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Primer Design with Primer3Plus and Primer-Blast

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Primer Design with Primer3Plus and Primer-Blast

Yustina C.F. Salsinha

(Doctoral Student of Faculty of Biology Universitas Gadjah Mada)

Pendahuluan

Forward

Primer AGCCCTCCAGGA

Complementary TCGGGAGGTCCT

Copy Strand

Forward

5’ AGCCCTCCAGGACAGGCTGCATCAGAAGAGGCCATCAAGCAGGTCTGTTCCAAGGGCCTTTGCGTCAGGT
GGGCTCAGGATTCCAGGGTGGCTGGACCCCAGGCCCCAGCTCTGCAGCAGGGAGGACGTGGCTGGGCTCG
TGAAGCATGTGGGGGTGAGCCCAGGGGCCCCAAGGCAGGGCACCTGGCCTTCAGCCTGCCTCAGCCCTGC
CTGTCTCCCAGATCACTGTCCTTCTGCCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTG
GCCCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAG
CTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACCCGCCGGGAGGCAGAGGACCT
GCAGGGTGAGCCAACTGCCCATTGCTGCCCCTGGCCGCCCCCAGCCACCCCCTGCTCCTGGCGCTCCCAC
CCAGCATGGGCAGAAGGGGGCAGGAGGCTGCCACCCAGCAGGGGGTCAGGTGCACTTTTTTAAAAAGAAG
TTCTCTTGGTCACGTCCTAAAAGTGACCAGCTCCCTGTGGCCCAGTCAGAATCTCAGCCTGAGGACGGTG
TTGGCTTCGGCAGCCCCGAGATACATCAGAGGGTGGGCACGCTCCTCCCTCCACTCGCCCCTCAAACAAA
TGCCCCGCAGCCCATTTCTCCACCCTCATTTGATGACCGCAGATTCAAGTGTTTTGTTAAGTAAAGTCCT
GGGTGACCTGGGGTCACAGGGTGCCCCACGCTGCCTGCCTCTGGGCGAACACCCCATCACGCCCGGAGGA
GGGCGTGGCTGCCTGCCTGAGTGGGCCAGACCCCTGTCGCCAGGCCTCACGGCAGCTCCATAGTCAGGAG
ATGGGGAAGATGCTGGGGACAGGCCCTGGGGAGAAGTACTGGGATCACCTGTTCAGGCTCCCACTGTGAC
GCTGCCCCGGGGCGGGGGAAGGAGGTGGGACATGTGGGCGTTGGGGCCTGTAGGTCCACACCCAGTGTGG
GTGACCCTCCCTCTAACCTGGGTCCAGCCCGGCTGGAGATGGGTGGGAGTGCGACCTAGGGCTGGCGGGC
AGGCGGGCACTGTGTCTCCCTGACTGTGTCCTCCTGTGTCCCTCTGCCTCGCCGCTGTTCCGGAACCTGC
TCTGCGCGGCACGTCCTGGCAGTGGGGCAGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGC
CCTTGGCCCTGGAGGGGTCCCTGCAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT
CTACCAGCTGGAGAACTACTGCAACTAGACGCAGCCCGCAGGCAGCCCCACACCCGCCGCCTCCTGCACC 3’
GAGAGAGATGGAATAAAGCCCTTGAACCAGC

Reverse

Reverse

5’ GCCTCCTGCACC 3’ Complemn

Copy

3’ CGAGGTCGTGG 5’ Primer

Pendahuluan

Hal-hal yang perlu diperhatikan:

- Primer lenght
- GC Content
- Melting temperature (Tm)
- Self Complementarity

Lenght: 18-24 bp

- Lower: lack of specifity
- Long: Annealing Efficiency Reduced (might not bind)

GC Content: 50-60%

- Lower: Annealing Efficiency Reduced
- Long: Tm Higher

Melting Temperature (Tm)

- Must be 3-5°C higher than annealing Temp
- Take a look on opt. Tempt

Primer sense complementary

- No hairpin loop
- No primer dimers
- No over complementary between forward and reverse primer

