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Validation of Hamilton® STARlet C for Capillary Electrophoresis Setup

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Document Details

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Abstract

Capillary Electrophoresis (CE) plate preparation within Forensic DNA Analysis involves the manual aliquoting of reagents and transfer of PCR product into a 96 well CE plate via a hand held pipette. PCR product is transferred from a 96 well PCR plate to the CE plate.

Currently, plate preparation occurs in two ways:

1. Whole Plate transfer, where the sample positions on the amplification plate map and the CE plate map are identical. The operator uses an 8 channel pipette to transfer an entire column at a time from the amplification plate to the corresponding column in the CE plate.
2. Re-CE, where amplified product from multiple amplification plates are transferred to one CE plate. The operator uses a single channel pipette to transfer one sample at a time from multiple amplification plates to one CE plate.

As CE plate preparation is a manual process there is a risk of pipetting error, with Re-CE plates having a higher risk than whole plate transfers. This risk can be mitigated through the use of a liquid handler for CE plate preparation. The liquid handler would prepare CE plates using imported plate maps, thereby mitigating the risk of operator error.

The purpose of this project was to validate the Hamilton® STARlet (Instrument C) for:

- Preparation of Capillary Electrophoresis (CE) plates; and
- Preparation of Re-CE plates.

A total of six experiments were performed in the validation of the Hamilton® STARlet (Instrument C):

1. Verification with ARTEL MVS.
2. Plate Seal Assessment.
3. Whole Plate Transfer PowerPlex®21.
4. Re-CE PowerPlex®21.
5. Repeatability and Reproducibility.
6. Seal Piercing Tool Assessment.

The STARlet C passed pipetting verification using the ARTEL at the tested volumes of 1 µL and 50 µL. Of the plate seals that were tested, the Hamilton® Foil seal has met all criteria and can be used with the automated plate sealer to reduce manual handling of the plate. Programs developed for Whole Plate Transfer and Re-CE performed as expected and no cross-contamination detected. A repeatability and reproducibility experiment demonstrated the STARlet C is consistent when adding reagents and PCR product to the CE plate. The 4titude seal piercing tool met all criteria and can be used to pierce the Hamilton® Foil seals on the PCR product plates prior to use on the instrument.

Upon conclusion of the project, the Project Team recommends the STARlet C is implemented within Forensic DNA Analysis for preparation of whole CE plates and Re-CE plates. We also recommend Forensic DNA Analysis uses Hamilton® Foil Seals, the Automated Hamilton® Sealing instrument and the 4titude Piercing Tool for preparation and sealing of PCR plates.

Introduction

Background

Forensic DNA Analysis performs DNA fragment analysis using capillary electrophoresis (CE) as the last analytical process for casework and reference samples. There are currently two capillary electrophoresis instruments used within Forensic DNA Analysis, the AB 3130*xl* and the AB 3500xL Genetic Analysers (Applied Biosystems, ThermoFisher Scientific). Samples are loaded to both instruments in 96 well plate format which are prepared manually by Analytical team members.

The risk of pipetting error when preparing CE plates is increased due to the manual handling process, with Re-CE plates having a higher risk than whole plate transfers. The risk of operator error can be mitigated through the use of a liquid handler for CE plate preparation as plates would be prepared according to a plate map generated by the Laboratory Information Management System (LIMS).

Purpose

The purpose of this project was to develop and validate programs to use the STARlet (Instrument C) for preparation of Whole Plate Transfer and Re-CE 'cherry-picking' plates for processing through CE.

Scope

The scope of this project covered the verification of the pipetting performance of the STARlet, the development and verification of programs for the preparation of Whole Plate Transfers and Re-CE plates using the STARlet instrument, assessment of cross-contamination while using the STARlet instrument and the assessment of appropriate seals for use from PCR amplification to re-sealing after CE preparation. This project also included testing a plate piercing tool.

Resources

All reagents, materials and equipment used in this project were as specified in the approved in-house document Project Proposal #175: Validation of Hamilton® STARlet C for Capillary Electrophoresis Setup (September 2017) with the addition of the materials listed below. This document will be referred to as the Experimental Design.

Additional Materials:
- 4titude® Pierceable Seal (4titude® Limited, Wotton, Surrey, UK)
- 4titude® PCR Foil Seal Strong (4titude® Limited, Wotton, Surrey, UK)
- Hamilton® Foil Plate Seal (Hamilton®, Reno, NV, USA)

Additional Equipment:
- Hamilton® Automated Plate Sealer (Hamilton®, Reno, NV, USA)
- 4titude Pierce Plate (4titude® Limited, Wotton, Surrey, UK)

Experiments and Results

Sample Selection

Amplified product

Amplified product was chosen from casework and reference samples submitted by the Queensland Police Service for routine testing and in-house controls. The Project Team only selected samples and controls which had previously undergone amplification with PowerPlex®21 according to QIS 34052 Amplification of Extracted DNA using the PowerPlex21 System and QIS 34035 Forensic Register FTA Processing.

CTS extracts

12 single source extracts from Collaborative Testing Service (CTS) cases completed within Forensic DNA Analysis were selected by the Project Team.

Donor buccal cell samples

Buccal cells on FTA cards were donated by two volunteers. The cells were collected according to QIS 33333 Participant Information and Consent Form (PICF – Common Biological Samples.

Experiment 1: Verification of STARlet C with ARTEL MVS

Purpose

To use the ARTEL MVS to verify the pipetting performance of the STARlet C instrument using 50 µL CO-RE filter tips.

Method

Verification of the STARlet C using the ARTEL MVS was performed according to Section 5.1 of the Experimental Design. The 50 µL CO-RE tips were verified at pipetting 1 µL and 50 µL.

To perform a pipetting verification within Forensic DNA Analysis, each Piston Operated Volumetric Apparatus (POVA) is used to add the ARTEL reagents to 12 positions within a microtiter plate. The ARTEL instrument analyses each well and calculates the mean pipetted volume, Relative Inaccuracy (%inaccuracy), standard deviation and Coefficient of Variation (%CV). This data is used to verify the pipetting precision and accuracy of the POVA. The acceptance criteria for a POVA is a %CV and %inaccuracy of +/- 5 % for volumes ≥ 11 µL and 10 % for volumes ≤10 µL.

In the case of the STARlet, each 1000 µL-channel is an individual POVA. All 8 channels are assessed concurrently and each channel must pass to verify the instrument.

Results and Discussion

50 µL CO-RE Tips at 1 µL

An ARTEL verification of the STARlet C using the 50 µL CO-RE tips to pipette 1 µL produced the results shown in Table 1.

Table 1 ARTEL results for pipetting 1 µL using the 50 µL CO-RE tips. All values are in µL. Row A contains the results for Channel 1 and Row H contains the results for Channel 8. Individual values outside of the 10 % threshold are highlighted in yellow.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.0078	1.0095	0.9628	0.9628	0.9518	0.9765	0.9299	0.9429	0.9675	1.0208	0.9618	1.0035
B	0.9360	0.9034	0.9329	0.9229	0.9530	0.9084	0.8941	0.8904	0.9386	0.9155	0.9524	0.9178
C	0.9987	0.9422	0.9726	0.9859	0.9737	0.9647	0.9414	0.9279	0.9507	0.9784	0.9453	0.9342
D	0.9683	0.9061	0.9364	0.9422	0.9748	0.9782	0.9415	0.9120	0.9346	0.9866	0.9634	0.9433
E	1.0350	0.9820	1.0159	1.0262	0.9894	1.0038	1.0108	1.0162	1.0210	1.0159	1.0134	1.0209
F	0.9708	0.9572	0.9341	0.9551	0.9334	0.9285	0.9293	0.9693	0.9466	0.9449	0.9796	0.9545
G	1.1301	0.9269	0.9705	0.9874	1.0046	0.9781	0.9571	0.9698	0.9505	0.9781	0.9555	0.9490
H	0.9332	0.9351	0.9068	0.9734	0.9267	0.9034	0.9304	0.9310	0.9170	0.9334	0.9144	0.9451

The data in Table 1 was used to calculate the mean pipetted volume, %inaccuracy, standard deviation and %CV for each channel as shown in Table 2. From these values, each individual channel has performed within the 10 % threshold and resulted in a pass.

Table { SEQ Table * ARABIC } Channel statistics for 50 µL tips pipetting 1 µL calculated from the data in Table 1.

Channel	Mean Volume	% inaccuracy	Standard Deviation	%CV	Status
1	0.97480	-2.52	0.02909	2.98	Passed
2	0.92212	-7.79	0.02098	2.28	Passed
3	0.95964	-4.04	0.02243	2.34	Passed
4	0.94895	-5.11	0.02562	2.70	Passed
5	1.01254	1.25	0.01482	1.46	Passed
6	0.95028	-4.97	0.01715	1.80	Passed
7	0.97980	-2.02	0.05149	5.26	Passed
8	0.92916	-7.08	0.01864	2.01	Passed

The data in Table 1 was used to calculate the mean pipetted volume, %inaccuracy, standard deviation and %CV for all channels as shown in Table 3. The overall %CV is 3.96 % and %inaccuracy is -4.03 %, both of which fall within the 10 % threshold.

Table { SEQ Table * ARABIC } Run statistics calculated for 50 µL tips pipetting 1 µL from the data in Table 1, showing the overall mean volume, %inaccuracy and %CV for all channels.

Target volume (µL)	1
Target solution	Range D
Number of data points per channel	12
Mean volume for all channels (µL)	0.95966
%inaccuracy for all channels	-4.03
Standard deviation for all channels (µL)	0.03801
%CV for all channels	3.96
%inaccuracy pass/fail limit	10
%CV pass/fail limit	10
Status based on channel results	Passed

Status based on run statistics	Passed

According to these results, the STARlet has passed the verification of 50 µL CO-RE tips pipetting 1 µL based on both channel results and run statistics.

50 µL CO-RE Tips at 50 µL

An ARTEL verification of the STARlet C using the 50 µL CO-RE tips to pipette 50 µL produced the results shown in Table 4.

Table { SEQ Table * ARABIC } ARTEL results for pipetting 50 µL using the 50 µL CO-RE tips. All values are in µL. Row A contains the results for Channel 1 and Row H contains the results for Channel 8.

	1	2	3	4	5	6	7	8	9	10	11	12
A	49.69	49.85	49.80	49.77	49.79	49.84	49.76	49.80	49.73	49.81	49.80	50.12
B	49.70	49.76	49.83	49.77	49.82	49.83	49.85	49.82	49.84	49.82	49.81	49.75
C	49.66	49.78	49.82	49.77	49.78	49.85	49.80	49.86	49.78	49.86	49.70	49.82
D	49.68	49.75	49.81	50.20	49.85	49.83	49.80	50.13	49.89	49.80	49.82	49.79
E	49.70	49.84	49.83	49.81	49.78	49.84	49.84	49.93	49.81	49.83	49.80	49.83
F	49.65	49.69	49.77	49.65	49.74	49.68	49.72	49.67	49.67	49.70	49.71	49.65
G	49.60	49.67	49.68	49.69	49.70	49.81	49.75	49.79	49.79	49.76	49.67	49.71
H	49.73	49.72	49.78	49.66	49.78	49.77	49.78	49.78	49.82	49.77	49.86	49.81

The data in Table 4 was used to calculate the mean pipetted volume, %inaccuracy, standard deviation and %CV for each channel as shown in Table 5. From these values, each individual channel has performed within the 5 % threshold and resulted in a pass.

Table { SEQ Table * ARABIC } Channel statistics for 50 µL tips pipetting 50 µL calculated from the data in Table 4.

Channel	Mean Volume	% inaccuracy	Standard Deviation	%CV	Status
1	49.813	-0.37	0.106	0.21	Passed
2	49.800	-0.40	0.045	0.09	Passed
3	49.790	-0.42	0.061	0.12	Passed
4	49.863	-0.27	0.151	0.30	Passed
5	49.820	-0.36	0.052	0.10	Passed
6	49.692	-0.62	0.038	0.08	Passed
7	49.718	-0.56	0.062	0.12	Passed
8	49.772	-0.46	0.051	0.10	Passed

The data in Table 4 was used to calculate the mean pipetted volume, %inaccuracy, standard deviation and %CV for all channels as shown in Table 6. The overall %CV is 0.19 % and %inaccuracy is -0.43 %, both of which fall within the 5 % threshold.

Table { SEQ Table * ARABIC } Run statistics calculated for 50 µL tips pipetting 50 µL from the data in Table 4, showing the overall mean volume, %inaccuracy and %CV for all channels.

Target volume (µL)	50
Target solution	Range A
Number of data points per channel	12
Mean volume for all channels (µL)	49.783
%inaccuracy for all channels	-0.43
Standard deviation for all channels (µL)	0.093
%CV for all channels	0.19
%inaccuracy pass/fail limit	5
%CV pass/fail limit	5
Status based on channel results	Passed
Status based on run statistics	Passed

According to these results, the STARlet has passed the verification of 50 µL CO-RE tips pipetting 50 µL based on both channel results and run statistics.

Acceptance Criteria

As per NATA requirements, the pipetting accuracy of the STARlet C must be assessed against the following acceptance criteria for Forensic DNA Analysis POVAs: %CV and %inaccuracy of +/- 5 % (10 % for volumes ≤10 µL).

The results for 50 µL CO-RE tips tested at 1 µL and 50 µL passed these acceptance criteria and this ARTEL verification confirms the pipetting accuracy of the STARlet C.

Experiment 2: Plate Seal Assessment

Purpose

To assess and select a plate seal suitable for use with the STARlet for CE preparation by testing a set of seals for freezer storage, piercing on the STARlet and PCR thermalcycling.

Method

1. Sample selection –
   The following seals were tested:
   - X-Pierce™ Films for Automation (Excel Scientific)
   - 4titude® Pierceable Seal (4titude Pierceable)
   - ThinSeal™ Films for ELISA, Incubation & Storage (ThinSeal)
   - EZ-Pierce™ Films for ELISA, Incubation & Storage (EZ-Pierce)
   - AlumaSeal II™ Foils for PCR & Cold Storage (AlumaSeal II)
   - PlateMax™ Aluminum Sealing Film (PlateMax)
   - Eppendorf® PCR clean PCR Foil, self-adhesive (Eppendorf PCR)
   - 4titude® PCR Foil Seal Strong (4titude Foil)
   - Hamilton® Foil Plate Seal (Hamilton® Foil)

2. Assessment of frozen storage:

Seals were applied to SSI PCR plates and placed in the chest freezer and checked after one month of storage.

3. Assessment of piercing on the STARlet:
   Seals were applied to SSI PCR plates and pierced on the STARlet using 10 µL and 50 µL tips.

