

# Optimizing the Processing of Databasing Samples using the Hamilton® easyPunch™ STARlet

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## Introduction

This study evaluated the Hamilton easyPunch™ STARlet and its ability to improve the efficiency and throughput of a databasing section. The Hamilton easyPunch™ STARlet liquid handling workstation integrates sample collection card imaging, punching, and liquid handling capability all in one instrument. The system enables full sample tracking, including the recording of card images that completely account for the consumption of evidence.

Ninety (n=90) Bode Buccal 2 collected samples were processed using the Hamilton easyPunch™ STARlet. The resulting DNA profiles were analyzed using appropriate laboratory analytical and stochastic thresholds. The data metrics recorded and compared first amplification success rate (samples not requiring reamplification), average allelic peak height per locus, and average intra-color balance.

Three direct amplification chemistries manufactured by three different companies were evaluated as part of this study. GlobalFiler™ Express, Investigator® 24plex GO!, and PowerPlex® Fusion 6C were chosen as they all contain the CODIS core 20 loci and contain additional Y chromosome specific loci.

The optimized protocol provided a method to obtain a 96-well plate containing lysed sample punches and amplification master mix in under 2 hours with minimal human interaction. Zero to two clean punches were previously evaluated to prevent sample carryover, results indicated that one clean punch optimized sample integrity and punching efficiency. Samples were evaluated for profiles that met laboratory guidelines for each kit based on the analytical and stochastic thresholds. The results from this study show that a fully automated platform can increase a laboratory's efficiency without decreasing profile quality or success rates.

## Materials

### The Bode Buccal 2 Collector and Cassette



### The Hamilton easyPunch™ STARlet



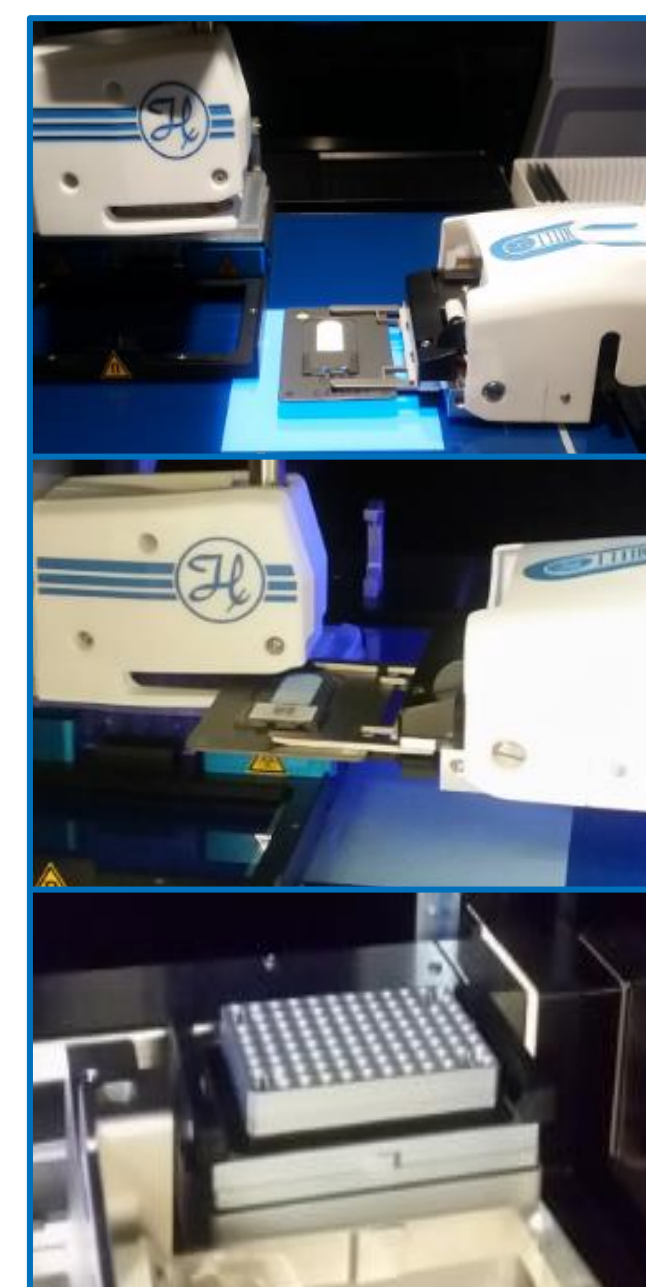
## Methods

### Preparation for Automated Processing

Ninety (90) buccal samples collected with Bode Buccal 2s and manually converted into cassettes.

