

Microzone Ltd

Human DNAOK!

Protocol:

- Mix together 7.5 μ l of Human DNAOK mix and 12.5 μ l of MegaMix~Gold
- Add 5 μ 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 30 times:
Step 1: 95°C for 30 secs
Step 2: 63°C for 30 secs
Step 3: 72°C for 45 secs

After cycling, load 10 μ 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:

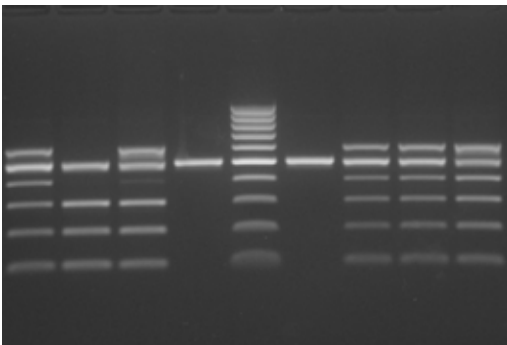
Expected fragment sizes: 100 bp, 200, 300, 400, 500 and 600 bp.

- **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

Example:

Human DNAOK! carried out on 25 ng of 7 different DNA samples

1 2 3 4 5 6 7 8 9



1. Good quality DNA
2. Bad quality DNA*
3. Bad quality DNA*
4. Bad quality DNA**
5. 100bp ladder
6. No human DNA control
7. 5 ng human DNA control
8. 10 ng human DNA control
9. 20 ng human DNA control

*Uneven amplification with bands missing - partially degraded DNA sample

**No amplification from human DNA - DNA sample fully degraded