Primer Task

Detection: find the target region
Especially is performed in PCR

Sequencing: design primer for sequencing

Cloning: isolating a specific DNA sequence to ligate in a vector

Primer list: every possible primer for a DNA sequence

Primer check: to get the information about your primer

Go find the sequence

https://www.ncbi.nlm.nih.gov/

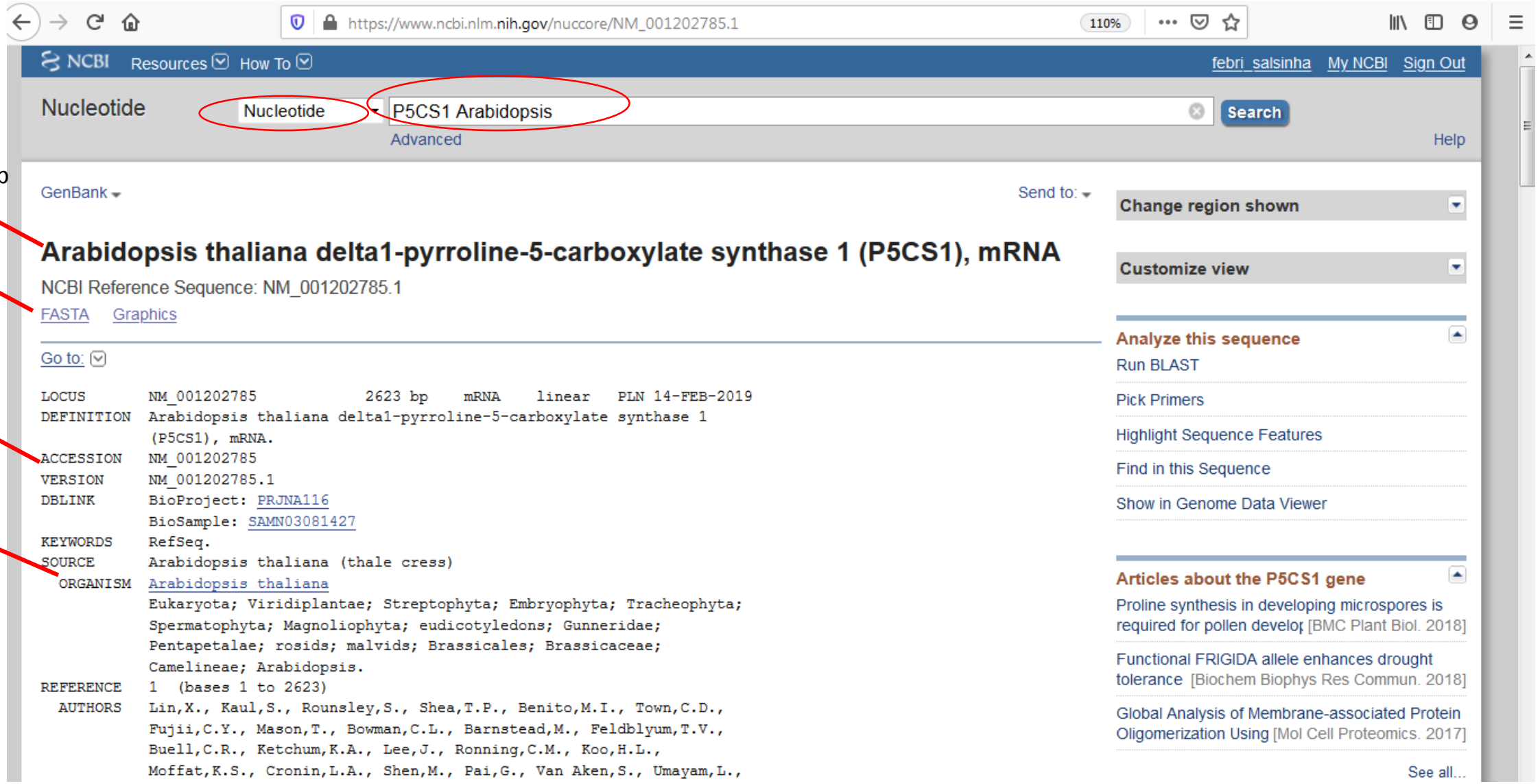
Go find the sequence

Title of Gene and Sp

Fasta Seq

Accesion number

Organism



Go find the sequence

```
FEATURES             Location/Qualifiers
     source          1..2623
                     /organism="Arabidopsis thaliana"
                     /mol_type="mRNA"
                     /db_xref="taxon:3702"
                     /chromosome="2"
                     /ecotype="Columbia"
     gene            1..2623
                     /gene="P5CS1"
                     /locus_tag="AT2G39800"
                     /gene_synonym="ATP5CS; delta1-pyrroline-5-carboxylate
                     synthase 1; T5I7.10; T5I7_10"
                     /note="encodes a delta1-pyrroline-5-carboxylate synthase
                     that catalyzes the rate-limiting enzyme in the
                     biosynthesis of proline. Gene is expressed in reproductive
                     organs and tissues under non-stress conditions but in the
                     whole plant under water-limiting condition. Expression is
                     also induced by abscisic acid and salt stress in a
                     light-dependent manner. encodes a
                     delta1-pyrroline-5-carboxylate synthase that catalyzes the
                     rate-limiting enzyme in the biosynthesis of proline. Gene
                     is expressed in reproductive organs and tissues under
                     non-stress conditions but in the whole plant under
                     water-limiting condition. Expression is also induced by
                     abscisic acid and salt stress in a light-dependent manner.
                     P5CS1 appears to be involved in salt stress responses
                     related to proline accumulation, including protection from
                     reactive oxidative species. P5CS1 appears to be present in
                     different cells and/or different subcellular locations
                     from P5CS2 in a tissue-dependent manner."
                     /db_xref="Araport:AT2G39800"
                     /db_xref="GeneID:818566"
                     /db_xref="TAIR:AT2G39800"
```

Also Known As: AT2G39800, ATP5CS, T5I7...

Homologs of the P5CS1 gene

The P5CS1 gene is conserved in human, chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, fruit fly, mosquito, C.elegans, rice, and frog.

Related information

- BioProject
- BioSample
- Protein
- PubMed
- Taxonomy
- Annotated Genomic
- BioSystems
- Functional Class
- Gene
- PubMed (RefSeq)
- PubMed (Weighted)
- SNP

Go find the sequence

```
                     from P5CS2 in a tissue-dependent manner."
                     /db_xref="Araport:AT2G39800"
                     /db_xref="GeneID:818566"
                     /db_xref="TAIR:AT2G39800"
CDS                  123..2276
                     /gene="P5CS1"
                     /locus_tag="AT2G39800"
                     /gene_synonym="ATP5CS; delta1-pyrroline-5-carboxylate
                     synthase 1; T5I7.10; T5I7_10"
                     /note="delta1-pyrroline-5-carboxylate synthase 1 (P5CS1);
                     CONTAINS InterPro DOMAIN/s: Glutamate 5-kinase
                     (InterPro:IPR001057), Glutamate 5-kinase, conserved site
                     (InterPro:IPR019797), Aspartate/glutamate/uridylate kinase
                     (InterPro:IPR001048), Aldehyde dehydrogenase, N-terminal
                     (InterPro:IPR016162), Gamma-glutamyl phosphate reductase
                     GPR, conserved site (InterPro:IPR020593), Aldehyde
                     dehydrogenase, C-terminal (InterPro:IPR016163),
                     Aldehyde/histidinol dehydrogenase (InterPro:IPR016161),
                     Delta l-pyrroline-5-carboxylate synthetase
                     (InterPro:IPR005766), Gamma-glutamyl phosphate reductase
                     GPR (InterPro:IPR000965), Aldehyde dehydrogenase
                     (InterPro:IPR015590), Glutamate 5-kinase, ProB-related
                     (InterPro:IPR005715); BEST Arabidopsis thaliana protein
                     match is: delta 1-pyrroline-5-carboxylate synthase 2
                     (TAIR:AT3G55610.1)."
                     /codon_start=1
                     /product="delta1-pyrroline-5-carboxylate synthase 1"
                     /protein_id="NP_001189714.1"
                     /db_xref="Araport:AT2G39800"
                     /db_xref="GeneID:818566"
                     /db_xref="TAIR:AT2G39800"
                     /translation="MEELDRSRAFARDVKRIVVKVGTAVVTGKGGRLALGRLGALCEQ
                     LAELNSDGFEVILVSSGAVGLGRQRLRYRQLVNSSFADLQKPQTELDGKACAGVGQSS
                     LMAYYETMFDQLDVTAAQLLVNDSSFRDKDFRKQLNETVKSMLDLRVIPIFNENDAIS
                     TRRAPYQDSSGIFWDNDSLAALLALELKADLLILLSDVEGLYTGPPSDPNSKLIHTFV
                     KEKHQDEITFGDKSRLGRGGMTAKVKAAVNAAYAGIPVIITSGYSAENIDKVLRGLRV
```

PubMed (Weighted)

SNP

UniGene

LinkOut to external resources

GenScript latest version of gene cDNA ORF Clone [GenScript latest version of g...]

