LGC Genomics

Comparison of the sbeadex® forensic kit with three other chemistries used in forensic labs

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Introduction

Crime scene samples come in a large variety of types and substrates and so present a challenge to any chemistry and methodology seeking to use a standard approach to extracting DNA. Furthermore, these samples may contain impurities which could affect the downstream processes. Having a standardised method for the majority of samples will greatly simplify the processing and will allow for easier automation of the process. However, any method used to process these precious samples needs to be robust and sensitive. This experiment compared three magnetic bead chemistries, which are fairly new on the market, to each other and to established chelex-based methods currently in use in our labs.

Experimental design

A range of samples covering the most common sample types expected to contain more than trace levels of DNA received by the Scene of Crime (SOC) laboratories were mocked up to be as similar to real samples as possible. Care was taken to ensure the replicates created for each method were as identical to each other as possible. Five replicates of each sample type per method were processed, a total of 160 samples (plus appropriate controls).

The samples were extracted according to the manufacturers' recommended protocols and the established chelex-based protocols, which include purification with a Microcon® YM-100 Centrifugal Filter Unit where necessary, in use in our labs. All extracts were quantified using the Applied Biosystems (AB) Quantifiler® Human DNA Quantification kit on the AB 7500 Fast Real Time PCR System, amplified using the AB AmpFISTR® SGM Plus® kit on an AB GeneAmp® PCR System 9700, electrophoresed on an ABI PRISM® 3100 Genetic Analyzer and analysed with the AB GeneMapper® ID v3.2 analysis software. Care was taken at all stages to reduce variability introduced by sources other then the extraction processes.

The results were assessed for DNA concentration, sample success (as defined by the United Kingdom National DNA Database [NDNAD] acceptability criteria), number of alleles and allelic peak height. The chemistries were also assessed for ease of use.

Sample Type	sbeadex®		Competitor kit 1		Competitor kit 2		Chelex	
	Mean (ng/µL)	% CV	Mean (ng/µL)	% CV	Mean (ng/µL)	% CV	Mean (ng/µL)	% CV
Blood swab	0.31	53.3	0.48	15.5	0.07	31.1	0.36	26.3
Cellular clothing (taping)	0.35	111.1	0.33	56.2	0.01	84.2	0.65	82.2
Cigarette	0.42	97.7	0.02	79.5	0.04	81.4	0.23	119.2
Hair	1.70	162.3	8.12	187.8	0.35	127.5	5.14	196.1
Drinks container	0.60	70.5	0.66	174.6	0.26	148.3	0.60	83.2
Saliva on clothing	2.05	46.2	4.73	72.5	0.54	56.0	0.06	172.2
Chewing gum	1.10	100.2	0.62	142.7	0.25	76.5	0.51	88.8
Saliva swab	2.11	64.8	2.18	63.6	0.55	68.0	0.87	79.0

Table 1: DNA Yield - Note: All samples were eluted in 50 µL of the appropriate elution buffer.

Results & Discussion

a DNA Yield

All samples from all the chemistries provided quantification values above zero. sbeadex® provided the highest number of samples (36) with sufficient DNA to allow the target amount of DNA (1ng) to be added to the subsequent 25µL PCR reaction. The Competitor and chelex methods provided 30, 10 and 31 such samples respectively.

Sample Type	Full Profile				Partial Profile			
	sbeadex®	Comp. 1	Comp. 2	Chelex	sbeadex®	Comp. 1	Comp. 2	Chelex
Blood swab	5	5	5	5				
Cellular clothing (taping)	4		1	3	1(1)		4(3)	2
Cigarette	5	1	3	4		4(4)	2(1)	1(1)
Hair	4	5	4	4	1(1)		1(1)	
Drinks container	5	3	5	3		2(1)		2(1)
Saliva on clothing	5	5	5					4(1)
Chewing gum	4	5	5	5	1			
Saliva swab	4	5	5	2	1			3
Total	**36**	**29**	**33**	**26**	**4(2)**	**6(5)**	**7(5)**	**12(3)**

Table 2: Sample success.
Note 1: Numbers in brackets indicate the number of partial profiles that are non-database-able.
Note 2: Empty cells indicate 0 samples.

