Queensland Health
HealthSupport Queensland

Project #192 - Validation of QIAsymphony SP for bone extraction

Supplementary Repeatability and Reproducibility

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Project #192 Validation of QIAsymphony SP for bone extraction – Supplementary Repeatability and Reproducibility

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Document sign off

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1. Purpose and Scope

1.1. Background

Project #192, the validation of bone extractions on the QIAsymphony SP using DNA Investigator Kit, was initially conducted and approved by Management Team in April 2018. Following this approval, recommendations were made that supplementary Repeatability and Reproducibility testing be performed to enable testing and comparison of an increased number of samples. This supplementary project will fulfil this requirement.

1.2. Purpose

To conduct supplementary Repeatability and Reproducibility experiments for the following protocols:

- QIAsymphony Pre-Lysis (5 hour incubation) with QIAsymphony SP Extraction
- QIAsymphony Pre-Lysis (overnight incubation) with QIAsymphony SP Extraction
- Organic Extraction (current validated protocol).

1.3. Scope

Samples used in this project will be sourced from bones which have been submitted to Forensic DNA Analysis for identification purposes.

All quantifications will be performed using Quantifiler® Trio DNA quantification kit.

All samples will be amplified with PowerPlex® 21 at 25µL total volume.

The 3130*xl* will be used for DNA fragment analysis of all samples.

This project will have both quantitative and qualitative acceptance criteria, and these criteria will be assessed on completion of the data analysis for each experiment performed.

2. Governance

Project Personnel

- **Project Manager:** Luke Ryan – Senior Scientist, Analytical Team
- **Project Collaborator:** Allan McNevin – Senior Scientist, Evidence Recovery Team
- **Project Officers:** Melissa Cipollone – Scientist, Analytical Team

Decision Making Group

The Management Team (in accordance with QIS 22871 22871 'Procedure for Change Management in Forensic DNA Analysis' and relevant quorum provisions) is the Decision Making Group for this project and is responsible for:

- Assessing and approving this experimental design and the proposed methodology
- Assessing and approving/rejecting any proposed changes to this document, including feedback from technical reviewers
- Using the acceptance/assessment criteria to make a final decision on whether this validation has been successful or not.

No changes to this document shall be made unless there is approval from the Decision Making Group, with a minimum quorum in accordance with Section 4.1 of QIS 22871 'Procedure for Change Management in Forensic DNA Analysis'.

Reporting

The Project Manager and Project Officer will meet with the Manager - Quality and Projects fortnightly to provide ongoing status updates.

3. Resources

The following resources are required for this validation/project:

3.1. Reagents

- 0.9 % saline solution (Baxter, Old Toongabbie, NSW, AU)
- 5 % v/v Bleach White N Bright (Ecolab, NSW, AU)
- 70 % v/v Ethanol (Recochem Incorporated, Wynnum, QLD, AU)
- 100 % v/v Ethanol (Sigma-Aldrich Pty. Ltd., Castle Hill, NSW, AU)
- Proteinase K (20 mg/mL) (Sigma-Aldrich Pty. Ltd., Castle Hill, NSW, AU)
- DTT (Dithiothreitol) 1 M (Sigma-Aldrich Pty. Ltd., Castle Hill, NSW, AU)
- 5 % v/v Trigene Advanced (CEVA DEIVET Pty. Ltd., Seven Hills, NSW, AU)
- 40 % v/v N-Lauroylsarcosine (Sarcosyl) (Sigma-Aldrich Pty. Ltd., Castle Hill, NSW, AU)
- QIAamp® DNA Investigator® Kit (QIAGEN Group, 40724 Hilden, DE)
- Quantifiler™ Trio DNA Quantification kits (Thermo Fisher Scientific, Foster City, CA, US)
- Promega PowerPlex® 21 system (Promega Corp., Madison, WI, US)
- Water, Amplification Grade (Promega Corp., Madison, WI, US)
- PowerPlex®21 2800M Control DNA (Promega Corp., Madison, WI, US)
- Promega WEN Internal Lane Standard 500 (Promega Corp., Madison, WI, US)
- Promega PowerPlex 5 Dye Matrix Standard (Promega Corp., Madison, WI, US)
- Promega PowerPlex® 21 Allelic Ladder Mix (Promega Corp., Madison, WI, US)
- Hi-Di™ Formamide (Thermo Fisher Scientific, Foster City, CA, US)

