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Primer Design with Primer3Plus and Primer-Blast

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National Research and Innovation Agency

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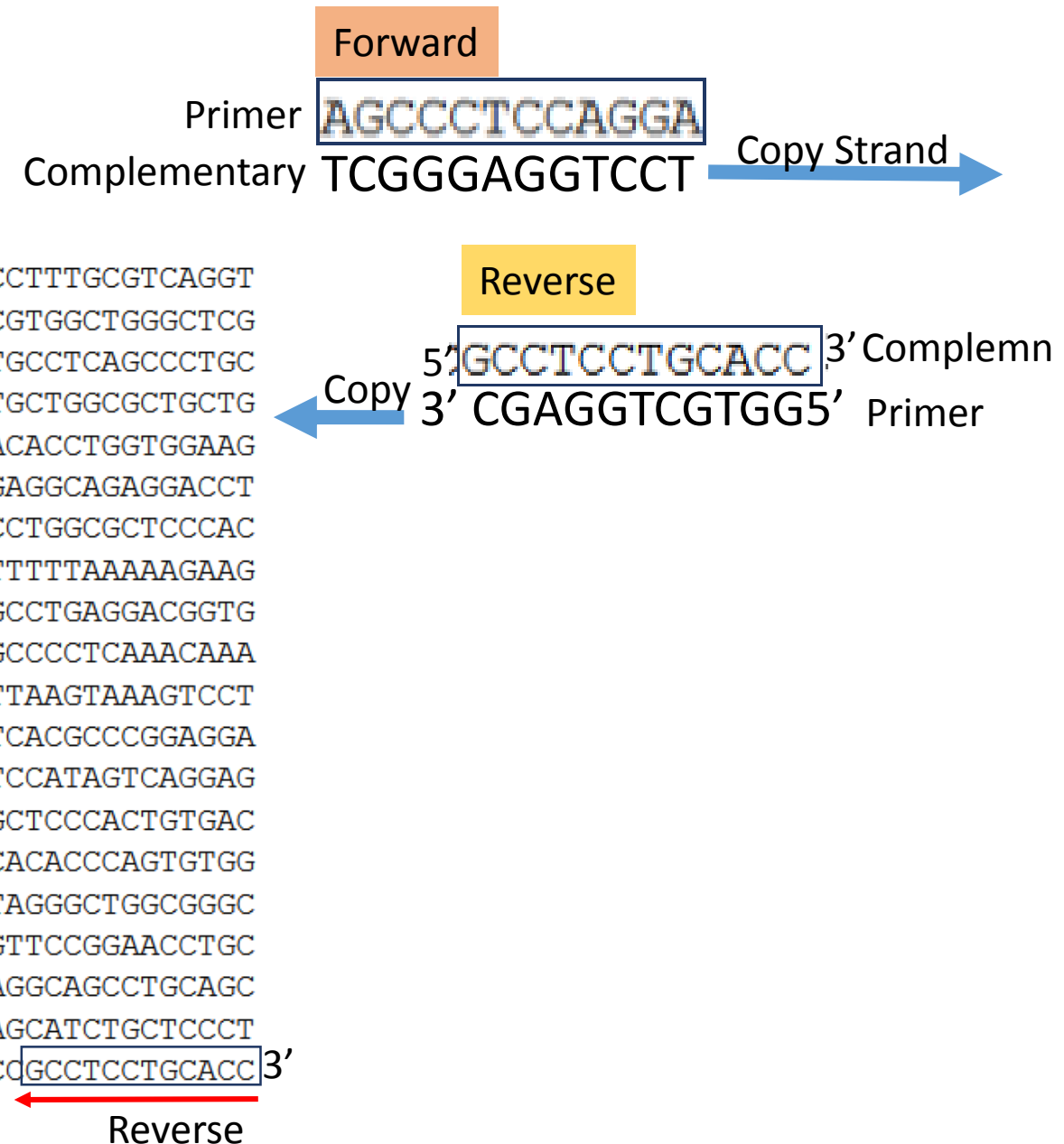
Yustina C.F. Salsinha

(Doctoral Student of Faculty of Biology Universitas Gadjah Mada)

