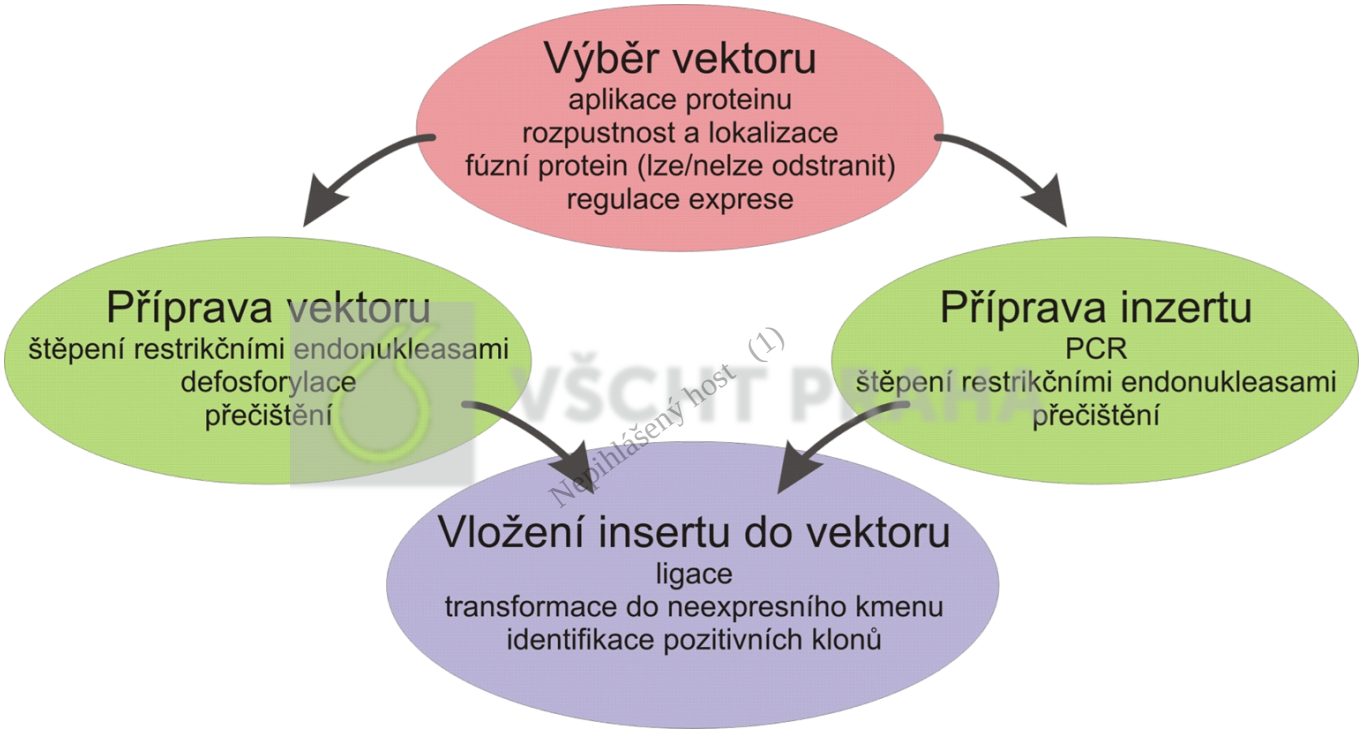


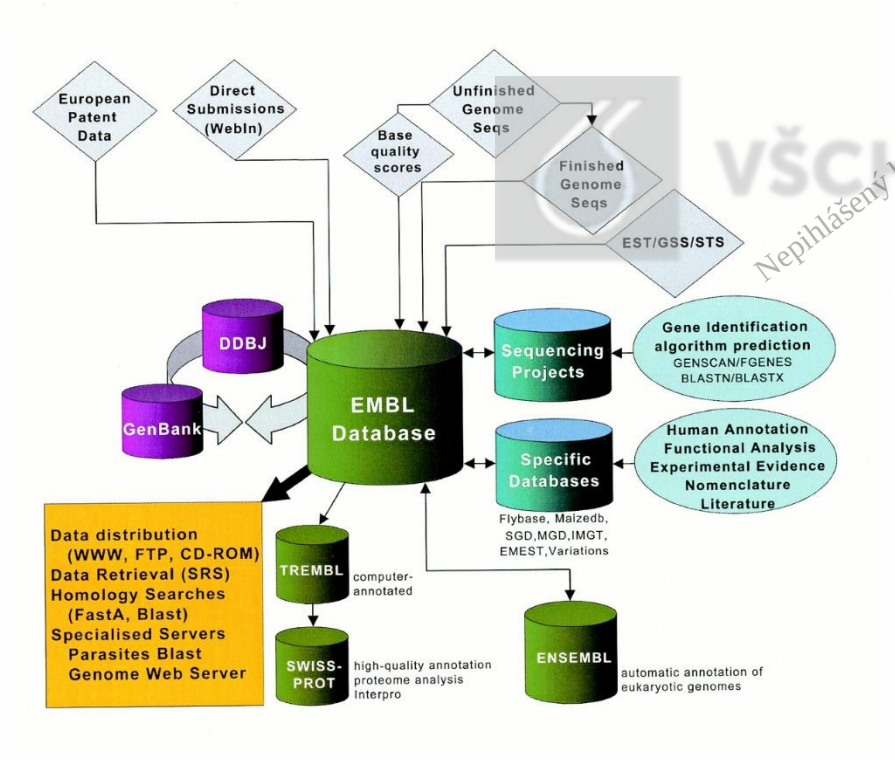
VŠCHT PRAHA
Nepřihlášený host (1)

Příklad: hexokinasa z *Aspergillus oryzae* *hxkA*

1. Najít sekvenci

– např. The International Nucleotide Sequence Database Collaboration (INSDC)

DDBJ (Japan), GenBank (USA) and the EMBL Nucleotide Sequence Database



VŠCHT PRAHA
Nepihlášený host (1)

https://www.uniprot.org

http://www.ddbj.nig.ac.jp/

http://www.ncbi.nlm.nih.gov/

http://www.ebi.ac.uk/embl/

UniProt

UniProtKB | hxka aspergillus oryzae | Advanced | Search

BLAST Align Retrieve/ID mapping Peptide search SPARQL Help Contact

UniProtKB 2021_04 results

Basket

UniProtKB consists of two sections:

Reviewed (Swiss-Prot) - Manually annotated
Records with information extracted from literature and curator-evaluated computational analysis.

Unreviewed (TrEMBL) - Computationally analyzed
Records that await full manual annotation.

The UniProt Knowledgebase (UniProtKB) is the central hub for the collection of functional information on proteins, with accurate, consistent and rich annotation. In addition to capturing the core data mandatory for each UniProtKB entry (mainly, the amino acid sequence, protein name or description, taxonomic data and citation information), as much annotation information as possible is added.

Help | UniProtKB help video | Other tutorials and videos | Downloads

Filter by

Reviewed (1)
Swiss-Prot

Unreviewed (1)
TrEMBL

Popular organisms

ASPOZ (1)

EMENI (1)

Search terms

Filter "oryzae" as:

BLAST | Align | Download | Add to basket | Columns

1 to 2 of 2 Show 25

Quote terms: "aspergillus oryzae"

Entry	Entry name	Protein names	Gene names	Organism	Length	Gene encoded by	Mass
P80581	HXK_EMENI	**Hexokinase**	**hxkA** AN7459	Emericella nidulans (strain FGSC A4 / ATCC 38163 / CBS 112.46 / NRRL 194 / M139) (Aspergillus nidulans)	490		53,925
Q9HGZ3	Q9HGZ3_ASPOZ	**Phosphotransferase**	**hxkA** OAory_01108740	Aspergillus oryzae (Yellow koji mold)	490		54,147

1 to 2 of 2 Show 25

VŠCHT PRAHA
Nepřihlášený host (1)

http://www.ebi.ac.uk/embl/

EMBL-EBI | Services | Research | Training | About us | EMBL-EBI

EBI Search

hxkA aspergillus oryzae

Examples: VAV_HUMAN, tp53, Sulston...

Build Query

Help & Documentation | About EBI Search | ORCID data claiming | Feedback

Search results for ***hxkA aspergillus oryzae***

Showing **5** results out of **5** in All results

Filter your results

Source

All results (6)

- Nucleotide sequences (2)
- Protein sequences (2)
- Enzymes (1)

VŠCHT PRAHA
Nepřihlášený host (1)

Nucleotide sequences (2 results)

Source: Sequence (ID: AB032268)

AB032268

Aspergillus oryzae hxkA mRNA for hexokinase, complete cds.

Cross References: Protein families (4) Nucleotide sequences (1) Samples & ontologies (1) + show more

Formats: in EMBL format in EMBL-SVA in FASTA format

Source: Coding (Standard) (ID: BAB12228)

BAB12228

Product: hexokinase
Gene: hxkA

Cross References: Protein families (4) Nucleotide sequences (2) Protein sequences (2) + show more

Formats: in EMBL format in FASTA format

Protein sequences (2 results)

Source: UniProtKB (ID: HXK_EMENI)

P80581

Coding: BAB12228.1

Aspergillus oryzae hexokinase

View: TEXT FASTA XML

Download: XML FASTA TEXT

Organism	Molecule type	Topology	Data class	Taxonomic Division
Aspergillus oryzae	mRNA	linear	STD	FUN

Sequence length	Sequence Version
1,473	1

Lineage

Eukaryota, Fungi, Dikarya, Ascomycota, Pezizomycotina, Eurotiomycetes, Eurotiomycetidae, Eurotiales, Aspergillaceae, Aspergillus

Navigation | Overview | Source Feature(s) | Sequence | Publications | Submission Details | Identical Sequences

Showing first 1 - 1000 of 1473

Find similar sequences

```
>ENA|BAB12228|BAB12228.1 Aspergillus oryzae hexokinase : Location:1..1000
ATGGTTGGAATTGGCCCCAAACGTCCCCCATCCCGCAAGGGATCGATGCATGAGCTGCCC
CAGAACCTGTTGGAGCAGATCAAGCAATTCGAGGACATCTTTACCGTGGACGGCGCCAAA
CTCAAGCAGATTGCGGACCATTTCGTGAAGGAGCTCGAAAAGGGTCTAAGCGTCGAGGGG
GGTAACATTCCCATGAATGTGACCTGGGTCATGGGATTCCCCGATGGCGACGAGCAGGGA
ACCTTCCTCGCTTTGGACATGGGCGGTACTAATCTGCGTGTCTGTGAGATCACTTTGACG
GAAGAGAAGGGCGCTTTCGACATCACCCAGTCCAAGTATCGTATGCCGGAGGAGTTGAAG
ACGGGTACAGCGGAGGAGCTTTGGGAATACATCGCCGACTGTTTGCAGCAGTTTATTGAA
TCCCACCACGAGAATGAGAAACTGTCTAAACTGCCATTGGGCTTTACCTTCTCCTATCCT
GCTACACAGGAATACATTGATCATGGTGTTCTCCAGCGTTGGACAAAGGGTTTCGACATT
GACGGAGTAGAGGGCCAGGATGTTGTGCCCCCATTGGAGGCAATCCTCAAGAAAAGAGGC
CTTCCTATCAAAGTTGCTGCGTTGATCAACGACACTACGGGCACCCTGATCGCCTCTTCT
TACACCGACTCAGATATGAAAATTGGCTGCATTTTCGGCACTGGTGTCAACGCCGCCTAC
ATGGAACATTGTGGCTCGGTTCCCAAGCTTGCACACAAGAATCTACCCCCAGACATGCCC
GTGGCCATCAACTGCGAGTACGGTGCCTTTGACAATGAGCACGTTGTTCTGCCCCTCACG
AAGTATGATATCATCATCGACCGCGACTCCCCTCGCCCAGGACAACAAGCTTTCGAGAAG
ATGACTGCAGGTCTTTACTTGGGAGAAATATTCCGTCTAGCCCTCTTGGATCTGTTGGAG
ACGAGGCCTGGTCTGATTTTCCAGGGCCAAGACACATCCC
...
```

