

Aluno:

Número:.....

Atividade Prática 1- O locus ribossomal 16S procarioto

Análises *in silico* importantes antes da reação de PCR *in vitro*

Passo 1: site NCBI

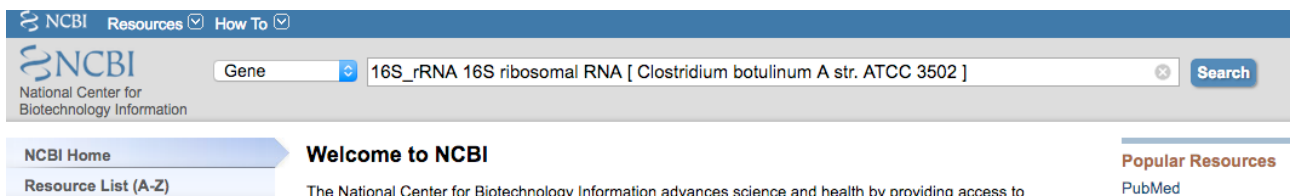
Obter a sequência do cDNA do *locus* RNA ribossomal de *E. coli*, salvar no formato fasta e anotar o tamanho de rRNA

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

à esquerda selecionar a opção gene e no campo aberto digitar o nome do gene de interesse, nesse caso, digitar:

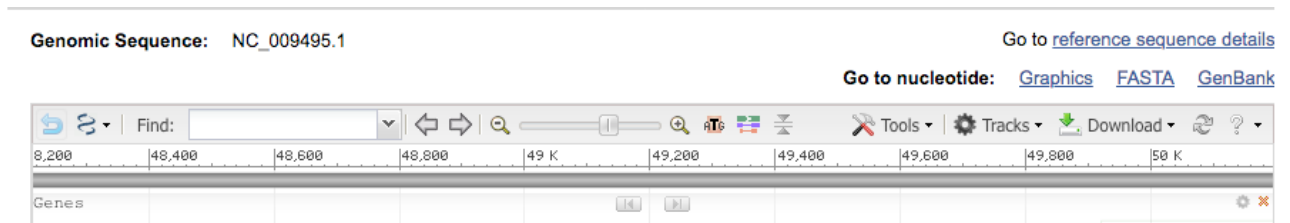
16S_rRNA 16S ribosomal RNA [Clostridium botulinum A str. ATCC 3502]

em seguida clicar *search*:



Na pagina seguinte, clicar em 16S rRNA – 16S ribosomal RNA.

Descer a pagina e obter o gene no formato fasta



>NC_009495.1:48444-49967 Clostridium botulinum A str. ATCC 3502 chromosome, complete genome

```
TTTAAATTTAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCG
ATGAAACTTCCTTCGGGGAGTGGATTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTCAAAGT
GGGGGATAGCCTTCCGAAAGGAAGATTAATACCGCATAATATAAGAGAATCGCATGATTTTCTTATCAAA
GATTTATTGCTTTGAGATGGACCCGCGCGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACG
ATGCGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGAACCTGAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGCGGTGATGAAGGTCTT
CGGATTGTAAAGCCCTGTTTTCTAGGACGATAATGACGGTACTAGAGGAGGAAGCCACGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTGTCCGATTTACTGGGCGTAAAGGGTGCGTAGGC
GGATGTTTAAAGTGGGATGTGAAATCCCCGGGCTTAACCTGGGGGCTGCATTCCAACTGGATATCTAGAG
TGCAGGAGAGGAAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGAGATTAGGAAGAACACCAGTGGC
GAAGGCGGCTTTCTGGACTGTAACGACGCTGAGGCACGAAAGCGTGGGTAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGGATACTAGGTGTAGGGGGTATCAACTCCCCCTGTGCCGCAGTTAA
CACAATAAGTATCCCGCCTGGGGAGTACGGTCGCAAGATTAAGAACTCAAAGGAATTGACGGGGGGCCGCA
CAAGCAGCGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGACTTGACATCCCTTGCA
TAGCCTAGAGATAGGTGAAGCCCTTCGGGGCAAGGAGACAGGTGGTGCATGGTTGTCTCGTCAGCTCGTGTG
GTGAGATGTTAGGTTAAGTCTGCAACGAGCGCAACCCTTGTTATTAGTTGCTACCATTAAAGTTGAGCAC
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TCTAATGAGACTGCCTGGGTAACCAGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCC
AGGGCTACACACGTGCTACAATGGTAGGTACAATAAGACGCAAGACCGTGAGGTGGAGCAAACTTATAA
AACCTATCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAGTTGCTAGTAATCGCGAAT
CAGAAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGCTGGTAACA
CCCGAAGTCCGTGAGGTAACCGTAAGGAGCCAGCGGCCGAAGGTGGGATTAGTGATTGGGGTGAAGTCGT
AACAAGGTAGCCGTAGGAGAACCTGCGGCTGGATCACCTCCTTTCTAAGGAGAA
```

