Compatibility of Bode Armor™ and Direct Amplification Chemistries

Allie Flores, BS; Dan Watsula, MS; Robert Bever, PhD; Jangbir Sangha, MA

Bode Cellmark Forensics, Inc. 10430 Furnace Road, Suite 107, Lorton, VA 22079

Introduction

Preservation of DNA databanking samples is critically important to enable the laboratory to retest reference samples when a CODIS hit occurs. Bode Buccal DNA Collection devices, through an ongoing stability study; have shown stability of buccal samples at the ten year time point. Through storage in The Bode Vault and treatment with the preservative Bode Armor, accelerated studies have yielded data indicating stability of buccal samples for greater than 30 years. Bode Armor acts to preserve DNA by inhibiting the most common factors that cause DNA degradation.

Direct amplification of reference samples eliminates the need for the time consuming extraction and quantification steps encountered in routine processing. During direct amplification, a 1.2mm punch is placed directly into the well with the reaction mix. Due to this direct interaction, Bode Armor must not only preserve DNA but it must also not inhibit the amplification reactions. Bode Buccal DNA collection devices were treated with Bode Armor after sample collection.

This presentation will describe the studies and results performed with Promega's PowerPlex® Fusion and Fusion 6C, Thermo Fisher's GlobalFiler™ Express Kit and Qiagen's Investigator® 24Plex GO! Kit. Bode Buccal DNA collectors were collected from volunteers. Each collector was treated with the preservative Bode Armor. 1.2 mm punches were removed from each collector and amplified with all four STR kits. Data was analyzed comparing RFU values, inter locus and intra color balance, and success rates. The results indicated 100% concordance between the Bode Armor treated collectors and extracted DNA. Bode Armor successfully enhances buccal sample stability without impacting DNA profiling success.

Application of Bode Armor

Step 1

Place Bode Armor in reservoir boat and aliquot using P200 multichannel.

Step 2

Dispense 100µL of Bode Armor on Bode Buccal 2 Collector.

Step 3

Dry prior to processing.

Promega PowerPlex® Fusion

Pre Amplification Procedure

- Ten 1.2mm punches placed into an amplification tray.
- Added 5µL of PunchSolution to each well.
- Incubated at 95°C for 10 minutes.
- Prepared master mix as listed below.
- Ran on thermal cycler following PPF parameters at 25 cycles.

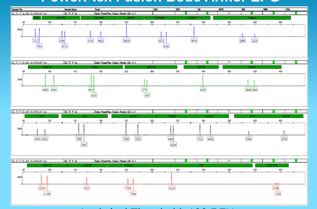
Amplification Master Mix	
Reagent	**Volume per Reaction (µL)**
Amplification Grade Water	15
5X Master Mix	5
5X Primer Pair Mix	5
Total Volume	25

Post Amplification Procedure

- Added 10µL master mix to CE tray.
- Added 1µL of amplification product.
- Loaded on 3500xL.
- Injected at 1.2kV for 19s.

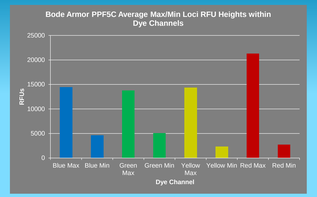
CE Master Mix	
Hi-Di Formamide	WEN-500 ILS
10µL	0.25µL

PowerPlex Fusion Bode Armor EPG



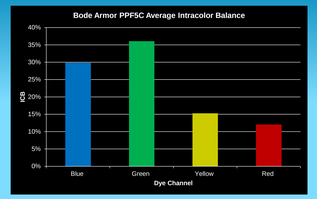
Analytical Threshold: 100 RFUs
Stochastic Threshold: 500 RFUs

Bode Armor PowerPlex Fusion Loci RFU Heights



Heterozygote Balance >50%

Bode Armor PowerPlex Fusion Intracolor Balance



Thermo Fisher GlobalFiler™ Express

Pre Amplification Procedure

- Twenty-five 1.2mm punches placed into an amplification tray.
- 2µL of Prep-n-Go Buffer to each well.
- Prepared master mix as listed below.
- Ran on thermal cycler following GFE parameters at 27 cycles.

Amplification Master Mix	
Reagent	**Volume per Reaction (µL)**
AmpFISTR PCR Enhancer	2.5
GlobalFiler Express Master Mix	6
GlobalFiler Express Primer Set	6
Total Volume	14.5

Post Amplification Procedure

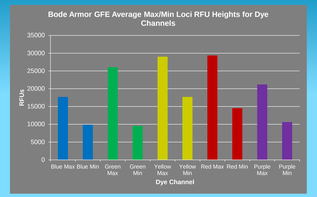
- Added 10µL master mix to CE tray.
- Added 1µL of amplification product.
- Loaded on 3500xL.
- Injected at 1.2kV for 23s.