Show full sequence

VŠCHT PRAHA
Nepihlášený host (1)

Search NCBI databases

Help

aspergillus oryzae hexokinase Search

Results found in 11 databases for "aspergillus oryzae hexokinase"

Literature

Books	0	books and reports
MeSH	0	ontology used for PubMed indexing
NLM Catalog	0	books, journals and more in the NLM Collections
PubMed	3	scientific & medical abstracts/citations
PubMed Central	116	full-text journal articles

Health

ClinVar	0	human variations of clinical significance
dbGaP	0	genotype/phenotype interaction studies
GTR	0	genetic testing registry
MedGen	0	medical genetics literature and links
OMIM	0	online mendelian inheritance in man
PubMed Health	0	clinical effectiveness, disease and drug reports

Genomes

Assembly	9	genome assembly information
BioProject	0	biological projects providing data to NCBI
BioSample	48	descriptions of biological source materials
Clone	0	genomic and cDNA clones
dbVar	0	genome structural variation studies
Genome	1	genome sequencing projects by organism
GSS	0	genome survey sequences
Nucleotide	118	DNA and RNA sequences
Probe	0	sequence-based probes and primers
SNP	0	short genetic variations
SRA	53	high-throughput DNA and RNA sequence read archive
Taxonomy	0	taxonomic classification and nomenclature catalog

Genes

EST	2	expressed sequence tag sequences
Gene	6	collected information about gene loci
GEO DataSets	0	functional genomics studies
GEO Profiles	0	gene expression and molecular abundance profiles
HomoloGene	0	homologous gene sets for selected organisms
PopSet	0	sequence sets from phylogenetic and population studies
UniGene	0	clusters of expressed transcripts

Proteins

Conserved Domains	0	conserved protein domains
Protein	27	protein sequences
Protein Clusters	0	sequence similarity-based protein clusters
Structure	0	experimentally-determined biomolecular structures

Chemicals

BioSystems	234	molecular pathways with links to genes, proteins and chemicals
PubChem BioAssay	0	bioactivity screening studies
PubChem Compound	0	chemical information with structures, information and links
PubChem Substance	0	deposited substance and chemical information

VŠCHT PRAHA
Nepihlášený host (1)

LOCUS XM_001819150 1473 bp mRNA linear PLN 12-MAR-2008
DEFINITION **Aspergillus oryzae (AO090001000710) mRNA, complete cds.**
ACCESSION XM_001819150
VERSION XM_001819150.1 GI:169769463
KEYWORDS .
SOURCE Aspergillus oryzae RIB40
ORGANISM Aspergillus oryzae RIB40
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
Eurotiomycetes; Eurotiomycetidae; Eurotiales; Trichocomaceae;
mitosporic Trichocomaceae; Aspergillus.
REFERENCE 1
AUTHORS Machida,M., Asai,K., Sano,M., Tanaka,T., Kumagai,T., Terai,G., Kusumoto,K., Arima,T., Akita,O., Kashiwagi,Y., Abe,K., Gomi,K., Horiuchi,H., Kitamoto,K., Kobayashi,T., Takeuchi,M., Denning,D.W., Galagan,J.E., Nierman,W.C., Yu,J., Archer,D.B., Bennett,J.W., Bhatnagar,D., Cleveland,T.E., Fedorova,N.D., Gotoh,O., Horikawa,H., Hosoyama,A., Ichinomiya,M., Igarashi,R., Iwashita,K., Juvvadi,P.R.,Kato,M., Kato,Y., Kin,T., Kokubun,A., Maeda,H., Maeyama,N., Maruyama,J., Nagasaki,H., Nakajima,T., Oda,K., Okada,K., Paulsen,I., Sakamoto,K., Sawano,T., Takahashi,M., Takase,K., Terabayashi,Y., Wortman,J.R., Yamada,O., Yamagata,Y., Anazawa,H.,Hata,Y., Koide,Y., Komori,T., Koyama,Y., Minetoki,T., Suharnan,S., Tanaka,A., Isono,K., Kuhara,S., Ogasawara,N. and Kikuchi,H.
TITLE Genome sequencing and analysis of Aspergillus oryzae
JOURNAL Nature 438 (7071), 1157-1161 (2005)
PUBMED 16372010
REFERENCE 2 (bases 1 to 1473)
AUTHORS Sawano,T., Tanaka,T., Machida,M. and Yamada,O.
TITLE Direct Submission
JOURNAL Submitted (03-JUN-2004) Toshihiko Sawano, National Institute of Technology and Evaluation, Genome Analysis Center, Department Of Biotechnology; 2-49-10 Nishihara, Shibuya-ku, Tokyo 151-0066, Japan (E-mail:bio@nite.go.jp, Tel:81-3-3481-1933)
COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final NCBI review. This record is derived from an annotated genomic sequence (NW_001884663).
COMPLETENESS: incomplete on both ends.

```
FEATURES             Location/Qualifiers
     source          1..1473
                     /organism="Aspergillus oryzae RIB40"
                     /mol_type="mRNA"
                     /db_xref="taxon:510516"
                     /note="SC001"
     gene            1..1473
                     /locus_tag="AO090001000710"
                     /db_xref="GeneID:5991173"
     CDS             1..1473
                     /locus_tag="AO090001000710"
                     /old_locus_tag="AO070287000066"
                     /codon_start=1
                     /product="hexokinase"
                     /protein_id="XP_001819202.1"
                     /db_xref="GI:169769464"
                     /db_xref="GeneID:5991173"
                     /translation="MVGIGPKRPPSRKGSMHELPQNLLEQIKQFEDIFTVDGAKLKQI
                     ADHFVKELEKGLSVEGGNIPMNVTWVMGFPDGDEQGTFLALDMGGTNLRVCEITLTEE
                     KGAFDITQSKYRMPEELKTGTAEELWEYIADCLQQFIESHHENEKLSKLPLGFTFSYP
                     ATQEYIDHGVLQRWTKGFDIDGVEGQDVVPPLEAILKKRGLPIKVAALINDTTGTLIA
                     SSYTDSDMKIGCIFGTGVNAAYMEHCGSVPKLAHKNLPPDMPVAINCEYGAFDNEHVV
                     LPLTKYDIIIDRDSPRPGQQAFEKMTAGLYLGEIFRLALLDLLETRPGLIFQGQDTSQ
                     LRKPYLLDASFLAAIEDDPYENLQETQELMERKLNIKATQQELEMIRRLAELIGTRAA
                     RLSACGVAAICKKKNIESCHVGADGSVFTKYPHFKARGAQALREILDWAPNEKDKVVI
                     MAAEDGSGVGAALIAALTLKRVKAGISCGIRDMADMQSLI"
```

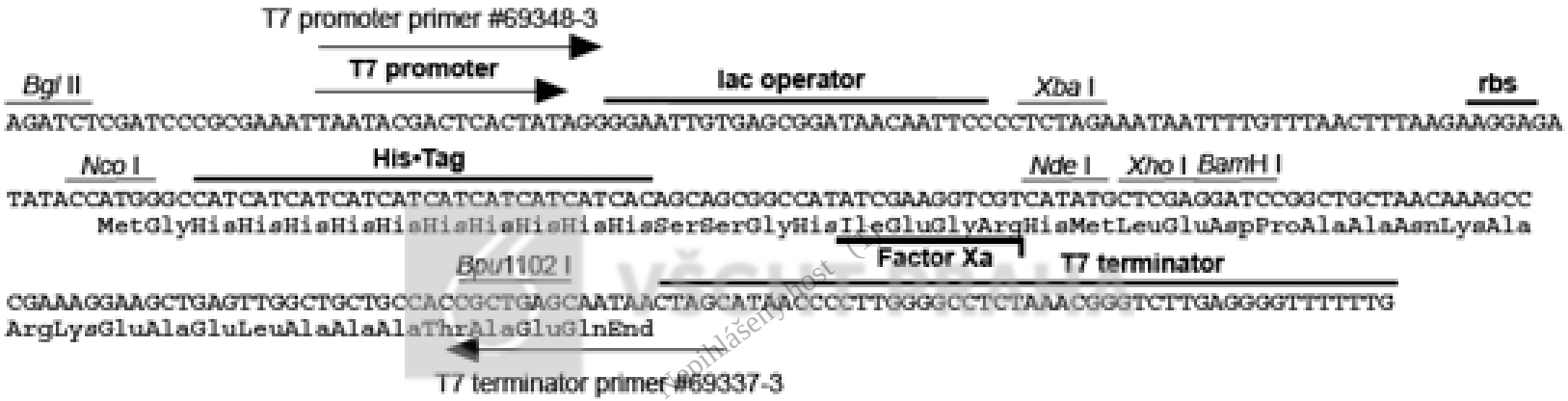
VŠCHT PRAHA
Neoprávněný host (1)

```
   1 atggttggaa ttggccccaa acgtccccca tcccgcaagg gatcgatgca tgagctgccc
  61 cagaacctgt tggagcagat caagcaattc gaggacatct ttaccgtgga cggcgccaaa
 121 ctcaagcaga ttgcggacca tttcgtgaag gagctcgaaa agggtctaag cgtcgagggg
 181 ggtaacattc ccatgaatgt gacctgggtc atgggattcc ccgatggcga cgagcaggga
 241 accttcctcg ctttggacat gggcggtact aatctgcgtg tctgtgagat cactttgacg
 301 gaagagaagg gcgctttcga catcacccag tccaagtatc gtatgccgga ggagttgaag
 361 acgggtacag cggaggagct ttgggaatac atcgccgact gtttgcagca gtttattgaa
 421 tcccaccacg agaatgagaa actgtctaaa ctgccattgg gctttacctt ctcctatcct
 481 gctacacagg aatacattga tcatggtgtt ctccagcgtt ggacaaaggg tttcgacatt
 541 gacggagtag agggccagga tgttgtgccc ccattggagg caatcctcaa gaaaagaggc
 601 cttcctatca aagttgctgc gttgatcaac gacactacgg gcaccctgat cgcctcttct
 661 tacaccgact cagatatgaa aattggctgc attttcggca ctggtgtcaa cgccgcctac
 721 atggaacatt gtggctcggt tcccaagctt gcacacaaga atctaccccc agacatgccc
 781 gtggccatca actgcgagta cggtgccttt gacaatgagc acgttgttct gcccctcacg
 841 aagtatgata tcatcatcga ccgcgactcc cctcgcccag gacaacaagc tttcgagaag
 901 atgactgcag gtctttactt gggagaaata ttccgtctag ccctcttgga tctgttggag
 961 acgaggcctg gtctgatttt ccagggccaa gacacatccc agctccggaa accttacttg
1021 ctggacgcgt ccttcctcgc agctattgag gatgatccgt acgagaactt gcaggaaact
1081 caggagctca tggagcgcaa gctgaacatc aaggccaccc agcaggagct ggaaatgatc
1141 cgtcgcttgg cggagttgat cggcactcgt gcagctcgtc tgtcggcgtg tggtgtggct
1201 gccatctgca agaagaagaa cattgagtct tgccatgtgg gcgccgacgg ctctgtcttt
1261 acgaaatatc cccacttcaa ggcccgtgga gcacaggctc tgcgcgagat cttggactgg
1321 gcacctaacg agaaggacaa ggtggtcatc atggctgctg aagacggttc tggtgttgga
1381 gcggctctta ttgctgcatt gacattgaag cgggtcaagg caggcatcag ctgcggtatc
1441 cgagatatgg ccgatatgca gagtctcatt taa
```

vši Praha
Nepřístupný obsah (1)

