

UFABC

Bacharelado em Biotecnologia

Construção do Vetor Recombinante

Proteínas recombinantes

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Etapas construção primers

- 1 – Verificar a sequência de DNA do gene de interesse se não há sítios de restrição para as enzimas que serão utilizadas. Por análise de restrição.
- 2 – Construção do primer sense – FW. Cópia da sequência 5' da fita codificadora acrescida do sítio de restrição e outros nucleotídeos complementares, se necessário.
- 3 – Construção do primer reverso. Cópia de sequência 5' da fita reversa complementar da fita codificadora, acrescida do sítio de restrição e outros nucleotídeos complementares, se necessário.
- 4 – Verificar se o fragmento a ser inserido no vetor estará “in frame” com o sistema de expressão para produzir a proteína corretamente.
- 5 – Checar os primers – T_m , formação de hairpin, complementaridade entre primers.

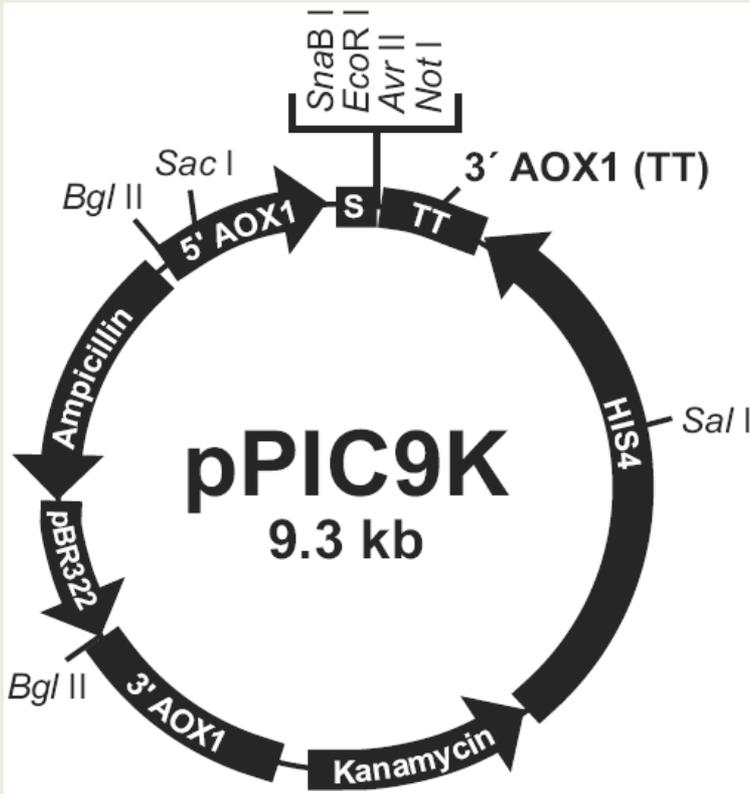
Exemplo: Clonar a proteína Boophilina no vetor pPIC9K

Boophilus microplus mRNA for boophilin, isoform H2
GenBank: AJ304447.1

GenBank Graphics

```
>AJ304447.1 Boophilus microplus mRNA for boophilin, isoform H2
ATTGGCCAGGCACTCAAGATGAAGTGCATCATTCTTCTAGCCGTTCTAGGAACAGCGTTTGCGCAGAGAA
ATGGATTCTGCCGACTGCCGGCAGATGAAGGCATCTGCAAGGCCCTCATACTCGCTTCTACTTCAACAC
TGAAACCGGAAAATGTACCATGTTTTCTATGGAGGCTGTGGAGGCAACGAGAACAACCTTCGAGACCATA
GAGGAGTGTCAAAAGGCGTGCGGAGCTCCGGAGCGAGTGAACGACTTCGAAAGCGCCGATTTCAAGACTG
GCTGTGAGCCGGCGGCCGACAGCGGCTCTTGCGCTGGTCAACTGGAGCGCTGGTTTTTACAATGTTCAATC
GGGAGAGTGCGAGACATTCGTCTACGGGGGCTGCGGGGGCAACGACAACAATTACGAAAGCGAGGAGGAG
TGTGAACCTCGTCTGCAAGAACATGTAATTATCCATACCTAGCGGACTTCAAAGTGCCTTCCCTCATGGAT
CGCATCATGCGTCATAAAGATACCGAAGTGTACCTTGTGAATAAAATGAA
```

