PRIMER DESIGNS, ARS-GIFVL

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VarMap #5: **FvH4_4g12840** Primer Design

***: Two Set of Primers

Note [1] **(9/20/2023):** This is the 1st & second set of primers; they must be pushed out upstream past repat sequence (outside gene), and closer on the downstream. Rember, Gene is on Minus Strand.
Note [2] (9/20/2023): Green Highlighting is FP1, Red Text is FP2; Red Highlighting is RP1, Green Text is RP2
Note [3] (9/20/2023): This will be the **Third** set of primers added to Chris' independent 'Chart O Primers'.

Gene: **FvH4_4g12840 SNP**: 16497002
Sequence of Interest: 250 nt within 5' UTR upstream on intron in UTR,
>Fvb4_v4.0.a1 Fvb4_v4.0.a1:16496950..16497199
ACGAAGGCAATTGCGTTGTTTCAAATGAATAAGGCGAATCAGGGAATATGGA**T**AGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
GAGAGAGAGCGCAGCGGTTTGGGGGAGAGAGAGAGAGATGAGTTAGGGTTCGGATTTTTATTTTTTATTTTTTTGATATATTTTCGTTTTATTAGG
GTTAAGAGATTTGAGGACTTTTAAACTTTGGGAAATTTTCCTATTCCACATCATACAGACCG

Fuchsia = Transcription Start Site; **Yellow** = SNP
Forward Primer **1**: **AGGCGAATCAGGGAATATGGA** ... **Reverse** Primer **1**: **CGGTCTGTATGATGTGGAATAGG**
Forward Primer **2**: **TGAATAAGGCGAATCAGGGAA**_... **Reverse** Primer **2**:_**TCCGAACCCTAACTCATCTCTC**

Primer3 Output:

```
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         32   21  58.66   47.62    0.00   0.00     0.0 AGGCGAATCAGGGAATATGGA
RIGHT PRIMER       250   23  58.44   47.83    0.00   0.00     0.0 CGGTCTGTATGATGTGGAATAGG
SEQUENCE SIZE: 250
INCLUDED REGION SIZE: 250

PRODUCT SIZE: 219, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

    1 ACGAAGGCAATTGCGTTGTTTCAAATGAATAAGGCGAATCAGGGAATATGGATAGAGAGA
                                     >>>>>>>>>>>>>>>>>>>>>


   61 GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGCGCAGCGGTTTGGGGGAGA


  121 GAGAGAGAGATGAGTTAGGGTTCGGATTTTTATTTTTTATTTTTTTGATATATTTTCGTT


  181 TTATTAGGGTTAAGAGATTTGAGGACTTTTAAACTTTGGGAAATTTTCCTATTCCACATC
                                                     <<<<<<<<<<<<<

  241 ATACAGACCG
      <<<<<<<<<<

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

ADDITIONAL OLIGOS
                     start  len     tm     gc%  any_th  3'_th hairpin seq

 1 LEFT PRIMER          26   21  57.08   42.86    0.00   0.00    0.00
TGAATAAGGCGAATCAGGGAA
   RIGHT PRIMER        146   22  58.71   50.00    0.00   0.00    0.00
TCCGAACCCTAACTCATCTCTC
```

```
   PRODUCT SIZE: 121, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

 2 LEFT PRIMER          33    23    58.55    47.83     0.00    0.00     0.00
GGCGAATCAGGGAATATGGATAG

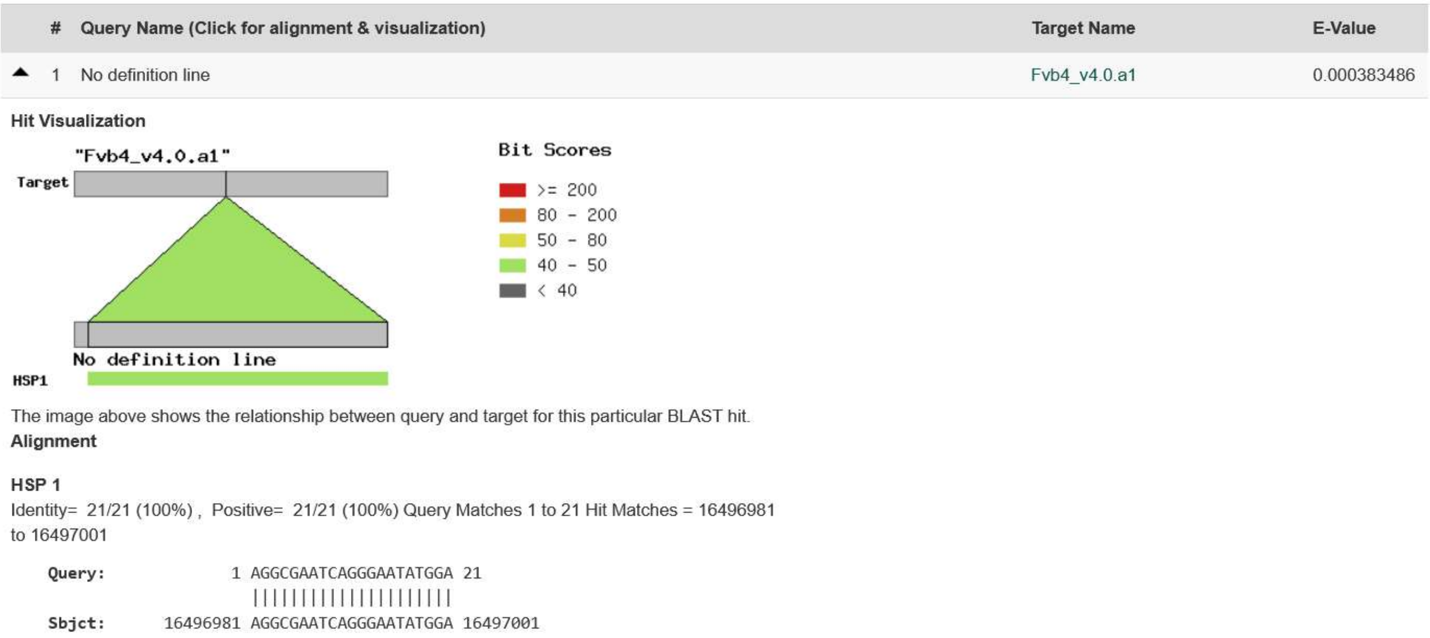
   RIGHT PRIMER        144    23    58.80    47.83     0.00    0.00     0.00
CGAACCCTAACTCATCTCTCTCT
   PRODUCT SIZE: 112, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

 3 LEFT PRIMER          21    23    57.24    39.13     0.00    0.00     0.00
TCAAATGAATAAGGCGAATCAGG
   RIGHT PRIMER        150    23    57.56    39.13     0.00    0.00     0.00
AAAATCCGAACCCTAACTCATCT
   PRODUCT SIZE: 130, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

Statistics
         con   too    in    in   not          no    tm    tm   high  high  high
high
         sid  many   tar  excl    ok   bad    GC   too   too any_th 3'_th hair-  poly
end
        ered    Ns   get   reg   reg   GC% clamp   low  high  compl compl   pin     X
stab    ok
Left     799     0     0     0     0   108     0   480    49      0     0    29    26
0   104
Right    797     0     0     0     0   337     0   323    48      0     0     0    29
0    52
Pair Stats:
considered 26971, unacceptable product size 26967, primer in pair overlaps a primer in a
better pair 6505, ok 4
libprimer3 release 2.4.0
```

Primer Set #1

Blast Forward Primer #1 Against *Fragaria vesca* V4 @ GDR



#	Query Name (Click for alignment & visualization)	Target Name	E-Value
▲ 1	No definition line	Fvb4_v4.0.a1	0.000383486

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

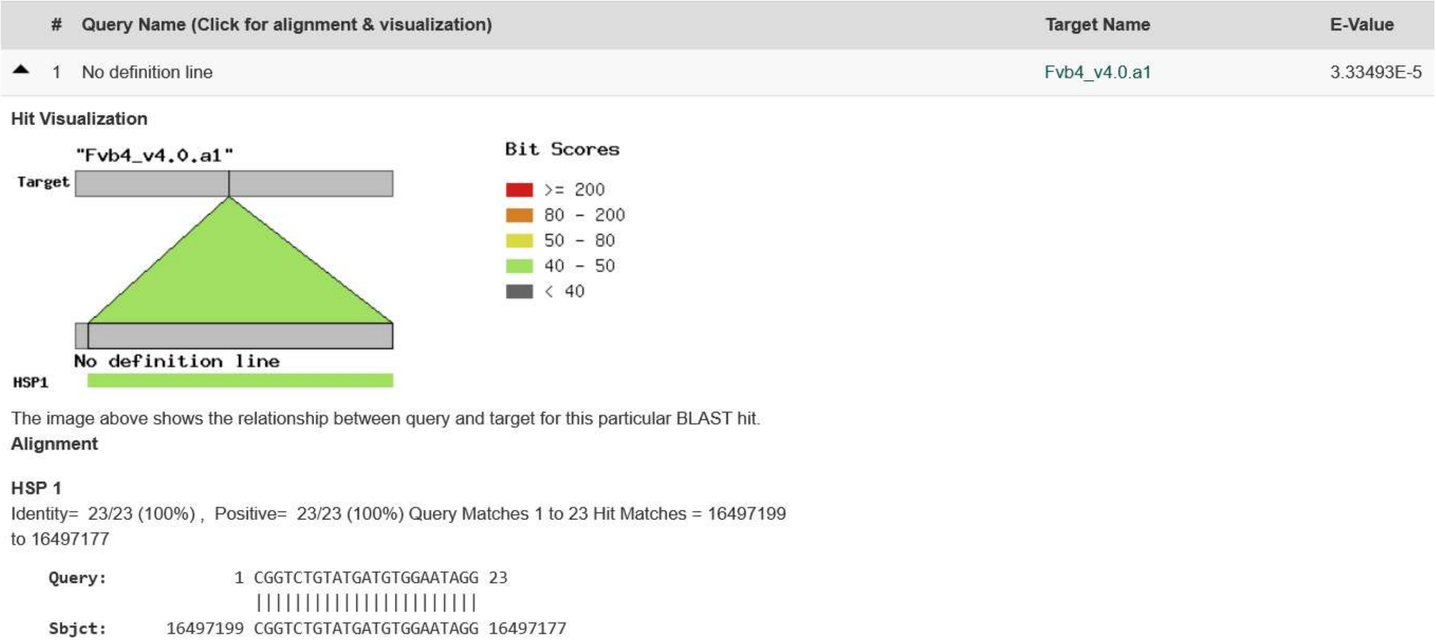
Alignment

HSP 1

Identity= 21/21 (100%) , Positive= 21/21 (100%) Query Matches 1 to 21 Hit Matches = 16496981 to 16497001

```
Query:            1 AGGCGAATCAGGGAATATGGA 21
                    |||||||||||||||||||||
Sbjct:     16496981 AGGCGAATCAGGGAATATGGA 16497001
```

Blast Reverse Primer #1 Against *Fragaria vesca* V4 @ GDR



#	Query Name (Click for alignment & visualization)	Target Name	E-Value
▲ 1	No definition line	Fvb4_v4.0.a1	3.33493E-5

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

Alignment

HSP 1

Identity= 23/23 (100%) , Positive= 23/23 (100%) Query Matches 1 to 23 Hit Matches = 16497199 to 16497177

```
Query:            1 CGGTCTGTATGATGTGGAATAGG 23
                    |||||||||||||||||||||||
Sbjct:     16497199 CGGTCTGTATGATGTGGAATAGG 16497177
```

