Developing primers to amplify honey bee viruses - teacher

Viral RNA Sequences - ANSWERS

DWV-A
5'-
UGGCUAACCGUCGUAAGGCGAAUGAAUCGUUUAAGAUGCGUGUGGAUGAAAUGCAAAUGUUACGUAUGGAUGAAC
CAUUGGAAGGUGAUAAUAUUCUCAAUAAGUAUGUUGAAGUUAAUCAGCGCUUAGUGGAGGAAAUGAAGGCAUUUA
AGGAGCGUACACUAUGGUCAGAUUUACAUCGCGUAGGUGCGGAAAUUAGU-3'
DWV-B
5'-
UGGCUAAUCGACGUAAAGCAAAUGAAUCGUUUAAGAUGCGUGUUGAUGAAAUGCAAAUGUUGCGUAUGGAUGAGC
CCUUGGAAGGCGAUAAUAUUUUAAAUAAGUAUGUUGAAGUUAAUCAGCGCUUAGUUGAGGAAAUGAAAGCUUUUA
AAGAGCGAACCCUCUGGGCUGAUUUACAACGUGUUGGCUCAGAGAUUAGU-3'

STEPS 3,4, and 5 shown below:

Align RNA sequences and use the find & replace function to change the Us for Ts. Next find and bold all differences between the red and black single-stranded DNA sequences. Last, highlight 15-20bps in each single-stranded DNA sequence that have 5-7 bp differences. This is where you will develop primers

5'-TGGCTAA**C**CG**T**CGTAA**G**GC**G**AATGAATCGTTTAAGATGCGTGT**G**GATGAAATGCAAATGTT**A**CGTAT
5'-TGGCTAA**T**CG**A**CGTAA**A**GC**A**AATGAATCGTTTAAGATGCGTGT**T**GATGAAATGCAAATGTT**G**CGTAT

GGATGA**A**CC**A**TTGGAAGG**T**GATAATATT**C**T**C**AATAAGTATGTTGAAGTTAATCAGCGCTTAGT**G**GAGGAA
GGATGA**G**CC**C**TTGGAAGG**C**GATAATATT**T**T**A**AATAAGTATGTTGAAGTTAATCAGCGCTTAGT**T**GAGGAA

ATGAA**G**GC**A**TTTAA**G**GAGCG**T**AC**A**CT**A**TGG**T**C**A**GATTTACA**T**CG**C**GT**A**GG**T**G**C**G**A**AATTAGT-3'
ATGAA**A**GC**T**TTTAA**A**GAGCG**A**AC**C**CT**C**TGG**G**C**T**GATTTACA**A**CG**T**GT**T**GG**C**T**C**A**G**AGATTAGT-3'

cDNA

Steps 6,7, and 8 below

Convert to double-stranded complementary DNA (cDNA). To fill in the complementary bases, I used a simple web-based program: https://www.bioinformatics.org/sms/rev_comp.html and select 'complementary' bases in the dropdown box after cutting and pasting in the sequence of each

virus. Highlight the complementary strand in purple font.

DWV-A

5′-TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCAAATGTTACGTAT

3′-ACCGATTGGCAGCATTCCGCTTACTTAGCAAATTCTACGCACACCTACTTTACGTTTACAATGCATA

GGATGAACCATTGGAAGGTGATAATATTCTCAATAAGTATGTTGAAGTTAATCAGCGCTTAGTGGAGGAA

CCTACTTGGTAACCTTCCACTATTATAAGAGTTATTCATACAACTTCAATTAGTCGCGAATCACCTCCTT

ATGAAGGCATTTAAGGAGCGTACACTATGGTCAGATTTACATCGCGTAGGTGCGGAAATTAGT-3′

TACTTCCGTAAATTCCTCGCATGTGATACCAGTCTAAATGTAGCGCATCCACGCCTTTAATCA-5′

DWV-B

5′-TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGTTGCGTAT

3′-ACCGATTAGCTGCATTTCGTTTACTTAGCAAATTCTACGCACAACTACTTTACGTTTACAACGCATA

GGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTTGAAGTTAATCAGCGCTTAGTTGAGGAA

CCTACTCGGGAACCTTCCGCTATTATAAAATTTATTCATACAACTTCAATTAGTCGCGAATCAACTCCTT

ATGAAAGCTTTTAAAGAGCGAACCCTCTGGGCTGATTTACAACGTGTTGGCTCAGAGATTAGT-3′

TACTTTCGAAAATTTCTCGCTTGGGAGACCCGACTAAATGTTGCACAACCGAGTCTCTAATCA-5′

Primer Development

Steps 9 and 10 below

Develop primer pairs (forward and reverse) for each variant (DWV-Type-A and DWV-Type-B). Note that the reverse primer should be the reverse complement of the top sequence. Use blue font for your primer sequences, make sure to provide orientation (5′ and 3′).

DWV-Type-A

DWV-A Forward Primer: 5′-AACCGTCGTAAGGCGAA-3′

DWV-A Reverse Primer: 5′-TTCCGCACCTACGCGATG-3′

Developing and diagnosing honey bee killers

DWV-A

5′-TGGCTAACCGTCGTAAGGCGAATGAATCGTTTAAGATGCGTGTGGATGAAATGCAAATGTTACGTAT

5′-AACCGTCGTAAGGCGAA-3′ Nj **PCR direction**
3′-ACCGATTGGCAGCATTCCGCTTACTTAGCAAATTCTACGCACACCTACTTTACGTTTACAATGCATA

GGATGAACCATTGGAAGGTGATAATATTCTCAATAAGTATGTTGAAGTTAATCAGCGCTTAGTGGAGGAA
CCTACTTGGTAACCTTCCACTATTATAAGAGTTATTCATACAACTTCAATTAGTCGCGAATCACCTCCTT

ATGAAGGCATTTAAGGAGCGTACACTATGGTCAGATTTACATCGCGTAGGTGCGGAAATTAGT-3′
lj **PCR direction** 3′-GTAGCGCATCCACGCCTT-5′

TACTTCCGTAAATTCCTCGCATGTGATACCAGTCTAAATGTAGCGCATCCACGCCTTTAATCA-5′

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DWV-B

DWV-B Forward Primer: 5′-AATCGACGTAAAGCAAA-3′
DWV-B Reverse Primer: 5′-CTCTGAGCCAACACGTTG-3′

5′-TGGCTAATCGACGTAAAGCAAATGAATCGTTTAAGATGCGTGTTGATGAAATGCAAATGTTGCGTAT

5′-AATCGACGTAAAGCAAA-3′ Nj **PCR direction**
3′-ACCGATTAGCTGCATTTCGTTTACTTAGCAAATTCTACGCACAACTACTTTACGTTTACAACGCATA

GGATGAGCCCTTGGAAGGCGATAATATTTTAAATAAGTATGTTGAAGTTAATCAGCGCTTAGTTGAGGAA
CCTACTCGGGAACCTTCCGCTATTATAAAATTTATTCATACAACTTCAATTAGTCGCGAATCAACTCCTT

ATGAAAGCTTTTAAAGAGCGAACCCTCTGGGCTGATTTACAACGTGTTGGCTCAGAGGATTAGT-3′
lj **PCR direction** 3′-GTTGCACAACCGAGTCTC-5′

TACTTTCGAAAATTTCTCGCTTGGGAGACCCGACTAAATGTTGCACAACCGAGTCTCTAATCA-5′