المعمل الثامن:

تصميم البادىء لتفاعل إنزيم البلمرة التسلسلي
Designing PCR Primers

251Mbio

Amal Alghamdi

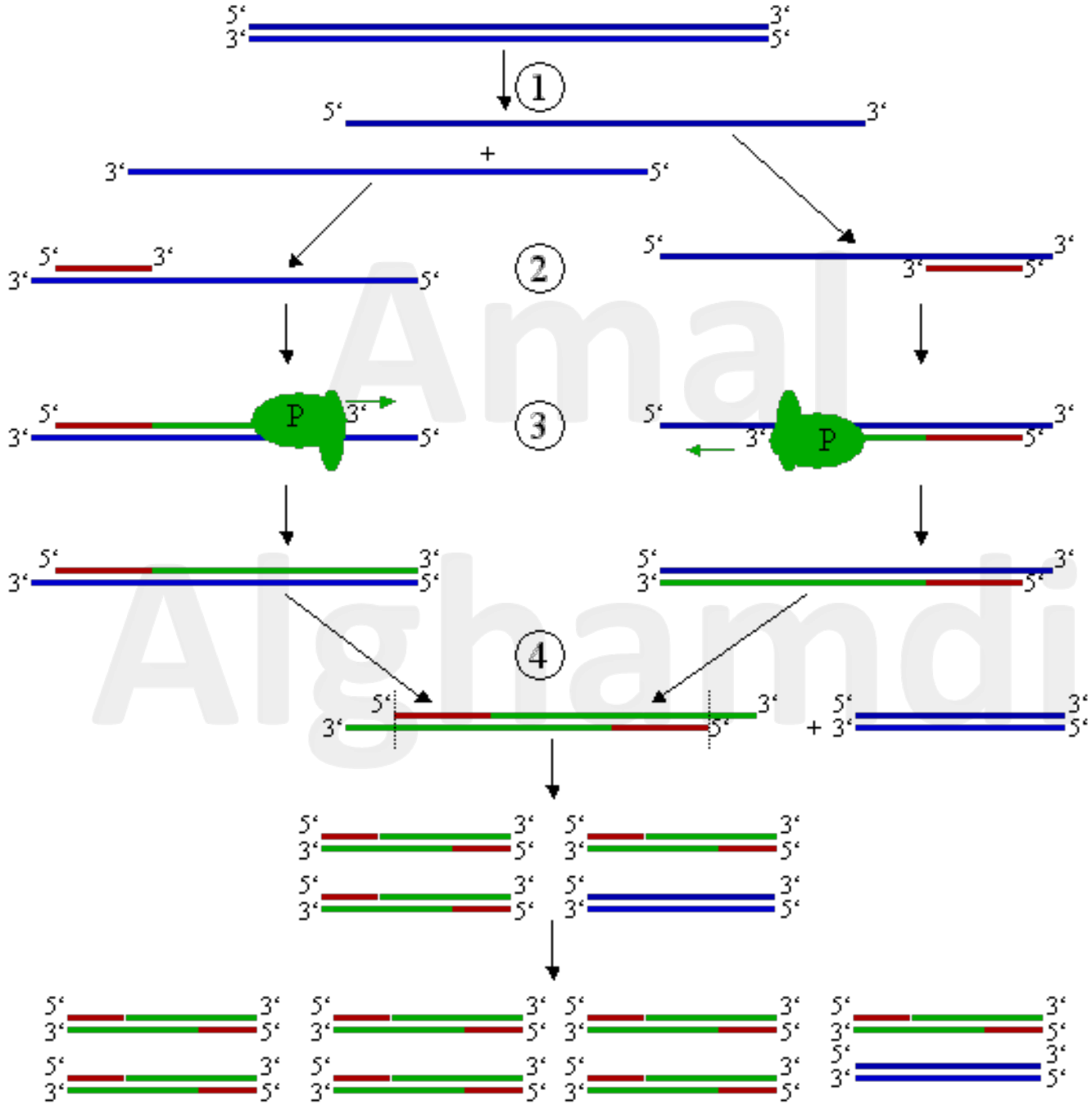
2018

بعض المصطلحات الهامة Terminologies

- البادييء Primer .
- تتابع القالب Template sequence.
- درجة الذوبان (Tm) Melting Temperature .
- درجة الإرتباط (Ta) Annealing Temperature .
- تفاعل انزيم البلمرة التسلسليي PCR.

