



## Human DNAOK!

### Protocol:

- Mix together 7.5  $\mu$ l of Human DNAOK mix and 12.5  $\mu$ l of MegaMix~Gold
- Add 5  $\mu$ l DNA (5 to 50 ng). Adjust volume with water if less is added
- Overlay with mineral oil if necessary.
- Place in a Thermal Cycler

### Cycling profile:

Initial denaturation step: 95°C for 5 mins

Then cycle 33 times:

Step 1: 95°C for 30 secs

Step 2: 62°C for 30 secs

Step 3: 72°C for 45 secs

After cycling, load 10  $\mu$ l onto 1.75% agarose gel and electrophorese alongside a 100 bp DNA Ladder (not supplied). Make sure that the sample hasn't evaporated during cycling, as this will distort the results.

### Interpretation of results:

**Expected fragment sizes: 100 bp, 200, 300, 400, 500 and 600 bp.**

- **If all 6 fragments are observed the DNA is more than likely to be okay**
- The 500 bp fragment is derived from an internal control and should always be present (even in negative controls). If not, PCR has failed and needs repeating
- Different band intensities can represent different amount of DNA.
- If less than 6 fragments are observed the DNA is likely to not be okay
- If only the control fragment is observed then the DNA is more than likely to not be okay or not added