Analyze Forward Primer #1 @ IDT

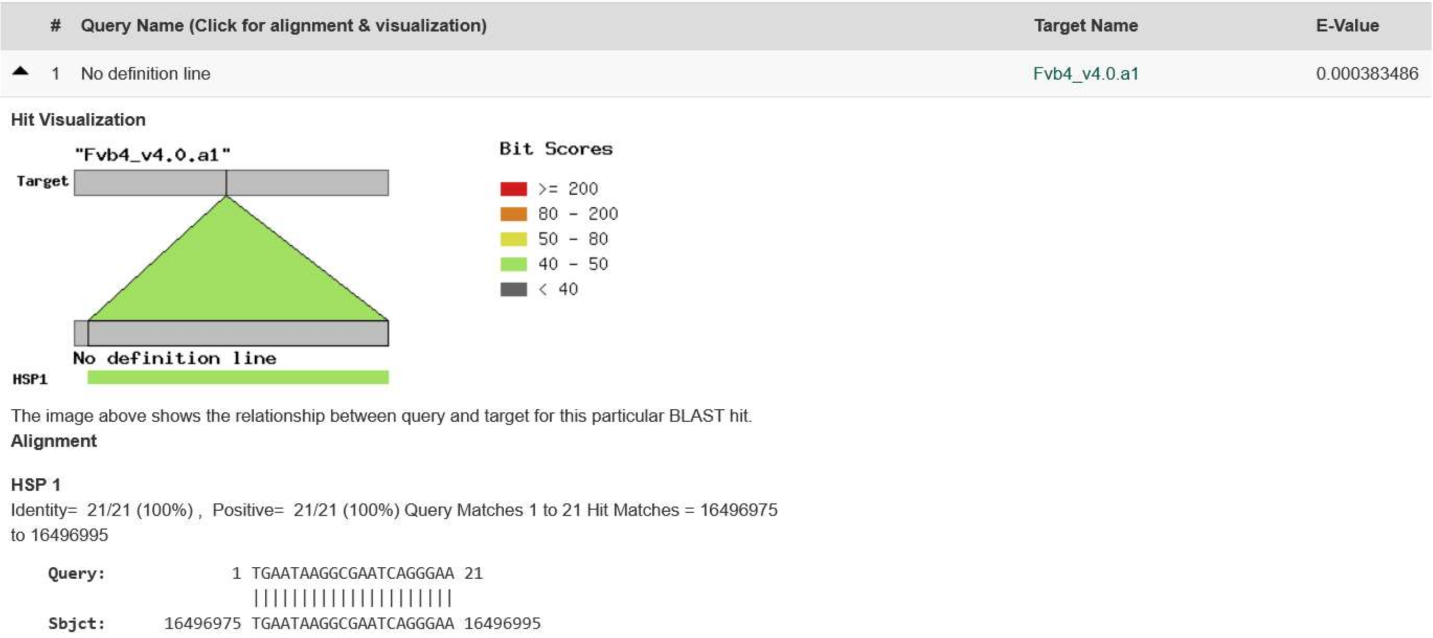
Nothing Major to Note …

Analyze Reverse Primer #1 @ IDT

Nothing Major to Note …

Primer Set #2

Blast Forward Primer #2 Against *Fragaria vesca* V4 @ GDR



	#	Query Name (Click for alignment & visualization)	Target Name	E-Value
▲	1	No definition line	Fvb4_v4.0.a1	0.000383486

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

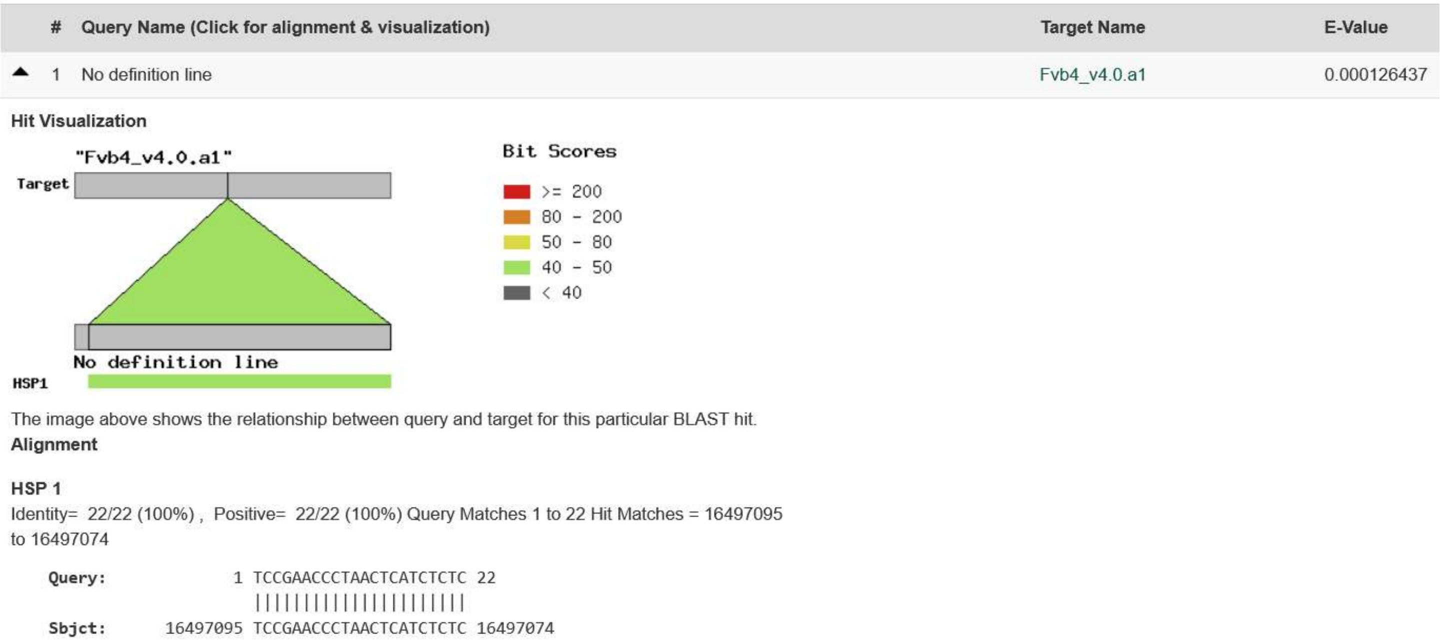
Alignment

HSP 1

Identity= 21/21 (100%) , Positive= 21/21 (100%) Query Matches 1 to 21 Hit Matches = 16496975 to 16496995

```
Query:          1 TGAATAAGGCGAATCAGGGAA 21
                  |||||||||||||||||||||
Sbjct:   16496975 TGAATAAGGCGAATCAGGGAA 16496995
```

Blast Reverse Primer #2 Against *Fragaria vesca* V4 @ GDR



	#	Query Name (Click for alignment & visualization)	Target Name	E-Value
▲	1	No definition line	Fvb4_v4.0.a1	0.000126437

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

Alignment

HSP 1

Identity= 22/22 (100%) , Positive= 22/22 (100%) Query Matches 1 to 22 Hit Matches = 16497095 to 16497074

```
Query:          1 TCCGAACCCTAACTCATCTCTC 22
                  ||||||||||||||||||||||
Sbjct:   16497095 TCCGAACCCTAACTCATCTCTC 16497074
```

Analyze Forward Primer #2 @ IDT

Nothing Major to Note …

Analyze Reverse Primer #2 @ IDT

Nothing Major to Note …

VarMap #6: **FvH4_5g09470** Primer Design

***: Two Set of Primers

Note [1] (9/20/2023)**:** This is the 1st & 2nd set of primers; they are not in a repast section and should theoretically be the easiest of the last four VarMap Primers (#5,6,9,10)
Note [2] (9/20/2023): Green Highlighting is FP1, Red Text is FP2; Red Highlighting is RP1, Green Text is RP2
Note [3] (9/20/2023): This will be the **Fourth** set of primers added to Chris' independent 'Chart O Primers'.

Note [4] (9/20/2023): No Results from Blast against F. v. V4 or H4 NAU @ GDR for Forward Primers …. Blast @ NCBI Instead …

Gene: **5g09470 SNP:** 5498224
Sequence of Interest: 400 nt surrounding SNP, in middle of single large exon, extending into 3' UTR
>Fvb5_v4.0.a1 Fvb5_v4.0.a1:5498024..5498423
AAAAAGGCTGTTCTGCTAATGGCTGTACCTATAACACAATTATCCGGGGGTTTATCAATAATAATGAGATATCGGAGG
CTATGGCACTTTT*TCAAGAAATGCGTAGGAGGGGTT*TCTCTGGAGATGCATCAACTATGGAATTGATTGTTAATTTGT
TGTTGAAGGATACAGTAGATCCTGTATTATTAGCATGGCTTAAA**G**ATTCAGTCTCAAGTTAATTGAATATTTGGGATA
AATATTTTGCAGTATCACTCTACAATTTGAAAC*TGTGTCCAAAGTTAATATCCATGTC*TTAAACAAGTGAATTATCCA
TAGTCCACTTTCTAACTGTTTGTTGCATAATATCATGATCACTCTACAATCGTGGAACTGTTTGTTGCATAATATCAT
GATAGTAAAG

Fuchsia = Translation Stop Signal; **Yellow** = SNP
~~**Forward** Primer 1: ATGCGTAGGAGGGGTTTCTC~~ … ~~**Reverse** Primer 1: ACTTTGGACACAGTTTCAAATTGT~~
Forward Primer **2**: AGAAATGCGTAGGAGGGGTT … **Reverse** Primer **2**: TTTGGACACAGTTTCAAATTGTAGA
Forward Primer **3**: *TCAAGAAATGCGTAGGAGGG* … **Reverse Primer 3**: *GACATGGATATTAACTTTGGACACA*

Primer3 Output:

```
PRIMER PICKING RESULTS FOR Fvb5_v4.0.a1 Fvb5_v4.0.a1:5498024..5498423
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         99   20  59.17   55.00    0.00    0.0     0.0 ATGCGTAGGAGGGGTTTCTC
RIGHT PRIMER       279   24  57.96   33.33    0.00    0.0     0.0 ACTTTGGACACAGTTTCAAATTGT
SEQUENCE SIZE: 400
INCLUDED REGION SIZE: 400; First Set wasn't specific enough, as there were too many exact
matching hits on GDR for the reverse primer #1

PRODUCT SIZE: 181, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

    1 AAAAAGGCTGTTCTGCTAATGGCTGTACCTATAACACAATTATCCGGGGGTTTATCAATA


   61 ATAATGAGATATCGGAGGCTATGGCACTTTTTCAAGAAATGCGTAGGAGGGGTTTCTCTG
                                            >>>>>>>>>>>>>>>>>>>>

  121 GAGATGCATCAACTATGGAATTGATTGTTAATTTGTTGTTGAAGGATACAGTAGATCCTG


  181 TATTATTAGCATGGCTTAAAGATTCAGTCTCAAGTTAATTGAATATTTGGGATAAATATT


  241 TTGCAGTATCACTCTACAATTTGAAACTGTGTCCAAAGTTAATATCCATGTCTTAAACAA
                     <<<<<<<<<<<<<<<<<<<<<<<<

  301 GTGAATTATCCATAGTCCACTTTCTAACTGTTTGTTGCATAATATCATGATCACTCTACA
```

```
  361 ATCGTGGAACTGTTTGTTGCATAATATCATGATAGTAAAG


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

ADDITIONAL OLIGOS
                    start  len      tm     gc%  any_th  3'_th hairpin seq

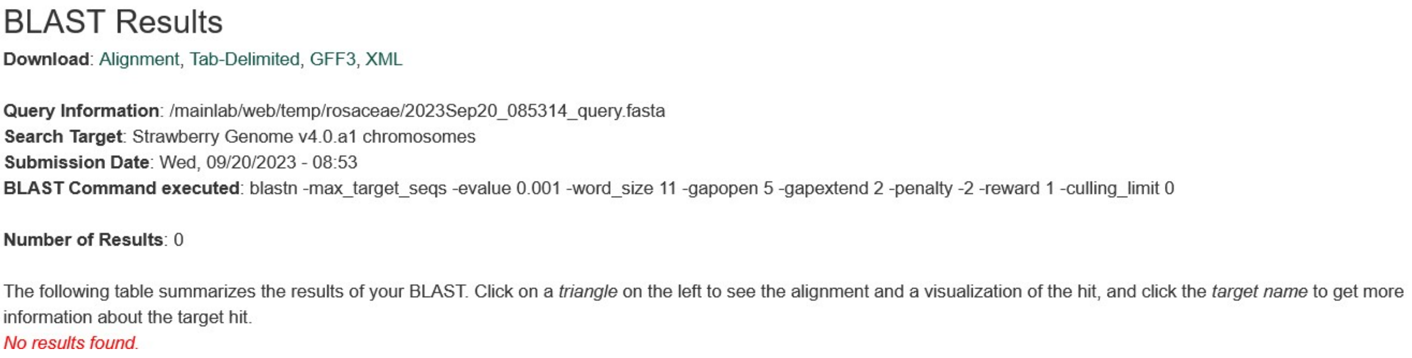
 1 LEFT PRIMER         95   20   58.71   50.00    0.00   0.00    0.00
AGAAATGCGTAGGAGGGGTT
   RIGHT PRIMER       277   25   57.60   32.00    0.00   0.00    0.00
TTTGGACACAGTTTCAAATTGTAGA
   PRODUCT SIZE: 183, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

 2 LEFT PRIMER         92   20   57.31   50.00    0.00   0.00    0.00
TCAAGAAATGCGTAGGAGGG
   RIGHT PRIMER       292   25   57.38   36.00    0.00   0.00    0.00
GACATGGATATTAACTTTGGACACA
   PRODUCT SIZE: 201, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

 3 LEFT PRIMER         27   23   57.76   43.48    0.00   0.00    0.00
ACCTATAACACAATTATCCGGGG
   RIGHT PRIMER       195   25   57.22   40.00    0.00   0.00    0.00
GCCATGCTAATAATACAGGATCTAC
   PRODUCT SIZE: 169, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

 4 LEFT PRIMER         61   24   57.34   41.67    0.00   0.00    0.00
ATAATGAGATATCGGAGGCTATGG
   RIGHT PRIMER       266   25   57.02   32.00    0.00   0.00    0.00
TTTCAAATTGTAGAGTGATACTGCA
   PRODUCT SIZE: 206, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00
```