4. Assessment of PCR:
   PCR seals were applied to a soccerball test plate containing six amplification controls, six extraction positive controls and 33 negative amplification controls according to Figure 1. Each test plate was prepared and amplified according to QIS 34052 using with the AlumaSeal, PlateMax and Hamilton® Foil seals. The amplified products were then processed through CE and analysed using the GeneMapper® *ID-X* software according to QIS 34062 and QIS 34112. The profiles were assessed for concordance and quality, while the blank positions were checked for evidence of cross-contamination.

Figure { SEQ Figure * ARABIC } Example of amplification plate layout for testing PCR foil seals. 2800M control was amplified in positions A01, E01, C03, G03, A05 and E05. Extracted positive control has been amplified in positions C01, G01, A03, E03, C05 and G05.

Results and Discussion

Currently within Forensic DNA Analysis, plastic seals are used to seal 96-well half skirted PCR plates for PCR, these are then removed for CE preparation and a new plastic seal is applied for long-term freezer storage. Each time a sample on the plate requires Re-CE, the plastic seal is again removed and a new one applied following processing. This practice carries a risk of sample to sample cross-contamination during the removal of a seal, thus requiring very careful and somewhat difficult manual handling.

This experiment aimed to identify a seal or sealing practice that will reduce the risk of cross-contamination. The ideal seal would be suitable for PCR, could be pierced during CE preparation on the STARlet and re-sealed for long-term freezer storage.

X-Pierce and 4titude Pierceable are plastic films with an 'X' pre-cut across each of the 96 wells. These films are not suitable for PCR and require a separate plastic seal for storage. The Excel Scientific X-Pierce films performed well for piercing with the STARlet using 10 µL and 50 µL tips, however a clamp would be required to ensure the plate would not lift if the seal caught on a tip (when processing on the STARlet). The 4titude® films did not perform well for piercing with the STARlet, as the tips frequently caught on the seal and lifted the plate. As shown in Table 7, both these seals were rejected.

ThinSeal™ and EZ-Pierce™ are plastic seals and after one month in the freezer, both these seals had warped and partially lifted from the plate and are therefore not fit for purpose.

Neither of these seals performed well for piercing with the STARlet, as the adhesive stuck to the tips causing the plate to lift and the pierced edges of the seal to pull out of the well as the tips were manually removed. As shown in Table 7, both these seals were rejected.

The 4titude® Foil seal did not perform well for piercing with the STARlet using 10 µL and 50 µL tips, as the strong adhesive stuck to the tips and required moderate force to remove the sealed plate from the tips. Observation of the behaviour of the adhesive with the tips indicated that there would always be a 'sticking' risk when trying to pierce this seal. As shown in Table 7, this seal was rejected.

Eppendorf PCR is a foil seal. This seal was acceptable for storage in the freezer and appeared to perform well for piercing with the STARlet using 10 µL and 50 µL tips. However, aspirating and dispensing was not consistent and foil "plugs" were observed within the tips and within the wells of the plate. This seal was not tested for PCR at this time.

AlumaSeal II™, PlateMax™ and Hamilton® Foil are all foil seals. All three of these seals were acceptable for performing PCR and for storage in the freezer. The PCR for each plate resulted in the expected profiles with no evidence of cross-contamination. None of these seals performed well for piercing with the STARlet using 10 µL and 50 µL tips, as the foil often 'plugged' the end of the tip which interferes with pipetting.

Testing of four foil seals for piercing directly on the STARlet using 10 µL and 50 µL tips demonstrated that direct piercing of a seal and aspiration of liquid is not a reliable procedure. While many contributing factors are likely to be involved, such as change in internal tip pressure due to the impact of piercing the seal, the most obvious issue in this testing is the foil "plugging" the tip. The vendor did suggest a work-around by pre-piercing the seals multiple times around each well, however this strategy would be considerably time-consuming and was rejected as a potential fix for this issue.

Due to these results, the criteria to pierce the seals using the tips on the STARlet during aspiration was rejected and replaced with the ability for the seal to be pierced prior to placement on the STARlet. In this case, as shown in Table 7, the AlumaSeal II™, PlateMax™ and Hamilton® Foil were accepted as potential seals for use within Forensic DNA Analysis. The Eppendorf PCR seal was tentatively accepted as the PCR function was not tested, however the Project Team expected this seal to pass.

Table { SEQ Table * ARABIC } Results of testing of seals. Boxes marked with N/T indicate that the seal was "Not Tested" for the specific quality.

Seal Name	X-Pierce™ Films for Automation	4titude® Pierceable Seal	ThinSeal™ Films for ELISA, Incubation & Storage	EZ-Pierce™ Films for ELISA, Incubation & Storage	4titude® PCR Foil Seal Strong	AlumaSeal II™ Foils for PCR & Cold Storage	PlateMax™ Aluminum Sealing Film	Eppendorf® PCR clean PCR Foil, self-adhesive	Hamilton® Foil Plate Seal
Brand	Excel Scientific	4titude	Excel Scientific	Excel Scientific	4titude	Excel Scientific	Axygen	Eppendorf	Hamilton®
Material	Plastic X-cut	Plastic X-cut	Plastic	Plastic	Foil	Foil	Foil	Foil	Foil
Freezer	Requires separate seal	Requires separate seal	Fail	Fail	N/T	Pass	Pass	Pass	Pass
Pierce	Pass - Requires clamp to retain plate	Fail	Fail	Fail	Fail	Fail	Fail	Fail	Fail
PCR	Not Applicable	Not Applicable	N/T	N/T	N/T	Pass	Pass	N/T	Pass

Overall Result	Reject	Reject	Reject	Reject	Reject	Accept	Accept	Tentative Accept	Accept

During this project, the Hamilton® Automated Plate Sealer was purchased for use within Forensic DNA Analysis for re-sealing PCR amplification plates after prepping on the STARlet C. The vendor has only supported the use of the Hamilton® Foil seals with this instrument (seals are loaded in a cartridge format).

Given the Hamilton® Foil seals have been assessed by the Project Team as an acceptable seal for use within Forensic DNA Analysis, further testing was only performed using this seal. The original PCR plate prepared for testing the Hamilton® foil seal had also been re-sealed using another Hamilton® Foil seal after prepping for CE and was stored within the freezer for approximately one month. This PCR plate was thawed and re-prepped for CE on the STARlet C instrument. This final experiment resulted in all the expected profiles with no evidence of cross-contamination.

Acceptance Criteria

Three seals (AlumaSeal II™, PlateMax™ and Hamilton® Foil) met the following acceptance criteria:

- Suitable for long-term frozen storage.
- Suitable for PCR without evidence of cross-contamination.
- Pierceable prior to preparation on the STARlet.

As such, all three seals can be used with the STARlet, however the project team recommends the Hamilton® Foil Plate Seal to ensure compatibility with the Hamilton® Plate Sealer and ongoing support from the vendor.

Experiment 3: Whole Plate Transfer PowerPlex® 21

Purpose

To develop and verify a protocol for the preparation of whole plate transfer for CE of casework and reference PP21 samples.

Method

Note: Within the Experimental Design, Experiment 3 includes the repeatability and reproducibility, however these experiments have been separated into Experiment 5 for this report.

Development of STARlet Program

A STARlet method for Whole Plate Transfer of casework and reference PCR amplified plates for processing through CE was programmed by BioStrategy in consultation with the Project Team according to Section 4.1 of the Experimental Design.

Verification of STARlet Whole Plate Transfer Program

The three CE plates listed below were prepared on the STARlet and processed through CE to compare allele concordance and quality of the STARlet prepared profiles to the original manually prepared profiles (as per Section 5.2 of the Experimental Design).

- CCE20180628-01 (Casework)

- RCE20180725-01 (Direct Amplification Reference)
- RCE20180723-03 (Extracted Reference)

DNA Fragment Analysis and Profile Interpretation

All plates were processed through CE and underwent profile interpretation according to Sections 4.4.1 and 4.5 of the Experimental Design. All profiles were checked for concordance with their original run and any indications of cross-contamination.

Data Analysis

Data analysis using qualitative and concordance assessments were performed according to Section 5.2 of the Experimental Design with the exception of the percentage change of the Size Standard. This was not performed as early assessment of this data analysis indicated extreme variation in percentage change that did not correlate with the profile quality. This data was deemed unsuitable by the Project Team.

Number of alleles were compared as well as the core reader comments, thereby including both qualitative and quantitative assessment. Samples that were NSD or NAD were excluded from the analysis.

Results

Development of STARlet Program

The Project Team performed the verification of the protocol, including defining accurate liquid classes for each liquid transfer, assessing required liquid volumes for reagents on the deck, defining the information required on batch plate map files and managing error controls throughout the program.

See Figure 2 for the STARlet deck setup for the Whole Plate Transfer program.

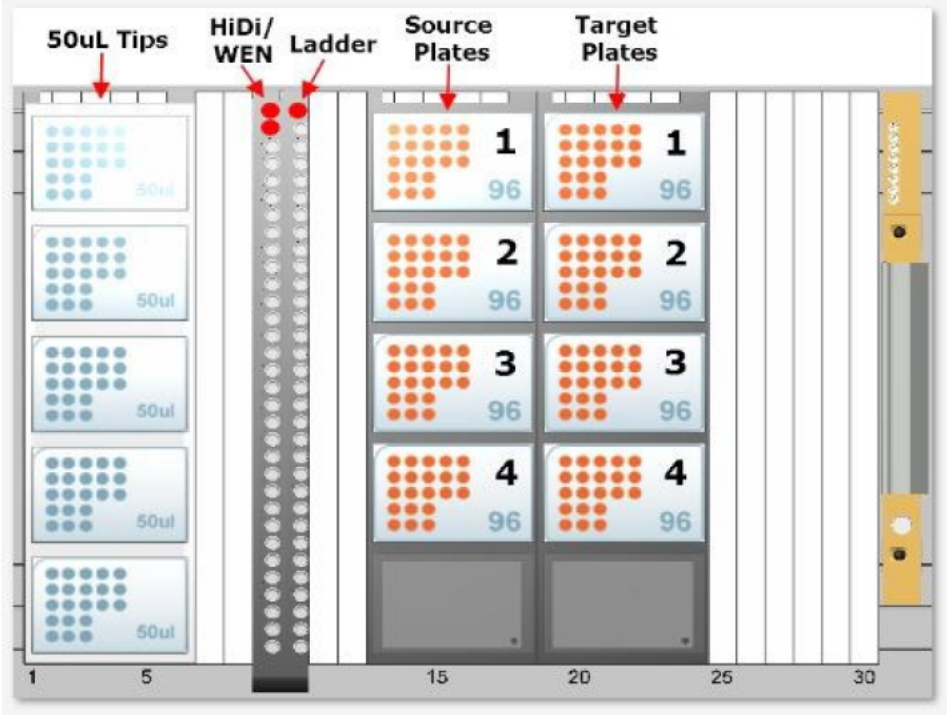

Figure { SEQ Figure * ARABIC } STARlet deck setup for whole plate transfer. The numbers on the Source Plate carrier correlate to those of the Target Plate carrier where, for example, PCR product

from Source Plate 1 must be transferred to Target Plate 1. The sequence checking function using the STARlet barcode reader ensures that the correctly labelled Source and Target plates are in each position of the carriers.

Sequence Checking

Checking the order of plates in the Source and Target Plate carriers on the STARlet deck is a crucial element of the Whole Plate Transfer program. Sequence checking each plate label on both carriers is performed using an integrated barcode reader. Forensic Register creates a platemap with the source plates, target plates and volumes. This file is uploaded to the STARlet which then scans the plates as they are loaded and compares the barcodes to the file. When a discrepancy arises between the labelled plates on the carriers with the barcodes expected by the program according to the platemap/s, the run is paused and the user is notified by an error message

If there is a sequence mismatch on the Source Plate carrier, an error will flag like the example in Figure 3.

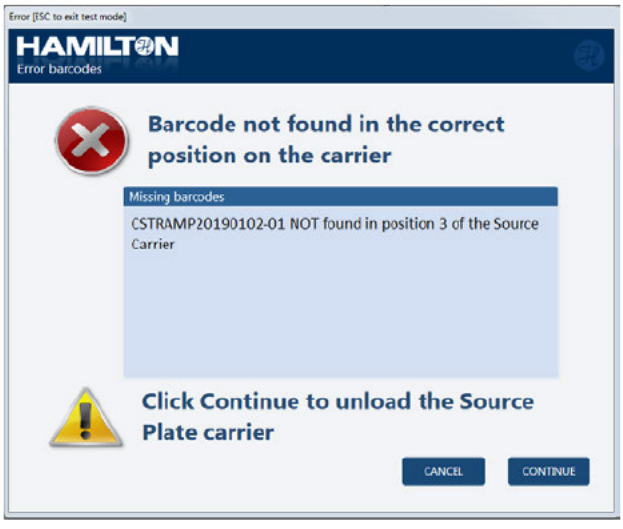

Figure { SEQ Figure * ARABIC } Whole Transfer Program Source Plate Error. This error specifies the position on the Source Plate carrier that has the incorrect plate.

The STARlet unloads the Source Carrier and provides a user prompt to load the correct plate into the correct position on the Source Carrier, like the example in Figure 4.

Figure { SEQ Figure * ARABIC } Whole Transfer Program Source Plate Error. This user prompt specifies the expected plate barcode and position which must be corrected.

Once the program is resumed, the barcode scanner will perform the sequence check of the Source Plate carrier again. The same process is applied to the Target Plate carrier and the program will not continue until all expected source and target plates are in the correct carrier positions.

Pipetting Accuracy

The pipetting of PCR product from Casework, Reference and FTA amplification plates was assessed by the Project Team and defined using the Liquid Class function of the Venus software. Of particular concern was whether the FTA card punch within the wells of the FTA amplification plates would interfere with accurate aspiration of PCR product. Within the program, PCR product is preferentially aspirated from 1 mm below the liquid level detected within the well and does not make contact with the FTA card punch in the bottom of the well.

However, in instances where the liquid level is too low for the instrument to detect, the aspiration must occur from the bottom of the well. In all instances observed of aspiration from the bottom of a well containing an FTA punch, the card did not interfere with pipetting accuracy.

Verification of STARlet Whole Plate Transfer Program

When interpreting the results of this experiment, it is important to consider that the pipetting %inaccuracy of the STARlet has been verified at ±10 % at 1uL, and while the handheld multi-channel pipette has also been verified according to that criteria manual preparation is more susceptible to well to well variation. Additionally, slight differences between each CE run contribute to variation between results.

CCE20180628-01

Casework plate CCE20180628-01 was prepared on the STARlet and processed through CE on the 3130*xl*. These results were compared to those of the manually prepared run. All samples were fully concordant and negative controls were NSD. Table 8 shows there were 88 samples available for comparison on this plate. Of these samples, 27 % had no change in

number of alleles and 18 % had less alleles above LOR on the STARlet prepared run. However, 55 % had more alleles above LOR on the STARlet prepared run.

