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AUTOMATED GENOMIC DNA EXTRACTION FROM WHOLE BLOOD ON THE RoboAmp4200 USING MAGNETIC BEAD SEPARATION



APPLICATION



AUTOMATION



RESULT

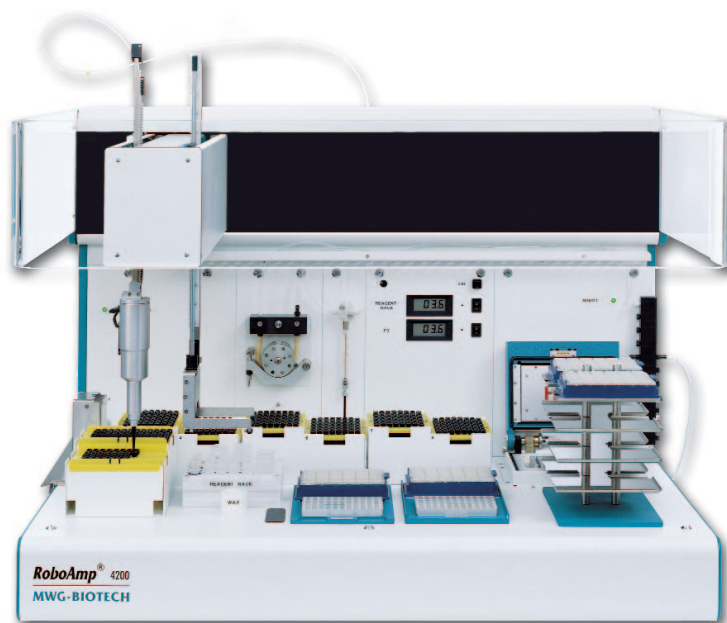


Always a Result ahead.

Overview

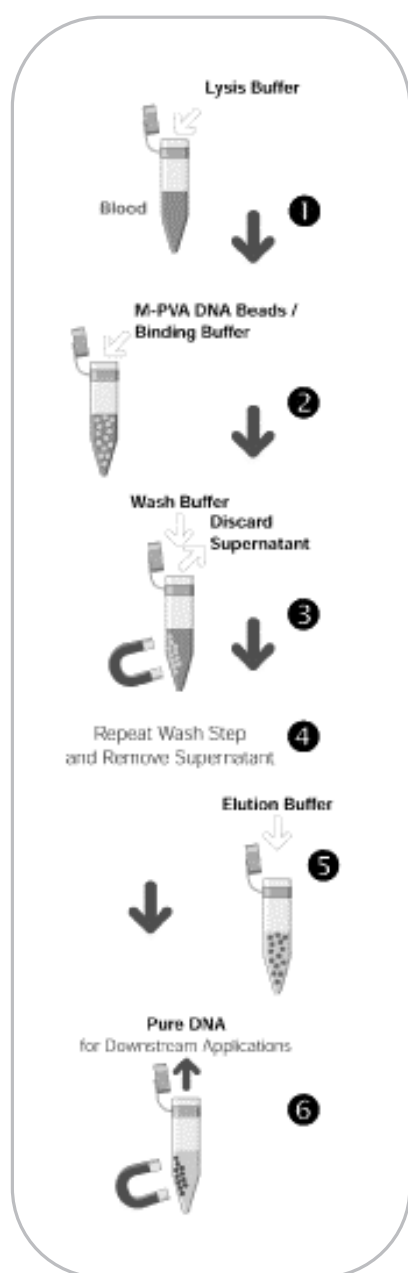
Genomic DNA extraction is required for a wide range of applications including PCR, sequencing, AFLP and SNP analysis. Traditional methods based on CTAB or phenol-chloroform extraction are both laborious and time consuming, while silica matrix column extraction kits are expensive and not automation-friendly, typically incorporating centrifugation steps requiring manual intervention. These kits also carry a risk of downstream PCR inhibition due to the presence of silica or ethanol.

Application Note: Automated Genomic DNA Extraction / Release October 2002, AN023



THE MWG RoboAmp4200 features non-cross-contamination (NCC) technology

Here we describe a fully automated method for genomic DNA extraction using magnetic particles (beads) coated with a DNA binding polymer. The method requires minimal starting material and is both cost-effective and user friendly, with no manual intervention steps from start to finish. We describe its use with human and animal blood, but the method has been successful in the automated extraction of DNA from other sources including amniotic fluid and fish eggs. The method is based on Bilatec's DNA 2 OS kit with some minor modifications.