The IPC C_t values of all the samples were assessed and no indication of inhibition was seen in any samples other then the Competitor 1 taping samples. This inhibition is believed to be due to an inappropriate lysis method being used for these samples as previous work on the Competitor 1 kit showed that it is possible to obtain almost full profiles, with some indication of inhibition, from these samples. A different method was tried here in an attempt to completely remove the inhibitors but did not result in improved results so the results quoted in table 3 include the figures with and without the assumption of full profiles for all Competitor 1 taping samples (indicated [ ]*).

Chemistry	Success rate
sbeadex®	95%
Comp 1	75% [88%]*
Comp 2	88%
Chelex	88%

Table 3: Success rates

Success rate calculation:

$$[(FP + DPP)/n] \times 100$$

FP = No. of full profiles
DPP = No. of database-able partial profiles
n = Total number of samples

b Sample success

sbeadex® provided the highest overall success rate for these sample types, see table 3.

c Number of alleles

sbeadex® also provided the highest total number of alleles for these sample types. The total number of alleles seen for each chemistry is given in brackets in the key of Figure 1.

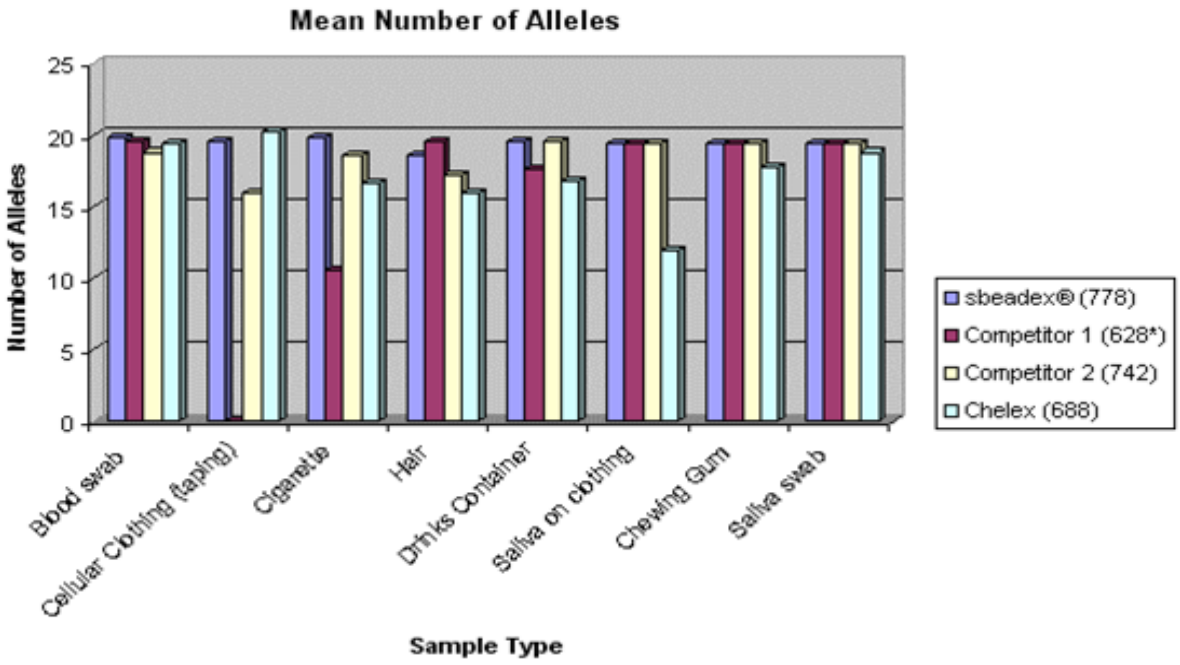

Figure 1: Mean number of alleles seen per profile, displayed by sample type.

Allelic Peak Height

The Competitor 2 chemistry provided the highest mean allelic peak heights for most sample types, even though it did not provide the highest number of alleles. This may be due to improved purification of the extracts allowing a more efficient amplification of the DNA that is present. The chelex samples consistently provided (on average) the lowest allelic peak heights while sbeadex provided good, intermediate, allelic peak heights.