Copiar somente os nucleotídeos da sequência no formato fasta e alinhar no *primer show*

Passo 2: site Primer Show

Confirmar que os *primers* universais *forward* e *reverse* do *locus* ribossomal 16S de procaríoto têm homologia com o organismo de interesse e obter informações sobre o tamanho do *amplicon*

https://www.bioinformatics.org/sms/primer_show.html

Colocar no primeiro campo a sequência de DNA e depois no segundo campo a sequência dos *primers*

Primer Show

Primer Show accepts a DNA sequence along with a set of primer sequences and returns a textual map showing the annealing positions of the primers. The translation of the DNA sequence can be shown in the reading frames you specify. You can also choose the number of bases per line of the map, and whether to show the DNA in its single-stranded or double-stranded form. The primer sequences you enter can contain "wild card" bases, a feature that allows Primer Show to handle degenerate primers. Use this program to produce a useful reference figure, particularly when you have designed a large number of primers for a particular template.

Paste the raw or FASTA sequence into the text area below.

```
>mRNA /gene="fem-2" (exons in uppercase)
TTTAAATTTAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGCGCTTAAACATGC
AAGTCGAGCG
ATGAAACTTCCTTCGGGGAGTGGATTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCT
GCCTCAAAGT
GGGGGATAGCCTTCCGAAAGGAAGATTAATACCGCATAATATAAGAGAATCGCATGATT
```

Enter the patterns in the 5' to 3' direction. An example pattern is: /ac[gt]agcct/ (My pattern's name). The two slashes mark the boundary of the pattern and the round brackets surround the name of the pattern. The square brackets surround possible bases at a degenerate site. You can enter multiple patterns separated by commas. Primer Show automatically constructs a reverse-complement version of each primer sequence so that matches on the reverse strand can be shown. Incorrect entry of the patterns may produce errors.

```
/ACGTTTGATCCTGGCTCAG/ (PS1),
/ACGGCTACCTTGTACGACTT/ (PS2),
/AT[AC]GT[CG]ATTGGATGT[CG]ATATTGG/ (PS3),
/aacagctatgacctg/ (reverse primer),
/attaaccctcactaaag/ (T3 primer),
/cgaggtcgacgtatcg/ (KS primer),
```

SUBMIT CLEAR

- Show 60 bases per line.
- Show the translation for none
- Show the DNA sequence as single-stranded DNA.