الأنواع المختلفة لتفاعل إنزيم البلمرة التسلسلي

- Standard PCR.
- Nested PCR
- Touch down PCR
- Sequencing PCR
- Intersequence--specific PCR (ISSR)
- والكثير غيرها.



مصدر التتابع القالب
Origin of the Template Sequence

- تتابع خاص بالباحث Self-generated.

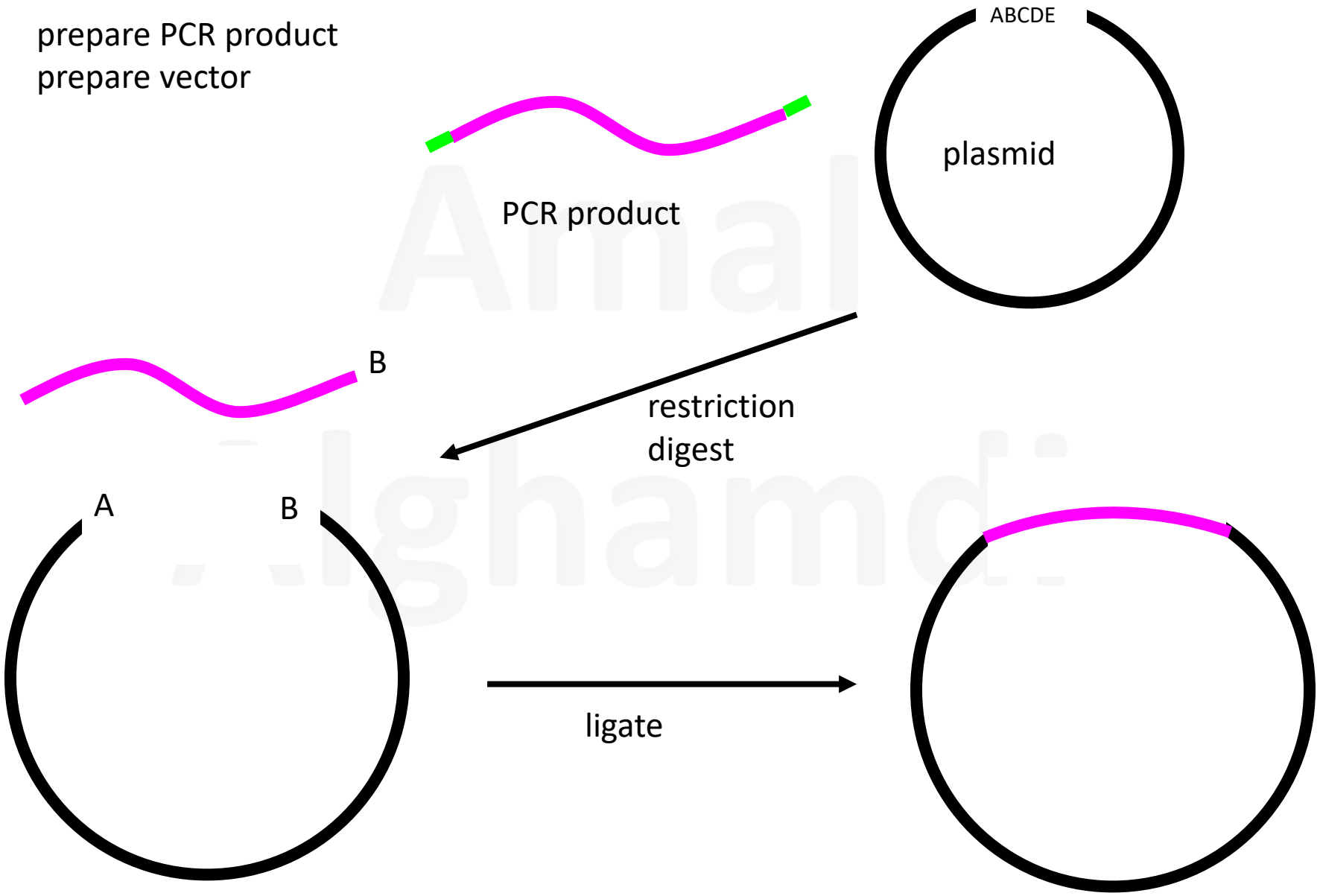
- تتابع نيوكليوتيدي من قاعدة بيانات التتابعات النيوكليوتيدية Nucelotide Sequence Databases مثل (NCBI's و GenBank).