2. Zvolit plasmid – např. pET16b

http://www.merckbiosciences.co.uk/html/NVG/pETTable.html



pET-16b cloning/expression region

3. Zkontrolovat restrikční místa a místo pro proteasu

http://tools.neb.com/NEBcutter2/index.php

NEBcutter - Opera

Soubor Editovat Zobrazit Záložky Pomůcky Nástroje Nápověda

Nový list | NEBcutter

http://tools.neb.com/NEBcutter2/cutshow.php?name=73f9c80a-

NEW ENGLAND BioLabs Inc. NEBcutter

Linear Sequence: ***unnamed sequence***

Help | Comments

Display: - NEB single cutter restriction enzymes
- Main non-overlapping, min. 100 aa ORFs

GC=53%, AT=47%

Cleavage code	Enzyme name code
blunt end cut	Available from NEB
5′ extension	Has other supplier
3′ extension	Not commercially available
cuts 1 strand	*: cleavage affected by CpG meth.
	#: cleavage affected by other meth.
	(enz.name): ambiguous site

490 aa

1 — 1473

NsiI, PshAI, Tsp45I, HphI, *BtgZI, BpmI, *AvaII, *#BspDI, *#ClaI, *BsmFI, *HincII, TspRI, MscI, NspI, EcoRV, *BsiWI, AflIII, *MluI, BsaWI, *BspEI, *BsmBI, BfaI, SspI, PstI, AhdI, SfcI, BspMI, BfuAI, EcoNI, *BsiEI, BsaXI, BsgI, EciI, BglII, CspCI, *BglI, *BsrBI, AcuI, MseI, BciVI, PvuII

Nepihlášený host (1)

Main options: New DNA, Custom digest, View sequence, ORF summary, Save project, Print

Availability: All commercial, All

Display: 2 cutters, 3 cutters

Zoom: Zoom in, More...

List: 0 cutters, 1 cutters, All sites, Save all sites, Flanking enzymes

Minimum ORF length to display: 100 a.a. OK

start | Microsoft Outl... | 2 Windows E... | Windows Medi... | pETmanual.pd... | konecna vers... | Microsoft Pow... | NEBcutter - O... | EN | 16:42

Design primerů

1. délka 17-28 bazí
2. 50-60% G+C
3. na 3´ konci G nebo C
4. T_m primerů (melting point) mezi 55-80°C
5. 5'-konec primeru nemusí být komplementární

VSCHT PRAHA
Nepihlášený host (1)

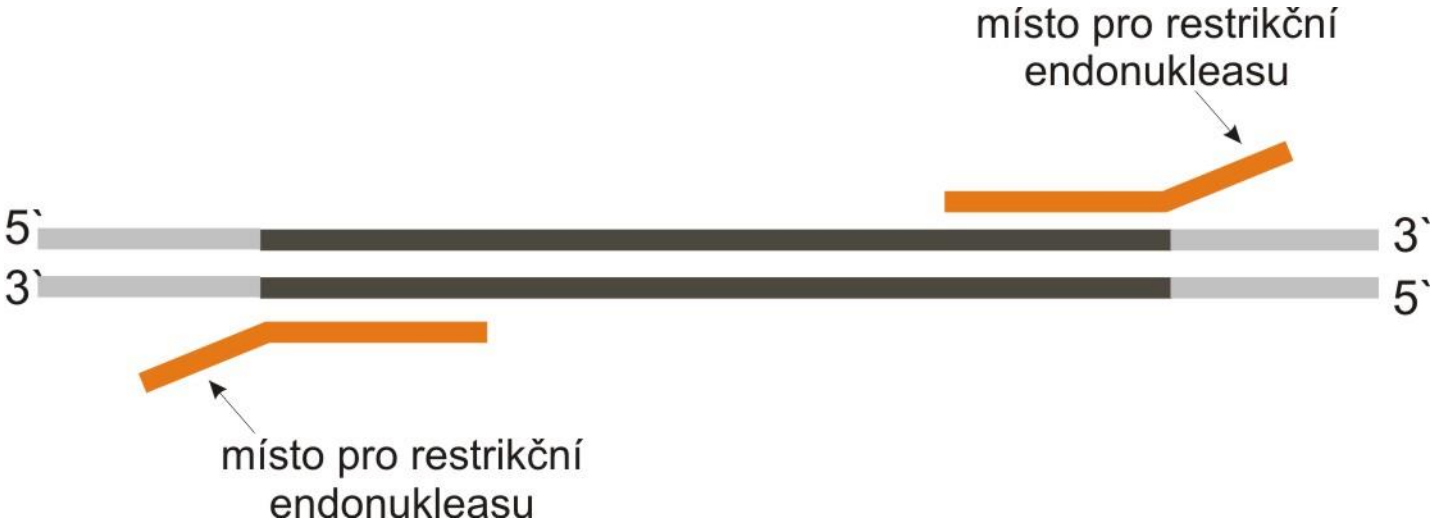
T_m = 4°C x (počet G a C v primeru) + 2°C x (počet A a Tv primeru)

T_m = 64.9°C + 41°C x (počet G a C v primeru - 16.4)/N

http://tmcalculator.neb.com/#!/

4. Navrhnout primery

VŠCHT PRAHA
Nepihlášený host (1)



NdeI CATATG
GTATAC

5` cacacaccatatggttggaattggccccaaac 3`

BamHI GGATCC
CCTAGG

5` tctctctcggatccttaaatgagactctgcatatcgg 3`

VŠCHT PRAHA
nepřihlášený host (1)

Clone Manager - [Seq Assembly 1]

File View Clone Map Primer Align Discover Operations Window Help

pET16b b-gal P.t - 7464 bp

1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 1510 1520

Reference	1336	atcgcgggtatggtggggttcgaaccaatggtcgaaccagccgttccaatattccatgcacatgagcgggccatccggttggtactccttcagcttgtcgaacgcttccttcggtcgggaaccgaagttaacggtcgcaagcacgccttccgccatgcccccctgcagcatgtcatcctgagggccgtccgag
03AH52	412C	atcgcgggtatggtggggttcgaaccaatggtcgaaccagccgttccaatattccatgcacatgagcgggccatccggttggtactccttcagcttgtcgaacgcttccttcggtcgggaaccgaagttaacggtcgcaagcacgccttccgccatgcccccctgcagcatgtcatcctgagggccgtccgag
03AH51	513C	atcgcgggtatggtggggttcgaaccaatggtcgaaccagccgttccaatattccatgcacatgagcgggccatccggttggtactccttcagcttgtcgaacgcttccttcggtcgggaaccgaagttaacggtcgcaagcacgccttccgccatgcccccctgcagcatgtcatcctgagggccgtccgag

complement

VŠCHT PRAHA
Nepřihlášený host (1)

TCCCCCCGTGCACCATATAGAAATTAACGGAGGCGCCCATGCCGAGCATGTCGTCAAGCACGCGAGCCGCATCCTTCGCATCGCGGGTATGGTGGGGTTCGAACCAATGGTCGAACCAGCCGTTCCAATATTCCATGCACA

Clone Manager - [Seq Assembly 1]

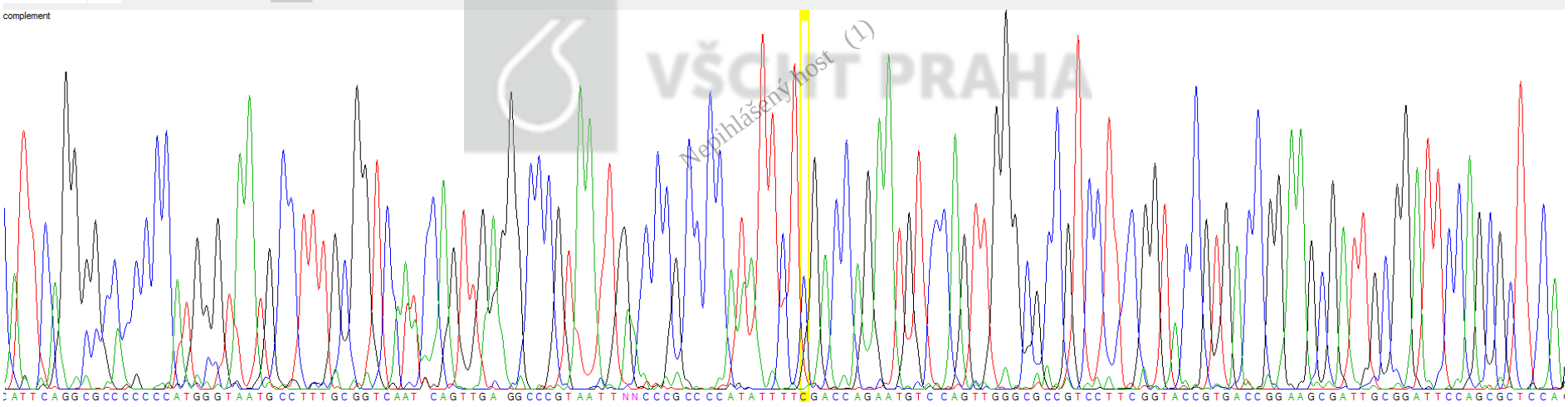
File View Clone Map Primer Align Discover Operations Window Help

pET16b b-gal P.t - 7464 bp

680 690 700 710 720 730 740 750 760 770 780 790 800 810 820 830 840 850 860 870 880

Reference	672	aatggaacaggaactgtccattcaggcg-cacccatgcgtaatgcctttgcggtcatacagttgaggcccgtaattgacccgccccatattctcgaccagaatgtccagttgggcgccgtcctccggtaccgtgaccggaagcgattgcggattccagcgctccacgacgccaatcagcttgcgatcgagaaacaccagtgcccgatcccgcac
03AH52	1076C	aatggaacaggaactgtccattcaggcg-cacccatgcgtaatgcctttgcggtcatacagttgaggcccgtaattgacccgccccatattctcgaccagaatgtccagttgggcgccgtcctccggtaccgtgaccggaagcgattgcggattccagcgctccacgacgccaatcagcttgcgatcgagaaacaccagtgcccgatcccgcac
03AH51	1178C	aatggaacaggaacnntccattcaggcgcccccccatgggtaatgcctttgcggtcaatcagttgaggcccgtaattnnncccgccccatatttttcgaccagaatgtccagttgggcgccgtccttcggtaccgtgaccggaagcgattgcggattccagcgctccaggacgccaatcagcttgcgatcgagaaacaccagtgcccgatcccgcac

