PCR Primers Design

Dr Murtakab Y Al-Hejjaj

PhD Molecular Biology and Biotechnology

What are primers?

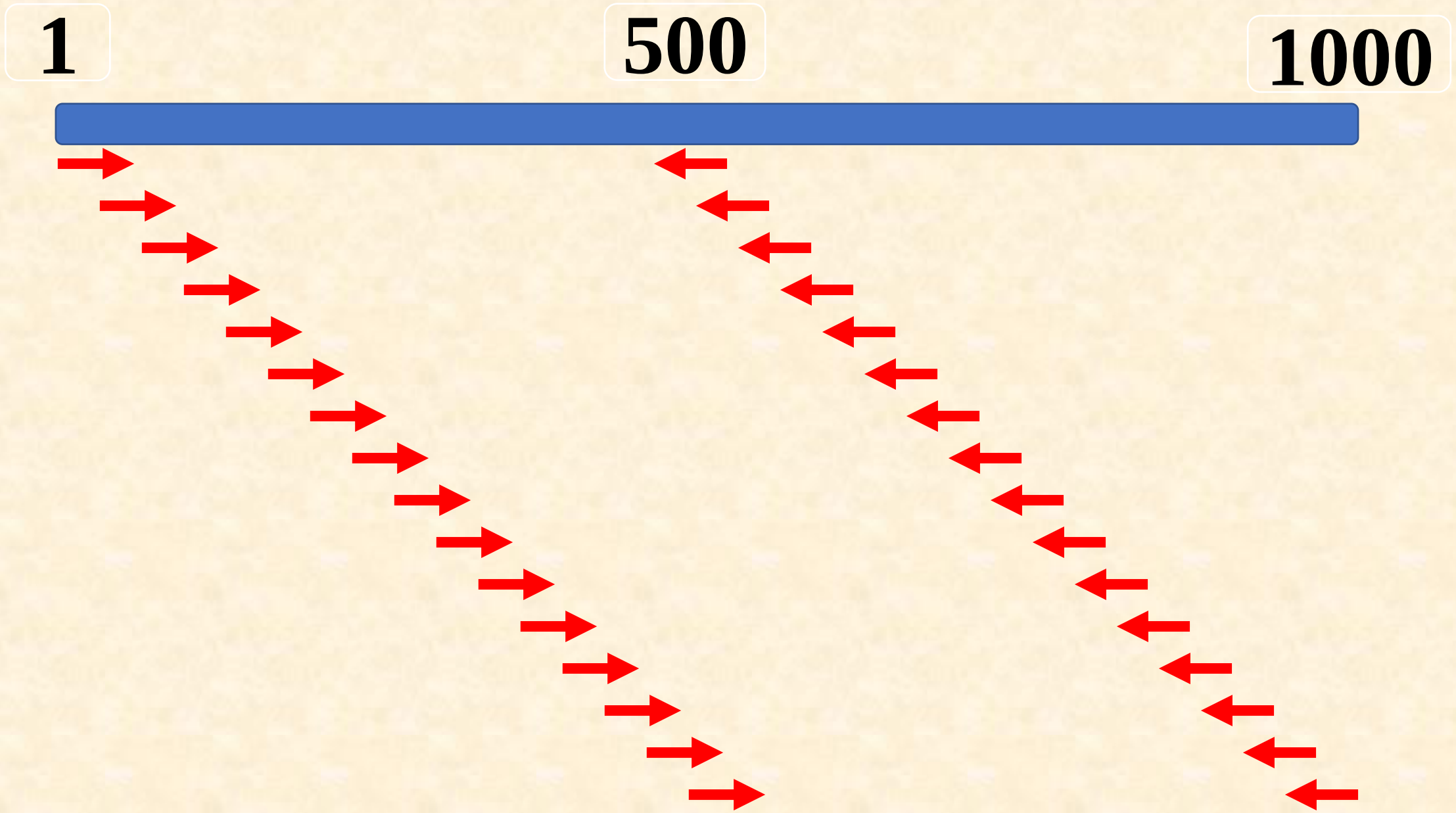
Primers

- Primers are short pieces of DNA or RNA
- Serves as a starting point for DNA synthesis.
- They are required for DNA replication, because the enzymes that catalyse this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA.
- In vivo DNA replication utilises short strands of RNA called RNA primers to initiate DNA synthesis on both the leading and lagging strands.

