FACULDADE DE MEDICINA DE RIBEIRÃO PRETO – USP

Disciplina RCB 300 – Tópicos em Biotecnologia III - 19/02/2020

Aluno: ..... Número:.....

Atividade Prática 1- **O locus ribossomal 16S procarioto**

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

Passo 1: site NCBI

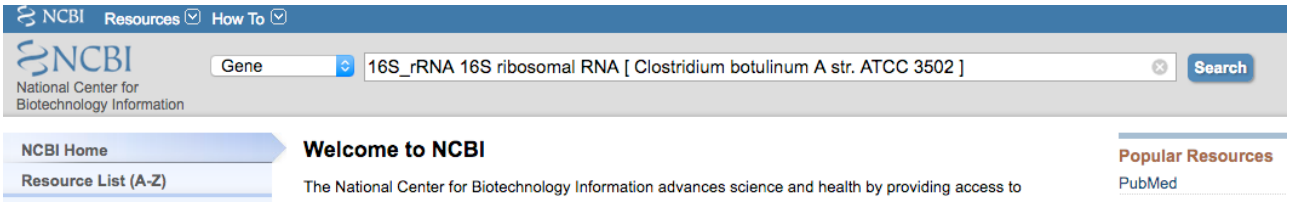
Obter a sequência do cDNA do *locus* RNA ribossomal de *E. coli*, salvar no formato fasta e anotar o tamanho de rRNA

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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>NC_009495.1:48444-49967 Clostridium botulinum A str. ATCC 3502 chromosome,
complete genome
TTTAAATTTAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCG
ATGAAACTTCCTTCGGGGAGTGGATTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTCAAAGT
GGGGGATAGCCTTCCGAAAGGAAGATTAATACCGCATAATATAAGAGAATCGCATGATTTTCTTATCAAA
GATTTATTGCTTTGAGATGGACCCGCGGCGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACG
ATGCGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGGGTGATGAAGGTCTT
CGGATTGTAAAGCCCTGTTTTCTAGGACGATAATGACGGTACTAGAGGAGGAAGCCACGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTGTCCGGATTTACTGGGCGTAAAGGGTGCGTAGGC
GGATGTTTAAGTGGGATGTGAAATCCCCGGGCTTAACCTGGGGGCTGCATTCCAAACTGGATATCTAGAG
TGCAGGAGAGGAAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGAGATTAGGAAGAACACCAGTGGC
GAAGGCGGCTTTCTGGACTGTAACTGACGCTGAGGCACGAAAGCGTGGGTAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGGATACTAGGTGTAGGGGGTATCAACTCCCCCTGTGCCGCAGTTAA
CACAATAAGTATCCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCA
CAAGCAGCGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGACTTGACATCCCTTGCA
TAGCCTAGAGATAGGTGAAGCCCTTCGGGGCAAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTAGGTTAAGTCCTGCAACGAGCGCAACCCTTGTTATTAGTTGCTACCATTAAGTTGAGCAC
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TCTAATGAGACTGCCTGGGTAACCAGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGTCC
AGGGCTACACACGTGCTACAATGGTAGGTACAATAAGACGCAAGACCGTGAGGTGGAGCAAAACTTATAA
AACCTATCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAGTTGCTAGTAATCGCGAAT
CAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGCTGGTAACA
CCCGAAGTCCGTGAGGTAACCGTAAGGAGCCAGCGGCCGAAGGTGGGATTAGTGATTGGGGTGAAGTCGT
AACAAGGTAGCCGTAGGAGAACCTGCGGCTGGATCACCTCCTTTCTAAGGAGAA
```

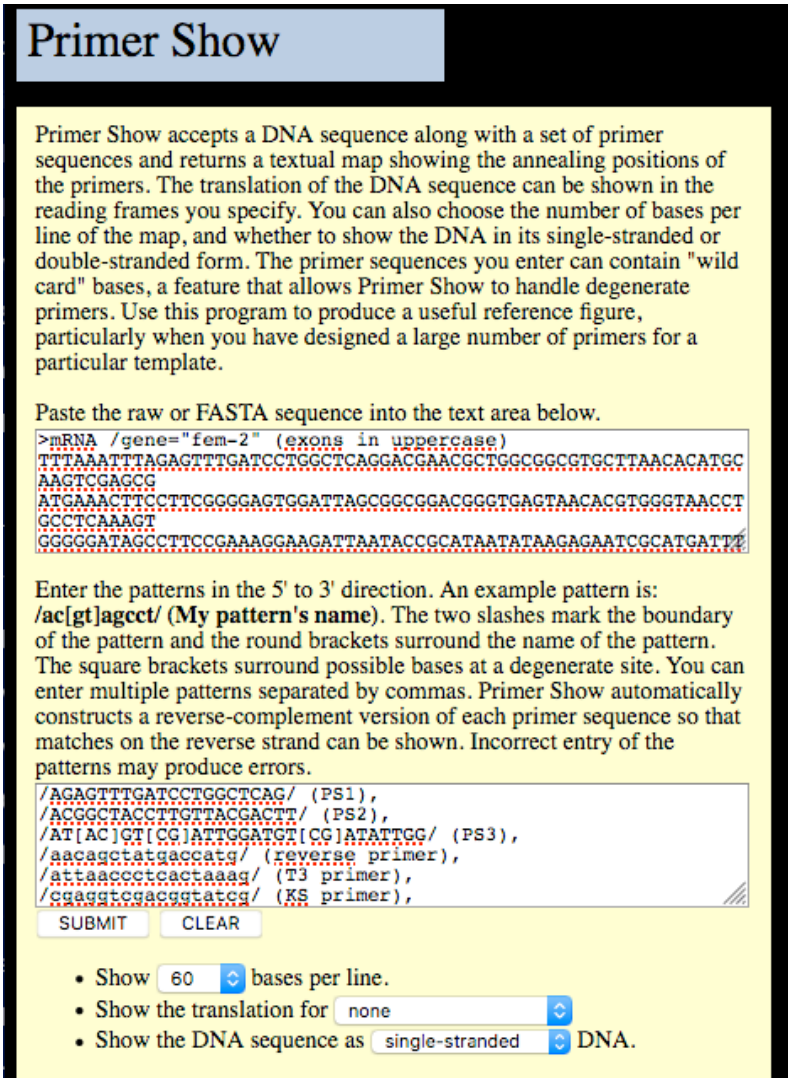
Copiar somente os nucleotídeos da sequencia no formato fasta e alinhar no *primer show*

Passo 2: site Primer Show

Confirmar que os *primers* universais *forward* e *reverse* do *locus* ribossomal 16S de procarioto 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*



The Sequence Manipulation Suite: Primer Show

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