Pendahuluan

Forward

5' AGCCCTCCAGGA CAGGCTGCATCAGAAGAGGCCATCAAGCAGGTCTGTTCCAAGGGCCTTTGCGTCAGGT
GGGCTCAGGATTCCAGGGTGGCTGGACCCAGGCCCCAGCTCTGCAGCAGGGAGGACGTGGCTGGGCTCG
TGAAGCATGTGGGGGTGAGCCCAGGGGGCCCCAAGGCAGGGCACCTGGCCTTCAGCCTGCCTCAGCCCTGC
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CCTTGGCCCTGGAGGGGTCCCTGCAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT
CTACCAGCTGGAGAACTACTGCAACTAGACGCAGCCCGCAGGCAGCCCCACACCCGCCGCCTCCTGCACC 3'
GAGAGAGATGGAATAAAGCCCTTGAACCAGC



Pendahuluan

Hal-hal yang perlu diperhatikan:

- Primer length
- GC Content
- Melting temperature (T_m)
- Self Complementarity

Length: 18-24 bp

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

GC Content: 50-60%

- Lower: Annealing Efficiency Reduced
- Long: T_m Higher

Melting Temperature (T_m)

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

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

Accession
number

Organism

NCBI Resources How To

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Nucleotide Nucleotide P5CS1 Arabidopsis Search

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

NCBI Reference Sequence: NM_001202785.1

[FASTA](#) [Graphics](#)

Go to: ☐

LOCUS NM_001202785 2623 bp mRNA linear PLN 14-FEB-2019

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

ACCESSION NM_001202785

VERSION NM_001202785.1

DBLINK BioProject: [PRJNA116](#)
BioSample: [SAMN03081427](#)

KEYWORDS RefSeq.

SOURCE Arabidopsis thaliana (thale cress)

ORGANISM [Arabidopsis thaliana](#)
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
Spermatophyta; Magnoliophyta; eudicotyledons; Gunneridae;
Pentapetalae; rosids; malvids; Brassicales; Brassicaceae;
Camelineae; Arabidopsis.

REFERENCE 1 (bases 1 to 2623)

AUTHORS Lin,X., Kaul,S., Rounsley,S., Shea,T.P., Benito,M.I., Town,C.D.,
Fujii,C.Y., Mason,T., Bowman,C.L., Barnstead,M., Feldblyum,T.V.,
Buell,C.R., Ketchum,K.A., Lee,J., Ronning,C.M., Koo,H.L.,
Moffat,K.S., Cronin,L.A., Shen,M., Pai,G., Van Aken,S., Umayam,L.,

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Articles about the P5CS1 gene

Proline synthesis in developing microspores is required for pollen develop [BMC Plant Biol. 2018]

Functional FRIGIDA allele enhances drought tolerance [Biochem Biophys Res Commun. 2018]

Global Analysis of Membrane-associated Protein Oligomerization Using [Mol Cell Proteomics. 2017]

See all...

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```
FEATURES             Location/Qualifiers
     source            1..2623
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                        /mol_type="mRNA"
                        /db_xref="taxon:3702"
                        /chromosome="2"
                        /ecotype="Columbia"
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                        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"
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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

UniProt

Go find the sequence

CDS

```
from P5CS2 in a tissue-dependent manner."
/db_xref="Araport:AT2G39800"
/db_xref="GeneID:818566"
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123..2276
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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 1-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)."
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S
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```

PubMed (Weighted)

SNP

UniGene

LinkOut to external resources

GenScript latest version of gene cDNA ORF

Clone [GenScript latest version of g...]

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Arabis 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...

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

NCBI Reference Sequence: NM_001202785.1

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