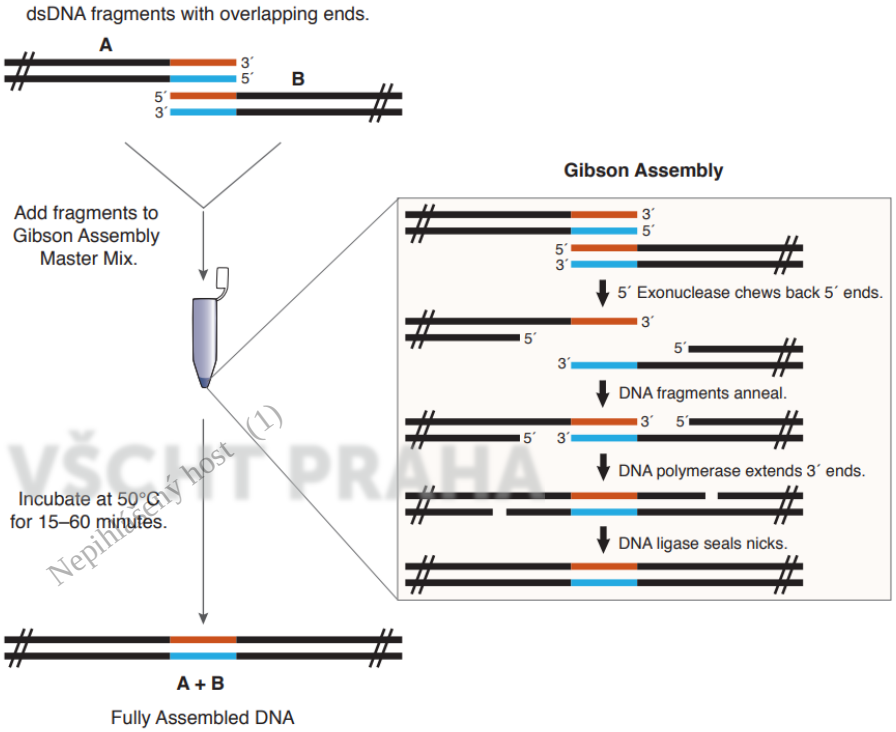
complement



VŠCHT PRAHA
Nepřihlášený host (1)

Gibson assembly

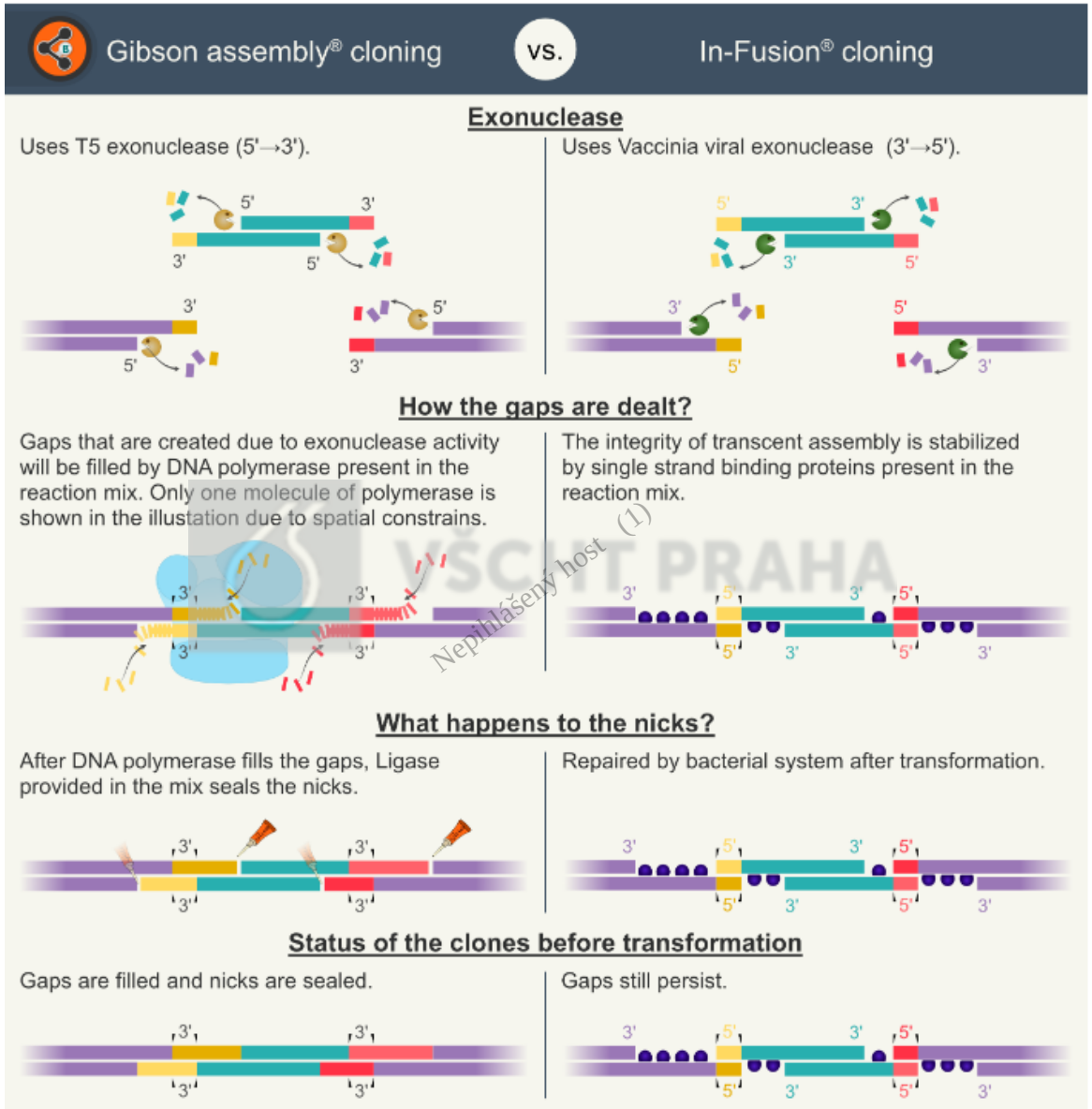
5´→ 3´ exonukleasová aktivita



VŠCHT PRAHA
Nepřenositelný host (1)

„In-fusion“ ligace nebo T4 DNA Polymerasa

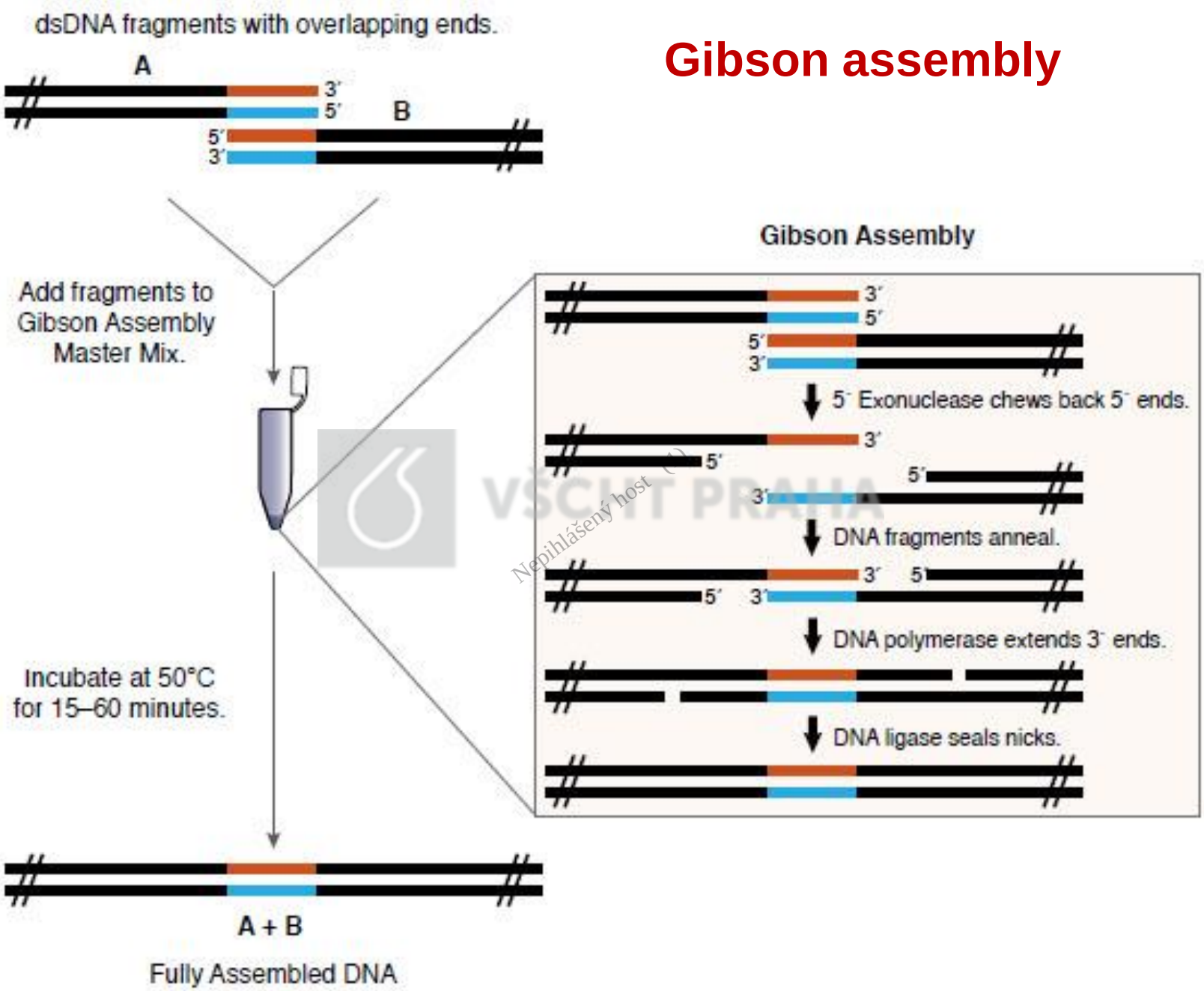
T4 DNA Polymerasa katalyzuje DNA syntézu ve směru 5´→ 3´, vyžaduje templát a primer. Má také **3´→ 5´ exonukleasovou aktivitu**



VŠCHT PRAHA
Nepřihlášený host (1)

