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Experiment No: 7 Experiment Result:

Primer design

Aim: To carry out primer designing using primer3.

Query

Introduction

Primer is a widely used program for designing PCR primers (PCR polymeric chain reaction). PCR is an essential & ubiquitous tool in genetics & molecular biology. Primer 3 can also design hybridization probes & sequencing primers. PCR is used for many different goals consequently, primer 3 has many different input parameters that you control & that tell primer 3 exactly what characteristics make good primer of your goals.

primer3 - the code the web interface & the documentation - are an open source community development project hosted by source forge.

Query: Alpha glucosidase / Alpha amylase
Uridophospholysase / e.t.c.

URL: primer 3. ut.ee

Procedure:

↓ log on to NCBI (www.ncbi.nlm.nih.gov/) & retrieve nucleotide sequence of your interest in FASTA format.

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2) Log onto primer 3.ut.ee which leads you to primer 3 software tool.
3) Paste the sequence in the Sequence Box.
4) Select pick left primer, pick right primer & pick hybridization on probes.
5) Click on pick primer option.
6) Results are obtained.

Result & Discussion:-

A primer design experiment is done with the help of primer 3 software tool. This tool allows the user to obtain the following results.

i) left primer (>>>>>)
Sequence : AATGTGGCTGCAAGTAGGA.
ii) Right primer (<<<<<)
Sequence: GTAGGCGTGTTGGTGATC
iii) Hybridization probe (^^^^^)
iv) GC %.
v) Tm value
vi) Hairpin Sequence
vii) Length of the Sequence.

P - 10
C - 10
V - 10
R - 10
40

6/2/24

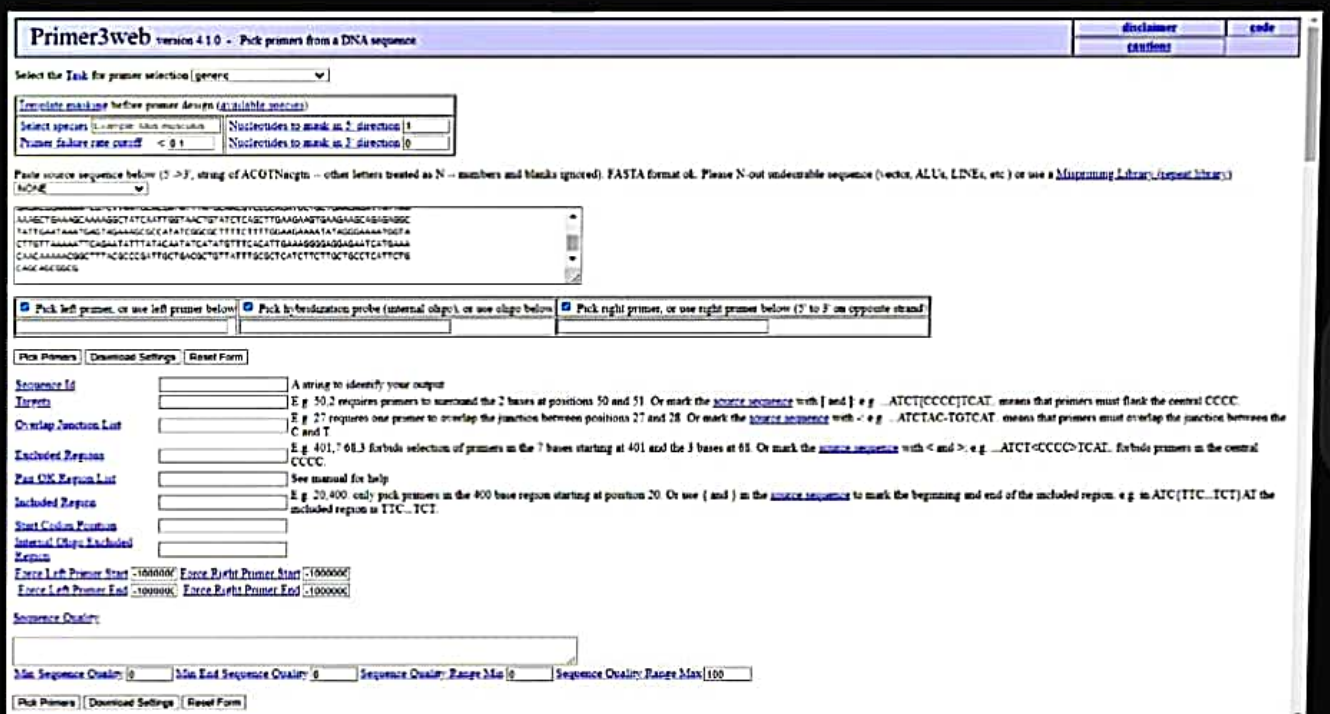
Bacillus licheniformis alpha-amylase (amyL) gene, 5' end

