BIMP CDS for USP (intron info gathered from IGV)

Atgatgaagaaggagaaaccgatgatgtcggtgaccgccatcatccaggggacccaggcccggcattggt

cccgtgggaacacttggttgagtctggacaacagtaacatgtccatgtcctcggtgggtccacaaagtcc

actggacatgaaacctgacacagcgagcctcatcaatccagggaatttcagtccttccggtcccaatagt

cctggatccttcaccgctggttgtcacagcaaccttctgagtacgtcgccgagtggacagaacaaagcag

ccgcaccctatccgccgaaccaccctctttccggaagcaagcatctttgctcgatctgcggtgaccgtgc

cagtggcaagcattatggtgtctatagctgcgagggttgcaagggctttttcaagaggaccgtacgcaag

gacctgtcgtacgcgtgtcgcgaggaaaaatcctgcatcatcgacaaacggcagagaaatcgatgtcagt

attgtagataccagaaatgcctggcgatgggcatgaagagagaagcagtacaggaagagcgtcaacgtac

caaggaaagggatcagagcgaggtggagagcaccagtagtctgcactcggacatgccgatcgagcgtatt

ctggaagccgagaaacgagtcgagtgcaaaatggaacagcaaggaaattacgagctgttccacgaaacgc

tcctccgattaattcggcatctcttgcaatgcacagaatgcagtgtcgcacatttgcaacgccacgaaca

aacagctgttccagctggtagcatgggcgaaacacatcccgcattttacgtcgttgccgctggaggatca

ggtacttctgctcagggccggctggaacgagttgctgatagcatccttttcccaccgttcgatcgacgtg

aaggacggtatcgtgctggcgacagggatcacggtgcaccgaaactccgcgcagcaggctggcgtgggca

cgatattcgaccgggtactttccgagcttgtatcgaaaatgcgcgaaatgaagatggacaggacggagct

tggttgcctgaggttgtccgaggactaaaatccatccaggaagtgaccctgctccgcgagaagatctacg

cagcgctggaaggctattgtcgcgtagcctggcccgacgatgctggaagattcgcgaaattactgttacg

tctgccggccatccgatccatcggattaaagtgtctggagtacctgttcttcttcaaaatgatcggcgac

gtgccgatcgacgatttcctcgtggagatgctagagtcacggtcggacccttag

Domain 1 begins at bp 108\*3 = after 324

Domain 2 begins at bp 358\*3 = after 1074

**To capture Domain 1 (zinc finger), first section:**

**Left Primer** design input (from exon 3 sequence)

tccttcaccgctggttgtcacagcaaccttctgagtacgtcgccgagtggacagaacaaagcag

ccgcaccctatccgccgaaccaccctctttccggaagcaagcat

**primer3 output:**

[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 12 20 59.73 50.00 5.00 0.00 tggttgtcacagcaaccttc

**Blast results;**

ref|XM\_006607873.1| PREDICTED: Apis dorsata retinoic acid rec...

ref|XR\_420612.1| PREDICTED: Apis dorsata retinoic acid recept...

ref|XM\_006607871.1| PREDICTED: Apis dorsata retinoic acid rec...

ref|XM\_006561553.1| PREDICTED: Apis mellifera ultraspiracle (...

ref|XM\_006561552.1| PREDICTED: Apis mellifera ultraspiracle (...

ref|XM\_006561550.1| PREDICTED: Apis mellifera ultraspiracle (...

ref|XM\_006561549.1| PREDICTED: Apis mellifera ultraspiracle (...

ref|XM\_006561548.1| PREDICTED: Apis mellifera ultraspiracle (...

ref|XM\_006561547.1| PREDICTED: Apis mellifera ultraspiracle (...

ref|XM\_006561546.1| PREDICTED: Apis mellifera ultraspiracle (...

ref|XM\_003690321.1| PREDICTED: Apis florea retinoic acid rece...

ref|XM\_003484415.1| PREDICTED: Bombus impatiens retinoic acid...