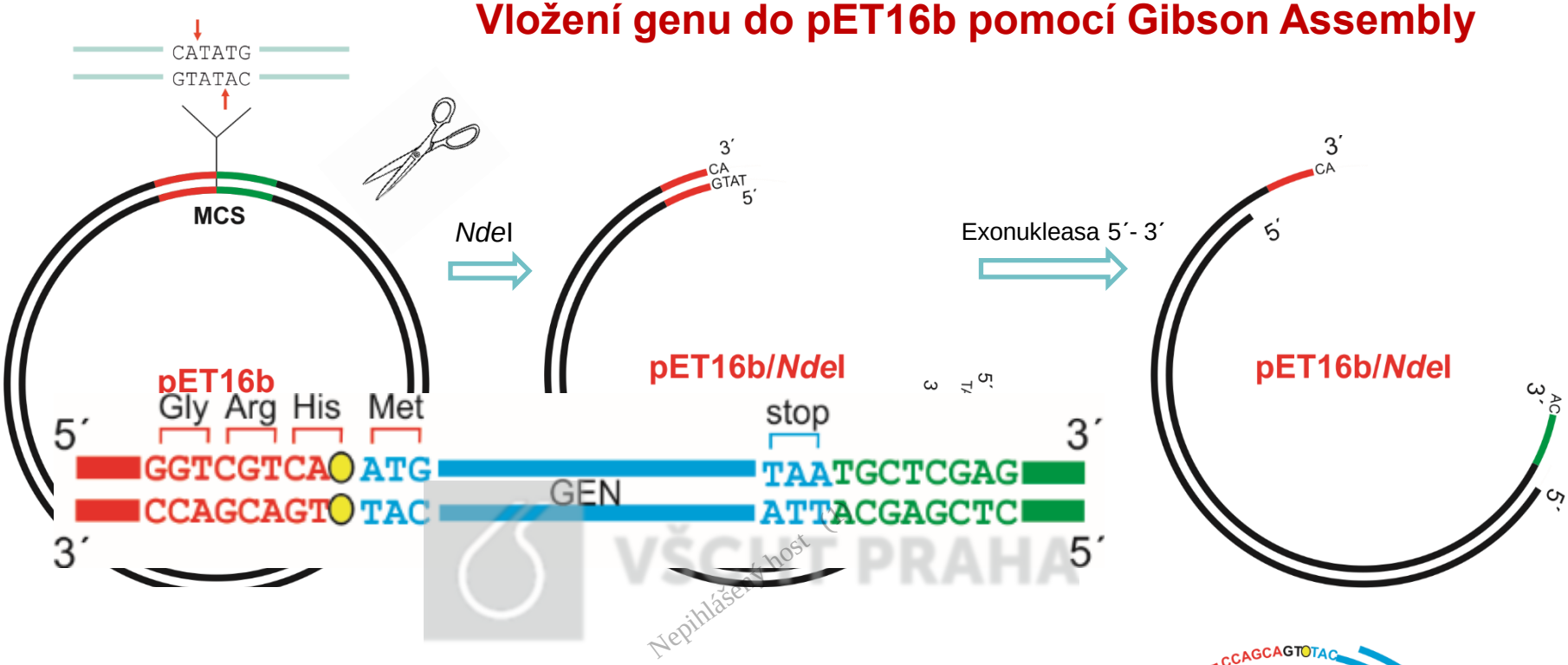
https://sharebiology.com/gibson-assembly-vs-in-fusion-cloning/

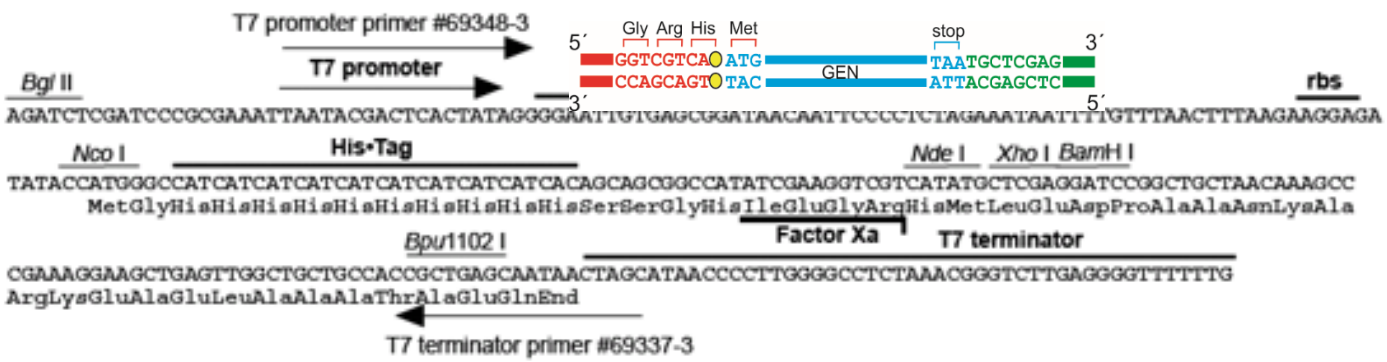
Gibson assembly

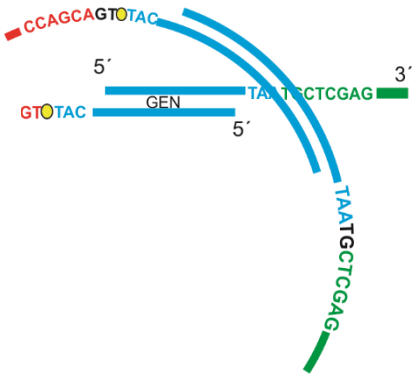


VŠCHT PRAHA
Nepřihlášený host

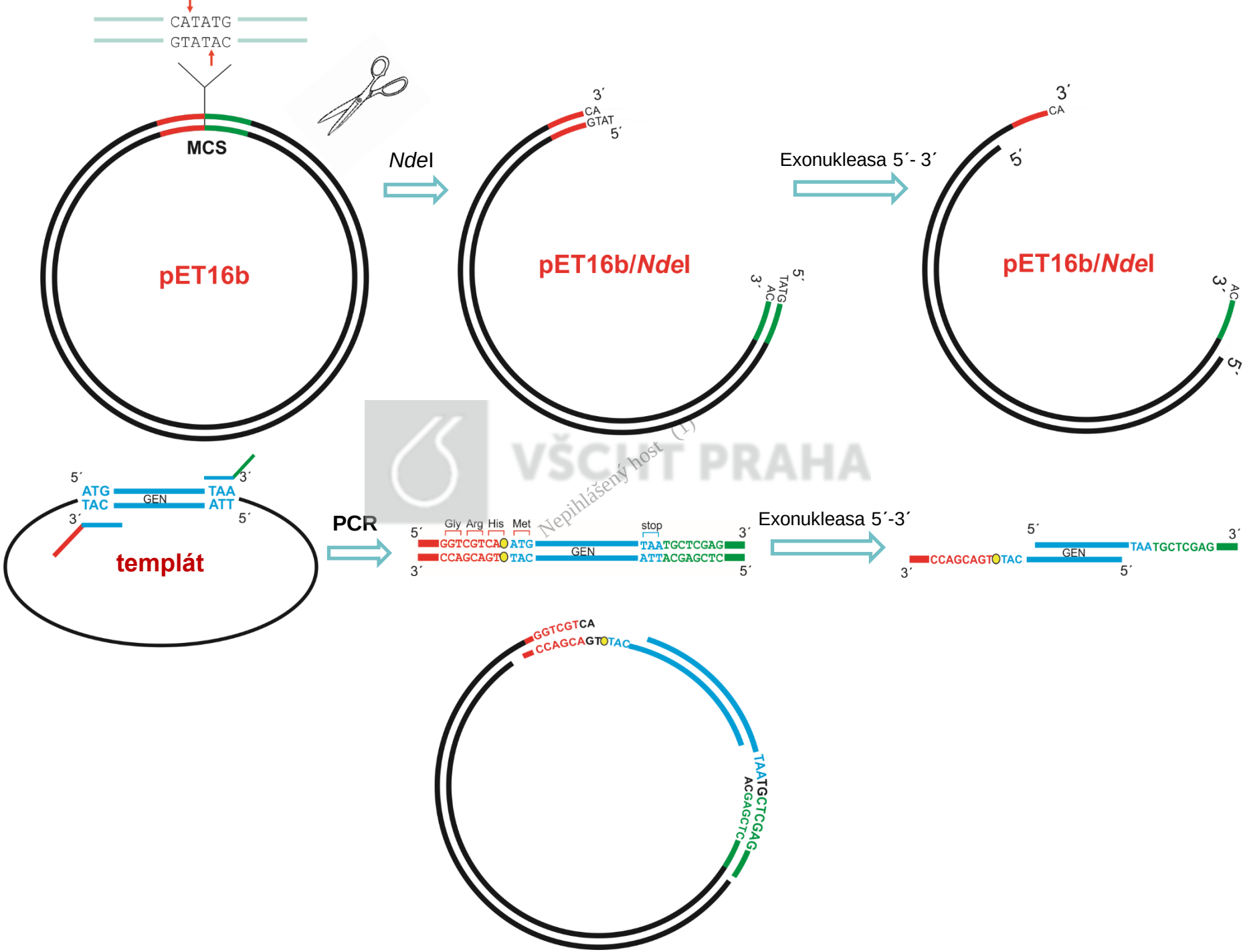
Vložení genu do pET16b pomocí Gibson Assembly







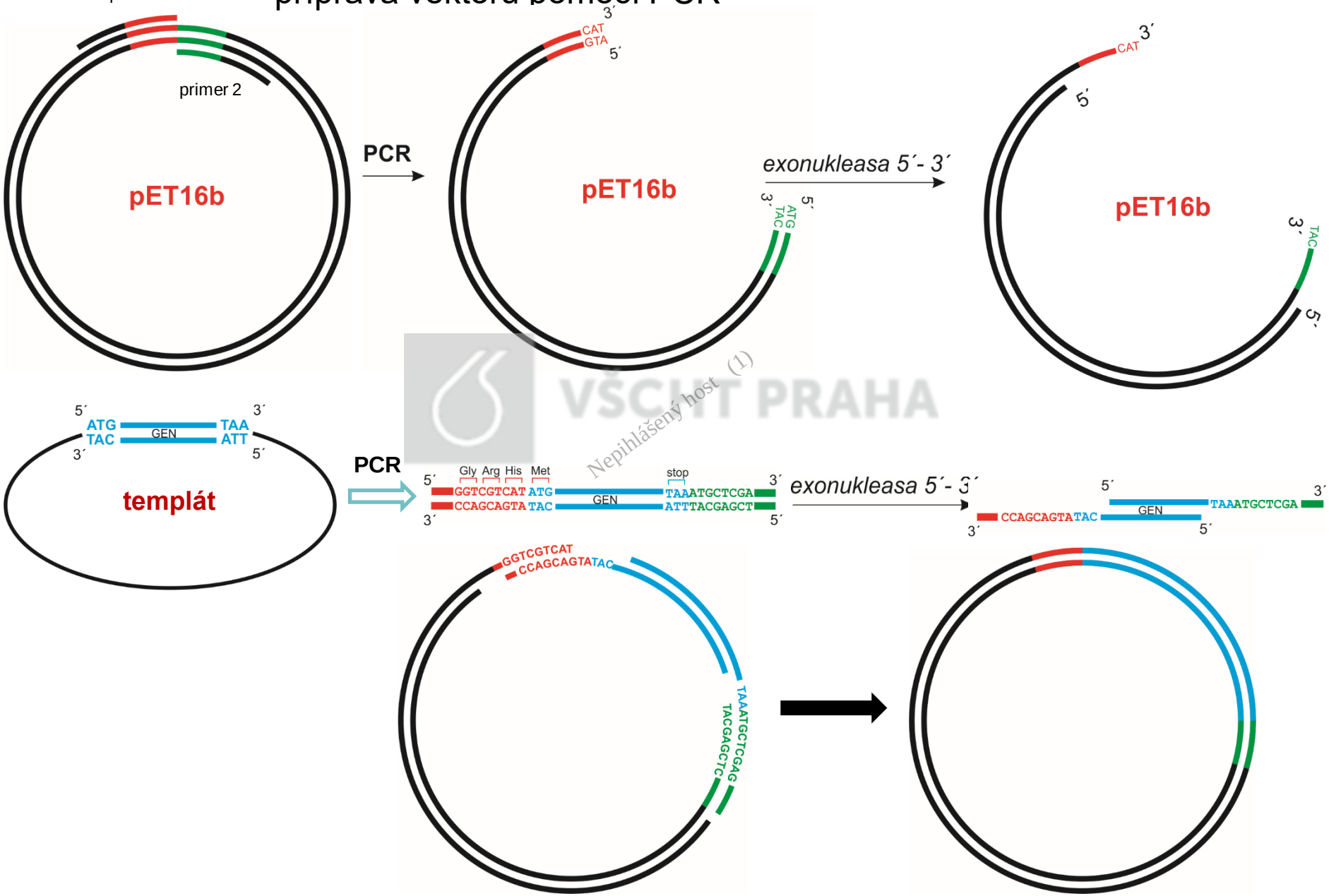
pET-16b cloning/expression region



VŠCHT PRAHA
Nepřihlášený host (1)