- 3130 POP-4™ Polymer (Thermo Fisher Scientific, Foster City, CA, US)
- Running Buffer (Gel Company, San Francisco, CA, US)
- Stain Extraction buffer (SEB) (Forensic DNA Analysis, QLD, AU)
- Phenol-Chloroform-Isoamyl Alcohol Solution (25:24:1) (Gibco BRL,Thermo Fisher Scientific, Life Technologies, Foster City, CA, US)

3.2. Materials

- Sterile 2 mL screw-cap tubes (Axygen® Scientific Inc., Union City, CA, US)
- Sterile spin baskets and 2 mL collection tubes (PM Separations, Brisbane, QLD, AU)
- Sterile 5 and 10 mL screw-cap tubes (Axygen® Scientific Inc., Union City, CA, US)
- Nunc CryoTubes Bank-It Cryobank Vials (Thermo Fisher Scientific Australia Pty. Ltd., Scoresby, Vic, AU)
- ART Filtered 1000 and 300 µL pipette tips (Molecular BioProducts Inc., San Diego, CA, US)
- F1-ClipTip pipette tips - 20µL, 50µL, 200µL & 1000 µL (Thermo Fisher Scientific Inc, 01621 Vantaa, FIN)
- Hamilton Conductive 50µL Filter Tips in Frames (Hamilton, Reno, NV, USA)
- Hamilton Conductive 300µL Filter Tips in Frames (Hamilton, Reno, NV, USA)
- MicroAmp® optical 96-well reaction plates (Thermo Fisher Scientific, Foster City, CA, US)
- MicroAmp® optical adhesive film (Thermo Fisher Scientific, Foster City, CA, US)
- SSI 96 well semi-skirt PCR plate (Interpath, Heidelberg West, VIC, AU)
- Reservoir septa (Gel Company, San Francisco, CA, US)
- Tape pads (Qiagen Pty. Ltd., Doncaster, VIC, AU)
- 96-well plate Septa mats (Thermo Fisher Scientific, Foster City, CA, US)
- Rediwipes (Cello Paper Pty. Ltd., Fairfield, NSW, AU)
- Sterile rayon swabs (Copan Diagnostics Inc., Murrieta, CA, US)
- QIAsymphony® Sample prep cartridges, 8-well (QIAGEN Group, 40724 Hilden, DE)
- QIAsymphony® 8-Rod Covers (QIAGEN Group, 40724 Hilden, DE)
- QIAsymphony® 2mL and 5mL conical tubes (QIAGEN Group, 40724 Hilden, GERMANY)
- QIAsymphony® Filter-tips 1500, 50 & 200 µL (QIAGEN Group, 40724 Hilden, GERMANY)

3.3. Equipment

- Milli-Q® Integral 3 (A10) System with Q-POD™ (Millipore™, Billerica, MA, US)
- Biological safety cabinet class II - Labculture (Esco Global, Singapore, SG)
- 50, 200 and 1000µl Pipettes (Finnpipette® Thermo Fisher Scientific Australia Pty. Ltd., Scoresby, Vic, AU)
- Minifuge (CS Bio Co. (ex-Tomy Tech US Inc.), Menlo Park, CA, US)
- ThermoMixer (Eppendorf South Pacific Pty. Ltd., North Ryde, NSW, AU)
- Labnet Shaker 20 (National Labnet Co., Woodbridge, NJ, USA)
- Vortex (Ratek Instruments Pty. Ltd., Boronia, VIC, AU)
- Hot-block (Ratek Instruments Pty. Ltd., Boronia, VIC, AU)
- Tube Centrifuge (Eppendorf South Pacific Pty. Ltd., North Ryde, NSW, AU)

- Refrigerators and freezers (Westinghouse Pty. Ltd., AU)
- STORstar instrument (Process Analysis & Automation, Hampshire, UK)
- Plate Centrifuge (Eppendorf South Pacific Pty. Ltd., North Ryde, NSW, AU)
- STARlet Automated Liquid Handler (Hamilton, Reno, NV, USA)
- 7500 Real Time PCR System (Life Technologies Applied Biosystems, Foster City, CA, US)
- GeneAmp PCR system 9700 (Life Technologies Applied Biosystems, Foster City, CA, US)
- ABI 3130*xl* Genetic Analyzer (Life Technologies Applied Biosystems, Foster City, CA, US)
- GeneMapper-*IDX* ver. 1.4 (Life Technologies Applied Biosystems, Foster City, CA, US)

4. Methods

4.1. Sample Selection

Samples used in this project will be selected from samples which have been submitted to Forensic DNA Analysis for testing.