**Right Primer** design input (primer begins at bp 54 on intron from IGV):

GGTAAGTCAGACATTAACATTTACAAGTTCACGAACGAACTGAATAAACGAACG**GACAATCGTATGCGAAGCAA**ATTTGCAAACTTTCCGAATTAATTTCCTTGCTAGTACGTTGATCAAGCTATTCAATTTCTGAACTGTGCGAAATAAAAATGTTCGAAAAAATACCTTTCTTTTCTATTATTTTTACGAATTTGCTATACGAGCTTAGAGATTCTCGTAGGGTTCCTTGTTTTATGAGGCCAATTTGTCAGGGTTTTCAGCAATGAATACAAATCGGTCTCGTTCCGGATATCGGACGATTTTTTCATCAGTCGTGACGGTC

**Primer3 output:**

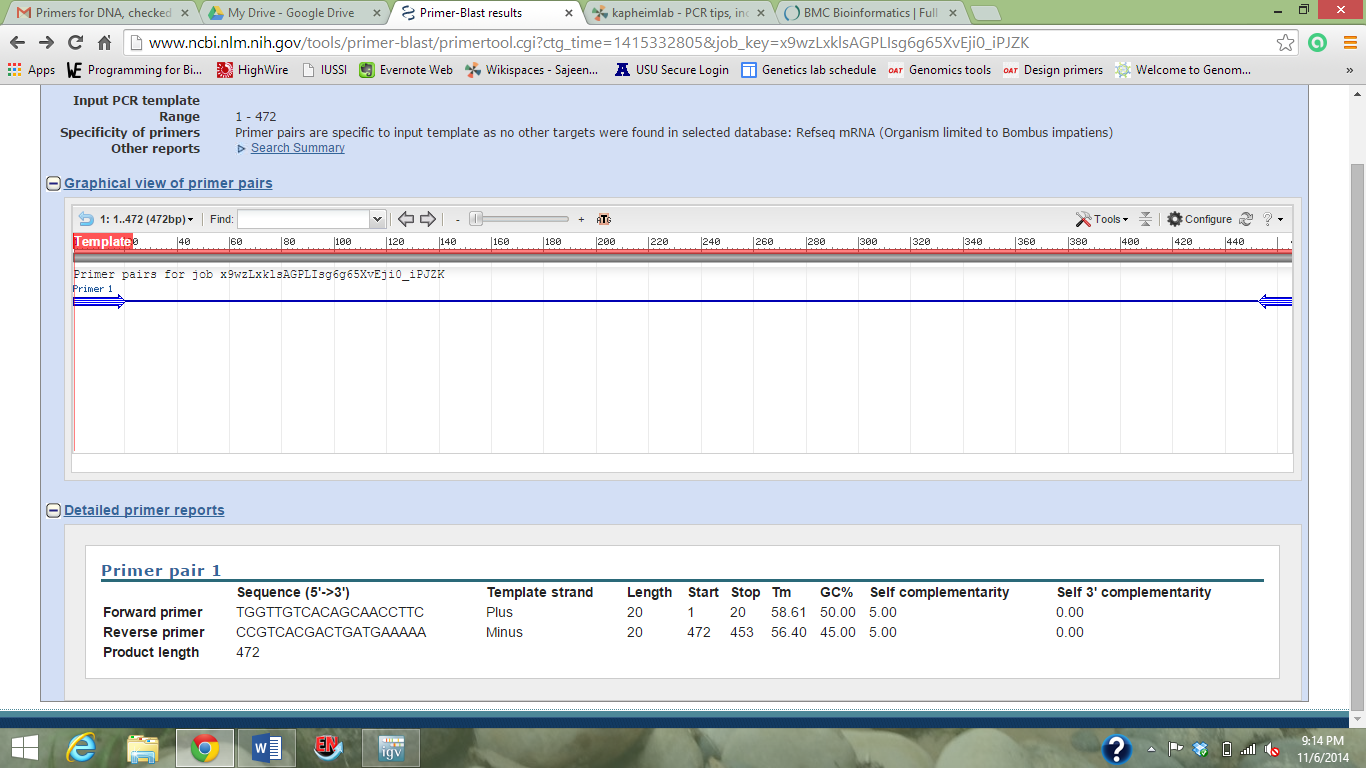
OLIGO [start](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_START)  [len](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://biotools.umassmed.edu/bioapps/primer3_www_results_help.html#PRIMER_GC)  [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)

