PROTOCOL

Thermocycle Testing Kit

Part Number:	20K-100
Batch:	1007-1
Storage:	Store @ -20ºC
Batch Number:	Marked on tube

Tube 1: OK Mix
Volume: 1.0 ml
Color of Lid: Yellow

Tube 2: DNA/Primers
Volume: 1.0 ml
Color of Lid: Green

Protocol:

1. Transfer 10 µl of OK mix (**Tube 1)** into the PCR tube. Add 10 µl DNA/Primers (**Tube 2**).
2. Overlay with mineral oil if necessary.
3. Place in a Thermal Cycler. If possible, set Thermal Cycler to **ramp at medium rate** (1.2 to 2.5ºC/sec)

Cycling Profile:

Initial denaturation step: 95°C for 5 mins

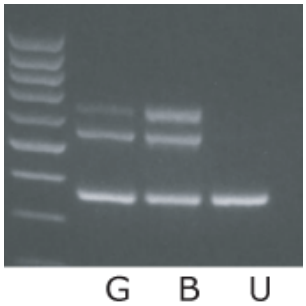
Then cycle 31 times:

Step 1: 95°C for 30 secs

Step 2: 66°C for 60 secs

Step 3: 72°C for 60 secs

After cycling, load 10 µl onto 1.7% agarose gel and electrophorese alongside a 100 bp DNA ladder.



Expected fragment sizes: 360 bp (bottom), 550 bp (middle) and 650 bp (top).
The 350 bp fragment should be the brightest, then the 550 and then the 650 bp.

G = Good machine: Top band just visible. Machine working correctly.

B = Bad machine: Middle and top band too strong.
Machine cooling below the required annealing temp.

U = Ugly machine: No top band and middle band very faint.
Machine not cooling to the required annealing temp.

For Research Only