Using ChargeSwitch® Technology for High-Throughput Purification of Forensic DNA Samples

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Introduction

This application note reports on the results of a first-stage validation study of ChargeSwitch® Technology (CST®) for purifying DNA from a wide variety of forensic sample types, including blood, saliva, hair, semen, cigarette butts, and DNA collected from various "touch" surfaces. This study was performed by LGC Forensics using the ChargeSwitch® Forensic DNA Purification Kit with 96-well plates on an automated liquid handling robot.

Overview

Forensic DNA samples are often highly variable in quality, making it difficult to validate a single purification protocol that will work for high-throughput screening of various sample types. ChargeSwitch® Technology is designed to purify samples of variable quality using a single protocol, without centrifugation, and without the introduction of reagents such as high-concentration salts or organic solvents that can interfere with downstream analytical protocols.

Experimental Design

For this study, 205 samples plus negative controls were processed using CST® in 96-well plates as described below.

Sample Types

The samples came from nine classes of forensic samples, as shown in Table 1 below.

Purification of DNA

DNA was purified from the samples using the ChargeSwitch® Forensic DNA Purification Kit following the standard protocol provided with the kit for automated isolation of genomic DNA. Samples were processed in 96-well plates on a Tecan Genesis® robot.

Quantitation of DNA

Following purification, we quantitated each sample using the following in-house procedures:

- DNA yield was measured by fluorescence using Quant-iT™ PicoGreen® dsDNA Reagent
- PCR analysis was performed using the AmpFLSTR® SGM Plus® PCR Amplification Kit and ABI PRISM® 377 Sequencer

Validation Criteria

The criteria used to validate the samples included:

- Concordance of allelic designations
- Success rates and full profile rates
- Profile quality, including stutter peaks, n-peaks, background, artifact peaks, and heterozygote balance
- Analysis of carryover and cross-contamination in the procedure

Table 1— Sample types analysed.

Sample class	Number of samples
Blood stains	30 light and 30 heavy
Saliva stains (drink cans)	30
Cigarette butts	30
Strip-removed cells	5 hats, 5 coats, 5 gloves
Hair follicles	30
Chewing gum	15
Touched items	5 tools, 5 mobiles, 5 microscope slides
Semen stains	5
Vaginal swabs	5

Figure 1—Function of CST®. At pH <6.5, charge is 'on'. At pH >8.0, charge is 'off'.

Description of ChargeSwitch® Technology

CST® is a novel magnetic bead–based technology that uses a pH-dependent ionic switch for the purification of nucleic acids (Figure 1). In low pH conditions (pH <6.5), the CST® beads have a positive charge that binds the negatively charged nucleic acid backbone. Proteins and other contaminants are not bound and are simply washed away in an aqueous wash buffer.

To elute nucleic acids, the charge on the surface of the bead is neutralized by raising the pH to 8.5 using a low-salt elution buffer. The purified DNA elutes instantly into this buffer and is ready for use in downstream applications (Figure 2).

Purification of DNA from Various Forensic Sample Types

The standard CST® protocol for automated isolation of genomic DNA was used to purify DNA from 205 forensic samples, plus negative controls. All samples were processed in 96-well plates using a Tecan Genesis® automated liquid handling robot.

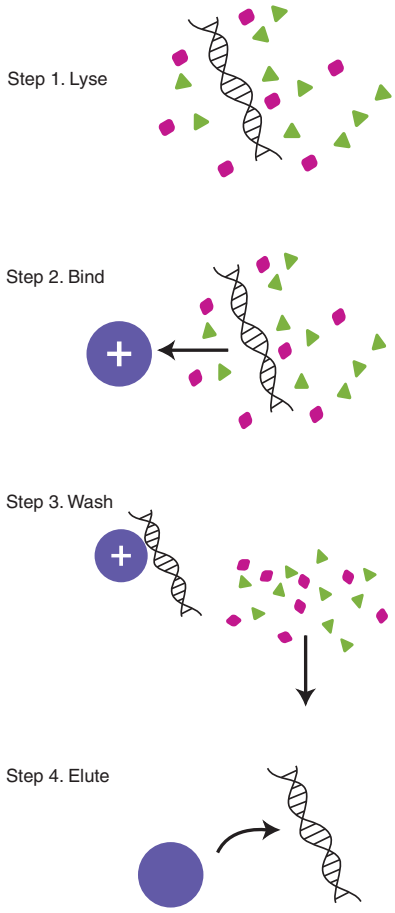

Figure 2—CST® protocol.