RIGHT\_PRIMER 74 20 59.84 45.00 6.00 2.00 TTGCTTCGCATACGATTGTC

Reverse GACAATCGTATGCGAAGCAA

**Blast results:** 

**Overall Primer-Blast results (putting in my primers with the template):**



**Amplicon for first section of domain 1(grey non-domain, yellow is domain) total bp 232**

**tggttgtcacagcaaccttc**tgagtacgtcgccgagtggacagaacaaagcagccgcaccctatccgccgaaccaccctctttccggaagcaagcatctttgctcgatctgcggtgaccgtgccagtggcaagcattatggtgtctataGGTAAGTCAGACATTAACATTTACAAGTTCACGAACGAACTGAATAAACGAACG**GACAATCGTATGCGAAGCAA**

**Domain 1 (zinc finger), second section:**

**Left primer** input (primer is 27 bp away from exon):

TCGTTCG**TGACGGTACATCTTGCCGTA**GTAACCTTTCCGTTCTTTCGCTCGTAG

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 8 20 60.13 50.00 4.00 2.00 TGACGGTACATCTTGCCGTA

SEQUENCE SIZE: 54

INCLUDED REGION SIZE: 54

**Blast results: all over the place**



**Right primer** input (exon 4):

gaagcagtacaggaagagcgtcaacgtaccaaggaaagggatcagagcgaggtggagagcaccagtagtctgcactcggacatgccgatcgagcgtattctggaagccgagaaacgagtcgagtgcaaaatggaacagcaaggaaattac

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)

RIGHT\_PRIMER 85 20 60.02 55.00 4.00 2.00 gcatgtccgagtgcagacta

SEQUENCE SIZE: 150

INCLUDED REGION SIZE: 15

(reverse complement: tagtctgcactcggacatgc)

**Blast results:**

ref|XM\_003484415.1| PREDICTED: Bombus impatiens retinoic acid... 40.1 0.14

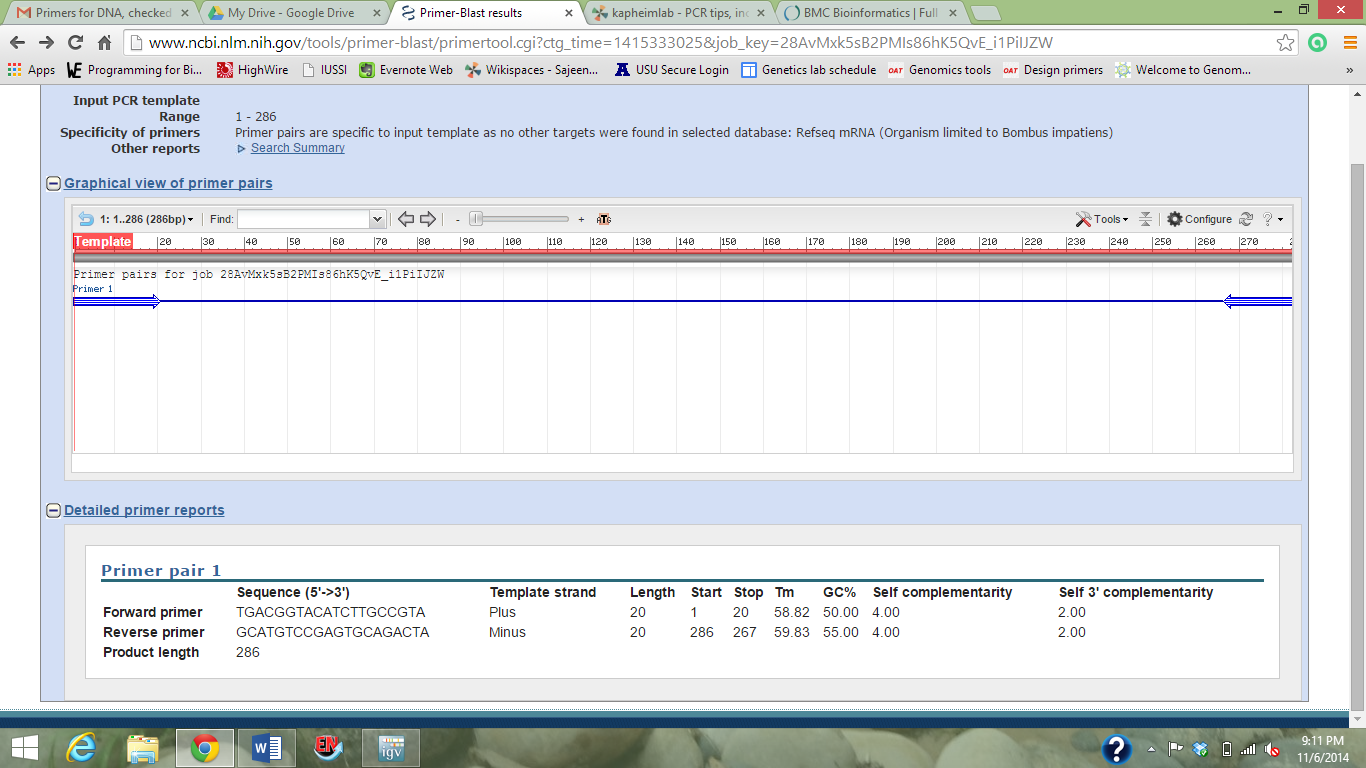
ref|XM\_003484414.1| PREDICTED: Bombus impatiens retinoic acid... 40.1 0.14

