

Developing primers tutorial

Continuation from Honey bee viral
sequence activity...

(using DNA subway is optional)

```
>Colony-299
TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGT
TGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTTGAAGTTAATC
AGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCTGGGCTGATTTACAAC
GTGTTGGCTCAGAGATTAGT
>Colony-300
TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGT
TGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTTGAAGTTAATC
AGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCTGGGCTGATTTACAAC
GTGTTGGCTCAGAGATTAGT
>NC_006494.1|varroa_destructor
TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGT
TGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTTGAAGTTAATC
AGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCTGGGCTGATTTACAAC
GTGTTGGCTCAGAGATTAGT
>NC_004830.2|deformed_wing
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGT
TACGTATGGATGAACCATTTGAAGGTGATAATTTCTCAATAAGTATGTTGAAGTTAATC
AGCGCTTAGTTGAGGAAATGAAGGCATTTAAGGAGCGTACACTATGGTCAGATTTACATC
GCGTAGGTGCGGAAATTAGT
```

The alignment may be downloaded in **FASTA** format on computer:

>Sequence Name1

ATCGATCG.....

>Sequence Name2

ATCGATCG.....

Cut and paste into a word or google doc for primer development

Courier New font is best

Use red font on Varroa Destructor Virus Sequence

```
>NC_006494.1|varroa_destructor/1-199  
TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA  
AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT  
GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCCTCT  
GGGCTG|ATTTACAACGTGTTGGCTCAGAGATTAG
```

```
>NC_004830.2|deformed_wing/1-199  
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTTGGATGAAATGCA  
AATGTTACGTATGGATGAACCATTTGGAAGGTGATAATATTCTCAATAAGTATGTT  
GAAGTTAATCAGCGCTTAGTTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT  
GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG
```

Manually re-align both sequences in the word processor

TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA
TGGCTAACCGTCGTAAAGGCGAATGAATCGTTTAAGATGCGTGTTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT
AATGTTACGTATGGATGAACCATTTGGAAGGTGATAATATTCTCAATAAGTATGTT

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCTCT
GAAGTTAATCAGCGCTTAGTTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG
GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

Find differences to make variant-specific primers for PCR

TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA

TGGCTAACCGTCGTAAAGGCGAATGAATCGTTTAAGATGCGTGTTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT

AATGTTACGTATGGATGAACCATTTGGAAGGTGATAATATCTCAATAAGTATGTT

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCTCT

GAAGTTAATCAGCGCTTAGTTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG

GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

|

Find differences to make variant-specific primers for PCR

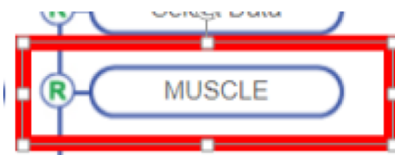
TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTAAATAAGTATGTT
AATGTTACGTATGGATGAACCATTGGAAGGTGATAATATTCTCAATAAGTATGTT

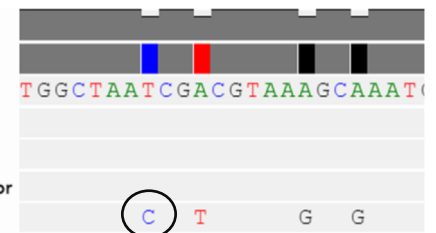
GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCTCT
GAAGTTAATCAGCGCTTAGTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

GGGCTTGATTTACAACGTGTTTGGCTAGAGGATTAG
GGTCAGATTTACATCGCGTAGGTGCGGAAAATTAG

As a cheat refer back to:



Sequence Conservation
Sequence Variation
Consensus
1. Colony-299
2. Colony-300
3. NC_006494.1|varroa_destructor
4. NC_004830.2|deformed_wing



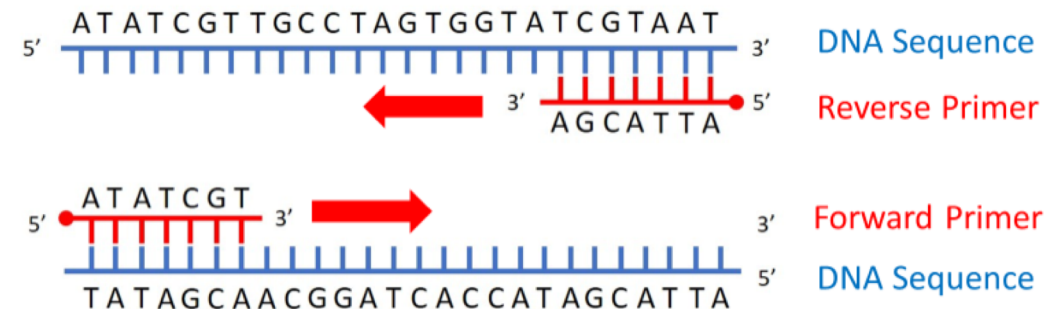
General guidelines for species-specific primer development

Target the differences between the variants!

Length of 18-30 nucleotides

GC content between 40-60% of total primer

Reverse primer is the complement to the sequence



CTAATCGACGTAAAGCAAAT

TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT
AATGTTACGTATGGATGAACCATTGGAAGGTGATAATATTCTCAATAAGTATGTT

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCT
GAAGTTAATCAGCGCTTAGTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

TTGCACAACCGAGTCTCT

GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG
GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

