

## DNA Isolation from Whole Blood

### DNA extraction from liquid animal blood using *prepGEM*<sup>™</sup>

This method is recommended for DNA extraction directly from whole liquid animal blood. Incubations can be performed either in a thermal cycler, water bath, or using an automated robotic workstation fitted with Peltier temperature-controlled heating blocks.

This method is intended to be a starting point for evaluating *prepGEM*<sup>™</sup>. The method should be optimised for the specific requirements of the test type.

### Extraction Method

1. Place 15  $\mu$ l liquid blood into a 96-well standard PCR plate or tube.
2. Add 40  $\mu$ l of *prepGEM*<sup>™</sup> buffer 3H and 1  $\mu$ l of *prepGEM*<sup>™</sup> to each sample [IMPORTANT - NOTE1; EDTA blood].
3. Incubate at 70-75°C for 15 minutes, then,  
Incubate at 95-99°C for 5-15 minutes [NOTE2].
4. Centrifuge the plate or tube at 5,000 rpm for 5 minutes to separate the phases (see Figure 1a.).
5. Take an aliquot of the clear liquid upper phase for PCR [NOTE3].

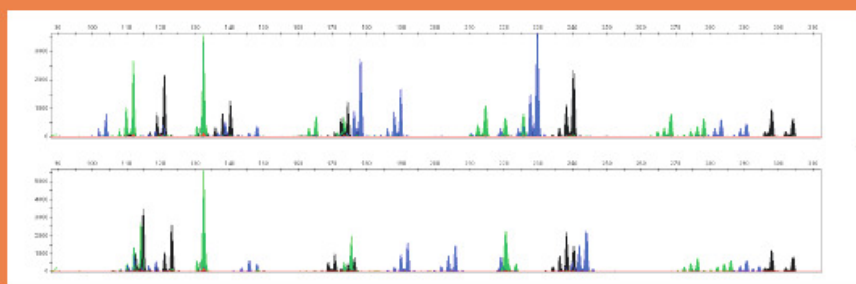
NOTE1: *prepGEM*<sup>™</sup> is Ca<sup>2+</sup> dependent, and so for EDTA stabilised blood, the buffer should be supplemented to a final concentration of 200  $\mu$ M CaCl<sub>2</sub>. This concentration will be effective for Vacutainers containing between 1 and 7 ml. *prepGEM*<sup>™</sup> and buffer can also be added as a master mix.

NOTE2: Both incubation times may be reduced further through optimisation.

