



BTE401: Bioinformatics

Summer 2020

Section : 02

QUIZ2

Date of submission: 09-08-20

Submitted to:

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Biotechnology Program

Department of Mathematics and Natural Sciences

BRAC University.

Submitted by:

Mahfuzul Hasan

ID: 17126024

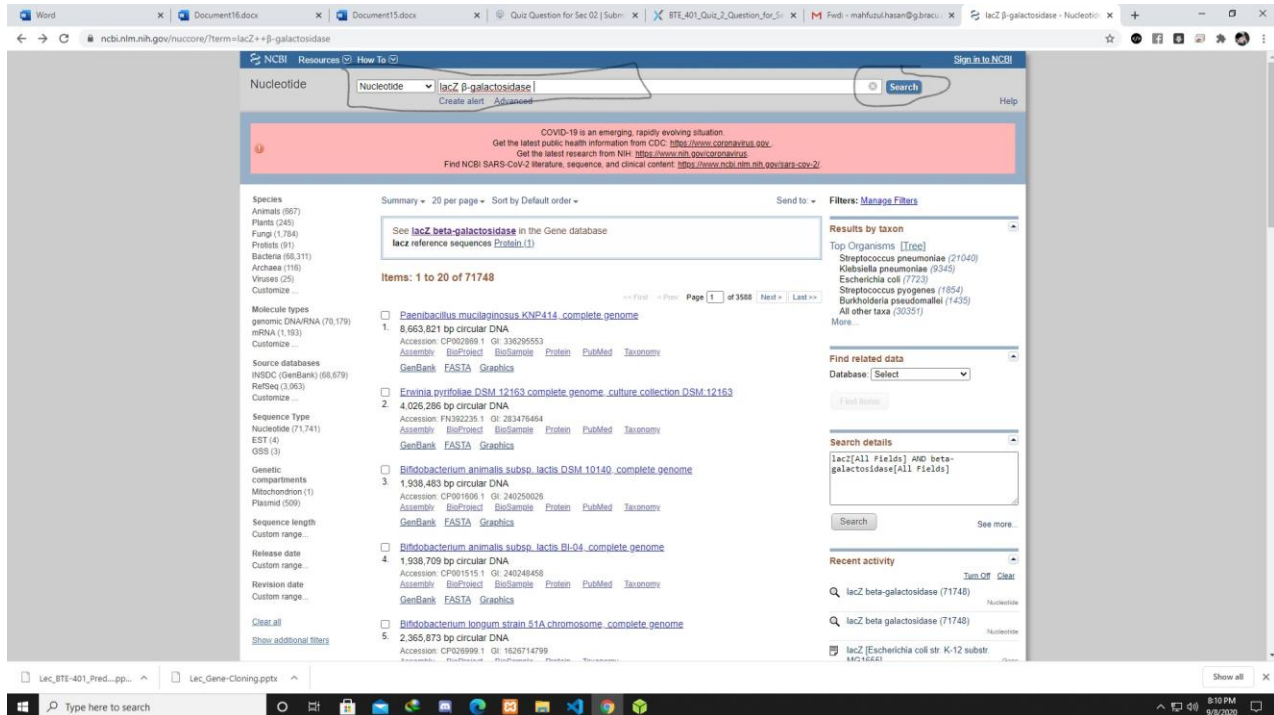
Microbiology program.

Department of Mathematics and Natural Sciences.

Answer to the question no 1:

Here, we extracted the FASTA sequence of lacZ gene from NCBI GenBank. For this purpose, the following steps were performed:

Step 1: searched for lac Z gene in the database:



Step2: after that, selected the desired gene from the database:

NCBI Resources How To Sign in to NCBI

Nucleotide Nucleotide Advanced Search Help

COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: <https://www.coronavirus.gov>.
Get the latest research from NIH: <https://www.nih.gov/coronavirus>.
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: <https://www.ncbi.nlm.nih.gov/sars-cov-2/>.

GenBank Send to: Change region shown Customize view

Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene, complete cds

GenBank: EU590652.1
[FASTA](#) [Graphics](#)

Go to: (v)

