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prepGEM™ Storage Card (Saliva)

DNA Extraction Using

rapid, enabling solutions for Biotechnology

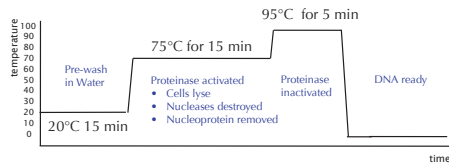
Zygem Quick-Start Guide

prepGEM™ Storage Card Saliva

The following method is recommended for extracting DNA from saliva 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



Preparation

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

Uneven application of the swab onto the storage card results in DNA yield variations. For the best results, punch in the centre of the area where the sample was applied.

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

Extraction

- Add:
 - 5 µl of 10x Buffer **SKY BLUE**
 - 44 µl of DNA-free water
 - 1 µl prepGEM™
- Incubate:
 - 75°C for 15 minutes
 - 95°C for 5 minutes

A thermal cycler can be used for this step

- Pipette the solution to a new tube
The DNA is in this solution - not the punch

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

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And then fold here

Technical tips for sample management

- prepGEM™ Storage Card Saliva is a preparative method for DNA extraction from most types of storage card. The prepGEM™ 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 prepGEM™ is largely single-stranded because of the 95°C heat step.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by prepGEM™ 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 prepGEM™ 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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