

TECHNICAL NOTE

Direct Amplification of Reference Samples Using the GlobalFiler™ PCR Amplification Kit

Introduction

FTA™ cards and associated sample collection devices can be useful for the collection, storage, and processing of biological samples. A small punch disc of the card containing the sample can be placed directly into an amplification tube, purified, and amplified, without transferring the disc. In addition, untreated substrates such as 903 cards and swabs can be used in combination with the Prep-N-Go™ lysis buffer to facilitate direct amplification processing of biological samples that are known to contain high quality DNA such as reference samples.

The GlobalFiler kit was designed and optimized for casework sample processing i.e. including a pre-amplification extraction and clean up step, with GlobalFiler Express as the partner kit specifically designed and optimized to perform direct amplification of reference samples. However, many laboratories have expressed a requirement for a single PCR amplification kit to process both sample types.

We have performed a study to evaluate the performance of the GlobalFiler PCR Amplification Kit using a direct amplification method to demonstrate that the kit can be used in this manner to support those laboratories that choose to use one kit for both applications.

In this Technical Note the results of this study are presented, along with protocols used. It is intended that this document is used as a guide for laboratories planning to use the GlobalFiler kit for direct amplification purposes.

Materials & Methods

Preliminary studies indicated that a 1.2-mm bloodstained disc contains approximately 5 to 20 ng of DNA.

Because of this high quantity of DNA, lower cycle numbers were pursued to produce on scale data.

Sample Types Evaluated

- Blood:
 - FTA™
 - Copan NUCLEIC-CARD™
 - Non-FTA:
 - Whatman™ Blood Stain Cards
 - Whatman™ 903 Sample Collection Cards
- Buccal:
 - FTA™ (EasiCollect)
 - Copan NUCLEIC-CARD™ COLOR
 - Non-FTA:
 - Bode Buccal DNA Collector™
 - Whatman™ 903 Sample Collection Cards
 - 4N6FLOQSwabs™

Blood Samples					
Substrate	FTA	NUCLEIC-CARD	Blood Stain Card	903 Card	
Sample age	5 years	3 days	4 months	1 day	
Substrate Type	Treated paper	Treated paper	Untreated paper	Untreated paper	
Number of samples	23	22	22	20	
Buccal Samples					
Substrate	EasiCollect (FTA)	NUCLEIC-CARD Color	Bode Collector	903 Card	4N6FLOQ Swabs
Sample age	4 months	8 months	4 months	2 months	2 months
Substrate Type	Treated paper	Treated paper	Untreated paper	Untreated paper	Untreated swab
Number of samples	22	20	22	20	23

Table 1 Sample Overview

Protocol

The GlobalFiler Express User Guide (P/N 4477672) protocols were followed for the preparation of samples before PCR.

In summary:

- 1.2mm treated paper punches were added to the 96 well plate along with 15µl of DNA Suspension Buffer (Low TE)
- 1.2mm untreated paper punches were added to the 96 well plate along 3µl of Prep-n-Go buffer and 12µl of DNA Suspension Buffer
- 400µl of Prep-N-Go buffer was added to swabs, incubated for 20 minutes at 90°C in a deep well plate. 2µl of the lysate was added to the 96 well plate along with 13µl of DNA Suspension Buffer.

The PCR reagents were prepared as per the GlobalFiler User Guide (P/N 4477604) and added to the 96 well plate wells containing samples giving a final reaction volume of 25µl.

PCR amplification was carried out as per the GlobalFiler User Guide but the cycle numbers were adjusted based on the sample type. Instead of 29 cycles, the following cycle numbers were used:

Blood Samples					
Substrate	FTA	NUCLEIC-CARD	Blood Stain Card	903 Card	
Cycle numbers	25	24	25	24	
Buccal Samples					
Substrate	EasiCollect (FTA)	NUCLEIC-CARD Color	Bode Collector	903 Card	4N6FLOQ Swabs
Cycle numbers	26	27	26	25	25

Table 2 Cycle Numbers for Different Sample Types

Capillary electrophoresis was carried out using a 3500xL Genetic Analyzer and data analysis utilized GeneMapper™ ID-X v1.5. Both steps in the workflow were carried out as per the GlobalFiler User Guide.

The following key analysis method key parameters were used:

- Global Cut-off Value of 0.2
- Peak Amplitude Threshold of 175 RFU for all dye channels

Results are presented as follows:

- Full Profile Success Rate- defined as the number of samples with a full profile divided by the total number of samples tested.
- Composite Genotype Quality (CGQ) Success Rate- defined as the number of samples with green CGQ flags divided by the total number of samples tested.
 - CGQ parameters used are shown in appendix B.
 - Flag descriptions are given for samples with a yellow or red CGQ outcome.
- Intracolor balance and average peak height data.