LOCUS EU590652 2017 bp DNA linear BCT 05-MAR-2010
DEFINITION Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene, complete cds.
ACCESSION EU590652
VERSION EU590652.1
KEYWORDS
SOURCE Lactobacillus acidophilus
ORGANISM [Lactobacillus acidophilus](#)
Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus.
REFERENCE 1 (bases 1 to 2017)
AUTHORS Pan,Q., Zhu,J., Liu,L., Cong,Y., Hu,F., Li,J. and Yu,X.
TITLE Functional identification of a putative beta-galactosidase gene in the special lac gene cluster of Lactobacillus acidophilus
JOURNAL Curr. Microbiol. 60 (3), 172-178 (2010)
PUBMED [19841976](#)
REFERENCE 2 (bases 1 to 2017)
AUTHORS Pan,Q., Hu,F., Li,J., Cong,Y., Liu,L. and Zhu,J.
TITLE Cloning, expression and characterization of a glycoside hydrolase family 42 beta-galactosidase from Lactobacillus acidophilus ATCC 4356
JOURNAL Unpublished
REFERENCE 3 (bases 1 to 2017)
AUTHORS Pan,Q., Hu,F., Li,J., Cong,Y., Liu,L. and Zhu,J.
TITLE Direct Submission
JOURNAL Submitted (25-MAR-2008) Microbiology, Third Military Medical University, Gaotanyan Street of Shapingba District, Chongqing, Chongqing 400038, China
FEATURES
source 1..2017
/organism="Lactobacillus acidophilus"

Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence

Related information
Protein
PubMed
Taxonomy
BioCollections
PubMed (Weighted)

LinkOut to external resources
Lactobacillus acidophilus [BacDive]
Order lacZ cDNA clone/Protein/Antibody/RNAi [OriGene]

Recent activity
Turn Off Clear
Lactobacillus acidophilus ATCC:4356 beta-

Step 3: finally, took the nucleotide sequence in FASTA format:

FASTA ▾

Send to: ▾

Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene, complete cds

GenBank: EU590652.1

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

```
>EU590652.1 Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene, complete
cds
TAGAGGAAATAAAAATGACACAATTATCACGTTTCTTTATGGTGGTGATTATAATCCTGACCAATGGCCA
GAAGAAACATGGTCGAAAGATATTACGATTTTAAAAAGCGGATATTAATTTCGGCAACGATTAAACATTT
TTTCTTGGGCATTGCTTGAACCAAGAGAAGGAAAAATATAATTTCTCAAAATTAGATAAAGTTGTACAACA
ATTATCTGATGCTAACTTTGATATTGTGATGGGAACAGCCACAGCAGCGATGCCAGCTTGGATGTTTAAA
AAATATCCCGATATTGCCAGAGTAGATTATCAAGACAGACGTCATGTATTGGTCAGCGGCATAACTTCT
GTCTTAATAGCTCAAAATATCAAAAGATTAGCTGGTGAATTAGTAAAGCAGTTAGTTGAAACGCTACAAGGA
TAATAAGCATATCGTAGTTTGGCACATAAAACAATGAATATGGTGGCAACTGTTATTGTGAGAATTGTCAA
AACGCTTTTAGAAAAATGGTTGAAGAATAAATAAAGACCGTTGAAGGCTTTAACAAGGCATGGAATATGA
ATGTATGGAGCCATACGATTATGACTGGGATGAAATTGTTGTTCTAATGAGTTAGGGGATGTATGGGG
AATAGAAGGTAGTGAACCTATTGTAGCTGGTCTTTCAATTGATTATGCTGCGTTTTCAATCTGAAAGTATG
CAAAATCTTTTCAAGATGGAAAAGAAGATTATAAAAAATATGATCCGGAAACTCTGTAAACGACTAATT
TCCATGGTTTGCCTAACCAAGATGGTTGATTATCAAAAGTGGGCAAAAGGTCAAGATATTATTTTCATATGA
TAGTTATCCAACCTATGATGCTCCTGCATATAAAGCGGCATTCTTGATGACTTAAAGCGAAGCTTGAAA
CATCAGCCATTTATGTTAATGGAATCTGCGCTTTCACAAGTTAACTGGCAACCATATAGTCCGCTTAAAG
GGCTGGACAAATGGGAAGCAACTGAATTTCAAGCTGTAGCCCATGGTGTGATACGGTACAATTCTTCCA
ATTAAAAACAAGCAGTTGGTGGCTCCGAAAAATTCACAGTGCAGTATTGCTCATTGCAAAAGAACCGAT
ACTAGAGTATTTAAAGAACTAGCTGATTAGGGGAAGAAATTAAGAATGCTGGACCAACGATTTTAGGGT
CAAGACTAAGGCAAGGTCGCAATTGCTTTGATTGGAGTAACTTCTGGTCGTATGAGTATGTGGACGG
AATTACTCAAGATTTGAACTATGTAGATTCTATTCTTGATTACTACCGTCAGTCTATGAACGCAATATT
CCAAC TGACATCATTGGGTAGACGATGACTTTAGCAACTATGATTGGTTGTAGCGCTGTGCTTTATA
TGTTTAAACATGGTCTTGATAAGAAGTCAACGACTATGTTGAAAACGGTGGTAACCTTTGTCACTACTTA
TATGTCAAGCATGGTGAACATCAGATAATGTATATCTTGGTGGCTATCCTGGTCATTGAAGGAAAGTT
ACAGGCATTTGGGTTGAAGAAAGTGATGCAGTAGTCCAGGACAAAGATTAAAGTCTTAATGAAGGTA
AGGATTATGATAGTGGTGTGATCTGTAACTTGATTCAATCAAAAGACGTAAGATTTTGGCAACTTATGC
GAGTGAATTTTATGCAAGGTACGCCAGCTGTACCGAAAAATCAATATGGCAAGGTAAGGCTTGGTATATT
GGTACAAGGCTTGAACATCAAGGGTTAACTCAATTATTCAATCATATTATTTTGAAGCAGGTTGGAAT
CACTGGTTTGCATAGTCATAAACTAGAAAACTAAGCGTGTACTGAAGATGGTAAGGAACCTTACTT
TGTGCTTAATATGAGTAATGAAGAAAGAACGTTACCAAGCAAGTTTCAAGGTTATGAAAGATATTTAACT
GGTAAAAAGCTCATAAAGATATGAAAGGTTGGGATGTTCAAGTATTGAGAAATTAG
```

