



Antarctica - the source of forensicGEM® (Upper Wright Valley, Ross Sea Region)

ForensicGEM® Saliva: validation of a modified DNA extraction method by the Kansas City Police Department.

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Introduction

The forensicGEM® Saliva DNA extraction kit uses a thermophilic proteinase to extract DNA in 20 minutes. The kit is suitable for forensic profiling as the extraction procedure is a single, closed-tube process which minimizes contamination and requires minimal handling by an analyst. The process is also suitable for automation. The extraction kit contains a buffer optimised for buccal swabs and is well-suited for rapid processing of reference samples.

The kit is manufactured by ZyGEM NZ Limited, Hamilton, New Zealand and is distributed in the United States and the UK by VWR.

Materials and methods

Standardised portions of buccal swabs were extracted using a modified forensicGEM Saliva extraction procedure. The extracts were then profiled to ensure that the DNA gave usable profiles and the correct alleles.

Extraction Mastermix (1x)

- 1 µl forensicGEM enzyme
- 10 µl 10 x Buffer BLUE
- 89 µl sterile H₂O

Procedure

- 1) Place a portion of a buccal swab into a tube
- 2) Add 100 µl of Mastermix
- 3) Heat at 75°C for 15 minutes
- 4) Heat at 95°C for 5 minutes

The samples were diluted for amplification using TE Buffer. The protocol modification was to use a smaller amount of sample than recommended to limit sample consumption.

Extraction sets

Five different extraction sets were performed with samples of known profiles. 4 sets were amplified using AmpF/STR® Identifier® (Applied Biosystems) and one set was amplified with Profiler Plus® and COfiler® (PP/CO) (Applied Biosystems). Quantitation, contamination, concordance, amplification quality and reproducibility were tested.

	# of samples	Tube type	ng used	Amplification kit
Set 1	4	1.5 ml microfuge	1	Identifier
Set 2	4	0.5 ml thin-wall PCR	1	Identifier
Set 3	9	0.5 ml thin wall PCR	Varied	Identifier
Set 4	13	0.5 ml thin wall PCR	Varied	Identifier
Set 5	5	0.5 ml thin wall PCR	6	PP/CO

DNA quantitation

Samples were quantified with Quantifiler® on an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems) to ensure that a sufficient amount was obtained from each sample for DNA amplification. Using standardised cuttings (Figure 1) DNA yields were seen from 34.5 ng to 494 ng.


Figure 1:

The standardised amount of swab cutting.

The Internal Positive Control (IPC) for the samples extracted with forensicGEM was compared to the IPC for the known quantitation standards for each quantitation run in order to determine possible inhibition.

Results

Quality of amplification/genetic profile

The quality of the genetic profile obtained was assessed using four criteria:

1. Total peak heights (compared to quantity of DNA input)

With the exception of one outlier, the total peak height corresponded to the quantity of DNA input.

2. Sister peak height ratio

The majority of the loci had sister peak height ratios within 70%. Only two samples had loci that fell outside this range.

3. Visual indication of degradation or inhibition

There was no indication of degradation or inhibition in any of the samples tested. None of the samples tested demonstrated the classic degradation electropherogram.

4. Presence of non-allelic peaks

The samples amplified with Identifiler contained an anomalous peak at approximately 100-103 bp. This peak is present in other Identifiler samples including the positive control and is associated with the amplification kit, not the *forensicGEM* extraction process. When high peak heights were observed, there was an increase in the number of anomalous peaks observed in samples extracted with the ZyGEM kit when compared to samples extracted organically.

Reproducibility and contamination

The quantitations and profiles for two samples were performed twice. No differences other than yield were observed, illustrating the variability in the collection method. No contamination was observed in the reagent blanks and all samples analysed were single source.

Indications of degradation or inhibition

There was no indication of degradation or inhibition in any of the samples tested. The IPC of all quantitations showed no inhibition. None of the samples tested demonstrated the classic degradation electropherogram, nor did any of the loci have significantly lower peak heights than the other loci within the sample.

Concordance

15 different genetic profiles were developed using the Identifiler amplification kit and 5 profiles were developed using the Profiler Plus and COfiler kits in conjunction. These profiles were previously developed from organically extracted samples. No discordances were observed in any of the 20 samples processed.

Conclusions

The *forensicGEM* Saliva extraction kit is a reliable and rapid extraction method to obtain DNA from buccal swab reference samples. The method provides DNA highly suitable for PCR STR amplification using industry standard methods. The protocol is quick, largely hands-free and can allow the high throughput processing of samples manually or using automation workstations.

The method does not cause degradation, inhibition, or other problems that might affect amplification.

The quantity of DNA obtained from a buccal swab is variable using any process and quantitation by qPCR should not be eliminated from the processing of buccal swabs for forensic analysis.

The full report can be downloaded at
<http://www.zygem.com/Products/Products-FG-Saliva.html>

For more information visit: www.zygem.com
or email: info@zygem.com