Results

Blood Samples					
Substrate	FTA	NUCLEIC-CARD	Blood Stain Card	903 Card	
Full Profile Success Rate	22/23 (95.7%)	20/20 (100%)	22/22 (100%)	20/20 (100%)	
CGQ Success Rate	22/23 (95.7%)	14/20 (70%)	19/22 (86.4%)	19/20 (95.0%)	
Flag Description	Amelogenin X dropout in one sample.	Off-scale peaks in six samples.	Off-scale peaks in two samples. One split peak in one sample.	Elevated stutter at one locus in one sample.	
Buccal Samples					
Substrate	EasiCollect (FTA)	NUCLEIC-CARD Color	Bode Collector	903 Card	4N6FLOQ Swabs
Full Profile Success Rate	19/22 (86.4%)	19/20 (95.0%)	21/22 (95.5%)	20/20 (100%)	23/23 (100%)
CGQ Success Rate	19/22 (86.4%)	16/20 (80.0%)	21/22 (95.5%)	19/20 (95.0%)	18/23 (78.3%)
Flag Description	Partial profiles in two samples. No profile in one sample.	Dropout in one sample. Low intralocus balance in three samples.	Dropout in one sample.	Low intralocus balance in one sample.	Off scale peaks in one sample. Pull up peaks in one sample. Low intralocus balance in three samples.

Table 3 Results by Sample Type and Substrate

Intracolor balance and average peak height data are presented in appendix A.

Conclusions

Our studies show that the GlobalFiler PCR Amplification Kit can be used to generate full profiles from reference samples prepared using direct amplification methods.

Because of the high quantity of DNA associated with this sample type, lower cycle numbers are required to produce on scale data. There are multiple sample and substrate combinations that can be encountered that require different cycle numbers to produce optimal data therefore we recommend that laboratories intending to implement a direct amplification workflow using the GlobalFiler kit determine the optimum cycle number for their workflow based on internal validation studies.