Blast Forward Primer #1 Against *Fragaria vesca* V4 @ GDR



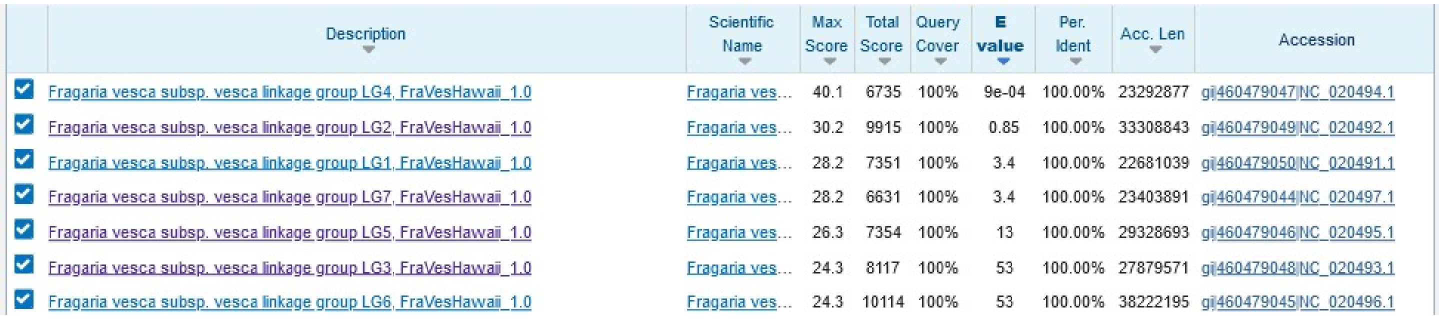
BLAST Results

Download: Alignment, Tab-Delimited, GFF3, XML

Query Information: /mainlab/web/temp/rosaceae/2023Sep20_085314_query.fasta
Search Target: Strawberry Genome v4.0.a1 chromosomes
Submission Date: Wed, 09/20/2023 - 08:53
BLAST Command executed: blastn -max_target_seqs -evalue 0.001 -word_size 11 -gapopen 5 -gapextend 2 -penalty -2 -reward 1 -culling_limit 0

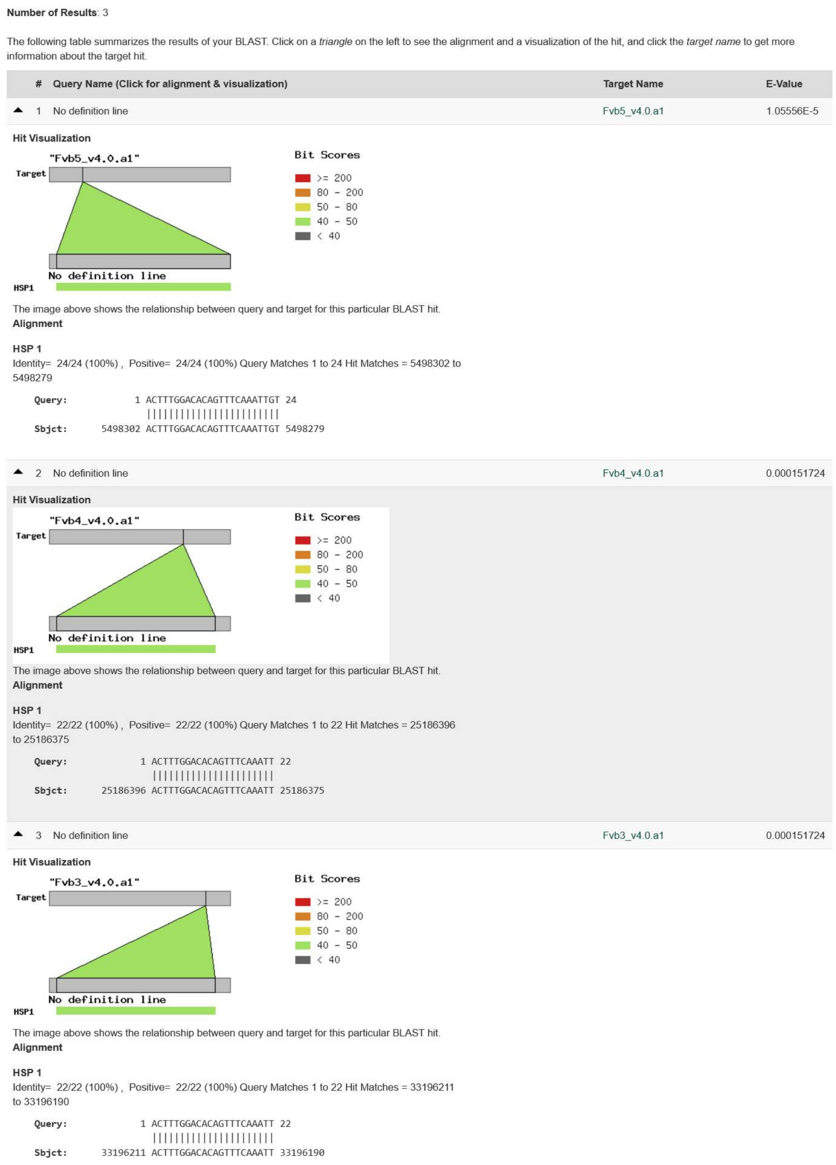
Number of Results: 0

The following table summarizes the results of your BLAST. Click on a *triangle* on the left to see the alignment and a visualization of the hit, and click the *target name* to get more information about the target hit.
No results found.



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria ves...	40.1	6735	100%	9e-04	100.00%	23292877	gi|460479047|NC_020494.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria ves...	30.2	9915	100%	0.85	100.00%	33308843	gi|460479049|NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria ves...	28.2	7351	100%	3.4	100.00%	22681039	gi|460479050|NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria ves...	28.2	6631	100%	3.4	100.00%	23403891	gi|460479044|NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria ves...	26.3	7354	100%	13	100.00%	29328693	gi|460479046|NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria ves...	24.3	8117	100%	53	100.00%	27879571	gi|460479048|NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria ves...	24.3	10114	100%	53	100.00%	38222195	gi|460479045|NC_020496.1

Blast Reverse Primer #1 Against *Fragaria vesca* V4 @ GDR



Number of Results: 3

The following table summarizes the results of your BLAST. Click on a *triangle* on the left to see the alignment and a visualization of the hit, and click the *target name* to get more information about the target hit.

	#	Query Name (Click for alignment & visualization)	Target Name	E-Value
▲	1	No definition line	Fvb5_v4.0.a1	1.05556E-5

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

Alignment

HSP 1

Identity= 24/24 (100%) , Positive= 24/24 (100%) Query Matches 1 to 24 Hit Matches = 5498302 to 5498279

```
Query:          1 ACTTTGGACACAGTTTCAAATTGT 24
                  ||||||||||||||||||||||||
Sbjct:    5498302 ACTTTGGACACAGTTTCAAATTGT 5498279
```

▲	2	No definition line	Fvb4_v4.0.a1	0.000151724

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

Alignment

HSP 1

Identity= 22/22 (100%) , Positive= 22/22 (100%) Query Matches 1 to 22 Hit Matches = 25186396 to 25186375

```
Query:           1 ACTTTGGACACAGTTTCAAATT 22
                   ||||||||||||||||||||||
Sbjct:    25186396 ACTTTGGACACAGTTTCAAATT 25186375
```

▲	3	No definition line	Fvb3_v4.0.a1	0.000151724

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

Alignment

HSP 1

Identity= 22/22 (100%) , Positive= 22/22 (100%) Query Matches 1 to 22 Hit Matches = 33196211 to 33196190

```
Query:           1 ACTTTGGACACAGTTTCAAATT 22
                   ||||||||||||||||||||||
Sbjct:    33196211 ACTTTGGACACAGTTTCAAATT 33196190
```

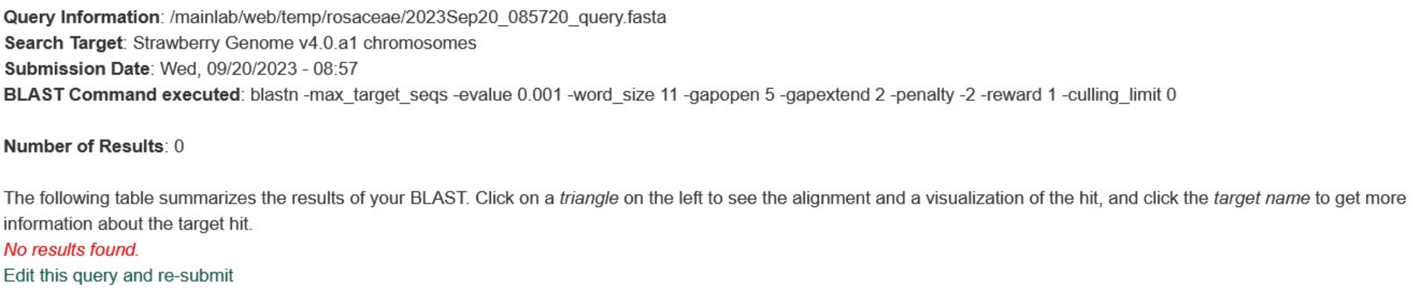
Analyze Forward Primer #1 @ IDT

Nothing Major to Note …

Analyze Reverse Primer #1 @ IDT

Nothing Major to Note …

Blast Forward Primer #2 Against *Fragaria vesca* V4 @ GDR



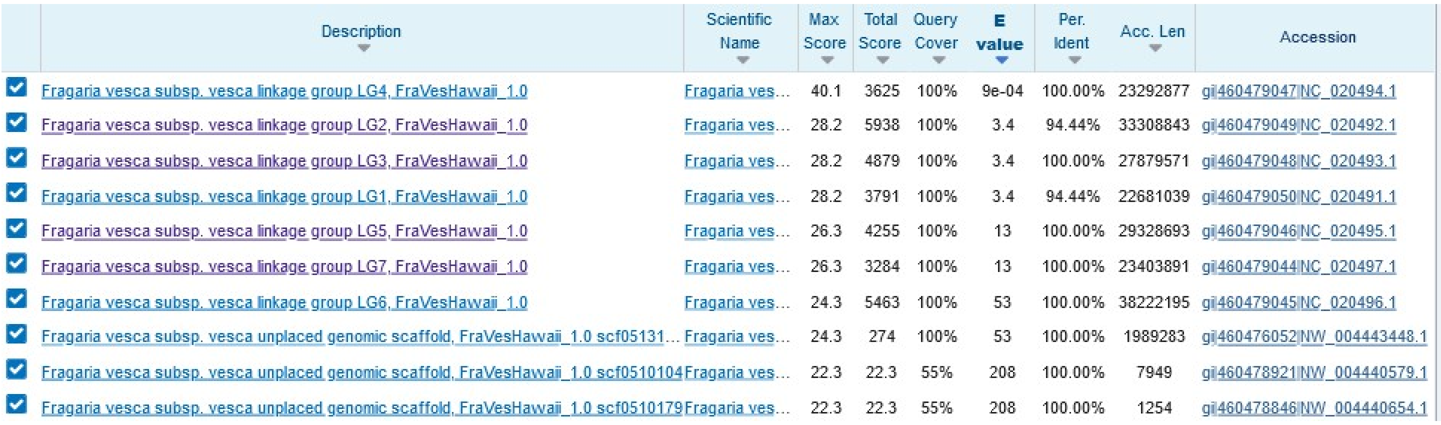
Query Information: /mainlab/web/temp/rosaceae/2023Sep20_085720_query.fasta
Search Target: Strawberry Genome v4.0.a1 chromosomes
Submission Date: Wed, 09/20/2023 - 08:57
BLAST Command executed: blastn -max_target_seqs -evalue 0.001 -word_size 11 -gapopen 5 -gapextend 2 -penalty -2 -reward 1 -culling_limit 0

Number of Results: 0

The following table summarizes the results of your BLAST. Click on a *triangle* on the left to see the alignment and a visualization of the hit, and click the *target name* to get more information about the target hit.

