October 8th, 2014

Redesigned 2nd set of primers

1st amplicon size: 521

2nd amplicon size: 655

atgatgaagaaggagaaaccgatgatgtcggtgaccgccatcatccaggggacccaggcccggcattggt

cccgtgggaacacttggttgagtctggacaacagtaacatgtccatgtcctcggtgggtccacaaagtcc

actggacatgaaacctgacacagcgagcctcatcaatccagggaatttcagtccttccggtcccaatagt

cctggatccttcaccgctggttgtcacagcaaccttctgagtacgtcgccgagtggacagaacaaagcag

ccgcaccctatccgccgaaccaccctctttccggaagcaagcatctttgctcgatctgcggtgaccgtgc

cagtggcaagcattatggtgtctatagctgcgagggttgcaagggctttttcaagaggaccgtacgcaag

gacctgtcgtacgcgtgtcgcgaggaaaaatcctgcatcatcgacaaacggcagagaaatcgatgtcagt

attgtagataccagaaatgcctggcgatgggcatgaagagagaagcagtacaggaagagcgtcaacgtac

caaggaaagggatcagagcgaggtggagagcaccagtagtctgcactcggacatgccgatcgagcgtatt

ctggaagccgagaaacgagtcgagtgcaaaatggaacagcaaggaaattacgagctgttccacgaaacgc

tcctccgattaattcggcatctcttgcaatgcacagaatgcagtgtcgcacatttgcaacgccacgaaca

aacagctgttccagctggtagcatgggcgaaacacatcccgcattttacgtcgttgccgctggaggatca

ggtacttctgctcagggccggctggaacgagttgctgatagcatccttttcccaccgttcgatcgacgtg

aaggacggtatcgtgctggcgacagggatcacggtgcaccgaaactccgcgcagcaggctggcgtgggca

cgatattcgaccgggtactttccgagcttgtatcgaaaatgcgcgaaatgaagatggacaggacggagct

tggttgcctgaggttgtccgaggactaaaatccatccaggaagtgaccctgctccgcgagaagatctacg

cagcgctggaaggctattgtcgcgtagcctggcccgacgatgctggaagattcgcgaaattactgttacg

tctgccggccatccgatccatcggattaaagtgtctggagtacctgttcttcttcaaaatgatcggcgac

gtgccgatcgacgatttcctcgtggagatgctagagtcacggtcggacccttag

**1st set of primers (from primer3):**

OLIGO [start](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_START)  [len](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_GC)  [any](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_OLIGO_SEQ)

LEFT PRIMER 11 20 60.07 50.00 3.00 2.00 aggagaaaccgatgatgtcg

RIGHT PRIMER 571 20 59.96 50.00 4.00 1.00 ccctttccttggtacgttga

*(Complement = tcaacgtaccaaggaaaggg)*

**2nd set of primers (from NCBI primer):**

Sequence (5'->3') Length Tm GC% Self complementarity Self 3' complementarity

Forward primer TAGTCTGCACTCGGACATGC 20 59.83 55.00 4.00 3.00 3.00

Reverse primer CATCTCCACGAGGAAATCGT 20 57.41 50.00 8.00 3.00 3.00

(*Complement = acgatttcctcgtggagatg)*

See below for NCBI primer blast results

Here are the NCBI primer blast results for the **second pair of primers**:

## Primer pair 1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Sequence (5'->3')** | **Length** | **Tm** | **GC%** | **Self complementarity** | **Self 3' complementarity** |
| **Forward primer** | TAGTCTGCACTCGGACATGC | 20 | 59.83 | 55.00 | 4.00 | 3.00 |
| **Reverse primer** | CATCTCCACGAGGAAATCGT | 20 | 57.41 | 50.00 | 8.00 | 3.00 |

**Products on target templates**

>[XM\_003484415.1](http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&id=350396163) PREDICTED: Bombus impatiens retinoic acid receptor RXR-alpha-A-like, transcript variant 2 (LOC100749223), mRNA

product length = 664

Forward primer 1 TAGTCTGCACTCGGACATGC 20

Template 853 .................... 872

Reverse primer 1 CATCTCCACGAGGAAATCGT 20

Template 1516 .................... 1497

>[XM\_003484414.1](http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&id=350396160) PREDICTED: Bombus impatiens retinoic acid receptor RXR-alpha-A-like, transcript variant 1 (LOC100749223), mRNA

product length = 664

Forward primer 1 TAGTCTGCACTCGGACATGC 20

Template 1104 .................... 1123

Reverse primer 1 CATCTCCACGAGGAAATCGT 20

Template 1767 .................... 1748

>[XM\_003397944.1](http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&id=340719094) PREDICTED: Bombus terrestris retinoic acid receptor RXR-alpha-A-like, transcript variant 2 (LOC100651492), mRNA

product length = 664

Forward primer 1 TAGTCTGCACTCGGACATGC 20

Template 853 ...C................ 872

Reverse primer 1 CATCTCCACGAGGAAATCGT 20

Template 1516 .................... 1497

>[XM\_003397943.1](http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&id=340719092) PREDICTED: Bombus terrestris retinoic acid receptor RXR-alpha-A-like, transcript variant 1 (LOC100651492), mRNA

product length = 664

Forward primer 1 TAGTCTGCACTCGGACATGC 20

Template 765 ...C................ 784

Reverse primer 1 CATCTCCACGAGGAAATCGT 20

Template 1428 .................... 1409

and results for the **first set of primers**:

## Primer pair 1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Sequence (5'->3')** | **Length** | **Tm** | **GC%** | **Self complementarity** | **Self 3' complementarity** |
| **Forward primer** | AGGAGAAACCGATGATGTCG | 20 | 57.41 | 50.00 | 3.00 | 2.00 |
| **Reverse primer** | CCCTTTCCTTGGTACGTTGA | 20 | 57.45 | 50.00 | 4.00 | 1.00 |

**Products on target templates**

>[XM\_003484414.1](http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&id=350396160) PREDICTED: Bombus impatiens retinoic acid receptor RXR-alpha-A-like, transcript variant 1 (LOC100749223), mRNA

product length = 561

Forward primer 1 AGGAGAAACCGATGATGTCG 20

Template 518 .................... 537

Reverse primer 1 CCCTTTCCTTGGTACGTTGA 20

Template 1078 .................... 1059