Vložení genu do pET16b pomocí Gibson Assembly

příprava vektoru pomocí PCR

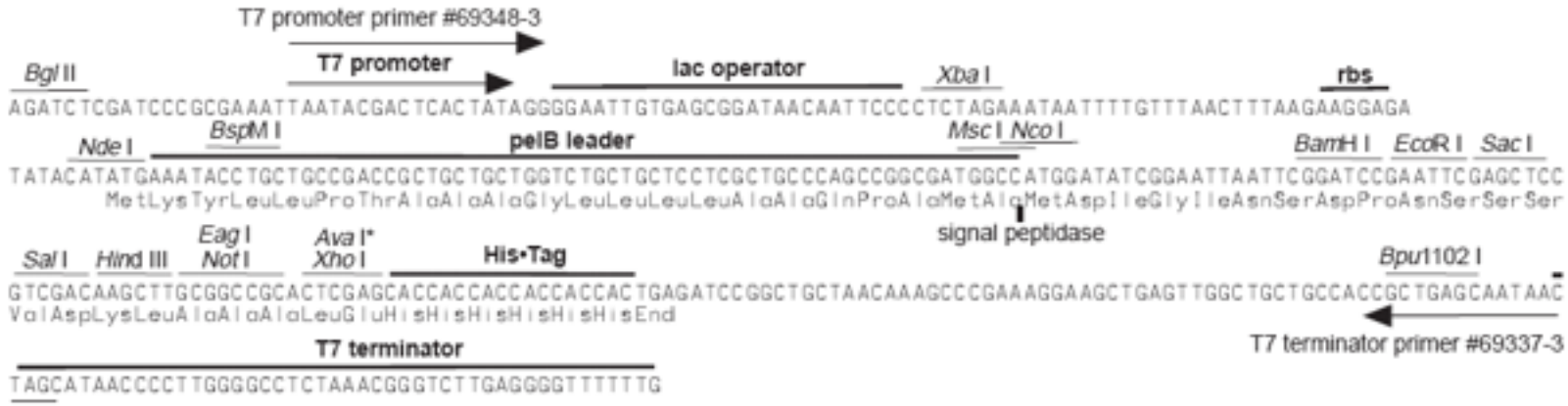


VŠCHT PRAHA
Nepihlášený host (1)

TBN1 nukleasa z rajčete

```
atgtggagcaaagaggggcatgtgatgacttgtcgaatcgcgcagggc
ttgttgaatgatgaggcagctcatgcagtcaagatgttgttgccggaatatgttaacggt
gacttatcggccctctgtgtgtggccagaccaagtcaggcactggtataagtataaatgg
acaagccctctacacttcattgatacaccagataaagcttgcaactttgattatgaaagg
gactgtcatgatcaacatggagtaaaggatatgtgtgttgctggtgcaattcagaacttt
actactcaactctctcattacagagagggaacttctgatcgtcgatataatatgacagag
gctttgctgtttttgtcacatttcatgggagatatccatcaacctatgcatgttggattt
acaagtgatgctggaggaaattctattgatttgcgctggtttaggcataaatcaaacttg
caccatgtgtgggatagggagataatactaacagctgcaaaagactactatgcgaaggat
ataaacctccttgaagaagacattgaagggaacttcactgacggaatttggtctgatgat
cttgcttcttggagagaatgtggcaatgtcttttcttgcgtaaacaagtttgcaacggaa
agtataaatatagcatgcaaatggggatacaaaggtgttgaagccggtgaaactttatca
gatgattacttcaattcaagacttccaatagtaatgaaacgagtagcacaaggtggaata
agattagcgatgcttttaaacaacgtttttggagcttctcaacaagaagattcagttgtt
gcaacttaa
```

VŠCHT PRAHA
Nepihlášený hosť (1)



pET-22b(+) cloning/expression region

NdeI

```
5'...CATATG... 3'
3'...GTATAC... 5'
```

atg tgg agc aaa gag ggg cat gtg atg

5` cacacaccatatgtggagcaaagaggggcatgtgatg 3`

XhoI

```
5'...CTCGAG... 3'
3'...GAGCTC... 5'
```

gat tca gtt gtt gca act taa

gat tca gtt gtt gca act ctc gag acacac

VŠCHT PRAHA
Nepihlášený host (1)

N-konec 5` cacacaccatatgtggagcaaagaggggcatgtgatg 3`

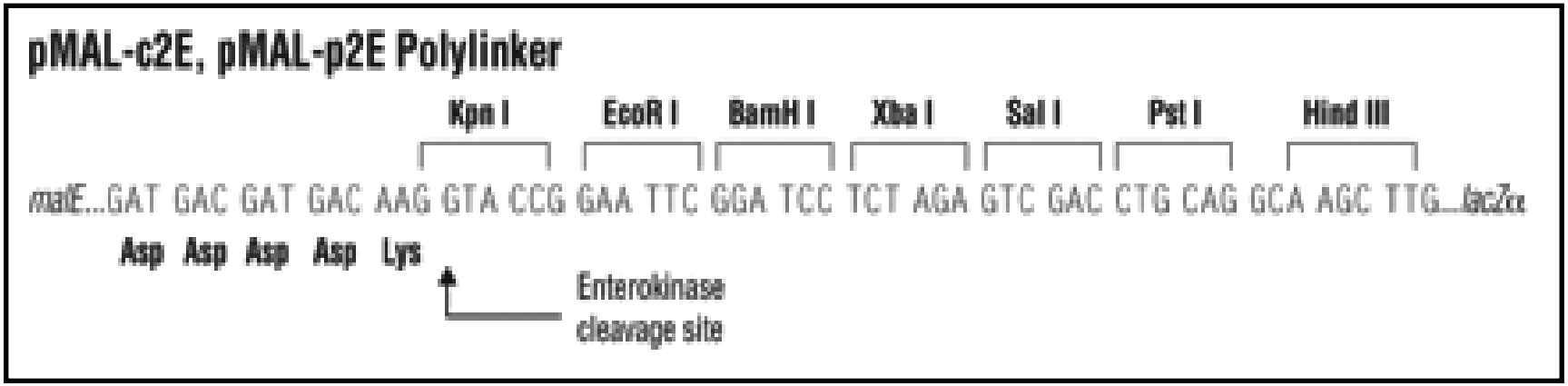
C-konec 5` gtgtgtctcgagagttgcaacaactgaatc3`

Úkol pMAL c2E – MBP a místo pro enterokinasu
E.coli EcoRI endonukleasa (J01675)

E.coli EcoRI endonukleasa (J01675)

```
                                                    atgtctaat aaaaaacagt
 361 caaataggct aactgaacaa cataagttat ctcaaggtgt aattgggatt tttggggatt
 421 atgcaaaagc tcatgatctc gctgttggtg aggtttcaaa attagtaaag aaagctctta
 481 gcaacgaata ccctcaatta tcatttcgat atagagatag tataaagaaa acagaaataa
 541 atgaagcttt aaaaaaaatt gaccctgatc ttggcggtac tttatttgtt tcaaattcca
 601 gcatcaaacc tgatggtgga attgtagagg tcaaagatga ttatggtgaa tggagagttg
 661 tacttgttgc tgaagccaaa caccaaggta aagatattat aaatataagg aatggtttgt
 721 tagttgggaa aagaggagat caagatttaa tggctgctgg taatgctatc gaaagatctc
 781 ataagaatat atcagagata gcgaatttta tgctctctga gagccacttt ccttacgtcc
 841 ttttcttaga ggggtctaac tttttaacag aaaatatctc aataacaaga ccagatggaa
 901 gggttgttaa tcttgagtat aattctggta tattaaatag gttagatcga ctaactgcag
 961 ctaattatgg aatgcctata aatagtaatc tatgtattaa caaatttgta aatcataaag
1021 acaaaagcat tatgctacaa gcagcatcta tatatactca aggagatggg agggagtggg
1081 attcgaaaat catgtttgaa ataatgtttg atatatcaac gacttcgctc agagtgttgg
1141 ggcgtgactt gtttgaacag cttacatcta agtga
```

VŠCHT PRAHA
Nepublikovaný text (1)



KpnI G ▲GTAC ▼C

atgtctaat aaaaaacagt c

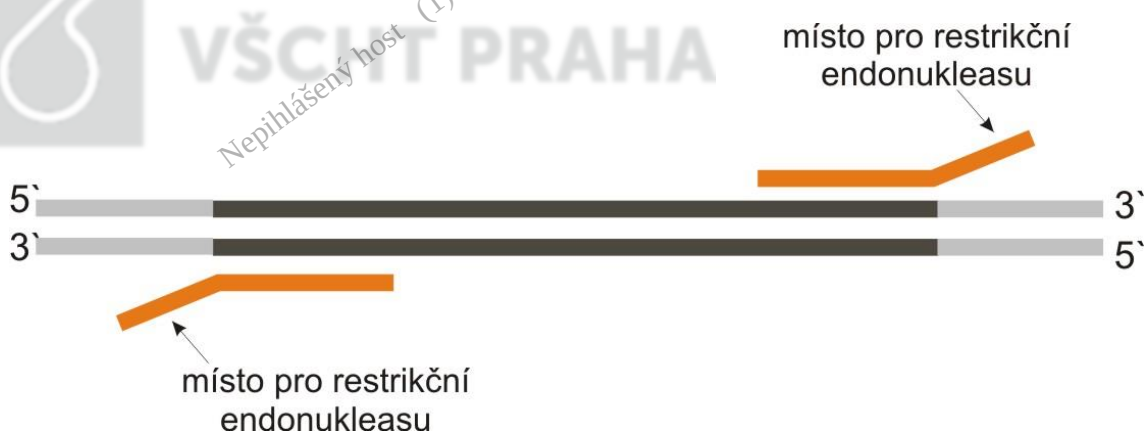
5` cacacacggtacccatgtctaataaaaaacagtc 3`

