LOW COST SAMPLING METHOD FOR DNA BASED TESTING

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SUMMARY

As part of a collaborative research project on DNA pedigreeing for the Australian sheep farming market we have developed a simple, non-technical method of sample collection, together with a low-cost DNA extraction method that can be used for polymerase chain reaction (PCR) based testing. Collection of various tissue samples and different DNA extraction methods were trialed to determine the most efficient in terms of cost, time and benefit.

Keywords: Pedigree, microsatellites, parentage, polymerase chain reaction, PCR.

INTRODUCTION

Breeding programs for livestock require accurate pedigree information. DNA based parentage is being researched as a reliable and cost effective method of pedigreeing for the Australian sheep farming market. Modern molecular genetic techniques make parentage testing in the laboratory an attractive alternative to time-consuming manual record keeping. However, a major requirement for DNA pedigreeing is a cheap and reliable method of sample collection and DNA extraction.

Polymerase chain reaction (PCR) based techniques and gene mapping research has generated a large number of DNA markers suitable for DNA profiling. These microsatellite markers can vary greatly between unrelated individuals making them extremely useful for parentage determination (Bruford and Wayne 1993). PCR analysis requires only very small quantities of sample to obtain a result and can be adapted to automated procedures (Budowle *et al.* 1995).

Current methods of DNA sampling and extraction suffer from several disadvantages. Collection, transport and storage of samples can be costly and time consuming. Extraction of DNA from the commonly utilised samples (eg. blood and tissue) often require several steps. The procedures may involve the use of hazardous and/or costly reagents, and provide opportunity for cross-transfer of samples or the introduction of contaminants (Walsh *et al.* 1991). A system whereby samples can be collected easily, and posted to a DNA laboratory for rapid DNA extraction and testing would result in major improvements.

We have investigated several methods of sample collection and DNA extraction to determine the most cost-effective system suitable for DNA based parentage in livestock species.

MATERIALS AND METHODS

Tissue sample collection.

1. Blood sampling using vacutainers: Currently the most common method in use. Requires two handlers, including one trained veterinary personnel, and is not suitable for large scale collection. Transportation costs are relatively high and the sample volume collected is more than that required.

2. Tail/testes tissue collection: In frequent use for research projects. This method has merit in that it utilises tissues normally discarded and does not require veterinary personnel. Transportation and laboratory storage costs are high and sampling is restricted to lambs before docking and castration.

3. Wool follicle sampling: A simple sample collection method consisting of plucking a small wool staple, placing the sample in an envelope and recording the eartag number on the envelope. Sampling does not require veterinary personnel and can be carried out at any time except just after shearing. Samples can be sent through the post and have minimum storage requirements.

4. Bloodstaining filter paper: A simple method involving the collection of a small sample of blood onto filter paper, recording the eartag number, air drying and placing paper in an individual plastic bag. Does not require veterinary personnel and can be carried out at any time on animals of all ages. Several filter papers (Schleicher and Schuell (S&S) Specimen Collection Paper 2992; S&S GB002; Bacto Clinical Test Paper; Whatman 1M, 4M, 3MM; blotting paper and S&S Isocode™ PCR DNA Sample Isolation Device) were trialed. Samples can be sent via post and have minimum storage requirements. Initial trials used previously frozen whole blood.

Laboratory processing. (wool follicle and bloodstain samples only) Wool follicle samples: Initial processing involves cutting of the wool staple and placing the root portion in a microcentrifuge tube. This procedure is best carried out under microscopy, is labour intensive and not suitable for processing large numbers of samples.

Bloodstain samples: A portion of the bloodstained filter paper is placed in a microcentrifuge tube prior to DNA extraction. The use of a hole punch to cut out a circle of paper was found to be the most efficient method, and large numbers of samples could be processed in a relatively short time. Unused stained filter paper can be stored for future DNA extractions.

DNA extraction methods trialed. (bloodstain samples only)

Proprietary methods:	Bresa-Clean™ DNA purification kit
	Cleanmix DNA extraction kit
Other methods:	Chelex®100 procedure (Walsh *et al.* 1991)
	Boiling procedure (adapted from Isocode™ protocol)

Qualitative success of extraction was determined visually following electrophoresis.

PCR. Reactions were carried out in a total volume of 10µl following established methods.

Storage. Extracted DNA was stored at -20^{0}C or 4^{0}C. Samples were stored at -20^{0}C with and without the addition of reagents (Proteinase K, 50ng/ml, or 100mM EDTA) routinely added to inactivate possible DNA contaminants.