4.2. Bone/Teeth Crushing

Bones and teeth will be prepared and crushed according to QIS 34300 'Examination of post mortem and associated samples from deceased persons'.

Aliquots of bone will be prepared consisting of 100 mg of bone powder.

4.3. Organic DNA Extraction

Bones/teeth will undergo organic DNA extraction according to QIS 34039 'Extracting DNA from Bone and Teeth'.

4.4. QIAGEN Pre-Lysis

Samples for "QIAGEN" Pre-Lysis will undergo Pre-Lysis as per QIS 33758 'DNA Extraction and Quantitation of casework and reference samples using the QIAsymphony SP/AS". The Pre-Lysis incubation step will be extended (to either 5 hours or overnight) as specified in each Experimental Design.

4.5. QIAsymphony SP Extraction

Bones will undergo DNA extraction on the QIAsymphony SP according to QIS 33758 'DNA Extraction and Quantitation of casework and reference samples using the QIAsymphony SP/AS".

4.6. Quantification

All samples will be quantified and setup as per QIS 34045 'Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit'.

4.7. Amplification

Amplified samples will be setup on STARlet A or B and PCR performed as per QIS 34052 'Amplification of Extracted DNA using the PowerPlex® 21 System'.

4.8. DNA Fragment Analysis

Plates for DNA fragment analysis on the 3130*xl* will be prepared and the PCR fragments separated by capillary electrophoresis (CE) according to QIS 34311 'Procedure for the Use and Maintenance of the AB 3130*xl* Genetic Analysers'. Analysis will be performed as per QIS 34131 "Capillary Electrophoresis Quality (CEQ) Check".

4.9. Profile Interpretation

All samples amplified with PowerPlex® 21 will be interpreted according to QIS 34112 ' STR Fragment Analysis of PowerPlex® 21 profiles using GeneMapper® ID-*X* software'.

Where reference to "qualitative" profile assessment are made, this will be performed by an FRIT member who is a competent coronial reporter.

5. Experimental Design

5.1. Repeatability

Intent

To conduct Repeatability Experiments for the following protocols:

- QIAsymphony Pre-Lysis (5 hour incubation) and QIAsymphony SP Extraction
- QIAsymphony Pre-Lysis (overnight incubation) and QIAsymphony SP Extraction
- Organic Extraction

Experimental Design

Table 1 below describes the Experimental Design for the Repeatability Experiment. Each aliquot will contain 100 mg of bone powder. The availability of bone samples for use in this project will likely be limited by the low numbers of bones which are submitted for forensic testing and also the small size of individual bone fragments which are submitted. In addition, because bones are required, staff donor samples and proficiency testing materials are not viable samples for use in this validation.

Following sample selection, crushing and determination of the number of available bone aliquots, this experimental design will be reviewed and the following modifications may be made:

- Increase or decrease the number of samples used for this experiment
- Increase or decrease the number of aliquots tested for each sample (please note that different samples may have different numbers of aliquots used in this experiment)

Final sample numbers and aliquots will be presented to the Decision Making Group for approval prior to commencement. The sample set will also be reviewed to ensure samples with a range of quality and DNA concentrations have been included.

Table 1: Repeatability Experiment

Sample	Aliquot	Day	Operator	Protocol
1	1-5	1	1	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
	6-10	1	1	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
	11-15	1	1	Organic Extraction
2	1-5	1	1	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
	6-10	1	1	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
	11-15	1	1	Organic Extraction
3	1-5	1	1	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
	6-10	1	1	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
	11-15	1	1	Organic Extraction
4	1-5	1	1	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
	6-10	1	1	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
	11-15	1	1	Organic Extraction
5	1-5	1	1	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
	6-10	1	1	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
	11-15	1	1	Organic Extraction

All samples will undergo Extraction, Quantification (in duplicate), Amplification, DNA Fragment Analysis and Profile Interpretation as per the Methods section.

Data Analysis

Repeatability for each protocol will be assessed by comparing the Quantification results and DNA profile results (allele count and qualitative profile assessment) for all replicates.

Acceptance Criteria

The QIAsymphony protocols will be accepted if they have comparable or better repeatability than the Organic protocol.

