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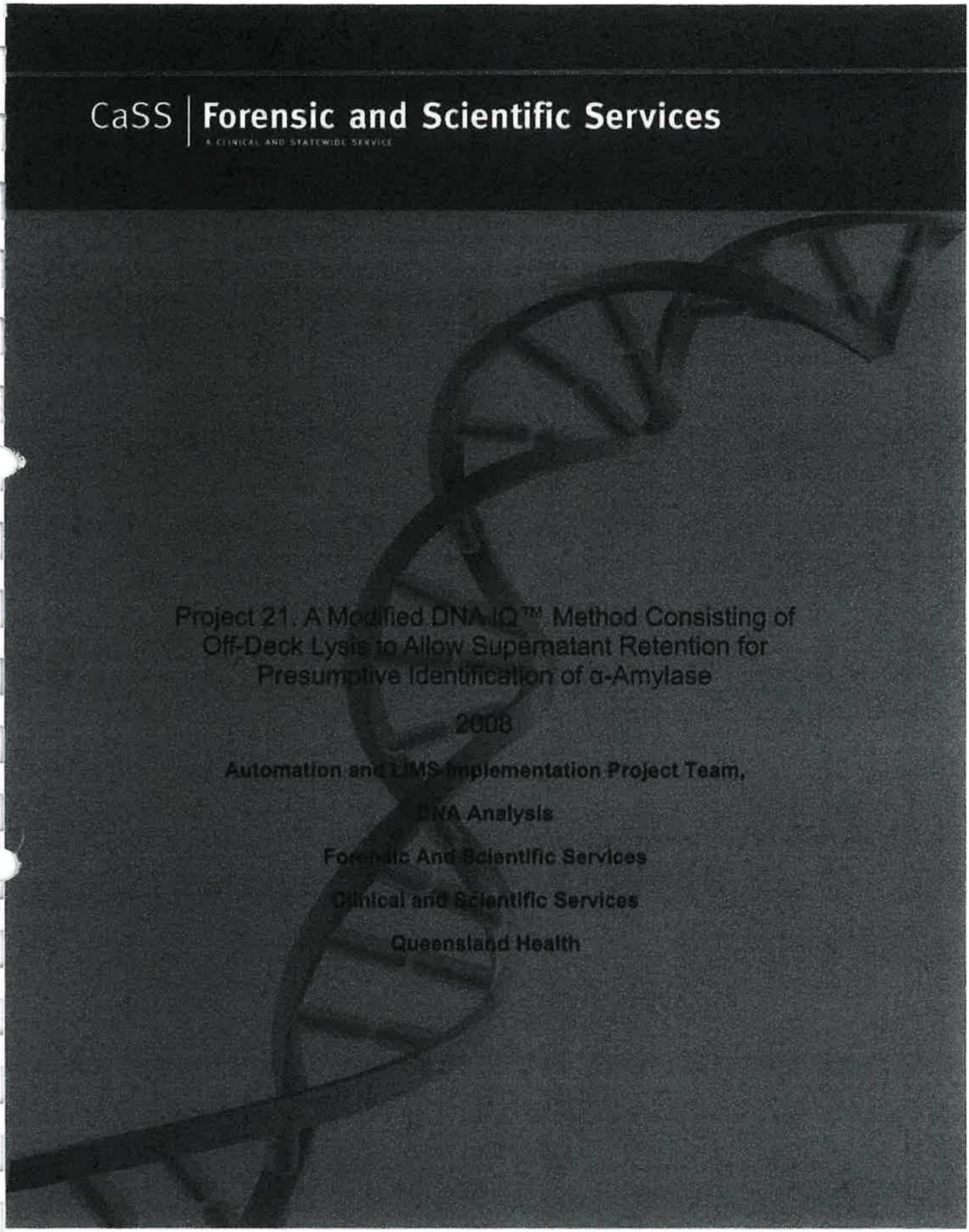

Project 21. A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase

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Project 21. A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase

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1. Abstract

The current in-house DNA IQ™ method for extracting forensic DNA samples of different types in the Slicprep™-96 Device does not allow for some sample types to be processed, e.g. fluffy swab heads or materials with static attraction. Furthermore, sample preparation for the Slicprep™ 96 Device is labour intensive and time consuming. In addition, the current DNA IQ™ method does not allow for samples where presumptive testing on the supernatant is required, such as for α-amylase testing.

We have investigated a modified DNA IQ™ protocol that incorporates off-deck lysis of forensic samples in 1.5mL tubes prior to automated extraction on the MultiPROBE® II PLUS HT EX platform. The off-deck lysis method allows for an increase in the range of sample types that can be processed using the automated DNA IQ™ method. The off-deck lysis method also incorporates the option to retain supernatant for use in presumptive identification procedures.

DNA samples where supernatant was or was not retained for presumptive testing generated comparable results. Importantly, the retained supernatant could be used to perform presumptive testing for α-amylase and produced the expected presumptive and DNA profile results.