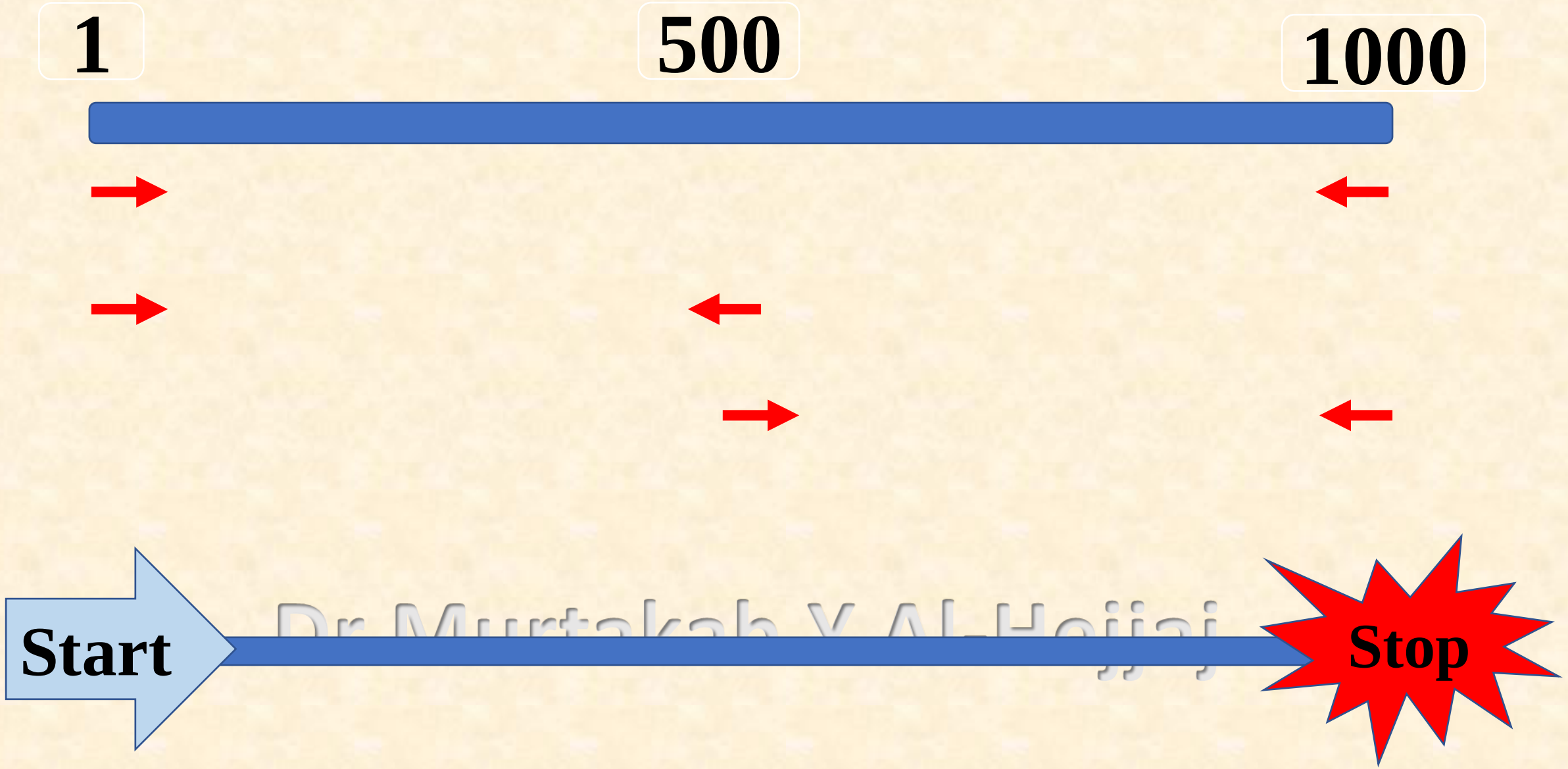
PCR primers usage

- DNA detection
- Cloning
- Making a cDNA
- etc

DNA detection



DNA cloning



Primer characterisation (properties)

- Single strand of DNA or RNA
- They are always specified 5' to 3', left to right
- **Generally**, about 18 to 24-30 bp in length.
- Should have a GC content between 40 and 60%.
- It is better to start with G or C and end by two Gs, Cs or GC/CG
- It is better not to contain more than three repeats of a single base or dinucleotide repeats (for example, ACCCC or ATATATAT) as this can cause primer mispriming.
- Annealing temperature (Ta) should range between 55°C and 65°C.
- Primer pairs should have similar Tm with a maximum difference of 5°C and should not be complementary to each other.

Examples

GCTAGAACTGATCGAGTCAG GCTAAAACTGATCGAGTCAT

Length Base(s) repeats start and end bases
Tm= 60-70˚C Ta = 50-65 ˚C Primer dimers GC% 40-60%

GCTACTACTACTACGAGTCAG GCTAGAACTGATCGAGTCAG

What do good primers look like?

Primer types (as detectors)

- Universal primers
- Genus specific primers
- Species specific primers

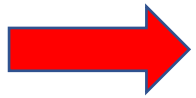
Choosing Primers

- An existing primers (designed by someone else)
- Your own primers (designed by your self)

How to: Design PCR primers and check them for specificity

Getting the target DNA sequence

- Obtain the sequence of the gene of interest from the database. Which is available for free in National Center for Biotechnology Information (NCBI) GenBank.



Primer designing tool | ncbi - Google Search

google.com/search?q=ncbi&rlz=1C1GGRV_enGB754GB754&oq=ncbi&aqs=chrome..69i57j0l6j69i60.1865j0j15&sourceid=chrome&ie=UTF-8

Apps | dictyBase Home | NetPrimer | free pri... | Overview | flyCRISPR | Gene Synthesis &... | The Higher Commit... | AVG Antivirus Free... | BSCB :: The British S... | Patient Access Patie...

