

Atividade Prof. Aparecida - RCB300

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Exercícios

1. Desenhar um conjunto de *primers* para amplificar a região constante do 16S rRNA de quatro espécies de bactérias:
2. Determinar se o primer “universal” tem 100% homologia com os 5 procariotos:
Forward primer (5'-AGAGTTTGATCCTGGCTCAG-3') e
Reverse primers (5'-ACTGCTACCTTGTACGAACTT-3')
3. Obter as informações para a tabela abaixo:

	sequência	Tamanho amplicon	Tm	Homologia com primer universal
Primer Forward				
Primer reverse				

Espécie escolhida: *Clostridium*

Clostridium butyricum strain KNU-L09 chromosome 1, complete sequence

NCBI Reference Sequence: NZ_CP013252.1

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

>NZ_CP013252.1:c90265-88742 Clostridium butyricum strain KNU-L09 chromosome 1, complete sequence

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TTTTTATTGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCG
ATGAAGCTCCTTCGGGAGTGGATTAGCGGCGGACGGGTGAGTAACACGTGGGTAACTGCCTCATAGAGG
GGAATAGCCTTTTCGAAAGGAAGATTAAATACCGCATAAGATTGTAGTACCGCATGGTACAGCAATTAAGG
AGTAATCCGCTATGAGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTACCAAGGCGACG
ATGCGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGGGAAACCTGATGCAGCAACGCCGCTGAGTGATGACGGTCTT
CGGATTGTAAGCTCTGTCTTTAGGGACGATAATGACGGTACCTAAGGAGGAAGCCACGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTACTGGGCGTAAAGGGAGCGTAGGT
GGATATTTAAGTGGGATGTGAAATACCCGGGCTTAACCTGGGTGCTGCATTCCAAACTGGATATCTAGAG
TGCAGGAGAGGAAAGGAGAATTCTAGTGTAGCGGTGAAATGCGTAGAGATTAGGAAGAATACCAAGTGGC
GAAGGCGCCTTTCTGGACTGTAACCTGACACTGAGGCTCGAAAGCGTGGGGAGCAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAACCGATGAATACTAGGTGTAGGGGTTGTCATGACCTCTGTGCCGCCGCTAAC
GCATTAAGTATTCCGCTGGGGAGTACGGTCGCAAGATTAAGCAAAAGGAATTGACGGGGGCCGAC
AAGCAGCGGAGCATGTGGTTAATTGGAAGCAACGCAAGAACCTTACCTAGACTTGACATCTCCTGAAT
TACTCTGTAATGGAGGAAGCCACTTCGGTGGCAGGAAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCTTATGTTAGTTGCTACCATTTGGTTGAGCA
CTCTAGCGAGACTGCCCGGTTAACCAGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGT
CTAGGGCTACACACGTGCTACAATGGTACGATGCAACCTCGCGAGAGTGAGCAAACTATA
AAACCGATCTCAGTTCGGATTGTAGGCTGAACTCGCCTACATGAAGCTGGAGTTGCTAGTAATCGCGAA
TCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGACACACCGCCCGTCACACCATGAGAGTTGGCAAT
ACCCAAAGTTGTCGAGCTAACGCGTAAGCGAGGACGACCTAAGGTAGGGTCAGCGATTGGGGTGAAGT
CGTAACAAGGTAGCCGAGGAGAACCTGCGGCTGGATCACCTCCTTCTATGGA
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The Sequence Manipulation Suite: Primer Show

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

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1      AGAGTTTGATCCTGGCTCAG 3' PFU
1      TTTTATTGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGCGTCTTAACACATGC
61     AAGTCGAGCGATGAAGCTCCTTCGGGAGTGGATTAGCGGCGGACGGGTGAGTAACACGTG
121    GGTAAACCTGCCTCATAGAGGGGAATAGCCTTTCGAAAGGAAGATTAAATACCGCATAAGAT
181    TGTAGTACCGCATGGTACAGCAATTAAGGAGTAATCCGCTATGAGATGGACCCGCGTCG
241    CATTAGCTAGTTGGTGAGGTAACGGCTACCAAGGCGACGATGCGTAGCCGACCTGAGAG
301    GGTGATCGGCCACATTGGGACTGAGACACGGCCAGACTCTACGGGAGGCAGCAGTGGG
361    GAATATTGCACAATGGGGGAAACCTGATGCAGCAACGCCGCGTGAGTGATGACGGTCTT
421    CGGATTGTAAAGCTCTGTCTTTAGGGACGATAATGACGGTACCTAAGGAGGAAGCCACGG
481    CTAACCTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCGGATTTACTG
541    GGCGTAAAGGGAGCGTAGGTGGATTTAAGTGGGATGTGAAATACCCGGGCTTAACCTG
601    GGTGCTGCATTCCTAACTGGATATCTAGAGTGCAGGAGAGGAAGGAGAATTCCTAGTGT
661    AGCGGTGAAATGCGTAGAGATTAGGAAGAATACCAAGTGGCGAAGGCGCCTTCTGGACTG
721    TAACGACACTGAGGCTCGAAAGCGTGGGGAGCAAAACAGGATTAGATACCTGGTAGTCC
781    AGGGGTTGTCATGACCTCTG 3' PF
781    ACGCCGTAACCGATGAATACTAGGTGTAGGGGTTGTCATGACCTCTGTGCCGCGCTAAC
841    GCATTAAGTATTCGCGCTGGGGAGTACGGTCGCAAGATTAAACTCAAAGGAATTGACGG
901    TTCGTTGCGCTTCTTGAAT 5'
901    GGGCCCCGACAAGCAGCGGAGCATGTGTTTAATTCGAAGCAACGCGAAGAACCTTACCT
961    PR
961    AGACTTGACATCTCCTGAATTACTCTGTAATGGAGGAAGCCACTTCGGTGGCAGGAAGAC
1021   AGGTGGTGCATGGTTGTCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGA
1081   GCGCAACCCCTATTGTTAGTTGCTACCATTTGGTTGAGCACTCTAGCGAGACTGCCCGGG
1141   TTAACCGGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTAGGGCTAC
1201   ACACGTGCTACAATGGTCGGTACAATGAGATGCAACCTCGCGAGAGTGAGCAAACTATA
1261   AAACCGATCTCAGTTTCGGATTGTAGGCTGAACTCGCTACATGAAGCTGGAGTTGCTAG
1321   TAATCGCAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCGTC
1381   ACACCATGAGAGTTGGCAATACCAAAAGTTCGTGAGCTAACGCGTAAGCGAGGCAGCGAC
1441   TTCAGCATTGTTCCATCGGCA 5' PRU
1441   CTAAGGTAGGGTCAGCGATTGGGGTGAAATCGTAACAAGGTAGCCGTAGGAGAACCTGCG
1501   GCTGGATCACCTCCTTCTATGGA
  
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	Sequência	Tamanho do amplicon	Tm (°C)	Tm (°C) (Software) 500nM Kit Q5 High Fidelity Polymerase	Ta (°C)	Homologia com o Primer universal
Primer Forward	AGGGGT TGTCATG ACCTCTG	190pb	62	67	58	
Primer Reverse	TAAGGTT CTTCGC GTTGCTT	190pb	58	64	54	
Primer Foward Universal	AGAGTTT GATCCT GGCTCA G	1477pb	60	64	56	
Primer Reverse Universal	TTCAGCA TTGTTCC ATCGGC A	1477pb	62	67	58	