Clade (Clade II, IV, VII, VIII) Specific PCR

Master Mix:

Reagent	Volume/rxn
ddH_2O	18.3µl
10x buffer	2.5 µl
dNTPs	2.0 µl
MgCl (25mM)	0.5 µl
F primer (25pmol/µl)	0.5 µl
R primer (25pmol/µl)	0.5 µl
Taq	0.2 µl
	24.5ul

1. Prepare master mix based on number of samples being processed (include controls and 1 extra, to be sure you have enough volume).
2. Mix thoroughly
3. Dispense 24.5 µl into PCR tubes or plate
4. Add 0.5 µl template DNA
5. Place in thermocyler and run appropriate program

Clade	Thermocycler Program
II	TD62
IV	TD61
VII	TD61
VIII	TD61

Thermocycler Conditions

TD61

Heated Lid 105

1. 95 2 min
2. 95 30 sec
3. 61 30 sec
4. 72 1 min
5. GOTO step 2
6. 95 30 sec
7. 59 30 sec
8. 72 1 min
9. GOTO step 6
10. 95 30 sec
11. 57 30 sec
12. 72 1 min
13. GOTO step 10 REPEAT 30 times
14. 72 2 min
15. 4 HOLD

TD62

Heated Lid 105

1. 95 2 min
2. 95 30 sec
3. 62 30 sec
4. 72 1 min
5. GOTO step 2
6. 95 30 sec
7. 60 30 sec
8. 72 1 min
9. GOTO step 6
10. 95 30 sec
11. 58 30 sec
12. 72 1 min
13. GOTO step 10 REPEAT 30 times
14. 72 2 min
15. 4 HOLD

Gel Imaging

1. PCR products are run on a 1.5% agarose gel in 1x TAE
   (ex. 0.75g agarose in 50ml 1x TAE)
2. Melt agarose/TAE in microwave (Be careful not to boil over!).
3. Add 3-5ul Ethidium Bromide (ETBR- use gloves and caution when using)
4. Allow to cool slightly
5. Pour into prepared gel tray and allow to solidify
6. Place gel into gel box
7. Load 5 ul sample + 1 ul loading dye to gel
8. Load appropriate ladder (100bp)
9. Run gel for approximately 20 minutes at 100 volts
10. View gel under UV light and image

Notes:
Our lab is currently using TaKaRa Taq and its included dNTP mix and 10x buffer. (#RR001A)
Be sure to wear gloves throughout the procedure, particularly when handling ETBR