RCB0300 - Biotecnologia III - Desenho de Primer

Grupo: Ana Clara L. Cressoni, Catarina S. Gomes, Gabriel M. C. Manso, Giovanna O. Nogueira, Giseli F. Correa

(1) Obter a sequência do RNA ribossomal específico: NCBI gene ou EMBL gene

(a) Espécie: *Propionibacterium acnes*

(b) https://www.ncbi.nlm.nih.gov/gene/2932196

(c) https://www.ncbi.nlm.nih.gov/nuccore/NC_006085.1?report=fasta&from=606152&to=607688

(2) Desenhar um conjunto de primers para amplificar a região constante do 16S rRNA da espécie escolhida

PRIMER DESENHADO COM PRIMER 3:

OLIGO	start	len	tm	gc%	any_th	3'_th	hairpin
LEFT PRIMER(forward)	797	20	59.03	55.00	0.00	0.00	0.00
AACGGTGGGTACTAGGTGTG							
RIGHT PRIMER(reverse)	1003	20	58.96	55.00	0.00	0.00	0.00
CATCTCTGAGCACTCCCGAT							

SEQUENCE SIZE: 1537

INCLUDED REGION SIZE: 1537

(3) Verificar se os primers tem homologia como a região constante (primer universal):

Primer show

The Sequence Manipulation Suite: Primer Show

```
Results for 1537 residue sequence "NC_006085.1:606152-607688 Propionibacteriu
      1           AGAGTTTGATCCTGGCTCAG 3' PUF
      1 TTTTTCATTGGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACAT
     61 GCAAGTCGAACGGAAAGGCCCTGCTTTTGTGGGGTGCTCGAGTGGCGAACGGGTGAGTAA
    121 CACGTGAGTAACCTGCCCTTGACTTTGGGATAACTTCAGGAAACTGGGGCTAATACCGGA
    181 TAGGAGCTCCTGCTGCATGGTGGGGGTTGGAAAGTTTCGGCGGTTGGGGATGGACTCGCG
    241 GCTTATCAGCTTGTTGGTGGGGTAGTGGCTTACCAAGGCTTTGACGGGTAGCCGGCCTGA
    301 GAGGGTGACCGGCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGT
    361 GGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCAACGCCGCGTGCGGGATGACGGC
    421 CTTCGGGTTGTAAACCGCTTTCGCCTGTGACGAAGCGTGAGTGACGGTAATGGGTAAAGA
    481 AGCACCGGCTAACTACGTGCCAGCAGCCGCGGTGATACGTAGGGTGCGAGCGTTGTCCGG
    541 ATTTATTGGGCGTAAAGGGCTCGTAGGTGGTTGATCGCGTCGGAAGTGTAATCTTGGGGC
    601 TTAACCCTGAGCGTGCTTTCGATACGGGTTGACTTGAGGAAGGTAGGGGAGAATGGAATT
    661 CCTGGTGGAGCGGTGGAATGCGCAGATATCAGGAGGAACACCAGTGGCGAAGGCGGTTCT
    721 CTGGGCCTTTCCTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGCTTAGATACCCT
    781                AACGGTGGGTACTAGGTGTG 3' PF
    781 GGTAGTCCACGCTGTAAACGGTGGGTACTAGGTGTGGGGTCCATTCCACGGGTTCCGTGC
    841 CGTAGCTAACGCTTTAAGTACCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAG
    901 GAATTGACGGGGCCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGTAG
    961                      TAGCCCTCACGAGTCTCTAC 5' PR
    961 AACCTTACCTGGGTTTGACATGGATCGGGAGTGCTCAGAGATGGGTGTGCCTCTTTTGGG
   1021 GTCGGTTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT
   1081 CCCGCAACGAGCGCAACCCTTGTTCACTGTTGCCAGCACGTTATGGTGGGGACTCAGTGG
   1141 AGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTAT
   1201 GTCCAGGGCTTCACGCATGCTACAATGGCTGGTACAGAGAGTGGCGAGCCTGTGAGGGTG
   1261 AGCGAATCTCGGAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCTCATGAAG
   1321 TCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGGCTTGT
   1381 ACACACCGCCCGTCAAGTCATGAAAGTTGGTAACACCCGAAGCCGGTGGCCTAACCGTTG
   1441                                  TTCAGCATTGTTCCATCGGCA
   1441 TGGGGGAGCCGTCGAAGGTGGGACTGGTGATTAGGACTAAGTCGTAACAAGGTAGCCGTA
   1501 5' PUR
   1501 CCGGAAGGTGCGGCTGGATCACCTCCTTTCTAAGGAG
```

Houve homologia dos primers universais com a região da sequência 16S da espécie escolhida.

(4) Determinar a temperatura de melting e de anelamento dos primers desenhados e dos primers universais:

(a) NEB Tm calculator (Q5 High-Fidelity DNA Polymerase):

Primer Universal forward (PUF): 5’ AGAGTTTGATCCTGGCTCAG 3' (**Tm: 64 °C)**
Primer Universal reverse (PUR): 3’ TTCAGCATTGTTCCATCGGCA 5**’ (Tm: 66ºC)**

Temperatura de anelamento: 65°C

Primer Personalizado forward (PF): 5’ AACGGTGGGTACTAGGTGTG 3’ **(Tm: 66 °C)**
Primer Personalizado reverse (PR): 5’ CATCTCTGAGCACTCCCGAT 3’ **(Tm: 66 °C)**

Temperatura de anelamento: 67ºC

(b) Fórmula tradicional:

Primer Universal forward (PUF): 5’ AGAGTTTGATCCTGGCTCAG 3' **(Tm 60ºC)**
Primer Universal reverse (PUR): 3’ TTCAGCATTGTTCCATCGGCA 5**’ (Tm: 62ºC)**

Primer Personalizado forward (PF): 5’ AACGGTGGGTACTAGGTGTG 3’ **(Tm: 62 °C)**
Primer Personalizado reverse (PR): 5’ CATCTCTGAGCACTCCCGAT 3’ **(Tm: 62 °C)**

(5) Determinar o tamanho do amplicon

(a) Tamanho do gene que codifica o 16S ribossomal do procarioto selecionado: 1537 bp
(b) Entre o Primer Universal forward e o Primer Universal reverse: 1488 bp
(c) Entre Primer Personalizado forward e o Primer Personalizado reverse: 207 bp