```
TCCACCTTTCCCTCACCTGATATTTATTTTCTTACCTTAAATACGACGGTGCTTCACTGAGTCCGACTCA
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AAGATCCAATCGGCCGTGTTTTAAAGAAAAACAGAGGTGGCAGATGGTCTTGCTTAGAGAAGACCTCATC
-----
```

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Articles about the P5CS1 gene

Proline synthesis in developing microspores is required for pollen develop [BMC Plant Biol. 2018]

Functional FRIGIDA allele enhances drought tolerance [Biochem Biophys Res Commun. 2018]

Global Analysis of Membrane-associated Protein Oligomerization Using [Mol Cell Proteomics. 2017]

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Primer3plus

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

For PCR
use detection

Primer3Plus
pick primers from a DNA sequence

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[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: No file selected.

Mark selected region:

[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).

Paste Nucleotide
Sequence here

No primer
in selected
region

Primers
include
entire
selected
region

Primers in
selected region

Insert our own primer

Primer3Plus

pick primers from a DNA sequence

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Task: Detection ▼

Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.

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150-250 100-300 301-400 401-500 501-600 601-700 701-850 851-1000

Lenght of PCR Product

Important Properties

[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](#)

Primer3Plus

pick primers from a DNA sequence

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Pair 1:

☒ Left Primer 1:

Sequence:

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

☒ Right Primer 1:

Sequence:

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	GTAACTCGT	TCCTCTCTCT	GTGTGTGGTT
101	TTGGTAGACG	ACGACGACGA	TAATGGAGGA	GCTAGATCGT	TCACGTGCTT
151	TTGCCAGAGA	CGTCAAACGT	ATCGTCGTTA	AGGTTGGGAC	AGCAGTTGTT
201	ACTGGAAAAG	GTGGAA	GATT	GGCTCTTGGT	CGTTTAGGAG
251	ACAGCTTGCG	GAATTAAACT	CGGATGGATT	TGAGGTGATA	TTGGTGTCA
301	CTGGTGCGGT	TGGTCTTGGC	AGGCAAAGGC	TTCGTTATCG	ACAATTAGTC
351	AATAGCAGCT	TTGCGGATCT	TCAGAAGCCT	CAGACTGAAC	TTGATGGGAA
401	GGCTTGTGCT	GGTGTGGAC	AAAGCAGTCT	TATGGCTTAC	TATGAGACTA
451	TGTTTGACCA	GCTTGATGTG	ACGGCAGCTC	AACTTCTGGT	GAATGACAGT
501	AGTTTTAGAG	ACAAGGATTT	CAGGAAGCAA	CTTAATGAAA	CTGTCAAGTC
551	TATGCTTGAT	TTGAGGGTTA	TTCCAATTTT	CAATGAGAAT	GATGCTATTA
601	GCACCCGAAG	AGCCCATAT	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
851	CAAGCTGCA	CTCAATGCG	CTTATGCTGC	GATTGCTCTC	ATCATTAAGC

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

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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

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Delete selected Primers

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

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Save File

Select	Name	Sequence	Designed on	Check!	BLAST!
<input checked="" type="checkbox"/>	Primer_F	TGGTCGTTTAGGAGCACTGTGT	28.02.2020	Check!	BLAST!
<input checked="" type="checkbox"/>	Primer_R	AGCCTTCCCATCAAGTTCAGTC	28.02.2020	Check!	BLAST!
<input checked="" type="checkbox"/>	Primer_1_F	GGTCGTTTAGGAGCACTGTGTG	28.02.2020	Check!	BLAST!
<input checked="" type="checkbox"/>	Primer_1_R	AGCCTTCCCATCAAGTTCAGTC	28.02.2020	Check!	BLAST!

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Primers_2020_02_28.fas - Notepad

FileEditFormatViewHelp

>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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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	?

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Organism *only top 20 will appear*

☐ exclude

Type common name, binomial, taxid or group name

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Percent Identity

E value

Query Coverage

to

to

to

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA	44.1	44.1	100%	0.028	100.00%	NM_001202786.1
<input checked="" type="checkbox"/>	Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA	44.1	44.1	100%	0.028	100.00%	NM_001202785.1
<input checked="" type="checkbox"/>	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/>

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


NCBI Reference Sequence: NM_001202785.1

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

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Proline synthesis in developing microspores is required for pollen develop [BMC Plant Biol. 2018]

Functional FRIGIDA allele enhances drought tolerance [Biochem Biophys Res Commun. 2018]

Global Analysis of Membrane-associated Protein Oligomerization Usin [Mol Cell Proteomics. 2017]

See all...

Primer-BLAST)(Via NCBI)

The image shows the Primer-BLAST web interface with several red boxes and arrows highlighting important features:

- PCR Template:**