We recommend the use of a modified DNA IQ™ method, incorporating an off-deck lysis protocol, to increase the range of sample types that can be extracted using the automated DNA IQ™ protocol, and to allow supernatant retention for presumptive testing.

2. Aim

- To investigate an off-deck lysis method that is compatible with the in-house automated DNA IQ™ protocol.
- To investigate the option of retaining supernatant to allow α-amylase presumptive testing, without compromising the ability to obtain DNA profile results from the same DNA extract.

3. Equipment and Materials

- DNA IQ™ System (Promega Corp., Madison, WI, USA)
- DNA IQ™ Spin Baskets (Promega Corp., Madison, WI, USA)
- TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- Proteinase K (20ng/μL)
- 20% w/v SDS
- Rayon swabs (Copan)

- Eppendorf 5415C centrifuge

4. Methods

4.1 Sample collection

Two donors were selected to provide saliva samples on swabs. The specific donors were selected based on the reactivity of their saliva to the presumptive α-amylase test using Phadebas: one donor was known to generate strong positive readings, while the other donor was known to generate weak positive readings.

A total of eight swabs were taken from each donor, collected over 2 days (4 swabs per person, per day, with two swabs collected from the left check and two from the right cheek).

Two samples each of negative controls, QC cells and QC blood sample types were also included in the test for quality control purposes.

All samples were split equally into two batches, to be processed under slightly different methods, designated Method 1 and Method 2.

4.2 Method 1: Off-deck lysis followed by automated DNA IQ™

All samples were placed in sterile 1.5mL tubes, and 500µL of TNE buffer was aliquoted into each tube and vortexed gently. To each sample, 25µL of 20ng/µL Proteinase K and 12.5µL 20% w/v SDS was added and vortexed briefly, before incubating at 37°C on a Thermomixer (Eppendorf) at 1000 rpm for 45 minutes. The sample substrate material was transferred to a DNA IQ™ Spin Basket and centrifuged for 2 minutes at room temperature at maximum speed (15800g). The centrifuged lysate, and the lysate in the original tube, were transferred and combined into a fresh 1.5mL tube. The samples were then incubated at 65°C on a Thermomixer (Eppendorf) at 1100 rpm for 10 minutes. After incubation, the lysate was added into a Slicprep™ 96 Device (without basket) using the STORstar instrument. Automated DNA IQ™ was then performed (without the automated addition of Extraction Buffer).

4.3 Method 2: Off-deck lysis with supernatant retention, followed by automated DNA IQ™

650µL of TNE buffer was aliquoted into each tube and vortexed gently. The sample was allowed to incubate at room temperature for 30 minutes, prior to vortexing and centrifuging at maximum speed for 3 minutes (15800g). From this tube, 150µL of supernatant was transferred to a fresh sterile 1.5mL tube and stored at -20°C to be used for presumptive testing. To each sample, 25µL of 20ng/µL Proteinase K and 12.5µL 20% w/v SDS was added and vortexed briefly, before incubating at 37°C on a Thermomixer (Eppendorf) at 1000 rpm for 45 minutes. The remainder of the off-deck lysis protocol was performed on all samples as per Method 1 above.

4.4 α-Amylase presumptive screening

Presumptive screening for the presence of α-amylase was performed as per QIS 17193.

4.5 DNA quantitation

All DNA extracts were quantified using the Quantifiler™ Human DNA Quantitation kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19977. Reaction setup was performed on the MultiPROBE® II PLUS HT EX (PerkinElmer) pre-PCR platform.

4.6 PCR amplification

DNA extracts were amplified using the AmpF*t*STR® Profiler Plus® kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19976. Reaction setup was performed on the MultiPROBE® II PLUS HT EX (PerkinElmer) pre-PCR platform.

4.7 Capillary electrophoresis and fragment analysis

PCR product was prepared for capillary electrophoresis using the manual 9+1 protocol as per QIS 19978. Capillary electrophoresis was performed on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) under the following conditions: 3kV injection voltage, 10 sec injection time, 15kV run voltage, 100µA run current, and 45min run time. Data Collection Software version 1.1 was used to collect raw data from the ABI Prism® 3100 Genetic Analyzer. Fragment size analysis was performed using GeneScan 3.7.1. Allele designation was performed using Genotyper 3.7, with thresholds for heterozygous and homozygous peaks at 150 and 300 RFU respectively. The allelic imbalance threshold is 70%.

5. Results and Discussion

The differences between the two off-deck lysis methods that were assessed are outlined in Table 1 below.

Table 1. Differences between two off-deck lysis methods.

