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DNA Extraction Using livestockGEM™ Storage Card (Blood)

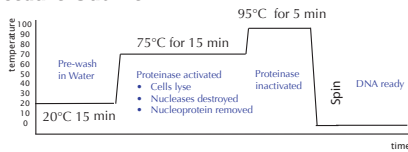
ZyGEM Quick-Start Guide

livestockGEM™ Storage Card Blood

The following method is recommended for extracting DNA from animal blood deposited on storage cards.

- All manipulations should be performed in a clean-room or a PCR hood.
- Use only certified DNA-free tubes and reagents.
- The Harris punch and cutting mat should be wiped with 0.05% hypochlorite bleach and rinsed with DNA-free water between samples.

Procedure Outline



Centrifugation Tips

The ZyGEM buffer is a proprietary formulation that precipitates PCR inhibitors. The solid material should not be disturbed when removing the supernatant.

Typically, 2 minutes at 13,000 r.c.f. is sufficient to give a well-packed pellet. Longer spins should be used for lower r.c.f. centrifugations. For example, a typical 96-well plate, swing out rotor rated at 3,000 r.c.f. should be spun for 10 minutes.



Technical tips for sample management

- livestockGEM™ Storage Card Blood is a preparative method for DNA extraction from most types of storage card. The livestockGEM™ method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can be used for many types of genotyping including SNP and STR analysis as well as quantitative, multiplex and end-point PCR.
- Storage cards contain preservatives that can inhibit Tag DNA polymerase. The pre-soak step is to remove these inhibitors and is essential.
- DNA extracted using livestockGEM™ is largely single-stranded because of the 95°C heat step.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by livestockGEM™ is approximately 90% single-stranded. If standard fluorescent chelating dyes are to be used for quantification, then this factor should be taken into consideration.
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are handled at 4°C, or on ice, before and after extraction.
- For long term storage of the extracted DNA, add TE buffer to 1x (10 mM Tris, pH 7.5, 1 mM EDTA) and store at -20°C.
- The livestockGEM™ reagents are stable at 4°C but after tubes have been opened and for longer term storage, reagents should be stored at -20°C.

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Preparation

1. Remove one to two 1.2 mm discs from the card-stored blood sample and place into a thin-walled PCR tube or a 96-well tray.

For the best results, punch in the centre of the area where the blood was applied.

2. Wash the disk in 100 µl of DNA-Free water by incubating at room temperature for 15 min. Aspirate the water from the disc(s) and discard.

Extraction

1. Add:
 - 5 µl of 10x Buffer **MAGENTA**
 - 44 µl of DNA-free water
 - 1 µl livestockGEM™

2. Incubate at:
 - 75°C for 15 minutes
 - 95°C for 5 minutes

A thermal cycler can be used for this step.

3. Centrifuge for 2 minutes at maximum speed and transfer the supernatant to a fresh tube (SEE CENTRIFUGATION TIPS)

The DNA is in the solution - not the punch

The sample is now ready for quantification and analysis. Typically, 2 - 5 µl should be used in PCR

To order livestockGEM™ Storage Card Blood visit www.zygem.com or email: info@zygem.com or contact your local distributor

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