### easyPunch™ STARlet Processing

Lysis Buffer Preparation					
Chemistry	Reagent	Diluent	Volume per Sample (µl)		
			Reagent	Diluent	Total
24plex GO!	Investigator STR GO! Lysis Buffer	Water	2	8	10
PPF6C	PunchSolution™	N/A	10	N/A	10
GFE	Prep-n-Go™ Buffer	Water	2	8	10



Lysis buffer is added to each well then sample is imaged and barcode (optional) read

1.2mm punch taken from each sample with one cleaning punch (discarded in waste trough) taken in between.

Heated lysis occurs on the integrated Hamilton Heater Shaker

Amplification Master Mix Prepared Manually and Robotically Dispensed				
Chemistry	Reagents	Volume per Sample (µl)	(+) Control	Cycle #
24plex GO!	Fast Reaction Mix 2.0	7.5		
	Primer Mix	12.5	9948	27
	Water	5		
PPF6C	6C 5X Master Mix	2.5	2800M	25
	6C 5X Primer Pair Mix	2.5		
	5X AmpSolution	2.5		
GFE	AmpFLSTR PCR Enhancer	2.5		
	GFE Master Mix	6	007	27
	GFE Primer Set	6		

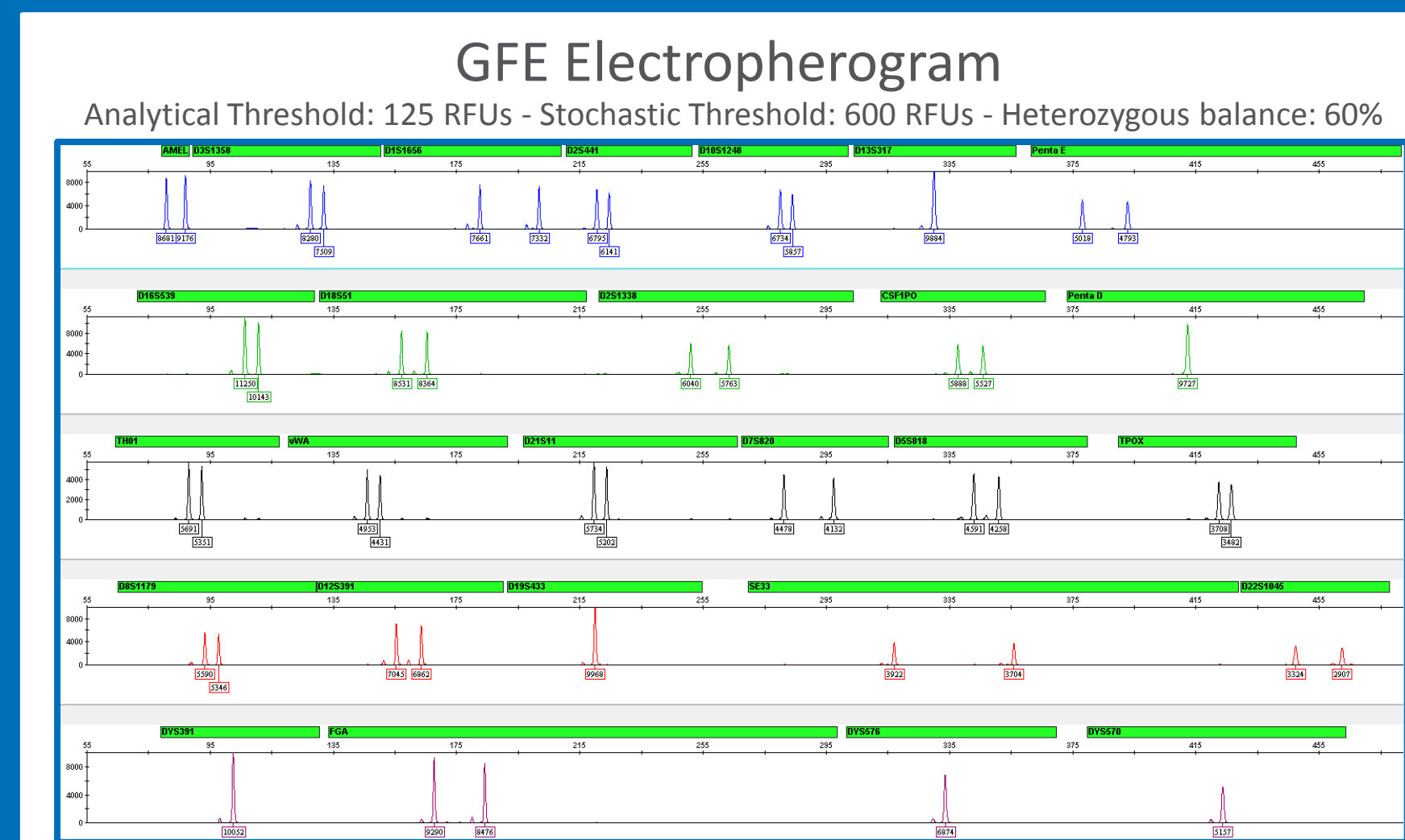
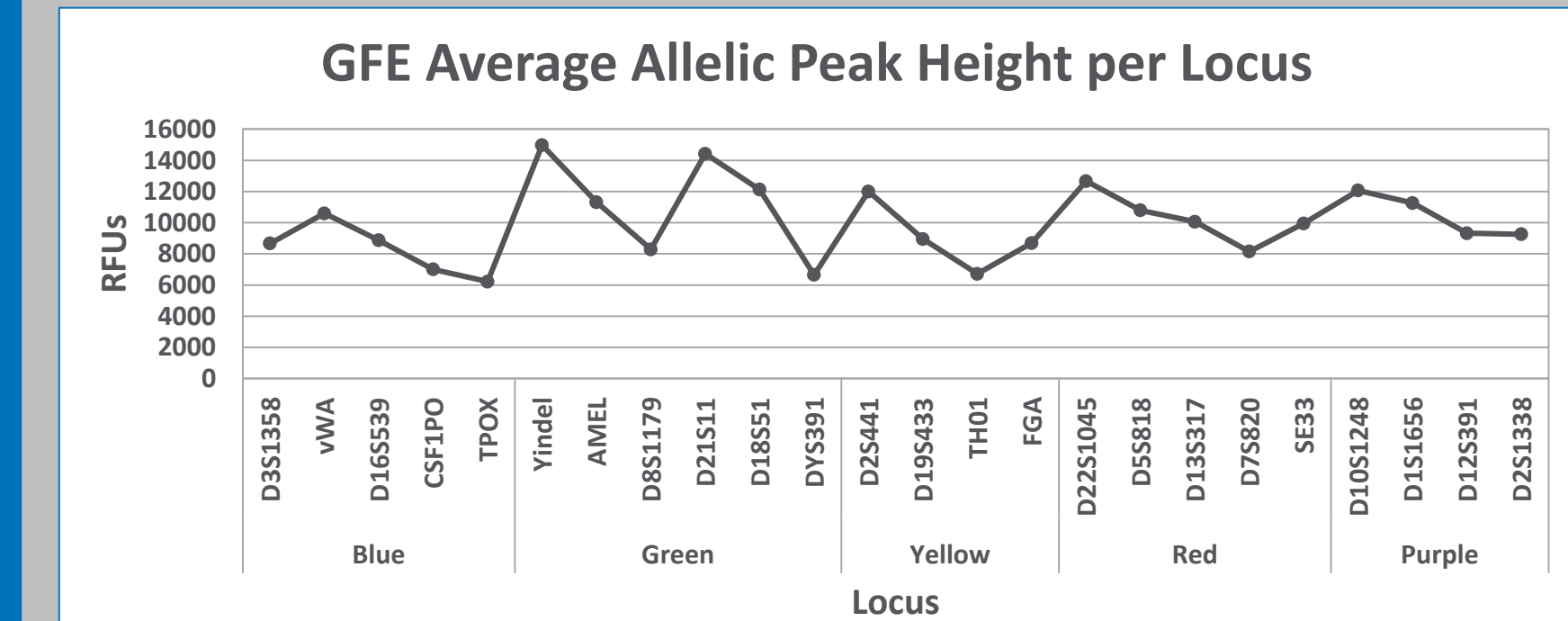
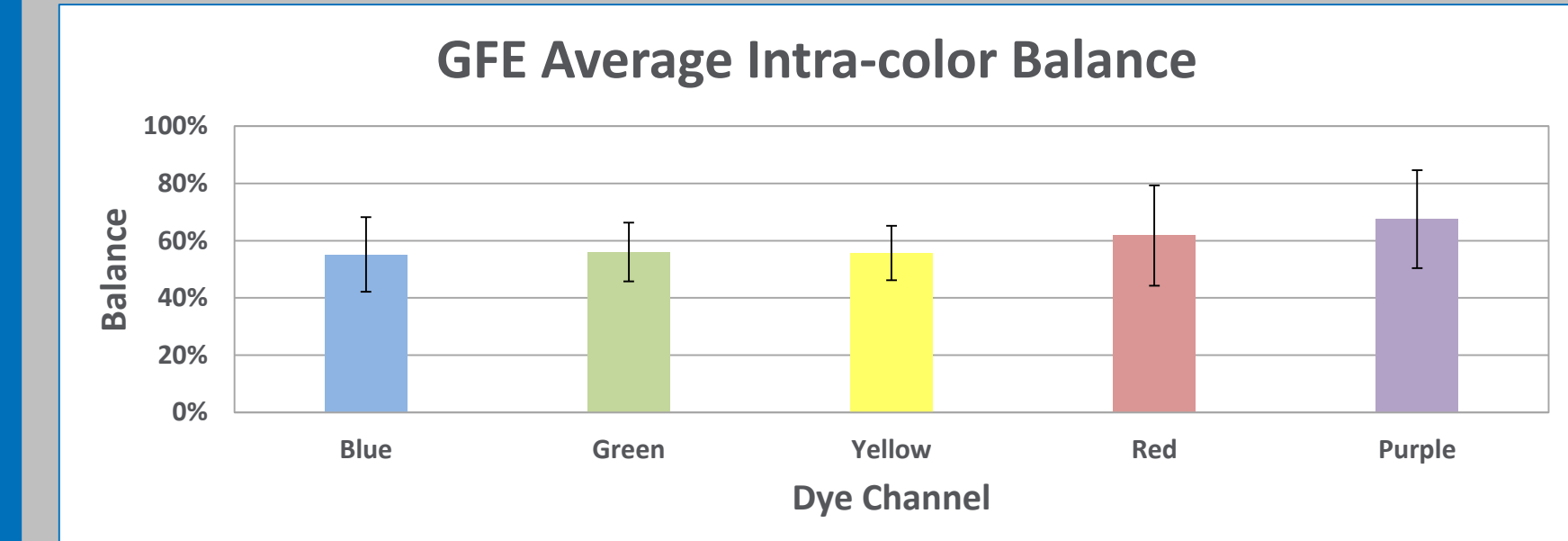
### Post-Amplification Processing

