Comparing Five Forensic DNA Extraction Methods

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Abstract

Many different extraction protocols are being used in the forensic community. These extraction methods work well for the majority of samples encountered. However, not all extraction methods perform equally well with heavily inhibited samples. A critical factor for Sorenson Forensics was to choose an extraction procedure that could extract DNA efficiently and had the ability to minimize the amount of inhibitors co-extracted with the sample. Organic extractions are well known for having high extraction efficiency as well as for removing a majority of the inhibitors. However, an organic extraction is a long, manual process that is not automatable. Organic extractions also possess a potential safety hazard and chemical disposal issues.

Four different extractions were chosen, DNAIQ (Promega), ChargeSwitch (Invitrogen), Qiagen MicroPrep (Qiagen), and ForensicGem (ZyGem) in an effort to find an extraction procedure capable of being automated. Each extraction method had to have the ability of organic extractions to reduce the amount of inhibitors being co-extracted, while still obtaining a suitable quantity of DNA for STR analysis. To evaluate the efficiency of the procedures, both quantity and quality of DNA were compared.

Initially, two magnetic bead systems, DNAIQ and ChargeSwitch, were run following the manufactures' protocol. Different fabric types with a variety of dilutions of blood were extracted. The quantitation results showed some evidence of inhibition. Modifications were made to each extraction protocol in an effort to optimize the extraction method. A second round of extractions was performed with a subset of the samples. The results showed reduction in inhibition, while increasing the amount of DNA isolated.

Subsequently, all five extraction methods were evaluated using challenging fabrics with blood, touched items, buccal swabs, hairs, and cigarette butts.

This poster demonstrates which non-organic extraction procedure maximizes extraction efficiency and inhibitor removal. The protocol was chosen based on the quality of data compared to our organic extraction method currently being used in our laboratory.

Introduction

This study was performed to evaluate the efficiency of four different DNA extraction protocols in comparison to the currently used procedure in our laboratory. The results will potentially allow for the automation of the new extraction method. The current method used in our laboratory is a modified organic extraction (phenol/phenol:chloroform:isoamyl alcohol) procedure. This method is very good at removing the inhibitors that are commonly found in forensic samples. However, the modified organic extraction procedure is a manual process that is not capable of being automated.

The new extraction procedure will allow for a higher extraction throughput. In order for this protocol to be used in our laboratory it had to demonstrate the ability to reduce the amount of inhibitors in the extract, as well as obtaining a suitable quantity of DNA for STR analysis. To evaluate the efficiency of the protocols, both the quantity and quality of the DNA were compared.

Methods

Five different extraction methods were examined: DNAIQ (Promega), ChargeSwitch (Invitrogen), Qiagen MicroPrep (Qiagen), ForensicGem (ZyGem), and an Organic extraction (phenol/phenol:chloroform:isoamyl alcohol).

Five different fabric types were used for this experiment (figure 1). These fabrics were chosen because they proved to be exceptionally difficult substrates from which to extract high quality DNA. Fabric 1 was a tan camouflage cotton/polyester blend. Fabric 2 was a teal nylon/polyester. Fabric 3 was blue denim. Fabric 4 was black silk with pink floral pattern. Fabric 5 was a dark maroon cotton blend. Different dilutions of fifty microliters of blood (1:10, 1:100, and 1:1000) were spotted on these fabrics, done in triplicate. The entire stain was taken for DNA extraction. Touched samples, hairs, cigarette butts, and buccal swabs were also examined. After extraction, samples were quantified using the ABI 7900HT, a real time PCR instrument, with the ABI Quantifiler Human® kit and then normalized to 0.15 ng/µl for amplification. One hundred and fifty nanograms of each sample was amplified in triplicate. Samples were amplified with the ABI Identifiler® PCR amplification kit and run on the ABI 3130 xl. Each extraction procedure was performed according to the manufacturer's protocol. Optimizations to the manufacturer's protocol were made when possible.

Results

In this experiment, three different dilutions were used (1:10, 1:100, and 1:1000) on five different fabrics (figure 1). Minimal to no DNA was obtained from the fabrics stained with a 1:1000 blood dilution; these samples were not amplified. The 1:10 dilution on fabrics 1, 3 and 5 (labeled 1:10, 3:10, and 5:10 respectively), and the 1:100 dilution on fabrics 2 and 4 (labeled 2:100 and 4:100 respectively) were amplified. Each sample was extracted in triplicate. The total mass, average mass, and standard deviation of the DNA isolated from each sample was calculated (figure 2 & table 1). The quantitation results for all dilutions on fabrics 3 and 4 for samples extracted with ChargeSwitch showed signs of inhibition. The amplified results were compared by examining the number of loci called properly (table 2) and the balance of the heterozygous peaks (table 3). The organic extraction was the only extraction protocol that successfully isolated DNA from fabric 1.

Buccal swabs, touch items, and cigarette butts were additionally amplified for each extraction protocol. Cigarette butts showed signs of inhibition when extracted with the ChargeSwitch and DNAIQ kits, while ForensicGem and Qiagen MicroPrep obtained good results. All protocols amplified well with extracted DNA from hair samples, buccal swabs, and touch samples.

As a final step in evaluating which DNA extraction method worked best for our lab, a cost per sample analysis was performed based on list price (table 4). ForensicGem was lowest in price, while Qiagen MicroPrep and Organic extractions were highest in price.