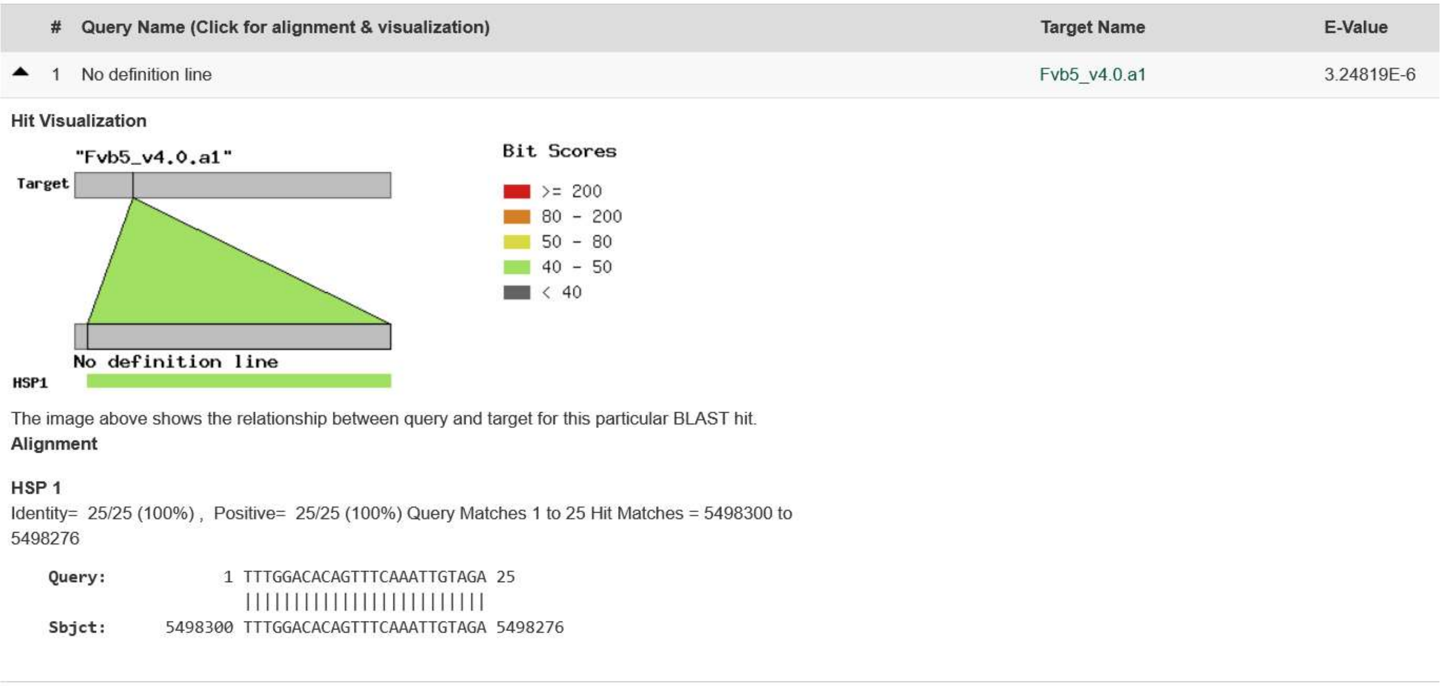
No results found.

Edit this query and re-submit



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria ves…	40.1	3625	100%	9e-04	100.00%	23292877	gi|460479047|NC_020494.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria ves…	28.2	5938	100%	3.4	94.44%	33308843	gi|460479049|NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria ves…	28.2	4879	100%	3.4	100.00%	27879571	gi|460479048|NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria ves…	28.2	3791	100%	3.4	94.44%	22681039	gi|460479050|NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria ves…	26.3	4255	100%	13	100.00%	29328693	gi|460479046|NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria ves…	26.3	3284	100%	13	100.00%	23403891	gi|460479044|NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria ves…	24.3	5463	100%	53	100.00%	38222195	gi|460479045|NC_020496.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf05131…	Fragaria ves…	24.3	274	100%	53	100.00%	1989283	gi|460476052|NW_004443448.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510104	Fragaria ves…	22.3	22.3	55%	208	100.00%	7949	gi|460478921|NW_004440579.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510179	Fragaria ves…	22.3	22.3	55%	208	100.00%	1254	gi|460478846|NW_004440654.1

Blast Reverse Primer #2 Against *Fragaria vesca* V4 @ GDR



	#	Query Name (Click for alignment & visualization)	Target Name	E-Value
▲	1	No definition line	Fvb5_v4.0.a1	3.24819E-6

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

Alignment

HSP 1

Identity= 25/25 (100%) , Positive= 25/25 (100%) Query Matches 1 to 25 Hit Matches = 5498300 to 5498276

```
Query:          1 TTTGGACACAGTTTCAAATTGTAGA 25
                  |||||||||||||||||||||||||
Sbjct:    5498300 TTTGGACACAGTTTCAAATTGTAGA 5498276
```

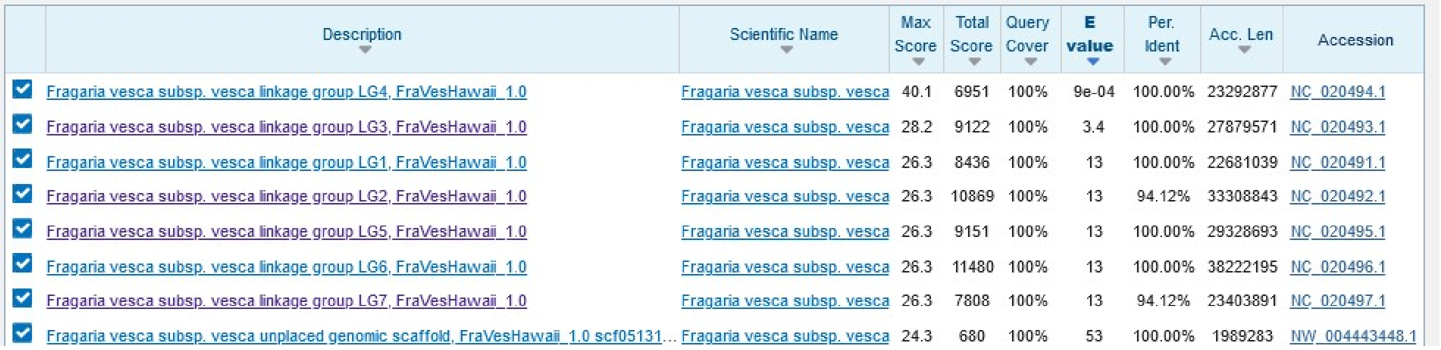
Analyze Forward Primer #2 @ IDT

Nothing Major to Note …

Analyze Reverse Primer #2 @ IDT

Nothing Major to Note …

Blast Forward Primer #3 Against *Fragaria vesca* V4 @ GDR / NCBI



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	40.1	6951	100%	9e-04	100.00%	23292877	NC_020494.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	9122	100%	3.4	100.00%	27879571	NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	26.3	8436	100%	13	100.00%	22681039	NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	26.3	10869	100%	13	94.12%	33308843	NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	26.3	9151	100%	13	100.00%	29328693	NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	26.3	11480	100%	13	100.00%	38222195	NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	26.3	7808	100%	13	94.12%	23403891	NC_020497.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf05131…	Fragaria vesca subsp. vesca	24.3	680	100%	53	100.00%	1989283	NW_004443448.1

Blast Reverse Primer #3 Against *Fragaria vesca* V4 @ GDR / NCBI

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	50.1	6697	100%	2e-06	100.00%	23292877	NC_020494.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	38.2	6717	100%	0.006	100.00%	27879571	NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	32.2	5878	100%	0.39	100.00%	22681039	NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	32.2	8292	100%	0.39	100.00%	33308843	NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	30.2	9077	100%	1.5	100.00%	38222195	NC_020496.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0511328	Fragaria vesca subsp. vesca	30.2	30.2	76%	1.5	94.74%	1200	NW_004441791.1
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	7243	100%	6.1	100.00%	29328693	NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	26.3	5697	100%	24	94.12%	23403891	NC_020497.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510373	Fragaria vesca subsp. vesca	26.3	26.3	52%	24	100.00%	1257	NW_004440848.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0511058	Fragaria vesca subsp. vesca	26.3	26.3	52%	24	100.00%	1595	NW_004441521.1

Analyze Forward Primer #3 @ IDT

Nothing Major to Note …

Analyze Reverse Primer #3 @ IDT

Nothing Major to Note …

VarMap #9: **FvH4_5g28580** Primer Design

***: Two Set of Primers

Note [1] (9/20/2023)**:** This is the 1st & second set of primers; they must be pushed out far upstream, past repeat sequence, and closer on the downstream.

Note [2] (9/20/2023): Green Highlighting is FP1, Red Text is FP2; Red Highlighting is RP1, Green Text is RP2

Note [3] (9/20/2023): This will be the **Fifth** set of primers added to Chris' independent 'Chart O Primers'.

Note [4] (9/20/2023): No Results from Blast against F. v. V4 or H4 NAU @ GDR …. Blast @ NCBI Instead …

Gene: **FvH4_5g28580 SNP**: **19740249**

Sequence of Interest: 300nt

>Fvb5_v4.0.a1 Fvb5_v4.0.a1:19740148..19740447
GTAGACGTTGGCCGATCGTACGGCGTCGTAAGGGAGGTTCTCCGGAAGG**GAGTCTTCCGGAACTTGCC**GCCCGCCCTAAGGGGCTGCTTGTGCTG
CTGCTC**T**GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGGGGGGGGGGGGGCGGGCGAAGCTAGAGTTTTGGATTGGGAAA
TAATTGGGACGGGGGCTGGCGGTAGGGGGGTGTAAAGGAGGCAAGGCTTGAGAGGTAGGTGTAGAGATTGAGCGAGCTTTGGATAAATAAATA
TAGAGGTGATGGTGATCTTG

Fuchsia = Splice Site; **Yellow** = SNP

Forward Primer **1**: GAGTCTTCCGGAACTTGCC … **Reverse** Primer **1**: CTACCTCTCAAGCCTTGCCT

Forward Primer **2**: CCTAAGGGGCTGCTTGTG … **Reverse** Primer **2**: TCTCAAGCCTTGCCTCCTTT

