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## Primer design

Aim:- To carry out primer designing using primer3.

### Query Introduction

Primer 3 is a widely used program for designing PCR primers (PCR polymerase 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 primers 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  
Uridiphosphorylase / U.S.

URL: primer 3. ut-ec

### Procedure:

1) log on to NCBI ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) & retrieve nucleotide sequence of your interest in FASTA format.

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Experiment Result.....

- 2) log onto primer 3 .ut.cc 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 : AATGTCCTGCAAGTAAAGAA

ii) Right primer (←←←←←)

Sequence : GTAGGCTGTTGGTCGATC

iii) hybridization probe (NNNNN)

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  
TGATCATTCTTTCGCGTGTGCGTTAATCTCCTGATACGCTTTTTCGTTTGACAGCCTTGTCATAAACGG  
ATG  
GTCCAGGAATGGTTGCCGACTTCGTTTCCTTCCTTCAGCATCCGTTTTACGTTTCGGGATAATATT  
GGG  
CTCTGCTTCCAAGCACAAAGAAGGTCGATGCCCTTCATGCTCTGTAAAGCGTTTAATATTTTATTTCG  
TTG  
TAGCGGGATTTCGGACCGTCATCAAATGTGAGGGCAATCACGTTTTTCATCGGGATTAATTTTCGCTT  
GCT  
TCGGAAGCGGAACAGGCTCCTGATCAGTGATTCCGTCGCTCGCTTTCCAATCTGAAGGTTTCATTG  
TGG  
GATGTTGATCCGGAAGATTGGAAGTACAAAAATAAGCAAAAGATTGTCAATCATGTCATGAGCCATG  
CGG  
GAGACGGAAAAATCGTCTTAATGCACGATATTTATGCAACGTCGCGCAGATGCTGCTGAAGAGATTAT  
TAA  
AAAGCTGAAAGCAAAGGCTATCAATTGGTAACTGTATCTCAGCTTGAAGAAGTGAAGAAGCAGAGA  
GGC  
TATTGAATAAATGAGTAGAAAGCGCCATATCGGCGCTTTTCTTTTGGGAAGAAAATATAGGGAAAATG  
GTA  
CTTGTTAAAAATTGAGAATATTTATACAATATCATATGTTTCACATTGAAAGGGGAGGAGAATCATG  
AAA  
CAACAAAACGGCTTTACGCCCGATTGCTGACGCTGTTATTTGCGCTCATCTTCTTGCTGCCTCATT  
CTG  
CAGCAGCGGCG

Primer3web version 4.1.0 - Pick primers from a DNA sequence		disclaimer	code
Select the Task for primer selection (generic)		cautions	
Template available before primer design (available sources)			
Select species (Example: Mus musculus)	Nucleotides to mask in 2' direction (1)		
Primer failure rate cutoff (< 0.1)	Nucleotides to mask in 2' direction (0)		
Paste source sequence below (2-3, string of ACGTNaGts - other letters treated as N - numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mapping Library (repeat library)			
ACG			
<div>ACGCTGAAAGCAAAGGCTATCAATTGGTAACTGTATCTCAGCTTGAAGAAGTGAAGAAGCAGAGAGGC TATTGAATAAATGAGTAGAAAGCGCCATATCGGCGCTTTTCTTTTGGGAAGAAAATATAGGGAAAATG CTTGTTAAAAATTGAGAATATTTATACAATATCATATGTTTCACATTGAAAGGGGAGGAGAATCATG CAACAAAACGGCTTTACGCCCGATTGCTGACGCTGTTATTTGCGCTCATCTTCTTGCTGCCTCATTCTG CAGCAGCGGCG</div>			
<input checked="" type="checkbox"/> Pick left primer, or use left primer below <input checked="" type="checkbox"/> Pick hybridization probe (internal oligo), or use oligo below <input checked="" type="checkbox"/> Pick right primer, or use right primer below (2' to 3' on opposite strand)			
<div>Pick Primers Download Settings Reset Form</div>			
Sequence Id	A string to identify your output		
Targets	E.g. 50-2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [ ] and   e.g. ...ATCT[CCCC]TCAT... means that primers must flank the central CCCC.		
Overlap Junction List	E.g. 27 requires one primer to overlap the junction between positions 27 and 28. Or mark the source sequence with < and > e.g. ...ATCTAC-TGTCAT... means that primers must overlap the junction between the C and T		
Excluded Regions	E.g. 401..7 683 Excludes selection of primers in the 7 bases starting at 401 and the 3 bases at 683. Or mark the source sequence with < and > e.g. ...ATCT<CCCC>TCAT... Excludes primers in the central CCCC.		
See OK Region List	See manual for help		
Included Region	E.g. 20-400 only pick primers in the 400 base region starting at position 20. Or use [ ] and   in the source sequence to mark the beginning and end of the included region. e.g. in ATC(TTC...TCT)AT the included region is TTC...TCT.		
Start Codon Position			
Internal Oligo Excluded Region			
Force Left Primer Start	<100000	Force Right Primer Start	>100000
Force Left Primer End	<100000	Force Right Primer End	>100000
Sequence Quality			
Min Sequence Quality	0	Max Sequence Quality	100
Sequence Quality Range Min	0	Sequence Quality Range Max	100
<div>Pick Primers Download Settings Reset Form</div>			