Table { SEQ Table * ARABIC } Casework whole plate transfer qualitative assessment results.

CCE20180628-01 – STARlet vs Manual		
TOTAL SAMPLES FOR COMPARISON	88	100%
TOTAL SAMPLES WITH NO CHANGE IN # ALLELES	24	27%
SAMPLES WITH MORE ALLELES ON STARlet PREPARED RUN	48	55%
SAMPLES WITH LESS ALLELES ON STARlet RUN	16	18%
SAMPLES WHERE CORE COMMENT CHANGED*	3	3%

***Note:** All Core Comment changes were due to extra alleles labelling on the STARlet run and changing the comment from SS to MIX or from MIX to COMPLEX. Please keep in mind that interpretation for these Core Comments can be subjective.

For each sample with a reduced number of alleles on the STARlet prepared run, the alleles are clearly visible below LOR on the profile. These alleles were close to LOR on the manually prepared run and this change in allele height (below threshold) is not unexpected. Given the accuracy of the ARTEL verification, this variation may be due to CE variation rather than the pipetting performance of the STARlet. In all instances where fewer alleles were labelled on the STARlet run, the core comment was unchanged.

RCE20180725-01

Direct Amplification Reference plate RCE20180725-01 was prepared on the STARlet and processed through CE on the 3500xL. The results were compared to those of the manually prepared run. All samples were concordant and negative controls were NSD. Table 9 shows there were 92 samples available for comparison on this plate. Of these samples, 60 % had no change in number of alleles and 14 % had more alleles above LOR on the STARlet prepared run. However, 26 % had less alleles above LOR on the STARlet prepared run.

Table { SEQ Table * ARABIC } Direct amplification reference whole plate transfer qualitative assessment results.

RCE20180725-01 – STARlet vs Manual		
TOTAL SAMPLES FOR COMPARISON	92	100%
TOTAL SAMPLES WITH NO CHANGE IN # ALLELES	55	60%
SAMPLES WITH MORE ALLELES ON STARlet PREPARED RUN	13	14%
SAMPLES WITH LESS ALLELES ON STARlet PREPARED RUN	24	26%
TOTAL REWORKS ON MANUAL PREPARED RUN	36	39%
TOTAL REWORKS ON STARlet PREPARED RUN	37	40%
SAMPLES WHERE CORE COMMENTS CHANGED	17	18%

Of the 17 samples that had a core comment change on the STARlet run, four were caused by CE running issues, e.g. NAD ReCE or RPT due to broad peaks, and cannot be attributed to STARlet performance. Two of the remaining 13 samples had more alleles on the STARlet run, so the core comment changed from NSD to PP and from PP to OK PP. One sample changed from OK to XS. Five samples changed from XS to OK and five samples from OK to PP. The five samples which changed from OK to PP had on average one or two alleles less than the original run, and these alleles were visible below LOR. These core comment changes produced a total of one additional rework on the STARlet prepared plate compared to the manually prepared plate.

RCE20180723-03

Extracted Ref plate RCE20180723-03 was prepared on the STARlet and processed through CE on the 3500xL. Due to reagent preparation miscalculation, the last folder was not used for comparison as the HiDi levels were not optimal.

The results were compared to those of the manually prepared run. All samples were concordant and negative controls were NSD. Table 10 shows there were 46 samples available for comparison on this plate. Of these samples, 50 % had no change in the number of alleles and 22 % had less alleles on the STARlet prepared run. However, 28 % of the samples had more alleles on the STARlet prepared run.

Table { SEQ Table * ARABIC } Extracted reference whole plate transfer results. All comparisons refer to the number of samples on the STARlet prepared CE run unless otherwise stated.

RCE20180723-03 – STARlet vs Manual		
TOTAL SAMPLES FOR COMPARISON	46	100%
TOTAL SAMPLES WITH NO CHANGE IN # ALLELES	23	50%
SAMPLES WITH MORE ALLELES ON STARlet PREPARED RUN	13	28%
SAMPLES WITH LESS ALLELES ON STARlet PREPARED RUN	10	22%
TOTAL REWORKS ON MANUAL PREPARED RUN*	17	51%
TOTAL REWORKS ON STARlet PREPARED RUN*	14	42%
SAMPLES WHERE CORE COMMENTS CHANGED	4	8%

***Note:** All reworks on both runs were due to excessive amplification.

Of the four samples that had a core comment change, three changed from EVDRW XS to OK. One sample changed from NOT OK OLs to OK due to a CE running issue and therefore cannot be attributed to STARlet performance. These samples account for the change in reworks.

On the manually prepared run, 51 % of samples required reworking, while on the STARlet prepared run, 42 % of samples required reworking. All reworks from both runs of this plate were due to excessive template for amplification.

Discussion

All three whole plates prepared on the STARlet C were consistent with the manually prepared run and resulted in comparable or more information overall. The programming of the STARlet C includes LIMS integration, barcode sequence checking which reduces the possibility of preparation errors, such as missing ladders or pipetting PCR product into the incorrect column of the CE plate. This will therefore increase the reliability of all CE results and reduce the amount of resources currently used in re-preparing CE plates.

While less allelic information in samples on the casework (18 %) and the direct amplification reference (26 %) plates was observed, this was outweighed by the increased allelic information, or no change in other samples on the same plates (82 % and 74 % respectively). The differences between runs on the direct amplification reference plate resulted in one extra rework sample overall.

The results of the extracted reference plate prepared on the STARlet outperformed those of the manual run due to overall better profile quality and less reworking required due to excessive profiles.

All samples on the casework plate were interpreted by the same reader and only three samples had the Core Comment change due to additional alleles on the STARlet run. These Core Comments can also be subjective and are used only to send the samples to a Profile Data Analysis list for peer review and further interpretation.

As stated previously, when assessing these results, it is important to consider that the variation inherent within the CE procedure and the susceptibility of manual preparation to well to well variation are both contributors to differences observed between profiles prepared on the STARlet C and those prepared manually. The results of Experiment 5 (Repeatability and Reproducibility) support the reliability of the STARlet C in preparing casework, reference and FTA CE plates.

Acceptance Criteria

These results show that preparation of whole CE plates using the STARlet C gives profiles that are concordant and overall comparable to or better than manual preparation. There was no indication of critical assay failure, sub-optimal assay preparation or cross-contamination. The project team recommends that this procedure is implemented within Forensic DNA Analysis for preparing whole casework, direct amplification reference and extracted reference CE plates.

Experiment 4: Re-CE PowerPlex®21

Purpose

To develop and verify a protocol for the preparation of cherry-picked samples for Re-CE of casework and reference PP21 samples.

To perform a contamination check by preparing and analysing a Re-CE plate in a checkerboard format.

Method

Development of STARlet Re-CE Program

A STARlet method for cherry-picking samples from casework or reference PCR amplified plates for reprocessing through CE was programmed by BioStrategy in consultation with the Project Team according to Section 4.1 of the Experimental Design.

See Figure 5 for the STARlet deck setup for the ReCE program.

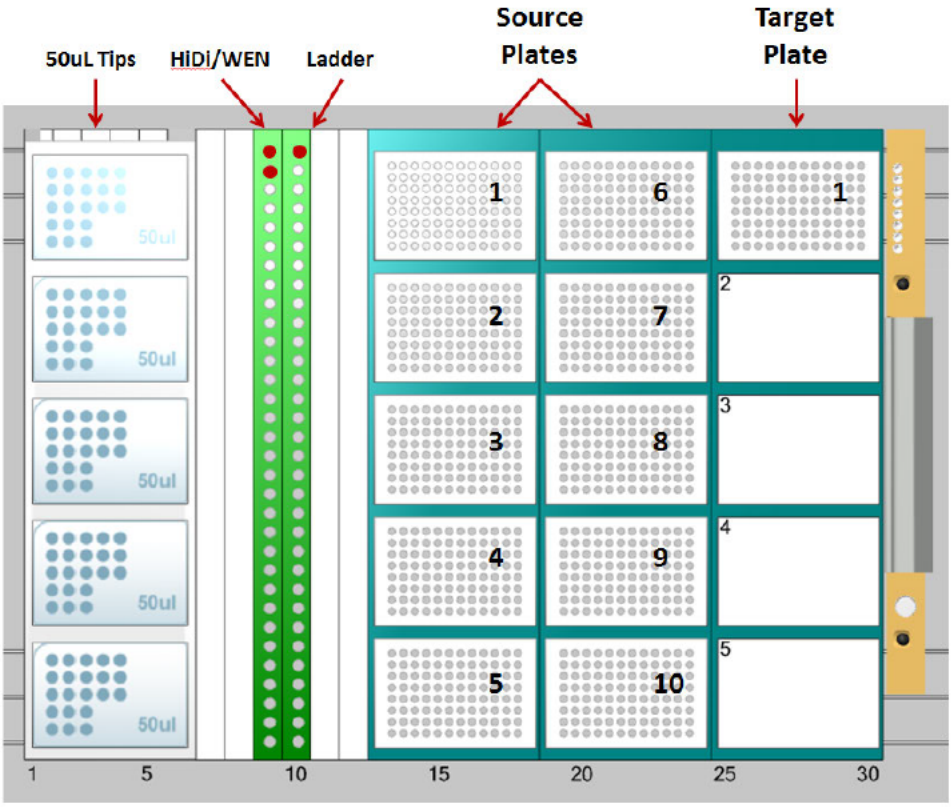

Figure { SEQ Figure * ARABIC } STARlet deck setup for Re-CE. There are two Source Plate carriers that have up to 10 positions. There is one Target Plate carrier and only one Target Plate position. The sequence checking function using the STARlet barcode reader ensures that all of the correctly labelled Source plates and Target plate are on the correct positions on the carriers.

Sequence Checking

Checking the order of plates in the Source and Target Plate carriers on the STARlet deck is a crucial element of the ReCE program. Sequence checking each plate label on both carriers is performed using an integrated barcode reader. Forensic Register creates a platemap with the source plates, target plates and volumes. This file is uploaded to the STARlet which then scans the plates as they are loaded and compares the barcodes to the file. When a discrepancy arises between the labelled plates on the carriers with the barcodes expected by the program according to the platemap/s, the run is paused and the user is notified by an error message.

If there is an issue with the barcodes on the Source Plate carriers, an error is flagged like the example in Figure 6. In this instance, there is an incorrect plate on the carrier. The error message specifies the barcode of the plate that needs to be removed from the carrier and the barcode of the plate that is missing from the carrier.

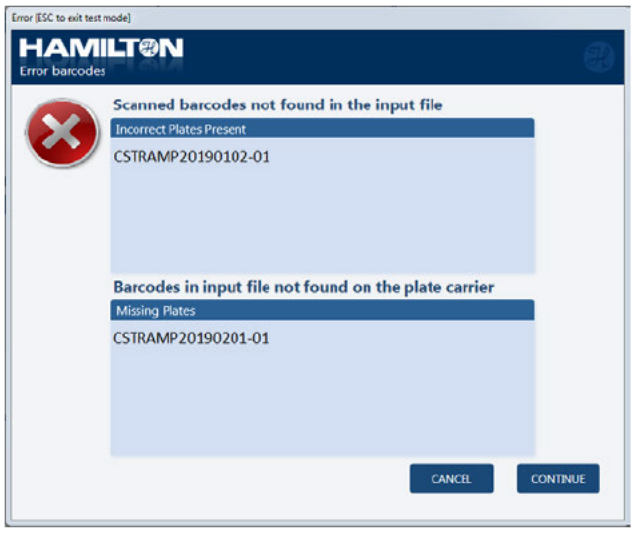

Figure { SEQ Figure * ARABIC } Re-CE Program Source Plate Error. In this example, there is an incorrect plate on the Source Plate carrier and there is an expected plate missing from the carrier.

The STARlet will then unload the Source Carriers and prompt the user to remove the incorrect plate/s and/or load the correct plate/s onto the Source Carrier, like the example in Figure 7.

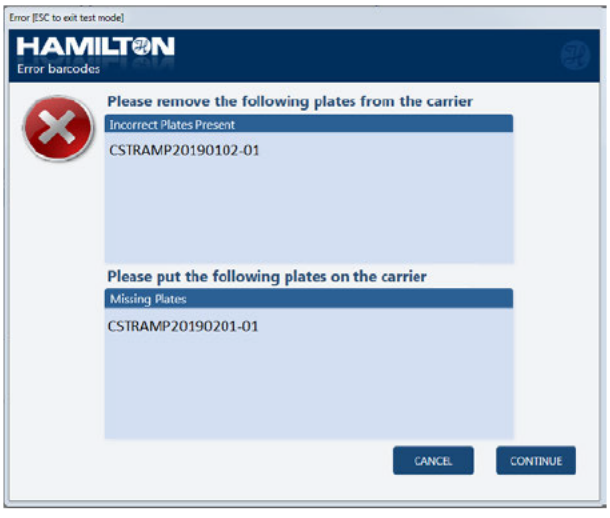

Figure { SEQ Figure * ARABIC } Re-CE Program Source Plate Error. In this example, the prompt specifies which plate must be removed and which plate must be added to the carrier for the program to proceed.

Once the program is resumed, the barcode scanner will perform the sequence check of the Source Plate carriers again. The same sequence checking process is applied to the Target Plate carrier and the program will not continue until all expected source and target plates are in the correct carrier positions.

Verification of STARlet Re-CE Program

To verify the accuracy of the program, ten plates were selected containing Extracted Reference samples that had already been manually prepared and undergone CE as part of routine processing within Forensic DNA Analysis. 45 samples from these ten plates, listed in

Table 11, were re-prepped on the STARlet and processed through CE to verify concordance of the re-prepped profiles to the original profiles.

Table { SEQ Table * ARABIC } Re-CE PowerPlex21 Source Plate and Target Plate Positions.

Source Plate	Source Position	Target Position
RSTRAMP20180621-02	A01	A01
	B01	B01
	E01	D01
	H02	F01
	B03	H01
	E03	E02
	D04	G02
	E04	B03
RSTRAMP20180626-01	B02	C02
	D02	D03
	F02	F03
	B03	H03
	C04	A04
	H04	C04
	B05	E04
RSTRAMP20180702-01	G01	G04
RSTRAMP20180706-01	H02	B05
	F07	D05
	D08	F05
	E08	H05
	A09	A06
	H10	C06
RSTRAMP20180710-01	B02	E06
	C02	G06
	E02	B07
	G02	D07
RSTRAMP20180718-01	D03	F07
	D06	H07
	B06	G08
	G03	B09
	D05	D09
	F01	F09
RSTRAMP20180712-02	C01	G10
RSTRAMP20180712-03	D02	E08
	E03	H09
	C02	A10
	F02	C10
	G01	E10
RSTRAMP20180723-01	D03	B11
	A09	D11
	G07	F11
	C05	H11
RSTRAMP20180725-01	F03	C08
	F01	A12
	H03	C12
	A06	E12
	D04	G12

Reagents and samples were added by the STARlet C to the Re-CE plate according to Table 12.