Google

ncbi

All | Images | Books | Videos | News | More | Settings | Tools

About 77,600,000 results (0.51 seconds)

www.ncbi.nlm.nih.gov

National Center for Biotechnology Information

Welcome to **NCBI**. The **National Center for Biotechnology Information** advances science and health by providing access to biomedical and genomic information.

BLAST

BLASTn - Protein BLAST - Blastx - Nucleotide BLAST - ...

Gene

Gene. Gene integrates information from a wide range of species. A ...

Nucleotide

Advanced - RefSeq - Batch Entrez - ...

Protein

Protein. The Protein database is a collection of sequences from ...

PubMed

PubMed® comprises more than 30 million citations for biomedical ...

SRA

Sequence Read Archive (SRA) data, available through multiple ...

More results from nih.gov »

People also ask

National Center for Biotechnology Information

Company

ncbi.nlm.nih.gov

The National Center for Biotechnology Information is part of the United States National Library of Medicine, a branch of the National Institutes of Health. The NCBI is located in Bethesda, Maryland and was founded in 1988 through legislation sponsored by Senator Claude Pepper. Wikipedia

Founder: Claude Pepper

Founded: November 4, 1988

Headquarters location: Bethesda, Maryland, United States

Search | ENG | 19:22 07/12/2020

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/.

NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials

Welcome to NCBI

The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.

About the NCBI | Mission | Organization | NCBI News & Blog

Submit

Deposit data or manuscripts into NCBI databases

Download

Transfer NCBI data to your computer

Learn

Find help documents, attend a class or watch a tutorial

Develop

Use NCBI APIs and code libraries to build applications

Analyze

Identify an NCBI tool for your data analysis task

Research

Explore NCBI research and collaborative projects

Popular Resources

PubMed
Bookshelf
PubMed Central
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem

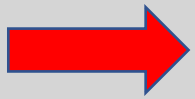
NCBI News & Blog

Recent enhancements in Genome Workbench version 3.4.1
06 Aug 2020

New Features Version 3.4.1 of Genome
New interaction data, downloads and track hub available for RefSeq Functional Elements
04 Aug 2020

We've added several new enhancements
Major update for the NCBI RefSeq mouse GRCm38.p6 annotation
30 Jul 2020

We have updated our annotation for the



Gene sources
Genomic

Categories
Alternatively spliced
Annotated genes
Protein-coding

Sequence content
CCDS
Ensembl
RefSeq
RefSeqGene

Status clear
✓ Current

Clear all

Show additional filters

Tabular 20 per page Sort by Relevance Send to:

Hide sidebar >>

ANTIMICROBIAL RESISTANCE GENE

Was this helpful?

PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA gene family

This gene family can be found in the set of reference sequences used to annotate antimicrobial resistance genes from the National Database of Antibiotic Resistant Organisms (NDARO).

RefSeq genomic (10) RefSeq protein (10)

Pathogen Isolate Browser | Reference Gene Catalog | Download

Search results

Items: 6

See also 72 discontinued or replaced items

Filters: Manage Filters

Results by taxon

Top Organisms [Tree]
Staphylococcus aureus *(5)*
Homo sapiens *(1)*

Find related data

Database: Select

Find items

Search details

```
(("Staphylococcus aureus"[Organism] OR staphylococcus aureus[All Fields]) AND mecA[All Fields]) AND alive[prop]
```

Search See more...

Search results

Items: 6

See also 72 discontinued or replaced items.

Name/Gene ID	Description	Location	Aliases	MIM
mecA ID: 59699569	adaptor protein MecA [*Staphylococcus aureus*]	NC_021670.1 (1031043..1031762)	SABB_RS05060, SABB_00966	
mecA ID: 59698631	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA [*Staphylococcus aureus*]	NC_021670.1 (68945..70954, complement)	SABB_RS00325, SABB_01772	
NEWENTRY ID: 2828033	Record to support submission of GeneRIFs for a gene not in Gene (Micrococcus aureus; Micrococcus pyogenes; Staphlococcus pyogenes citreus; Staphylococcus pyogenes aureus. Use when strain, subtype, isolate, etc. is unspecified, or when different from all specified ones in Gene.). [*Staphylococcus aureus*]			

mecA adaptor protein MecA [*Staphylococcus aureus*]

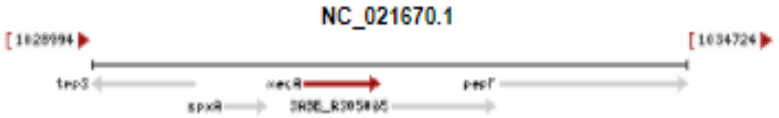
Gene ID: 59699569, updated on 21-Nov-2020

Summary

Gene symbol	mecA
Gene description	adaptor protein MecA
Locus tag	SABB_RS05060
Gene type	protein coding
Organism	Staphylococcus aureus (strain: Bmb9393)
Lineage	Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae; Staphylococcus
Old locus tag	SABB_00966

Genomic context

Sequence: NC_021670.1 (1031043..1031762)

