRNA](#) [SELECTION CRITERIA](#) [SIRNA DESIGN GUIDELINES](#)

Selection of siRNA/shRNA targets

InvivoGen's siRNA Wizard™ is a software designed to help you select siRNA/shRNA sequences targeting your gene(s) of interest. This program selects siRNA/shRNA sequences that match criteria suggested by studies of RNA interference and which will have the best expression rate in psiRNA vectors.

siRNA Wizard™ is composed of 3 parts:

Find siRNA/shRNA sequence :

Two types of searches can be performed to find siRNA/shRNA sequences:

Standard search utilizes a default set of criteria to analyze your gene of interest and provide the best sequences to silence gene expression.

Advanced search lets you manually set the criteria for selecting the sequences against your target gene.

Motif size:

21

nt

mRNA Database:

human

miRNA SEED Database:

human

Search

Reset

6 siRNA candidate target sequences of 21 nt found :

There is non putative siRNA without homology to other genes.

Check the list of homolog genes to each putative siRNA to choose siRNA corresponding to your needs.

Sequence	Start	GC%	# of Blast homologs	Design	Scrambled
GGGATAGCAAGTGCAAGGTCA	77	52.38	3	<input type="checkbox"/>	<input type="checkbox"/>
GGATAGCAAGTGCAAGGTCAA	78	47.62	4	<input type="checkbox"/>	<input type="checkbox"/>
GCCCAAGAGCACACCTGTAA	1320	52.38	2	<input type="checkbox"/>	<input type="checkbox"/>
GGCTGGCATCGAACTCATCAT	1656	52.38	2	<input type="checkbox"/>	<input type="checkbox"/>
GGAAGTCTCTGGCTCTTGACA	1748	52.38	4	<input type="checkbox"/>	<input type="checkbox"/>
GAAGTCTCTGGCTCTTGACAT	1749	47.62	5	<input type="checkbox"/>	<input type="checkbox"/>

For generating the hairpin insert :

Loop sequence: TCAAGAG

psiRNA Vector for cloning :

psiRNA-h7SK G1 (cloning sites: BbsI/BbsI)

For generating scrambled siRNA, select database :

human

Design hairpin

Generate scrambled siRNA

Reset

portals.broadinstitute.org/gpp/public/seq/search



GPP Web Portal

[Home](#) | [Search by Gene](#) | [Search by Clone](#)

Design Hairpins to Target a Transcript Sequence

Scoring of candidate shRNA sequences available in 2 ways:

1. If the desired transcript is listed in NCBI RefSeq, you can find hairpin designs by:
 - [Searching for an NCBI gene or transcript here](#) or selecting "Search by Gene" from the Navigation bar above
 - Clicking on the "Transcript ID" link on the Search Results page to navigate to the "Transcript Details" page
 - Under the "Hairpin Candidate Sequences" heading, clicking the link titled "Show high scoring hairpin designs for this transcript"

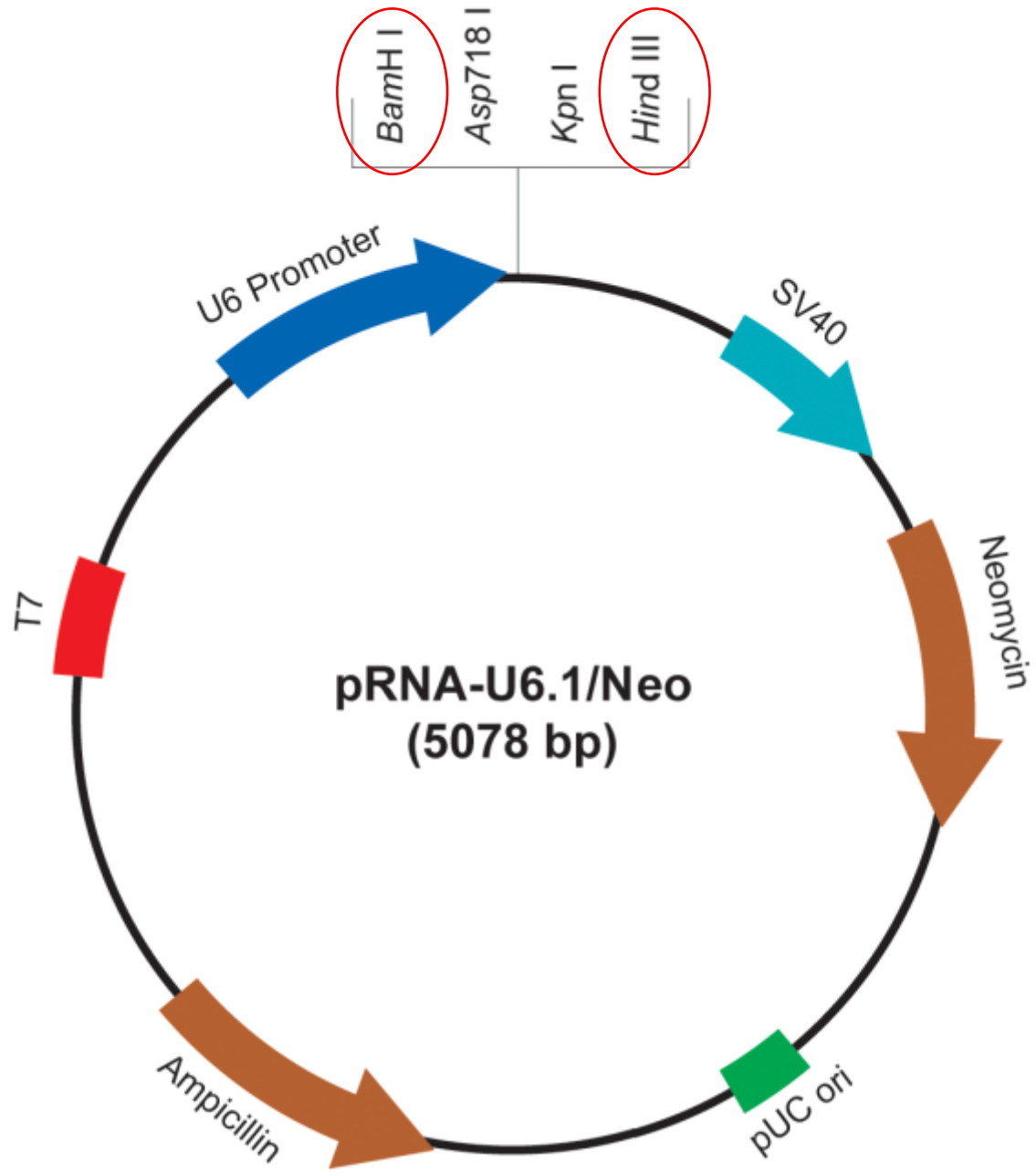
This will yield a table of the top shRNA designs for this transcript, pre-scored for predicted knockdown efficacy and for specificity vs. other genes of the same taxon.