Recent activity

Turn Off Clear

Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), n Nucleotide

P5CS1 (64) Nucleotide

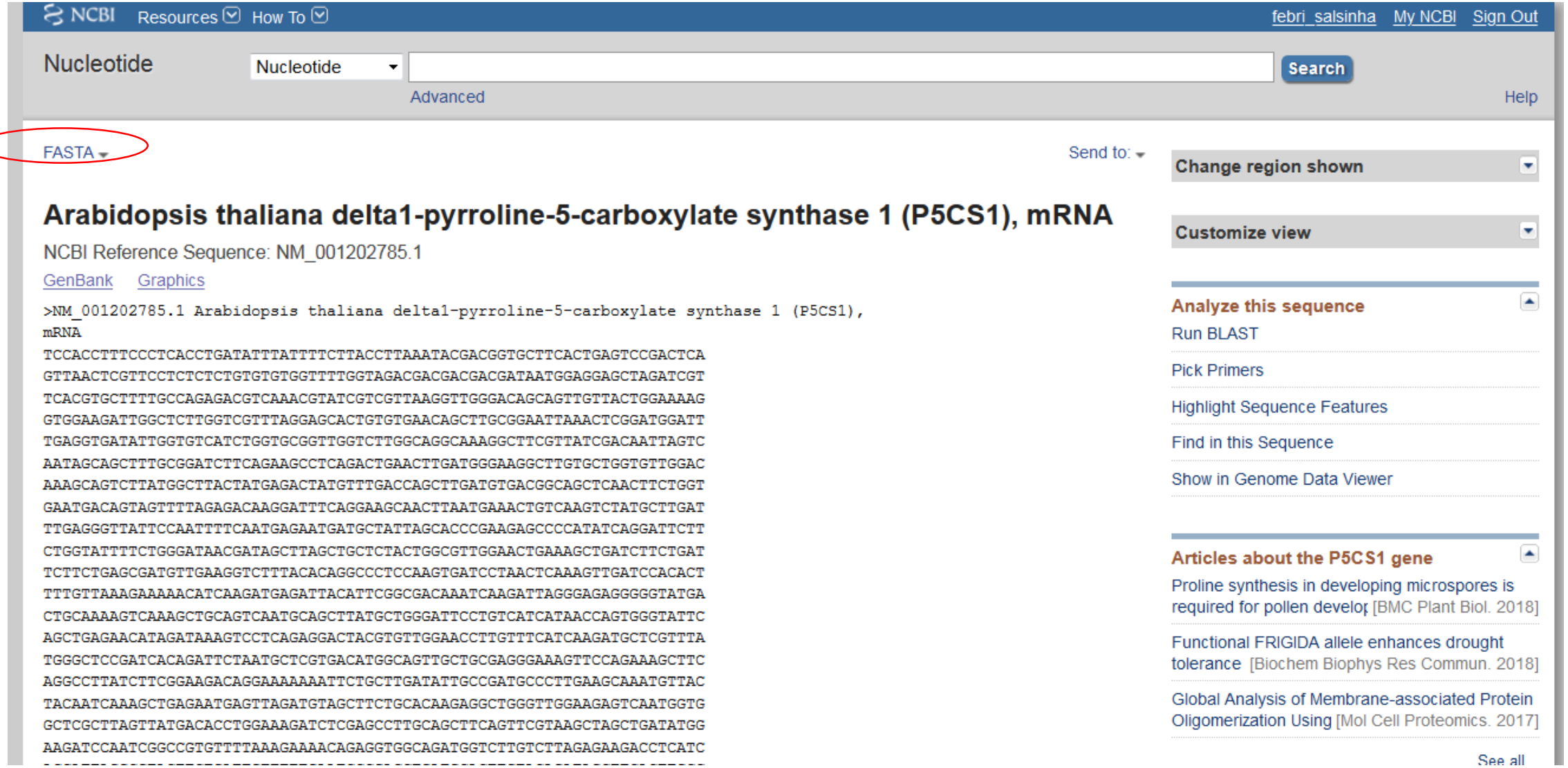
INS insulin [Homo sapiens] Gene

(insulin homo) AND "Homo sapiens"[porgn] AND ((alive[prop] OR rep... (2012) Gene

(insulin homo) AND "Homo sapiens"[porgn] AND (alive[prop]) (1949) Gene

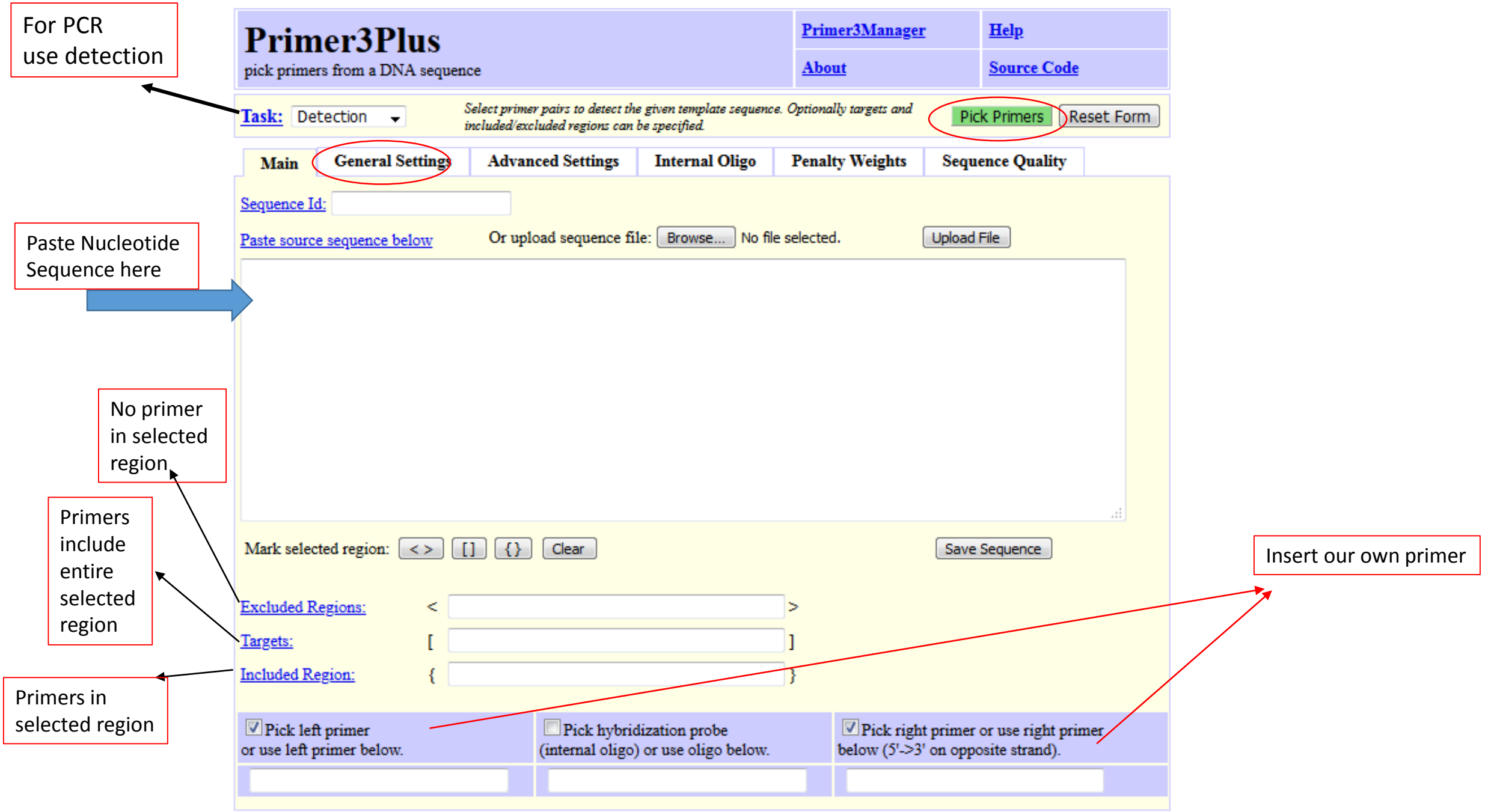
See more...

