PCR to detect bee viruses

Today, you and your partner will quantify the presence of cDNA using a nanodrop, then conduct PCR. As always, we advocate a PCR negative control with no DNA to confirm your lab reagents, technique, and setup are uncontaminated.

Determine the concentration of cDNA in your sample using the nanodrop. Discuss with your instructors whether you should dilute your cDNA. Whether you dilute or not, create a new 1.5 ml tube for your cDNA for long term storage/use, and transfer your entire sample into the new tube.

For a positive control, we'll confirm that your cDNA sample contains bee cDNA from a gene called *rps18*, a "housekeeping gene" expressed in all bees and used by Scharlaken et al (2008) to confirm the presence of cDNA.

Table 1. Positive control genes for bee cDNA from Scharlaken et al 2008

Primer	Gene	Sequence 5' to 3'	Product length	Ta
actin-f	actin	TGCCAACACTGTCCTTTCTG	155	61
actin-r		AGAATTGACCCACCAATCCA		
rps18-f	RPS18	GATTCCCGATTGGTTTTTGA	149	61
rps18-r		CCCAATAATGACGCAAACCT		

Each team will also screena mixed cDNA sample against all five viruses for which we have primers (Table 2) in a 'multiplex PCR.'

Table 2. Virus primers from Sguazza et al 2013

Primer	Virus	Sequence 5' to 3'	Product length	Ta
AIVf	IAPV	GGTGCCCTATTTAGGGTGAGGA	158	56
IAPVr		GGGAGTATTGCTTTCTTGTTGTG		
DWVf	DWV	TGGTCAATTACAAGCTACTTGG	269	56
DWVr		TAGTTGGACCAGTAGCACTCAT		
SBVf	SBV	CGTAATTGCGGAGTGGAAAGATT	342	56
SBVr		AGATTCCTTCGAGGGTACCTCATC		
BQCVf	BQCV	CTTTATCGAGGAGGAGTTCGAGT	536	56
BQCVr		GCAATAGATAAAGTGAGCCCTCC		
CBPVf	CBPV	AACCTGCCTCAACACAGGCAAC	774	56
CBPVr		ACATCTCTTCTTCGGTGTCAGCC		

Work out the volumes for your master mixes, noting also which items should *not* be added to the master mix, then obtain PCR tubes and label with your group letter and the numbers 1-5 as below:

1 = *rps18* positive control gene + cDNA from last week (pos ctrl)
2 = *rps18* positive control gene + no cDNA (neg ctrl)
3 = a multiplex of the 5 viruses + Virus cDNA mix provided by TAs (pos ctrl)
4 = a multiplex of the 5 viruses + cDNA from last week (experimental sample)
5 = a multiplex of the 5 viruses + no cDNA (neg ctrl)

Set up your PCR tubes with a 25 ul reaction volume according to the table below. Place your PCR tubes in the thermal cycler as directed by the TAs.

Literature Cited

Scharlaken, B. et al. 2008. Reference gene selection for insect expression studies using quantitative real-time PCR: The head of the honeybee, Apis mellifera, after a bacterial challenge. Journal of Insect Science, 8:33, insectscience.org/8.33, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3061606/

Sguazza, G. H. et al. 2013. Simultaneous detection of bee viruses by multiplex PCR. Journal of Virological Methods 194:102-106, http://dx.doi.org/10.1016/j.jviromet.2013.08.003.

Simplex PCR to confirm that bee cDNA is present

PCR Reagent with final concentration	Stock Concentration	1x (ul)	____X Master Mix (ul)
H_2O	N/A	11	
PCR Buffer	10X	2.5	
2.5 mM MgCl2	25 mM	1.5	
0.2 mM dNTPs	2.5 mM	2	
5 U *Taq* DNA Polymerase	5 units/ul	0.5	
1 uM Forward Primer	10 µM	2.5	
1 uM Reverse Primer	10 µM	2.5	
cDNA	25–100 ng	2.5	-
Final Volume		25	-

Multiplex PCR pilot on known

PCR Reagent with final concentration	Stock Concentration	1x (ul)	____X Master Mix (ul)
H_2O	N/A	3.5	
PCR Buffer	10X	2.5	
2.5 mM MgCl2	25 mM	1.5	
0.2 mM dNTPs	2.5 mM	2	
2.5 U *Taq* DNA Polymerase	5 units/ul	0.5	
0.5 uM Forward Primer Mix (contains F primers for all 5 viruses)	10 µM	6.25	
0.5 uM Reverse Primer Mix (contains R primers for all 5 viruses)	10 µM	6.25	
cDNA	25–100 ng	2.5	-
Final Volume		25	-

PCR Conditions

Cycles	Step	Function
1	94 °C for 5 min	Initial prolonged heating fully denatures genomic DNA
35	94 °C for 30 sec	DNA denatures
	56 °C for 30 sec	PCR primers anneal to DNA
	72 °C for 45 sec	*Taq* polymerase extends DNA strand from primer
1	72 °C for 10 min	Final extension ensures all PCR products are completed extended

Gel electrophoresis (next week)

Electrophorese your PCR products on a 2.0% agarose gel at 100 V for 45-60 minutes, or until good size separation for PCR products ranging from 150–775 bp. Record the gel number and gel lanes that contain your samples. Visualize using UV, photograph for your lab notebook and lab report, and record your results.

Note: To fit two groups onto a single gel, plan to combine both of your negative control samples in the same well in the gel.