Change region shown ▾

Customize view ▾

Analyze this sequence ▴

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Related information ▴

Protein

PubMed

Taxonomy

BioCollections

PubMed (Weighted)

LinkOut to external resources ▴

Lactobacillus acidophilus


[BacDive]

Order lacZ cDNA clone/Protein/Antibody/RNAi

[OriGene]

Recent activity ▴

Turn Off Clear

 Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene, complete Nucleotide

 lacZ beta-galactosidase (71748)

Nucleotide

For detection of vector contamination, we used VecScreen
(<https://www.ncbi.nlm.nih.gov/tools/vecsreen/>).

In vecscreen, the FASTA format of the sequence was pasted in query box

NCBI Resources How To Sign in to NCBI

VecScreen All Databases Search

VecScreen UniVec Contamination

COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: <https://www.coronavirus.gov>.
Get the latest research from NIH: <https://www.nih.gov/coronavirus>.
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: <https://www.ncbi.nlm.nih.gov/sars-cov-2/>.

VecScreen: Screen a Sequence for Vector Contamination

VecScreen is a system that quickly finds segments of a nucleic acid sequence that may be of vector origin. It helps researchers identify and remove any segments of vector origin before they analyze or submit sequences. [more...](#)

Enter your query sequence below as an Accession or [FASTA](#).

```
>EU590652.1 Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene, complete cds
TAGAGGAAATAAAATGACACAAATATCACGTTTCTTTATGGTGGTATTATACTCTGACCAATGGCCA
GAAGAAACATGGTCAAAGATATTACGTTATTTAAAGGCGGATTAATTCGGCAACGATTAACATTT
TTTCTTGGGCATTGCTTGAACCAAGAGAAGGAAATAAATTTCTCAAAATAGATAAAGTTGTACACAA
ATTATCTGATGCTAACTTTGATATTGTGATGGGAACAGCCACAGCAGCGATGCCAGCTTGGATGTTTAA
AAATATCCCGATATTGCCAGATAGATTATCAAGACAGCGCTCATGATTGGTCAGCGGCATCAACTTCT
GTCCTAATAGCTCAAATATCAAGATTAAGCTGGTGAATTAGTAAAGCAGTTAGTTGAACGCTACAAAGGA
TAATAAGCATATCGTAGTTTGGCACATAAACAATGAATATGGTGGCACTGTATTGTGAGAAATGTCAA
AACGCTTTTAGAAAATGGTTGAAGAATAAAATATAAGACGTTGAAGGCTTAAACAAGGATGGAATATGA
ATGATGGAGCCATACGATTATGACTGGGATGAAATGTTGTTCTCAATGAAGTAGGGGATGTATGGGG
AATAGAAGGTAGTGAACCTATTGACTGGTCTTTCAATTGATTATCTGCGTTTCAATCTGAAAGTATG
CAAAATCTTTCAAGATGGAAGAAAGATTATTAATAATATGATCCGGAACCTCTGTAAACGACTAATT
TCCATGTTTGGCTAACAAAGATGGTGTATTATCAAAAGTGGCAAAAGGTCAAGATATTATTTCAATGA
TAGTTATCAACTTATGATGCTCTGCTATATAAGCGCATTCCTTATGATGACCTAATGCGAAGCTGAAA
CATCAGCAATTTATGATTAATGAACTGGCTTCAAGATTAAGTGGCAACATATAGTCCGCTTAAGC
GGCTGGCAAAATGGAAGCAACTGAATTTCAAGCTGTAGCCCATGGTGTCTGATACGTAATCTTCAAC
ATTAAACAAGCAGTTGGTGGCTCGAAAAATCCACAGTGAATTTGCTCATTCGCAAGAACCGAT
ACTAGATATTAAAGAACTAGCTGATTAGGGAAGAAATTAAGAAATGCTGGACCAACGATTTTAGGGT
```