	Method 1: Off-deck lysis	**Method 2: Off-deck lysis with supernatant retention**
Sample format	In 1.5mL tube	In 1.5mL tube
Volume of TNE buffer added to sample	500µL	650µL
Incubation at room temperature		30 minutes
Supernatant transfer		150µL
Proteinase K	25µL (20ng/µL)	25µL (20ng/µL)
20% w/v SDS	12.5µL	12.5µL
Sample lysis 37°C	37°C, 45 minutes at 1100 rpm	37°C, 45 minutes at 1100 rpm
Substrate transfer	DNA IQ™ Spin Basket, all lysate transferred to fresh 1.5mL tube	DNA IQ™ Spin Basket, all lysate transferred to fresh 1.5mL tube
Inactivate Proteinase K	65°C, 10 minutes at 1100 rpm	65°C, 10 minutes at 1100 rpm
Automated protocol	Transfer lysate to Slicprep™ using STORstar, then present to MP II	Transfer lysate to Slicprep™ using STORstar, then present to MP II

The primary difference between both methods is the addition of 150µL extra TNE buffer in Method 2 that allows 150µL of supernatant to be retained. The sample is incubated at room temperature for 30 minutes with the additional TNE buffer, which is then transferred to a fresh tube for use in presumptive testing procedures. The remainder of the Method 2 protocol from this point onwards is identical to Method 1 in order to achieve off-deck lysis, with identical lysis volumes for both methods of 500µL. The off-deck lysis protocol differs from the automated DNA IQ™ lysis protocol in the following aspects:

- Lysis at 37°C is performed on a Thermomixer at 1100 rpm, instead of the DPC shaker with heater tiles controlled by the automated heater controller.
- Sample substrates in each individual tube are separated from the lysate using the DNA IQ™ Spin Basket, instead of using the Slicprep™ basket with collar attached.
- Proteinase K is inactivated at 65°C on a Thermomixer at 1100 rpm, instead of the DPC shaker.

Quantitation results (ng/µL) for Method 2, where the supernatant was retained, was comparable to the results for Method 1, where supernatant was not retained (Table 2). Importantly, α-amylase presumptive screening was able to be performed on the retained supernatant and generated the expected results (Table 2).

Table 2. Quantitation (ng/µL) and α-amylase presumptive testing results for samples extracted using Method 1 (off-deck lysis) and Method 2 (off-deck lysis with retained supernatant option).

Sample	Method 1 Q'filer	Method 2 Q'filer	Phadebas Test	Saliva kit 5 min	Saliva kit 10 min
NegCtl	0.0000	0.0000	Negative	Neg	Neg
QC Cells	0.1030	0.0582	Negative	Neg	Weak
QC Blood	0.0700	0.0991	Negative	Neg	Neg
Donor 1 Right Cheek Day 1†	0.9190	1.2600	3+	V. Strong	V. Strong
Donor 1 Right Cheek Day 2†	0.6990	1.7000	2+	V. Strong	V. Strong
Donor 1 Left Cheek Day 1†	1.9700	0.9350	1+	V. Strong	V. Strong
Donor 1 Left Cheek Day 2†	3.0600	1.7000	1+	V. Strong	V. Strong
Donor 2 Right Cheek Day 1‡	0.6860	1.4300	1+	V. Strong	V. Strong
Donor 2 Right Cheek Day 2‡	2.0300	1.4000	3+	V. Strong	V. Strong
Donor 2 Left Cheek Day 1‡	0.7290	1.9800	2+	V. Strong	V. Strong
Donor 2 Left Cheek Day 2‡	0.7630	1.7300	2+	V. Strong	V. Strong

†Donor 1: strong positive α-amylase
‡Donor 2: weak positive α-amylase

Variable

DNA profiles were obtained for all samples (Table 3). In general, full profiles were generated by all samples, but less allelic imbalance was observed in samples processed using Method 2.

16 swabs from 2 donors

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Table 3. DNA profile results for all samples processed using Method 1 and Method 2.

Sample	Method 1	Method 2
NegCtl	NSD	NSD
QC Cells	OK	PA*
QC Blood	AI @ D13 ~69%	OK
Donor 1 Right Cheek Day 1†	OK	OK
Donor 1 Right Cheek Day 2†	OK	OK
Donor 1 Left Cheek Day 1†	OK	OK
Donor 1 Left Cheek Day 2†	AI @ D18 ~63%	OK
Donor 2 Right Cheek Day 1‡	OK	OK
Donor 2 Right Cheek Day 2‡	AI @ D7 ~66%	OK
Donor 2 Left Cheek Day 1‡	OK	OK
Donor 2 Left Cheek Day 2‡	OK	OK

†Donor 1: strong positive α-amylase
‡Donor 2: weak positive α-amylase
*Resolved alleles were consistent with the expected profile

only because easy to type

6. Conclusion and Recommendations

The processing of forensic samples using Method 2, where supernatant is retained, was satisfactory for both α-amylase testing and for obtaining quantitation and DNA profile results. A volume of 650µL of TNE buffer is recommended for addition to samples where supernatants are retained for testing.

7. Acknowledgements

Phadebas testing was performed by Rhys Parry, Rebecca Gregory and Kirsten Scott (DNA Analysis, FSS). Experiments were performed by the Automation/LIMS Implementation Project team, with the assistance of the Analytical Section.

8. References

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