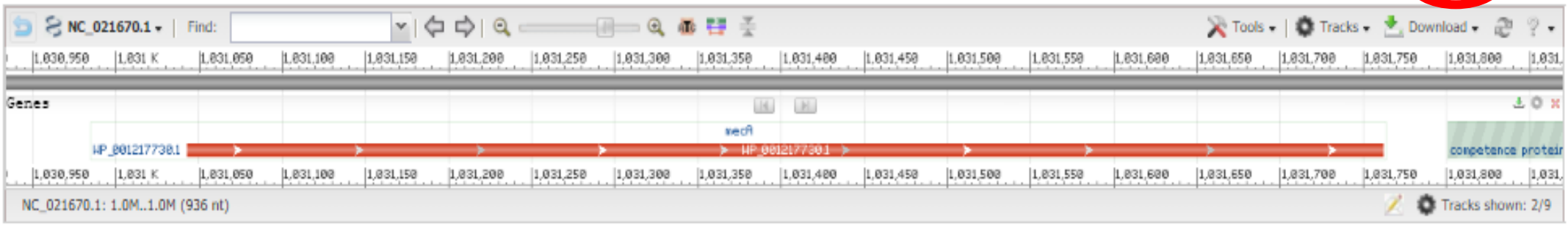


Genomic regions, transcripts, and products

Genomic Sequence: NC_021670.1

Go to reference sequence details

Go to nucleotide: Graphics FASTA GenBank



Bibliography

Select all the gene nucleotides sequence and copy them

FASTA Send to:

Staphylococcus aureus, complete sequence

NCBI Reference Sequence: NC_021670.1

GenBank Graphics

```
>NC_021670.1:1031043-1031762 Staphylococcus aureus, complete sequence
ATGAGAATAGAACGAGTAGATGATACAACTGTAAAATTGTTTATAACATATAGCGATATCGAGGCCCGTG
GATTTAGTCGTGAAGATTTATGGACAAATCGCAAACGTGGCGAAGAATTCTTTTGGTCAATGATGGATGA
AATTAACGAAGAAGAAGATTTTGTTGTAGAAGGTCCATTATGGATTCAAGTACATGCCTTTGAAAAAGGT
GTCGAAGTCACAATTTCTAAATCTAAAAATGAAGATATGATGAATATGTCTGATGATGATGCAACTGATC
AATTTGATGAACAAGTTCAAGAATTGTTAGCTCAAACATTAGAAGGTGAAGATCAATTAGAAGAATTATT
CGAGCAACGAACAAAAGAAAAAGAAGCTCAAGGTTCTAAACGTCAAAAGTCTTCAGCACGTAAAAATACA
AGAACAATCATTGTGAAATTTAACGATTTAGAAGATGTTATTAATTATGCATATCATAGCAATCCAATAA
CTACAGAGTTTGAAGATTTGTTATATATGGTTGATGGTACTTATTATTATGCTGTATATTTTGATAGTCA
TGTTGATCAAGAAGTCATTAATGATAGTTACAGTCAATTGCTTGAATTTGCTTATCCAACAGACAGAACA
GAAGTTTATTTAAATGACTATGCTAAAATAATTATGAGTCATAACGTAACAGCTCAAGTTCGACGTTATT
TTCCAGAGACAACTGAATAA
```

Change region shown

- Whole sequence
- Selected region

from: 1031043 to: 1031762

Update View

Customize view

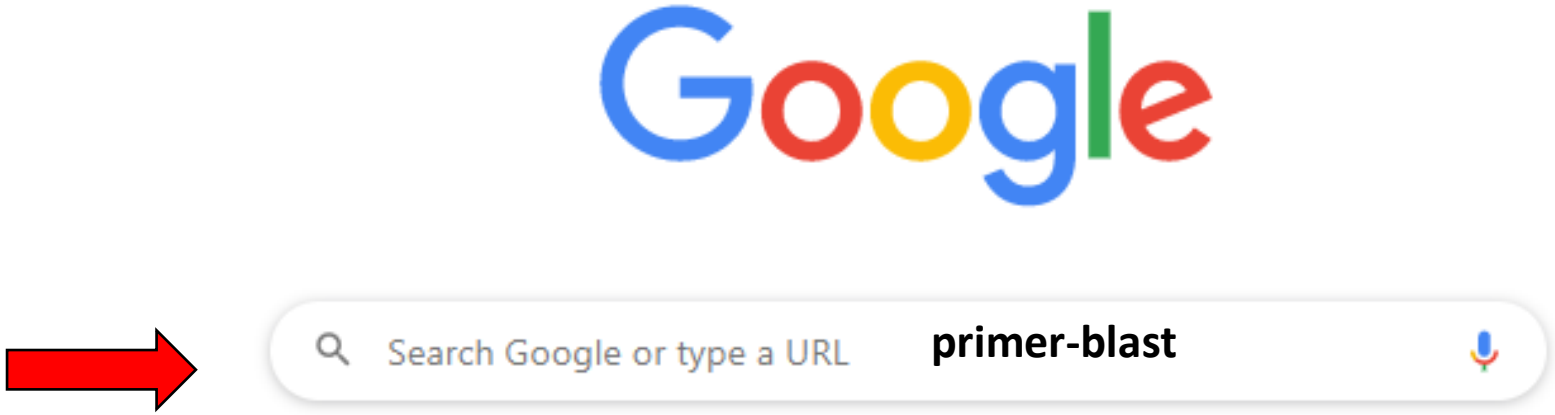
Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Using new web site, Go to primer blast web site



google.com/search?q=Primer-Blast&rlz=1C1GGRV_enGB754GB754&oq=primer&aqs=chrome.0.69i59l2j46j0l4j69i60.7583j0j8&sourceid=chrome&ie=UTF-8

Apps | dictyBase Home | NetPrimer | free pri... | Overview | flyCRISPR | Gene Synthesis &... | The Higher Commit... | AVG Antivirus Free... | BSCB :: The British S... | Patient Access Patie...

