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Research article

Evaluation of the use of freezer mill to improve DNA retrieval from dried cotton swabs

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Abstract

This investigation evaluated the use of a freezer mill to improve retrieval of DNA from dried cotton swabs (using 100 μl saliva) compared to uncrushed swabs and whole saliva by measuring DNA yield and profile average peak height. Three treatments were tested; short, medium (as for a bone sample) and extended. The samples subjected to the freezer mill had the powder that remained on the freezer mill components collected (using a water and agitation method). All freezer mill samples returned a lower average DNA yield than either uncrushed swabs or whole saliva. The powder from the crushed swabs comprised an average of 35% of the total DNA yield, whereas the powder from the components comprised 65%. Allele drop-out was observed in samples exposed to extended treatments. Both short and medium treatments provided significantly higher peak heights than uncrushed cotton swabs with equal quantities of DNA ($P < 0.05$). Using a freezer mill on dried cotton swabs does not increase the DNA yield. This investigation suggests collecting powder from the freezer mill components will increase DNA yield, especially from trace samples.

Keywords: Trace DNA; Freezer mill; Cotton swab

1. Introduction

The usefulness of DNA samples in implicating or exonerating suspects largely depends on the quantity and quality of the DNA retrieved from a crime scene. This is in part determined by the collection and extraction processes used, as well as the amplification and typing processes that follow. Many of our trace biological samples are collected using cotton swab sticks. It was found by van Oorschot et al., that the standard protocols of cutting and teasing the cotton off the swab stick, followed by chelex extraction only yields approximately 24–49% of the available DNA [1].

There is potential to increase DNA recovery by improving the pre-extraction process, possibly by means of cryogenic grinding. The cryogenic grinding procedure, using a freezer mill, incorporates using liquid nitrogen and a magnetically driven impactor to grind a sample into a powder. The sample is placed in a vial which is suspended in a liquid nitrogen bath, to cool the sample so it becomes brittle, as well as keeping the mill from overheating during grinding [2,3]. Previous research has found that subjecting samples such as teeth [4,5], bones [6], cartilage [7], microscope slides, latex gloves and shoelaces [8] to freezer mill pre-extraction can improve the DNA yield compared to conventional methods. Here we evaluate the use of a freezer mill to improve recovery of DNA from dried cotton swabs.

2. Methods

2.1. Samples and freezer mill

One hundred microlitres of saliva was applied directly to a cotton swab and left to dry ($n = 25$). All saliva originated from one donor. The dried swabs were removed from the stick and placed in the freezer mill vial. A 6750 SPEX CertiPrep Freezer Mill (SPEX CertiPrep, Methucen, NJ, USA) and 6751 grinding vial set (including magnetic stainless steel end caps, impactor rods and five polycarbonate tubes) were used to grind the cotton swabs. The vials were assembled according to the manufac-

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turer's instructions and cleaned prior to reuse (0.5% hypochlorite, 70% ethanol and autoclaved). Equipment control swabs were taken from the inside of each vial and quantitated to assess if the vial was contaminated prior to experimentation (all returned negative results).

Three crushing times were investigated: short treatment (ST) 30 s grind, 1 cycle ($n = 4$); medium treatment (MT) 30 s grind, 2 cycles ($n = 5$); extended treatment (ET) 1 min grind, 2 cycles ($n = 4$). Note that the MT is our laboratory's standard treatment for small pieces of bone. Each sample was subjected to an impact frequency of 10, 7.5 min pre-cool and 2.5 min cooling time between cycles where necessary. To collect the powder left on the components, 200 μl aliquots of sterile water (up to approximately 1.5 ml) was used to moisten the area and agitated with a pipette tip. The water droplets were recycled over the components before collecting the powder/water in a 1.5 ml Eppendorf tube. The water/powder sample was separated into approximately 1 ml water supernatant and an approximately 500 μl powder/water portion. Two whole saliva samples and six dried saliva cotton swabs not receiving freezer mill treatment were used as controls and for comparisons.

2.2. DNA extraction, profiling and analysis

The samples were extracted with Chelex 100 (Bio-Rad Laboratories, Hercules, CA) [9]. All samples subjected to the freezer mill were concentrated using a Centricon centrifugal filter device (Millipore). The human-specific DNA concentration was measured using the Quantifiler™ Human DNA Quantification kit (Applied Biosystems, Foster City, CA, USA), substituting the standard for a commercial DNA (K562 high-molecular-weight DNA, Promega, Madison, WI, USA) and an ABI PRISM® 7500 real-time PCR instrument (Applied Biosystems).

DNA samples were profiled using the AmpF*l*STR Profiler Plus multiplex PCR Amplification kit (Applied Biosystems) (1 ng in 50 μl reactions) and a PE9700 thermal cycler. The amplified products were genotyped using an ABI3100 genetic analyser and GeneMapper™ ID software (Applied Biosystems). Data were analysed using GenALEx and XLstatistics statistical analysis programs to 95% confidence levels.

