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DNA Extraction Using forensicGEM™ Cigarette

Zygem Quick-Start Guide

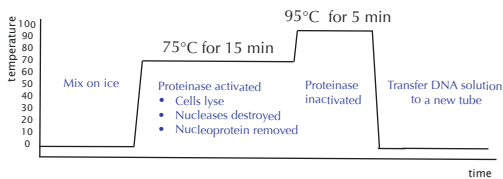
forensicGEM™ Cigarette

forensicGEM™ is specifically formulated and validated for forensic DNA extractions. Validation data can be obtained from www.zygem.com

The following method is recommended for extracting DNA from cigarette butts.

- All manipulations should be performed in a clean-room or a PCR hood.
- Use only certified DNA-free tubes and reagents.
- Wash any equipment that will come into contact with the sample in 0.05% hypochlorite bleach. Rinse thoroughly with DNA-free water.

Procedure Outline



- forensicGEM™ Cigarette is a preparative method for DNA extraction. The forensicGEM™ 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.
- DNA extracted using forensicGEM™ is largely single-stranded because of the 95°C heat step.
- Yields will vary significantly from sample to sample. The method provides sufficient DNA enough DNA for forensic profiling from most freshly-smoked butts. However, in some cases, a concentration step may be necessary to obtain a profile.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by forensicGEM™ 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, for best results prepare and manage samples at 4°C, or on ice, before and after extraction.
- When storing the sample after extraction, aspirate the supernatant from the precipitated residue and store separately.
- 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 forensicGEM™ reagents are stable at 4°C but after tubes have been opened and for longer term storage, reagents should be stored at -20°C.

Technical tips for sample management

Preparation

1. Remove a 1 cm strip of paper from the cigarette butt and cut into quarters. These can be processed separately as replicates.
2. Cut each sample of paper into smaller pieces (3 mm squares) and place in a thin-walled PCR tube or 96-well PCR tray.

Extraction Method

1. Add:
 - 89 µl DNA-free water.
 - 10 µl of 10x Buffer **ORANGE**
 - 1 µl forensicGEM™
2. Incubate at:
 - 75°C for 15 minutes
 - 95°C for 5 minutes

A thermal cycler can be used for this step

3. Aspirate the extract away from the paper immediately and transfer to a fresh tube

The DNA is in this solution. **Do not discard**. The paper can be discarded

The sample is now ready for quantification and analysis.

Fold here

And then fold here

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