Appendix A

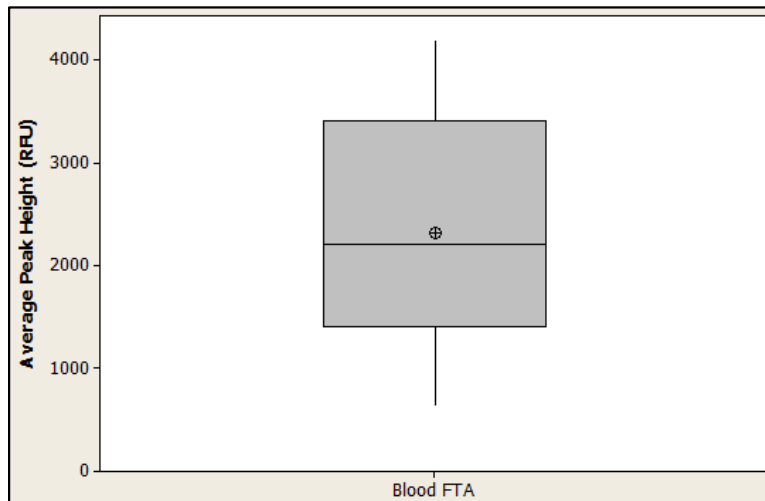


Figure 1 Box and Whisker Plot of Average Peak Height: Blood on FTA

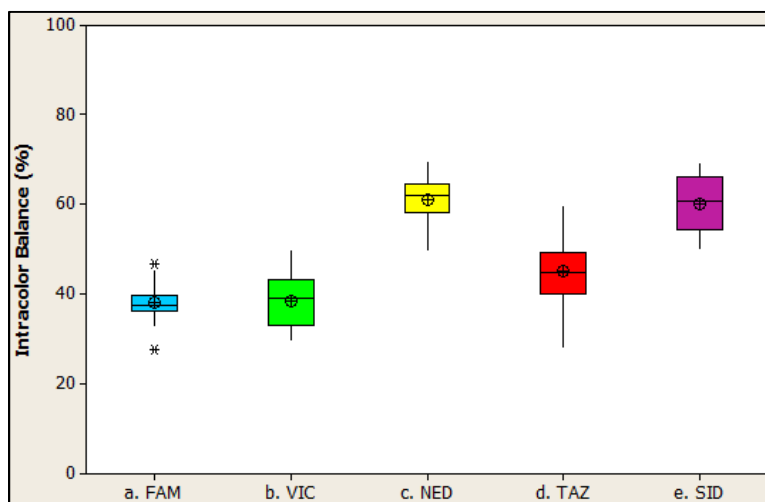


Figure 2 Box and Whisker Plot of Intracolor Balance: Blood on FTA

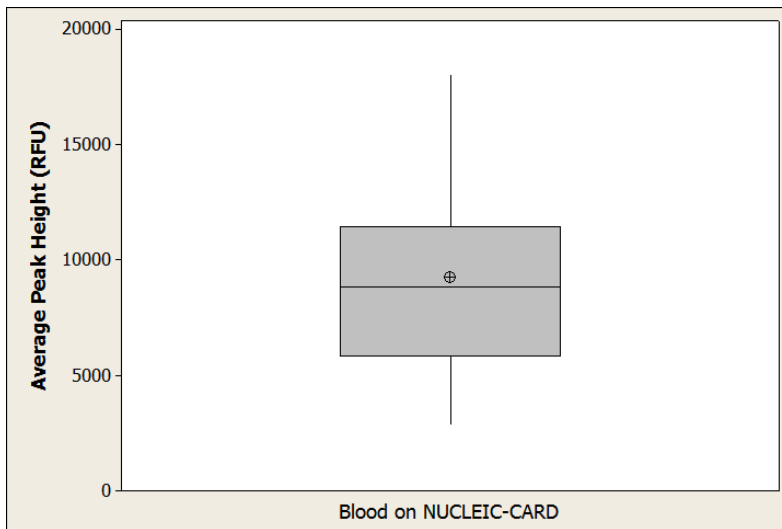


Figure 3 Box and Whisker Plot of Average Peak Height: Blood on NUCLEIC-CARD

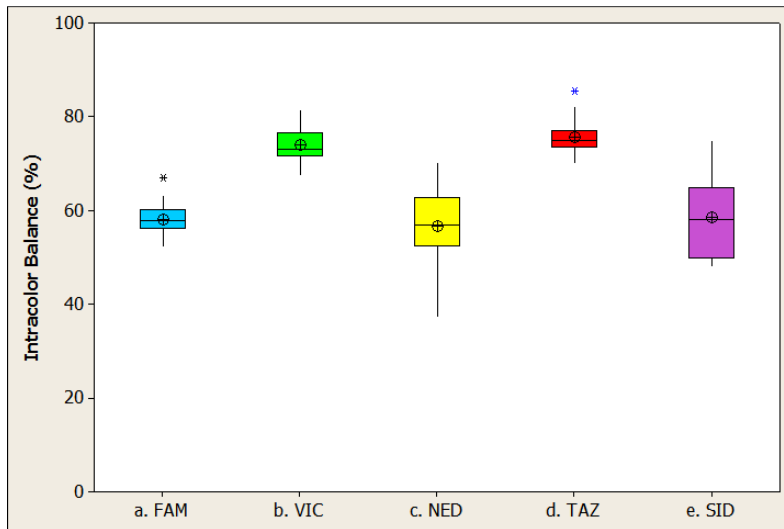


