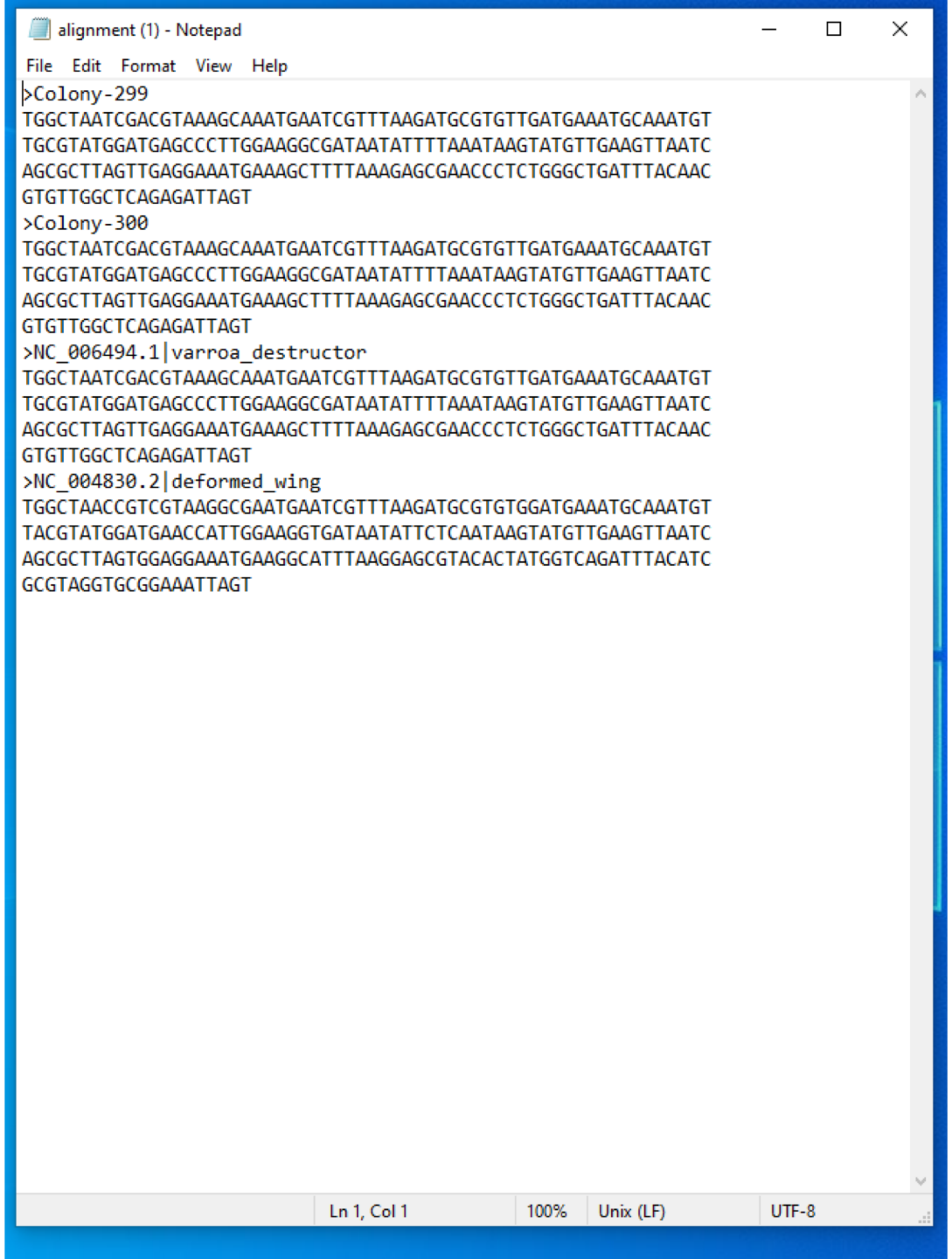
Developing primers tutorial

Continuation from Honey bee viral sequence activity…

(using DNA subway is optional)



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
GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCTCT
GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG

>NC_004830.2|deformed_wing/1-199
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCA
AATGTTACGTATGGATGAACCATTGGAAGGTGATAATATTCTCAATAAGTATGTT
GAAGTTAATCAGCGCTTAGTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT
GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

