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Faculdade de Medicina de Ribeirão Preto
Curso de Ciências Biomédicas
RCB0300 - Tópicos em Biotecnologia III
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Desenho de *primers* para amplificação do gene 16S rRNA de *Staphylococcus aureus*

1. Obtenção da sequência do gene 16S rRNA da bactéria *Staphylococcus aureus* no formato FASTA:

Staphylococcus aureus strain ATCC 12600 16S ribosomal RNA, complete sequence

NCBI Reference Sequence: NR_118997.2

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

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>NR_118997.2 Staphylococcus aureus strain ATCC 12600 16S ribosomal RNA, complete sequence
TTTATGGAGAGTTTGATCTCGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAA
CGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAAACCACCTATAA
GACTGGGATAAATTCGGGAAACCGGAGCTAATACCGGATAATATTTGAACCGCATGGTTCAAAGTGAA
AGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA
AGGCAACGATACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACAGAGACACGGTCCAGACTCCT
ACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGTCTTCGGATCGTAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACGTGCACATCTTGAC
GGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTA
TCCGGAATTATTGGGCGTAAAGCGCGCTAGGCGGTTTTTAAAGTCTGATGTGAAAGCCACGGCTCAAC
CGTGGAGGGTCATTGGAACTGGAAACTTGAGTGCAGAAGAGGAAAGTGGAAATTCATGTGTAGCGGTG
AAATGCGCAGAGATATGGAGGAACACCAAGTGGCGAAGGCGACTTCTGGTCTGTAAGTACGCTGATGTG
CGAAAGCGTGGGATCAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTGCTAAGTGT
TAGGGGGTTTTCCGCCCTTAGTGCTCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAG
GTTGAACTCAAAGGAATTGACGGGACCCGCACAAGCGGTGGAGCATGTGGTTAATTGAAAGCAACGC
GAAGAACCCTTACCAATCTTGACATCCTTGACAACCTAGAGATAGAGCCTTCCCTTCGGGGGACAAA
GTGACAGGTGGTGCATGTTGTCGTCAGCTCGTGTGAGATGTTGGTTAAGTCCCACAACGAGCGCA
ACCTTAAGCTTAGTGCCATCATTAAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAG
GTGGGGATGACGTCAAATCATCATGCCCTTATGATTTGGGTACACAGTGTCTACAATGGACAATAACA
AGGGCAGCGAAACCGGAGGTCAAGCAAATCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACT
CGACTACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTATT
GTACACACCGCCGTCACACCAGAGATTTGTAACACCGAAGCCGGTGGAGTAACCTTTTAGGAGCTA
GCCGTGAAGGTGGGACAAATGATTGGGGTGAAGTCGTAACAAGTAGCCGTATCGGAAGGTGCGGCTGG
ATCACCTCCTT
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2. Desenho dos *primers* para a sequência apresentada utilizando o *software*
Primer 3:
PFA: *primer forward*
PRA: *primer reverse*

OLIGO	start	len	tm	gc%	any	3'	seq
LEFT PRIMER	503	20	59.90	55.00	6.00	2.00	GAAAGCCACGGCTAACTACG
RIGHT PRIMER	705	20	59.99	50.00	4.00	2.00	CATTTACCGCTACACATGG

SEQUENCE SIZE: 1552
INCLUDED REGION SIZE: 1552

3. Verificação de homologia entre os *primers* obtidos pelo Primer 3 com o primer universal pelo *software* Primer Show:

PFU (primer foward universal): AGAGTTTGATCCTGGCTCAG

PRU(primer reverse universal): ACGGCTACCTTGTTACGACTT

The Sequence Manipulation Suite: Primer Show

Results for 1552 residue sequence "mRNA /gene="fem-2" (exons in uppercase)" starting "TTTATGGAGA".