Vetor pPIC9K



Vetor pPIC9K

Gene® FEATURES VIEWER RESOURCES SUPPORT PRICING MY ACCOUNT FREE TRIAL

GCACAAATAACGGGTTATTGTTTATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATC 1190
CGTGTTTATTGCCAATAACAAATATTTATGATGATAACGGTCGTAACGACGATTTCTTCTTCCCATAG

S T N N G L L F I N T T I A S I A A K E E G V S
pro region
α-factor secretion signal

SnaBI EcoRI AvrII NotI EagI

TCTCGAGAAAAGAGAGGCTGAAGCTTACGTAGAATTCCTAGGGCGGCCGCGAATTAATTCGCCTTAGAC 1260
AGAGCTCTTTTCTCTCCGACTTCGAATGCATCTTAAGGGATCCCGCCGGCGCTTAATTAAGCGGAATCTG

L E K R E A E A Y V E F P R A A A N *
pro region (in frame with α-factor secretion signal)

Tradução da sequência com ferramenta EXPASY translate:

<https://web.expasy.org/translate/>

The screenshot shows the ExPASy Translate tool interface. At the top, there are browser tabs for 'ExPASy - Translate tool', 'SignalP - 6.0 - Services - DTU Health Te...', and 'NEBcutter'. The address bar shows 'web.expasy.org/translate/'. Below the browser, the ExPASy logo and 'Translate' header are visible. A 'Programmatic access' dropdown is on the left. The main content area has a light blue background and contains the following text:

Translate is a tool which allows the translation of a nucleotide (DNA/RNA) sequence to a protein sequence.

DNA or RNA sequence

```
atggccaggcactcaagatgaagtcattcttctagcgttctaggaacagcgtttgcccagagaaatggattctgccgac
tgccggcagatgaaggcatctgcaagccctcaccctcttacttcaactgaaaccggaaaatgtaccatgttttcccta
tggaggctgtggaggcaagagaacacttcagaccatagaggagtgtcaaaaggctgaggagctcggagcagtgaaacgac
ttcgaagcggcatttcaagactggctgtgagccggcggcgcacagcggccttgcgctggcgaactggagcgtggtttaca
atgtcaatcgggagagtgagacattcgtctacggggcgcgggggcaacgacacaattacgaaagcaggaggagtgatga
actcgtctgaagaacatgtaattaccatactagcggacttcaaaagtgcttccctcatggatcgcacatcgtcataaaga
```

Output format

- Verbose: Met, Stop, spaces between residues
- Compact: M, -, no spaces
- Includes nucleotide sequence
- Includes nucleotide sequence, no spaces

DNA strands

- forward
- reverse

Genetic codes - See NCBI's genetic codes

Standard

reset TRANSLATE!

Results of translation

- Open reading frames are highlighted in red
- Select your initiator on one of the following frames to retrieve your amino acid sequence

Download all the translated frames

The Windows taskbar at the bottom shows the date as 20/07/2022 and the time as 23:28. The system tray includes icons for weather (17°C, Pred. nublado), network, and volume.

Tradução da sequência com ferramenta EXPASY translate:

<https://web.expasy.org/translate/>

Results of translation

- Open reading frames are highlighted in red
- Select your initiator on one of the following frames to retrieve your amino acid sequence

Download all the translated frames

5'3' Frame 1

```
att ggc cag gca ctc aag atg aag tgc atc att ctt cta gcc gtt cta gga aca gcg ttt
I G Q A L K M K C I I L L A V L G T A F
gcg cag aga aat gga ttc tgc cga ctg ccg gca gat gaa ggc atc tgc aag gcc ctc ata
A Q R N G F C R L P A D E G I C K A L I
cct cgc ttc tac ttc aac act gaa acc gga aaa tgt acc atg ttt tcc tat gga ggc tgt
P R F Y F N T E T G K C T M F S Y G G C
gga ggc aac gag aac aac ttc gag acc ata gag gag tgt caa aag gcg tgc gga gct ccg
G G N E N N F E T I E E C Q K A C G A P
gag cga gtg aac gac ttc gaa agc gcc gat ttc aag act ggc tgt gag ccg gcg gcc gac
E R V N D F E S A D F K T G C E P A A D
agc gcc tct tgc gct ggt caa ctg gag cgc tgg ttt tac aat gtt caa tcg gga gag tgc
S G S C A G Q L E R W F Y N V Q S G E C
gag aca ttc gtc tac ggg ggc tgc ggg ggc aac gac aac aat tac gaa agc gag gag gag
E T F V Y G G C G G N D N N Y E S E E E
tgt gaa ctc gtc tgc aag aac atg taa tta tcc ata cct agc gga ctt caa agt gcc ttc
C E L V C K N M - L S I P S G L Q S A F
cct cat gga tcg cat cat gcg tca taa aga tac cga agt gta cct tgt gaa taa aat gaa
P H G S H H A S - R Y R S V P C E - N E
```