- *الحصول على التتابع الصحيح من قاعدة البيانات*
- *ينسخ التتابع في قاعدة البيانات لإستخراج التتابعات المشابهة من نفس الكائن الحي تحت الدراسة.*

ما هو الباديء Primer؟

- هو تتابع قصير من النيوكليوتيدات، يتم تصميمه طبقاً لتتابع معاكس ومكمل لمنطقة محددة من جينوم الحمض النووي DNA المستهدفة.

prepare PCR product
prepare vector



أهم مواصفات تتابع البادىء Primer

- يتراوح طوله بين 18 – 28 نيوكليوتيدة.
- نسبة GC له 50-60 %.
- يحتوي توزيع متوازن من القواعد النيتروجينية G/C و A/T.

تتابع صالح

5’ ATGCACTCAGACGTACAACG TGAC 3’

24 bases

AT: 12

GC: 12 (50% GC)

Balanced distributon

Ta = 65°C

تتابع غير صالح

5’ AACAAACGATTTTTT 3’

17 bases

AT: 14

GC: 3 (18 % GC)

Unbalanced distributon

Ta= 38°C

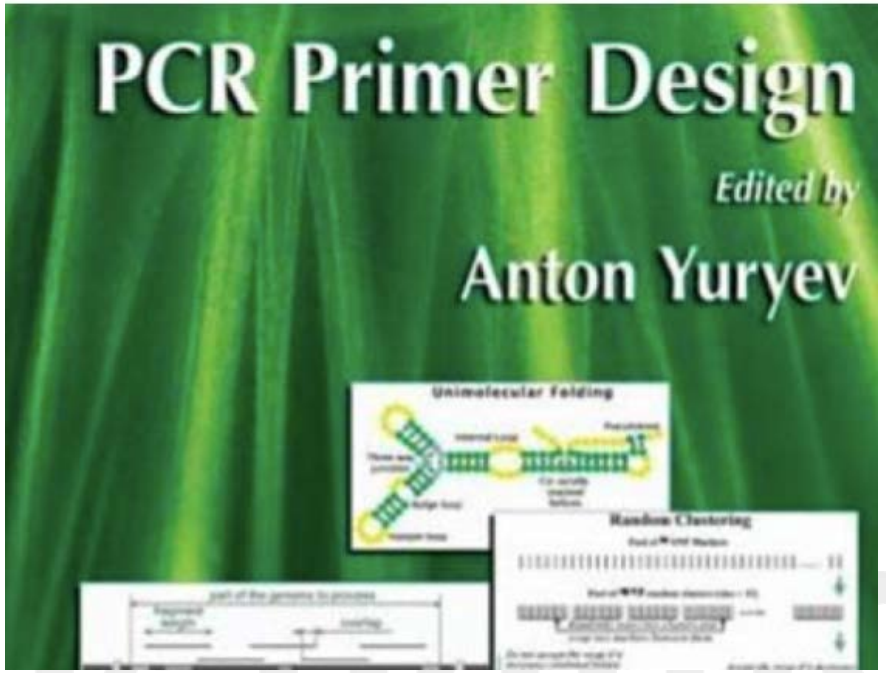
الغرض من تصميم البادىء Purpose

- لغرض الكلونة Gene cloning.
- للإختبارات التشخيصية باستخدام تقنية PCR.
- للتقدير الكمي للجينات أو نسخ الحمض النووي RNA (Transcript).

مصادر تصميم البادىء على الإنترنت
Sources of Primer Design

- CLC Workbench
- Primer3
- Primer3 Plus
- PrimerZ
- PerlPrimer
- وغيره االكثير بالبحث في محرك البحث (Google).

Reference المرجع



- **Freely accessible Primer Design Book**
  - Explains the design of complex primers blow by blow:

hmp://vetbiotech.um.ac.ir/parameters/vetbiotech/filemanager/new_admin/books/PCR%20Primer%20Design.pdf

مثال: جين lacZ gene في جينوم بكتيريا Escherichia coli

- يحتوي البلازميد pUC18 على تتابع لجين Lac Z الذي يشفر إنزيم الجالاكتوسيداز b-galactosidase.
- يحتوي هذا البلازميد على جين المقاومة للمضاد الحيوي Ampicillin.