Figure 4 Box and Whisker Plot of Intracolor Balance: Blood on NUCLEIC-CARD

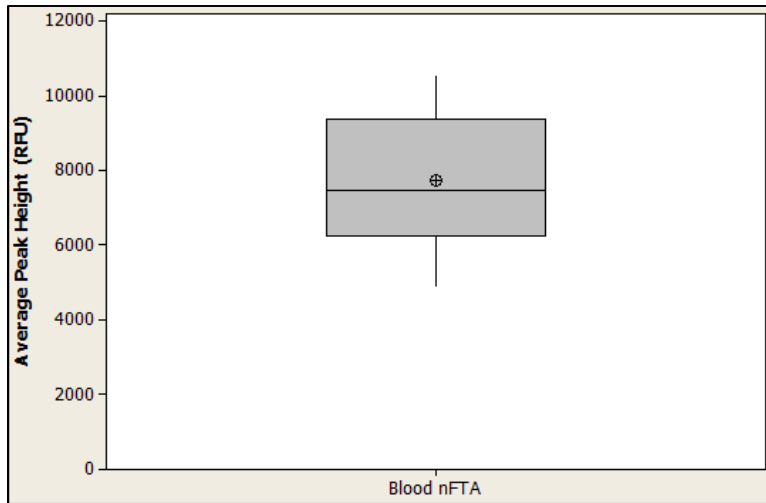


Figure 5 Box and Whisker Plot of Average Peak Height: Blood on Blood Stain Card

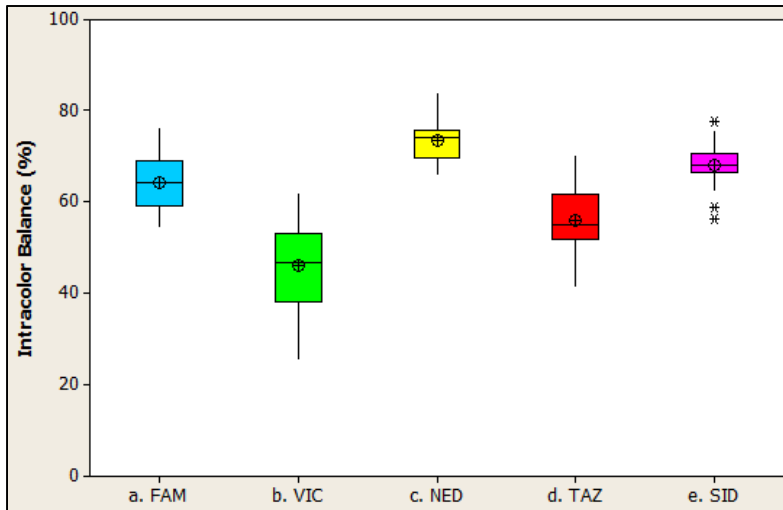


Figure 6 Box and Whisker Plot of Intracolor Balance: Blood on Blood Stain Card

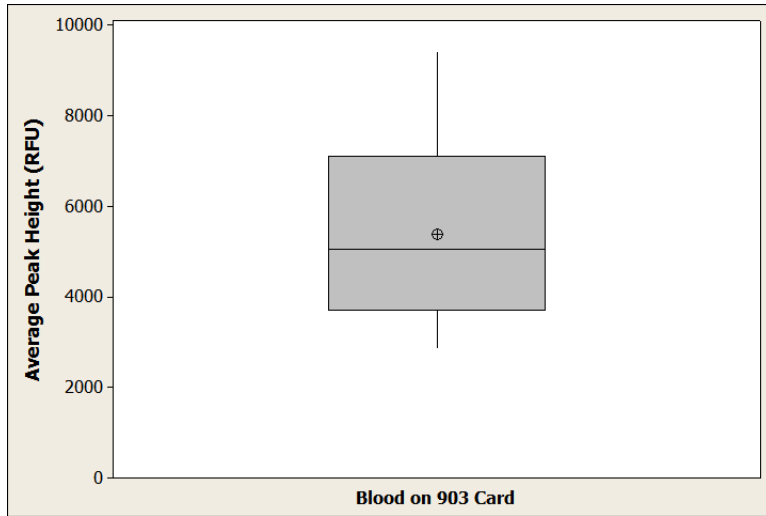


Figure 7 Box and Whisker Plot of Average Peak Height: Blood on 903 Card