Google

Primer-Blast

All | Images | Videos | Books | News | More | Settings | Tools

About 44,800,000 results (0.49 seconds)

www.ncbi.nlm.nih.gov › tools › primer-blast

Primer-BLAST - NCBI - NIH

Primer-BLAST A tool for finding specific primers. Finding primers specific to your PCR template (using Primer3 and BLAST). Reset page Save search parameters ...

Primer-Blast results

Primer-BLAST was developed at NCBI to help users make ...

Publication

Primer-BLAST was developed at NCBI to help users make ...

More results from nih.gov »

blast.ncbi.nlm.nih.gov

BLAST: Basic Local Alignment Search Tool

The Basic Local Alignment Search Tool (**BLAST**) finds regions of local similarity between sequences. ... **Design primers** specific to your PCR template.

www.ncbi.nlm.nih.gov › guide › howto › design-pcr-p...

Design PCR primers and check them for specificity - NCBI - NIH

Primer BLAST performs only a specificity check when a target template and both primers are provided. In the Primer Pair Specificity Checking Parameters section, ...

Type here to search

ENG 10:51 10/08/2020

Here you have three choices

- **You try to make primers for a known DNA sequence region**
- You already have your primers and would like to check their specificity
- You have your primers and the sequence of DNA, you would like to check the fitting of this primer onto the DNA.

Primer-BLAST

A tool for finding specific primers

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) Clear

Range: From / To

Forward primer

Reverse primer Clear

Or, upload FASTA file: Choose file No file chosen

Primer Parameters

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

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

	Min	Max
PCR product size	70	1000
# of primers to return	10	

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match

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

Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range

Min	Max
1000	1000000

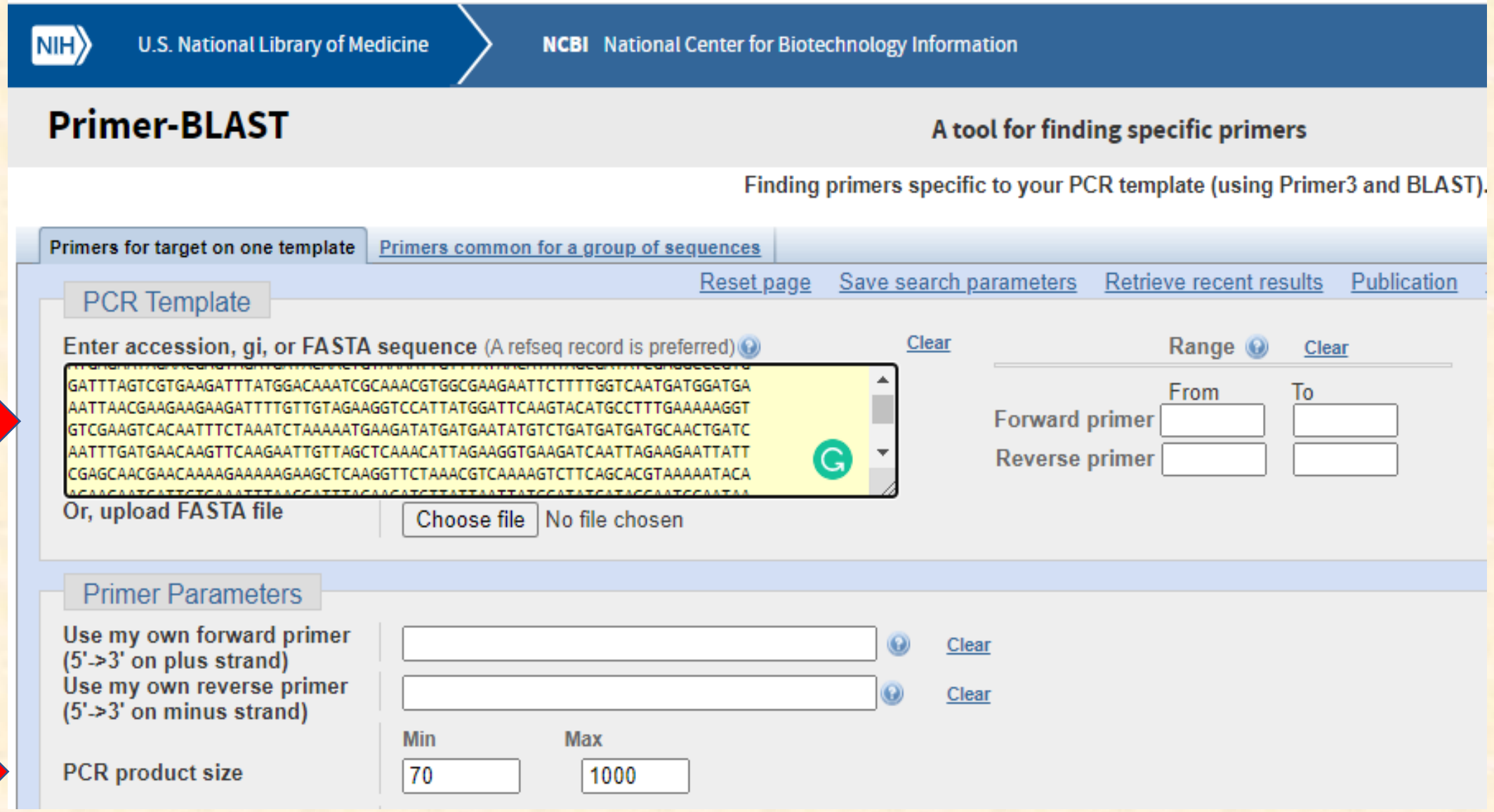
Primer Pair Specificity Checking Parameters