مثال: جين lacZ gene في جينوم بكتيريا Escherichia coli

5’ end of lacZ gene

3’ end of lacZ gene

```
5’ atgaccatga ttacggattc actggccgtc    5’ gctaccatta  ccagttggtc tggtgtcaaa aataa 3’
3’ tactggtact aatgcctaag tgaccggcag    3’ cgatggtaat  ggtcaaccag accacagttt ttatt 5’
```

```
atg acc atg att acg gat tca ctg gcc gtc          cag ttg gtc tgg tgt caa aaa taa
M   T   M   I   T   D   S   L   A   V            Q   L   V   W   C   Q   K   *
```

Full Report ▾ Send to: ▾

lacZ beta-D-galactosidase [*Escherichia coli str. K-12 substr. MG1655*]

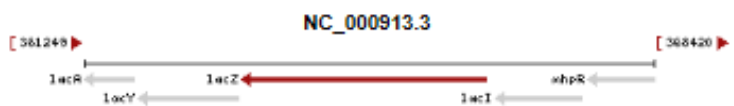
Gene ID: 945006, updated on 4-Feb-2018

Summary

Gene symbol	lacZ
Gene description	beta-D-galactosidase
Primary source	EcoGene:EG10527
Locus tag	b0344
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Escherichia coli str. K-12 substr. MG1655 (strain: K-12, substrain: MG1655)
Lineage	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia
Also known as	ECK0341; JW0335
Summary	Repressed during biofilm formation. [More information is available at EcoGene: EG10527]. galacotsidase is a metalloenzyme exhibiting broad substrate specificity. [More information is available at EcoCyc: EG10527].

Genomic context

Sequence: NC_000913.3 (363231..366305, complement)



Genomic regions, transcripts, and products

Genomic Sequence: NC_000913.3

Go to reference sequence details

Go to nucleotide: Graphics FASTA GenBank

1- البحث عن التتابع النيوكليوتيدي للجين PuC 18 :

- تم اختيار **Synthetic construct DNA, plasmid vector pUC18 including artificial sequence** (GenBank: LC129268.1).
- https://www.ncbi.nlm.nih.gov/nuccore/LC129268.1?&feature=any

- بعد اختيار FASTA يتم اختيار highlight sequence feature
- يتم تظليل exons الخاصة بالجين
- يتم نسخ التتابعات المظللة ثم نقلها إلى Primer3web

Synthetic construct DNA, plasmid vector pUC18 including artificial sequence

GenBank: LC129268.1

FASTA Graphics

Go to:

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  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 2808)
  AUTHORS   Takahashi,M. and Goto-Yamamoto,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (03-MAR-2016) Contact:Masayuki Takahashi National
            Research Institute of Brewing, Technology Development Research
            Division; 3-7-1 Kagamiyama, Higashi-hiroshima, Hiroshima 739-0046,
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      301 tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt
      361 tttcccagtc acgacgttgt aaaacgacgg ccagtgccaa gcttgcatgc ctgcaggtcg
```

Customize view

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

Related information
- Taxonomy

Recent activity

Turn Off Clear

- Synthetic construct DNA, plasmid vector pUC18 including artificial sequence — Nucleotide
- pUC18 vector (1388) — Nucleotide
- pUC18 cloning vector — Nucleotide
- Highlight Sequence Features - NCBI Video Vault
- pUC18 (19816) — Nucleotide

See more...

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439..560
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NCBI Resources How To — Sign in to NCBI

Nucleotide — Nucleotide — Search — Advanced — Help

FASTA — Send to:

Synthetic construct DNA, plasmid vector pUC18 including artificial sequence

GenBank: LC129268.1

GenBank Graphics

```
>LC129268.1:439-560 Synthetic construct DNA, plasmid vector pUC18 including artificial
sequence
AACTAATACGACTCACTATAGGGTCCGATCTTCCGAGGTCTCATATCGATCGGTAGGGCATCTAATGGCT
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Change region shown
- Whole sequence
- Selected region

from: 439 to: 560

Update View

Customize view

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

Related information
- Taxonomy

NCBI Resources How To

Nucleotide Nucleotide Advanced

GenBank

E. coli gene lacZ coding for beta-galactosidase (EC 3.2.1.23)

GenBank: V00296.1

FASTA Graphics

Go to:

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REFERENCE   1  (bases 1 to 3078)
  AUTHORS   Kalnins,A., Otto,K., Ruther,U. and Muller-Hill,B.
  TITLE     Sequence of the lacZ gene of Escherichia coli
  JOURNAL   EMBO J. 2 (4), 593-597 (1983)
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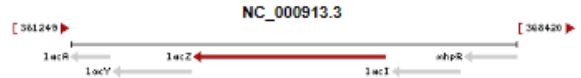
Genomic context

Sequence: NC_000913.3 (363231..366305, complement)

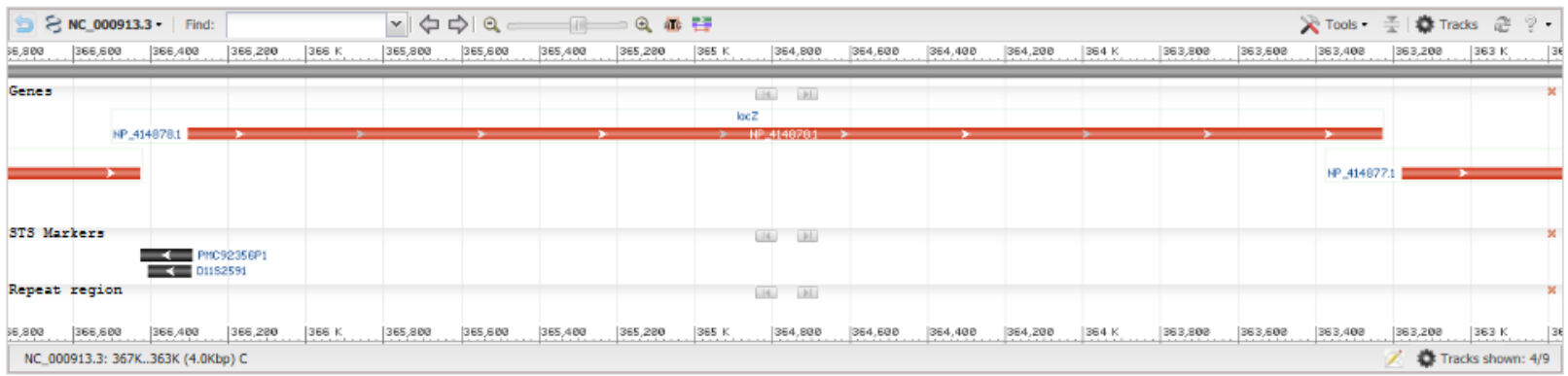


Genomic regions, transcripts, and products

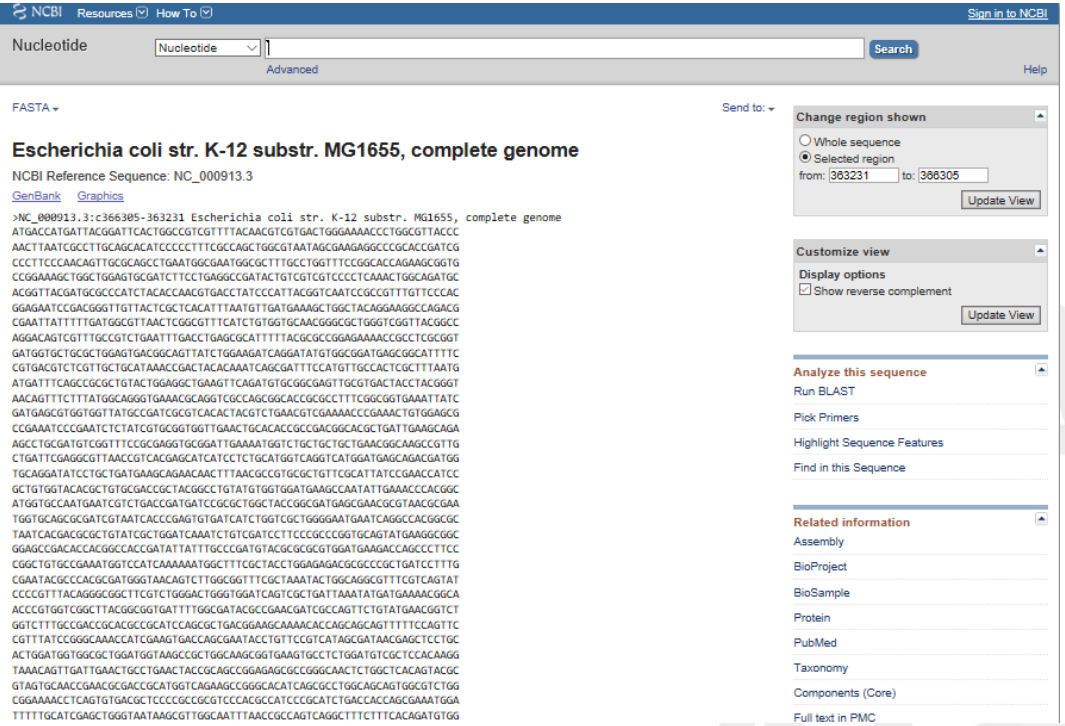
Genomic Sequence: NC_000913.3

Go to reference sequence details

Go to nucleotide: Graphics FASTA GenBank



Bibliography


NCBI Resources How To Sign in to NCBI
Nucleotide
Nucleotide Search
Advanced Help
FASTA Send to:
Escherichia coli str. K-12 substr. MG1655, complete genome
NCBI Reference Sequence: NC_000913.3
GenBank Graphics
>NC_000913.3:c366305-363231 Escherichia coli str. K-12 substr. MG1655, complete genome
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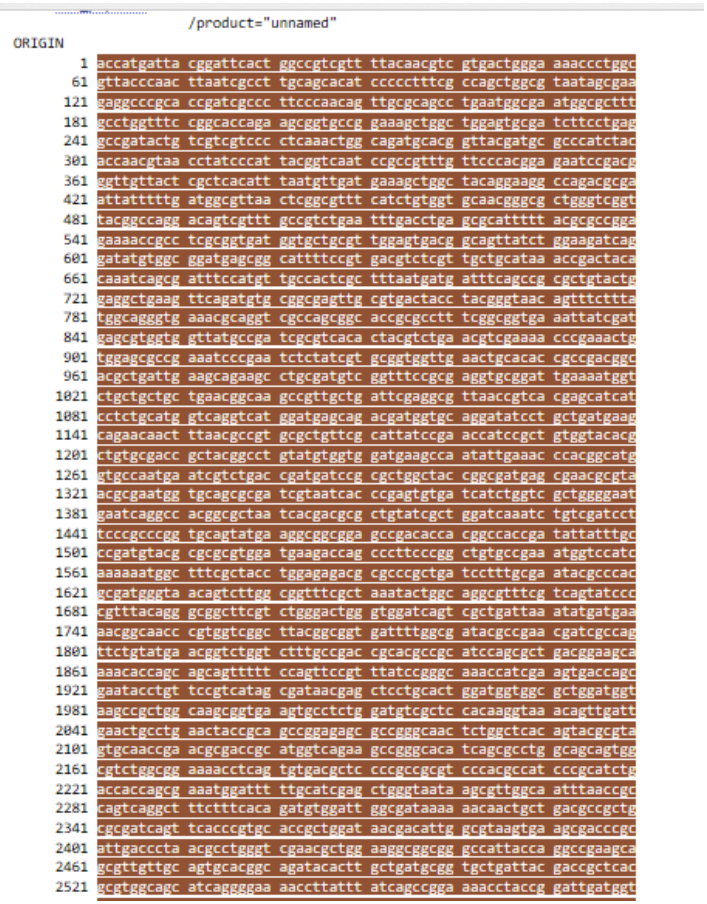
Change region shown
Whole sequence
Selected region
from: 363231 to: 366305
Update View
Customize view
Display options
Show reverse complement
Update View
Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence
Related information
Assembly
BioProject
BioSample
Protein
PubMed
Taxonomy
Components (Core)
Full text in PMC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2- النسخ في Primer3web

- يتم لصق التتابع الخاص في المكان المخصص
- يتم اختيار Pick Primer
- تظهر تتابع الباديء الأمامي والعكسي.
- http://primer3.ut.ee/cgi-bin/primer3/primer3web_results.cgi

Primer3 Input
primer3.ut.ee/
All rights reserved. This file is part of the **primer3web** suite. The primer3 suite and libraries are free software; you can redistribute them and/or modify them under the terms of the GNU General Public License as published by the Free Software Foundation; either version 2 of the License, or (at your option) any later version.
Department of Bioinformatics · Primer3Web Release 4.1.0 ... · Primer3 k-mer lists

People also search for ×

primer3 manual netprimer
primer3 download ucsc in silico pcr
snp check primerblast

Primer3 Input (version 0.4.0)
bioinfo.ut.ee/primer3-0.4.0/
There is a newer version of Primer3 available at http://primer3.ut.ee. Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N ...