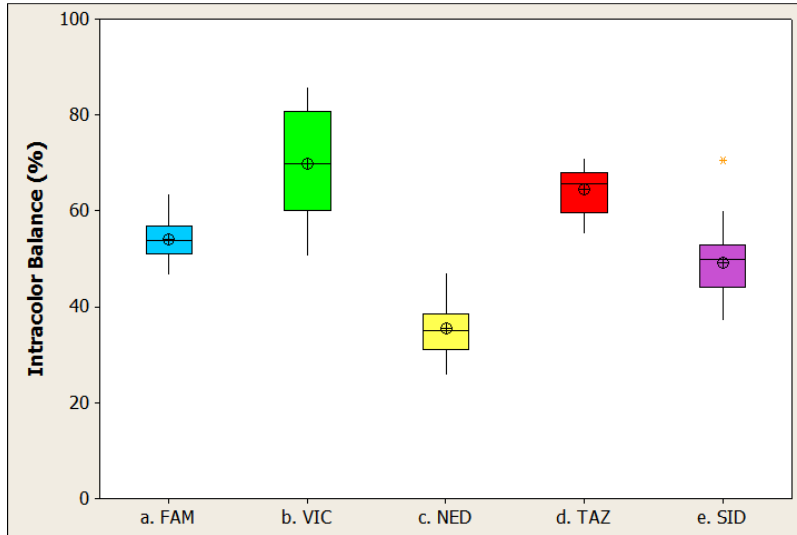


Figure 8 Box and Whisker Plot of Intracolor Balance: Blood on 903 Card

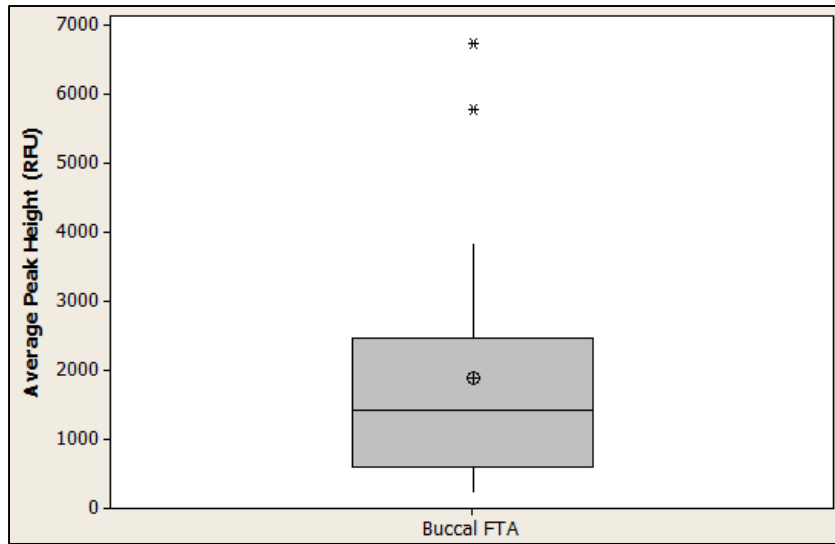


Figure 9 Box and Whisker Plot of Average Peak Height: Buccal on EasiCollect (FTA)

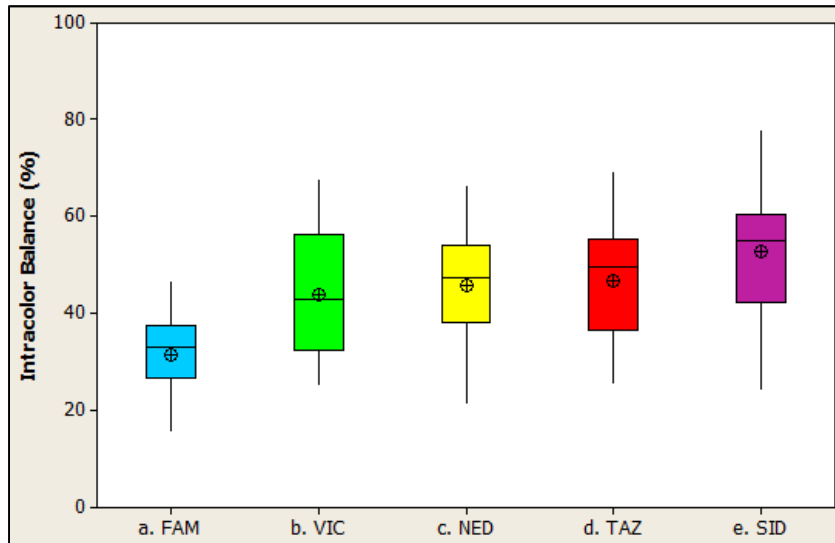


Figure 10 Box and Whisker Plot of Intracolor Balance: Buccal on EasiCollect (FTA)