2. If the desired target transcript is not found in NCBI RefSeq, or is from a taxon other than mouse or human (e.g. Rat) you can paste the sequence you wish to target in the box below (in DNA or RNA form), and receive a list of matching shRNA sequence designs, scored according to predicted knockdown efficacy. Note that specificity versus other targets not is not considered in this score.

For a description of the candidate-picking process and rules, please see the following link: [shRNA design process](#)

Target transcript sequence (in DNA or RNA form):

e.g. 'ACGTATTGATGCCACAGACGTATTGATGCCACAGACGTATTGATGCCACAG', etc.



B-Myb shRNA

BamH I

5' GATCCGCCATGGACCAAAGAGGAAATTCAAGAGATTCCTCTTTGGTCCATGGCTTTTTT GG 3'
| Sense | Loop | Antisense | Termination Signal

5' AGCTCCAAAAAAGCCATGGACCAAAGAGGAA TCTCTTGAATTCCTCTTTGGTCCATGGCCG 3'
Hind III

BamH I

GATCCG **GCCATGGACCAAAGAGGAA** ATTCAAGAGATTCCTCTTTGGTCCATGGCTTTTTT GG
GCCGGTACCTGGTTTCTCCTTAAGTTCTCTAAGGAGAAACCAGGTACCGAAAAACCTCGA
Hind III

ligace, transformace E. coli ligační směsí, expanze klonů, izolace plazmidové DNA,
sekvenace

další postup ...

- přenos plazmidu do eukaryotických buněk (přechodná transfekce)
- analýza exprese cílového genu na úrovni proteinu (westernův přenos)

Organizace cvičení (shRNA)

- skupiny po 12 lidech (2x 6 hod praktických cvičení)

Každá skupina

... před praktickou částí cvičení ...

- přidělen jeden cílový gen (stejný jako pro CRISPR)
- návrh siRNA/shRNA sekvence (miniprojekt)
- zaslání **nejpozději** týden před začátkem cvičení na **email vyučujícího dané skupinky**

... po praktické části cvičení ...

- výsledky získané na cvičeních budou zpracovány do tohoto miniprojektu

Miniprojekt (.doc)

- stručný úvod + cíl
 - detailní popis návrhu gRNA a siRNA/shRNA sekvencí pro vybraný gen
-

- stručný popis výsledků z praktických cvičení:
 - a) dílčích kroků mutageneze cílového genu
 - b) dílčích kroků posttranskripčního umlčování cílového genu
-

- analýza výstupů ze sekvenace genomové DNA získaných klonů
- závěr

Cílové geny

Mus musculus !!!!!!!!!!!!!

- Tacstd2 (Gene ID: 56753) - 23. 3. 2022 - Knopfová (knopfova@sci.muni.cz)
- Myb (Gene ID: 17863) - 30. 3. 2022 - Beneš (pbenes@sci.muni.cz)
- Mybl2 (Gene ID: 17865) - 13. 4. 2022 - Beneš (pbenes@sci.muni.cz)
- Cxcl1 (Gene ID: 14825) - 20. 4. 2022 - Kohoutek (jiri.kohoutek@sci.muni.cz)
- Icam1 (Gene ID: 15894) - 27. 4. 2022 - Kohoutek (jiri.kohoutek@sci.muni.cz)
- Tnfsf9 (Gene ID: 21950) - 4. 5. 2022 - Navrátilová (22031@mail.muni.cz)

Přihlašování v ISu dnes od 17.00