5'3' Frame 2

```
attg gcc agg cac tca aga tga agt gca tca ttc ttc tag ccg ttc tag gaa cag cgt ttg
L A R H S R - S A S F F - P F - E Q R L
cgc aga gaa atg gat tct gcc gac tgc cgg cag atg aag gca tct gca agg ccc tca tac
R R E M D S A D C R Q M K A S A R P S Y
ctc gct tct act tca aca ctg aaa ccg gaa aat gta cca tgt ttt cct atg gag gct gtg
L A S T S T L K P E N V P C F P M E A V
ggg gca agg agg agg agt tgg agg gca tag agg agt gtc aaa agg ggt ggg gaa gtc ggg
```

Análise se a proteína tem peptídeo sinal por meio do uso do Signal P.

<https://services.healthtech.dtu.dk/service.php?SignalP>

DTU.dk > Departments and Centers | > Shortcuts | Contact | Dansk

DTU Health Tech

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SignalP - 6.0

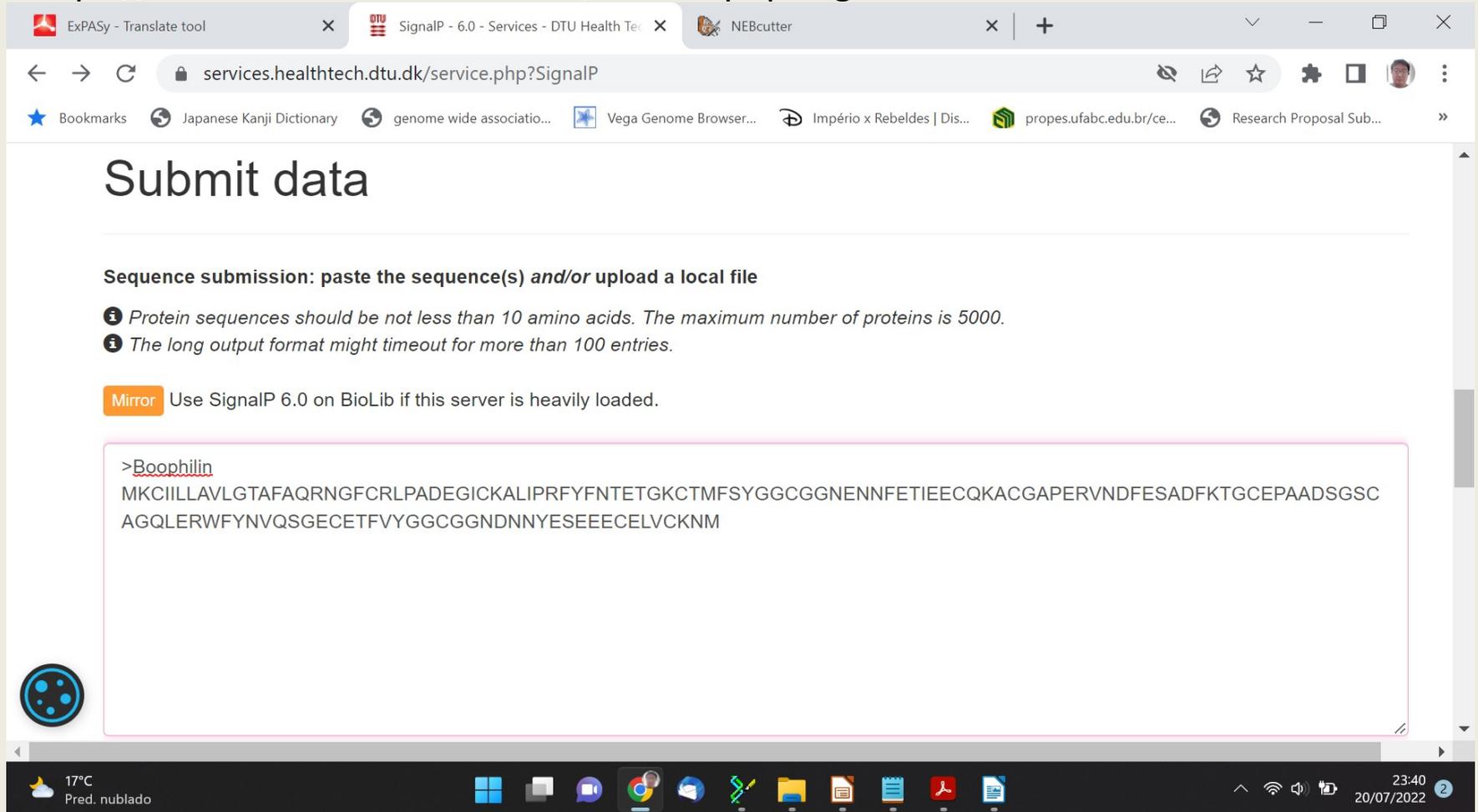
Signal peptides and their cleavage sites in all domains of life