GenBank: M26412.1

GenBank Graphics

>M26412.1 Bacillus licheniformis alpha-amylase (amyL) gene, 5' end

TGATCATTTCTTGCGTGTCGTTAATCTCCTGATACGCTTTTTCGTTTGACAGCCTTGTCAATAACGG
ATG
GTCCCAGGAATGGTTGCCGACTTCGTTTCCTTCCTTCAGCATCCGTTTTACGTTTCGGGATAATATT
GGG
CTCTGCTTCCAAGCACAAAGAAGGTCGATGCCCTTCATGCTCTGTAAAGCGTTTAATATTTTATTCG
TTG
TAGCGGGATTCGGACCGTCATCAAATGTGAGGGCAATCACGTTTTTCATCGGGATTAATTTTCGCTT
GCT
TCGGAAGCGGAACAGGCTCCTGATCAGTGATTCCGTCCGCTCGCTTTCCAATCTGAAGGTTTCATTG
TGG
GATGTTGATCCGGAAGATTGGAAGTACAAAAATAAGCAAAAGATTGTCAATCATGTCATGAGCCATG
CGG
GAGACGGAAAAATCGTCTTAATGCACGATATTTATGCAACGTCCGCAGATGCTGCTGAAGAGATTAT
TAA
AAAGCTGAAAGCAAAAGGCTATCAATTGGTAACTGTATCTCAGCTTGAAGAAGTGAAGAAGCAGAGA
GGC
TATTGAATAAATGAGTAGAAAGCGCCATATCGGCGCTTTTCTTTTGGAAGAAAATATAGGGAAAATG
GTA
CTTGTTAAAAATTCAGAATATTTATACAATATCATATGTTTCACATTGAAAGGGGAGGAGAATCATG
AAA
CAACAAAAACGGCTTTACGCCCGATTGCTGACGCTGTTATTTGCGCTCATCTTCTTGCTGCCTCATT
CTG
CAGCAGCGGCG



Primer3 Output

```
PRIMER PICKING RESULTS FOR M26412.1 Bacillus licheniformis alpha-amylase (amyL) gene, 5' end

Template masking not selected
No mispriming library specified
No internal oligo mishyb library specified
Using 1-based sequence positions
OLIGO            start  len      tm      gc%   any_th   3'_th  hairpin seq
LEFT PRIMER         75   20   59.20   55.00     0.00    0.00     0.00 CAGGAATGGTTGCCGACTTC
RIGHT PRIMER       244   20   59.27   55.00     0.00    0.00     0.00 GCCCTCACATTTGATGACGG
INTERNAL OLIGO      98   20   59.95   50.00     0.00    0.00     0.00 TCCTTCCTTCAGCATCCGTT
SEQUENCE SIZE: 781
INCLUDED REGION SIZE: 781

PRODUCT SIZE: 170, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

    1 TGATCATTTCTTGCGTGTCGTTAATCTCCTGATACGCTTTTTCGTTTGACAGCCTTGTCA


   61 ATAACGGATGGTCCCAGGAATGGTTGCCGACTTCGTTTCCTTCCTTCAGCATCCGTTTTA
                  >>>>>>>>>>>>>>>>>>>>   ^^^^^^^^^^^^^^^^^^^^


  121 CGTTTCGGGATAATATTGGGCTCTGCTTCCAAGCACAAAGAAGGTCGATGCCCTTCATGC


  181 TCTGTAAAGCGTTTAATATTTTATTCGTTGTAGCGGGATTCGGACCGTCATCAAATGTGA
                                                 <<<<<<<<<<<<<<<<<<<<


  241 GGGCAATCACGTTTTTCATCGGGATTAATTTTCGCTTGCTTCGGAAGCGGAACAGGCTCC
      <<<<


  301 TGATCAGTGATTCCGTCCGCTCGCTTTCCAATCTGAAGGTTTCATTGTGGGATGTTGATC


  361 CGGAAGATTGGAAGTACAAAAATAAGCAAAAGATTGTCAATCATGTCATGAGCCATGCGG


  421 GAGACGGAAAAATCGTCTTAATGCACGATATTTATGCAAECGTCCGCAGATGCTGCTGAAG


  481 AGATTATTAAAAAGCTGAAAGCAAAAGGCTATCAATTGGTAACTGTATCTCAGCTTGAAG


  541 AAGTGAAGAAGCAGAGAGGCTATTGAATAAATGAGTAGAAAGCGCCATATCGGCGCTTTT


  601 CTTTTGGAAGAAAATATAGGGAAAATGGTACTTGTTAAAAATTCAGAATATTTATACAAT
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