Please note this platemap was amended from the Experimental Design to run on the 3500xL rather than the 3130*xl*.

Table { SEQ Table * ARABIC } Platemap for Re-CE of REF extracted samples amended for 3500xl (= 45 samples total).

Plate	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos	Ladder 1	Blank	Sample	Ladder 2	Sample	Blank	Ladder 3	Blank	Sample	Ladder 4	Sample
B	Neg	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank
C	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample
D	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank
E	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample
F	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank
G	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample
H	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank	Sample	Blank

A total of 45 samples were cherry-picked from 10 amplification plates, selected so that they were spread randomly across the plates (see Figure 8 below). All selected samples gave full profiles on the first run.

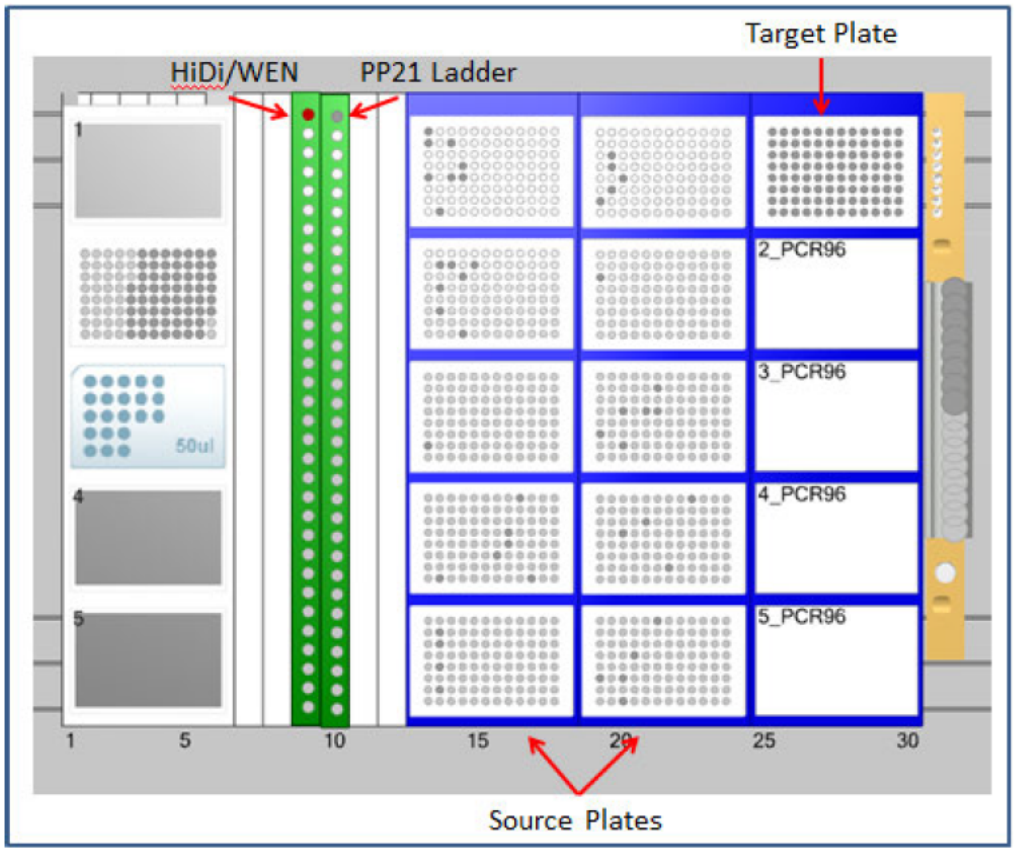

Figure { SEQ Figure * ARABIC } STARlet deck setup for whole plate transfer where the dark grey well positions on the 10 source plates indicate where samples have been selected for addition to the target Re-CE plate.

Results and Discussion

All profiles on the Re-CE plate prepared on the STARlet C were concordant with the expected manually prepared profile and no blanks gave DNA profile results. Table 13 shows the Re-CE STARlet results compared to the original manually prepared run.

Table { SEQ Table * ARABIC } Re-CE results compared to their original manually prepared run.

VCE20180727-01		
TOTAL SAMPLES FOR COMPARISON	46	100%
TOTAL SAMPLES WITH NO CHANGE IN # ALLELES	46	100%
SAMPLES WITH MORE ALLELES ON STARlet PREPARED RUN	0	0%
SAMPLES WITH LESS ALLELES ON STARlet PREPARED RUN	0	0%
SAMPLES WHERE CORE COMMENTS CHANGED	0	0%

Twenty of the samples processed had lower peak heights than the original run, and twenty-six had higher peak heights. These results demonstrate that the STARlet is suitable for preparing a Re-CE plate from up to 10 source plates without error or cross-contamination.

Acceptance Criteria

The Re-CE plate has accurately prepared a checkerboard Re-CE plate of extracted reference samples. Manually preparing these plates has a high risk of error, however the LIMs integration and barcode matching features of the STARlet C significantly decreases the chance of errors. The Project Team recommend that this process can be implemented for preparation of casework, direct amplification reference and extracted reference CE plates within Forensic DNA Analysis.

Experiment 5: Repeatability and Reproducibility

Purpose

The purpose of this experiment is to assess the Repeatability and Reproducibility performance for preparation of CE plates using the STARlet C.

Experiment 5.1 - Repeatability Method

An amplification plate consisting of 12 CTS samples was prepared on the Pre-PCR STARlet and run on the GeneAmp PCR system 9700 CW program according to the plate map in Table 14.

Table { SEQ Table * ARABIC } Plate layout for Amplification and Reproducibility plates.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Amp Pos	Ladder 1	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Amp Neg	Sample 7	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
C	Sample 1	Sample 8	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
D	Sample 2	Sample 9	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
E	Sample 3	Sample 10	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
F	Sample 4	Sample 11	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	Sample 5	Sample 12	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
H	Sample 6	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

The STARlet C prepared the PCR product from the 12 amplified CTS samples into a CE plate seven times according to the plate map in Table 15.

Table { SEQ Table * ARABIC } Plate layout for CE preparation of Repeatability.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Amp Pos	Ladder 1	Sample 2	Ladder 2	Sample 5	Ladder 3	Sample 8	Ladder 4	Sample 11	Ladder 5	Sample 2	Ladder 6
B	Amp Neg	Sample 7	Sample 3	Sample 10	Sample 6	Sample 1	Sample 9	Sample 4	Sample 12	Sample 7	Sample 3	Sample 10
C	Sample 1	Sample 8	Sample 4	Sample 11	Sample 7	Sample 2	Sample 10	Sample 5	Sample 1	Sample 8	Sample 4	Sample 11
D	Sample 2	Sample 9	Sample 5	Sample 12	Sample 8	Sample 3	Sample 11	Sample 6	Sample 2	Sample 9	Sample 5	Sample 12
E	Sample 3	Sample 10	Sample 6	Sample 1	Sample 9	Sample 4	Sample 12	Sample 7	Sample 3	Sample 10	Sample 6	Blank
F	Sample 4	Sample 11	Sample 7	Sample 2	Sample 10	Sample 5	Sample 1	Sample 8	Sample 4	Sample 11	Sample 7	Blank
G	Sample 5	Sample 12	Sample 8	Sample 3	Sample 11	Sample 6	Sample 2	Sample 9	Sample 5	Sample 12	Sample 8	Blank
H	Sample 6	Sample 1	Sample 9	Sample 4	Sample 12	Sample 7	Sample 3	Sample 10	Sample 6	Sample 1	Sample 9	Blank

Experiment 5.1 - Repeatability Results and Discussion

The profiles obtained from the seven replicates for each of the 12 amplified CTS samples were concordant with the expected profile. This indicates the STARlet C is preparing the CE plates as expected.

Allele peak heights from each of the seven replicates were graphed according to dye (Fig 9, 10, 11 and 12). Figure 9 shows an example graph of one allele from one sample and this is the format for all subsequent graphs in Experiment 5.

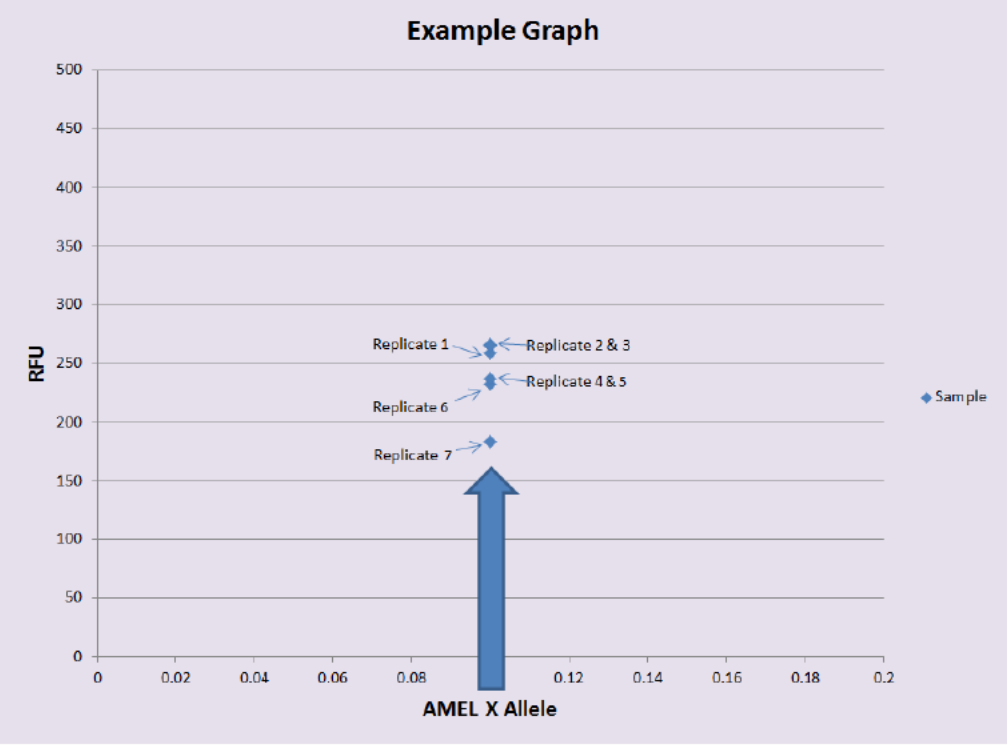

Figure { SEQ Figure * ARABIC } Example graph showing a plot of the RFU height of seven replicates of one allele of the Amelogenin locus (X). In the following graphs for Experiment 5, all replicates of each allele within a sample are plotted on the same x-axis.

The X-axis represents the individual alleles in the profile and is arranged by locus base pair size (smallest to largest i.e. for the green dye - Amel to Penta E). The Y-axis is the RFU of these alleles, please note homozygous alleles have been averaged. As each sample was on the CE plate seven times, there are seven points in each data series. As with Replicates 2 & 3 and 4 & 5 in this example, these replicates are sometimes so similar in height that it is impossible to differentiate between them. Each sample has a unique marker colour and shape. The closer the markers are grouped, the closer the alleles are in RFU.

Each of the twelve samples are represented by unique markers on the graphs in Figures 10, 11, 12 and 13. Although some samples appear to have a wider spread than others this reflects the variability of CE and quality/behaviour of the sample (as the volume of PCR product in the amplification plate decreases for each preparation) rather than the pipetting of the STARlet C which has been proven with the ARTEL verification results. Please note that samples have been manually ordered from left to right - according to allele RFUs - for ease of readability, not for trend analysis.

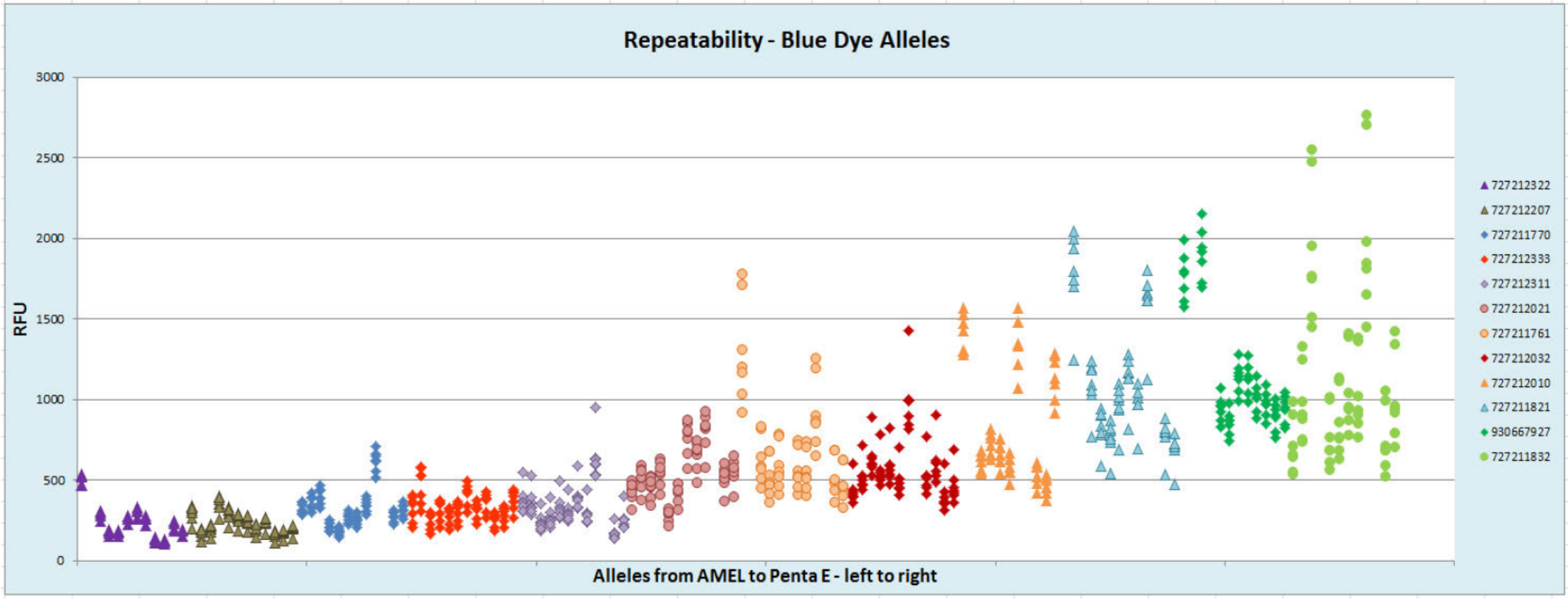

Figure { SEQ Figure * ARABIC } Blue Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