Run VecScreen Clear Input

Links

- [About VecScreen](#)
- [Interpretation of Results](#)
- [Contamination](#)
- [The UniVec Database](#)
- [Current UniVec Statistics](#)
- [Current UniVec Content](#)

You are here: NCBI Support Center

GETTING STARTED	RESOURCES	POPULAR	FEATURED	NCBI INFORMATION
NCBI Education	Chemicals & Bioassays	PubMed	Genetic Testing Registry	About NCBI
NCBI Help Manual	Data & Software	Bookshelf	GenBank	Research at NCBI
NCBI Handbook	DNA & RNA	PubMed Central	Reference Sequences	NCBI News & Blog
Training & Tutorials	Domains & Structures	BLAST	Gene Expression Omnibus	NCBI FTP Site
Submit Data	Genes & Expression	Nucleotide	Genome Data Viewer	NCBI on Facebook
	Genetics & Medicine	Genome	Human Genome	NCBI on Twitter
	Genomes & Maps	SNP	Mouse Genome	NCBI on YouTube
	Homology	Gene	Influenza Virus	Privacy Policy
	Literature	Protein	Primer-BLAST	
	Proteins	PubChem	Sequence Read Archive	
	Sequence Analysis			

Then pressed “Run VecScreen” and the report was viewed:

Word Document16.docx Document15.docx Quiz Question for Sec 02 BTE_401_Quiz_2_Question... Fwd - mahfuzulhasan@... NCBI Blast Lactobacillus acidophilus ATCC 4356

blast.ncbi.nlm.nih.gov/Blast.cgi

U.S. National Library of Medicine NCBI National Center for Biotechnology Information Sign in to NCBI

BLAST® → blastn suite Home Recent Results Saved Strategies Help

Format Request

Query EU590652.1 Lactobacillus acidophilus ATCC 4356

Database screen:UniVec

Job title EU590652.1 Lactobacillus acidophilus ATCC 4356

Request ID NF50KCEA814 View report Show results in a new window

Format Alignment View Show aligned seq HTML

Alignment View Parameters

Display ☒ Graphical Overview ☐ Linkout ☐ Sequence Retrieval ☐ NCBI-gi ☐ CDS feature

Masking Character Lower Case Color Blast

Limit results Descriptions 100 Graphical overview 5 Alignments 1000 Line length 60

Organism Type common name, binomial, taxid, or group name. Only 25 top taxa will be shown. Enter organism name or taxid - completions will be suggested. Exclude

Enter query

Expect Min Expect Max

Percent Identity Min Percent Identity Max

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NCBI National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA Policies and Guidelines Contact

Support center Mailing list

NIH USA.gov

Activate Windows

Lac_BTE-401_Pred...pptx Lac_Gene-Cloning.pptx

Type here to search

8:29 PM 5/6/2020

The report showed that the nucleotide sequence did not contain any vector contamination:

Word Document16.docx Document15.docx Quiz Question for Sec 02 BTE_401_Quiz_2_Question... Fred - mahfuzulhasan@... NCBI Blast EU590652.1 Lac... Lactobacillus acidophilus ATCC:4356

blast.ncbi.nlm.nih.gov/blast.cgi

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information Sign in to NCBI

BLAST⁺ » vector contamination » RID-NF50KCEA014 Home Recent Results Saved Strategies Help

» Formatting options » Download

BLAST Results

Vecscreen

Job title: EU590652.1 Lactobacillus acidophilus ATCC:4356

RID: NF50KCEA014 (Expires on 09-09 22:24 pm)

Query ID: lclQuery_26445

Description: EU590652.1 Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene, complete cds

Molecule type: dna

Query Length: 2017

Database Name: screen/Univec

Description: Univec (built 10.0)

Program: BLASTN 2.10.1+ » Citation

Interpretation of VecScreen Results

No significant similarity found. For reasons why, click here

Other reports: » Search Summary

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Support center Mailing list

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Activate Windows Questions/comments

Lec_BTE-401_Pred...ppt Lec_Gene-Cloning.pptx

Show all

Type here to search

8:25 PM 9/9/2020

Answer to the question no: 02

For finding promoter of Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene, we used BPRM because the organism is prokaryotic. First, went to the website:

<http://linux1.softberry.com/berry.phtml>

Here, selected BPRM from menu:

Word Document16.docx Document15.docx Quiz Question for Sec BTE_401_Quiz_2_Questi Fwd:-

Not secure linux1.softberry.com/berry.phtml

SoftBerry

This is outdated Softberry web archive, use the current Softberry Web Site (www.softberry.com)

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TEST ON LINE

- GENE FINDING in Eukarya
- GENE FINDING WITH SIMILARITY
- OPERON AND GENE FINDING IN BACTERIA
- GENE FINDING IN VIRUSES
- NEXT GENERATION Sequences&Genomes
- ALIGNMENT
- GenomeSequence EXPLORERIntegene
- SEARCH FOR MOTIFS /promoters&functional
- PROTEIN LOCATION /patterns/Epitops
- RNA STRUCTURE COMPUTING
- PROTEIN STRUCTURE
- PROTEIN / DNA 3D-Visual Works
- SEGMAN
- MULTIPLE ALIGNMENTS
- ANALYSIS OF EXPRESSION DATA
- PLANT PROMOTERS DATABASE
- REPEATS /find&map repeats
- SNP Extracting known SNPs
- Proteomics

CASE STUDIES:

- Annotation of animal genomes: genes, promoters, functional motifs, protein sub-cellular localization: Softberry software, solutions and services.
- Annotation of plant genomes: genes, promoters, functional motifs, protein sub-cellular localization: Softberry software, solutions and services.
- Annotation of Bacterial Genomes and Community Sequences: Genes, Operons, Promoters, Terminators, Protein Sub-Cellular Localization.
- Analyze RNASeq Next Generation Sequencing Data:
 - Accurate alignment of high-throughput RNA-seq data to a reference genome (ReadsMap);
 - De novo transcriptome reads assembly into RNA transcripts (TransSeq);
- Transomics - pipeline to map RNAseq data, assemble them into transcripts and quantify the abundance of these transcripts in particular datasets.
- Analyze Genomic Next Generation Sequencing Data:
 - De novo reconstruction (assembling) of genomic sequence;
 - Reconstruction of sequences using reference genome;
 - Mutation profiling and SNP discovery (OligoZip Assembler);
 - Functional analysis of SNP (SNP-effect);

For ACADEMIA UNIVERSITY Research

For BIOTECH and PHARMA Companies

Publications by Topics
Publications by program

Recent News

August 10, 2013. Softberry developed parameters for 30 new genomes, for use with FGENESH suite of gene prediction programs on its own or in conjunction with Transomics pipeline, which uses next generation sequencing data analysis to discover alternative splice variants.

December 24, 2012. Softberry provides free download of about 100 genome and protein analysis programs for use in research academic research. [Download Page.](#)

>All news...

Software Summary Software manuals Applied in hundreds Publications nature

Automatic genome annotation
Eukaryotic animal, plant, fungi
Bacterial and bacterial community DNA
Visualization of annotations
GenomeSequence Explorer
Visualization of Bacterial genome comparison and annotation

Sequence comparison
Alignment of genomic sequences
Multiple alignment and tree construction
Fast search in genomes

Analysis of Gene Regulation
Promoter prediction for animal and plant genes

Protein 3D-structure analysis and modeling
Assignment of secondary structure and accessibility
Gradients of 3D structures
Isotopic coordinates

linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb

Lec_BTE-401_Pred...pptx Lec_Gene-Cloning.pptx

Type here to search

Here, we pasted the sequence in FASTA format

Word Document16.docx Document15.docx Quiz Question for Sec BTE_401_Quiz_2_Questi Fwd

Not secure linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb

SoftBerry This is outdated Softberry web archive, use the current Softberry Web Site (www.softberry.com)

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TEST ON LINE

- GENE FINDING in Eukaryota
- GENE FINDING WITH SIMILARITY
- OPERON AND GENE FINDING IN BACTERIA
- GENE FINDING IN VIRUSES
- NEXT GENERATION
- ALIGNMENT /Sequences&genomes
- GenomeSequence EXPLORER/Infogene
- SEARCH FOR MOTIFS /promoters&functional
- PROTEIN LOCATION /patterns/Epitops
- RNA STRUCTURE COMPUTING
- PROTEIN STRUCTURE
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- SEQMAN
- MULTIPLE ALIGNMENTS
- ANALYSIS OF EXPRESSION DATA
- PLANT PROMOTERS DATABASE
- REPEATS /find&map repeats
- SNP Extracting known SNPs
- Proteomics

BPRom

Reference: V. Solovyev, A. Salamov (2011) Automatic Annotation of Microbial Genomes and Metagenomic Sequences. In Metagenomics and its Applications in Agriculture, Biomedicine and Environmental Studies (Ed. R.W. Li), Nova Science Publishers, p. 61-78

BPRom - Prediction of bacterial promoters

BPRom is bacterial sigma70 promoter recognition program with about 80% accuracy and specificity. It is best used in regions immediately upstream from ORF start for improved gene and operon prediction in bacteria.

