fisher biotec australia

MORE THAN 20 YEARS IN BUSINESS

Established 1997

Definitive Fingerprinting Teaching System (Cat # FNT-1)

Kit Components

- 6 x 250uL 2 x Apo B Reaction Buffer
- 1 x 1mL Taq Dilution Buffer
- Taq DNA Polymerase (250U) (Cat # TAQ-1)
- 5 x VNTR Control DNA
- 1 x 1mL 2 x Gel Loading Buffer

Suggested Protocol for Fingerprinting Teaching System

Buccal Cell Preparation

Additional Material required:

- NET Buffer (20mM Tris-HCl, pH 8.0, 10mM EDTA, 154mM NaCl)
- 0.1% Triton X-100

1. Rinse mouth with water.
2. Scrape the inside cheek with a plastic spoon (two sweeps should be adequate).
3. With the aid of micropipette suspend the collected material in 1mL NET buffer.
4. Transfer the contents to 1.5mL microfuge tube and centrifuge at full speed for 30 seconds.
5. Remove all traces of supernatant with a micropipette.
6. Resuspend the pellet in 1mL of 0.1% Triton X-100, vortex vigorously.

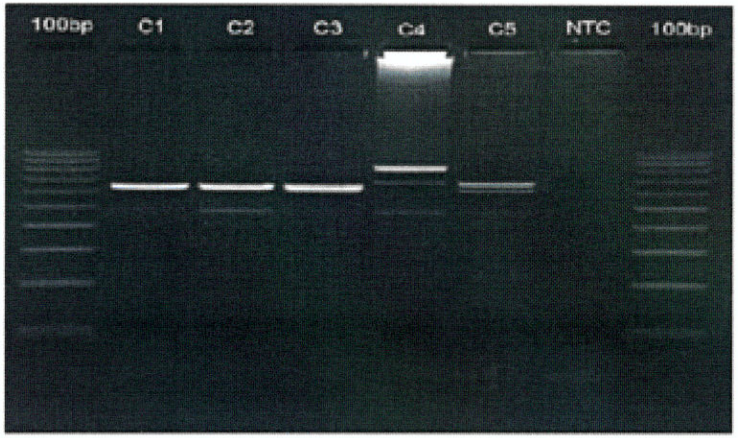
Note:
1. Several cycles of freezing and thawing appear to enhance the amplification signal.
2. DNA samples obtained from smokers using the following protocol tend to produce inconsistent results.

Suggested Protocol for DNA-PCR Amplification of the ApoB gene

1. Add to a sterile tube the following:
   - 10uL 2 x Apo B Reaction Buffer
   - 5uL Buccal cell extract or 1uL Control DNA
   - Make up final volume to 20uL with PCR grade water (Cat # UPW-100)
2. Add 1uL Taq DNA pol (1U/uL) (Cat # TAQ-1 diluted 1:4 with Taq Dilution Buffer)
3. PCR amplify using the conditions overleaf

PCR Amplification Cycles

Primary Denaturation x 1	94°C for 5 minutes 58°C for 1 minute
↓	
Repetitive Cycles x 38	94°C for 1 minute 58°C for 6 minutes
↓	
Chase Cycle x 1	94°C for 1 minute 58°C for 15 minutes 25°C for 1 minute
↓	
Amplicon detection	Analyse 5uL of reaction mix on a 3% Agarose gel with DNA size markers (Cat # M-DNA-100bp)



C1 - C5 Samples, No template Control, 100bp ladder – 3% Agarose Gel