emb|LN590677.1| Cyprinus carpio genome assembly common carp g... 36.2 2.1

emb|FO906003.1| Leptosphaeria maculans lepidii ibcn84\_scaffol... 34.2 8.4

ref|XM\_009814061.1| PREDICTED: Gavia stellata LETM1 domain co... 34

**Overall Primer Blast results:**



Amplicon of Domain 1, second section, bp 286:

Checked in Primer blast.

**TGACGGTACATCTTGCCGTA**GTAACCTTTCCGTTCTTTCGCTCGTAGctgcgagggttgcaagggctttttcaagaggaccgtacgcaaggacctgtcgtacgcgtgtcgcgaggaaaaatcctgcatcatcgacaaacggcagagaaatcgatgtcagtattgtagataccagaaatgcctggcgatgggcatgaagagagaagcagtacaggaagagcgtcaacgtaccaaggaaagggatcagagcgaggtggagagcaccag**tagtctgcactcggacatgc**

**Domain 2 (hormone receptor):**

**Left primer input** (from intron, 25 bp away from exon):

A**TCGAGCAATTAATCACAAACAGA**TCTATAATACTCTTCAACCCCGAAG

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 2 23 59.77 34.78 6.00 0.00 TCGAGCAATTAATCACAAACAGA

SEQUENCE SIZE: 49

INCLUDED REGION SIZE: 49

**Blast results:**



**Right primer input** (exon 6):

ggcgacgtgccgatcgacgatttcctcgtggagatgctagagtcacggtcggacccttag

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)

RIGHT\_PRIMER 36 20 60.07 50.00 8.00 3.00 catctccacgaggaaatcgt

SEQUENCE SIZE: 60

INCLUDED REGION SIZE: 60

(reverse complement: acgatttcctcgtggagatg)