Specificity check: Enable search for primer pairs specific to the intended PCR template

Search mode: Automatic

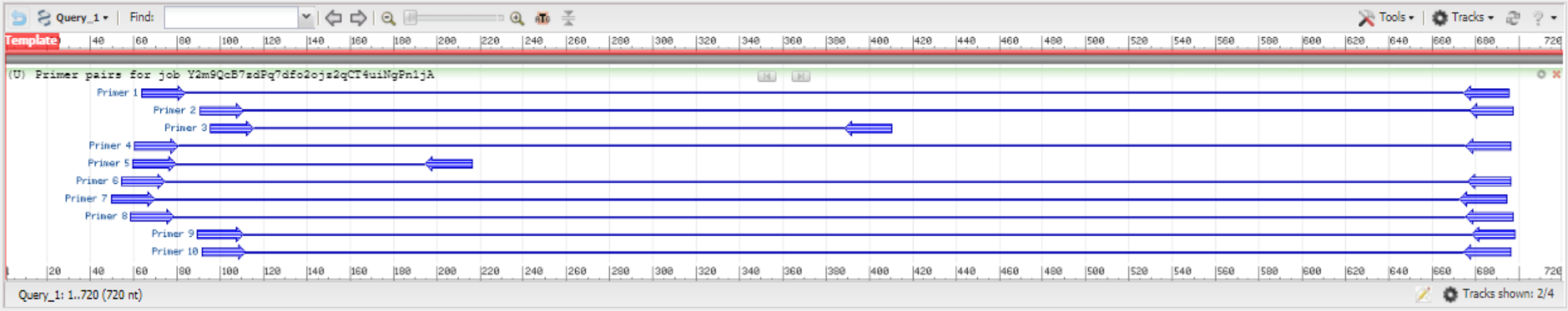
Database: Refseq mRNA

- Paste the selected sequence in the FASTA sequence field
- Insert the require size of the PCR product
- Click the Get Primers button



Input PCR template	lcl|Query_1
Range	1 - 720
Specificity of primers	Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports	▷ Search Summary

Graphical view of primer pairs



Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCCCGTGGATTTAGTCGTGA	Plus	20	64	83	60.11	55.00	3.00	2.00
Reverse primer	CGTCGAACTTGAGCTGTTACG	Minus	21	695	675	59.62	52.38	4.00	2.00
Product length	632								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGGACAAATCGCAAACGTGG	Plus	20	91	110	59.69	50.00	4.00	0.00
Reverse primer	AACGTCGAACTTGAGCTGTT	Minus	20	697	678	58.06	45.00	4.00	3.00
Product length	607								

Primer pair 3

Check the newly designed primers

- The primers themselves (such as Tm, dimers and hairpin)
- Useful for the purpose they are designed for
- They do not miss match their target (inside: in the same gene, out side: in other gene or intergenic space)

Checking the primers themselves

PREMIER Biosoft
Accelerating Research in Life Sciences

HOME COMPANY PRODUCTS ORDERING SUPPORT

Net Primer

Free! Primer Analysis Software

"I wish I had analyzed my primers before ordering for the third time. Now I check all my primers with NetPrimer before ordering" -*Stacey McCann, Cancer Biology Program, Stanford University*