```
       1          AGAGTTTGATCCTGGCTCAG 3' PS1
       1 TTTAAATTTAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGC
      61 AAGTCGAGCGATGAAACTTCCTTCGGGGAGTGGATTAGCGGCGGACGGGTGAGTAACACG
     121 TGGGTAACCTGCCTCAAAGTGGGGGATAGCCTTCCGAAAGGAAGATTAATACCGCATAAT
     181 ATAAGAGAATCGCATGATTTTCTTATCAAAGATTTATTGCTTTGAGATGGACCCGCGGCG
     241 CATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCGTAGCCGACCTGAGAG
     301 GGTGATCGGCCACATTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG
     361 GAATATTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGGGTGATGAAGGTCTT
     421 CGGATTGTAAAGCCCTGTTTTCTAGGACGATAATGACGGTACTAGAGGAGGAAGCCACGG
     481 CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTGTCCGGATTTACTG
     541 GGCGTAAAGGGTGCGTAGGCGGATGTTTAAGTGGGATGTGAAATCCCCGGGCTTAACCTG
     601 GGGGCTGCATTCCAAACTGGATATCTAGAGTGCAGGAGAGGAAAGCGGAATTCCTAGTGT
     661 AGCGGTGAAATGCGTAGAGATTAGGAAGAACACCAGTGGCGAAGGCGGCTTTCTGGACTG
     721 TAACTGACGCTGAGGCACGAAAGCGTGGGTAGCAAACAGGATTAGATACCCTGGTAGTCC
     781 ACGCCGTAAACGATGGATACTAGGTGTAGGGGGTATCAACTCCCCCTGTGCCGCAGTTAA
     841 CACAATAAGTATCCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACG
     901 GGGGCCCGCACAAGCAGCGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC
     961 TGGACTTGACATCCCTTGCATAGCCTAGAGATAGGTGAAGCCCTTCGGGGCAAGGAGACA
    1021 GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTAGGTTAAGTCCTGCAACGAG
    1081 CGCAACCCTTGTTATTAGTTGCTACCATTAAGTTGAGCACTCTAATGAGACTGCCTGGGT
    1141 AACCAGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGTCCAGGGCTACAC
    1201 ACGTGCTACAATGGTAGGTACAATAAGACGCAAGACCGTGAGGTGGAGCAAAACTTATAA
    1261 AACCTATCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAGTTGCTAGT
    1321 AATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
    1381 CACCATGAGAGCTGGTAACACCCGAAGTCCGTGAGGTAACCGTAAGGAGCCAGCGGCCGA
    1441                          TTCAGCATTGTTCCATCGGCA 5' PS2
    1441 AGGTGGGATTAGTGATTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGAGAACCTGCGGCT
    1501 GGATCACCTCCTTTCTAAGGAGAA
```

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 ^{o}C a menos da Tm dos primers.

A Tm é dependente da composição do *primer*, da concentracão do sal na reação e da termodinamica da reação.

Com base nessas informações, construa uma tabela no excell contend a Tm dos primers *forward* e *reverse* universais de 16S ribosomal 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'-	ACGGCTACCTTGTTACGACTT				-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.