Paste nucleotide sequence here (plain or in fasta format):

```
AGGTTATGAAGATATTTTAAC
GGTCAAAAGCTCATAAGATATGAAAGGTTGGGATGTTCAAGTATTG
GGAATAG
```

Alternatively, load a local file with sequence:

Local file name: Choose File No file chosen

PROCESS **RESET**

[\[Help\]](#)
[\[Example\]](#)

Return to page with other programs of group: [Operon and gene finding in bacteria](#)

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Last modification date: 24 Sep 2013

Document16 (1).pdf Document16.pdf Lec_BTE-401_Pred....pp... Lec_Gene-Cloning.pptx

Type here to search

Then after pressing "process" option, we could view the possible promoters for this sequence. Here, the sequence suggested 5 possible promoters. Moreover, the site also shows some TF binding sites, which influence the transcription process.


```
Word x Document16.docx x Document15.docx x Quiz Question for Sec 0 x BTE_401_Quiz_2_Questi x Fwd: - ma
Not secure | linux1.softberry.com/cgi-bin/programs/gfindb/bprom.pl

>EU590652.1 Lactobacillus acidophilus ATCC:4356 beta-galactosidase (lacZ) gene,
Length of sequence- 2017
Threshold for promoters - 0.20
Number of predicted promoters - 5
Promoter Pos: 62 LDF- 5.06
-10 box at pos. 47 GATTATAAT Score 76
-35 box at pos. 23 TTATCA Score 30
Promoter Pos: 866 LDF- 4.86
-10 box at pos. 851 ACTTATGAT Score 58
-35 box at pos. 832 TTCATA Score 38
Promoter Pos: 1852 LDF- 4.64
-10 box at pos. 1837 TCATAAACT Score 51
-35 box at pos. 1815 TTGAAT Score 55
Promoter Pos: 524 LDF- 3.53
-10 box at pos. 509 TTGAAGAAT Score 34
-35 box at pos. 484 TTGTCA Score 53
Promoter Pos: 1497 LDF- 2.58
-10 box at pos. 1482 TGGTGAAT Score 39
-35 box at pos. 1458 TTGTCA Score 53

Oligonucleotides from known TF binding sites:

For promoter at 62:
ihf: AAATAAAA at position 7 Score - 13
ihf: AATAAAAT at position 8 Score - 10
fis: ACAATTAT at position 19 Score - 8
For promoter at 866:
rpoD16: ATTTCATA at position 830 Score - 11
rpoD19: TTTCATAT at position 831 Score - 11
dnaA: AGTTATCC at position 842 Score - 6
For promoter at 1852:
lrp: ATAACTAA at position 1850 Score - 14
tus: TAACTAAG at position 1851 Score - 12
For promoter at 524:
rpoD17: AATAAATA at position 515 Score - 11
fnr: ATAAATAT at position 516 Score - 9
For promoter at 1497:
fadR: AACTCATC at position 1487 Score - 5
```

Answer to the question no : 03

For finding out which restriction enzymes can cut this sequence, we used NEBCUTTER version 2 (<http://www.labtools.us/nebcutter-v2-0/>).

Word Document16.docx Quiz Question for Sec 02 BTE_401_Quiz_2_Question Fed - mahfuzulhasan@... Primer-Blast results Lactobacillus acidophilus X NEBcutter V2.0 - LabTools

Not secure labtools.us/nebutter-v2-0/

NEBcutter V2.0 [Program Guide](#) [Help](#) [Comments](#)

This tool will take a DNA sequence and find the large, non-overlapping open reading frames using the E. coli genetic code and the sites for all Type II and commercially available Type III restriction enzymes that cut the sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence and "submit". Further options will appear with the output. **The maximum size of the input file is 1 MByte, and the maximum sequence length is 300 KBases.**
[What's new in V2.0](#) [Using NEBcutter](#)