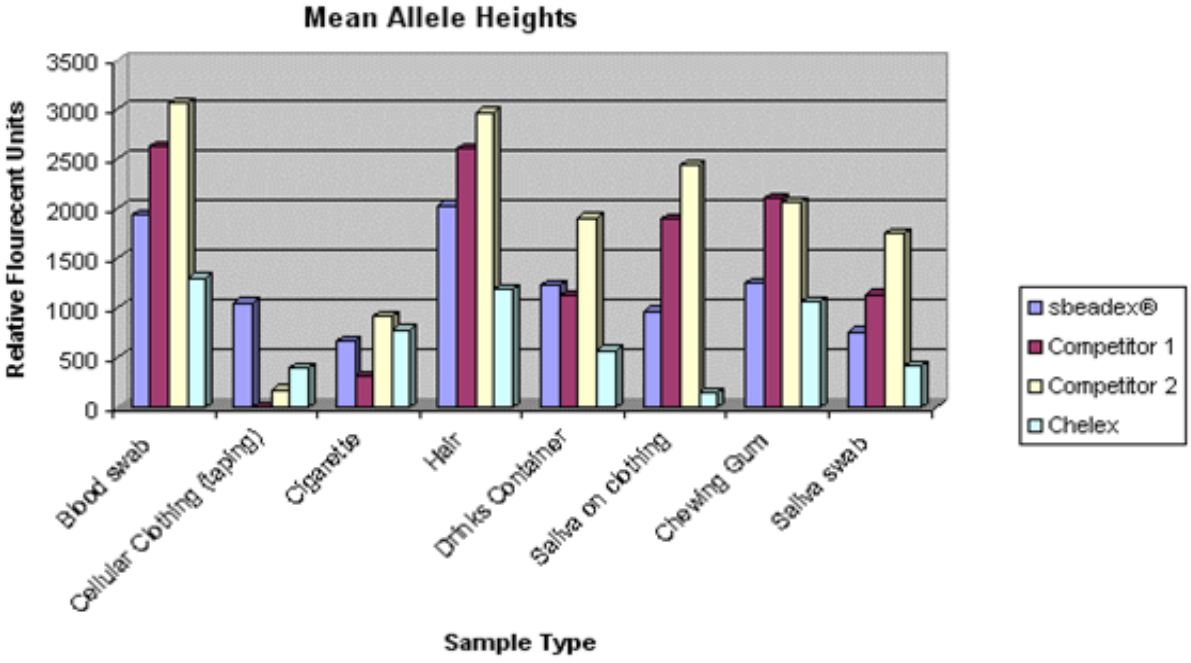

Figure 2: Mean peak heights displayed by sample type.

Ease of use

The sbeadex®, Competitor 1 and chelex methods were performed manually and the ease of use of each assessed:

- Chelex methods are relatively simple to perform, however a large number of optimised methods are required to successfully extract DNA from a range of sample types. This results in sub-optimal batching, increased operational complexity and increased training requirements. Chelex also does not remove any impurities from the DNA solution and so a further purification is required which adds to the complexity of the methods.
- The Competitor 1 and 2 chemistries are magnetic bead based chemistries with lysis buffers that are capable of lysing most sample types with little variation between methods. However, the magnetic beads of the Competitor 1 chemistry proved extremely difficult to handle as they tended to stick to the inner walls of the tubes and tended to clump, making them very difficult to re-suspend, which led to increased pipetting and excessive vortexing. This difficulty resulted in this chemistry being excluded from our considerations for use despite the promising results it provided.
- The purification stage of the Competitor 2 chemistry is carried out on a proprietary automated platform so the beads were not handled manually.
- The sbeadex® lysis buffer was also capable of lysing most sample types with little variation between methods and the beads were very easy to use. When placed on the magnetic rack the beads quickly formed tight pellets which we were able to re-suspend with minimal pipette mixing, minimal vortexing or even just a flick of the tube. This chemistry was seen to be very amenable to manual use while still offering the automation benefits inherent in magnetic bead systems.

Conclusions

This comparison has shown that the sbeadex® forensic kit provides higher success rates for the samples processed than two competitor magnetic bead-based kits and chelex based methods. While sbeadex® did not provide the highest quantification values or allelic peak heights it did provide DNA of sufficient quality to give the best chance of obtaining the most alleles and hence the highest evidential value. Along with the ease of use this chemistry provides and the relatively straightforward automation potential it presents, these results show that the sbeadex® forensic kit is suitable for use with routine Scene of Crime samples.

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