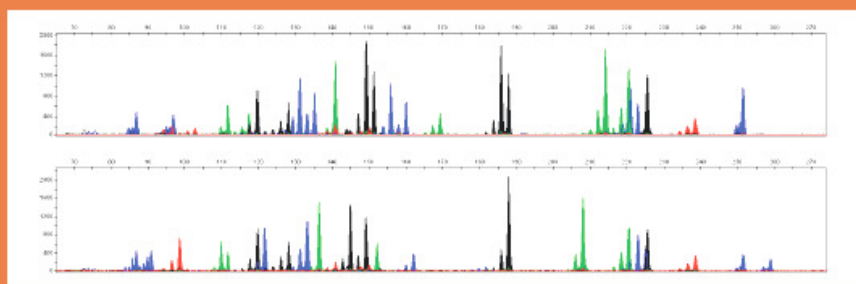
## WHOLE BLOOD



Centrifuged samples showing phase separation



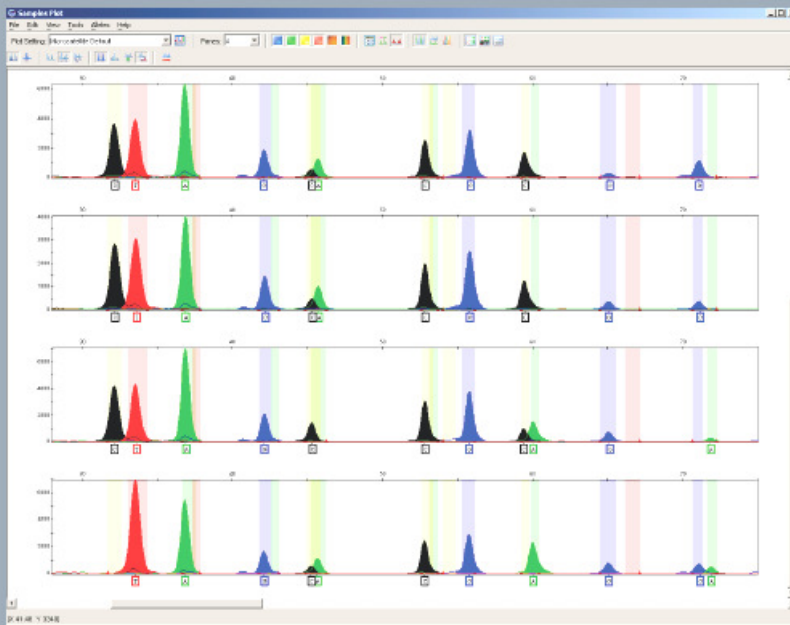
14-plex sheep panel (15  $\mu$ l blood in 40  $\mu$ l of buffer)



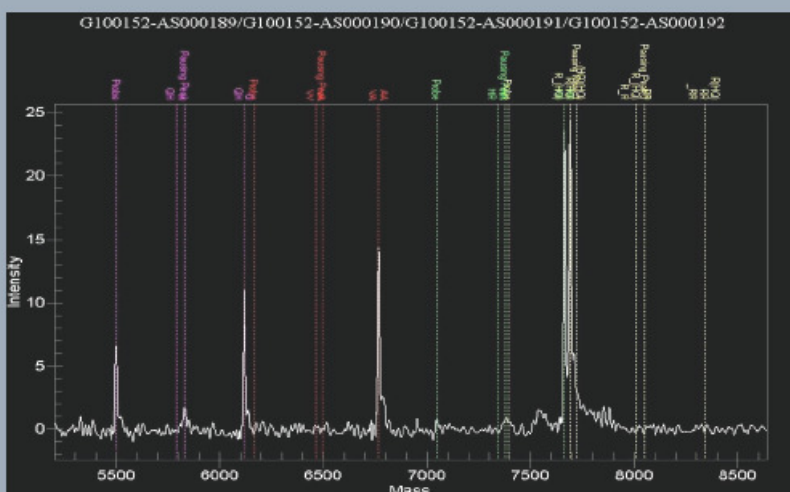
14-plex cattle panel (15  $\mu$ l blood in 90  $\mu$ l of buffer)

# ANIMAL BLOOD

prepGEM™ also delivers excellent results for SNP analysis. The figures below show results from a 9-plex single base pair extension assay using dye labeled terminators from 4 amplicons, performed on an ABI automated sequencer and a scrapie resistance hME assay performed on a Sequenom Autoflex Mass Array.



9-plex SNaPshot™ reaction. 2 µl of extract (15 µl of blood in 40 µl of buffer) was used for PCR.



Scrapie resistance hME assay spectra. 0.5 – 1 µl (15 µl blood:60 µl buffer) was used for PCR.

NOTE3: 1-2 µl is sufficient for a 5-12 µl reaction. DNA can also be dried down for delayed amplification. The mixture does not necessarily need to be centrifuged; the DNA performs as well (although brown in colour) when used directly after the incubation steps. It can be diluted further, but is more difficult to pipette. For a more rapid extraction, the centrifugation step can be removed by adding >90 µl of prepGEM™ buffer and 1 µl of prepGEM™ to 15 µl of liquid blood, and reducing incubation times [NOTE2] (see Figure 1b). The larger buffer volume makes the sample more amenable to automatic pipetting. Optimisation of your test will need to be carried out if the centrifugation step is removed. Microsatellite and SNP analysis has been successfully carried out using DNA extracted with and without the centrifugation step.