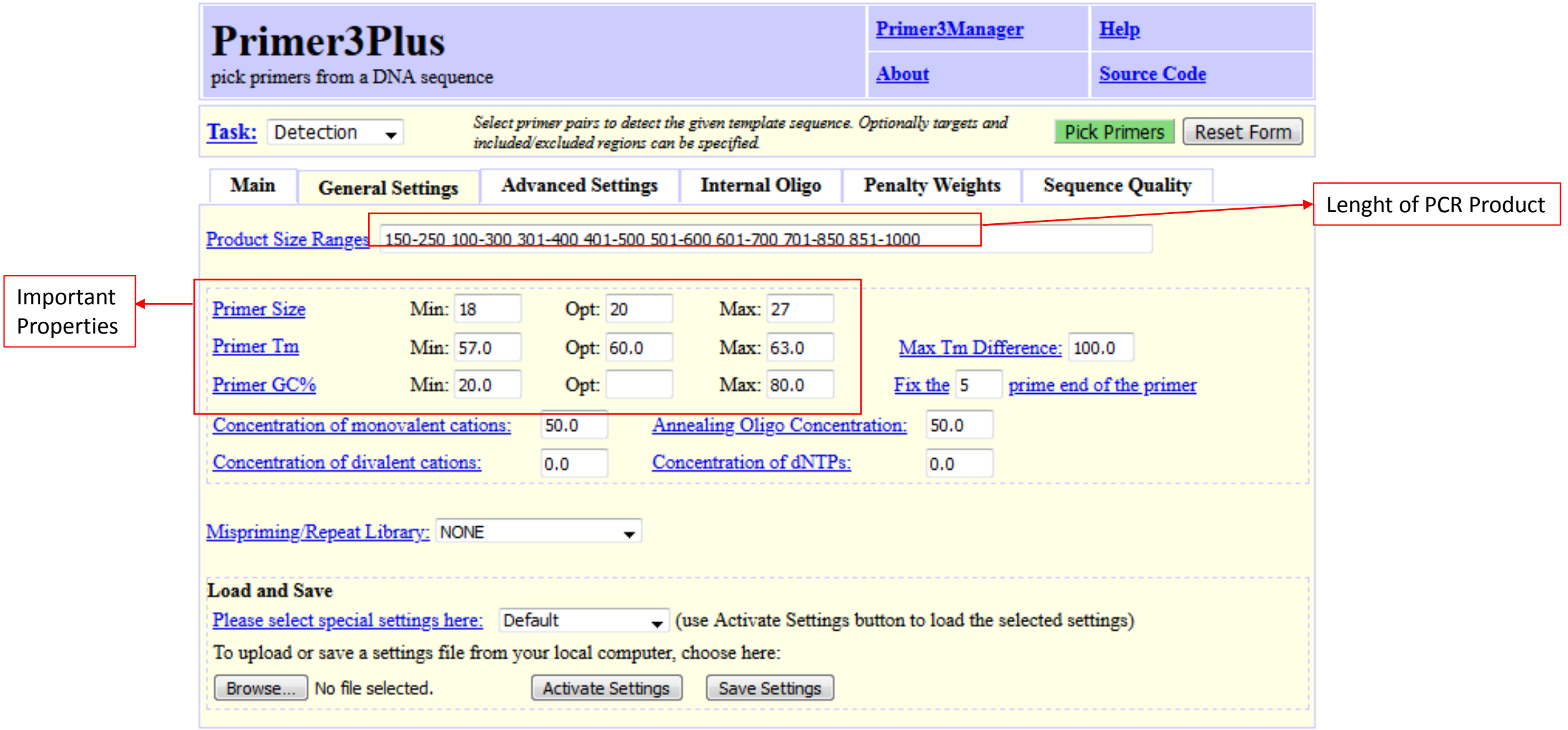
Go find the sequence



Primer3plus

http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi


For PCR use detection
Primer3Plus
pick primers from a DNA sequence
Primer3Manager
Help
About
Source Code
Task:
Detection
Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.
Pick Primers
Reset Form
Main
General Settings
Advanced Settings
Internal Oligo
Penalty Weights
Sequence Quality
Sequence Id:
Paste source sequence below
Or upload sequence file:
Browse...
No file selected.
Upload File
Paste Nucleotide Sequence here
No primer in selected region
Primers include entire selected region
Primers in selected region
Mark selected region:
< >
[ ]
{ }
Clear
Save Sequence
Insert our own primer
Excluded Regions:
Targets:
Included Region:
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'->3' on opposite strand).


Primer3Plus
pick primers from a DNA sequence
Primer3Manager
Help
About
Source Code
Task: Detection
Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.
Pick Primers
Reset Form
Main
General Settings
Advanced Settings
Internal Oligo
Penalty Weights
Sequence Quality
Product Size Ranges 150-250 100-300 301-400 401-500 501-600 601-700 701-850 851-1000
Primer Size Min: 18 Opt: 20 Max: 27
Primer Tm Min: 57.0 Opt: 60.0 Max: 63.0
Max Tm Difference: 100.0
Primer GC% Min: 20.0 Opt: Max: 80.0
Fix the 5 prime end of the primer
Concentration of monovalent cations: 50.0
Annealing Oligo Concentration: 50.0
Concentration of divalent cations: 0.0
Concentration of dNTPs: 0.0
Mispriming/Repeat Library: NONE
Load and Save
Please select special settings here: Default (use Activate Settings button to load the selected settings)
To upload or save a settings file from your local computer, choose here:
Browse... No file selected.
Activate Settings
Save Settings
Lenght of PCR Product
Important Properties

Primer3Plus

pick primers from a DNA sequence

Primer3Manager | Help | About | Source Code

< Back

Pair 1:

Left Primer 1: Primer_F

Sequence: TGGTCGTTTAGGAGCACTGTGT

Start: 227 Length: 22 bp Tm: 62.0 °C GC: 50.0 % ANY: 5.0 SELF: 3.0

Right Primer 1: Primer_R

Sequence: AGCCTTCCCATCAAGTTCAGTC

Start: 404 Length: 22 bp Tm: 61.9 °C GC: 50.0 % ANY: 3.0 SELF: 1.0

Product Size: 178 bp Pair Any: 4.0 Pair End: 0.0

Send to Primer3Manager | Reset Form

```
1     TCCACCTTTC CCTCACCTGA TATTTATTTT CTTACCTTAA ATACGACGGT
51    GCTTCACTGA GTCCGACTCA GTTAACTCGT TCCTCTCTCT GTGTGTGGTT
101   TTGGTAGACG ACGACGACGA TAATGGAGGA GCTAGATCGT TCACGTGCTT
151   TTGCCAGAGA CGTCAAACGT ATCGTCGTTA AGGTTGGGAC AGCAGTTGTT
201   ACTGGAAAAG GTGGAAGATT GGCTCTTGGT CGTTTAGGAG CACTGTGTGA
251   ACAGCTTGCG GAATTAAACT CGGATGGATT TGAGGTGATA TTGGTGTCAT
301   CTGGTGCGGT TGGTCTTGGC AGGCAAAGGC TTCGTTATCG ACAATTAGTC
351   AATAGCAGCT TTGCGGATCT TCAGAAGCCT CAGACTGAAC TTGATGGGAA
401   GGCTTGTGCT GGTGTTGGAC AAAGCAGTCT TATGGCTTAC TATGAGACTA
451   TGTTTGACCA GCTTGATGTG ACGGCAGCTC AACTTCTGGT GAATGACAGT
501   AGTTTTAGAG ACAAGGATTT CAGGAAGCAA CTTAATGAAA CTGTCAAGTC
551   TATGCTTGAT TTGAGGGTTA TTCCAATTTT CAATGAGAAT GATGCTATTA
601   GCACCCGAAG AGCCCCATAT CAGGATTCTT CTGGTATTTT CTGGGATAAC
651   GATAGCTTAG CTGCTCTACT GGCGTTGGAA CTGAAAGCTG ATCTTCTGAT
701   TCTTCTGAGC GATGTTGAAG GTCTTTACAC AGGCCCTCCA AGTGATCCTA
751   ACTCAAAGTT GATCCACACT TTTGTTAAAG AAAAACATCA AGATGAGATT
801   ACATTCGGCG ACAAATCAAG ATTAGGGAGA GGGGGTATGA CTGCAAAAGT
```

Primer forward

The region included

Select all Primers

Pair 2:

Left Primer 2: Primer_1_F

Sequence: GGTCGTTTAGGAGCACTGTGTG

Start: 228 Length: 22 bp Tm: 62.0 °C GC: 54.5 % ANY: 5.0 SELF: 3.0

Right Primer 2: Primer_1_R

Sequence: AGCCTTCCCATCAAGTTCAGTC

Start: 404 Length: 22 bp Tm: 61.9 °C GC: 50.0 % ANY: 3.0 SELF: 1.0

Product Size: 177 bp Pair Any: 4.0 Pair End: 1.0

Send to Primer3Manager Reset Form

Pair 3:

Left Primer 3: Primer_2_F

Sequence: GGTCGTTTAGGAGCACTGTGTG

Start: 228 Length: 22 bp Tm: 62.0 °C GC: 54.5 % ANY: 5.0 SELF: 3.0

Right Primer 3: Primer_2_R

Sequence: GCCTTCCCATCAAGTTCAGTCT

Start: 403 Length: 22 bp Tm: 61.9 °C GC: 50.0 % ANY: 3.0 SELF: 3.0

Product Size: 176 bp Pair Any: 4.0 Pair End: 1.0

Send to Primer3Manager Reset Form

Primer3Manager

manage your primer library

Primer3Plus | Help | About | Source Code

Order selected Primers | Refresh | Reset Form | Delete selected Primers

To upload or save a primer file from your local computer, choose here:

Browse... No file selected. Upload File Save File

Select	Name	Sequence	Designed on	Check!	BLAST!
☑	Primer_F	TGGTCGTTTAGGAGCACTGTGT	28.02.2020	Check!	BLAST!
☑	Primer_R	AGCCTTCCCATCAAGTTCAGTC	28.02.2020	Check!	BLAST!
☑	Primer_1_F	GGTCGTTTAGGAGCACTGTGTG	28.02.2020	Check!	BLAST!
☑	Primer_1_R	AGCCTTCCCATCAAGTTCAGTC	28.02.2020	Check!	BLAST!

Order selected Primers | Refresh | Reset Form | Delete selected Primers

Primers_2020_02_28.fas - Notepad

File Edit Format View Help

```
>Primer_F       |X|28.02.2020
TGGTCGTTTAGGAGCACTGTGT

>Primer_R       |X|28.02.2020
AGCCTTCCCATCAAGTTCAGTC

>Primer_1_F     |X|28.02.2020
GGTCGTTTAGGAGCACTGTGTG

>Primer_1_R     |X|28.02.2020
AGCCTTCCCATCAAGTTCAGTC
```

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febri_salsinha

BLAST® » blastn suite » results for RID-5GUZDVAA016

Home | Recent Results | Saved Strategies | Help

Edit Search | Save Search | Search Summary

How to read this report? | BLAST Help Videos | Back to Traditional Results Page

Your search parameters were adjusted to search for a short input sequence.

Job Title	**Nucleotide sequence (26 letters)**
RID	5GUZDVAA016 *Search expires on 02-29 08:25 am* Download All
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_10459
Description	None
Molecule type	nucleic acid
Query Length	22
Other reports	Distance tree of results MSA viewer

Filter Results

Organism *only top 20 will appear* exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity to **E value** to **Query Coverage** to

Filter | Reset

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download | Manage Columns | Show 100