The average yield among all sample types was 48 ng (Figure 3, Table 2). This compares to an average of 18 ng when the same sample types were processed manually (data not shown). Especially high yields were observed for chewing gum and touched mobile phones, which also generated good downstream STR (short tandem repeat) data. Semen samples also had much higher DNA yields than were previously achieved using manual methods.

STR Analysis

PCR reactions were performed, and the products were loaded on 4% acrylamide gels and run on the ABI PRISM® 377 sequencer. Success was defined as either a full or partial STR profile. The results were variable, in keeping with expected sample type quality (Table 2). However, these results exceeded results obtained using current sample processing methods.

Three subclasses of sample failed to produce successful STRs, including touched microscope slides, touched new work tools, and material removed from clothing by adhesive strips. It is unclear whether these failures are significant, given the nature of these samples, environmental degradation, and other variables. These sample types may require a smaller volume of elution buffer to increase DNA concentration, and further development work will be performed in this area.

STR Profile Quality

The quality of the STR profile provides a final measure of performance for any purification procedure. Figure 4 shows an example for a sample processed using CST®. Combined peak analysis of the TH01 allele reflected the performance trend across all sample types.

Of the 205 samples profiled, we analysed the size of stutter peaks, n-peaks, and peaks at heterozygous loci.

- 3 had stutter peaks that were greater than 15% of the allelic peak
- 6 had one of the peaks at a heterozygous locus that was less than 50% of the second peak area (Table 3)

The combined quality failure rate of 4.4% is lower than the expected failure rate for other purification methods.

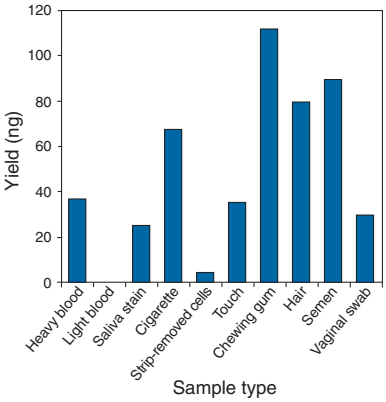

Figure 3—Average DNA yields by sample type.

Table 2—DNA yields and percent STR success from forensic sample processing.

Sample type	No. of samples	Average DNA yield (ng)	Percent STR success
Heavy blood	30	36.53	97%
Light blood	30	0.85	87%
Saliva stain	30	24.55	77%
Cigarette	30	68.12	87%
Strip-removed cells	15	4.03	0%
Touched mobile phones	5	160.07	86%
Touched microscope slides	5	0	0%
Touched new work tools	5	0	0%
Chewing gum	15	112.57	60%
Hair	30	79.06	77%
Semen	5	88.20	100%
Vaginal swab	5	29.81	100%

Table 3—Nine samples that showed STR profile quality issues.

Sample ID	Sample type	Locus	Observation
D-H2	Heavy blood	D2	Stutter peak greater than 15%
D2-C8	Cigarette	D3	Stutter peak greater than 15%
D2-C14	Cigarette	D8	Stutter peak greater than 15%
D-S3	Drink container	D18	Peak area difference
D-S18	Drink container	D16	Peak area difference
D-S24	Drink container	VWA	Peak area difference
D-S29	Drink container	D18	Peak area difference
D2-C5	Cigarette	FGA	Peak area difference
D2-C10	Cigarette	VWA	Peak area difference