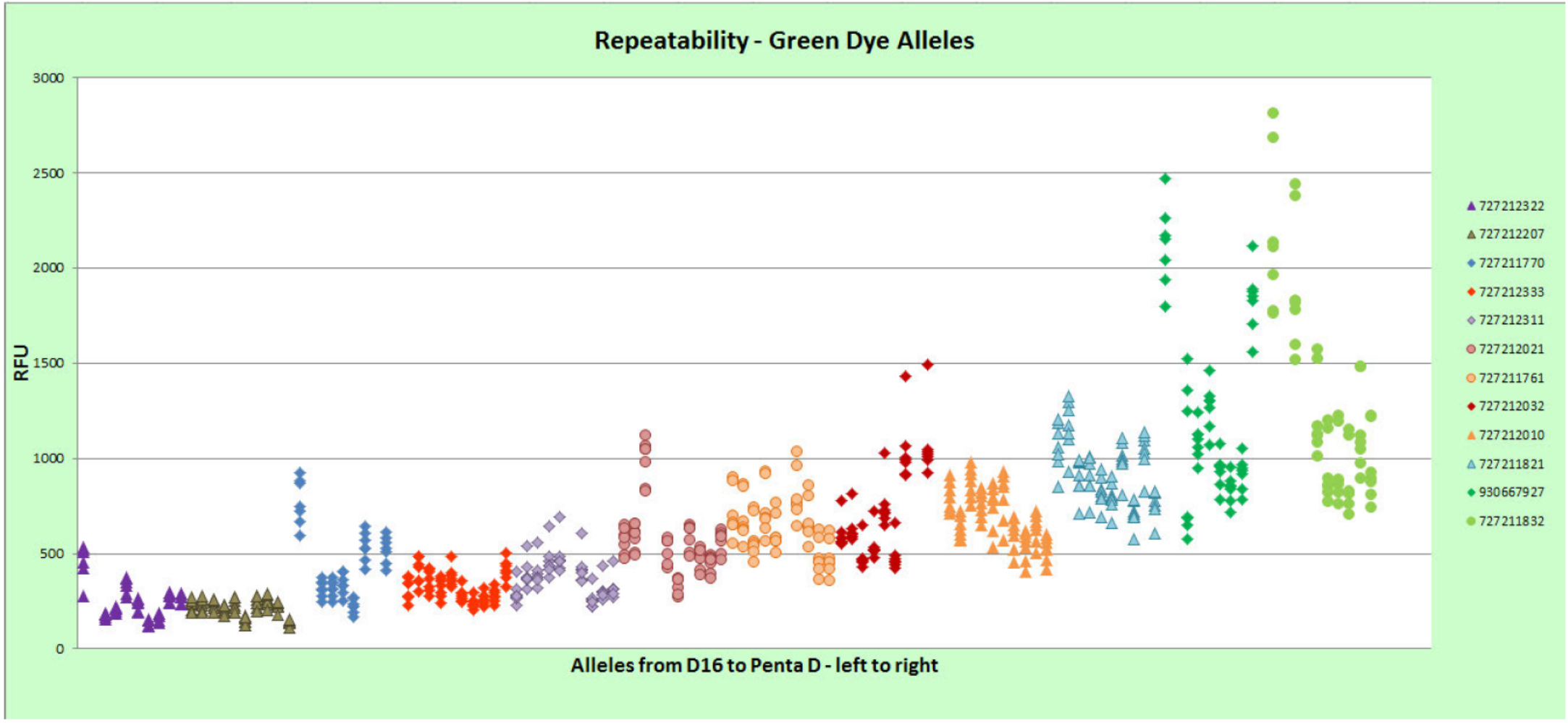

Figure { SEQ Figure * ARABIC } Green Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

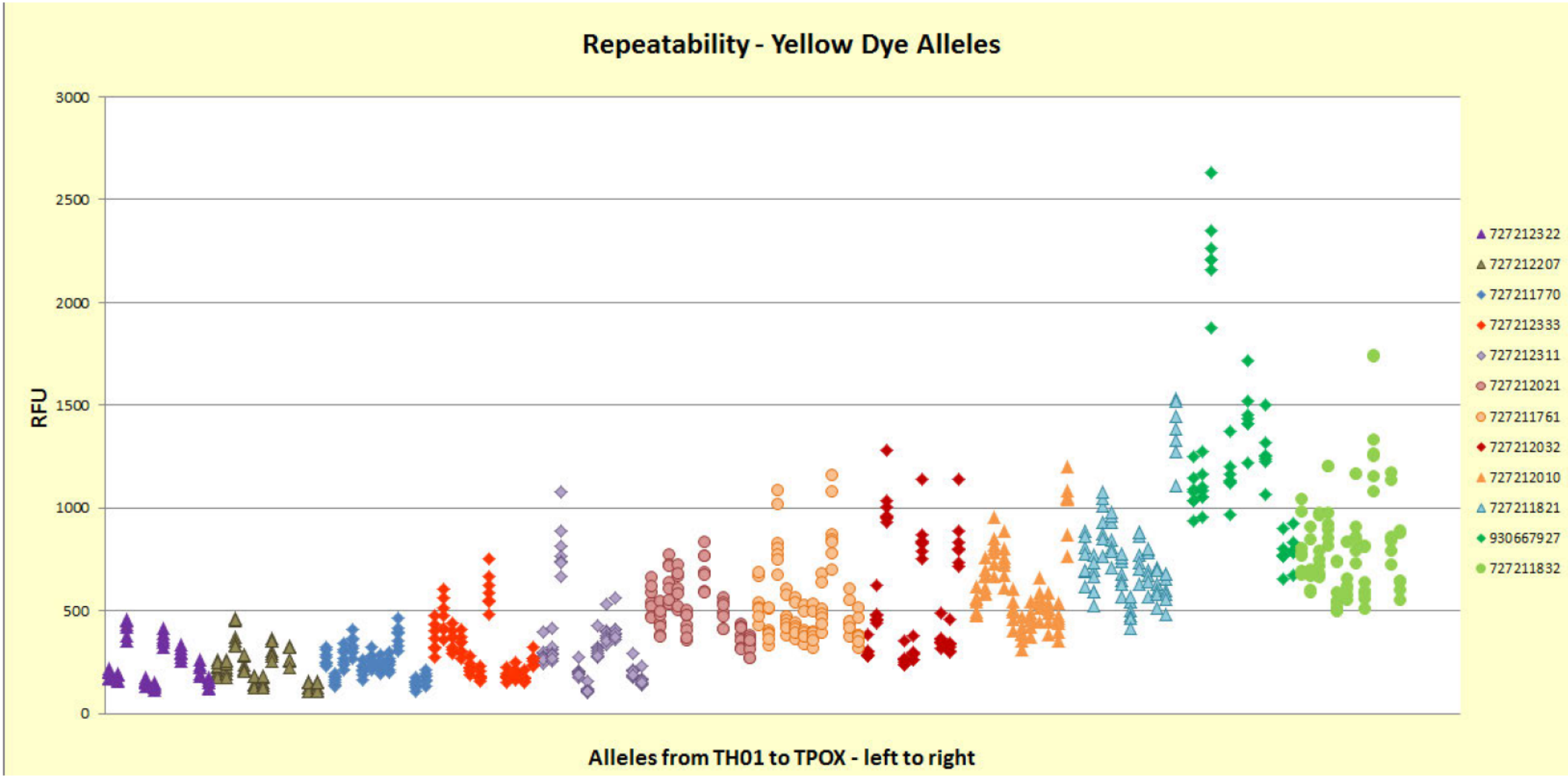

Figure { SEQ Figure * ARABIC } Yellow Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

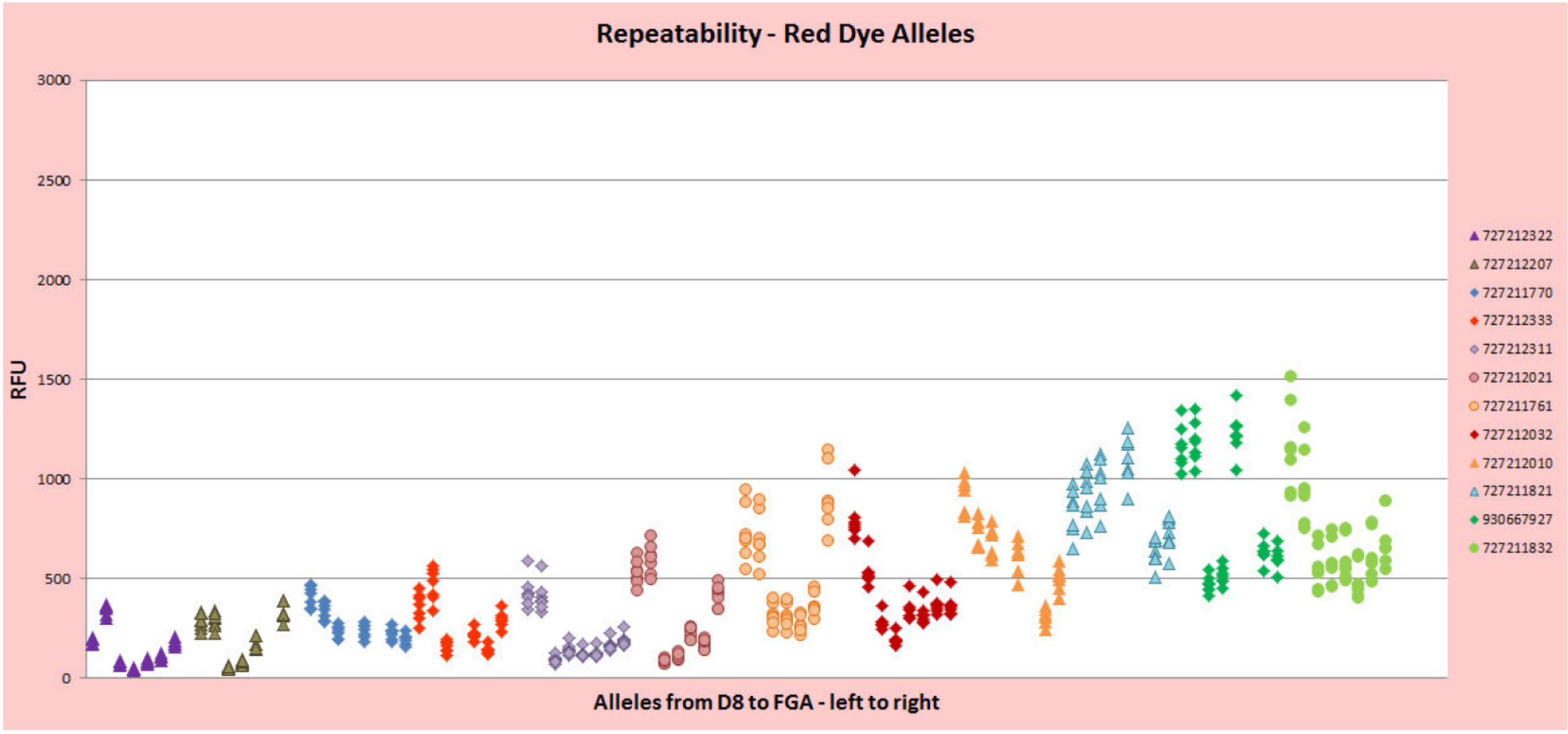

Figure { SEQ Figure * ARABIC } Red Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

Experiment 5.2 - Reproducibility Method

An amplification plate consisting of 12 CTS samples was prepared on the Pre-PCR STARlet and run on the GeneAmp PCR system 9700 CW program according to the plate map in Table 14.

The STARlet C prepared the PCR product from the 12 amplified CTS samples into a CE plate once (i.e. a single replicate for each of the 12 samples) according to the plate map in Table 14. This was performed on each of the five consecutive work days after the Repeatability plate has been prepared, resulting in five Reproducibility plates. To assess reproducibility each sample on the Reproducibility plates (Experiment 5.2) were compared to each other and the plate from Experiment 5.1 (Repeatability).

Experiment 5.2 - Reproducibility Results and Discussion

The profiles obtained from the five reproducibility plates for each of the 12 amplified CTS samples were concordant with the expected profile. This indicates the STARlet C is preparing the CE plates as expected.

Allele peak heights from each of the five replicates were graphed according to dye. Each of the twelve samples are represented by unique markers on the graphs in Figures 14, 15, 16 and 17. Although some samples appear to have a wider spread than others this reflects the quality/behaviour of the sample as the volume of PCR product in the amplification plate decreases for each preparation and CE rather than the pipetting of the STARlet C. Please note that samples have been manually ordered to match the Repeatability graphs for ease of readability, not for trend analysis.

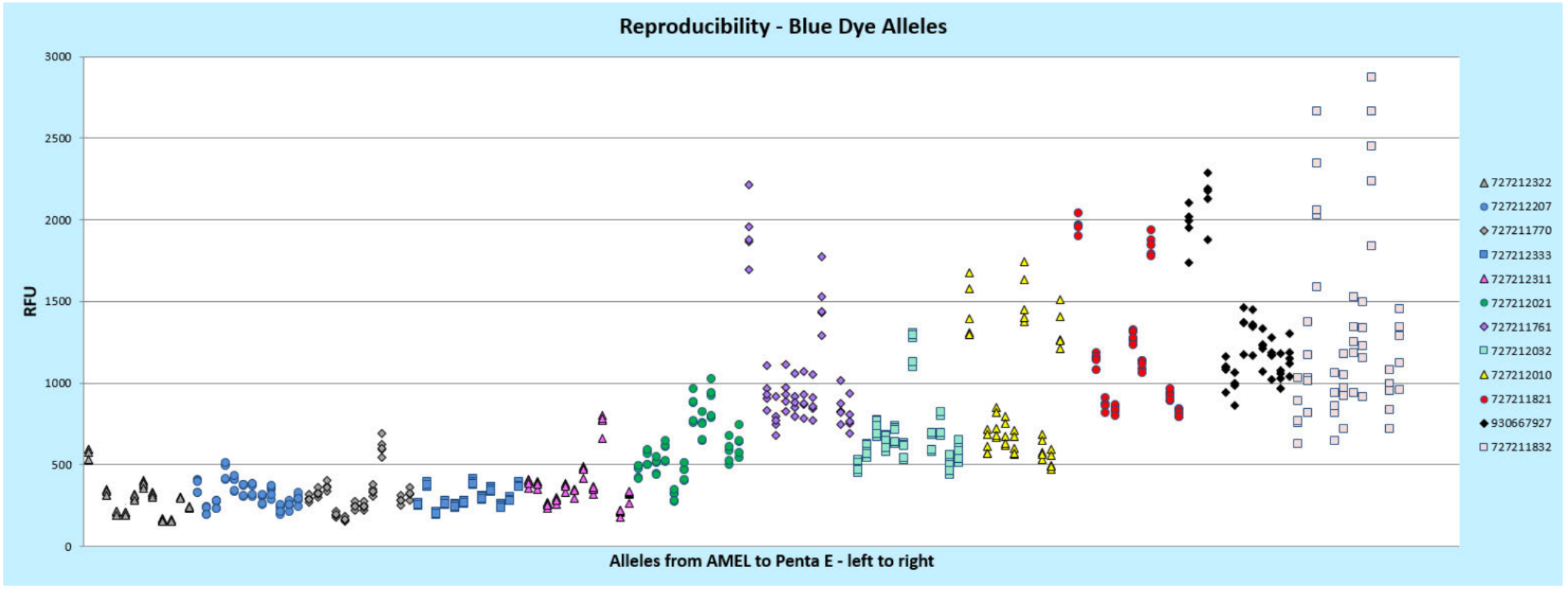

Figure { SEQ Figure * ARABIC } Blue Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