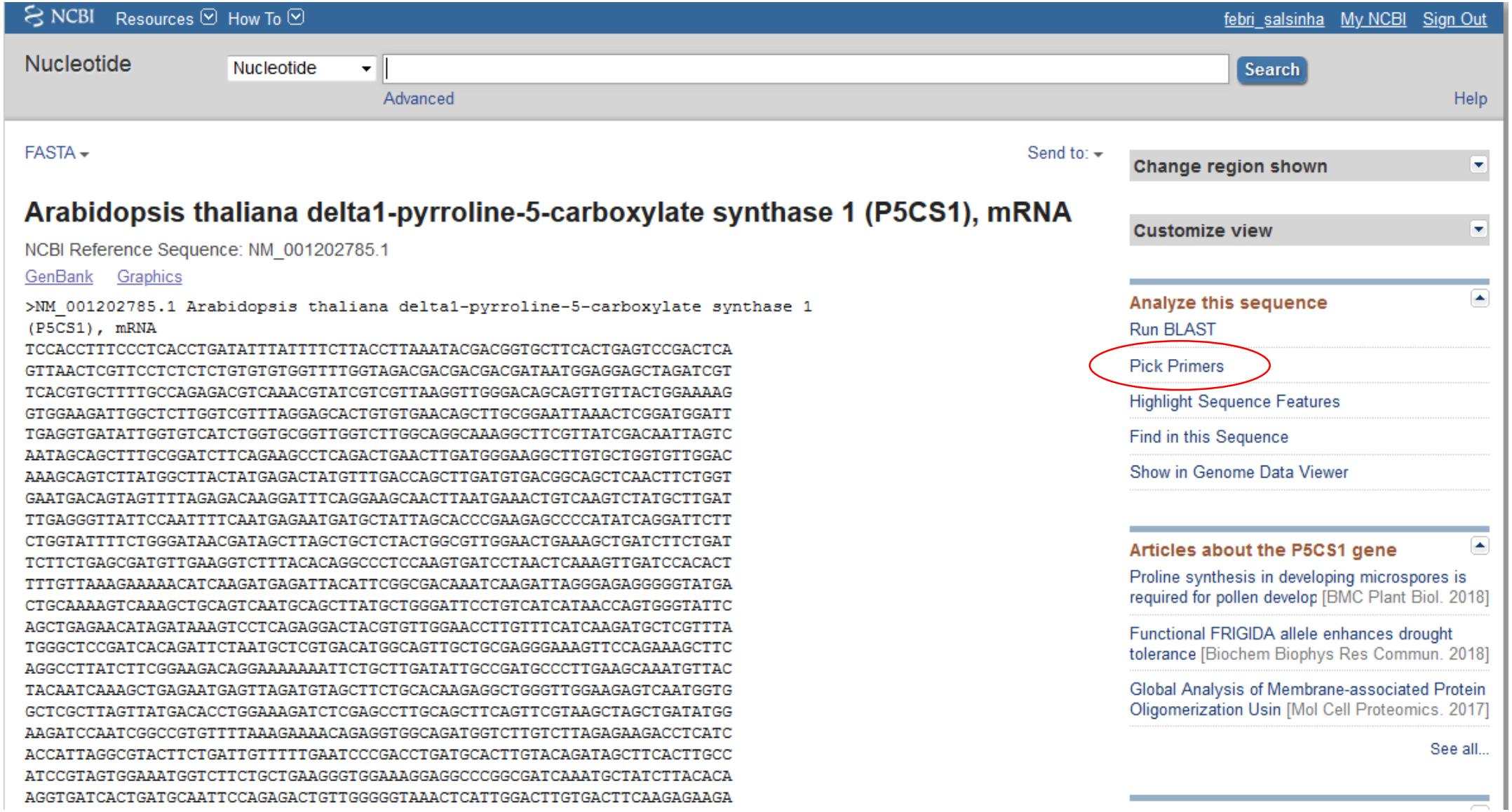
select all *98 sequences selected* GenBank | Graphics | Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA	44.1	44.1	100%	0.028	100.00%	NM_001202786.1
Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA	44.1	44.1	100%	0.028	100.00%	NM_001202785.1
Arabidopsis thaliana AT2G39800 mRNA, complete cds, clone: RAFL05-01-F23	44.1	44.1	100%	0.028	100.00%	AK316756.1

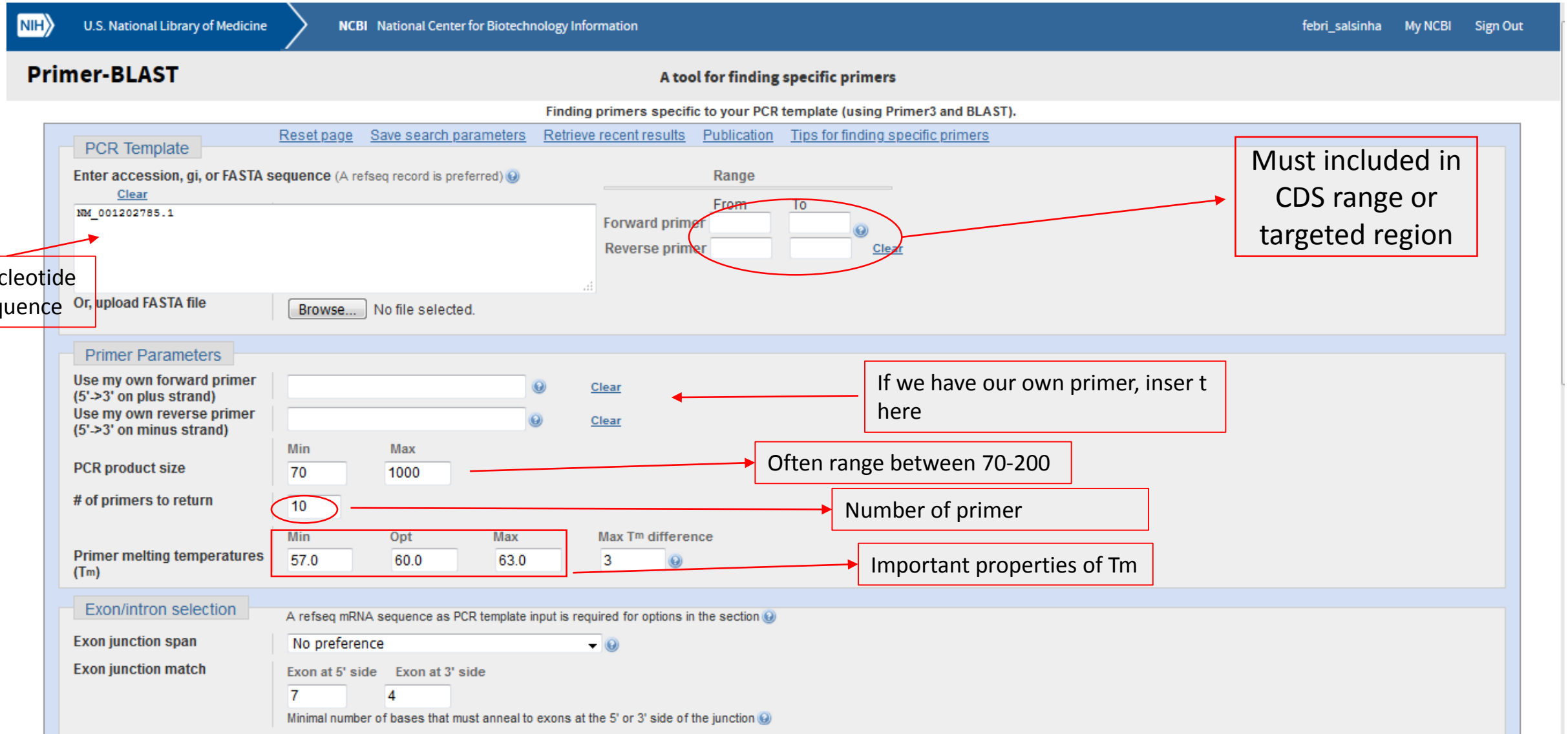
Primer-BLAST)(Via NCBI)

https://www.ncbi.nlm.nih.gov/

Primer-BLAST)(Via NCBI)



Primer-BLAST)(Via NCBI)



Exon junction match | Exon at 5' side: 7 | Exon at 3' side: 4

Minimal 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: 1000 Max: 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 Refseq mRNA

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

Organism 3702

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)

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.