RESULTS

Sample collection using filter paper was the method of choice in terms of simplicity, ease and laboratory processing. The following on-farm sampling procedure was trialed at CSIRO Division of Animal Production, Prospect, using the filter papers which had proved to be the most successful during initial laboratory testing. Blood was collected via vacutainer before filter paper staining. Sample strips were 25mm X 100mm with a printed mid-line. Filter papers trialed were S&S Specimen Collection Paper 2992, CTP, and the S&S Isocode™ PCR DNA Sample Isolation Device.

On-farm sampling Procedure.

1. Expose a small amount of blood on the animal, remove a single sample strip from the bag, ensuring you only handle the strip at the end where the animal number will be written.
2. Place the strip on the exposed blood until an area covering the size of a 20 cent piece is stained.
3. Air dry the blood on the sample strip, making sure the blood stain does not come into contact with anything whilst wet. This should take approximately 2 minutes.
4. When the blood is dry, place strip in individual sample bags leaving one end exposed.
5. Record animal number on exposed end and place all of strip in bag.
6. When sample collection is complete, place individual sample bags containing sample strips into large self-sealing bags (approximately 100 samples per bag) and return to the testing laboratory.

Sample strips were sent through the mail and stored in an airtight bag at either room temperature or 4^{0}C . One or more extractions were performed on each strip.

The Chelex®100 and boiling procedure methods of DNA extraction were more successful than both proprietary kits, giving higher yields and more consistent PCR amplification. Both methods are simple and inexpensive, the boiling method being more suitable for large scale processing and adaptable for automative equipment.

DNA could be successfully extracted from sample filter paper strips stored for up to three months at either room temperature or 4^{0}C. The S&S Specimen Collection Paper 2992 produced the most consistent results, confirmed by qualitative determination of the success of DNA extraction and PCR amplification. Table 1 suggests that the boiling method is slightly more reliable than the Chelex procedure, however the difference is not statistically significant. Only samples collected using the on-farm sampling procedure were considered in this analysis.

Table 1. Percentage success of extraction and subsequent positive PCR result

Chelex®100	Boiling	Cleanmix
98.4%	100%	40.0%

Extracted DNA samples, stored at -20°C for up to six weeks, could be defrosted and refrozen several times, and amplified successfully using PCR. The addition of EDTA had no effect on amplification results but samples stored with Proteinase K could not be amplified. PCR amplification was unsuccessful on extracted DNA samples kept at 4°C for longer than two weeks.

DISCUSSION

We have developed a simple and inexpensive procedure for the collection of blood samples onto filter paper for DNA extraction and use in PCR based analyses. Only a small amount of blood is required and samples can be collected at any time. This method is reliable, efficient and less invasive than other methods of sample collection. The samples can be sent through the post to a DNA testing laboratory and have minimum storage requirements. DNA recovery is achieved rapidly, without the use of dangerous reagents, and in a single tube, ready for PCR analysis.

Laboratory processing involves punching a circle out of the bloodstained filter paper and extracting the DNA using a simple boiling method. The resulting DNA yield is sufficient for a minimum of 20 small scale PCR reactions and unused sample paper can be stored for further DNA extraction if required. DNA extraction methods using either Chelex®100 or a boiling procedure are easy to perform and lend themselves to automation. They both reliably yield DNA which is suitable for use in PCR based analyses. The Chelex method removes PCR inhibitors, uses few reagents, and can be performed in a single tube (Walsh *et al.* 1991). However, the amplification of extra bands following Chelex extraction has been reported (Comey *et al.* 1994). The boiling method does not require any reagents, is also performed in a single tube, and can be completed in less than 35 minutes.

Extracted DNA, using either Chelex or the boiling method, can be successfully amplified after storage at -20°C for several weeks. The addition of Proteinase K before storage decreased the amplification success, while storage with EDTA did not seem to affect it. Successful amplification of DNA stored at 4°C could only be achieved within two weeks of the extraction date.

The sampling procedure has been trialed following recommended sampling procedure instructions (see results). This trial involved sampling blood following vacutainer collection. A follow up trial on-farm will be carried out to test the efficacy of sampling blood during normal farm procedures. As only a small amount of blood is required sampling can be carried out during any procedure where slight bleeding normally occurs, e.g. tail docking or ear-tagging.

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REFERENCES

Bruford, M.W. & Wayne, R.K. (1993) *Curr. Opin. Genet. Develop.* **3**:939.
Comey, C.T., Koons, B.W., Presley, K.W., Smerick, J.B., Sobieralski, C.A., Stanley, D.M. & Baechtel, F.S. (1994) *J. Forensic Sciences.* **39**:1254.
Budowle, B., Baechtel, F.S., Comey, C.T., Giusti, A.M. & Klevan, L. (1995) *Electroph.* **16**:1559.
Walsh PS, Metzger DA, Higuchi R. (1991) *Biotechniques.* **10**:506.