

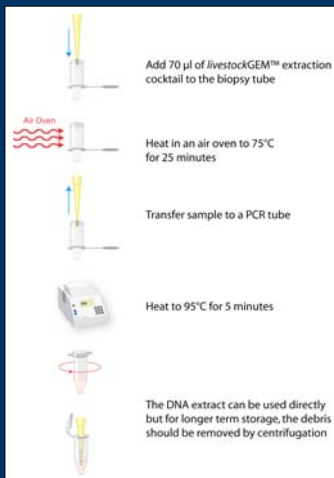


OBJECTIVES

Most manual or automated methods for DNA extraction require multiple steps. Some procedures require long extraction times, or purification steps to make the extracted DNA compatible with downstream applications. As the complexity of a procedure increases, there is an increased likelihood of failure and cross-contamination. Furthermore, a more complex procedure is more difficult to automate on standard, programmable robotic platforms.

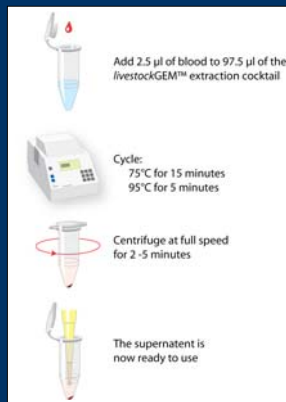
The *livestockGEM*[™] product range from ZyGEM Corporation are based on the unique characteristics of a proteinase isolated from a thermophilic bacterium from mount Erebus in Antarctica. The goal of this work was to develop the *livestockGEM*[™] product line to make it suitable for the animal testing industry. The *livestockGEM*[™] reagents and procedures provide a fast, reliable, robust and easily automated DNA extraction method from a variety of livestock samples including ear punches, blood and blood deposited on storage cards.

livestockGEM[™] Ear Punch



METHODS

livestockGEM[™] Blood



livestockGEM[™] Storage Card Blood



PCR & qPCR data

Figures 1 & 2 demonstrate the efficiency *livestockGEM*[™] at extracting DNA for processing by the PCR. Consistency of the results is demonstrated in the agarose gels shown in Figure 1 and the qPCR traces in Figure 2. The concentration of DNA in the extracts was as follows: [A] Ear punches >20 ng/µl, [B] Blood DNA extracts >20 ng/µl [C] Storage Card Blood DNA 0.5 - 2 ng/µl.

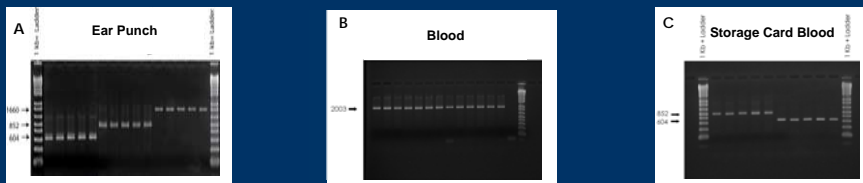


Figure 1. Standard PCR of DNA extracted using *livestockGEM*[™]. A PCR (25 µl) was performed using 5 µl of a 1:10 dilution of DNA extracted from each tissue type. 5 pmol of each primer was used (*Bos taurus* GAPDH for Ear Punch & Storage Card Blood extracted samples, mitochondrial primers for blood samples), 0.5 Units AmpII *Taq* Gold DNA polymerase was used in buffers provided by the manufacturer. PCR conditions used were: 10 min at 95°C, followed by 35 cycles of 95°C, 30 sec, 57°C, 30 sec & 72°C, 1 min. 10 µl of amplified DNA was visualised on a 1.5% agarose gel.

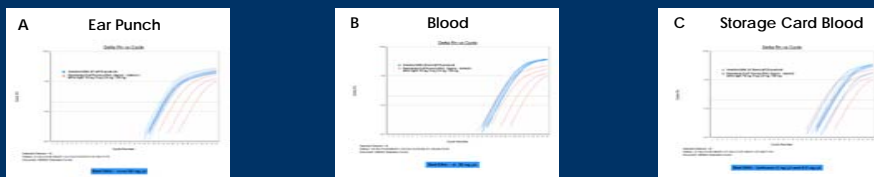


Figure 2. qPCR traces of DNA extracted from different sample types using *livestockGEM*[™] kits. PCR was carried out in a final volume of 20 µl with 5 µl of a 1:10 dilution of the DNA extract, 10 µl of SYBR GREEN PCR Master Mix (Applied Biosystems) and 5 pmol of each primers were used. The PCR was performed in an ABI 7300 PCR System. PCR conditions were: 10 min, 95°C, followed by 35 cycles of 95°C, 30 sec, 57°C, 30 sec and 72°C, 1 min. Calf Thymus DNA (Sigma-Aldrich) was used as a standard.

RESULTS

Single nucleotide polymorphisms (SNPs) analysis is becoming common practice in livestock breeding programs and in meat tracing. Figure 3 shows an example of the results from a 36-plex Mass Spectroscopy array of SNPs using DNA extracted from ear punches using *livestockGEM*[™] Ear Punch. Thirty samples were tested - ten each from ear tags, liquid blood and blood deposited on FTA cards and each sample was tested in triplicate. All provided results of sufficient quality to call >35 of the 36 alleles.



Figure 3. Mass array data for DNA extracted from cow ear punches using the Zee Tags ear punch and *livestockGEM*[™] Ear Punch. Of the 30 samples tested, all gave data of sufficient quality to call at least 35 of the 36 alleles.

CONCLUSIONS

- Eliminates time-consuming purification steps that contribute to sample loss
- One step, closed tube procedure - less chance of cross contamination
- The simplicity of the procedure makes it amenable for high throughput processing of DNA samples
- DNA is of a quality suitable for a range of downstream applications including: PCR, qPCR, multiplex STR and SNP genotyping
- Can be fully or partially automated on any liquid handling workstation
- Cost effective

SELECTED PUBLICATIONS

Saul DJ, Williams LC, Toogood HC, Daniel RM, Bergquist PL (1996) Sequence of the gene encoding a highly thermostable neutral proteinase from *Bacillus* sp. strain EA1: expression in *Escherichia coli* and characterization. *Biochim Biophys. Acta*, 1308,74-80

Daniel R, Toogood HC, Bergquist PL (1996) Thermostable proteases. *Biotechnology Genet. Eng. Rev.* 13, 51-100