SalI G ▼TCGA ▲C

gtttgaacag cttacatcta agtga

gtttgaacagcttacatctaagtgagtcgacgtgtgtgtgt

VŠCHT PRAHA
Nepihlášený host (1)



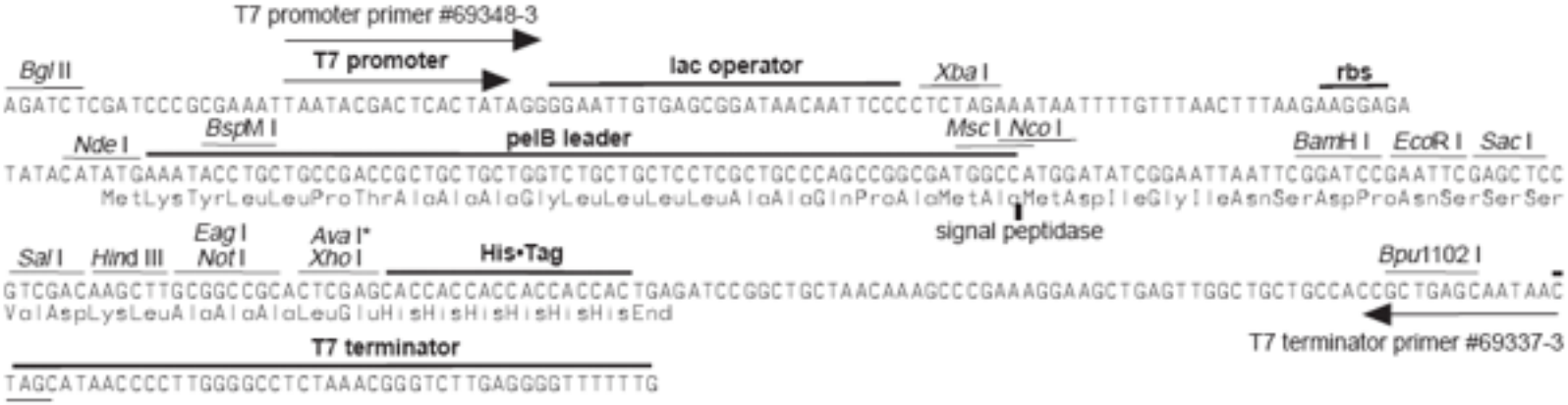
N-konec 5` cacacacggtacccatgtctaataaaaaacagtc 3`

C-konec 5` acacacacacgtcgactcacttagatgtaagctgttcaaac 3`

Bacillus subtilis alkoholdehydrogenasa (AAB80874)

```
atgaaggcag taacgtatca aggcattaaa aatgttgttg tcaaagatgt ccccgatcca      60
aagattgaaa aatccgatga catgattatc aaagtcacca gtacagccat ttgcggatca     120
gatttacatt taatccatgg attcattccg aatatgcaag aagactatgt caccggccat     180
gaaccgatgg gaatcgtcga agaagttggt tctggggtga ctaaactaaa aaagggagat     240
cgggtaatta ttccctttaa tatagcatgc ggggaatgct ttttttgtaa aaaccagctg     300
gaaagccaat gtgatcagtc taatgacaat ggggaaatgg gtgcttattt cggctattca     360
gggcaaactg gcggttatcc aggcgggcaa gctgaatatt taagagtgcc gtttgcgaat     420
tttacccatt ttaaaatccc tgaatcttgt gaggaacccg atgagaaatt aagcgtgatt     480
gccgatgcca tgaccaccgg cttttggagt gtggataatg ccggcgtaaa aaaaggcgat     540
acagttatcg ttctcggctg cggaccagtc ggcctgtttg ctcaaaagtt ttgttggcta     600
aaaggcgcaa aacgcgtcat agcagttgac tatgtaaact atcgcttaca gcatgcgaaa     660
cgtacaaaca aagtagaaat cgttaatttt gaagaccatg agaatacagg gaattattta     720
aaggaaatca cgaaaggcgg agcggatgta gtcattgacg ctgttgggat ggatggtaaa     780
atgagcgatc tcgagttcct tgccagcggc ttaaagcttc atggcggaac gatgagtgca     840
ttggtcattg cttcccaagc tgttcgcaaa ggaggaacca tacaaattac aggtgtgtat     900
ggaggcagat ataacggttt tccgttagga gatattatgc agcgaaacgt caatatacgc     960
tctggacagg ctccagtgat
```

VISCH-HUNH
Nepiny xenyhosz (1)



pET-22b(+) cloning/expression region

NdeI

```
5,...CA TA TG...3'
3,...GT AT AC...5'
```

atgaaggcag taacgtatca aggc

5` cacacaccatatgaaggcagtaacgtatcaaggc 3`

NotI

```
5'...GC GGCC GC... 3'
3'...CG CCGG CG... 5'
```

cgc tctggacagg ctccagtgat

cgctctggacaggctccagtgcggccgcacacac

není dobrý primer asi jiné místo

VŠCHT PRAHA
Nepihlášený host (1)

N-konec 5` cacacaccatatgaaggcagtaacgtatcaaggc 3`

C-konec 5` gtgtgtcgccggcgatcactggagcctgtccagagcg 3`

Úsek DNA z *Arthrobacter pocusus*

```
ccgggcaccgcgtgcgctggagcgatctggaattggaccgtgccggtgcaccgtggttgg
atcgacgccgaacccgatgccgccggacgccggcccgggttctcgctgccggatgtctgg
ccgttgcccgacttccgcccggaggcaagctcagacctgcgcagcatctgcacccccctag
ccggcccatctacaccaacaagaaggagacgcacagatggcaagaaggagacgcccagtg
atgaccaccgcagatgtctcctacctcaccgaccagggccccggctccggccggcgcgtg
ccggcccgctcctggctgcacagcgacgcaccggcgctctccctcaacggggactggcgc
ttccgcctgctccccgccgcaccgggcaccgcgggcgccggctccgtccttccctccggg
gaaactgtggagggcgttgccggccgcccgagtcctacgacgacctgggacaccctgccc
gtcccctcccactgggtgatgggacaagacggcaaatacggccggcccatctacaccaac
gtccagtacccgttcccgatcgatcctacgtgcccgacccccgccaaccccacaggagac
ttccgccgccgcttcgacgtccccgcccagtggtttgaaagcacgacggcggcactcacc
ctgcgctttgacggggtcgaatcccgctacaaggtgtgggtcaacggccaggaaatcggg
tccaacctgctggtggtgcgggtgcatcaatggtccgccgccagctacctggaagaccag
gaccagtggtggctgcccggcatctttgacgccgtgacgttgcaagcgcgccccgccggc
gggatcaccgacgcctggctgcgcaccggctggtccgccagatcgggggccggcaccggc
acgattgacccggaaatcaccgcggacgcaacggcattccccgtcactttgtccgtgccc
gaattgggcgtcaacgtcacctggaaaaagaagtccgccgtggctccgctggccctggag
aacgtggagccctggtctgcggaggtcccgcgcctctacgaagcctcggtgtccagcgct
gccgagagcatctccgttcgcctcggtttccgcaccgtaaggatcgttggcgaccagttc
ctggtgaatgggcgccgggtggtgttccacggcgtcaaccgccacgagacacaccccgac
cgcggccgcgtctttgacgaagcgcccgcgagcggcgacctggcgctgatgaagcggttc
aacgtcaacgccatccgcaccagccactacccgccccacccgcgcctgctggacctggcg
gacgagatgggcttctgggtgatcctggaatgtgatctggaaacccacggctttgaagcc
ggcggatgggtggagaatcccagcgacgtccccgcctggcgtgacgccttggtcgaccgg
atggagcgcacggtcacccgtccatcggagcgggacaaaaacgtcatgtggtccctgggc
aacgaatcgctccaacctggccgccatggcggcctgcggcaccgggcccatgcccgggac
agctcccggcccgtccactacgagggcgactacacgggcgcctacaccgacgtgtactcc
cgcatccatcccggagacggactccattggccgcaagtactcattgactcccacgccctg
ctccttggctgcgactccgccgaatccgcccgccagcgcaccaaaccgttcatcctgtgc
gagtacgtgcacgccatggcgccatggacgcaacggcccgggcagtacgaggcgctcctg
gatgcacggcggatttcaaatatccgcggcgcgaccacggcatacgccgtgtgggaatgc
acgcgcactgcggaaggcatggagttctttgcctacggcggtgattttggcgaggtggtg
cacgattcgaactttgtcacgattccggcattggatttcggtcccactcccacgcctggc
ctgtacgaattcaagcagatcgtctcccccattcgcctcggactctccctgcccgcggga
gggaagccgaccctgggcggcacacggccgacgcgtccgcagtggccaacctacgtcgta
ctgcgctggcgagtggaacacgatggcgcagtggcggcctccggtgaggttgccgcggaa
cgcggccgcgtctttgacgaagcgcctggcgctgatgaagcccgcgagcggcgacggttc
ggcagcgacggcccgctgcgggcgggggaatcggccacgatcgcccttccggccatgccg
gccgcaccgctcggggaaacgtggttgaccgtgggctgcgggacgccaggccgtaccggc
tgggcacccgccggccacgtgcaactggcccgctgggcgcatctttcggccccggccgtg
ccgacgcggtcgccccggccggccacgccgctcgacggcgcgctccccgtttccctgggc
cctgccacgttcgacgccggaaccctggtctcgctggcaggacagcccgtgtccggtccc
cggctcgagctgtggcgtgccccaggcgccgcaccgacaacgatcggctttggcgcctac
ggcccgggcgatccttggctcaacagcggcaggggcgtgccggcgccgtcgtccgaggcc
gtgtggaagcaggctggactggaccggctgacccggagggtggaggacgtggcggcgctg
ccggacgggatccgcgtcgccgacagcacccattccggcgcacacgctacgcagctggcg
gtcgaggaaaactggcagctggatgggggcgaactgtgtctgcgcatcgacatcacgccc
agtgcgggctggaacctcgtgtggccccgcattggcgtgcggtgggacctgcccacggac
gtggacggtgcggccggcccgcgggagtccctggtttggcgctacccggacagcatgcat
gccactgtggtggcccggcatgccgcttcgctcgaggagctcaacgtcccgtatgcccgg
ccccaggaaccgggcaccgcgtgcgctggagcgatctggaatgtggaccgtgccggtgca
ccgtggttgcggatcgacgccgaacccgatgccgccggacgccggcccgggttctcgctg
ccggccgacttccgctaggaggcccatgtctggccgttgcccgacttccgcccggaggcc
ctagagcgggacaaaaacgtcatgtggtccctgggcaacgaatcgctccaacctggccgc
catggcggcctgcggcaccgggc
```