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1      AGAGTTTGATCCTGGCTCAG 3' PFU
1  F M E S L I L A Q D E R W R R A * Y M Q
1  TTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGCGTGCCTAATACATGCAA
21 V E R T D E K L A S L M L A A D G * V T
61 GTCGAGCGAACGGACGAGAAGCTTGCTTCTGTATGTTAGCGCGGACGGGTGAGTAACA
41 R G * P T Y K T G I T S G N R S * Y R I
121 CGTGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATA
61 I F * T A W F K S E R R S C C H L * M D
181 ATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGAT
81 P R C I S * L V R * R L T K A T I R S R
241 CCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATACGTAGCCGA
101 P E R V I G H T G T E T R S R L L R E A
301 CCTGAGAGGGTGATCGGCCACACTGGAAC TGAGACACGGTCCAGACTCCTACGGGAGGCA
121 A V G N L P Q W A K A * R S N A A * V M
361 GCAGTAGGGAATCTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTAGTGATG
141 K V F G S * N S V I R E E H M C K * L C
421 AAGGCTTCGGATCGTAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAAGTGTGC
481      GAAAGCCACGGCTAACTACG 3' PFA
161 T S * R Y L I R K P R L T T C Q Q P R *
481 ACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAA
181 Y V G G K R Y P E L L G V K R A * A V F
541 TACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGGTAGGCGGTTTTT
201 * V * C E S P R L N R G G S L E T G K L
601 TAAGTCTGATGTGAAAGCCACGGCTCAACCGTGAGGGTCAATTGGAACTGGAAAACTT
661      GGTACACATCGCCACTTTAC 5' PRA
221 E C R R G K W N S M C S G E M R R D M E
661 GAGTGCAAGAGAGAAAGTGGAATTCATGTGTAGCGGTGAAATGCGCAGAGATATGGAG
241 E H Q W R R R L S G L * L T L M C E S V
721 GAACACCAAGTGGCGAAGGCGACTTCTGGTCTGTAAGTACGCTGATGTGCGAAAGCGTG
261 G I K Q D * I P W * S T P * T M S A K C
781 GGGATCAACAGGATTAGATACCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGT
281 * G V S A P * C C S * R I K H S A W G V
841 TAGGGGGTTTCCGCCCTTAGTGTGTCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTA
301 R P Q G * N S K E L T G T R T S G G A C
901 CGACCGCAAGTTGAAACTCAAAGGAATTGACGGGGACCCGACAAAGCGGTGGAGCATGT
321 G L I R S N A K N L T K S * H P L T T L
961 GGTTTAATTCGAAGCAACGCAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTA
341 E I E P S P S G D K V T G G A W L S S A
1021 GAGATAGAGCCTTCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCT
361 R V V R C W V K S R N E R N P * A * L P
1081 CGTGTCTGAGATGTTGGTTAAGTCCCGCAACGAGCGCAACCTTAAGCTTAGTTGCCA
381 S L S W A L * V D C R * Q T G G R W G *
1141 TCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA
401 R Q I I M P L M I W A T H V L Q W T I Q
1201 CGTCAAATCATCATGCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAA
421 R A A K P R G Q A N P I K L F S V R I V

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1261 AGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTA
441 V C N S T T * S W N R * * S * I S M L R
1321 GTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGG
461 * I R S R V L Y T P P V T P R E F V T P
1381 TGAATACGTTCCCGGTATTGTACACACCGCCGTCACACCACGAGAGTTTGTAAACACC
481 E A G G V T F * E L A V E G G T N D W G
1441 GAAGCCGGTGGAGTAACCTTTTAGGAGCTAGCCGTCGAAGGTGGGACAAATGATTGGGGT
1501 TTCAGCATTGTTCCATCGGCA 5' PRU
501 E V V T R * P Y R K V R L D H L L
1501 GAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT

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4. Tamanho do amplicon

Sabendo-se que a sequência de estudo possui 1552 bp, calcula-se o tamanho do amplicon da seguinte forma:

Tamanho entre PFU e PRU (1522-8): 1514 bp

Tamanho entre PFA e PRA: 123 bp

5. Cálculo da temperatura de Melting e de annealing para amplificação da sequência de interesse:

a) Cálculo pelo site NEB Tm calculator utilizando a enzima Q5

High-Fidelity DNA Polymerase:

Forward primer universal (5'-AGAGTTTGATCCTGGCTCAG-3'): 64°C

Reverse primer universal (5'-ACGGCTACCTTGTTACGACTT-3'): 66°C

Temperatura de anelamento: 65°C

Forward primer *S. aureus* (5'-GAAAGCCACGGCTAACTACG-3'): 66°C

Reverse primer *S. aureus* (5'-CATTTACACGCTACACATGG-3'): 64°C

Temperatura de anelamento: 65°C

b) Cálculo manual:

Forward primer universal (5'-AGAGTTTGATCCTGGCTCAG-3'): 60°C

Reverse primer universal (5'-ACGGCTACCTTGTTACGACTT-3'): 62°C

Forward primer *S. aureus* (5'-GAAAGCCACGGCTAACTACG-3'): 62°C

Reverse primer *S. aureus* (5'-CATTTACACGCTACACATGG-3'): 60°C