Local sequence file: No file chosen

GenBank number: [Browse GenBank](#)

or paste in your DNA sequence: (plain or FASTA format)

```
>EU590652.1 Lactobacillus acidophilus ATCC:4356 beta-galactosidase  
[lacZ] gene, complete cds  
TAGAGGAATAAATBACACGATTATTCACGTTTCTTATBGTBGTGATTATCTGACCAATG  
GCCA  
GAAGAGACATGTCGAAAGATATTCACGTATTTAAAGAGCGGATTAATTCCGACACGATTAAAC  
ATTT  
TTTCTTGGGCTTGCTTGACCAAGAGAGAAATATAATTTCTCAAAATAGATAAGTTGAC  
AACCA
```

Standard sequences:
Plasmid vectors
Viral + phage

☒ NEB enzymes
☐ All commercially available specificities
Enzymes to use: ☐ All specificities
☐ All + defined oligonucleotide sequences
☐ Only defined oligonucleotide sequences
[\[define oligos\]](#)

The sequence is: ☒ Linear ☐ Circular

Minimum ORF length to display: 100 a.a.

Name of sequence: (optional)

Earlier projects:

Note: Your earlier projects will be deleted 2 days after they were last accessed.
You need to have cookies enabled in your browser for this feature to work.

☐ Disable NEBcutter cookies

Activate Windows
Go to Settings to activate Windows.

Document16 (1).pdf Document16.pdf LAC_401_Pred...pptx LAC_Gene-Cloning.pptx

Type here to search

9:53 PM 9/6/2020

Then after pressing "Submit", the tool provided a list of restriction enzymes which can be used for cutting this DNA sequence:



Linear Sequence: EU590652.1 Lactobac

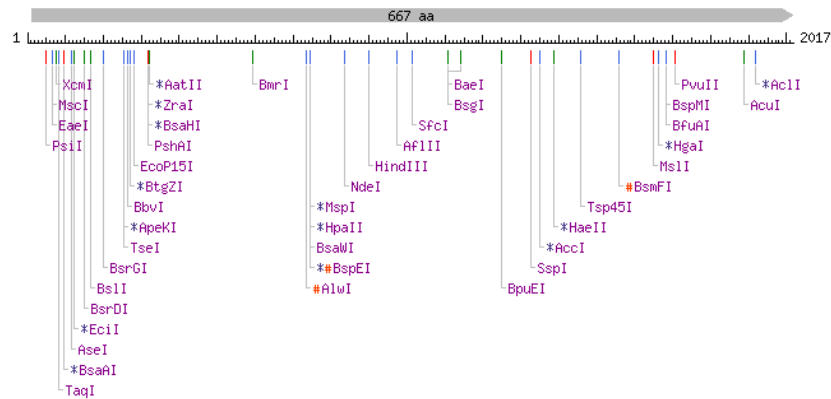
Help

Comments

Display: - NEB single cutter restriction enzymes
- Main non-overlapping, min. 100 aa ORFs

GC=37%, AT=63%

Cleavage code	Enzyme name code
blunt end cut	Available from NEB
5' extension	Has other supplier
3' extension	Not commercially available
cuts 1 strand	*: cleavage affected by CpG meth.
	#: cleavage affected by other meth.
	(enz.name): ambiguous site



Main options
New DNA
Custom digest
View sequence
ORF summary
Save project
Print

Availability
All commercial
All

Display
2 cutters
3 cutters

Zoom
Zoom in
More...

Minimum ORF length to display: 100 aa. OK

List
0 cutters
1 cutters
All sites
Save all sites
Flanking enzymes

Answer to the question no 04

Here, for designing primers for this gene, PRIMER-BLAST was used. First, visited the website:(
<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>)

The sequence obtained from NCBI-GenBank was placed on the query box.

Here, the default conditions provided by the database was used for primer detection,

Word Document16.docx Document15.docx Quiz Question for Sec 02 BTE-401_Quiz_2_Question... Fed - mahmud.hasan@g... Primer designing tool Lactobacillus acidophilus

ncbi.nlm.nih.gov/tools/primer-blast/

Finding primers specific to your PCR template (using Primer3 and BLAST).

Reset page Save search parameters Retrieve recent results Publication Tips for finding specific primers

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) [Help](#) [Copy](#)

FASTA sequence: `GAATGATTTTCTGAGAGAGGACGCTTTCACGAGATTCATATTCATGATATTTTAAAGAGGAGGCTTGTCTATTT
AGTCAAGGCTTGAACATCAGAGGCTTTCATATTCATGATATTTTAAAGAGGAGGCTTGTCTATTT
GATTTTCTGAGAGAGGACGCTTTCACGAGATTCATATTCATGATATTTTAAAGAGGAGGCTTGTCTATTT
TGTCTTATGATATTTTAAAGAGGAGGCTTTCACGAGATTCATATTCATGATATTTTAAAGAGGAGGCTTGTCTATTT`