Ignore targets that have 6 or more mismatches to the primer.

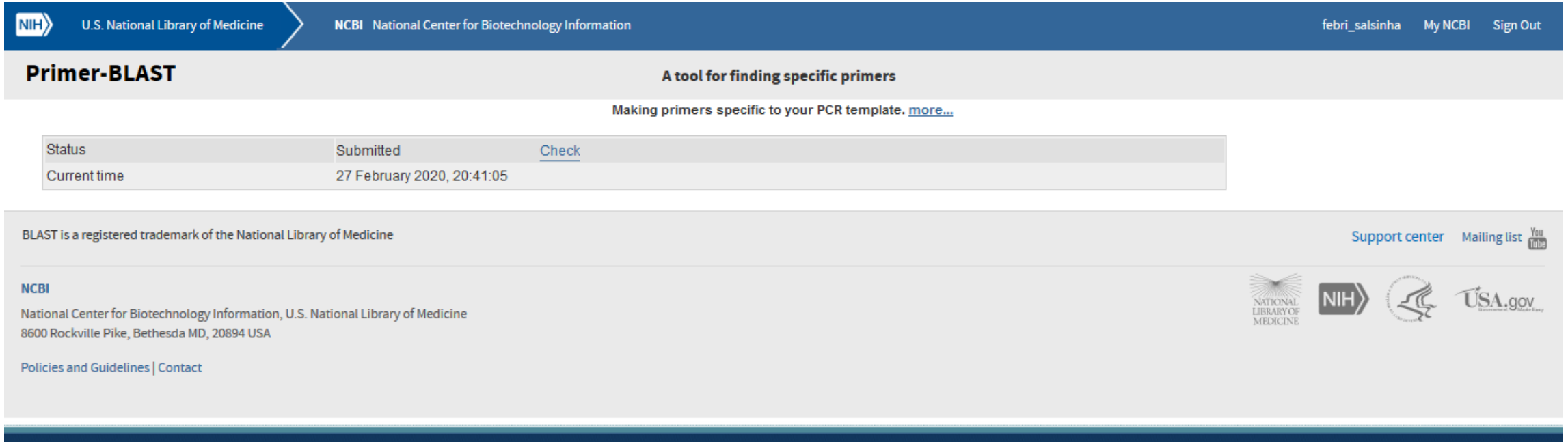
Max target size 4000

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

Get Primers ☐ Show results in a new window ☑ Use new graphic view

▶ Advanced parameters

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



Be patient, tho :P
It takes 3-5 minutes until the primer is ready

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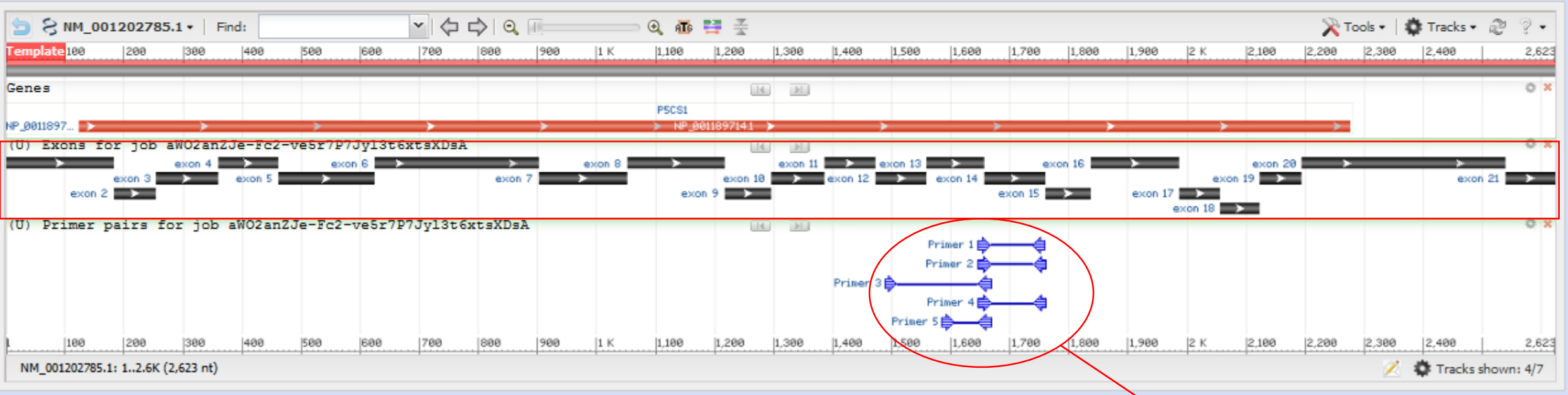
ner-BLAST» JOB ID:aWO2anZJe-Fc2-ve5r7P7Jyl3t6xtsXDsA

Primer-BLAST Results

Input PCR template NM_001202785.1 Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA
Range 1 - 2623
ecificity of primers Primers may **not** be specific to the input PCR template as targets were found in selected database:Refseq mRNA (Organism limited to Arabidopsis thaliana, Oryza sativa Japonica Group)...help on specific primers
Other reports ▷Search Summary

Graphical view of primer pairs



Primer designed

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Primer-BLAST» JOB ID:aWO2anZJe-Fc2-ve5r7P7Jyl3t6xtsXDsA

Primer-BLAST Results

Input PCR template NM_001202785.1 Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA
Range 1 - 2623
Specificity of primers Primers may **not** be specific to the input PCR template as targets were found in selected database:Refseq mRNA (Organism limited to Arabidopsis thaliana, Oryza sativa Japonica Group)...help on specific primers
Other reports ▷Search Summary

Graphical view of primer pairs

Detailed primer reports

You can re-search for specific primers by accepting some of the unintended targets, check the box(es) next to the ones you accept and try again to re-search for specific primers Submit