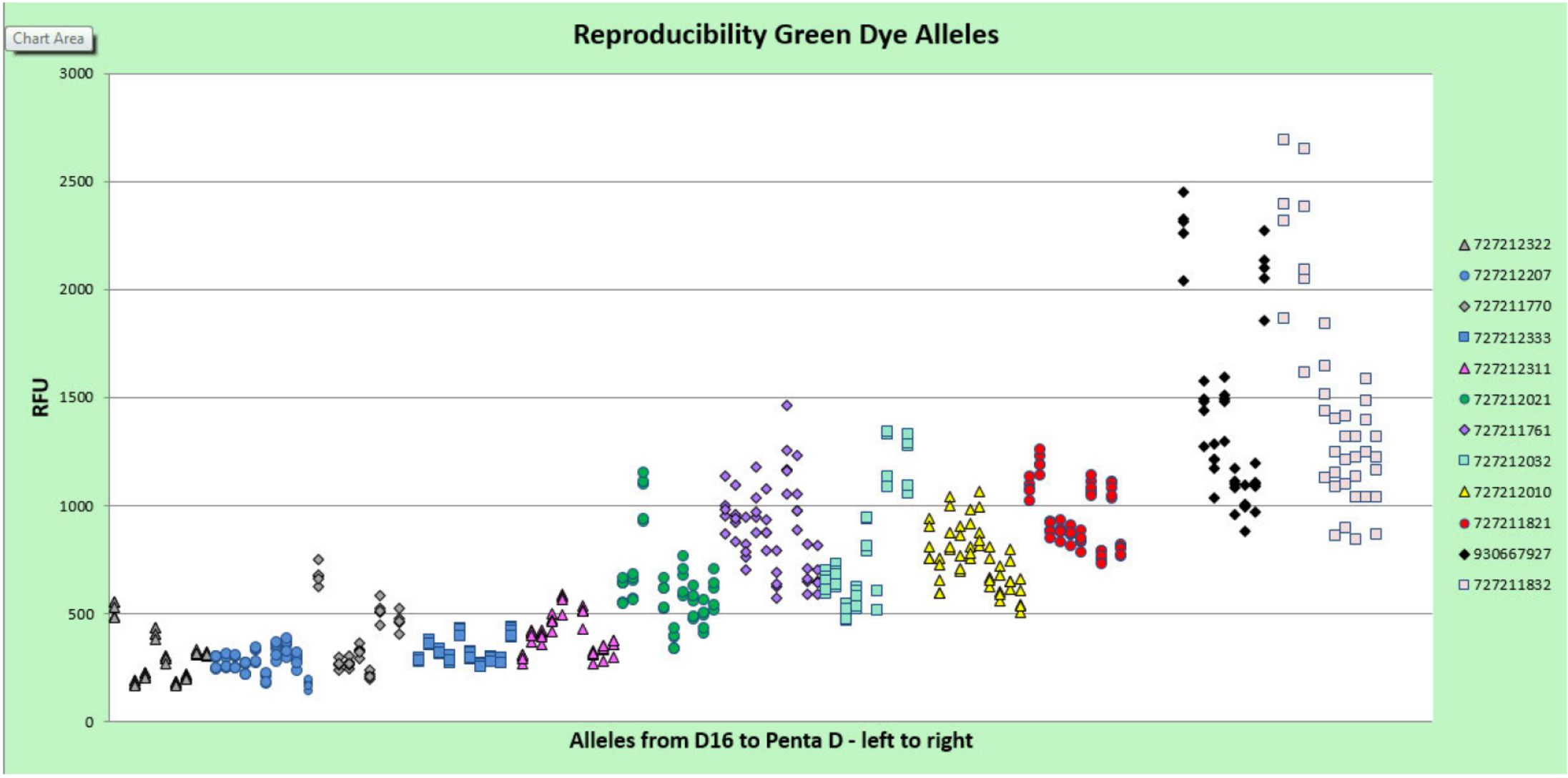

Figure { SEQ Figure * ARABIC } Green Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

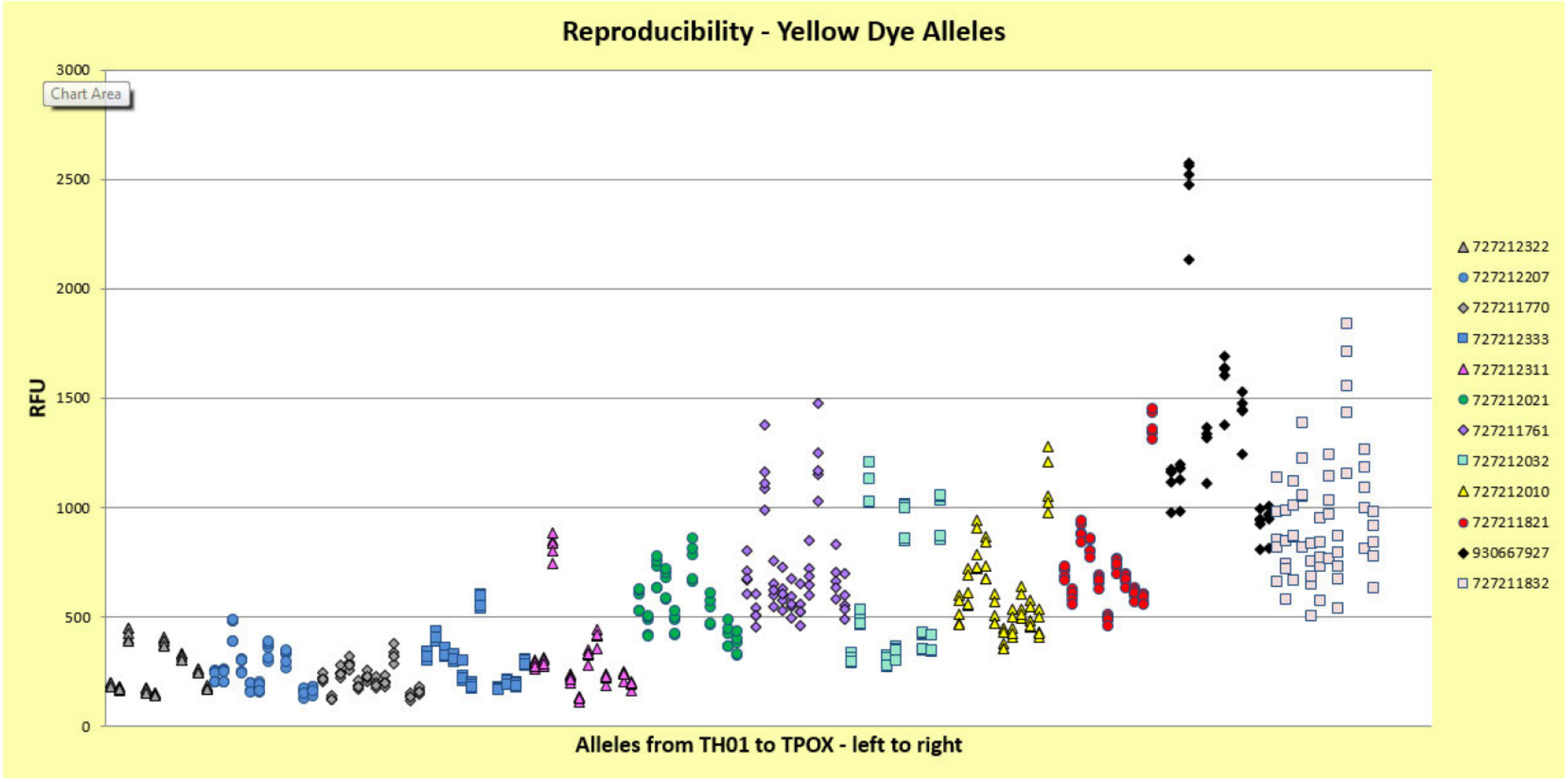

Figure { SEQ Figure * ARABIC } Yellow Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

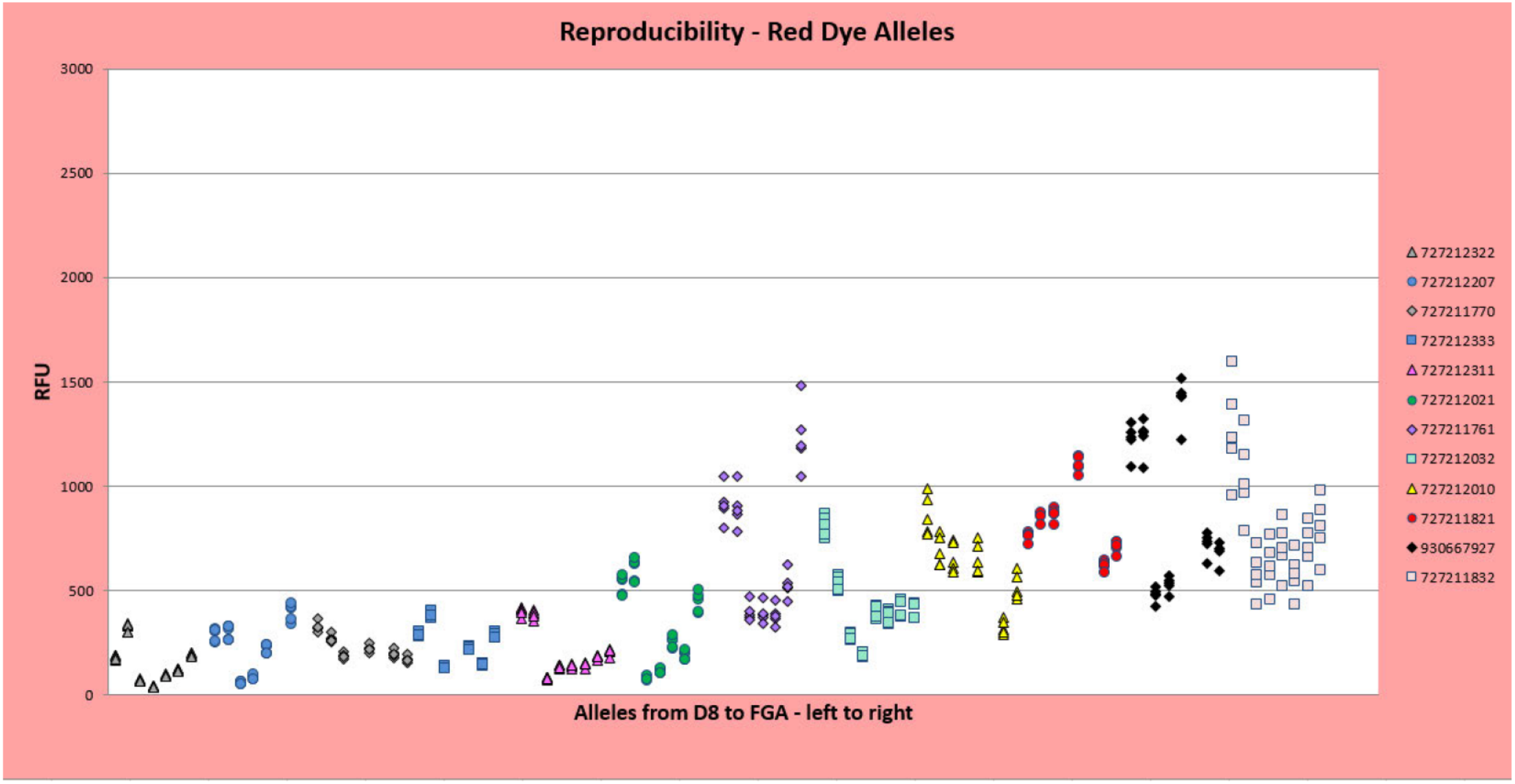

Figure { SEQ Figure * ARABIC } Red Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

Experiment 5 – Combined Repeatability & Reproducibility Results and Discussion

The Repeatability and Reproducibility results were overlaid on graphs in Figures 18, 19, 20 and 21 to compare the allele heights when preparing a sample for CE multiple times on the same day, as well as across multiple days.

Each of the twelve samples are represented by unique markers on the graphs in Figures 14-17. Although there seems to be a general trend of the peak heights being higher in the Reproducibility data sets (blue and grey markers), the spread of heights is very similar to the Repeatability as expected. Higher peak heights on the Reproducibility runs could be caused by the lower volumes of PCR product in the amplification plate after being run multiple times for the Repeatability data and the variability of CE. Allele heights were consistent for both Repeatability and Reproducibility (ie: high peaks on the Repeatability runs, were also high on the Reproducibility runs).

