Developing primers to amplify honey bee viruses

Background

Deformed Wing Viruses (DWV) are some of the most dangerous pathogens to honey bee colonies. As their name suggests, elevated levels of DWV within colonies lead to many adult bees displaying deformed or wrinkled wings. Such deformities reduce the life of individual bees and make them unable to forage for the colony. DWV spreads across colonies through an intermediate host, a parasitic mite, called *Varroa destructor*.

DWV is a complex of closely-related RNA viruses that are further separated into distinct types, the most common thought to be DWV Type A (DWV-A) in the United States. However, a recent viral genome was discovered which closely matched DWV-A, but showed enough nucleotide differences to be reclassified as DWV Type B (DWV-B). Once characterized, it becomes important to develop a diagnostic test to better understand the distribution of the new virus as well as study its effects within the commercial beekeeping industry.

Polymerase Chain Reaction (PCR) is an important technique that allows scientists to quickly identify infected bee colonies by amplifying viral genetic material. When creating a new diagnostic test using PCR, a critical step is finding unique regions within the DWV genome that can be used as primer sites. These regions are where single-stranded primers anneal (or hybridize) with the target sequence to begin the PCR amplification process necessary for detection.

Your task

A commercial beekeeping operation has **lost 10%** of their colonies after almond pollination. Word has spread that a new variant of DWV has been discovered and is transmitted by the parasitic mite, *Varroa destructor*. The National Beekeeping Association has raised money to give to the diagnostic lab to develop a PCR diagnostic test for DWV-B to help identify and track new infected colonies.

To help your efforts, a researcher at the university has provided a highly variable region of DWV-A and DWV-B genomes to aid in the PCR test development. In this activity, the goal is to develop one set of primers that will amplify only DWV-A and a second set of primers that will amplify only DWV-B.

You are expected to:

1. Convert viral genetic material into complementary DNA (cDNA) for PCR use
2. Align the DWV-A and DWV-B sequences in a word processor
3. Identify and highlight nucleotide differences between DWV-A and DWV-B sequences
4. Identify suitable primer sites as well as the primers for separate tests to detect each virus

Viral Sequences

DWV-A

5′-

UGGCUAACCGUCGUAAGGCGAAUGAAUCGUUUAAGAUGCGUGUGGAUGAAAUGCAAAUGUUACGUAUGGAUGAACCAUUGGAA
GGUGAUAAUAUUCUCAAUAAGUAUGUUGAAGUUAAUCAGCGCUUAGUGGAGGAAAUGAAGGCAUUUAAGGAGCGUACACUAUG
GUCAGAUUUACAUCGCGUAGGUGCGGAAAUUAGU-3′

DWV-B

5′-

UGGCUAAUCGACGUAAAGCAAAUGAAUCGUUUAAGAUGCGUGUUGAUGAAAUGCAAAUGUUGCGUAUGGAUGAGCCCUUGGAA
GGCGAUAAUAUUUUAAAUAAGUAUGUUGAAGUUAAUCAGCGCUUAGUUGAGGAAAUGAAAGCUUUUAAAGAGCGAACCCUCUG
GGCUGAUUUACAACGUGUUGGCUCAGAGAUUAGU-3′

Discovering and diagnosing honey bee killers

Question: Examine the DWV-A and DWV-B sequences. What type of genetic material are the viruses made of? How can you tell?

Cut and paste both viral sequences into a word processor document. (Microsoft Word or Google Doc). Maintain the differences in font colors to easily distinguish DWV-A from DWV-B.

1. Change the font of both sequences to `COURIER NEW` so that each nucleotide letter (A, T, C, and G) has identical spacing.
2. Align the sequences by placing DWV-A sequence on top and DWV-B sequence directly below. You will have several lines of this stacking pattern to complete the full alignment.

   5′-UGGCUAACCGU
   5′-UGGCUAAUCGA

4. Find and replace the Us for Ts in the alignment.

   5′-TGGCTAACCGT
   5′-TGGCTAATCGA

5. Bold font or underline the differences between the aligned sequences.

   5′-TGGCTAA**C**CG**T**
   5′-TGGCTAA**T**CG**A**

Question: Once all mismatches are located, how many total differences or mismatches are there?

6. Separate the two DWV sequences back to their full, individual sequences, while keeping the differences bolded or underlined.
7. Fill in the complementary strand (3'-5') to create double-stranded cDNA, which is necessary for PCR and primer development. [The use of the web-based program can help speed up the base pairing if needed https://www.bioinformatics.org/sms/rev_comp.html]. Carry over the previously bolded or underlined differences to the complementary strand and make the 3'-5' strand a different color.

   5′-TGGCTAA**C**CG**T**-3′
   3′-ACCGATT**G**GC**A**-5′

8. Examine each DWV-A and DWV-B sequences for locations where nucleotide differences are clustered within a span of approximately 15-20 base pairs. Look near the beginning and end of the cDNA sequences. Highlight these 15-20 bp regions to mark them as primer binding sites.

   5′-TGGCTAA**C**CG**T**-3′
   3′-ACCGATT**G**GC**A**-5′

9. Create a space between the DNA strands to fill in the nucleotides for the proposed primers. There are two primers per virus target - a forward and a reverse. The forward primer anneals to the 3'- 5' strand provided, whereas the reverse primer anneals to the provided 5'-3' strand. (Hint - Reverse primers are the reverse complement to the top strand - see deck slide). Use blue font for primers.

   5′-TGGCTAA**C**CG**T**-3′
   5′-CCGT
   3′-ACCGATT**G**GC**A**-5′

10. Check to make sure that the proposed primers for each virus will not anneal to the non-target virus. That is, the forward primer for DWV-A should not be able to anneal to the DWV-B sequence.