The SignalP 6.0 server predicts the presence of signal peptides and the location of their cleavage sites in proteins from Archaea, Gram-positive Bacteria, Gram-negative Bacteria and Eukarya. In Bacteria and Archaea, SignalP 6.0 can discriminate between five types of signal peptides:

17°C Pred. nublado 23:33 20/07/2022

Análise se a proteína tem peptídeo sinal por meio do uso do Signal P.

<https://services.healthtech.dtu.dk/service.php?SignalP>



ExPASy - Translate tool | SignalP - 6.0 - Services - DTU Health Tec | NEBcutter

services.healthtech.dtu.dk/service.php?SignalP

Submit data

Sequence submission: paste the sequence(s) *and/or* upload a local file

- i** Protein sequences should be not less than 10 amino acids. The maximum number of proteins is 5000.
- i** The long output format might timeout for more than 100 entries.

Mirror Use SignalP 6.0 on BioLib if this server is heavily loaded.

```
>Boophilin
MKCIILLAVLGTAFQRNGFCRLPADEGICKALIPRFYFNTETGKCTMFSYGGCGGNENNFETIEECQKACGAPERVNDNFESADFKTGCEPAADSGSC
AGQLERWFYINVQSGECETFVYGGCGGNDNNYESEEECELVCKNM
```

17°C Pred. nublado | 23:40 20/07/2022

Análise se a proteína tem peptídeo sinal por meio do uso do Signal P.

<https://services.healthtech.dtu.dk/service.php?SignalP>

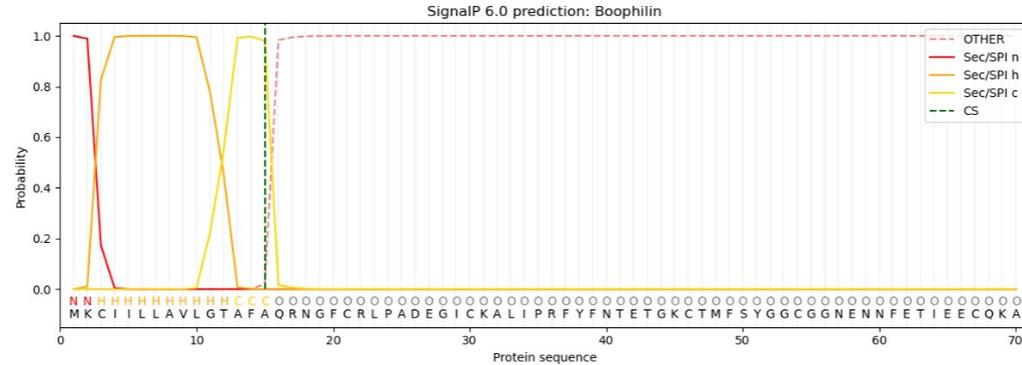
Boophilin

Prediction: Signal Peptide (Sec/SPI)

Cleavage site between pos. 15 and 16. Probability 0.980307

Protein type	Other	Signal Peptide (Sec/SPI)
Likelihood	0.0003	0.9997

Download: [PNG](#) / [EPS](#) / [Tabular](#)