Primer3 Output:

```
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         50   19  58.16   57.89   14.35   0.00    0.00 GAGTCTTCCGGAACTTGCC
RIGHT PRIMER       244   20  59.09   55.00    0.00   0.00    0.00 CTACCTCTCAAGCCTTGCCT
SEQUENCE SIZE: 300
INCLUDED REGION SIZE: 300

PRODUCT SIZE: 195, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

    1 GTAGACGTTGGCCGATCGTACGGCGTCGTAAGGGAGGTTCTCCGGAAGGGAGTCTTCCGG
                                                       >>>>>>>>>>>

   61 AACTTGCCGCCCGCCCTAAGGGGCTGCTTGTGCTGCTGCTCTGAGAGAGAGAGAGAGAGA
      >>>>>>>>

  121 GAGAGAGAGAGAGAGAGAGAGAGGGGGGGGGGGGGGCGGGCGAAGCTAGAGTTTTGGATT


  181 GGGAAATAATTGGGACGGGGGCTGGCGGTAGGGGGGTGTAAAGGAGGCAAGGCTTGAGAG
                                                  <<<<<<<<<<<<<<<<

  241 GTAGGTGTAGAGATTGAGCGAGCTTTGGATAAATAAATTATAGAGGTGATGGTGATCTTG
      <<<<

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

ADDITIONAL OLIGOS
```

```
                     start  len      tm     gc%  any_th  3'_th hairpin seq

 1 LEFT PRIMER          75    18    57.67    61.11     0.00    0.00     0.0 CCTAAGGGGCTGCTTGTG
   RIGHT PRIMER        239    20    59.23    50.00     0.00    0.00     0.0 TCTCAAGCCTTGCCTCCTTT

PRODUCT SIZE: 165, PAIR ANY_TH COMPL: 24.99, PAIR 3'_TH COMPL: 23.00

 2 LEFT PRIMER         114    23    57.86    47.83     0.00    0.00     0.00
AGAGAGAGAGAGAGAGAGAGAGA
   RIGHT PRIMER        263    20    58.15    55.00     0.00    0.00     0.00
GCTCGCTCAATCTCTACACC
   PRODUCT SIZE: 150, PAIR ANY_TH COMPL: 21.03, PAIR 3'_TH COMPL: 12.65

 3 LEFT PRIMER         110    23    57.86    47.83     0.00    0.00     0.00
AGAGAGAGAGAGAGAGAGAGAGA
   RIGHT PRIMER        269    20    57.98    50.00     0.00    0.00     0.00
TCCAAAGCTCGCTCAATCTC
   PRODUCT SIZE: 160, PAIR ANY_TH COMPL: 16.90, PAIR 3'_TH COMPL: 16.90

 4 LEFT PRIMER         162    23    58.41    43.48     0.00    0.00     0.00
GAAGCTAGAGTTTTGGATTGGGA
   RIGHT PRIMER        261    22    59.05    50.00     0.00    0.00     0.00
TCGCTCAATCTCTACACCTACC
   PRODUCT SIZE: 100, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

Statistics
         con   too     in    in   not          no    tm    tm   high  high  high
high
         sid  many    tar  excl    ok   bad    GC   too   too any_th 3'_th hair-   poly
end
        ered    Ns    get   reg   reg   GC% clamp   low  high  compl compl   pin      X
stab    ok
Left    1142     0      0     0     0   275     0   272   319      0     1   183     23
0    47
Right   1086     0      0     0     0   284     0   439    88      0     0     0     35
0   240
Pair Stats:
considered 12167, unacceptable product size 12157, primer in pair overlaps a primer in a
better pair 4690, ok 10
libprimer3 release 2.4.0
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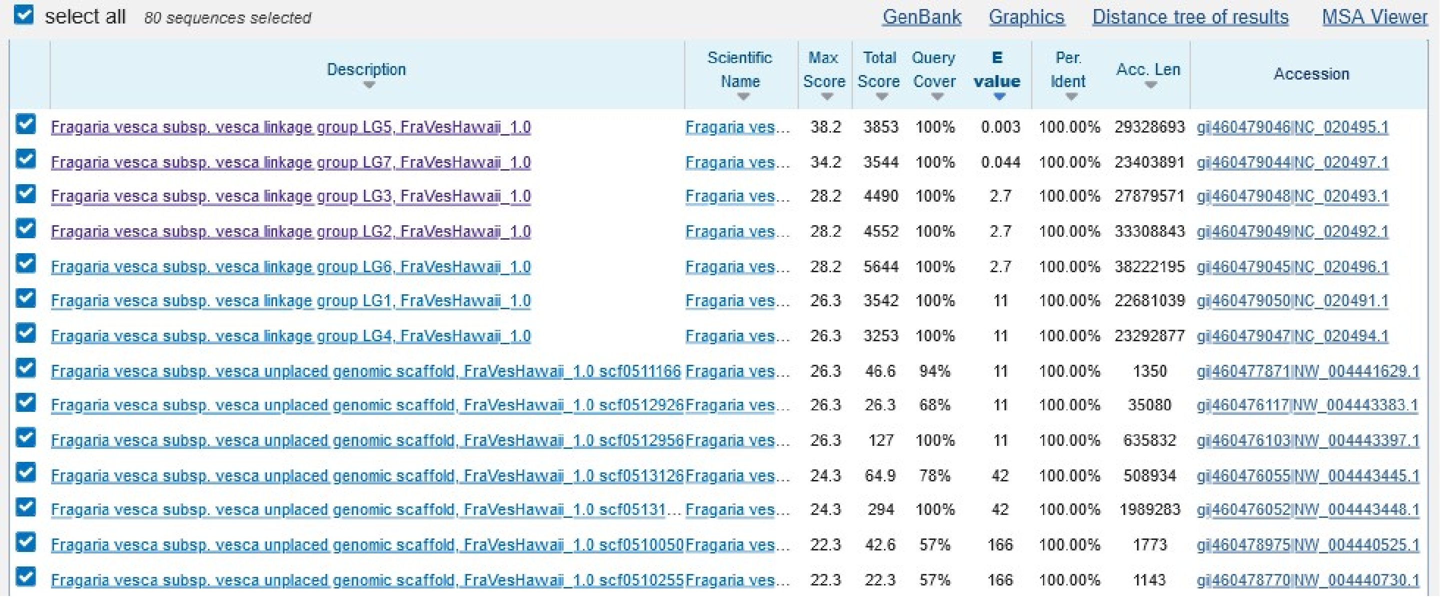
Primer Set #1

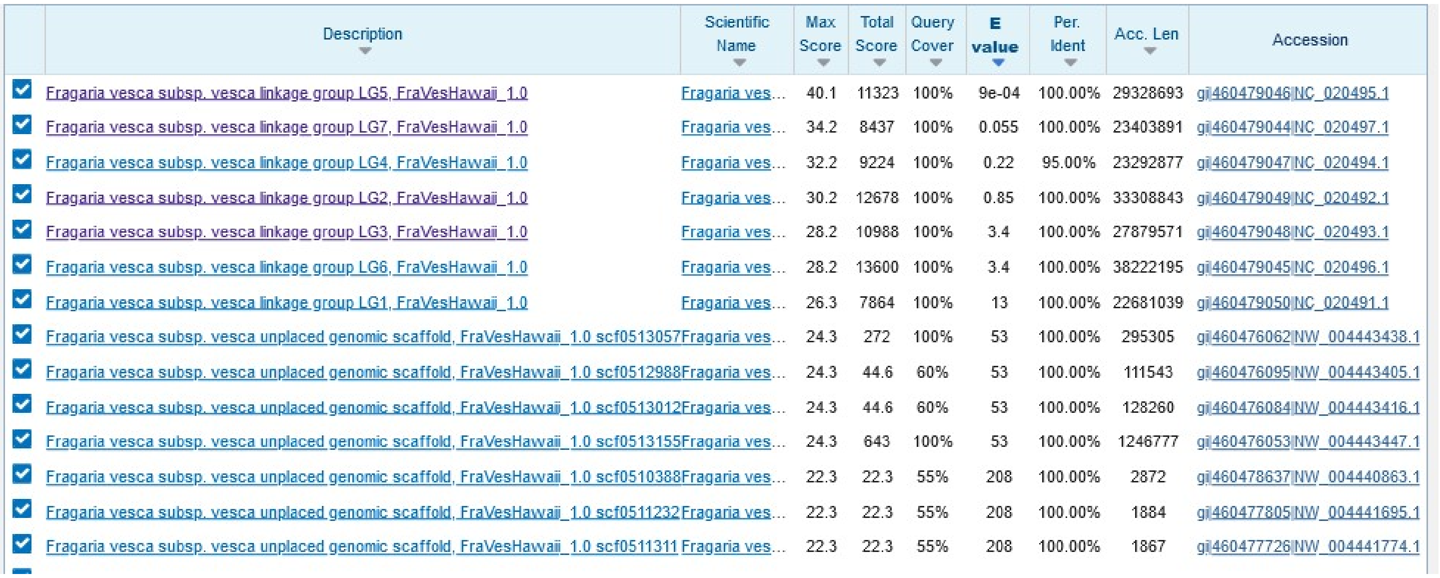
Blast Forward Primer #1 Against *Fragaria vesca* @ NCBI (refseq_genomes)



☑ select all 80 sequences selected

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria ves...	38.2	3853	100%	0.003	100.00%	29328693	gi|460479046|NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria ves...	34.2	3544	100%	0.044	100.00%	23403891	gi|460479044|NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria ves...	28.2	4490	100%	2.7	100.00%	27879571	gi|460479048|NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria ves...	28.2	4552	100%	2.7	100.00%	33308843	gi|460479049|NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria ves...	28.2	5644	100%	2.7	100.00%	38222195	gi|460479045|NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria ves...	26.3	3542	100%	11	100.00%	22681039	gi|460479050|NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria ves...	26.3	3253	100%	11	100.00%	23292877	gi|460479047|NC_020494.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0511166	Fragaria ves...	26.3	46.6	94%	11	100.00%	1350	gi|460477871|NW_004441629.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0512926	Fragaria ves...	26.3	26.3	68%	11	100.00%	35080	gi|460476117|NW_004443383.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0512956	Fragaria ves...	26.3	127	100%	11	100.00%	635832	gi|460476103|NW_004443397.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0513126	Fragaria ves...	24.3	64.9	78%	42	100.00%	508934	gi|460476055|NW_004443445.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf05131...	Fragaria ves...	24.3	294	100%	42	100.00%	1989283	gi|460476052|NW_004443448.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510050	Fragaria ves...	22.3	42.6	57%	166	100.00%	1773	gi|460478975|NW_004440525.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510255	Fragaria ves...	22.3	22.3	57%	166	100.00%	1143	gi|460478770|NW_004440730.1

Blast Reverse Primer #1 Against *Fragaria vesca* @ NCBI (refseq_genomes)



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria ves...	40.1	11323	100%	9e-04	100.00%	29328693	gi|460479046|NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria ves...	34.2	8437	100%	0.055	100.00%	23403891	gi|460479044|NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria ves...	32.2	9224	100%	0.22	95.00%	23292877	gi|460479047|NC_020494.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria ves...	30.2	12678	100%	0.85	100.00%	33308843	gi|460479049|NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria ves...	28.2	10988	100%	3.4	100.00%	27879571	gi|460479048|NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria ves...	28.2	13600	100%	3.4	100.00%	38222195	gi|460479045|NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria ves...	26.3	7864	100%	13	100.00%	22681039	gi|460479050|NC_020491.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0513057	Fragaria ves...	24.3	272	100%	53	100.00%	295305	gi|460476062|NW_004443438.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0512988	Fragaria ves...	24.3	44.6	60%	53	100.00%	111543	gi|460476095|NW_004443405.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0513012	Fragaria ves...	24.3	44.6	60%	53	100.00%	128260	gi|460476084|NW_004443416.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0513155	Fragaria ves...	24.3	643	100%	53	100.00%	1246777	gi|460476053|NW_004443447.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510388	Fragaria ves...	22.3	22.3	55%	208	100.00%	2872	gi|460478637|NW_004440863.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0511232	Fragaria ves...	22.3	22.3	55%	208	100.00%	1884	gi|460477805|NW_004441695.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0511311	Fragaria ves...	22.3	22.3	55%	208	100.00%	1867	gi|460477726|NW_004441774.1

Analyze Forward Primer #1 @ IDT

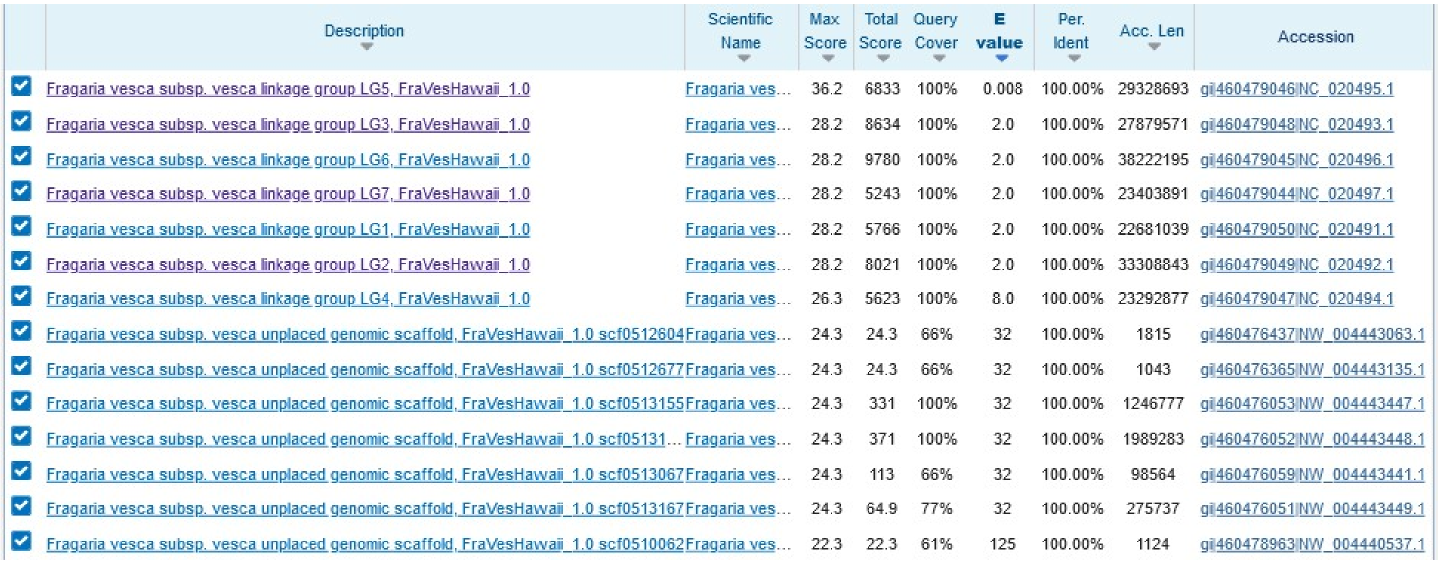
Nothing Major to Note …

Analyze Reverse Primer #1 @ IDT

Nothing Major to Note …

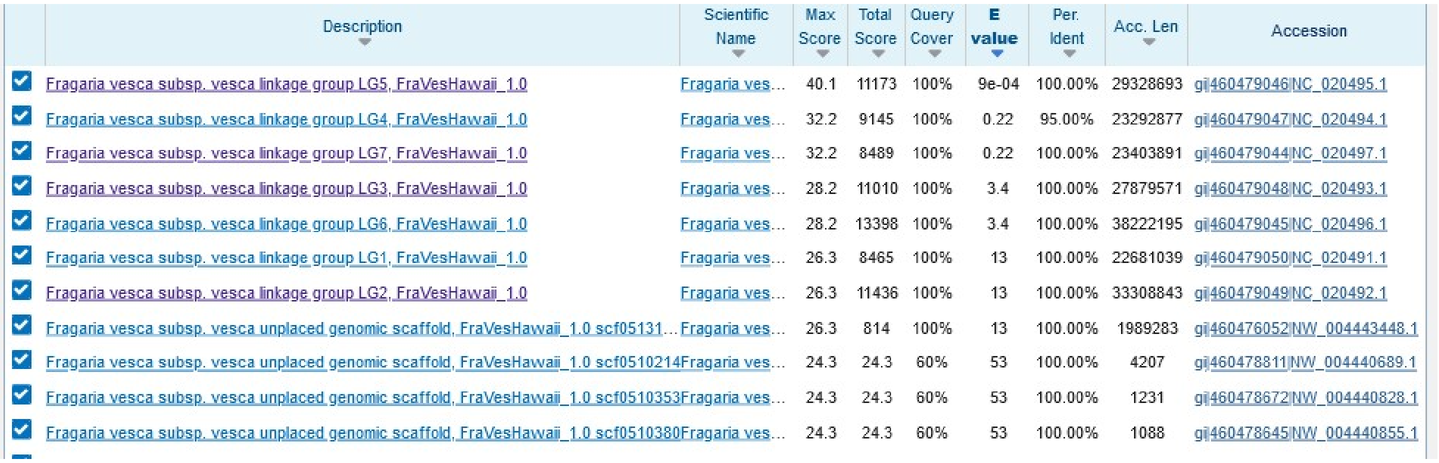
Primer Set #2

Blast Forward Primer #2 Against *Fragaria vesca* @ NCBI (refseq_genomes)



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria ves...	36.2	6833	100%	0.008	100.00%	29328693	gi|460479046|NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria ves...	28.2	8634	100%	2.0	100.00%	27879571	gi|460479048|NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria ves...	28.2	9780	100%	2.0	100.00%	38222195	gi|460479045|NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria ves...	28.2	5243	100%	2.0	100.00%	23403891	gi|460479044|NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria ves...	28.2	5766	100%	2.0	100.00%	22681039	gi|460479050|NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria ves...	28.2	8021	100%	2.0	100.00%	33308843	gi|460479049|NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria ves...	26.3	5623	100%	8.0	100.00%	23292877	gi|460479047|NC_020494.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0512604	Fragaria ves...	24.3	24.3	66%	32	100.00%	1815	gi|460476437|NW_004443063.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0512677	Fragaria ves...	24.3	24.3	66%	32	100.00%	1043	gi|460476365|NW_004443135.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0513155	Fragaria ves...	24.3	331	100%	32	100.00%	1246777	gi|460476053|NW_004443447.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf05131...	Fragaria ves...	24.3	371	100%	32	100.00%	1989283	gi|460476052|NW_004443448.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0513067	Fragaria ves...	24.3	113	66%	32	100.00%	98564	gi|460476059|NW_004443441.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0513167	Fragaria ves...	24.3	64.9	77%	32	100.00%	275737	gi|460476051|NW_004443449.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510062	Fragaria ves...	22.3	22.3	61%	125	100.00%	1124	gi|460478963|NW_004440537.1

Blast Reverse Primer #2 Against *Fragaria vesca* @ NCBI (refseq_genomes)



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria ves...	40.1	11173	100%	9e-04	100.00%	29328693	gi|460479046|NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria ves...	32.2	9145	100%	0.22	95.00%	23292877	gi|460479047|NC_020494.1
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria ves...	32.2	8489	100%	0.22	100.00%	23403891	gi|460479044|NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria ves...	28.2	11010	100%	3.4	100.00%	27879571	gi|460479048|NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria ves...	28.2	13398	100%	3.4	100.00%	38222195	gi|460479045|NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria ves...	26.3	8465	100%	13	100.00%	22681039	gi|460479050|NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria ves...	26.3	11436	100%	13	100.00%	33308843	gi|460479049|NC_020492.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf05131...	Fragaria ves...	26.3	814	100%	13	100.00%	1989283	gi|460476052|NW_004443448.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510214	Fragaria ves...	24.3	24.3	60%	53	100.00%	4207	gi|460478811|NW_004440689.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510353	Fragaria ves...	24.3	24.3	60%	53	100.00%	1231	gi|460478672|NW_004440828.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510380	Fragaria ves...	24.3	24.3	60%	53	100.00%	1088	gi|460478645|NW_004440855.1

Analyze Forward Primer #2 @ IDT

Nothing Major to Note …

Analyze Reverse Primer #2 @ IDT

Nothing Major to Note …

VarMap #10: **FvH4_7g15500** Primer Design

***: Two Set of Primers

Note [1] (9/20/2023)**:** This is the 1st & second set of primers; they must be pushed out far on right, past repeat sequence, and closer on the left.

Note [2] (9/20/2023): Green Highlighting is FP1, Red Text is FP2; Red Highlighting is RP1, Green Text is RP2

Note [3] (9/20/2023): This will be the **Fifth** set of primers added to Chris' independent 'Chart O Primers'.

Note [4] (9/20/2023): No Results from Blast against F. v. V4 or H4 NAU @ GDR .... Blast @ NCBI Instead (For Reverse Primers) ...

Note [5] (9/21/2023): Made the Product Size bigger and pushed primers out for alternative / additional primer, to #2, which is a bit to close in, having too small of a product size.

Gene: FvH4_7g15500 SNP: 13414877
Sequence of Interest: 200 nt (#1) 451 nt (#2)
>Fvb7_v4.0.a1 Fvb7_v4.0.a1:13414776..13414975
GTATTAAATGCAGCAGCTGGAAGCACCCAACTACTT**TCTCTCTCAAAA**CCACCTCCA**ATGGCTAAAACC**CCTCCCACCCTCCACCACCTCCTCCTCCA
ATG**T**CCCCCCTCCCTCCTCCCCCTCCTCAAATTACCTCACTACCCTCGGCCTCGGCTACGCCATAGCCATCGCC**CTCGGCTTCCTCGTCCTC****CTCTCCAC**
CC
>Fvb7_v4.0.a1 Fvb7_v4.0.a1:13414674..13415073
CAAAAAAACTGGCTGCCCAAAAAGGAGAAGGGGAAGAACTAGATGTCAACACCACTCACCACTCACAATTTCCCACCAAATTTAATGCAATCCCCT
CTGTGAGTATTAAATGCAGCAGCTGGAAGCACCCAACTACTTTCTCTCTCAAAACCACCTCCAATGGCTAAAACCCCTCCCACCCTCCACCACCTCCT
CCTCCAATG**T**CCCCCCTCCCTCCTCCCCCTCCTCAAATTACCTCACTACCCTCGGCCTCGGCTACGCCATAGCCATCGCCCTCGGCTTCCTCGTCCTCCT
CTCCACCCTCCTCCTCGCCTCCTACATCTGCTGCCGCGTCTCCCGCCGCTCCCAAACCCAAACCCGAACCCGAACCCAAAACCCCAACTCCTCCTCAG
AAGGTGTC

Fuchsia = Splice Site; **Yellow** = SNP
Forward Primer **1**: CCACCTCCAATGGCTAAAACC ... **Reverse** Primer **1**: GTGGAGAGGAGGACGAGGAA
Forward Primer **2**: **TCTCTCTCAAAACCACCTCCA** ... **Reverse** Primer **2**: **GAGGACGAGGAAGCCGAG**
Forward Primer **2**: AATGGCTAAAACCCCTCCCA... **Reverse** Primer **2**: CAGATGTAGGAGGCGAGGAG

Primer3 Output: #1 – For First & Second Set of Primers