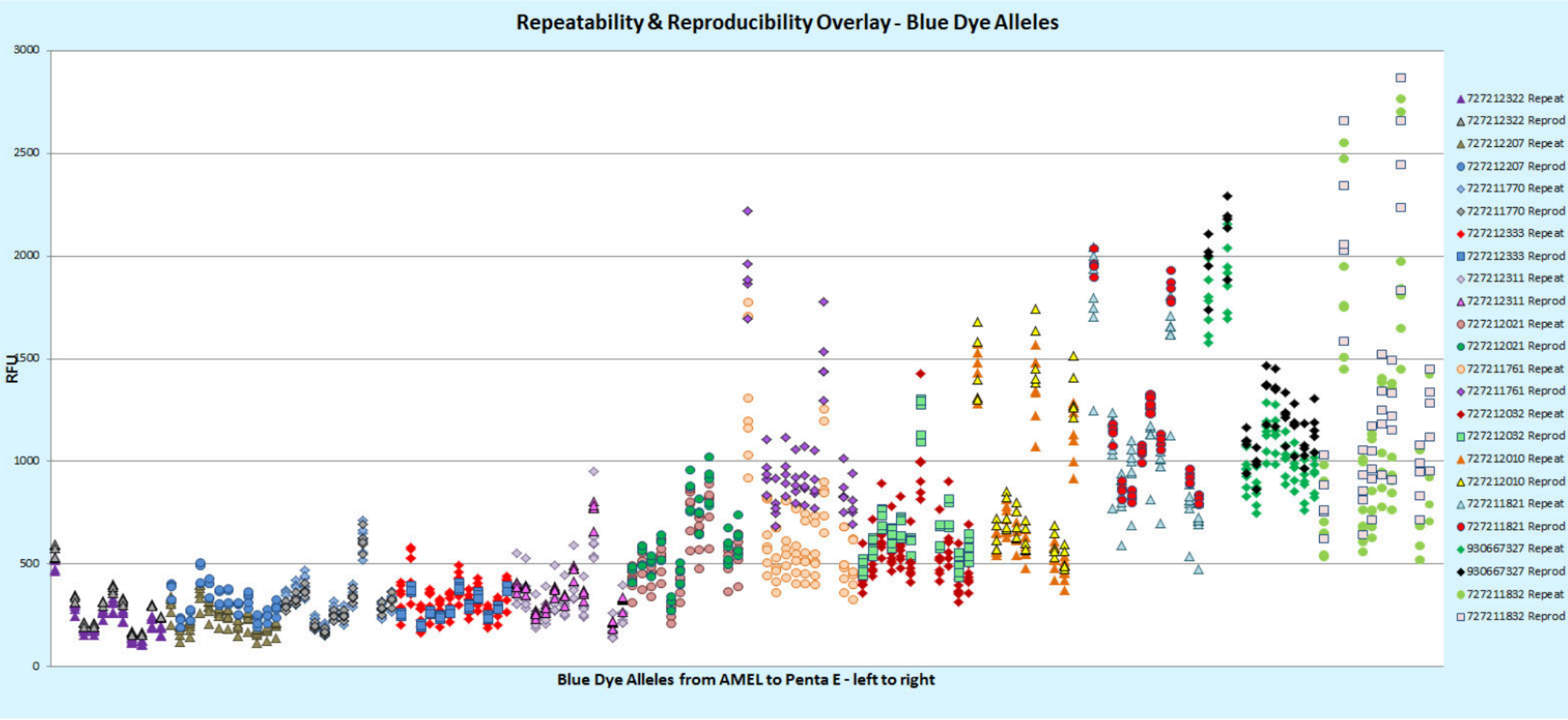

Figure { SEQ Figure * ARABIC } Blue Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

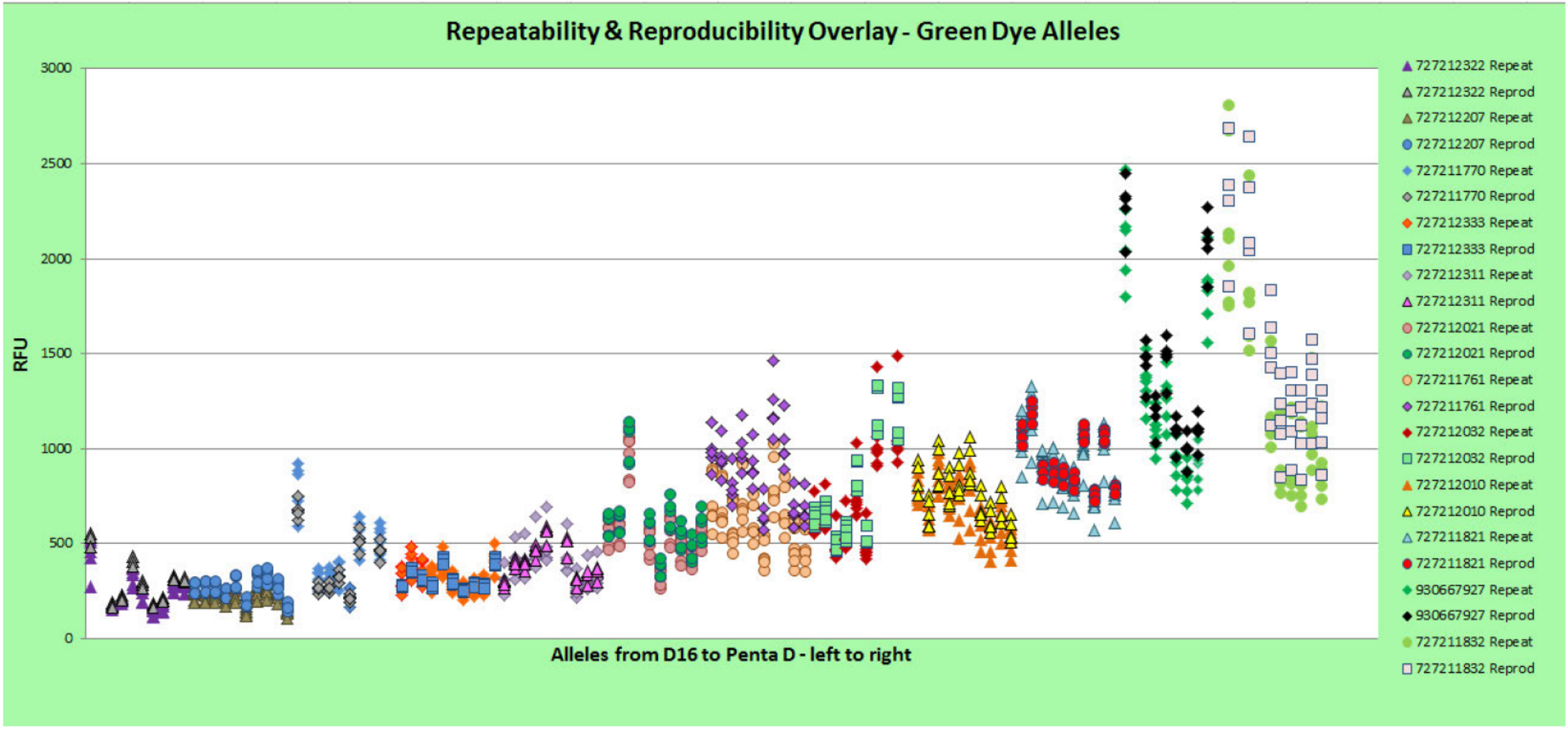

Figure { SEQ Figure * ARABIC } Green Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

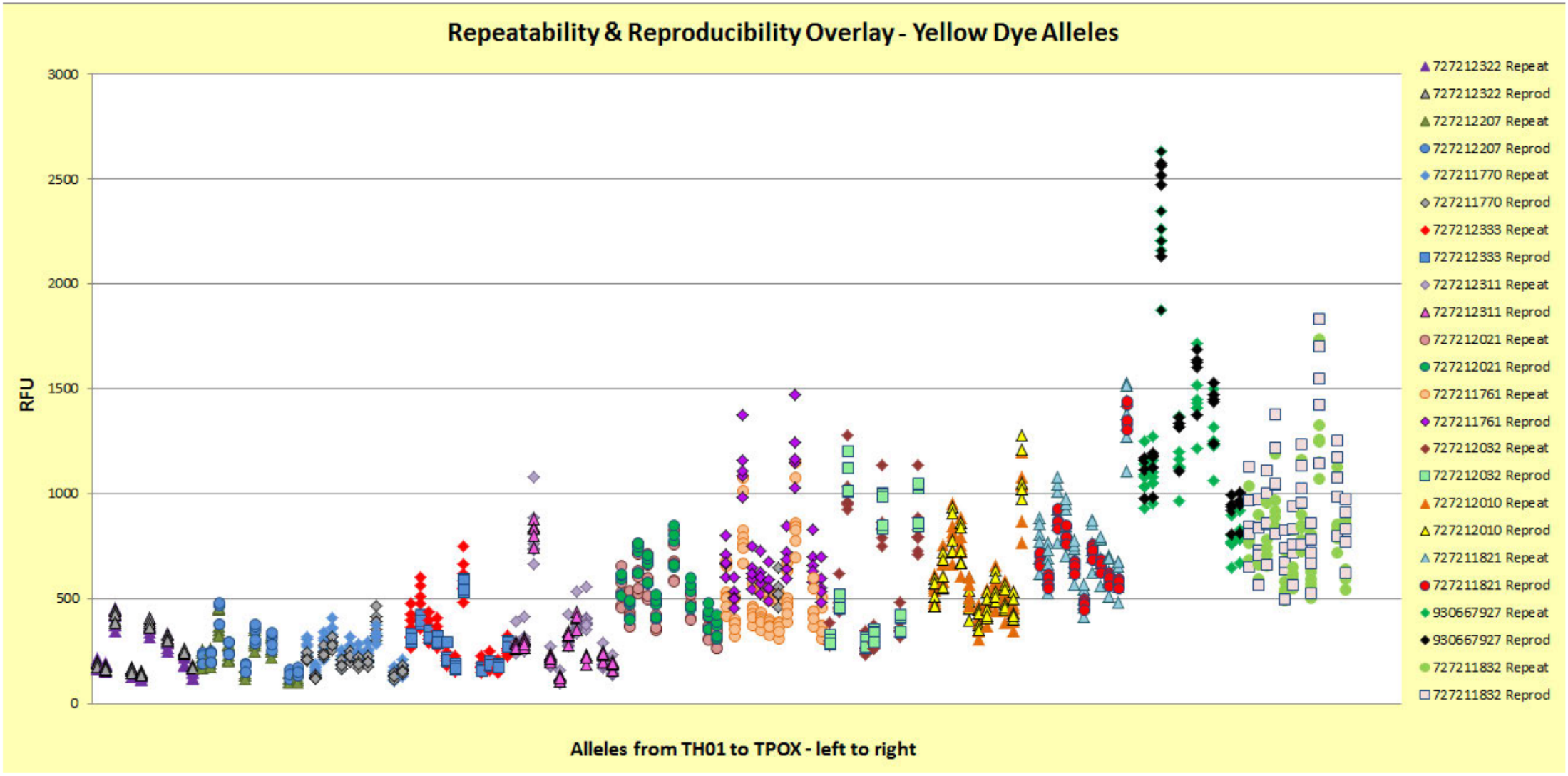

Figure { SEQ Figure * ARABIC } Yellow Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

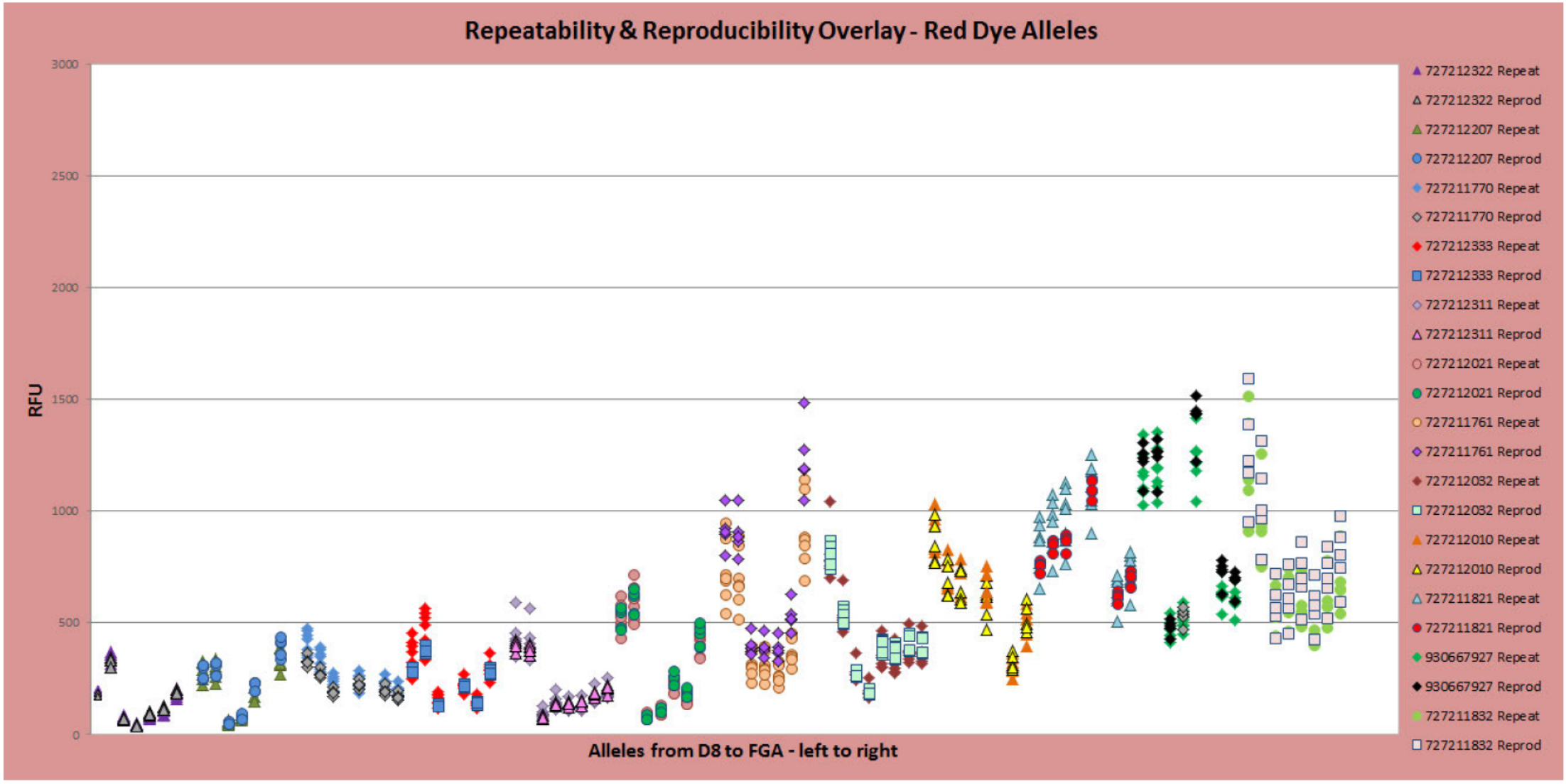

Figure { SEQ Figure * ARABIC } Red Dye Alleles. Allele RFUs have been graphed for each loci from left to right.

Acceptance Criteria

The expected results for this experiment would be grouped data sets for Repeatability and Reproducibility, and that these data sets would overlap. These graphs show grouped data sets which overlap, indicating the STARlet C is performing consistently across plates and days. There were no observed critical failures/differences observed in either Repeatability or Reproducibility data sets which would indicate that the STARlet C is unsuitable for use. The Project Team recommend the STARlet C be implemented for preparation of all casework, direct amplification reference and extracted reference CE plates within Forensic DNA Analysis.

Experiment 6: Plate Piercer Assessment

Purpose

To assess the suitability of the 4titude piercing tool for pre-piercing amplification plates prior to CE preparation on the STARlet.

To assess the risk of cross-contamination between CE preparations by the 4titude piercing tool both with and without a decontamination procedure.

Method

Testing STARlet C pipetting (water plate)

A “dummy” amplification plate prepared with 25 µL of Nanopure water in each well was sealed with a Hamilton® Foil seal and run on the GeneAmp PCR system 9700 CW program. This plate was then pierced with the 4titude piercing tool before loading onto the STARlet C and run through the whole plate transfer method to ensure the piercer produces holes which are compatible with the STARlet.