The Sequence Manipulation Suite: Primer Show

Results for 1524 residue sequence "mRNA /gene="fem-2" (exons in uppercase)" starting "TTTAAATTA".

```

1          AGAGTTTGATCCTGGCTCAG 3' PS1
1 TTTAAATTTAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTCTTAACACATGC
61 AAGTCGAGCGATGAAACTTCCTTCGGGGAGTGGATTAGCGGCGGACGGGTGAGTAACACG
121 TGGGTAACTTCGCTCAAAGTGGGGGATAGCCTTCGAAAGGAAGATTAATACCGCATAAT
181 ATAAGAGAATCGCATGATTTCCTTATCAAACATTTATTGCTTTGAGATGGACCCGCGCG
241 CATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCGTAGCCGACCTGAGAG
301 GGTGATCGGCCACATTGGAACAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG
361 GAATATTGCGCAATGGGGGAAACCCCTGACCGCAGCAACGCGCGCTGGGTGATGAAGGTCTT
421 CGGATTGTAAAGCCCTGTTTCTAGGACGATAATGACGGTACTAGAGGAGGAAGCCACGG
481 CTAAGTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTGTCGGATTACTG
541 GCGCTAAAGGGTGGCTAGCGGATGTTTAAGTGGGATGTGAAATCCCCGGGCTTAACCTG
601 GGGGCTGCATTCCAAACTGGATATCTAGAGTGCAGGAGAGGAAAGCGGAATTCCTAGTGT
661 AGCGGTGAAATGCGTAGAGATTAGGAAGAACACCAAGTGGCGAAGGCGGCTTTCTGGACTG
721 TAACTGACGCTGAGGCACGAAAGCGTGGGTAGCAAAACAGGATTAGATACCCCTGGTAGTCC
781 ACGCCGTAACGATGGATAGTAGGTGATGGGGGTATCAACTCCCCCTGTGCCGACAGTTAA
841 CACAATAAGTATCCCGCCTGGGGAGTACGGTCCGAAGATTAAACTCAAAGGAATTGACG
901 GGGGCCCCGACAAAGCAGCGGAGCATGTGGTTTAATTGCAAGCAACGCGAAGAACCTTACC
961 TGGACTTGACATCCCTTGCATAGCCTAGAGATAGGTGAAGCCCTTCGGGGCAGGAGACA
1021 GGTGCTGACATGCTTGTCTAGCTCGTGTCTGAGATGTTAGGTTAAGTCCCTGCAACGAG
1081 CGCAACCCCTGTTATTAGTTGCTACCATTAAGTTGAGCACTCTAATGAGACTGCGCTGGGT
1141 AACCAGGAGGAGGTGGGGATGACGCTCAATCATATGCCCTTATGTCAGGGCTACAC
1201 ACCTGCTACAATGCTAGGTACAATAAGACGCAAGACCGTGAGGTGGAGCAAACTTATAA
1261 AACCTATCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGCGAGTTGCTAGT
1321 AATCGCGAATCAGAAATGTCGCGGTGAATACGTTCCCGGGCCTGTACACACCGCCCTCA
1381 CACCATGAGAGCTGTAACACCCGAAGTCCGTGAGGTAAACGTAAGGAGCCAGCGGCCGA
1441          TTCAGCATGTTCCATCGGCA 5' PS2
1441 AGTGGGATTAGTGATTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGAGAACCTGCGGCT
1501 GATCACCTCCTTTCTAAGGAGAA

```

Passo 3: Determinar as temperaturas de *melting* (Tm), e de *annealing* (Ta) também denominada de pareamento

A Tm é a temperatura na qual metade dos fragmentos de DNA está na forma desnaturada, ou seja, não pareados, e a outra está pareada.

A Ta é a temperatura na qual ocorre o pareamento entre o *primer* e o *template*. Em geral, a Ta difere de 3 a 5 °C a menos da Tm dos primers.

A Tm é dependente da composição do *primer*, da concentração do sal na reação e da termodinâmica da reação.

Com base nessas informações, construa uma tabela no excel contendo a Tm dos primers *forward* e *reverse* universais de 16S ribossomal procarioto.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
									Tamanho Primer (bp)	G+C	A+T	(G+C) *4	(A+T) *2	°C = L + M	Tm - 4°C
Clostridium botulinum	PF_primer 3	5'-	GGCAGCAGTGGGGAATATTG					-3'	20	11	9	44	18	62	58
	PR_primer 3	5'-	TCCCACTTAAACATCCGCCT					-3'	20	10	10	40	20	60	56
	PF_universal	5'-	AGAGTTTGATCCTGGCTCAG					-3'	20	10	10	40	20	60	56
	PR_universal	5'-	ACGGCTACCTTGTACGACTT					-3'	21	10	11	40	22	62	58

Passo 4: Site New England BioLabs Tm calculator

<https://tmcalculator.neb.com/#!/main>

Determinar o tipo de enzima DNA polimerase que será utilizada na reação de PCR e a concentração dos *primers* e adicionar nos três primeiros campos. Adicionar as informações das sequências dos *primers* e realizar a pesquisa.

Product Group Taq DNA Polymerase	Anneal at 51 °C	
Polymerase/Kit Taq DNA Polymerase with Standard Taq Buffer		
Primer Concentration (nM) 200	Reset concentration	Primer 1 20 nt 50% GC Tm: 56 °C
Primer 1 AGAGTTTGATCCTGGCTCAG		
Primer 2 ACGGCTACCTTGTACGACTT		Primer 2 21 nt 48% GC Tm: 58 °C
Switch to batch mode	Clear Use example input	