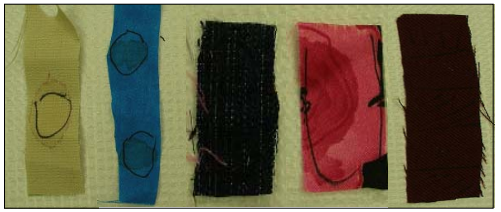

Figure 1: Various fabrics that were used in extraction procedure, from left to right, fabrics 1 thru 5.

Number of Loci Called Properly

Fabric	Organic	ChargeSwitch	DNAIQ	Qiagen	ForensicGem
1	48	0	0	0	0
2	42	48	42	0	48
3	32	48	34	0	30
4	47	3	46	0	0
5	43	48	48	0	48
TOTAL	212	147	170	0	126

Table 2: The total number of loci that were called properly for each of the fabrics, and for each of the different protocols used. 48 loci possible per fabric type, 240 total loci possible.

Total Mass DNA Isolated(ng)

ORGANIC	Replicate # A	B	C	AVG Mass	StDev
1:10	256.2	53.05	23.43	110.8933	126.70776
2:100	17.22	2.35	2.52	7.363333	8.5365469
3:10	121.38	21.45	40.74	61.19	53.010877
4:100	22.176	9.15	3.09	11.472	9.7525695
5:10	121.026	18.4	27.06	55.49533	56.916167
ChargeSwitch					
1:10	0	0	0	0	0
2:100	13.2	15.8	11.3	13.43333	2.2590558
3:10	52.95	68.95	34.1	52	17.444412
4:100	1.75	8.35	0.25	3.45	4.3092923
5:10	91.4	101.8	73.25	88.81667	14.44925
DNAIQ					
1:10	0	4.75	0	1.583333	2.7424138
2:100	7.45	3.95	1.8	4.4	2.8517538
3:10	34.45	16.4	28.3	26.38333	9.1763736
4:100	5.525	3.705	10.35	6.526667	3.4338766
5:10	49.4	41.25	29.3	39.98333	10.10969
QIAGEN					
1:10	4.41	3.474	7.2	5.028	1.9383529
2:100	0.7	3.5	0.7	1.633333	1.6165808
3:10	13.23	6.03	9.54	9.6	3.600375
4:100	0.7	0	0.61	0.436667	0.3808324
5:10	9.45	7.965	11.1798	9.5316	1.6089527
ForensicGem					
1:10	0	0	0	0	0
2:100	37	18.75	24.28	26.67667	9.3580785
3:10	8.4	14.6	32.75	18.58333	12.654281
4:100	0	0	0	0	0
5:10	44.2	123.2	102.8	90.06667	41.010405

Table 1: Total mass of DNA isolated from the various fabrics and different dilutions, using ABI Quantifiler Human®. The sample 1:10 is fabric 1 with whole blood diluted 1:10, sample 2:100 is fabric 2 with a 1:100 dilutions, sample 3:10 is fabric 3 with a 1:10 dilution, sample 4:100 is sample 4 with a 1:100 dilution, and sample 5:10 is fabric 5 with a 1:10 dilution. Each protocol was evaluated for the average mass of DNA as well as the standard deviation for each fabric type.

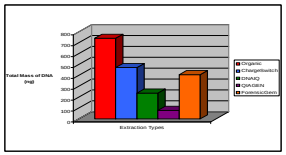

Figure 2: The total amount of DNA isolated (ng) from the five fabrics with different dilutions from each of the different extraction protocols.

Percentage of Imbalanced Peaks (<60%)

Fabric	Organic	ChargeSwitch	DNAIQ	Qiagen	ForensicGem
1	2.08	n/a	n/a	n/a	n/a
2	2.38	0.00	0.00	n/a	0.00
3	0.00	0.00	0.00	n/a	0.00
4	0.00	0.00	0.00	n/a	n/a
5	0.00	2.08	0.00	n/a	0.00

Table 3: The percentage of heterozygous peaks that were imbalanced less than 60%. This was calculated for each fabric and each of the different protocols used. Lower values are better.

	Cost Analysis
Organic	$3.17/sample
Qiagen	$3.12/sample
ChargeSwitch	$1.51/sample
DNAIQ	$1.49/sample
ForensicGem	$1.00/sample

Table 4: The cost of each of the different protocols per sample (according to manufactures' list price).

Conclusion

Overall, the organic extraction method had the highest amount of DNA recovered and demonstrated the best amplification results. The organic extraction method is clearly the best extraction procedure when working with difficult forensic samples.

When looking at the products that were capable of being automated, performance was similar between the ChargeSwitch, DNAIQ, and ForensicGem protocols. The Qiagen MicroPrep system was not successful in amplifying the samples from any of the fabrics. There were signs of inhibition that appeared to affect the amplification. When comparing the results of the fabric samples, DNAIQ had the highest number of loci that were called properly, as well as the most balanced peaks. ChargeSwitch and ForensicGem were not successful in obtaining a profile from fabric 4. ChargeSwitch and ForensicGem were very similar in the amount of DNA that was isolated, although ChargeSwitch showed signs of inhibition.

The ForensicGem successfully amplified touched samples, cigarette butts, buccal swabs, and hair samples. The ChargeSwitch and DNAIQ also successfully amplified touched samples, buccal swabs, and hair samples; however these protocols showed signs of inhibition with cigarette butts. Qiagen MicroPrep successfully amplified cigarette butts and hair samples.

The cost analysis showed that Qiagen MicroPrep and organic extractions were the most expensive, while ForensicGem was the least expensive. ChargeSwitch and DNAIQ were midpoints between the ForensicGem and organic extractions.

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