### Primer3 Output

PRIMER PICKING RESULTS FOR H26412.1 *Bacillus licheniformis* alpha-amylase (amyl) gene, 5' end

template masking not selected

No mispriming library specified

No internal oligo misht library specified

Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any th	1 <sup>st</sup> th	hairpin seq
LEFT PRIMER	75	20	59.20	55.00	0.00	0.00	CAGGATGATGTTGCGGACGC
RIGHT PRIMER	244	20	59.27	55.00	0.00	0.00	CGCTTCACATTGATGACG
INTERNAL OLIGO	98	20	59.95	50.00	0.00	0.00	TCCTTCATTCAAGATCCGT

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INTERNAL SIZE: 761
SEQUENCE SIZE: 761
INCLUDED REGION SIZE: 761

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PRODUCT SIZE: 170, PAIR ANY TH COMPL: 0.00, PAIR 3' TH COMPL: 0.00

1 TGATCATTTCCTGCGTGTCGTTAATCTCCTGATAAGCTTTTTCGTTTGACAGCCTTGTCA

61 ATACGGATGGTCCCAAGAAATGTTGCCBACTTCGTTCCTTCTTCAGCATCCGTTTA  
>>>>>>>>>>>>>>>> <<<<<<<<<<<<<<<<

121 CGTTTCGGGATAATATGGGCTCTGCTTCCAACACAAAGAAGGTGATGECCTTCATGC

181 TCTGTAAAGCGTTTAAATATTTTATTGGTTGAGCGGGATTCCGACCGTCATCAATGTGA  
<<<<<<<<<<<<<<<<<<

241 GGGCAATCACGTTTTTCATCGGATTAAATTTTCGTTGCTTCGGAGCGGAACAGTCTC  
 <<<<

381 TGATCAGTGAATCCGTCCGTCGCTTCCAAATCTGAAGGTTTCATTGTGGGATGTTGATC

361 CGGAGATTGGAGTACAAAATAGCAAAAGATTGTCAATCATGTCATGAGCCATGCGG

421 GAGACGGAAAAATCGTCTTAATGCAGATATTTATGCAAGTCCGCAGATGCTGCTGAGG

481 AGATTATTAAAGCTGAAGCAAAAGGCTATCAATTGGTAACTGTATCTCACTTGAAG

541 AAGTGAAGAGCAGAGAGGCTATTGATTAATGATAGAAAGCGCCATATCGGCGCTTTT

601 CTTTGGAAQAAAATATAGGGAATGUTACTTGTTAAAAATTCAGATATTTATACAAI