- 10µl of each blood sample was pipetted into each well of a 96 PCR microtiter plate on an ambient 96 well pipetting position.
- 80 ml of "Combined Lysis Buffer" containing beads, proteinase K and Lysis Buffer B was added to each sample and mixed thoroughly.
- The PCR plate was transferred to a heated position (55°C) for 15 minutes, to aid cell lysis.
- The PCR plate was then positioned on the separator magnet, and left for 2 minutes to allow the beads to gather at the side of the tube.
- Supernatant was discarded by gentle pipetting from the bottom of each well.
- The beads were washed by the addition of 85ml of wash buffer 5A while still on the magnet station. The pellets were not disturbed.
- Wash Buffer 5A was removed by gentle pipetting from the bottom of the wells.
- 85µl of Wash Buffer 5B was added, again while still on the separator magnet. The pellets were again not disturbed.
- Wash Buffer 5B was removed by gentle pipetting from the bottom of the wells. Special care was taken to remove all residual liquid.
- PCR plates were allowed to air dry for 5 minutes.
- Genomic DNA samples were 'eluted' by resuspending the beads in 70µl of Elution Buffer.
- Optional Step - isolated genomic DNA can be separated from the beads by heating the PCR plate (65°C) for 10 minutes, and then transferring the clear DNA solution to a clean plate.
- 2µl of eluted beads were used in a 20ml PCR reaction.

Results



Figure 1
Genomic DNA isolated from 5 different sheep blood samples

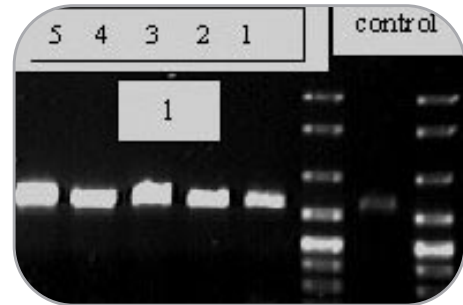


Figure 2
PrP gene PCR products (771 bases) amplified from the same samples

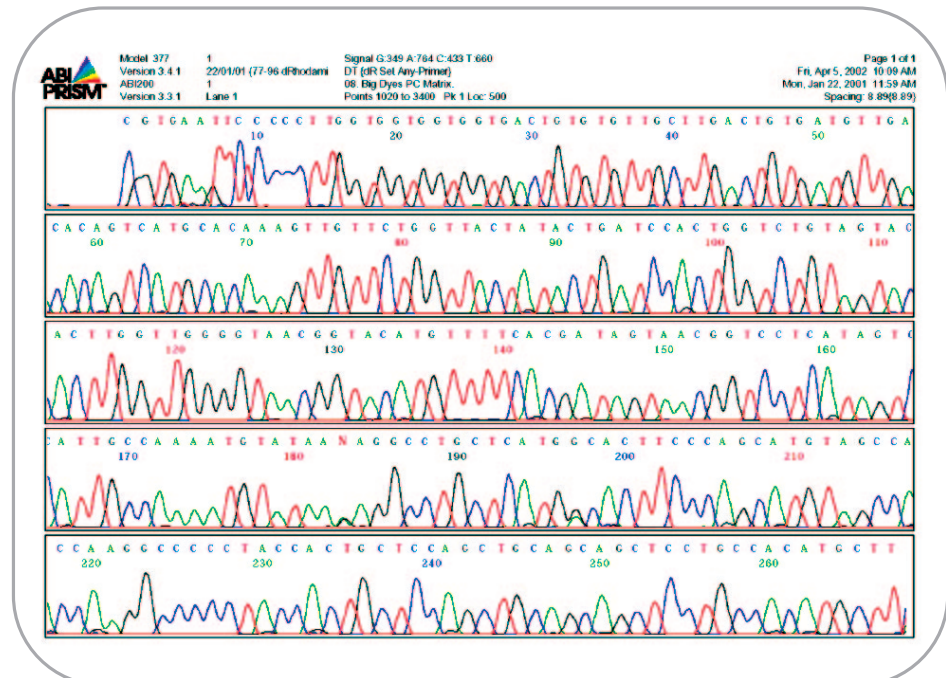


Figure 3
Sequence chromatogram of the sheep PrP gene, used to identify polymorphisms associated with resistance or susceptibility to scrapie at codons 136, 154 and 171

Discussion

- Blood samples that have been frozen prior to processing are less likely to separate into layers if left on the robot platform for extended periods, making the extraction more reproducible
- Eluting the DNA from the beads by heating at 65°C for 10 minutes makes the PCR reactions cleaner and prevents possible blockage of capillary based sequencers and genetic analysers
- PCR Methods will require optimisation for different sample types

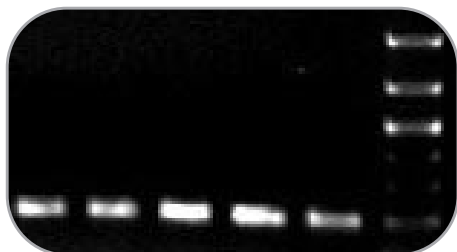


Figure 4
Human haemochromatosis PCR products
with primers H63D-FSK/H63D-RSK



Figure 5
Human haemochromatosis PCR products
with primers C282Y-FSK/C282Y-RSK

Acknowledgements

Gel images in Figure 4 and Figure 5 kindly provided by Dr. Stephen Keeney
Sequence chromatogram kindly provided (Natural History
Museum, London)

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