**Blast results:**

ref|XM\_003699982.1| PREDICTED: Megachile rotundata retinoic a... 40.1 0.14

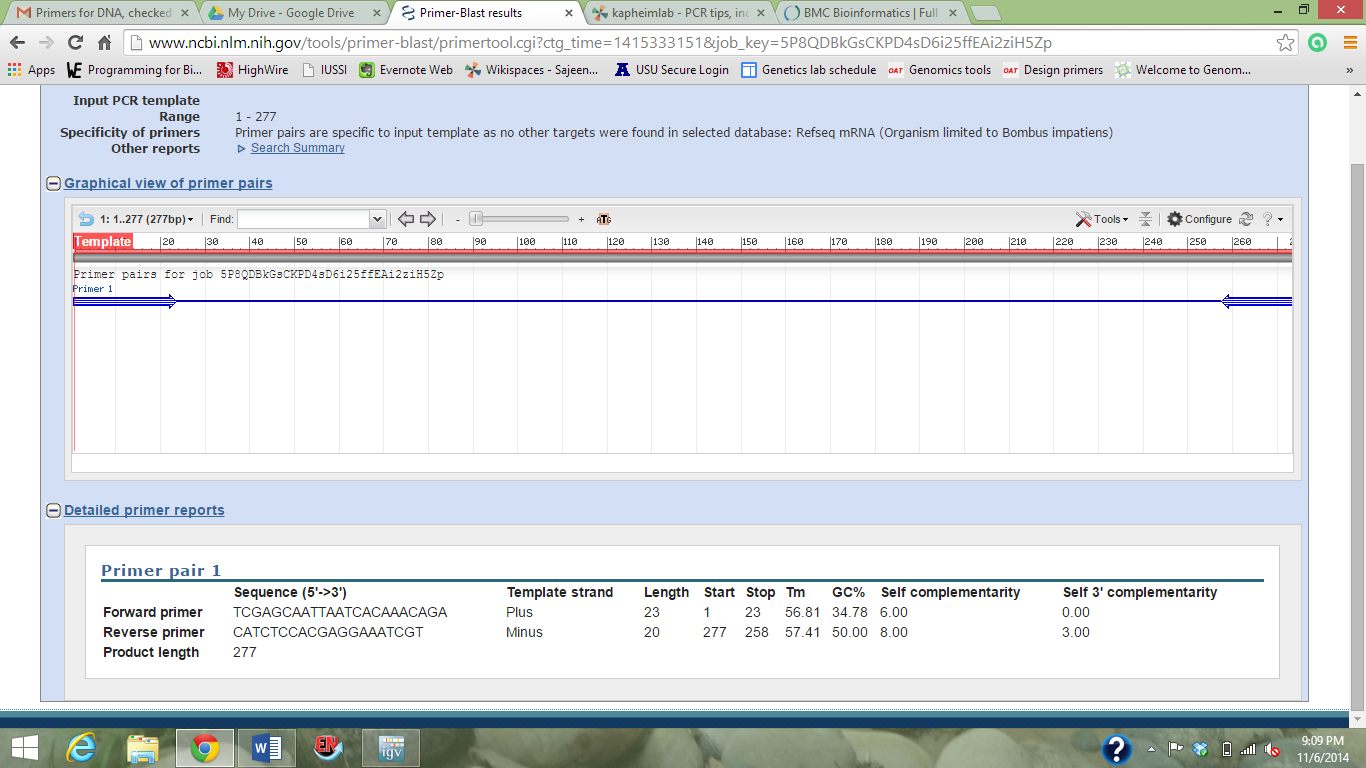
ref|XM\_003484415.1| PREDICTED: Bombus impatiens retinoic acid... 40.1 0.14

ref|XM\_003484414.1| PREDICTED: Bombus impatiens retinoic acid... 40.1 0.14

ref|XM\_003397944.1| PREDICTED: Bombus terrestris retinoic aci... 40.1 0.14

ref|XM\_003397943.1| PREDICTED: Bombus terrestris retinoic aci... 40.1 0.14

**Overall Primer-Blast results:**



**TCGAGCAATTAATCACAAACAGA**TCTATAATACTCTTCAACCCCGAAGggttgtccgaggactaaaatccatccaggaagtgaccctgctccgcgagaagatctacgcagcgctggaaggctattgtcgcgtagcctggcccgacgatgctggaagattcgcgaaattactgttacgtctgccggccatccgatccatcggattaaagtgtctggagtacctgttcttcttcaaaatgatcggcgacgtgccgatcg**acgatttcctcgtggagatg**

Notes:

Length of amplicon: http://gqinnovationcenter.com/documents/sequencing/UserGuide\_SangerSequencing\_en.pdf

PCR amplifications must be verified on an agarose gel and a copy of the gel picture must be sent to the sequencing service with the samples. PCR products of less than 250 bp result in lower quality sequences due to an oversaturation phenomenon whereby they appear much more intense than usual and to the compression of bases at the beginning of the sequencing read, which is an inherent limitation of the 3730xl technology.PCR products greater than 2000 bp can be difficult to sequence because the concentration of DNAsubmitted is generally too low. The longer the PCR products the higher DNA concentrations are required to achieve good quality sequencing.

Use igv to get introns and design primers within intron as close as possible to exons of interest.

When I load the .genome, also load the gff file to get gene information.

For bimp, load two gff files: annotated (just for usp) and original on igv.

Also, if I am downloading CDS then am only showing exons. Need to use the entire bimp fasta file,plus gff.

Carbonite backup or box storage.

Practice for melipona, use the genome fa and gff, then just usp gff.

exon for domain 1, section 1, right primer:

ATCCTTCACCGCTGGTTGTCACAGCAACCTTCTGAGTACGTCGCCGAGTGGACAGAACAAAGCAGCCGCACCCTATCCGCCGAACCACCCTCTTTCCGGAAGCAAGCATCTTTGCTCGATCTGCGGTGACCGTGCCAGTGGCAAGCATTATGGTGTCTATAG