Exymes

forensicGEM Universal

Antarctica - the source of *forensic*GEM
(Victoria Glacier, McMurdo Dry Valleys)

Improved elution of DNA from Whatman FTA™ cards using *forensic*GEM Universal extraction kits.

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Introduction

Whatman FTA Cards (Whatman, GE Healthcare, USA) are a popular choice for storing biological material. The cards allow tissue samples and DNA to be stored at room temperature and they are now the standard for diagnostic, R&D and forensic organisations.

The cards have a cellulose-paper base that has been impregnated with a surfactant, a chelating agent, buffer and a free radical trap [1]. On application of biological material, cells lyse and the DNA becomes tightly associate with the paper. Because of this strong association, the sample is generally analysed while bound to the paper. The preserving agents are inhibitory to most downstream reactions and so they are removed by using a series of washes.

However, in situations where it is preferable to elute the DNA from the paper (for example with qPCR), a number of chemical treatments are available. These include using alkali [2] or alternatively methanol to 'fix' the DNA. [3,4]. The intention of the latter method is to replace aqueous wash steps with methanol which can be removed by evaporation rather than pipetting. Although it is unclear how methanol evaporation removes the inhibitory preserving agents, the method does seem to work and it reduces the number of steps that are difficult to automate.

As an alternative to these methods, Exymes manufactures formulations within the *prep*GEM and *forensic*GEM ranges of DNA extraction products. These have been designed for extracting either blood or buccal swab smears from FTA cards.

Here we compare the Exymes methods with other elution methods and score for yield, efficiency, and factors such as time, potential for contamination and the number of tips required for each extraction.

Methods

Preparation of cards and punches

Blood: 40 µl of fresh blood from a healthy individual was spotted onto FTA cards. The cards were allowed to dry and were stored for 3 days. Two 1 mm^2 punches were used for each extraction.

Saliva: For each FTA card, a cotton medical swab (Cultiplast, Corban Laboratories) was used. The swabs were rubbed vigorously on the inside of the cheek for 30 seconds and then were rolled onto FTA cards applying pressure to squeeze out as much of the liquid as possible. Cards were allowed to dry and were stored for 3 days. Two 1 mm^2 punches were used for each extraction.

DNA extraction

A total of six different method variants were tested:

A. Whatman alkali method
B. Modified Whatman method with treatment at 65°C
C. Methanol method of Johanson *et al.* [4]
D. Methanol method of Johanson *et al.* with proteinase K.
E. Exymes Recommended method for Blood.
F. Exymes Recommended method for Saliva

Whatman extraction methods A and B

1. The punches were washed 4x as follows:
   a. 200 µl of FTA Wash solution (Cat # 2719978) was added to the punches and incubated for 5 minutes at room temperature with occasional mixing.
   b. The liquid was removed with a pipette.
2. The punches were washed 3x as follows:
   a. 200 µl of TE buffer was added (10 mM Tris pH 8.0, 1 mM EDTA) and again the tubes were incubate for 5 minutes at room temperature with occasional mixing.
   b. The liquid was removed with a pipette.
3. All the buffer was drained and 35 µl of alkaline incubation buffer added (0.1 N NaOH, 0.3 mM EDTA, pH 13.0).
4. The samples were incubated at room temperature (Method A) or 65°C (Method B) for 5 minutes.
5. 65 µL of neutralising solution (0.1 M Tris-HCl, pH 7.0) was added and the samples vortexed 5 times to mix.
6. Tubes were incubated for 10 minutes at room temperature.
7. The tubes were then 'flash' vortexed 10 times.
8. Punches were removed making sure to extract the maximum amount of eluate.

Methanol extraction methods C and D

1. The punches were overlaid with methanol as follows
   a. Drop 4 drops via pipette onto the punches and allowed to air-dry at room temperature for 20 minutes.
   b. Repeat twice more incubating at 37°C for 40 min each.
2. Once dry, 50 µl of 1x PCR II buffer (Applied Biosystems) was added (Method C) or 50 µl of 1x PCR II buffer containing proteinase K at 50 µg per ml (Method D)
3. The tubes were incubated at 60°C for 30 minutes and 99°C for 10 minutes and then held at 4°C
4. Punches were left in the extract.

*forensic*GEM features:

- Thermostable Enzyme & optimized buffer.
- Simple wash and two step temperature shift (75°C & 95°C).
- Closed-tube - secures the sample from contamination.
- DNA ready for PCR, qPCR, SNP testing in 20 - 30 min.
- No overnight incubations or centrifugation required.
- Compatible with PCR or profiling reagents

Application Note 003

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Exymes extraction method E (saliva)

1. 100 µl of water was added to punches. Samples were briefly vortexed and placed at room temperature for 15 minutes.
2. The tubes were vortexed and the liquid removed by pipette.
3. Punches were resuspended in:
   a. 44 µl Water
   b. 5 µl BLUE Buffer
   c. 1 µl *forensic*GEM
4. Tubes were placed in a thermal cycler and heated at 75°C for 15 minutes followed by 95°C for 5 minutes.
5. Liquid was decanted into new tubes.

Exymes extraction method F (blood)

1. 100 µl of water was added to punches. Samples were briefly vortexed and placed at room temperature for 15 minutes.
2. The tubes were vortexed and the liquid removed by pipette.
3. Punches were resuspended in:
   a. 44 µl Water
   b. 5 µl RED+ Buffer
   c. 1 µl *forensic*GEM
4. Tubes were placed in a thermal cycler and heated at 75°C for 15 minutes followed by 95°C for 15 minutes.
5. Tubes were centrifuged at 16,000 x *g* for 5 minutes.
6. The supernatant was decanted into new tubes.
7. Liquid was decanted into new tubes.