Run Primer3Web - Primer3Plus
https://primer3plus.com/primer3web/primer3web_input.htm
Pick left primer, or use left primer below, Pick hybridization probe (internal oligo), or use oligo below, Pick right primer, or use right primer below (5' to 3' on opposite strand). 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 ...

Primer3Plus
https://primer3plus.com/
Primer3 Development. On this place the efforts of developing Primer3, Primer3Plus and **Primer3Web** are coordinated. Please go there for bug reports, feature requests and support.

Primer3web version 4.1.0 - Pick primers from a DNA sequence.

disclaimer | code | cautions

Select the Task for primer selection generic

Template masking before primer design (available species)

Select species	Example: Mus musculu	Nucleotides to mask in 5' direction	1
Primer failure rate cutoff	< 0.1	Nucleotides to mask in 3' direction	0

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library) NONE

☑ Pick left primer, or use left primer below	☐ Pick hybridization probe (internal oligo), or use oligo below	☑ Pick right primer, or use right primer below (5' to 3' on opposite strand)

Pick Primers | Download Settings | Reset Form

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 -: e.g. ...ATCTAC-TGTCAT.. means that primers must overlap the junction between the C and T.
Excluded Regions		E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.
Pair 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

Primer3web version 4.1.0 - Pick primers from a DNA sequence.

disclaimer | code | cautions

Select the Task for primer selection generic

Template masking before primer design (available species)

Select species Example: Mus musculu | Nucleotides to mask in 5' direction 1

Primer failure rate cutoff < 0.1 | Nucleotides to mask in 3' direction 0

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library) NONE

```
1 accatgatta cggattcact ggccgtcgtt ttacaacgtc gtgactggga aaaccctggc
      61 gttacccaac ttaatcgcct tgcagcacat ccccctttcg ccagctggcg taatagcgaa
     121 gaggcccgca ccgatcgccc ttcccaacag ttgcgcagcc tgaatggcga atggcgcttt
     181 gcctggtttc cggcaccaga agcggtgccg gaaagctggc tggagtgcga tcttcctgag
     241 gccgatactg tcgtcgtccc ctcaaactgg cagatgcacg gttacgatgc gcccatctac
     301 accaacgtaa cctatcccat tacggtcaat ccgccgtttg ttcccacgga gaatccgacg
```

☑ Pick left primer, or use left primer below | ☐ Pick hybridization probe (internal oligo), or use oligo below | ☑ Pick right primer, or use right primer below (5' to 3' on opposite strand)

Pick Primers | Download Settings | Reset Form

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 -: e.g. ....ATCTAC-TGTCAT.. means that primers must overlap the junction between the C and T.
Excluded Regions		E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and >: e.g. ....ATCT<CCCC>TCAT.. forbids primers in the central CCCC.
Pair 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 -10000 | Force Right Primer Start -10000

Force Left Primer End -10000 | Force Right Primer End -10000

Sequence Quality

Primer3 Output

WARNING: Numbers in input sequence were deleted.

```
PRIMER PICKING RESULTS FOR

Template masking not selected
No mispriming library specified
Using 1-based sequence positions
OLIGO             start  len       tm      gc%  any_th  3'_th hairpin seq
LEFT PRIMER          26   20    58.04    55.00    0.97   6.85    0.00 acgactcactatagggtccg
RIGHT PRIMER        130   21    58.01    47.62    0.00   0.00    0.00 gcatacaaatctgacatggcg
SEQUENCE SIZE: 150
INCLUDED REGION SIZE: 150

PRODUCT SIZE: 105, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

     1 actctagaggatccccggaactaatacgactcactatagggtccgatcttccgaggtctc
                               >>>>>>>>>>>>>>>>>>>>

    61 atatcgatcggtagggcatctaatggcttcggagttcaagggctatattcgccatgtcag
                                                     <<<<<<<<<<

   121 atttgtatgccaaaggccgggtaccgagct
           <<<<<<<<<<
```

```
KEYS (in order of precedence):
>>>>>> left primer
<<<<<< right primer
```