3. Results and discussion

3.1. Testing observations

The cotton swabs (dried 100 μl saliva) were subjected to three different freezer mill grinding treatments. The powdering differed between treatments, where the ET was more powdered and the ST remained fibrous. This was also reflected in the amount of powder obtained for each treatment (ET gave least and ST the most), and the amount of powder left on the components (ET most, ST least). Static was exhibited on the inside of the plastic vial after crushing, and may have accounted for some powder remaining on the inside of the vial. An equivalent amount of powder has been observed to remain on the components when crushing bone samples.

3.2. DNA yield

The average total amount of DNA extracted from freezer mill crushed samples was less than from either uncrushed cotton swabs or whole saliva (Fig. 1). The crushed samples gave average total DNA yields which decreased with increasing crushing time: ST was not significantly different from uncrushed cotton swabs, but both MT and ET were significantly lower ($P < 0.05$). It has been suggested that freezer mill crushing results in a higher DNA yield than conventional methods (e.g. teeth), but may also damage DNA [4,6]. DNA damage may be an explanation for the decreased DNA yield from our freezer mill subjected samples, and could have a profound effect when testing and extracting from trace samples.

Fig. 1 indicates where the DNA was retrieved from, i.e. the main powder, versus the washed down powder from the inside of the vial and the components, versus the water supernatant from the washed down powder. The main powder source

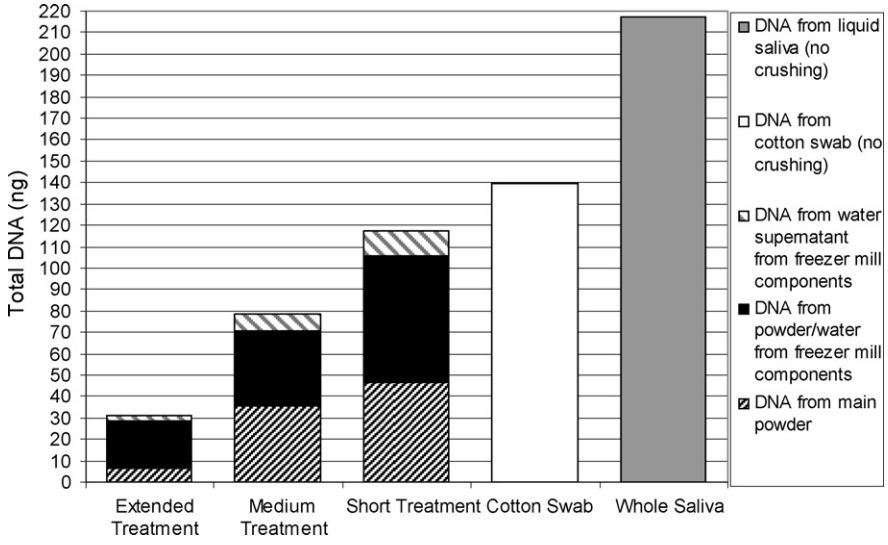

Fig. 1. The DNA yield from dried cotton swabs with 100 μl saliva subjected to freezer mill grinding compared to uncrushed swabs and 100 μl whole saliva.

provided an average of 35% of the total amount of DNA, whereas the washed vial and component powder produced an average of 55% and the water supernatant accounted for 10%. The normal procedure within our laboratory has been to clean the components and throw away the left over powder. These results strongly suggest that the powder residue on the components should be collected, especially when trace samples are used.

3.3. Profile results

The profiles were consistent across the samples with average peak heights ranging between 200 and 450 RFU. There was no inhibition, contamination or degradation, and the two alleles of a heterozygote were well balanced. Some allele drop-out was observed in ET due to lower amplification efficiency, possibly an effect of degradation on the DNA template. ST showed the highest average peak height. Both ST and MT returned results on a par with whole saliva (ST: 399 RFU, MT: 354 RFU, saliva: 372 RFU) and both also gave significantly higher peak heights (and, therefore, better profile) than uncrushed cotton swabs (226 RFU, $P < 0.05$). One explanation is that the Centricon not only concentrated the DNA but also enhanced the quality for amplification. Alternatively, the freezer mill pre-extraction treatment itself improved the amplification ability of the retrieved DNA. This phenomenon requires further investigation.

4. Conclusion

Subjecting dried cotton swabs to freezer mill pre-extraction does not increase DNA yield compared to samples not so subjected (conventional methods). The use of shorter rather than longer freezer mill treatments may provide better DNA profiling results. The amount of DNA retrieved from powdered samples can be improved by collecting the powder that adheres to the freezer mill components.

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Conflict of interest

None.

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