```
PRIMER PICKING RESULTS FOR Fvb7_v4.0.a1 Fvb7_v4.0.a1:13414776..13414975

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         49   21  59.18   52.38    0.00   0.00    0.00 CCACCTCCAATGGCTAAAACC
RIGHT PRIMER       198   20  60.32   60.00    0.00   0.00    0.00 GTGGAGAGGAGGACGAGGAA
SEQUENCE SIZE: 200
INCLUDED REGION SIZE: 200

PRODUCT SIZE: 150, PAIR ANY_TH COMPL: 11.38, PAIR 3'_TH COMPL: 2.23

    1 GTATTAAATGCAGCAGCTGGAAGCACCCAACTACTTTCTCTCTCAAAACCACCTCCAATG
                                                      >>>>>>>>>>>>

   61 GCTAAAACCCCTCCCACCCTCCACCACCTCCTCCTCCAATGTCCCCCCTCCCTCCTCCCC
      >>>>>>>>>

  121 CTCCTCAAATTACCTCACTACCCTCGGCCTCGGCTACGCCATAGCCATCGCCCTCGGCTT
                                                                <<

  181 CCTCGTCCTCCTCTCCACCC
      <<<<<<<<<<<<<<<<<<
```

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

ADDITIONAL OLIGOS
                    start  len      tm     gc%  any_th  3'_th hairpin seq

 1 LEFT PRIMER         37   21   58.31   47.62    0.00   0.0    0.0 TCTCTCTCAAAACCACCTCCA
   RIGHT PRIMER       190   18   59.20   66.67    0.00   0.00   0.0 GAGGACGAGGAAGCCGAG
   PRODUCT SIZE: 154, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

 2 LEFT PRIMER         19   22   58.92   50.00    0.00   0.00    0.00
GGAAGCACCCAACTACTTTCTC
   RIGHT PRIMER       193   18   58.79   66.67    0.00   0.00    0.00 GAGGAGGACGAGGAAGCC
   PRODUCT SIZE: 175, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

 3 LEFT PRIMER         22   22   58.76   45.45    0.00   0.00    0.00
AGCACCCAACTACTTTCTCTCT
   RIGHT PRIMER       200   18   59.64   66.67    0.00   0.00    0.00 GGGTGGAGAGGAGGACGA
   PRODUCT SIZE: 179, PAIR ANY_TH COMPL: 5.01, PAIR 3'_TH COMPL: 0.00

 4 LEFT PRIMER         39   22   58.25   45.45    0.00   0.00    0.00
TCTCTCAAAACCACCTCCAATG
   RIGHT PRIMER       148   20   58.59   55.00    0.00   0.00    0.00
GCCGAGGGTAGTGAGGTAAT
   PRODUCT SIZE: 110, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

Statistics
         con   too    in    in   not          no    tm    tm   high  high  high
high
         sid  many   tar  excl    ok   bad    GC   too   too any_th 3'_th hair-  poly
end
        ered    Ns   get   reg   reg   GC% clamp   low  high  compl compl  pin      X
stab    ok
Left     548     0     0     0     0    45     0   166   141      0     0    55    14
0   127
Right    499     0     0     0     0   105     0    60   212      0     1    14    27
0    80
Pair Stats:
considered 8046, unacceptable product size 8040, primer in pair overlaps a primer in a
better pair 2757, ok 6
libprimer3 release 2.4.0
```