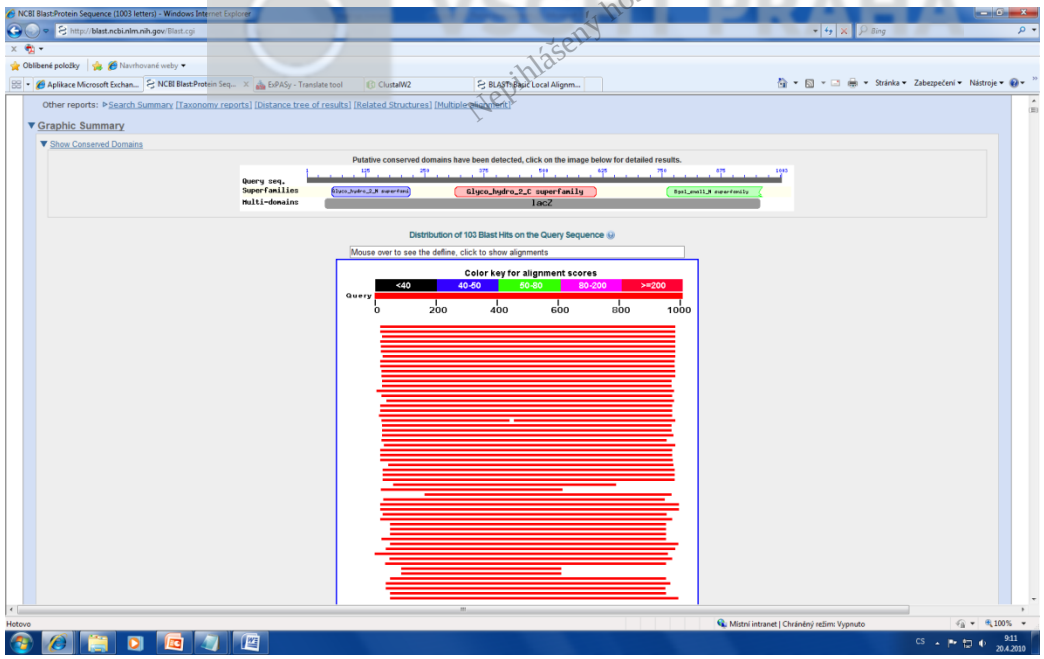
Nepřihlášený host (1)
VŠCHT PRAHA

Co to je???

Choose a BLAST program to run.

Program	Program Description
nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

VŠCHT PRAHA
Nepřihlášený host



http://blast.ncbi.nlm.nih.gov/Blast.cgi

Translate tool

Translate Tool

Please select one of the "Methionine" or one of the highlighted residues following a Stop codon (or the beginning of the sequence).

This will create a virtual Swiss-Prot entry, comprising the residues from your chosen start position up to the following Stop codon.

P G T A C A G A I W N W T V P V H R G W I D A E P D A A G R R P G F S L P D V W P L P D F R P E A S S D L R
S I C T P Stop P A H L H Q Q E G D A Q M A R R R R P V M T T A D V S Y L T D Q G P G S G R R V P A R S W L H
S D A P A L S L N G D W R F R L L P A A P G T A G A G S V L P S G E T V E G V A G R P S P T T T W D T L P V
P S H W V M G Q D G K Y G R P I Y T N V Q Y P F P I D P T C P T P A N P T G D F R R R F D V P A Q W F E S T
T A A L T L R F D G V E S R Y K V W V N G Q E I G S N L L V V R V H Q W S A A S Y L E D Q D Q W W L P G I F
D A V T L Q A R P A G G I T D A W L R T G W S A R S G A G T G T I D P E I T A D A T A F P V T L S V P E L G
V N V T W K K K S A V A P L A L E N V E P W S A E V P R L Y E A S V S S A A E S I S V R L G F R T V R I V G
D Q F L V N G R R V V F H G V N R H E T H P D R G R V F D E A P A S G D L A L M K R F N V N A I R T S H Y P
P H P R L L D L A D E M G F W V I L E C D L E T H G F E A G G W V E N P S D V P A W R D A L V D R M E R T V
T R P S E R D K N V M W S L G N E S L Q P G R H G G L R H R A H A R D S S R P V H Y E G D Y T G A Y T D V Y
S R I H P G D G L H W P Q V L I D S H A L L L G C D S A E S A R Q R T K P F I L C E Y V H A M A P W T Q R P
G Q Y E A L V D A R R I S N I R G A T T A Y A V W E C T R T A E G M E F F A Y G G D F G E V V H D S N F V T
I P A L D F G P T P T P G L Y E F K Q I V S P I R L G L S L P A G G K P T L G G T R P T R P Q W P T Y V V L
R W R V E H D G A V A A S G E V A A E R G R V F D E A P G A D E A R E R R R F G S D G P L R A G E S A T I A
L P A M P A A P L G E T W L T V G C G T P G R T G W A P A G H V Q L A R W A H L S A P A V P T R S P R P A T
P L D G A L P V S L G P A T F D A G T L V S L A G Q P V S G P R L E L W R A P G A A P T T I G F G A Y G P G
D P W L N S G R G V P A P S S E A V W K Q A G L D R L T R R V E D V A A L P D G I R V A D S T H S G A H A T
Q L A V E E N W Q L D G G E L C L R I D I T P S A G W N L V W P R I G V R W D L P T D V D G A A G P R E S L
V W R Y P D S M H A T V V A R H A A S L E E L N V P Y A R P Q E T G H R V R W S D L E L D R A G A P W L R I
D A E P D A A G R R P G F S L P D V W P L P D F R P E A L E R D K N V M W S L G N E S L Q P G R H G G L R H
R

© ÚSOLT PRAHA
Nedistribuovat (!)

http://www.expasy.ch/tools/dna.html

Porovnání a hledání začátku

```
Arthrobacter_sp20B      -------------MSQSTASGRTADVSYITDRGPGSGRRTAARSWLHTTA 37
Arthrobacter_SB         -------------MAQFTASPPAAGVSYITDRGPGSGRRTAARSWLHSNA 37
Arthrobacter_psychro    --------------------MTPADVSYITDQGPGSGLRVPARSWLNSDA 30
Arthrobacter pocusus    -PAHLHQQEGDAMARRRRPVMTTADVSYLTDQGPGSGRRVPARSWLHSDA 50
Arthrobacterc2-2        --------------------MTTADVSYLTDQGPGSGRRVPARSWLHSDA 30
                                              .*.***:**:***** *..*****:: *
```

VŠCHT PRAHA
Nepihlášený host (1)

http://www.ebi.ac.uk/Tools/clustalw2/index.html

cDNA z *Solarum salatum*

```
ttcggcacga gtctcgggcg ggagaagaag aagaattagt aaagtgtgat
cataatgtct gctaggatgc gcaccggaga tgaagataag aagcctaaag
atcagatggt tcatatcaat ctcaaggtta aaggacagga tcggaatgaa
gtttttttca ggatcaaacg tagcacacag atgcgcaagc tcatgaatgc
tagtagtgac cggcagtcag tggagctgaa ctcaattgca ttcttatttg
atgggcgcag gcttagggca gagcattctc ctgatgagct ggagcgggag
gagggtgatg aaatcgatgc aatgctacat caaactggag gcagttgctg
cacttgtttc tctaattttc ttggtttatg ttagtagatt gtttaggcgt
aatactttca actccctcat ctgctctaag atgggtaaat ttatgaattt
tagttttcag cagtattaga tgatgacact actaaatggt tcaattttca
tggcatttgt aaaagtttac tcttaatatg gttaaaaaaa aaaaaaaaaa
aaaaaaaaaa
```

VŠCHT PRAHA
Nepřihlášený host (1)

- najít co to je... - BLAST
- najít začátek a konec – přeložit do AK najít ORF
- vložit do plasmidu

vložit do pPICZ A

5′ end of *AOX1* mRNA — 5′ *AOX1* priming site

```
811   AACCTTTTTT TTTATCATCA TTATTAGCTT ACTTTCATAA TTGCGACTGG TTCCAATTGA

871   CAAGCTTTTG ATTTTAACGA CTTTTAACGA CAACTTGAGA AGATCAAAAA ACAACTAATT
```

Sfu I, *Eco*R I, *Pml* I, *Sfi* I, *Bsm*B I, *Asp*718 I, *Kpn* I, *Xho* I

```
931   ATTCGAAACG AGGAATTCAC GTGGCCCAGC CGGCCGTCTC GGATCGGTAC CTCGAGCCGC
```

Sac II, *Not* I, *Apa* I, *myc* epitope

```
991   GGCGGCCGCC AGCTT GGGCCC GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG
                              Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
```

Polyhistidine tag

```
1042  AAT AGC GCC GTC GAC CAT CAT CAT CAT CAT CAT TGA GTTTTAGCCT TAGACATGAC
      Asn Ser Ala Val Asp His His His His His His ***

1098  TGTTCCTCAG TTCAAGTTGG GCACTTACGA GAAGACCGGT CTTGCTAGAT TCTAATCAAG
```

3′ *AOX1* priming site

VŠCHT PRAHA
Nepihlášený host (1)

vložit do pPICZ A α

5′ end of *AOX1* mRNA — 5′ *AOX1* priming site

```
811   AACCTTTTTT TTTATCATCA TTATTAGCTT ACTTTCATAA TTGCGACTGG TTCCAATTGA

871   CAAGCTTTTG ATTTTAACGA CTTTTAACGA CAACTTGAGA AGATCAAAAA ACAACTAATT

931   ATTCGAAACG ATG AGA TTT CCT TCA ATT TTT ACT GCT GTT TTA TTC GCA GCA
                 Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala

983   TCC TCC GCA TTA GCT GCT CCA GTC AAC ACT ACA ACA GAA GAT GAA ACG GCA
      Ser Ser Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala
```

α-factor signal sequence

```
1034  CAA ATT CCG GCT GAA GCT GTC ATC GGT TAC TCA GAT TTA GAA GGG GAT TTC
      Gln Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe

1085  GAT GTT GCT GTT TTG CCA TTT TCC AAC AGC ACA AAT AAC GGG TTA TTG TTT
      Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu Phe
```

Xho I*

```
1136  ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG GTA TCT CTC
      Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val Ser Leu
```

Kex2 signal cleavage — *Eco*R I — *Pml* I — *Sfi* I — *Bsm*B I — *Asp*718 I

```
1187  GAG AAA AGA GAG GCT GAA GCT GAATTCAC GTGGCCCAG CCGGCCGTC TCGGATCGGT
      Glu Lys Arg Glu Ala Glu Ala
```

Ste13 signal cleavage

Kpn I — *Xho* I — *Sac* II — *Not* I — *Xba* I — *c-myc* epitope

```
1244  ACCTCGAGCC GCGGCGGCC GCCAGCTTTC TA GAA CAA AAA CTC ATC TCA GAA GAG
                                         Glu Gln Lys Leu Ile Ser Glu Glu
```

polyhistidine tag

```
1299  GAT CTG AAT AGC GCC GTC GAC CAT CAT CAT CAT CAT CAT TGA GTTTGTAGCC
      Asp Leu Asn Ser Ala Val Asp His His His His His His ***

1351  TTAGACATGA CTGTTCCTCA GTTCAAGTTG GGCACTTACG AGAAGACCGG TCTTGCTAGA
```

3′ *AOX1* priming site

```
1411  TTCTAATCAA GAGGATGTCA GAATGCCATT TGCCTGAGAG ATGCAGGCTT CATTTTTGAT
```

3′ polyadenylation site

```
1471  ACTTTTTTAT TTGTAACCTA TATAGTATAG GATTTTTTTT GTCATTTTGT TTCTTCTCGT
```

KŠ ATRAH
Nepřihlášený host (1)