5.2. Reproducibility

Intent

To conduct Reproducibility Experiments for the following protocols:

- QIAsymphony Pre-Lysis (5 hour incubation) and QIAsymphony SP Extraction
- QIAsymphony Pre-Lysis (overnight incubation) and QIAsymphony SP Extraction
- Organic Extraction

Experimental Design

Tables 2, 3 and 4 below describes the Experimental Design for the Reproducibility Experiments for the protocols being tested. Each aliquot will contain 100 mg of bone powder. The availability of bone samples for use in this project will likely be limited by the low numbers of bones which are submitted for forensic testing and also the small size of individual bone fragments which are submitted. In addition, because bones are required, staff donor samples and proficiency testing materials are not viable samples for use in this validation.

Following sample selection, crushing and determination of the number of available bone aliquots, this experimental design will be reviewed and the following modifications may be made:

- Increase or decrease the number of samples used for this experiment
- Decrease the number of days tested

Final sample numbers and test days will be presented to the Decision Making Group for approval prior to commencement. The sample set will also be reviewed to ensure samples with a range of quality and DNA concentrations have been included.

Table 2: Reproducibility QIAGEN Pre-Lysis (5 hours) QIAsymphony SP Extraction

Sample	Aliquot	Day	Operator	Protocol
1	16	2	2	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
2	16			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
3	16			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
4	16			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
5	16			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
1	17	3	3	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
2	17			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
3	17			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
4	17			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
5	17			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
1	18	4	4	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
2	18			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
3	18			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
4	18			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
5	18			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
1	19	5	5	QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
2	19			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
3	19			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
4	19			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction
5	19			QIAGEN Pre-Lysis (5 Hour) QIAsymphony SP Extraction

Table 3: Reproducibility QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction

Sample	Aliquot	Day	Operator	Protocol
1	20	2	2	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
2	20			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
3	20			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
4	20			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
5	20			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
1	21	3	3	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
2	21			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
3	21			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
4	21			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
5	21			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
1	22	4	4	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
2	22			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
3	22			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
4	22			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
5	22			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
1	23	5	5	QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
2	23			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
3	23			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
4	23			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction
5	23			QIAGEN Pre-Lysis (Overnight) QIAsymphony SP Extraction

Table 4: Reproducibility Organic Extraction

Sample	Aliquot	Day	Operator	Protocol
1	24	2	2	Organic Extraction
2	24			Organic Extraction
3	24			Organic Extraction
4	24			Organic Extraction
5	24			Organic Extraction
1	25	3	3	Organic Extraction
2	25			Organic Extraction
3	25			Organic Extraction
4	25			Organic Extraction
5	25			Organic Extraction
1	26	4	4	Organic Extraction
2	26			Organic Extraction
3	26			Organic Extraction
4	26			Organic Extraction
5	26			Organic Extraction
1	27	5	5	Organic Extraction
2	27			Organic Extraction
3	27			Organic Extraction
4	27			Organic Extraction
5	27			Organic Extraction

All samples will undergo Extraction, Quantification (in duplicate), Amplification, DNA Fragment Analysis and Profile Interpretation as per the Methods section.

Data Analysis

Reproducibility will be assessed by comparing the quantification and DNA profile results (allele count and qualitative profile assessment) for the replicates of each sample processed on days 1-5.

Acceptance Criteria

The QIAsymphony protocols will be accepted if they have comparable or better reproducibility than the Organic protocol.

5.3. Protocol Comparison

Intent

To compare the performance of the following protocols:

- QIAsymphony Pre-Lysis (5 hour incubation) and QIAsymphony SP Extraction
- QIAsymphony Pre-Lysis (overnight incubation) and QIAsymphony SP Extraction
- Organic Extraction

The protocols will be compared in terms of the Quantification results and final DNA profile results (allele count and qualitative profile assessment).

Experimental Design

Results from the Repeatability and Reproducibility experiments will be used for this experiment. Sample/replicate results from each of the three protocols will be compared and assessed in terms of:

- DNA quantification results
- Allele count in final DNA profile
- Qualitative assessment of profile quality

Acceptance criteria

The QIAsymphony protocols will be accepted if they have overall comparable or better performance when compared to the Organic protocol.

6. Results and Data Compilation

The results of each experiment will be assessed to determine if the QIAsymphony protocols have demonstrated comparable or better performance than the current Organic protocol and therefore can be validated and implemented.

If the Project Team forms the opinion that additional experiments are required before a final assessment can be made, application will be made to the Decision Making Group for a modification to this Experimental Design. The Decision Making Group is responsible for assessing this application and approving or rejecting it.

A final report will be produced which will compile all analyses, conclusion and recommendations. The final report will be prepared by the Project Group.