OR Refseq FASTA file [Choose File](#) No file chosen

Range

Forward primer: From To Clear

Reverse primer: From To Clear

Primer Parameters

Use my own forward primer (5' to 3' on plus strand) Clear

Use my own reverse primer (5' to 3' on minus strand) Clear

PCR product size

Min: 70 Max: 1000

of primers to return

Min: 10

Primer melting temperatures (T_m)

Min: 57.0 Opt: 60.0 Max: 63.0 Max T_m difference: 3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section [Help](#)

Exon junction span

No preference [Help](#)

Exon junction match

Min 5' match: 7 Min 3' match: 4 Max 3' match: 8

Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction [Help](#)

Intron inclusion

☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA [Help](#)

Intron length range

Min: 1000 Max: 1000000 [Help](#)

Primer Pair Specificity Checking Parameters

Specificity check

☒ Enable search for primer pairs specific to the intended PCR template [Help](#)

Search mode

Automatic [Help](#)

Database

Refseq mRNA [Help](#)

Exclusion

☐ Exclude predicted Refseq transcripts (accession with XM, XR prefix) ☐ Exclude uncultured/environmental sample sequences [Help](#)

Organism

Homo sapiens

Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type. [Add more organisms](#)

Entrez query (optional) [Help](#)

Primer specificity stringency

Primer must have at least 2 total mismatches to unintended targets, including

Max target size

4000 [Help](#)

Allow splice variants

☐ Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input) [Help](#)

Get Primers [Help](#)

☐ Show results in a new window ☒ Use new graphic view [Help](#)

Advanced parameters

Activate Windows

Document16.pdf Lec_BTE-401_Pred...pp... Lec_Gene-Cloning.pptx

Type here to search

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Then, clicked the option of “get primers”.

Word Document16.docx Document15.docx Quiz Question for Sec 02 BTE-401_Quiz_2_Question... Fed - mahmud.hasan@g... Primer designing tool Lactobacillus acidophilus

ncbi.nlm.nih.gov/tools/primer-blast/

Finding primers specific to your PCR template (using Primer3 and BLAST).

Reset page Save search parameters Retrieve recent results Publication Tips for finding specific primers

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) [Help](#) [Copy](#)

FASTA sequence: `GAATGATTTTCTGAGAGAGGACGCTTTCACGAGATTCATATTCATGATATTTTAAAGAGGAGGCTTGTCTATTT
AGTCAAGGCTTGAACATCAGAGGCTTTCATATTCATGATATTTTAAAGAGGAGGCTTGTCTATTT
GATTTTCTGAGAGAGGACGCTTTCACGAGATTCATATTCATGATATTTTAAAGAGGAGGCTTGTCTATTT
TGTCTTATGATATTTTAAAGAGGAGGCTTTCACGAGATTCATATTCATGATATTTTAAAGAGGAGGCTTGTCTATTT`

OR Refseq FASTA file [Choose File](#) No file chosen

Range

Forward primer: From To Clear

Reverse primer: From To Clear

Primer Parameters

Use my own forward primer (5' to 3' on plus strand) Clear

Use my own reverse primer (5' to 3' on minus strand) Clear

PCR product size

Min: 70 Max: 1000

of primers to return

Min: 10

Primer melting temperatures (T_m)

Min: 57.0 Opt: 60.0 Max: 63.0 Max T_m difference: 3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section [Help](#)

Exon junction span

No preference [Help](#)

Exon junction match

Min 5' match: 7 Min 3' match: 4 Max 3' match: 8

Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction [Help](#)

Intron inclusion

☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA [Help](#)

Intron length range

Min: 1000 Max: 1000000 [Help](#)

Primer Pair Specificity Checking Parameters

Specificity check

☒ Enable search for primer pairs specific to the intended PCR template [Help](#)

Search mode

Automatic [Help](#)

Database

Refseq mRNA [Help](#)

Exclusion

☐ Exclude predicted Refseq transcripts (accession with XM, XR prefix) ☐ Exclude uncultured/environmental sample sequences [Help](#)

Organism

Homo sapiens

Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type. [Add more organisms](#)

Entrez query (optional) [Help](#)

Primer specificity stringency

Primer must have at least 2 total mismatches to unintended targets, including

at least 2 mismatches within the last 5 bps at the 3' end [Help](#)

Ignore targets that have 6 or more mismatches to the primer [Help](#)

Max target size

4000 [Help](#)

Allow splice variants

☐ Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input) [Help](#)

Get Primers [Help](#)

☐ Show results in a new window ☒ Use new graphic view [Help](#)

Advanced parameters

Activate Windows

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The result suggested 10 pairs of primer.