16°C
Nublado



Seleciona-se a região de interesse a ser clonada:

```
cag aga aat gga ttc tgc cga ctg ccg gca gat gaa ggc atc tgc aag gcc ctc ata
Q  R  N  G  F  C  R  L  P  A  D  E  G  I  C  K  A  L  I
cct cgc ttc tac ttc aac act gaa acc gga aaa tgt acc atg ttt tcc tat gga ggc tgt
P  R  F  Y  F  N  T  E  T  G  K  C  T  M  F  S  Y  G  G  C
gga ggc aac gag aac aac ttc gag acc ata gag gag tgt caa aag gcg tgc gga gct ccg
G  G  N  E  N  N  F  E  T  I  E  E  C  Q  K  A  C  G  A  P
gag cga gtg aac gac ttc gaa agc gcc gat ttc aag act ggc tgt gag ccg gcg gcc gac
E  R  V  N  D  F  E  S  A  D  F  K  T  G  C  E  P  A  A  D
agc ggc tct tgc gct ggt caa ctg gag cgc tgg ttt tac aat gtt caa tcg gga gag tgc
S  G  S  C  A  G  Q  L  E  R  W  F  Y  N  V  Q  S  G  E  C
gag aca ttc gtc tac ggg ggc tgc ggg ggc aac gac aac aat tac gaa agc gag gag gag
E  T  F  V  Y  G  G  C  G  G  N  D  N  N  Y  E  S  E  E  E
tgt gaa ctc gtc tgc aag aac atg taa
C  E  L  V  C  K  N  M
```

Apenas os nucleotídeos

cag aga aat gga ttc tgc cga ctg ccg gca gat gaa ggc atc tgc aag gcc ctc ata
cct cgc ttc tac ttc aac act gaa acc gga aaa tgt acc atg ttt tcc tat gga ggc tgt
gga ggc aac gag aac aac ttc gag acc ata gag gag tgt caa aag gcg tgc gga gct ccg
gag cga gtg aac gac ttc gaa agc gcc gat ttc aag act ggc tgt gag ccg gcg gcc gac
agc ggc tct tgc gct ggt caa ctg gag cgc tgg ttt tac aat gtt caa tcg gga gag tgc
gag aca ttc gtc tac ggg ggc tgc ggg ggc aac gac aac aat tac gaa agc gag gag gag
tgt gaa ctc gtc tgc aag aac atg taa

Estabelecer a região dos primers:

cag aga aat gga ttc tgc cga ctg ccg gca gat gaa ggc atc tgc aag gcc ctc ata
cct cgc ttc tac ttc aac act gaa acc gga aaa tgt acc atg ttt tcc tat gga ggc tgt
gga ggc aac gag aac aac ttc gag acc ata gag gag tgt caa aag gcg tgc gga gct ccg
gag cga gtg aac gac ttc gaa agc gcc gat ttc aag act ggc tgt gag ccg gcg gcc gac
agc ggc tct tgc gct ggt caa ctg gag cgc tgg ttt tac aat gtt caa tcg gga gag tgc
gag aca ttc gtc tac ggg ggc tgc ggg ggc aac gac aac aat tac gaa agc gag gag gag
tgt gaa ctg gtc tgc aag aac atg taa

Analisar as regiões selecionadas

https://www.genscript.com/sms2/rev_comp.html (para obter sequência reversa complementar)

<https://www.thermofisher.com/br/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/tm-calculator.html>

<https://m4.igenetech.com/hairpin/a261de23-1897-47e0-b83f-e38c2e562c6d>

Forward:

5'cag aga aat gga ttc tgc c 3'

Tm: 53oC

Pré-reverso - fase 1:

5'c gtc tgc aag aac atg taa3'

>reverse primer reverse complement

5'ttacatggttcttgacagc 3'

Tm: 52,2oC

Sítios de restrição escolhidos

Enzyme Sequence

SnaBI TAC↑↓GTA

Enzyme Sequence Overhang

Properties

NotI GC↓GGCC↑GC 5' GGCC

Adicionar os sítios de restrição

Forward:

5' agcc TACGTA cag aga aat gga ttc tgc c 3'

'

Tm: 65,4oC

Reverso:

5' ttatt CGCGGCCGC tta cat gtt ctt gca gac g 3'

Tm: 69,8oC

Analisar se formam hairpin ou tem autocl complementaridade

<https://www.thermofisher.com/br/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html>

Forward:

5' agcc TACGTA cag aga aat gga ttc tgc c 3'

'

Tm: 65,4oC

Reverso:

5' ttatt CGCGGCCGC tta cat gtt ctt gca gac g 3'

Tm: 69,8oC

Outros programas

DNAMAN

BIOEDIT

<https://eurofinsgenomics.eu/en/ecom/tools/oligo-analysis/>

https://www.sigmaaldrich.com/BR/pt/technical-documents/technical-article/genomics/pcr/oligo-evaluator-for-tm-calculation-primer-analysis?srsId=AfmBOopVrLXMbjU_vN5RGOeaViN4XrXm_7IQnaIJWG5Kkv7BgHs4qzNd