Primer3 Output: #2 – For Third Set of Primers

```
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        159   20   58.91   50.00    0.00   0.00    0.00 AATGGCTAAAACCCCTCCCA
RIGHT PRIMER       324   20   59.04   60.00    0.00   0.00    0.00 CAGATGTAGGAGGCGAGGAG
SEQUENCE SIZE: 400
INCLUDED REGION SIZE: 400

PRODUCT SIZE: 166, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

    1 CAAAAAAACTGGCTGCCCAAAAAGGAGAAGGGGAAGAACTAGATGTCAACACCACTCACC


   61 ACTCACAATTTCCCACCAAATTTAATGCAATCCCCTCTGTGAGTATTAAATGCAGCAGCT


  121 GGAAGCACCCAACTACTTTCTCTCTCAAAACCACCTCCAATGGCTAAAACCCCTCCCACC
                                            >>>>>>>>>>>>>>>>>>>>

  181 CTCCACCACCTCCTCCTCCAATGTCCCCCCTCCCTCCTCCCCCTCCTCAAATTACCTCAC


  241 TACCCTCGGCCTCGGCTACGCCATAGCCATCGCCCTCGGCTTCCTCGTCCTCCTCTCCAC


  301 CCTCCTCCTCGCCTCCTACATCTGCTGCCGCGTCTCCCGCCGCTCCCAAACCCAAACCCG
          <<<<<<<<<<<<<<<<<<<<

  361 AACCCGAACCCAAAACCCCAACTCCTCCTCAGAAGGTGTC


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

ADDITIONAL OLIGOS
                    start  len     tm    gc%  any_th  3'_th hairpin seq

 1 LEFT PRIMER        164   20   59.38   60.00    0.00   0.00    0.00
CTAAAACCCCTCCCACCCTC
   RIGHT PRIMER       385   20   58.95   55.00    0.00   0.00    0.00
GGAGTTGGGGTTTTGGGTTC
   PRODUCT SIZE: 222, PAIR ANY_TH COMPL: 27.20, PAIR 3'_TH COMPL: 19.75

 2 LEFT PRIMER        231   20   58.59   55.00    0.00   0.00    0.00
ATTACCTCACTACCCTCGGC
   RIGHT PRIMER       392   20   58.93   55.00    0.00   0.00    0.00
CTGAGGAGGAGTTGGGGTTT
   PRODUCT SIZE: 162, PAIR ANY_TH COMPL: 9.66, PAIR 3'_TH COMPL: 0.25

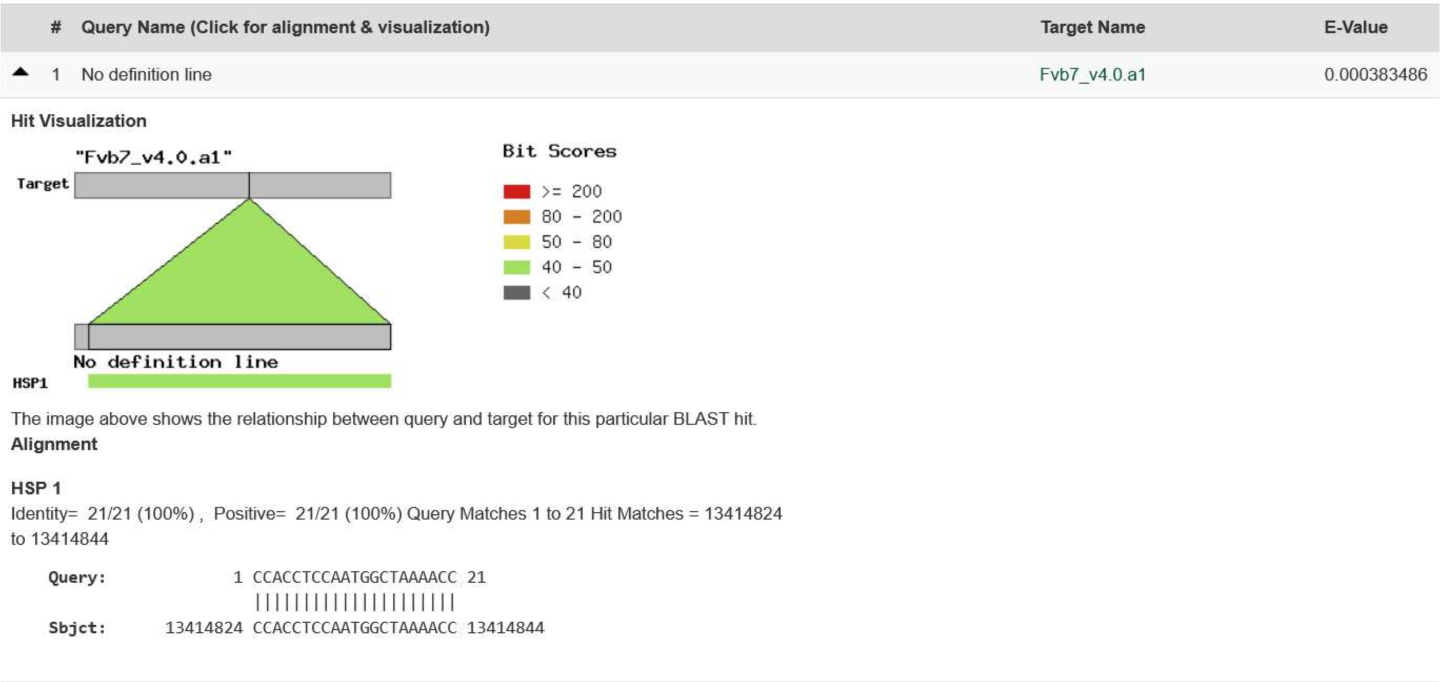
 3 LEFT PRIMER         49   20   59.09   50.00    0.00   0.00    0.00
ACACCACTCACCACTCACAA
```

```
   RIGHT PRIMER        250    20    58.59    55.00     0.00    0.00     0.00
GCCGAGGGTAGTGAGGTAAT
   PRODUCT SIZE: 202, PAIR ANY_TH COMPL: 6.66, PAIR 3'_TH COMPL: 1.55

 4 LEFT PRIMER          44    20    59.09    50.00     0.00    0.00     0.00
TGTCAACACCACTCACCACT
   RIGHT PRIMER        205    20    59.46    60.00     0.00    0.00     0.00
GACATTGGAGGAGGAGGTGG
   PRODUCT SIZE: 162, PAIR ANY_TH COMPL: 17.96, PAIR 3'_TH COMPL: 11.88

Statistics
         con    too    in    in    not          no    tm    tm   high  high  high
high
         sid   many   tar  excl    ok   bad    GC   too   too any_th 3'_th hair-   poly
end
        ered    Ns   get   reg    reg   GC% clamp   low  high  compl compl   pin     X
stab    ok
Left    1610     0     0     0      0   164     0   551   410      0     0     2    51
0   432
Right   1699     0     0     0      0   236     0   349   616      0     0     2    27
0   469
Pair Stats:
considered 265, unacceptable product size 255, primer in pair overlaps a primer in a
better pair 62, ok 10
libprimer3 release 2.4.0
```

Blast Forward Primer #1 Against *Fragaria vesca* @ GDR



#	Query Name (Click for alignment & visualization)	Target Name	E-Value
1	No definition line	Fvb7_v4.0.a1	0.000383486

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

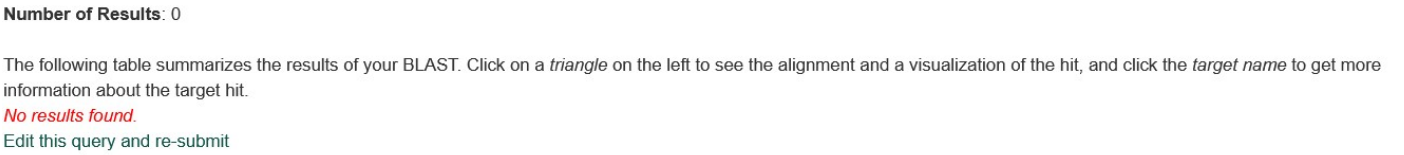
Alignment

HSP 1

Identity= 21/21 (100%) , Positive= 21/21 (100%) Query Matches 1 to 21 Hit Matches = 13414824 to 13414844

```
Query:          1 CCACCTCCAATGGCTAAAACC 21
                  |||||||||||||||||||||
Sbjct:   13414824 CCACCTCCAATGGCTAAAACC 13414844
```

Blast Reverse Primer #1 Against *Fragaria vesca* @ GDR & NCBI $^{(\text{refseq\_genomes})}$

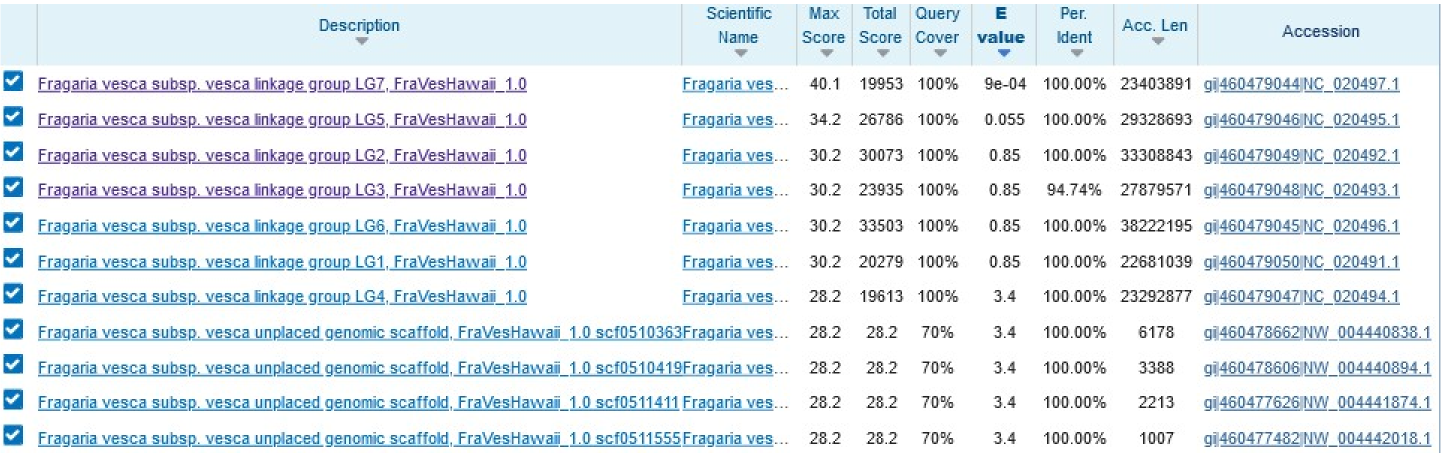


Number of Results: 0

The following table summarizes the results of your BLAST. Click on a *triangle* on the left to see the alignment and a visualization of the hit, and click the *target name* to get more information about the target hit.

No results found.