10 µl CE master mix and 1 µl amp product added to tray. Loaded on Applied Biosystems 3500xL Genetic Analyzer. Analyzed using GeneMapper ID-X v1.5 software.

Capillary Electrophoresis Preparation				
Chemistry	ILS	Volume per Sample (µl)		
		ILS	Hi-Di Formamide	Total
24plex GO!	550 BTO	1	10	10
PPF6C	WEN-500	0.25	10	10
GFE	GS-600 LIZ v2.0	0.4	10	10

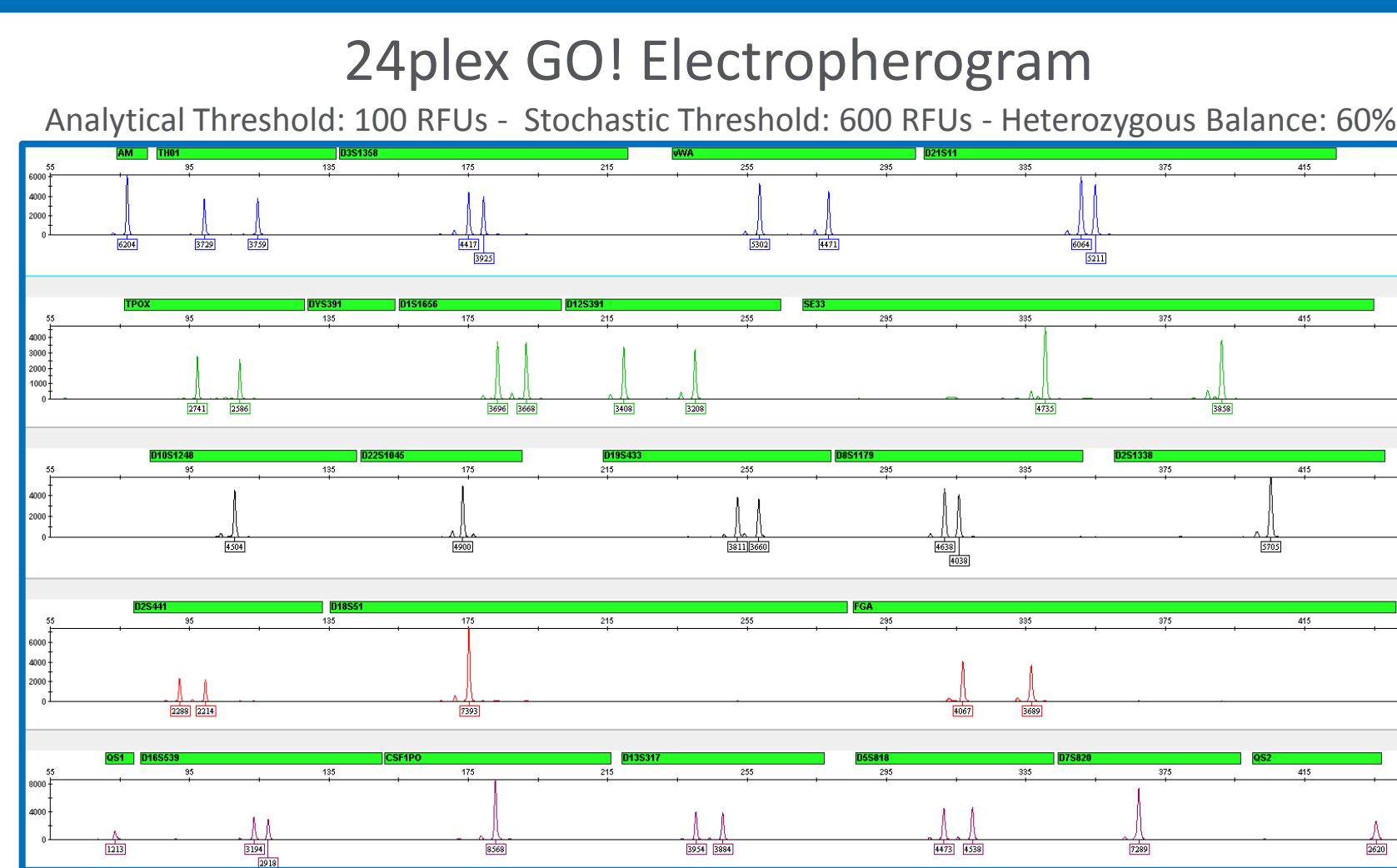
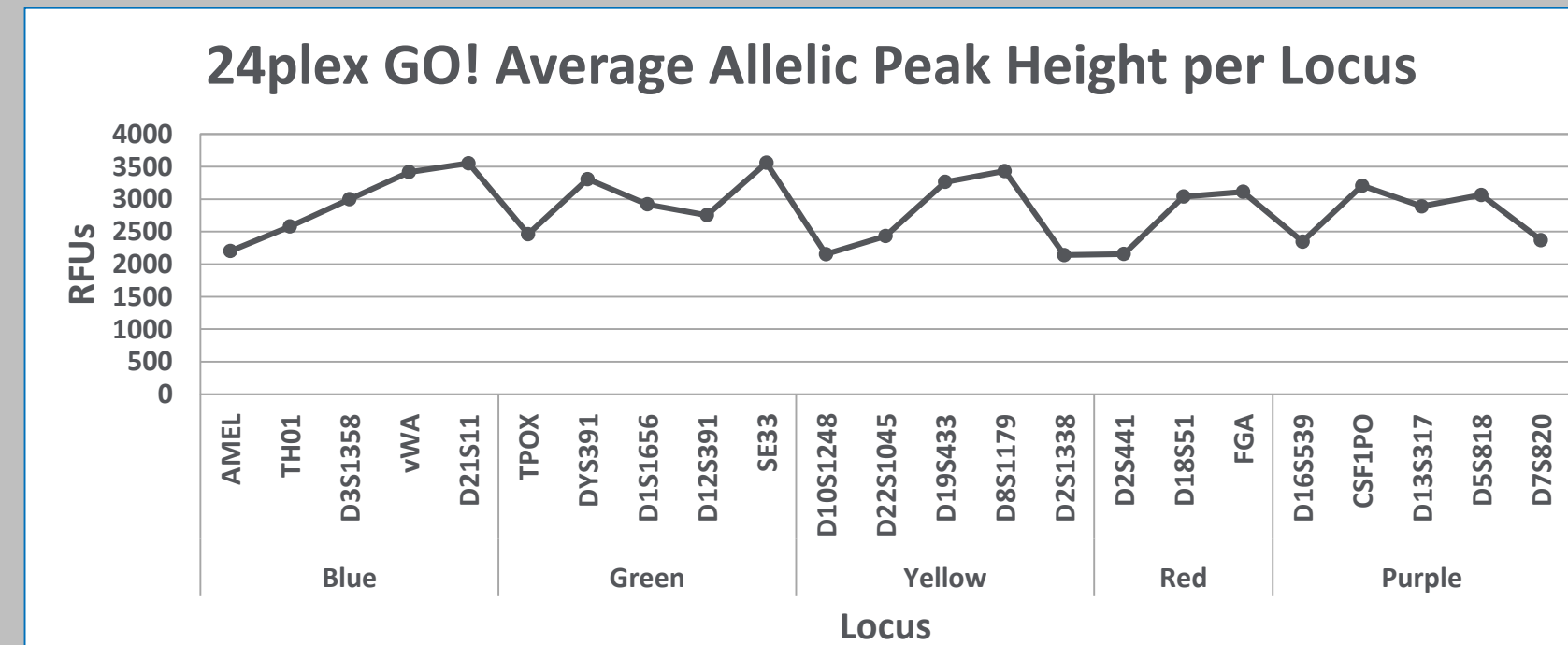
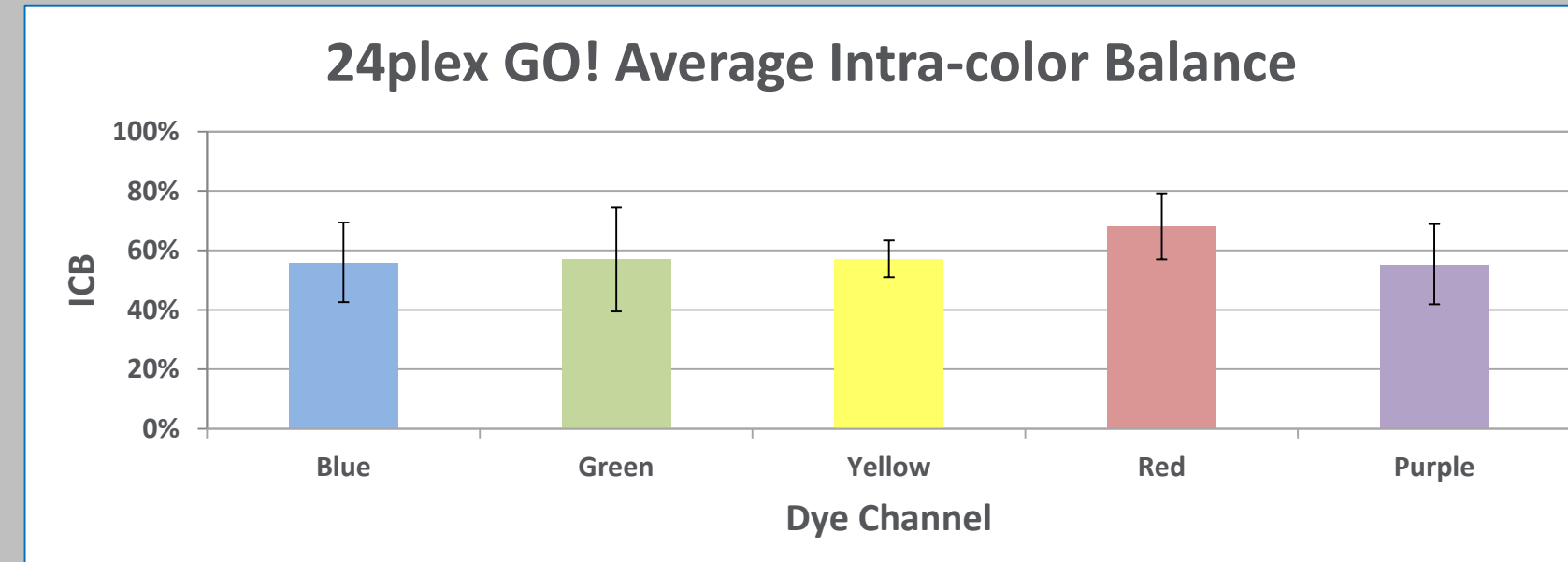
## Thermo Fisher GlobalFiler™ Express Results