Quantification was carried out using a qPCR with primers specific for the human GAPDH gene. The qPCR reagents used were the Invitrogen Platinum SYBR Green qPCR SuperMix-UDG with ROX.

Results and Discussion

A schematic of the methods is shown in Figure 1 and an analysis of of the methods and the yields of DNA are given in Table 1.

A key factor to consider when working with archived samples is that ideally, only a small amount of the archive material should be consumed. As a result, the amount of sample presented to the extraction process will be small and therefore extraction should be efficient and secure from extraneous contamination. Security is particularly important for crime samples or rare material.

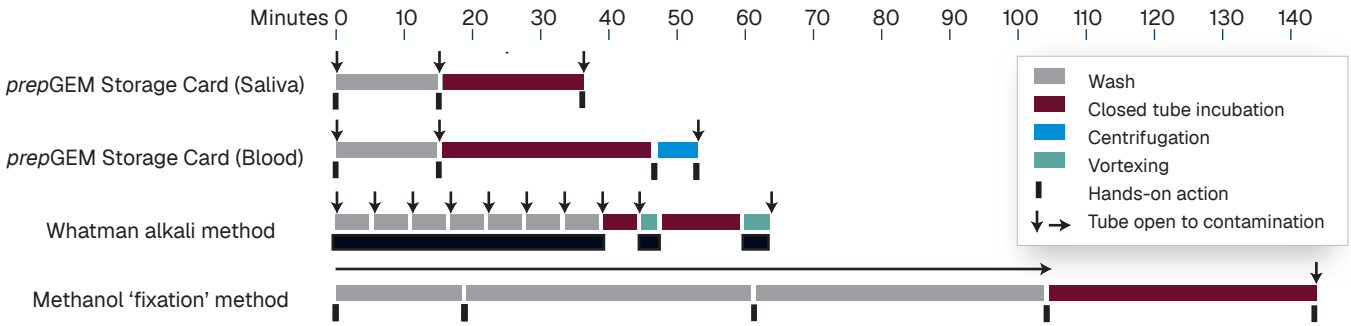
In our hands, the alkaline method offered by Whatman [2] repeatedly failed to yield measurable quantities of DNA. Furthermore the method is labour-intensive and requires multiple tube-openings that are undesirable for samples requiring rigorous security.

The methanol method [4] gave good yields of DNA but the procedure is protracted and most importantly, requires long periods of air drying where the tube is open to potential contamination. With over 100 minutes spent with the sample exposed to the environment (or at best a PCR hood) it is unlikely that the method is a viable option for laboratories requiring rigorous containment

The Exymes methods out-performed on almost all of the scored factors. Overall, the method:

- Takes less time to perform,
- Is simpler and so less prone to error,
- Requires less technical input and so frees staff for other tasks,
- Is the most secure of the methods tested.

While showing these advantages, the Exymes methods provided high yields of non-inhibitory DNA that performs well in polymerase -based reaations such as the PCR. In addtion, it is suited for forensic profiling.



Blood on FTA Cards	Whatman [A]	Whatman 65°C [B]	Methanol [C]	Methanol + PK [D]	*prep*GEM Blood [E]
Total laboratory time (min)	62	62	145	145	55
Hands-on time (min)	45	45	5	5	3
Number of manipulations	16	16	6	6	6
Number of tube openings	10	10	4	4	2
Open to potential contamination (min)	4	4	101	101	<1
Total number of tips	10	10	4	4	3
Final volume of extract (µl)	50	50	100	100	50
Concentration of DNA in extract (ng/µl)	0.0003	0.0098	0.0180	0.0043	0.2000
Total DNA yield (ng)	0.017	0.049	1.804	0.431	10.02

Saliva on FTA Cards	Whatman [A]	Whatman 65°C [B]	Methanol [C]	Methanol + PK [D]	*prep*GEM Saliva [F]
Total laboratory time (min)	62	62	145	145	37
Hands-on time (min)	45	45	5	5	2
Number of manipulations	16	16	6	6	6
Number of tube openings	10	10	4	4	2
Open to potential contamination (min)	4	4	101	101	<1
Total number of tips	10	10	4	4	3
Final volume of extract (µl)	50	50	100	100	50
Concentration of DNA in extract (ng/µl)	0.0005	0.0094	0.0987	0.0693	0.0811
Total DNA yield (ng)	0.025	0.472	9.875	6.387	4.055

Figure 1. (Above) Schematics of the stages in the methods. The diagram shows the stages in the procedures and when human input is required and also when the sample is exposed to potential contamination.

Table 1. (Left) An analysis of parameters relating to the six methods. Analysis covers aspects of the procedures and the DNA yields and concetrations in the extracts.. For each parameter, the method showing the best performance is highlighted in red text.

References

[1] US Patent No. 5985327

[2] www.whatman.com/Applicationnotes.aspx – Eluting Genomic DNA from FTA Cards Using Alkaline Conditions.

[3] Lin, Z., J.G. Suzow, J.M. Fontaine, and E.W. Naylor. (2005). A simple automated DNA extraction method for dried blood specimens collected on filter paper. J. Assoc. Lab. Autom. 10:310-314.

[4] Johanson, H.C., V. Hyland, C. Wicking, and R.A. Sturm (2009). DNA elution from buccal cells stored on Whatman FTA Classic Cards using a modified methanol fixation method

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