

Developing primers tutorial

Continuation from Honey bee viral
sequence activity...

(using DNA subway is optional)

```
alignment (1) - Notepad
File Edit Format View Help
>Colony-299
TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGT
TGCGTATGGATGAGCCCTTGAAGGCGATAAATATTTAAATAAGTATGTTGAAGTTAATC
AGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCTGGGCTGATTTACAAC
GTGTTGGCTCAGAGATTAGT
>Colony-300
TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGT
TGCGTATGGATGAGCCCTTGAAGGCGATAAATATTTAAATAAGTATGTTGAAGTTAATC
AGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCTGGGCTGATTTACAAC
GTGTTGGCTCAGAGATTAGT
>NC_006494.1|varroa_destructor
TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGT
TGCGTATGGATGAGCCCTTGAAGGCGATAAATATTTAAATAAGTATGTTGAAGTTAATC
AGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCTGGGCTGATTTACAAC
GTGTTGGCTCAGAGATTAGT
>NC_004830.2|deformed_wing
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGT
TACGTATGGATGAACCATTGGAAGGTGATAAATTTCTCAATAAGTATGTTGAAGTTAATC
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

TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGGAAGGCGATAATATTTTAAATAAGTATGTT

AATGTTACGTATGGATGAACCATTTGGGAAGGTGATAATATTCTCAATAAGTATGTT

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCT

GAAGTTAATCAGCGCTTAGTTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG

GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

|

Find differences to make variant-specific primers for PCR

TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA

TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT

AATGTTACGTATGGATGAACCATTGGAAGGTGATAATATTCCTCAATAAGTATGTT

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCCTCT

GAAGTTAATCAGCGCTTAGTTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG

GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

|

Find differences to make variant-specific primers for PCR

TGGCTAATTCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA

TGGCTAACCCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT

AATGTTACGTATGGATGAACCATTGGAAGGTGATAATATTTCTCAATAAGTATGTT

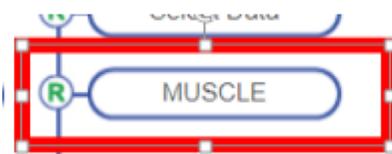
GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCT

GAAGTTAATCAGCGCTTAGTGGAGGAAATGAAGGCATTTAAGGAGCGTACTA

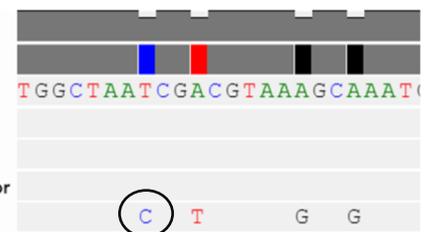
GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG

GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

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



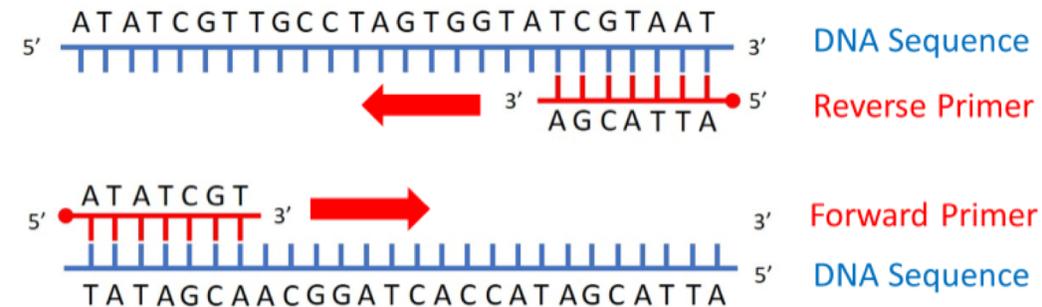
General guidelines for species-specific primer development

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

TGGCTAATTCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT
AATGTTACGTATGGATGAACCATTGGAAGGTGATAATATTTCTCAATAAGTATGTT

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCTCT
GAAGTTAATCAGCGCTTAGTGGAGGAAATGAAGGCATTTAAGGAGCGTACTAT

TTGCACAACCGAGTCTCT

GGGCTGATTTACAACGTGTTTGGCTCAGAGATTAG
GGTCAGATTTACATCGCGTAGGTGCGGAAAATTAG