Primer3 Output

WARNING: Numbers in input sequence were deleted.

```
PRIMER PICKING RESULTS FOR

Template masking not selected
No mispriming library specified
Using 1-based sequence positions
```

OLIGO	start	len	tm	gc%	any_th	3'_th	hairpin	seq
LEFT PRIMER	2729	20	59.05	50.00	0.00	0.00	0.00	ttactgccgcctgttttgac
RIGHT PRIMER	2890	20	59.00	55.00	13.86	0.00	0.00	gactgtagcggctgatgttg

```
SEQUENCE SIZE: 3072
INCLUDED REGION SIZE: 3072

PRODUCT SIZE: 162, PAIR ANY_TH COMPL: 23.88, PAIR 3'_TH COMPL: 8.15

    1 accatgattacggattcactggccgtcgttttacaacgtcgtgactgggaaaaccctggc

   61 gttacccaacttaatcgccttgcagcacatccccctttcgccagctggcgtaatagcgaa

  121 gaggcccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgaatggcgcttt

  181 gcctggtttccggcaccagaagcggtgccggaaagctggctggagtgcgatcttcctgag

  241 gccgatactgtcgtcgtcccctcaaactggcagatgcacggttacgatgcgcccatctac

  301 accaacgtaacctatcccattacggtcaatccgccgtttgttcccacggagaatccgacg

  361 ggttgttactcgctcacatttaatgttgatgaaagctggctacaggaaggccagacgcga

  421 attatttttgatggcgttaactcggcgtttcatctgtggtgcaacgggcgctgggtcggt

  481 tacggccaggacagtcgtttgccgtctgaatttgacctgagcgcatttttacgcgccgga

  541 gaaaaccgcctcgcggtgatggtgctgcgttggagtgacggcagttatctggaagatcag

 2521 gcgtggcagcatcaggggaaaaccttatttatcagccggaaaacctaccggattgatggt

 2581 agtggtcaaatggcgattaccgttgatgttgaagtggcgagcgatacaccgcatccggcg

 2641 cggattggcctgaactgccagctggcgcaggtagcagagcgggtaaactggctcggatta

 2701 gggccgcaagaaaactatcccgaccgccttactgccgcctgttttgaccgctgggatctg
                                   >>>>>>>>>>>>>>>>>>>

 2761 ccattgtcagacatgtataccccgtacgtcttcccgagcgaaaacggtctgcgctgcggg

 2821 acgcgcgaattgaattatggcccacaccagtggcgcggcgacttccagttcaacatcagc
                                                     <<<<<<<<<<

 2881 cgctacagtcaacagcaactgatggaaaccagccatcgccatctgctgcacgcggaagaa
      <<<<<<<<<<

 2941 ggcacatggctgaatatcgacggtttccatatggggattggtggcgacgactcctggagc

 3001 ccgtcagtatcggcggaattccagctgagcgccggtcgctaccattaccagttggtctgg

 3061 tgtcaaaaataa


KEYS (in order of precedence):
>>>>>> left primer
<<<<<< right primer

ADDITIONAL OLIGOS
```

		start	len	tm	gc%	any_th	3'_th	hairpin	seq
1	LEFT PRIMER	2599	20	59.05	50.00	0.00	0.00	0.00	accgttgatgttgaagtggc
	RIGHT PRIMER	2841	20	58.96	50.00	26.76	26.76	0.00	gccataattcaattcgcgcg
	PRODUCT SIZE: 243, PAIR ANY_TH COMPL: 7.49, PAIR 3'_TH COMPL: 14.73								
2	LEFT PRIMER	72	20	58.98	50.00	0.00	0.00	0.00	taatcgccttgcagcacatc
	RIGHT PRIMER	252	20	58.89	55.00	0.00	0.00	0.00	gacagtatcggcctcaggaa
	PRODUCT SIZE: 181, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00								
3	LEFT PRIMER	2871	20	59.00	55.00	0.00	0.00	0.00	caacatcagccgctacagtc
	RIGHT PRIMER	3021	20	58.87	55.00	0.00	0.00	0.00	gaattccgccgatactgacg
	PRODUCT SIZE: 151, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 5.17								
4	LEFT PRIMER	887	20	58.97	50.00	0.00	0.00	0.00	aaaacccgaaactgtggagc
	RIGHT PRIMER	1118	20	58.87	50.00	0.00	0.00	0.00	accatcgtctgctcatccat
	PRODUCT SIZE: 232, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00								