Access NetPrimer Request More View More Tools

About NetPrimer

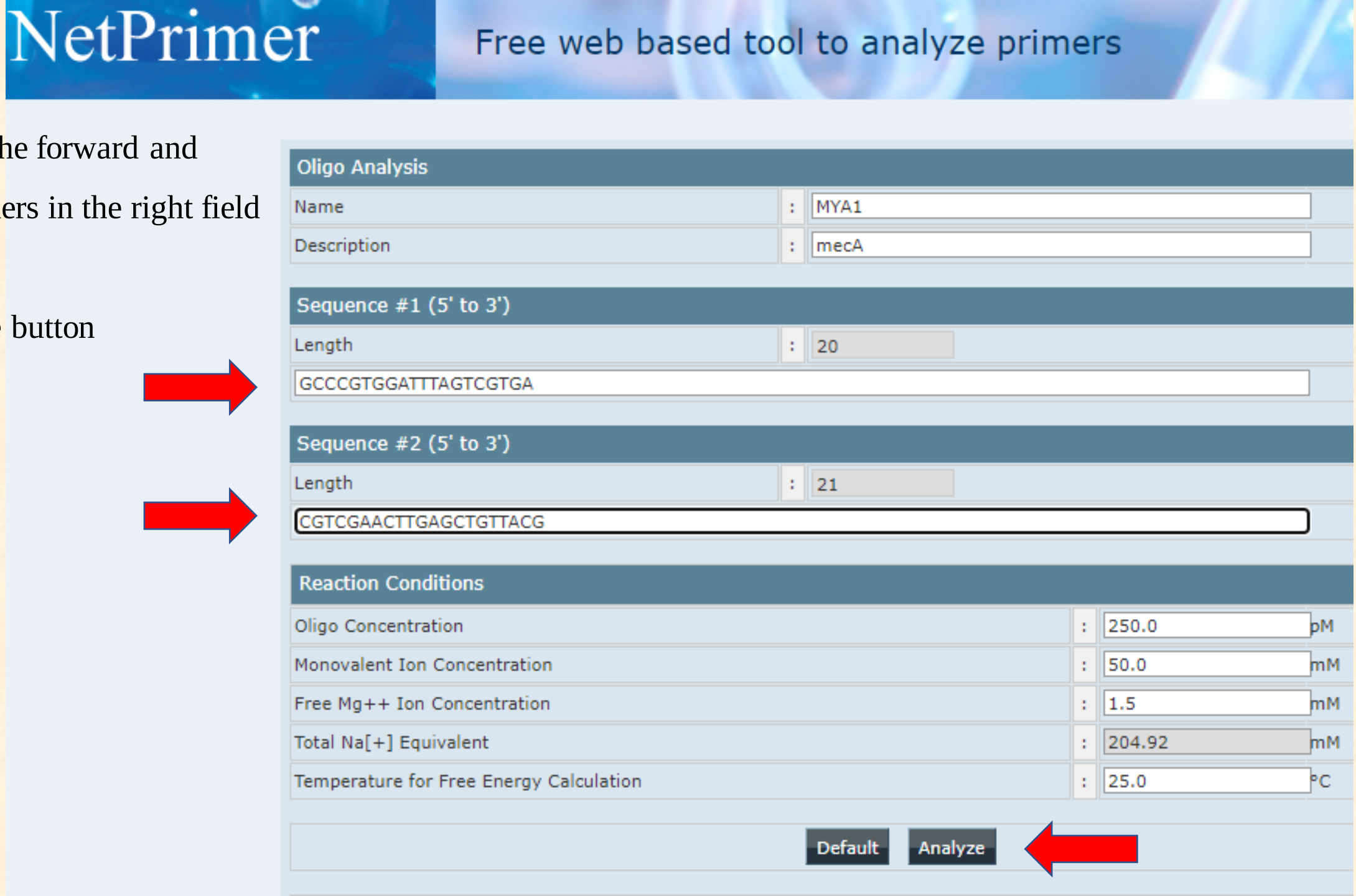
NetPrimer combines the latest primer analysis algorithms with a web-based interface allowing the user to analyze primers over the Internet. All primers are analyzed for **primer melting temperature** using the nearest neighbor thermodynamic theory to ensure accurate Tm prediction. Primers are analyzed for all **primer secondary structures** including hairpins, self-dimers, and cross-dimers in primer pairs. This ensures the availability of the primer for the reaction as well as minimizing the formation of primer dimer. The program eases quantitation of primers by calculating primer molecular weight and optical activity. To facilitate the selection of an optimal primer, each primer is given a rating based on the stability of its secondary structures. A comprehensive analysis report can be printed for individual primers or primer pairs.

Major Features

Copy and paste the forward and

The reverse primers in the right field

Click on Analyse button



Checking the results

Default Analyze

Analysis Results #1: GCCCGTGGATTTAGTCGTGA

Rating	:	100.0		3' end stability	:	-8
Molecular Wt.	:	6164.08		ΔH	:	-1
Tm	:	60.71	°C	ΔS	:	-0
GC%	:	55.0		5' end ΔG	:	-1
GC Clamp	:	2		Self Dimer (ΔG)	:	
nmol/A_{260}	:	5.2		Hairpin (ΔG)	:	
				Repeats (# of pairs)	:	
			kcal/mol	Run (# of bases)	:	3

Total Found = 2

1. ΔG = -5.95 kcal/mol (3' Cross Dimer)

```
5' GCCCGTGGATTTAGTCGTGA 3'
      |||          ||
   3' GCATTGTCGAGTTCAAGCTGC 5'
```

2. ΔG = -5.95 kcal/mol (3' Cross Dimer)

```
5' GCCCGTGGATTTAGTCGTGA 3'
                |||  |
             3' GCATTGTCGAGTTCAAGCTGC 5'
```