Edit this query and re-submit



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria ves...	40.1	19953	100%	9e-04	100.00%	23403891	gi|460479044|NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria ves...	34.2	26786	100%	0.055	100.00%	29328693	gi|460479046|NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria ves...	30.2	30073	100%	0.85	100.00%	33308843	gi|460479049|NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria ves...	30.2	23935	100%	0.85	94.74%	27879571	gi|460479048|NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria ves...	30.2	33503	100%	0.85	100.00%	38222195	gi|460479045|NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria ves...	30.2	20279	100%	0.85	100.00%	22681039	gi|460479050|NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria ves...	28.2	19613	100%	3.4	100.00%	23292877	gi|460479047|NC_020494.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510363	Fragaria ves...	28.2	28.2	70%	3.4	100.00%	6178	gi|460478662|NW_004440838.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510419	Fragaria ves...	28.2	28.2	70%	3.4	100.00%	3388	gi|460478606|NW_004440894.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0511411	Fragaria ves...	28.2	28.2	70%	3.4	100.00%	2213	gi|460477626|NW_004441874.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0511555	Fragaria ves...	28.2	28.2	70%	3.4	100.00%	1007	gi|460477482|NW_004442018.1

Analyze Forward Primer #1 @ IDT

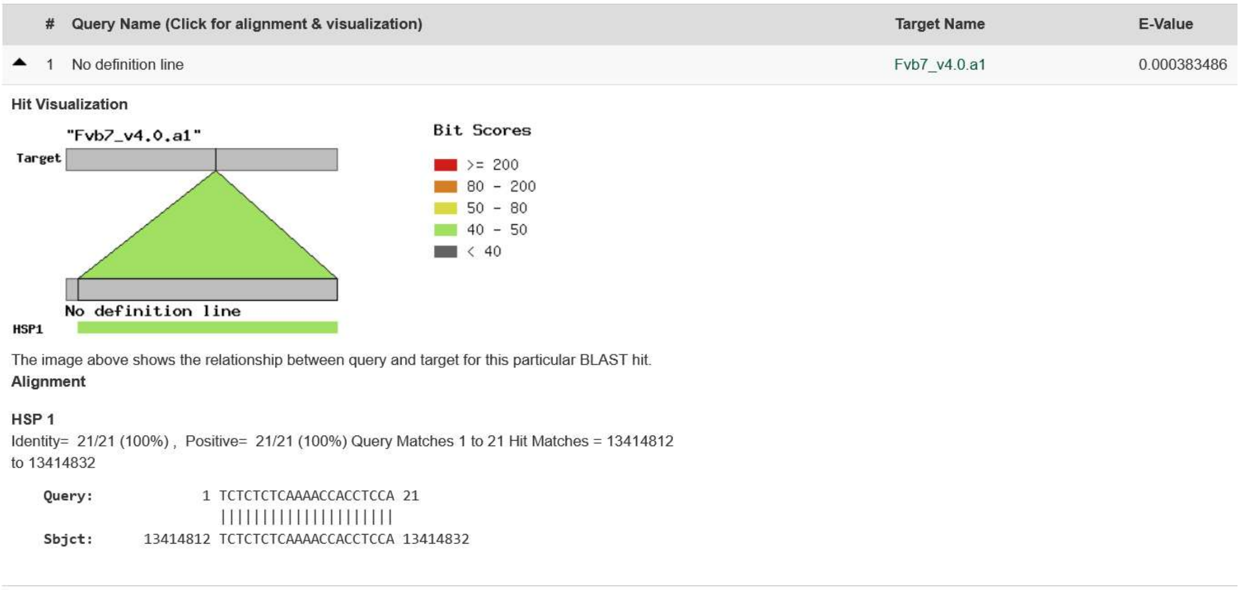
Nothing Major to Note …

Analyze Reverse Primer #1 @ IDT

Nothing Major to Note …

Primer Set #2

Blast Forward Primer #2 Against *Fragaria vesca* @ GDR



#	Query Name (Click for alignment & visualization)	Target Name	E-Value
▲ 1	No definition line	Fvb7_v4.0.a1	0.000383486

Hit Visualization

The image above shows the relationship between query and target for this particular BLAST hit.

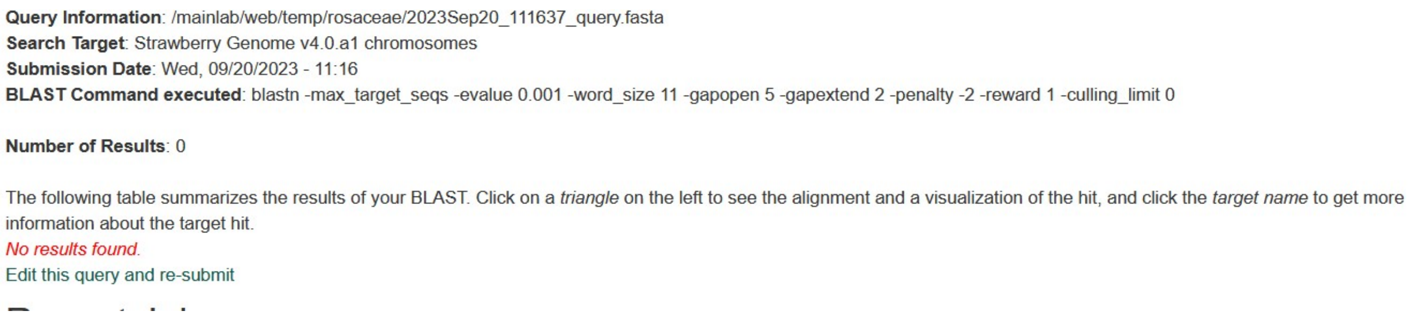
Alignment

HSP 1

Identity= 21/21 (100%) , Positive= 21/21 (100%) Query Matches 1 to 21 Hit Matches = 13414812 to 13414832

```
Query:          1 TCTCTCTCAAAACCACCTCCA 21
                  |||||||||||||||||||||
Sbjct:   13414812 TCTCTCTCAAAACCACCTCCA 13414832
```

Blast Reverse Primer #2 Against *Fragaria vesca* @ GDR & NCBI $^{\text{(refseq\_genomes)}}$



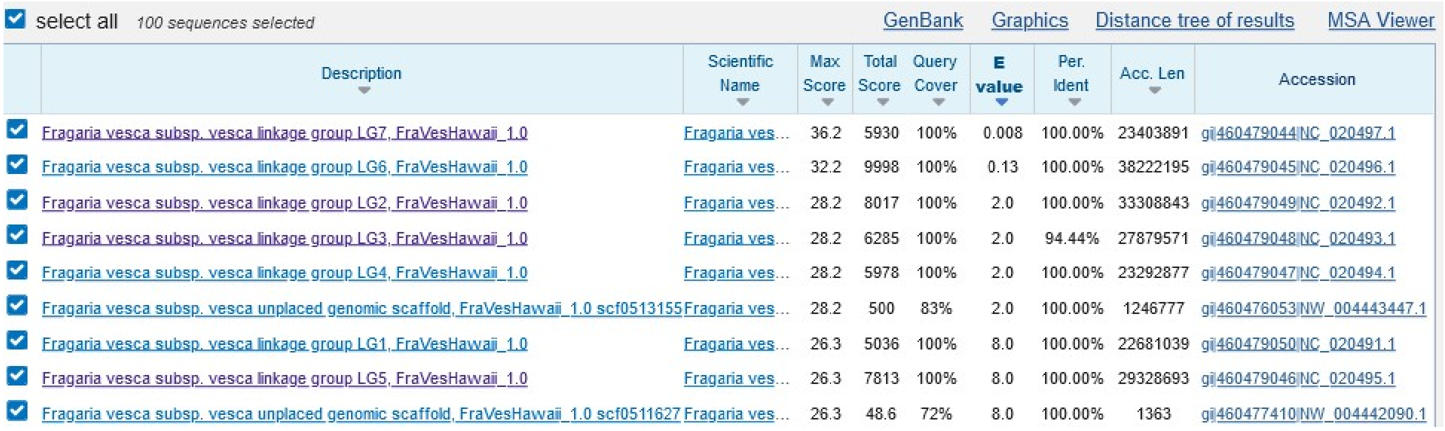
Query Information: /mainlab/web/temp/rosaceae/2023Sep20_111637_query.fasta
Search Target: Strawberry Genome v4.0.a1 chromosomes
Submission Date: Wed, 09/20/2023 - 11:16
BLAST Command executed: blastn -max_target_seqs -evalue 0.001 -word_size 11 -gapopen 5 -gapextend 2 -penalty -2 -reward 1 -culling_limit 0

Number of Results: 0

The following table summarizes the results of your BLAST. Click on a *triangle* on the left to see the alignment and a visualization of the hit, and click the *target name* to get more information about the target hit.

No results found.

Edit this query and re-submit



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria ves...	36.2	5930	100%	0.008	100.00%	23403891	gi|460479044|NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria ves...	32.2	9998	100%	0.13	100.00%	38222195	gi|460479045|NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria ves...	28.2	8017	100%	2.0	100.00%	33308843	gi|460479049|NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria ves...	28.2	6285	100%	2.0	94.44%	27879571	gi|460479048|NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria ves...	28.2	5978	100%	2.0	100.00%	23292877	gi|460479047|NC_020494.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0513155	Fragaria ves...	28.2	500	83%	2.0	100.00%	1246777	gi|460476053|NW_004443447.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria ves...	26.3	5036	100%	8.0	100.00%	22681039	gi|460479050|NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria ves...	26.3	7813	100%	8.0	100.00%	29328693	gi|460479046|NC_020495.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0511627	Fragaria ves...	26.3	48.6	72%	8.0	100.00%	1363	gi|460477410|NW_004442090.1

Analyze Forward Primer #2 @ IDT

Nothing Major to Note …

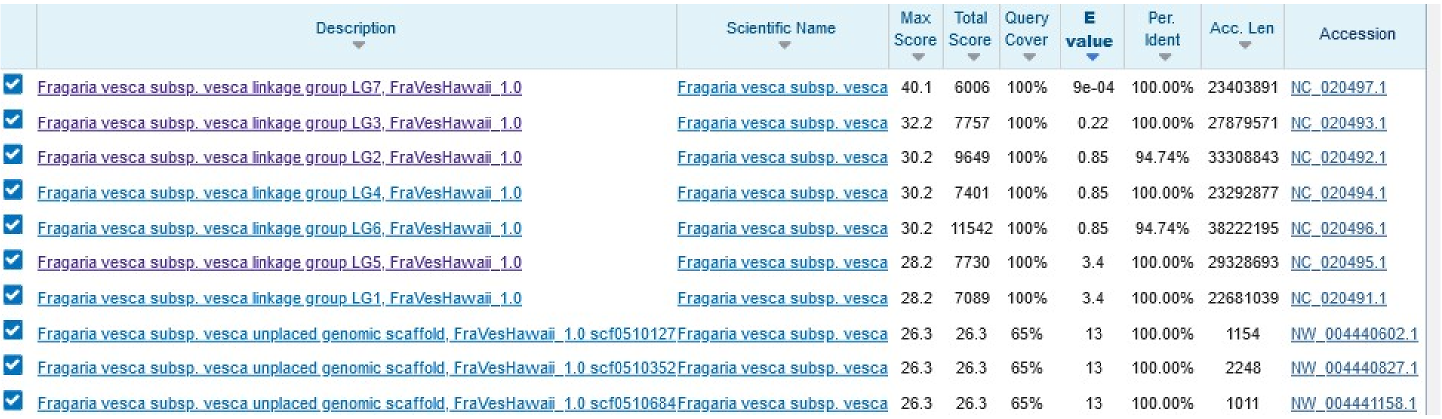
Analyze Reverse Primer #2 @ IDT

Nothing Major to Note …

Blast Forward Primer #3 Against *Fragaria vesca* @ NCBI

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	40.1	10518	100%	9e-04	100.00%	23403891	NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	9703	100%	3.4	100.00%	22681039	NC_020491.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	13011	100%	3.4	100.00%	27879571	NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	12188	100%	3.4	100.00%	29328693	NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	18233	100%	3.4	100.00%	38222195	NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	14761	100%	3.4	100.00%	33308843	NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	26.3	11141	100%	13	100.00%	23292877	NC_020494.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0512944	Fragaria vesca subsp. vesca	26.3	46.6	65%	13	100.00%	90737	NW_004443389.1

Blast Reverse Primer #2 Against *Fragaria vesca* @ NCBI



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fragaria vesca subsp. vesca linkage group LG7, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	40.1	6006	100%	9e-04	100.00%	23403891	NC_020497.1
☑	Fragaria vesca subsp. vesca linkage group LG3, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	32.2	7757	100%	0.22	100.00%	27879571	NC_020493.1
☑	Fragaria vesca subsp. vesca linkage group LG2, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	30.2	9649	100%	0.85	94.74%	33308843	NC_020492.1
☑	Fragaria vesca subsp. vesca linkage group LG4, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	30.2	7401	100%	0.85	100.00%	23292877	NC_020494.1
☑	Fragaria vesca subsp. vesca linkage group LG6, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	30.2	11542	100%	0.85	94.74%	38222195	NC_020496.1
☑	Fragaria vesca subsp. vesca linkage group LG5, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	7730	100%	3.4	100.00%	29328693	NC_020495.1
☑	Fragaria vesca subsp. vesca linkage group LG1, FraVesHawaii_1.0	Fragaria vesca subsp. vesca	28.2	7089	100%	3.4	100.00%	22681039	NC_020491.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510127	Fragaria vesca subsp. vesca	26.3	26.3	65%	13	100.00%	1154	NW_004440602.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510352	Fragaria vesca subsp. vesca	26.3	26.3	65%	13	100.00%	2248	NW_004440827.1
☑	Fragaria vesca subsp. vesca unplaced genomic scaffold, FraVesHawaii_1.0 scf0510684	Fragaria vesca subsp. vesca	26.3	26.3	65%	13	100.00%	1011	NW_004441158.1

Analyze Forward Primer #2 @ IDT

Nothing Major to Note …

Analyze Reverse Primer #2 @ IDT

Nothing Major to Note …