Manually re-align both sequences in the word processor

TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT
AATGTTACGTATGGATGAACCATTGGAAGGTGATAATATTCTCAATAAGTATGTT

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCTCT
GAAGTTAATCAGCGCTTAGTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG
GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

Find differences to make variant-specific primers for PCR

TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCA
TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCA

AATGTTGCGTATGGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTT
AATGTTACGTATGGATGAACCATTGGAAGGTGATAATATTCTCAATAAGTATGTT

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCTCT
GAAGTTAATCAGCGCTTAGTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG
GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG

Find differences to make variant-specific primers for PCR

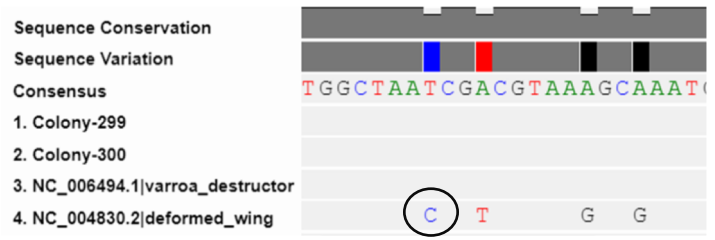
TGGCTAA**T**CG**A**CGTAA**A**GC**A**AATGAATCGTTTAAGATGCGTGT**T**GATGAAATGCA
TGGCTAA**C**CG**T**CGTAA**G**GC**G**AATGAATCGTTTAAGATGCGTGT**G**GATGAAATGCA

AATGTT**G**CGTATGGATGA**G**CC**C**TTGGAAGG**C**GATAATATT**T**T**A**AATAAGTATGTT
AATGTT**A**CGTATGGATGA**A**CC**A**TTGGAAGG**T**GATAATATT**C**T**C**AATAAGTATGTT

GAAGTTAATCAGCGCTTAGT**T**GAGGAAATGAA**A**GC**T**TTTAA**A**GAGCG**A**AC**C**CT**C**T
GAAGTTAATCAGCGCTTAGT**G**GAGGAAATGAA**G**GC**A**TTTAA**G**GAGCG**T**AC**A**CT**A**T

GG**G**C**T**GATTTACA**A**CG**T**GT**T**GG**CT**C**A**GA**G**ATTAG
GG**T**C**A**GATTTACA**T**CG**C**GT**A**GG**TG**C**G**GA**A**ATTAG

As a cheat refer back to:



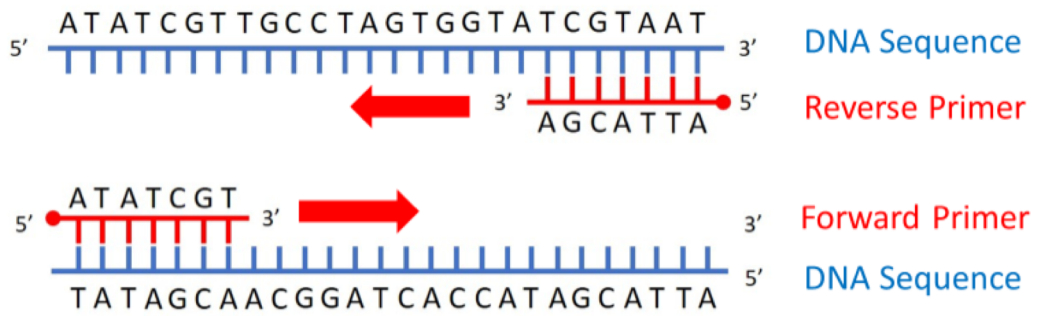
General guidelines for species-specific primer development

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

GAAGTTAATCAGCGCTTAGTTGAGGAAATGAAAGCTTTTAAAGAGCGAACCCTCT

GAAGTTAATCAGCGCTTAGTGGAGGAAATGAAGGCATTTAAGGAGCGTACACTAT

TTGCACAACCGAGTCTCT

GGGCTGATTTACAACGTGTTGGCTCAGAGATTAG

GGTCAGATTTACATCGCGTAGGTGCGGAAATTAG