CE Master Mix	
Hi-Di Formamide	GS-600 LIZ v2.0 ILS
10µL	0.4µL

GlobalFiler Express Bode Armor EPG

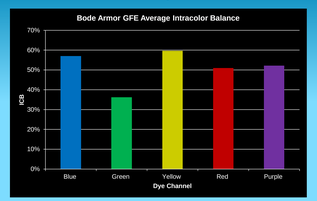
Analytical Threshold: 125 RFUs
Stochastic Threshold: 600 RFUs

Bode Armor GlobalFiler Express Loci RFU Heights



Heterozygote Balance >60%

Bode Armor GlobalFiler Express Intracolor Balance



Qiagen Investigator® 24plex GO!

Pre Amplification Procedure

- Twenty-five 1.2mm punches placed into amplification tray.
- 2µL of Investigator STR GO! Lysis Buffer to each well.
- Incubated at 95°C for 5 minutes.
- Prepared master mix as listed below.
- Ran on thermal cycler following 24plex GO! parameters at 24 cycles.

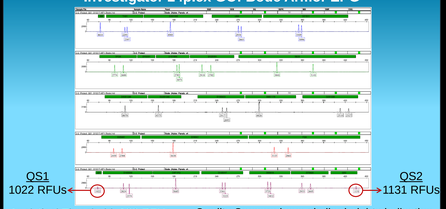
Amplification Master Mix	
Reagent	**Volume per Reaction (µL)**
Fast Reaction Mix 2.0	7.5
Primer Mix 24plex GO!	12.5
Total Volume	20

Post Amplification Procedure

- Added 12µL CE master mix to tray.
- Diluted amplification product 1:3.5 then added 1µL to CE tray.
- Loaded on 3500xL.
- Injected at 1.2kV for 24s.

CE Master Mix	
Hi-Di Formamide	550 BTO ILS
12µL	1.0µL

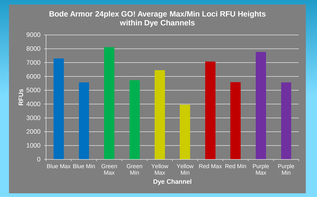
Investigator 24plex GO! Bode Armor EPG



Analytical Threshold: 100 RFUs
Stochastic Threshold: 600 RFUs

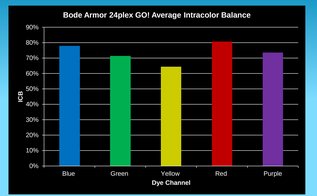
Quality Sensors have similar heights indicating no inhibition occurred during amplification.

Bode Armor Investigator 24plex GO! Loci RFU Heights



Heterozygote Balance >60%

Bode Armor Investigator 24plex GO! Intracolor Balance



Promega PowerPlex® Fusion 6C

Pre Amplification Procedure

- Ten 1.2mm punches placed into amplification tray well.
- 10uL of PunchSolution to each well.
- Incubate at 70°C for 30 minutes.
- Prepared master mix as listed below.
- Ran on thermal cycler following PPF6C paramaters at 24 cycles.

Amplification Master Mix	
Reagent	**Volume per Reaction (µL)**
5X AmpSolution	7.5
5X Master Mix	2.5
5X Primer Pair Mix	2.5
Total Volume	12.5

Post Amplification Procedure

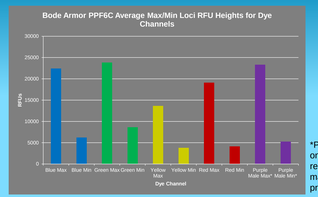
- Added 10µL master mix to CE tray.
- Added 1µL of amplification product to CE tray.
- Loaded on 3500xL.
- Injected at 1.2kV for 35s.

CE Master Mix	
Hi-Di Formamide	WEN-500 ILS
10µL	0.25µL

PowerPlex Fusion 6C Bode Armor EPG

Analytical Threshold: Blue/Yellow=100 RFUs, Green/Red=75 RFUs, Purple=90 RFUs
Stochastic Threshold: 600 RFUs

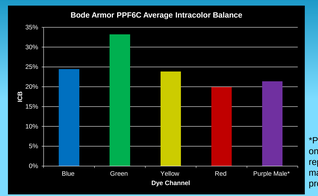
Bode Armor PowerPlex Fusion 6C Loci RFU Heights



*Purple dye only represents male profiles.

Heterozygote Balance >60%

Bode Armor PowerPlex Fusion 6C Intracolor Balance



*Purple dye only represents male profiles.

Conclusion

- Bode Armor is compatible with the direct amplification chemistries:
  - PowerPlex Fusion
  - GlobalFiler Express
  - Investigator 24plex GO!
  - PowerPlex Fusion 6C
- 100% genotype concordance was observed between samples treated with and without Bode Armor.
- Direct amplification of Bode Armor treated buccal collectors produced profiles that met operational acceptance criteria:
  - Intracolor balance
  - Peak height
  - Heterozygous balance