 - Enter accession, gi, or FASTA sequence:** A text box containing "NM_001202785.1". An arrow points to it with the label "Nucleotide sequence".
 - Range:** A section with "From" and "To" input fields for Forward and Reverse primers. An arrow points to these fields with the label "Must included in CDS range or targeted region".
 - Or, upload FASTA file:** A "Browse..." button and "No file selected." text.
- Primer Parameters:**
 - Use my own forward primer (5'→3' on plus strand):** An input field with a "Clear" button.
 - Use my own reverse primer (5'→3' on minus strand):** An input field with a "Clear" button.
 - PCR product size:** "Min" (70) and "Max" (1000) input fields. An arrow points to the "Max" field with the label "Often range between 70-200".
 - # of primers to return:** A dropdown menu set to "10". An arrow points to it with the label "Number of primer".
 - Primer melting temperatures (T_m):** "Min" (57.0), "Opt" (60.0), and "Max" (63.0) input fields. An arrow points to the "Max" field with the label "Important properties of T_m".
 - Max T_m difference:** An input field set to "3".
- Exon/intron selection:**
 - Exon junction span:** A dropdown menu set to "No preference".
 - Exon junction match:** "Exon at 5' side" (7) and "Exon at 3' side" (4) input fields.
 - Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction:** A note at the bottom.

Navigation links at the top include: NIH, U.S. National Library of Medicine, NCBI, National Center for Biotechnology Information, febr_salsinha, My NCBI, and Sign Out.

Exon junction match	Exon at 5' side	Exon at 3' side
	<input type="text" value="7"/>	<input type="text" value="4"/>
	Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction	
Intron inclusion	<input type="checkbox"/> Primer pair must be separated by at least one intron on the corresponding genomic DNA	
Intron length range	Min	Max
	<input type="text" value="1000"/>	<input type="text" value="1000000"/>

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

Primer Pair Specificity Checking Parameters


Specificity check	<input checked="" type="checkbox"/> Enable search for primer pairs specific to the intended PCR template
Search mode	<input type="text" value="Automatic"/>
Database	<input type="text" value="Refseq mRNA"/>
Exclusion	<input type="checkbox"/> Exclude predicted Refseq transcripts (accession with XM, XR prefix) <input type="checkbox"/> Exclude uncultured/environmental sample sequences
Organism	<input type="text" value="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)	<input type="text"/>
Primer specificity stringency	Primer must have at least <input type="text" value="2"/> total mismatches to unintended targets, including at least <input type="text" value="2"/> mismatches within the last <input type="text" value="5"/> bps at the 3' end. Ignore targets that have <input type="text" value="6"/> or more mismatches to the primer.
Max target size	<input type="text" value="4000"/>
Allow splice variants	<input type="checkbox"/> 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

 U.S. National Library of Medicine

NCBI National Center for Biotechnology Information

febri_satsinha My NCBI Sign Out

Primer-BLAST

A tool for finding specific primers

Making primers specific to your PCR template. [more...](#)

Status	Submitted	Check
Current time	27 February 2020, 20:41:05	

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NCBI

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

Primer-BLAST » JOB ID:aW02anZJe-Fc2-ve5r7P7Jyl3t6xtsXDsa

Primer-BLAST Results

Input PCR template

Range

Specificity of primers

Other reports

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

1 - 2623

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](#)

[Search Summary](#)

Graphical view of primer pairs



Primer designed

**Primer-BLAST** » JOB ID:aW02anZJe-Fc2-ve5r7P7Jyl3t6xtsXDsa

Primer-BLAST Results

Input PCR template Range [NM_001202785.1](#) Arabidopsis thaliana delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), mRNA
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

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								

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

Primer information

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

Copy the primer from Primer3Plus and paste it on Primer Blast

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

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](#)

NM_201912.1

Range

Forward primer [?](#)

Reverse primer [Clear](#)

Or, upload FASTA file No file selected.

Primer Parameters

Use my own forward primer (5'→3' on plus strand) [Clear](#)

Use my own reverse primer (5'→3' on minus strand) [Clear](#)

PCR product size

Min Max

of primers to return

Primer melting temperatures (T_m)

Min Opt Max Max T_m difference [?](#)

Exon/intron selection

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

Thank you