Total Found = 4

1. ΔG = -6.76 kcal/mol

```
          5' CGTCGAACTTGAGCTGTTACG 3'
                |||||
3' GCATTGTCGAGTTCAAGCTGC 5'
```

2. ΔG = -6.34 kcal/mol

```
5' CGTCGAACTTGAGCTGTTACG 3'
          ||||
    3' GCATTGTCGAGTTCAAGCTGC 5'
```

3. ΔG = -5.95 kcal/mol (3' Dimer)

```
5' CGTCGAACTTGAGCTGTTACG 3'
  |||   |  | | |  |   |||
3' GCATTGTCGAGTTCAAGCTGC 5'
```

4. ΔG = -4.29 kcal/mol (3' Dimer)

```
5' CGTCGAACTTGAGCTGTTACG 3'
       |||       |||
  3' GCATTGTCGAGTTCAAGCTGC 5'
```

ACTTGAGCTGTTACG

				3' end stability	:	-7
			27	ΔH	:	-157.9
			°C	ΔS	:	-0.41
				5' end ΔG	:	-10.14
				Self Dimer (ΔG)	:	-6.76
				Hairpin (ΔG)	:	-0.19
ug/A_{260}	:	32.59		Repeats (# of pairs)	:	
ΔG	:	-35.06	kcal/mol	Run (# of bases)	:	

Total Found = 2

1. ΔG = -0.19 kcal/mol (3' Hairpin)

```
┌GTTCAAGCTGC 5'
A   |||
└GCTGTTACG 3'
```

2. ΔG = -0.15 kcal/mol (3' Hairpin)

```
┌TTCAAGCTGC 5'
G   |   |||
└AGCTGTTACG 3'
```

Cross Dimer (ΔG)	:	-5.95	kcal/mol

Print

Delta G value

- ΔG is the energy required to break the secondary structure, and larger negative values indicate stable, undesirable structures, that can adversely affect the reaction.

In general

- **Delta G value for self-dimer and hetero-dimer**
- **Between** -5.00 kcal/mol to -7.00 kcal/mol: refers to a good primer
- **Below** -5.00 kcal/mol: refers to a better primer
- **Above** -7.00 kcal/mol: refers to an unsuitable primer, it would be better to change it (them).

Primer-BLAST

A tool for finding specific primers

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) Clear

Range: From / To

Forward primer

Reverse primer Clear

Or, upload FASTA file: Choose file No file chosen

Primer Parameters

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

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

	Min	Max
PCR product size	70	1000
# of primers to return	10	

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match

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

Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range

Min	Max
1000	1000000

Primer Pair Specificity Checking Parameters

Specificity check: Enable search for primer pairs specific to the intended PCR template

Search mode: Automatic

Database: Refseq mRNA

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range Clear

From To

Forward primer

Reverse primer

Or, upload FASTA file Choose file No file chosen

Primer Parameters

Use my own forward primer (5'->3' on plus strand) GCCCGTGGATTTAGTCGTGA Clear

Use my own reverse primer (5'->3' on minus strand) CGTCGAACTTGAGCTGTTACG Clear

	Min	Max
PCR product size	70	1000

# of primers to return: 10

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match

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

Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range

Min	Max
1000	1000000

Note: Parameter values that differ from the default are highlighted in yellow

Primer Pair Specificity Checking Parameters

Specificity check: Enable search for primer pairs specific to the intended PCR template

Search mode: Automatic

Database: nr

Exclusion: Exclude predicted Refseq transcripts (accession with XM, XR prefix) Exclude uncultured/environmental sample sequences

Organism

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

Notes

- Enter one or both primer sequences in the Primer Parameters section of the form. If only one primer is available, a template sequence is also required. See "A Target Template Sequence"
- In the Primer Pair Specificity Checking Parameters section, select the appropriate source Organism and the smallest Database that is likely to contain the target sequence. These settings give the most precise results. For broadest coverage, choose the nr database and do not specify an organism

Primer-BLAST » JOB ID:__Ugg5M6npK5qA6tA80qn3nWO61UxSCwVQ

Primer-BLAST Results

Input PCR template none
Specificity of primers Target templates were found in selected database: Nucleotide collection (nt)
Other reports ▷Search Summary

Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCCCGTGGATTTAGTCGTGA	20	60.11	55.00	3.00	2.00
Reverse primer	CGTCGAACTTGAGCTGTTACG	21	59.62	52.38	4.00	2.00

Products on target templates

>CP055225.1 Staphylococcus aureus LAC

```
product length = 632
Forward primer  1        GCCCGTGGATTTAGTCGTGA  20
Template        1003228  ....................  1003247

Reverse primer  1        CGTCGAACTTGAGCTGTTACG  21
Template        1003859  ....................  1003839
```

>CP063990.1 Staphylococcus aureus strain WMC_NY

In summary

1. Go to the **Primer BLAST** submission form.
2. Enter the target sequence in FASTA format or an accession number of an NCBI nucleotide sequence in the PCR Template section of the form.
3. If one or both primer sequences are to be used in the search, enter these in the Primer Parameters section of the form.
4. Primer BLAST performs only a specificity check when a target template and both primers are provided.
5. In the Primer Pair Specificity Checking Parameters section, select the appropriate source Organism and the smallest Database that is likely to contain the target sequence.
6. For broadest coverage, choose the nr database and do not specify an organism.
7. Click the "Get Primers" button to submit the search and retrieve specific primer pairs.