Testing STARlet C pipetting (FTA plate)

A soccerball FTA punch plate containing buccal samples from two donors was prepared on the BSD and sealed with a Hamilton® Foil seal. See Table 16 for the soccerball sample layout. This plate was run on the GeneAmp PCR system 9700 FTA program and then pierced using the 4titude piercing tool before prepping for CE on the STARlet C. The CE plate was processed on the 3500xL and the profiles were qualitatively assessed.

Table { SEQ Table * ARABIC } Plate layout for FTA soccerball amplification plate. Note that position A09 in this layout differs from the Experimental Design.

Plate	1	2	3	4	5	6	7	8	9	10	11	12
A	Amp Pos	Ladder 1	Donor 1	Blank	Ladder 2	Blank	Donor 1	Ladder 3	Donor 2	Blank	Ladder 4	Blank
B	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
C	Donor 1	Blank	Donor 2	Blank	Donor 1	Blank	Donor 2	Blank	Donor 1	Blank	Donor 2	Blank
D	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
E	Donor 2	Blank	Donor 1	Blank	Donor 2	Blank	Donor 1	Blank	Donor 2	Blank	Donor 1	Blank
F	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	Donor 1	Blank	Donor 2	Blank	Donor 1	Blank	Donor 2	Blank	Donor 1	Blank	Donor 2	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

Testing STARlet C pipetting (CW plate)

A soccerball CW plate containing Amplification Positive Controls and Extraction Positive Controls was prepared on the pre-PCR STARlet and sealed with a Hamilton® Foil seal. See Table 17 for the soccerball sample layout. This plate was run on the GeneAmp PCR system 9700 CW program and then pierced using the 4titude piercing tool before prepping for CE on the STARlet C. The CE plate was processed on the 3130xl and the profiles were qualitatively assessed.

Table { SEQ Table * ARABIC } Plate layout for CW soccerball amplification plate.

Plate	1	2	3	4	5	6	7	8	9	10	11	12
A	Amp Pos	Ladder 1	Ext Pos	Ladder 2	Amp Pos	Ladder 3	Ext Pos	Ladder 4	Amp Pos	Ladder 5	Ext Pos	Ladder 6
B	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
C	Ext Pos	Blank	Amp Pos	Blank	Ext Pos	Blank	Amp Pos	Blank	Ext Pos	Blank	Amp Pos	Blank
D	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
E	Amp Pos	Blank	Ext Pos	Blank	Amp Pos	Blank	Ext Pos	Blank	Amp Pos	Blank	Ext Pos	Blank
F	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	Ext Pos	Blank	Amp Pos	Blank	Ext Pos	Blank	Amp Pos	Blank	Ext Pos	Blank	Amp Pos	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

Contamination check of piercing tool with decontamination procedure

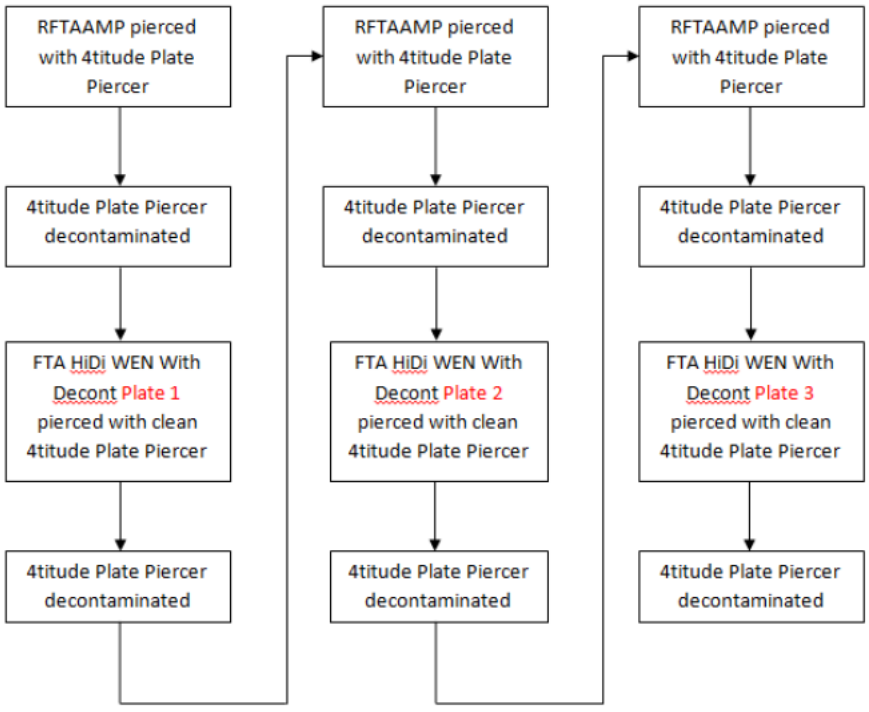

Figure { SEQ Figure * ARABIC } Contamination Check of 4titude Plate Piercer with Decontamination flow chart.

1. A checkerboard FTA plate (RFTAAMP20181106-01) using two different donors was prepared on the BSD and sealed with a Hamilton® Foil Seal
2. This plate was run on the FTA program on the GeneAmp PCR system 9700
3. Plate RFTAAMP20181106-01 was pierced with the 4titude handheld plate piercer
4. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.
5. Plate FTA HiDiWEN With Decont Plate 1 was pierced with the 4titude handheld plate piercer
6. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.
7. RFTAAMP20181106-01 was resealed with a Hamilton® Foil Seal and pierced with the 4titude handheld plate piercer
8. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.
9. FTA HiDiWEN With Decont Plate 2 was pierced with the 4titude handheld plate piercer.
10. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.
11. RFTAAMP20181106-01 was resealed with a Hamilton® Foil Seal and pierced with the 4titude handheld plate piercer
12. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.
13. FTA HiDiWEN With Decont Plate 3 was pierced with the 4titude handheld plate piercer
14. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.

These steps were repeated with the CW checkerboard plate VSTRAMP20181106-01, resulting in CW HiDiWEN With Decont Plates 1-3.

Contamination check of piercing tool without decontamination procedure

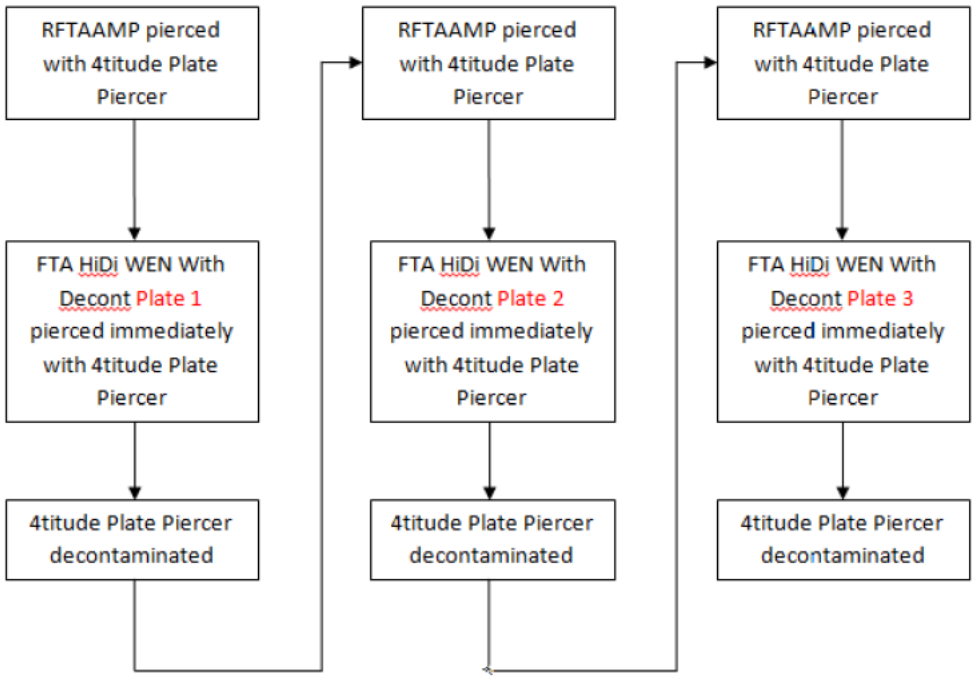

Figure { SEQ Figure * ARABIC } Contamination Check of 4titude Plate Piercer without Decontamination flow chart.

1. The same checkerboard FTA plate (RFTAAMP20181106-01) using two different donors was used for this experiment
2. Plate RFTAAMP20181106-01 was pierced with the 4titude handheld plate piercer
3. FTA HiDiWEN With No Decont Plate 1 was pierced with the 4titude handheld plate piercer
4. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.
5. RFTAAMP20181106-01 was resealed with a Hamilton® Foil Seal and pierced with the 4titude handheld plate piercer
6. FTA HiDiWEN With No Decont Plate 2 was pierced with the 4titude handheld plate piercer
7. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.
8. RFTAAMP20181106-01 was resealed with a Hamilton® Foil Seal and pierced with the 4titude handheld plate piercer
9. FTA HiDiWEN With No Decont Plate 3 was pierced with the 4titude handheld plate piercer
10. The piercer was scrubbed with 5% Trigene, rinsed in nH2O and then 70% ethanol and allowed to air dry.

These steps were repeated with the CW checkerboard plate VSTRAMP20181106-01, resulting in CW HiDiWEN With No Decont Plates 1-3.

Results and Discussion

Testing STARlet C pipetting (water plate)

During the preparation of the "dummy" amplification plate some errors were observed when using the STARlet C with the Hamilton® Foil Seals pierced by the 4titude handheld plate piercer. The pipetting channels were not moving through the pierced wells smoothly and were catching on the side of the foil seal. These errors were resolved by re-teaching the position of the amplification plate and allowing the STARlet C to pipette from the middle of the well. New versions of all programs were saved with the updated positions to the amplification plate. No pipetting parameters were changed.

Testing STARlet C pipetting (FTA plate)

All profiles of the two donors on the checkerboard FTA plate were concordant with the expected profiles with no indication of contamination. Two blank controls displayed peaks suspected to be CE carry-over. These controls were processed through Re-CE and resulted in NSD profiles confirming the peaks originally observed were carry over. All other blank controls gave NSD profiles as expected.

Testing STARlet C pipetting (CW plate)

All profiles of the positive amplification and extraction controls on the checkerboard CW plate were concordant with the expected profiles with no indication of contamination. All blank controls gave NSD profiles as expected.

Contamination check of piercing tool with decontamination procedure

All six plates (3x FTA HiDiWEN With Decont Plate, 3x CW HiDiWEN With Decont) were analysed using current negative control thresholds and all samples an each plate gave NSD profiles with no signs of contamination from the 4titude handheld plate piercer.

Contamination check of piercing tool without decontamination procedure

All HidiWEN With No Decont plates were analysed using current negative control thresholds.

FTA HiDiWEN With No Decont Plate 1 – Two blank wells in positions B02 and B09 displayed low level peaks. The low level peaks observed did not match any alleles from the donor profiles on RFTAAMP20181106-01. If these were processed as routine negative controls, these would be accepted as EXTN<3 peaks. No evidence of contamination from the 4titude handheld plate piercer was observed.

FTA HiDiWEN With No Decont Plate 2 – One blank well in position B01 displayed a low-level profile. The profile did not match the donor profiles on RFTAAMP20181106-01 and did not match any profiles when a quality search was performed. Another blank well in position

B10 displayed CE carry-over from the previously run CE plate. No evidence of contamination from the 4titude handheld plate piercer was observed.

FTA HiDiWEN With No Decont Plate 3 – One blank in position well B02 displayed two low level peaks which do not match any profiles on RFTAAMP20181106-01. If these were processed as routine negative controls, these would be accepted as <3 peaks. No evidence of contamination from the 4titude handheld plate piercer was observed.

All three CW HiDiWEN With No Decont plates were analysed using current negative control thresholds and gave NSD profiles with no signs of contamination from the 4titude handheld plate piercer.

Acceptance Criteria

As these results have shown no evidence of contamination from the 4titude handheld plate piercer when using the decontamination procedure, the Project Team recommend purchasing a minimum of four additional 4titude handheld plate piercers (to reduce decontamination procedure drying times) and implement this as the routine procedure in CE.

Conclusion

The STARlet C was verified with the ARTEL at all critical volumes in Experiment 1 (i.e. 50 µL tips at 1 µL and 50 µL volumes) and is therefore pipetting accurately within verification thresholds.

Experiment 2 showed four seals (AlumaSeal II, PlateMax, Eppendorf PCR and Hamilton® Foil) met the acceptance criteria and can be used with the STARlet however the project team recommends the Hamilton® Foil Plate Seal to ensure compatibility with the Hamilton® Plate Sealer and ongoing support from the vendor.

This validation study has determined the STARlet C using the methods developed in conjunction with Bio-Strategy is suitable for routine preparation of Capillary Electrophoresis PowerPlex® 21 casework and reference assays in the Forensic DNA Analysis laboratory. It has also shown STARlet C gave results comparable to the current manual method. No evidence of cross contamination between samples (between runs or between samples within a run) was identified in this study.

The Repeatability and Reproducibility experiments (see Experiment 5) demonstrate the STARlet C performs consistently across plates and days.

The Seal Piercing experiment (see Experiment 6) has shown there is minimal risk of contamination when decontaminating the 4titude handheld plate piercer using the recommended procedure. It also showed the Hamilton® Foil Seals and 4titude handheld plate piercer can be used with the STARlet C without issues.

Recommendations

1. Hamilton® Foil Seals be implemented into Pre-PCR and the Hamilton® plate sealer implemented into CE ;
2. Additional hand-held piercers be purchased and stocked in CE;
3. The STARlet C be implemented for the preparation of PowerPlex® 21 casework assays;

4. The STARlet C be implemented for the preparation of PowerPlex® 21 reference assays;
5. The STARlet C be implemented for the preparation of PowerPlex® 21 casework and reference Re-CE assays;
6. A selection of staff undergoes training in programming the STARlet C.

Appendix 1 Final Versions of Protocols for Implementation

- CE_preparation_Re-CE_v1.0
- CE_preparation_Whole plate to plate_v1.0

Appendix 2 Abbreviations

AI	Allelic Imbalance
%CV	Coefficient of Variance
%inaccuracy	Relative Inaccuracy
CE	Capillary Electrophoresis
µL	microliter
ng	nanogram
PA	Preferential Amplification
PP21	Powerplex®21
Re-CE	Repeat Capillary Electrophoresis
STARlet	Hamilton® Microlab® STARlet and LabElite® Integrated I.D. Capper™

References

Ryan, L., Mathieson, M., Dwyer, T., Darmanin, A. and Allen, C. (2017) Project Proposal #175: Validation of Hamilton® ID STARlet C for Capillary Electrophoresis Setup. [Experimental Design].