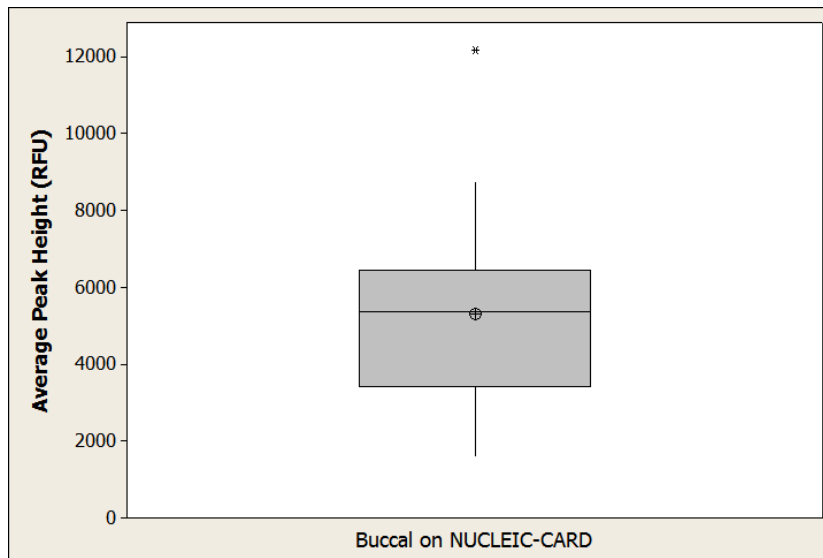


Figure 11 Box and Whisker Plot of Average Peak Height: Buccal on NUCLEIC-CARD

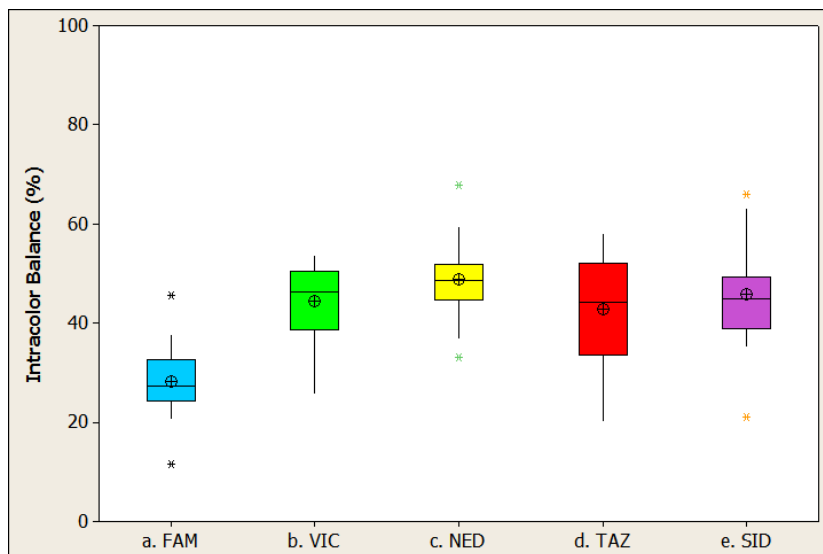


Figure 12 Box and Whisker Plot of Intracolor Balance: Buccal on NUCLEIC-CARD

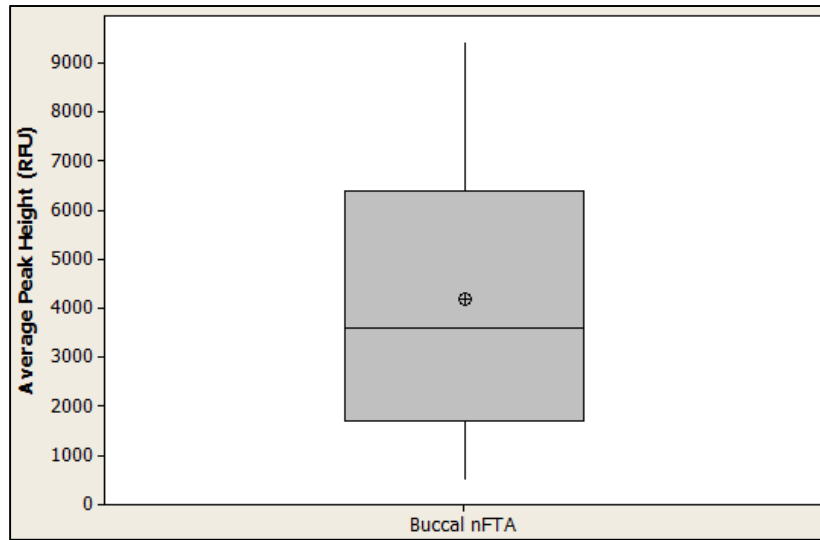


Figure 13 Box and Whisker Plot of Average Peak Height: Buccal on Bode Collector

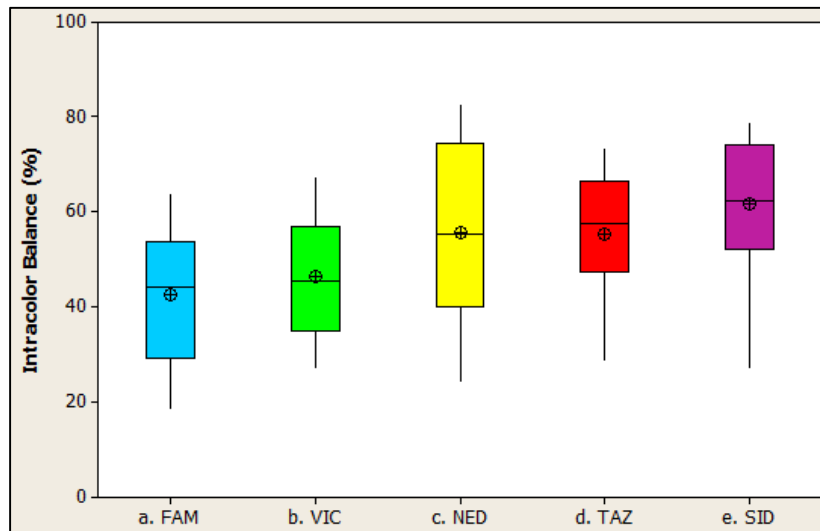


Figure 14 Box and Whisker Plot of Intracolor Balance: Buccal on Bode Collector

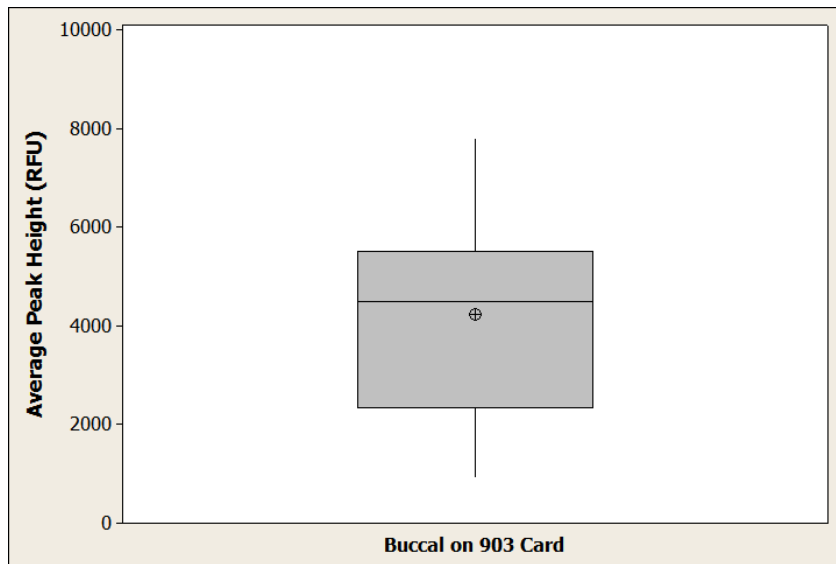


Figure 15 Box and Whisker Plot of Average Peak Height: Buccal on 903 Card

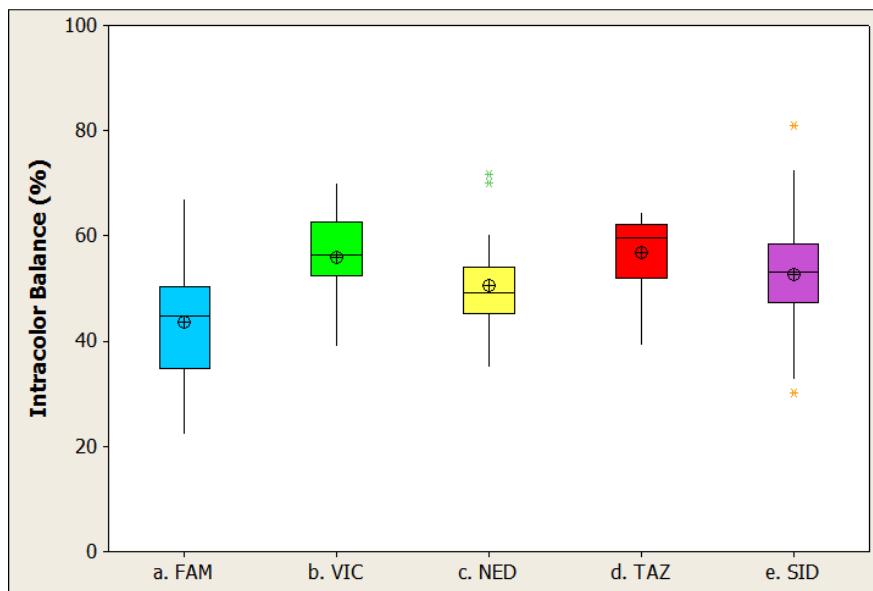


Figure 16 Box and Whisker Plot of Intracolor Balance: Buccal on 903 Card

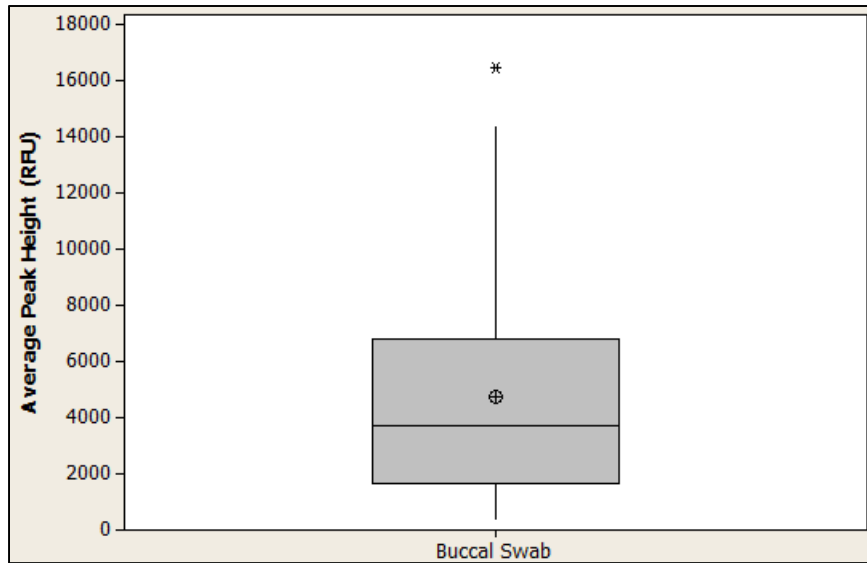


Figure 17 Box and Whisker Plot of Average Peak Height: Buccal on 4N6FLOQSwabs

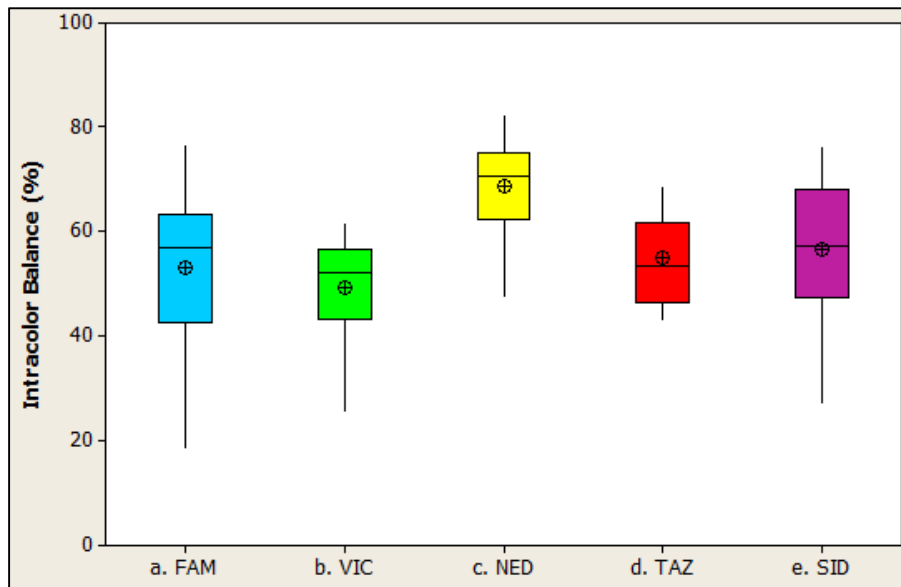


Figure 18 Box and Whisker Plot of Intracolor Balance: Buccal on 4N6FLOQSwabs

Appendix B

General	Allele	Peak Detector	Peak Quality	SQ & GQ Settings	General	Allele	Peak Detector	Peak Quality	SQ & GQ Settings
