PROTOCOL: Pilot PCR on Bee cDNA and

Objective: Using isolated DNA and existing primers for a specific gene or set of genes, practice bench skills like pipetting and sterile technique to amplify the gene(s) of interest in the positive control but not the negative control PCR tube. Each pair will make a master mix and set up PCR tubes for a bee today in class.

A. Prepare your PCR master mix. The PCR components are listed below along with a worksheet to help you calculate the amount of each component you will be adding. Always make at least 1 extra aliquot's worth (e.g., if you need 6 PCR tubes, then mix 7X instead of 6X). Be careful not to add DNA into your master mix.

Reagent	Stock Concentration	Volume (µl) 1X	Master Mix (µl) (N+1)X	Final Concentration
H_2O	N/A			
PCR Buffer	10X			
$MgCl_2$	25 mM	1.5		
dNTPs	2.5 mM	2		
Taq DNA Polymerase	5 units/ml	0.5		
Forward Primer	10 µM	2.5		
Reverse Primer	10 µM	2.5		
Bee cDNA	25–100 ng	2.5	NOT IN THE MM!	
Final Volume		25		

B. Label 0.2 ml PCR tubes top and side (just below the rim) with your assigned PCR codes: one tube is to amplify DNA (positive control) and the other is a DNA-free tube (negative control). PCR tubes are thin-walled to better transfer heat but are therefore easy to crush or bend.

C. Add Master Mix to each PCR tube.

D. Add 2.5 µl of the relevant DNA to the bottom of each PCR tube.
When you have combined the components into a single tube, vortex the master mix for about three seconds. Then add the appropriate amount of master mix to each PCR tube. Be careful not to add DNA into your negative control tube. Finally, spin your tubes briefly in a PCR tube centrifuge to collect all the reagents at the bottom.

E. Place your tubes in the PCR thermal cycler and (once all class samples are loaded) start the cycling program. We use the following cycling standard profile, where you need to determine the annealing temperature at which your primers anneal optimally.

Cycles	Step	Function
1	94 °C for 2 min	Initial prolonged heating fully denatures genomic DNA
30	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

Place your samples in the PCR thermal cycler and ensure that the proper program is loaded. Once all the samples are loaded, close the lid (careful, the lid is hot!), and start the thermal cycler. The PCR cycling profile will take about 2-3 hours. The TAs will remove your reactions and place them at –20 °C until next week.