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGGTCATGCTGATGGAATCTGT	Plus	22	1646	1667	59.49	45.45	4.00	1.00
Reverse primer	TCGCATTACAGGCTGCTGGA	Minus	20	1758	1739	61.90	55.00	5.00	3.00
Product length	113								

Primer information

Products on intended targets

>NM_001202785.1 Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA

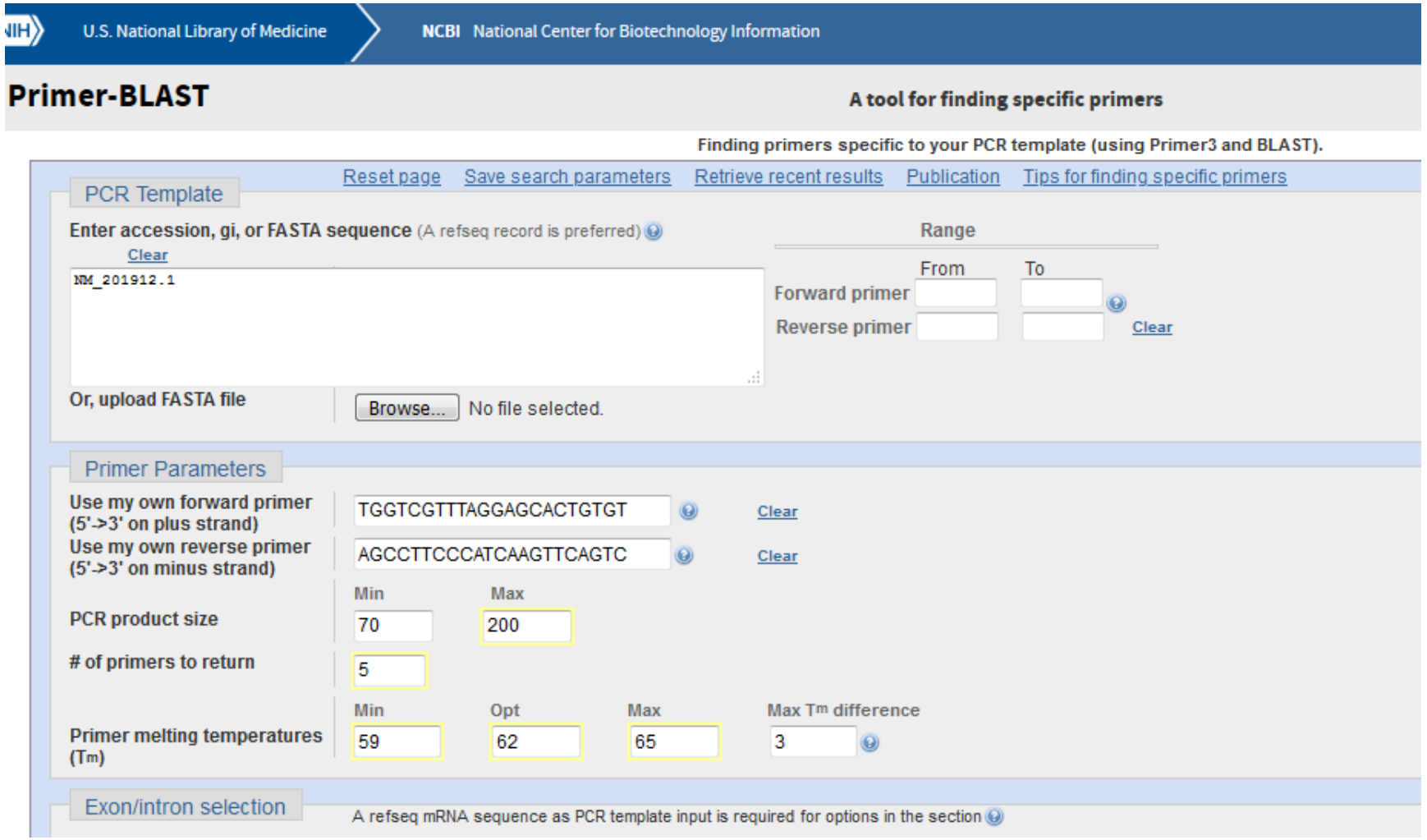
```
product length = 113
Forward primer  1     AGGTCATGCTGATGGAATCTGT  22
Template        1646  ......................  1667

Reverse primer  1     TCGCATTACAGGCTGCTGGA  20
Template        1758  ....................  1739
```

Products on potentially unintended templates

☐ >NM_201912.1 Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA

We can use Primer-Blast as combination with primer 3plus

Copy the primer from Primer3Plus and paste it on Primer Blast

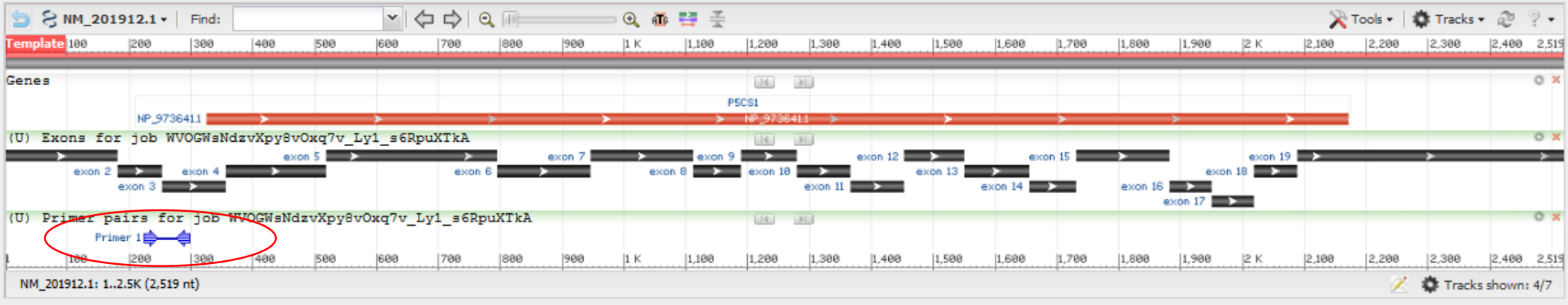


Primer-BLAST» JOB ID:WVOGWsNdzvXpy8vOxq7v_Ly1_s6RpuXTkA

Primer-BLAST Results

Input PCR template NM_201912.1 Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA
Range 1 - 2519
Specificity of primers Primers may **not** be specific to the input PCR template as targets were found in selected database:Refseq mRNA (Organism limited to Arabidopsis thaliana)...help on specific primers
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	TGGTCGTTTAGGAGCACTGTGT	Plus	22	225	246	61.86	50.00	5.00	3.00
Reverse primer	AGCCTTCCCATCAAGTTCAGTC	Minus	22	298	277	60.29	50.00	3.00	1.00
Product length	74								

Thank you

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