Min/Max Peak Height (LPH/MPH) Homozygous min peak height: <input type="text" value="200.0"/> Heterozygous min peak height: <input type="text" value="100.0"/> Max Peak Height (MPH): <input type="text" value="30000.0"/> Peak Height Ratio (PHR) Min peak height ratio: <input type="text" value="0.7"/> Broad Peak (BD) Max peak width (basepairs): <input type="text" value="1.5"/> Allele Number (AN) Max expected alleles: For autosomal markers & AMEL: <input type="text" value="2"/> For Y markers: <input type="text" value="1"/> Allelic Ladder Spike Spike Detection: <input type="text" value="Enable"/> Cut-off Value: <input type="text" value="0.2"/> Sample Spike Detection Spike Detection: <input type="text" value="Enable"/>					Quality weights are between 0 and 1. Sample and Control GQ Weighting Broad Peak (BD): <input type="text" value="0.8"/> Allele Number (AN): <input type="text" value="1.0"/> Out of Bin Allele (BIN): <input type="text" value="0.8"/> Low Peak Height (LPH): <input type="text" value="0.3"/> Overlap (OVL): <input type="text" value="0.8"/> Max Peak Height (MPH): <input type="text" value="0.3"/> Marker Spike (SPK): <input type="text" value="0.3"/> Off-scale (OS): <input type="text" value="0.8"/> AMEL Cross Check (ACC): <input type="text" value="0.0"/> Peak Height Ratio (PHR): <input type="text" value="0.3"/> Control Concordance (CC) Weight = 1.0 (Only applicable to controls) SQ Weighting Broad Peak (BD): <input type="text" value="0.5"/> Allelic Ladder GQ Weighting Spike (SSPK/SPK): <input type="text" value="1"/> Off-scale (OS): <input type="text" value="1"/> SQ & GQ Ranges Pass Range: Pass Range: Low Quality Range: Sizing Quality: From <input type="text" value="0.75"/> to <input type="text" value="1.0"/> From <input type="text" value="0.0"/> to <input type="text" value="0.25"/> Genotype Quality: From <input type="text" value="0.75"/> to <input type="text" value="1.0"/> From <input type="text" value="0.0"/> to <input type="text" value="0.25"/>				

Revision History

Revision	Date	Description
A	29-Apr-2016	Initial publication.
B	13-Jul-2016	Updated to include NUCLEIC-CARD data. Addition of revision history and CGQ analysis parameters.

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