Profile Quality	# Samples	First Amplification Success Rate
Complete	88	98%
Requires reamplification	2	2%



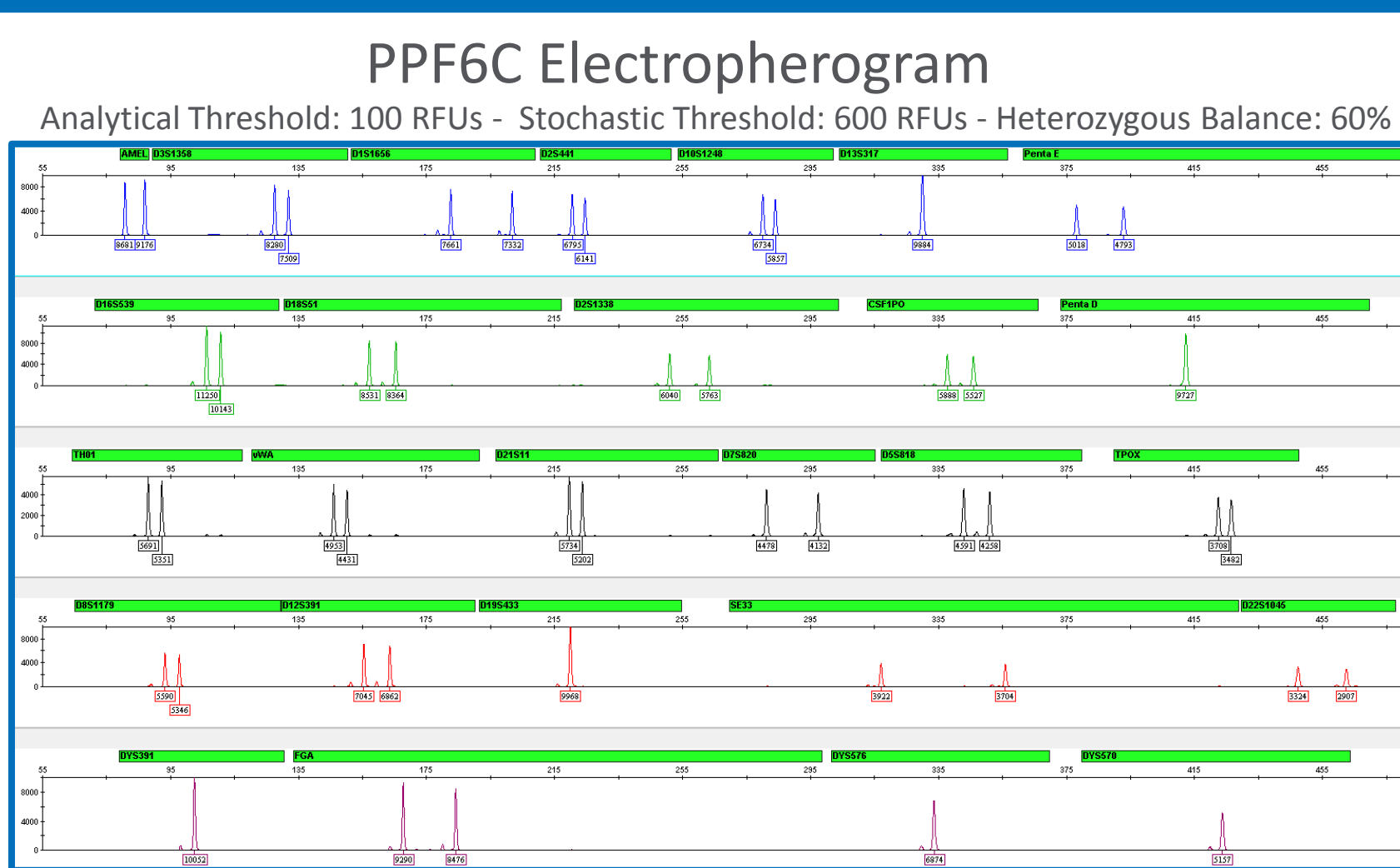
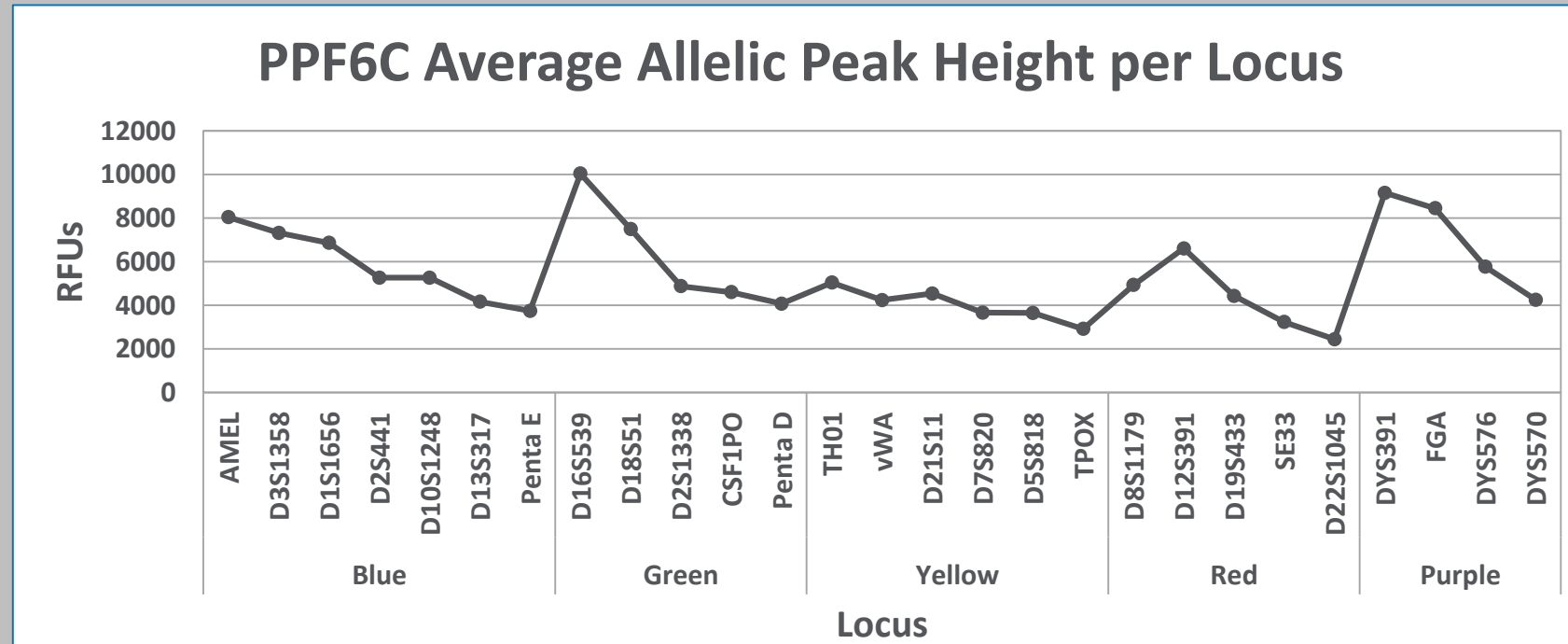
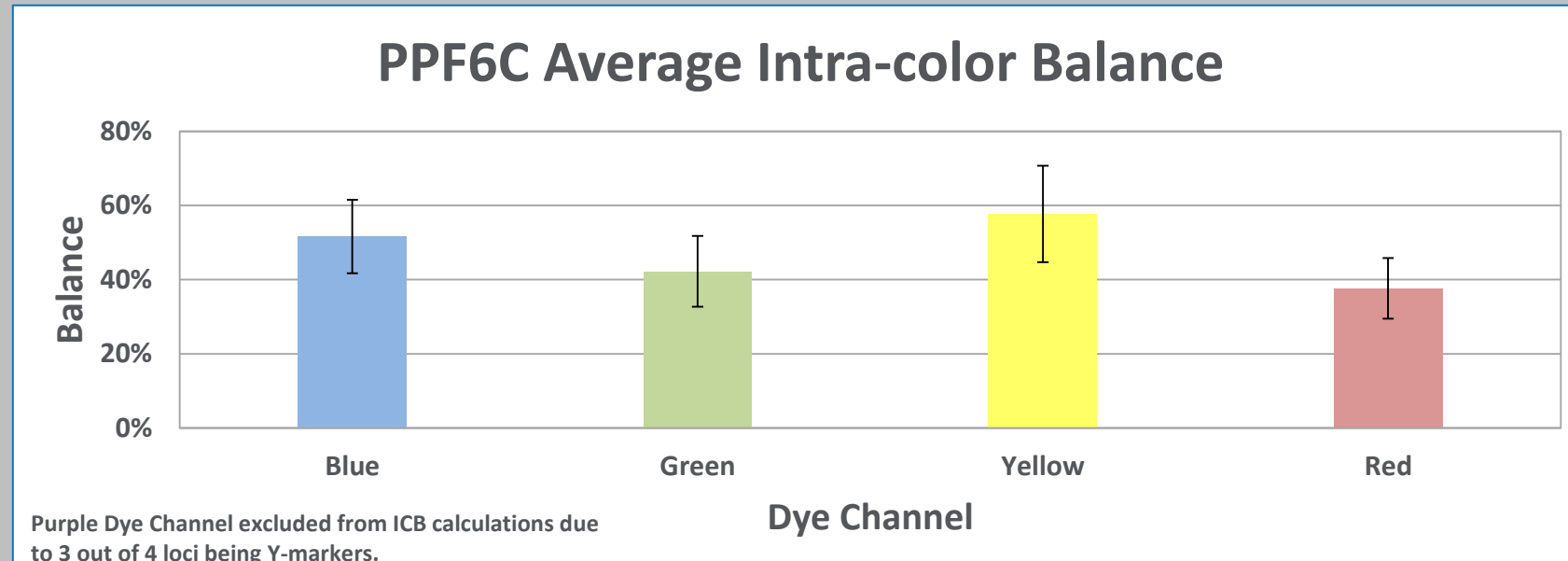
## Qiagen Investigator® 24plex GO! Results

Profile Quality	# Samples	First Amplification Success Rate
Complete	90	100%



## Promega PowerPlex® Fusion 6C Results

Profile Quality	# Samples	First Amplification Success Rate
Complete	89	99%
easyPunch™ STARlet Error	1	1%



## Conclusion

- Universal Bode Buccal 2 and easyPunch™ STARlet script is compatible with multiple direct amplification chemistries:

- GlobalFiler Express
- Investigator 24plex GO!
- PowerPlex Fusion 6C

- Reduces laboratory workflow of a full tray from 3.5 hours to 2 hours.

- One clean punch between each sample prevents carryover contamination by >99% of the >2000 loci analyzed for each amplification kit.

- 99% of DNA profiles, including reloaded samples, met operational acceptance criteria from the first amplification.