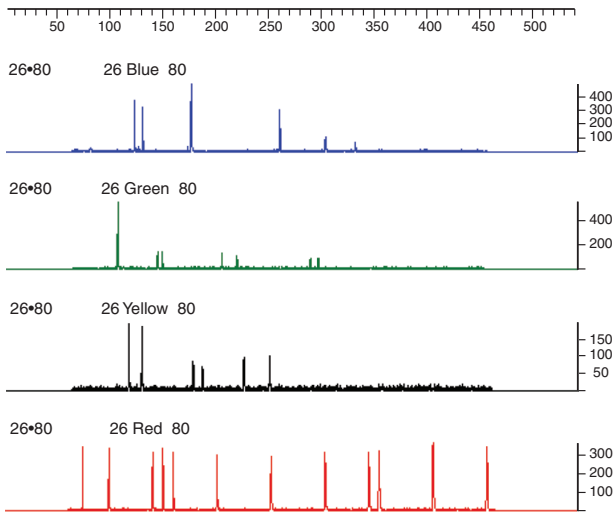

Figure 4—STR profile from touched mobile phone sample.

Contamination Testing

Due to the nature of forensic sample analysis, great care must be taken to avoid cross-contamination of samples. To evaluate the risk of sample contamination when using a liquid handling robot with ChargeSwitch® Technology:

- Negative control samples were included in the validation study. None of these controls showed any evidence of cross-contamination.
- A matrix of positive and negative samples was processed on a separate 96-well plate (Figure 5). The results showed no cross-contamination between wells.

Of the 205 test samples in the study, 10 were found to be contaminated due to the intrinsic nature of the samples themselves. These samples were from items that had been worn/touched by more than one individual, and therefore a mixed profile was expected. Two test samples showed contamination from a neighbouring well (Table 4). Additional studies will be performed to determine the cause of this cross-contamination.

The automated CST® methodology avoids many manual handling steps and does not require centrifugation, thereby significantly reducing the potential for contamination.

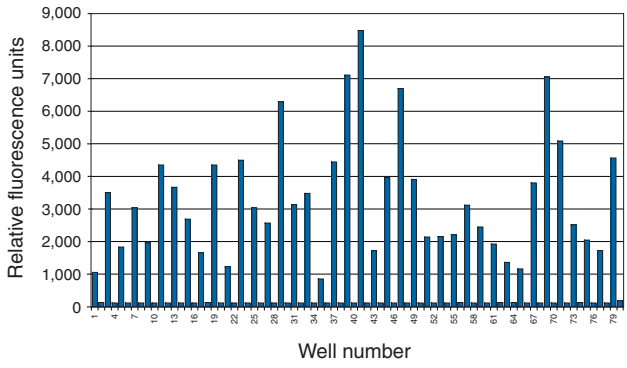

Figure 5—Results from positive/negative matrix samples processed on a 96-well plate.

Conclusions

We validated the ChargeSwitch® Technology with an automated liquid handling protocol on a range of forensic samples and found a very high level of performance. The single protocol was found to be highly flexible and could handle the full range of sample types tested. This has important consequences both in the time and cost associated with validating a new purification method, and potentially considerable savings in handling time if the method is fully adopted. The protocol does not require centrifugation and avoids the use of high-concentration salts and organic solvents, which can be problematic when used with liquid handling robots.

The results of this validation study suggest that CST® may be an appropriate chemistry for widespread use in the forensics arena.

Table 4—Contaminated samples.

Sample ID	Sample type	Comment
D-H3	Heavy blood	Contaminated original sample
D-L5	Light blood	Contaminated original sample (same item as D-L6)
D-L6	Light blood	Contaminated original sample (same item as D-L5)
D-L11	Light blood	Contaminated original sample (same item as D-L30)
D-S1	Drink container	Contaminated original sample
D-S7	Drink container	Contaminated original sample
D2-G9	Chewing gum	Cross-contamination with neighbour sample D2-I2
D2-I15	Hair	Cross-contaminated with neighbour sample D2-I14
D2-MG5	Cellular material (glove)	Contaminated original sample
D2-TP2	Touch DNA (mobile phone)	Multiple phone users—expected mixture
D2-TP3	Touch DNA (mobile phone)	Multiple phone users—expected mixture
D2-TP4	Touch DNA (mobile phone)	Multiple phone users—expected mixture