

Introduction

DNA stability of a buccal sample is difficult to predict. The stability of a DNA sample can also vary amongst individuals as each sample is unique in the amount of DNA recovered, the bacteria or enzymes that are collected with the sample, and the storage conditions. The bacteria and nucleases collected with the sample can degrade the DNA and decrease the number of reportable loci.

Bode's newest development to enhance DNA stability is Bode Armor, a proprietary preservative solution that can be applied after sample collection. Bode Armor prevents DNA degradation by inhibiting nucleases and preventing the growth of bacteria as well as other factors associated with DNA degradation. This presentation will detail the developmental studies providing evidence of DNase and bacteria inhibition as well as long term accelerated studies displaying the enhanced DNA stability.

Accelerated stability studies were performed by placing Bode Armor treated Buccal DNA Collector samples in a 56°C incubator for nearly 3 years. At the 3 year time point, samples were removed from the selected storage conditions and processed using standard DNA analysis procedures. Using an accelerated aging calculation, storage for approximately 3 years at 56°C equates to storing at room temperature (22°C) for thirty (30) years.

This study demonstrates the next step in enhancing buccal sample stability; Bode Armor. Bode Armor, when applied to collected samples, prevents naturally occurring enzymes, bacteria, and additional factors collected from the individual's mouth from affecting DNA yields and profile success rates. Combining low humidity storage (The Bode Vault) and a preservative solution (Bode Armor), Bode's scientists have shown stability of a buccal DNA sample up to 30 years during an accelerated study.

Experimental Design

A. Anti-Microbial Property

- Collect 10 paired swabs.
- Apply 100µL Bode Armor to one of the paired samples, leaving the other sample untreated.
- Place swabs in 2.0 ml Nutrient Broth for 15 minutes then vortex briefly.
- Add 0.2mL to Nutrient Agar plate.
- Incubate at 37°C for 16-48 hours.

B. Inhibit DNase I Activity

- Digest 350ng of 9947 DNA with 0.01 units of Mo Bio Max Kit DNase I.
- Incubate at 37°C for 15 minutes.
- Extract samples with Qiagen EZ1 DNA Investigator Kit.
- Quantify using ABI Quantifiler™ Trio.

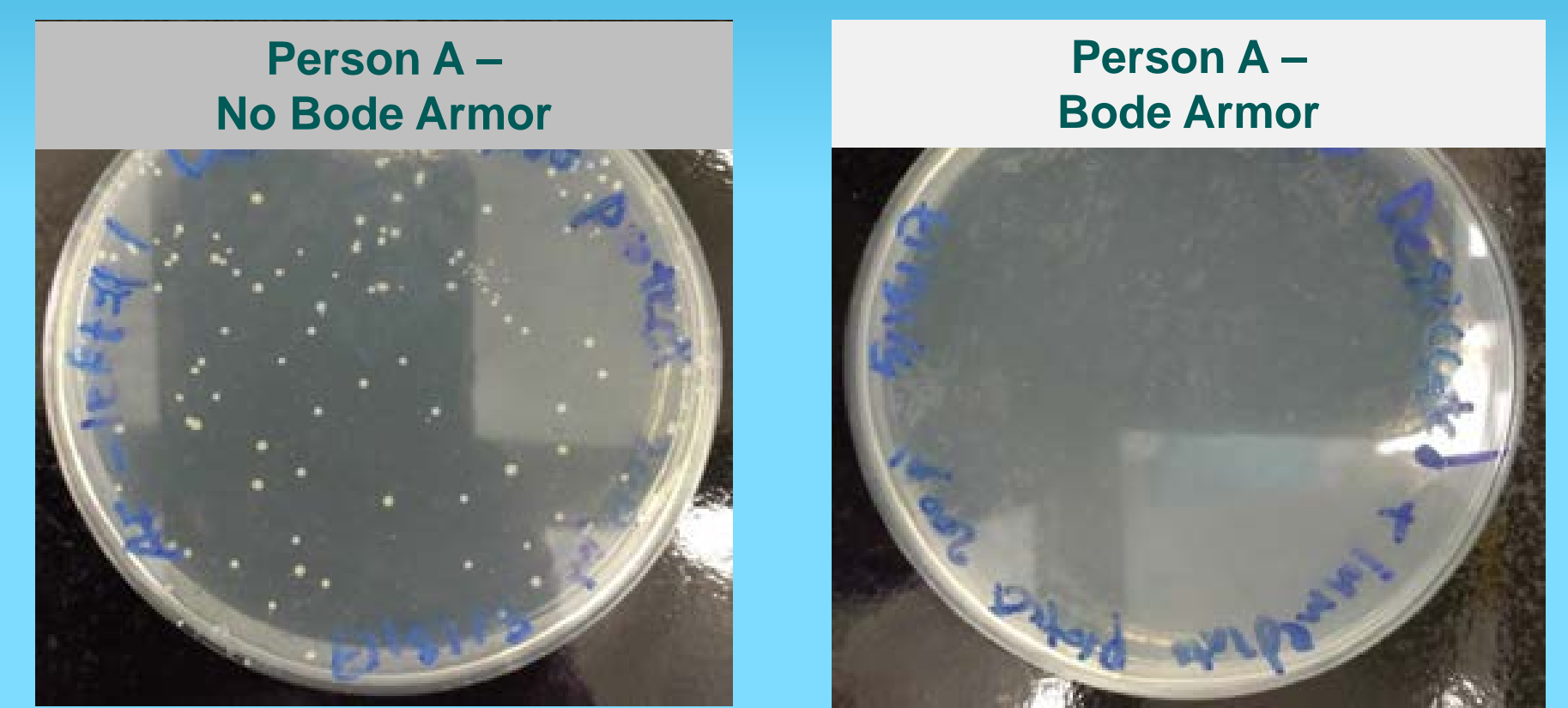
C. DNA Stability Study

- Collect Swabs from volunteers and apply 100µl Bode Armor.
- Store swabs in Bode Vault or Humidity Chamber for 2.8 years at 22°C and 56°C.
- Collect 2 x 4.7mm punches per reaction.
- Extract samples with Qiagen QiaSymphony DNA Investigator® Kit.
- Quantify with ABI Quantifiler™ Trio DNA
- Amplify with Promega PowerPlex® Fusion (1ng target, 25 µL reaction at 29 cycles).
- Separate and analyze with ABI 3500xL Genetic Analyzer and Gene Mapper ID-X.

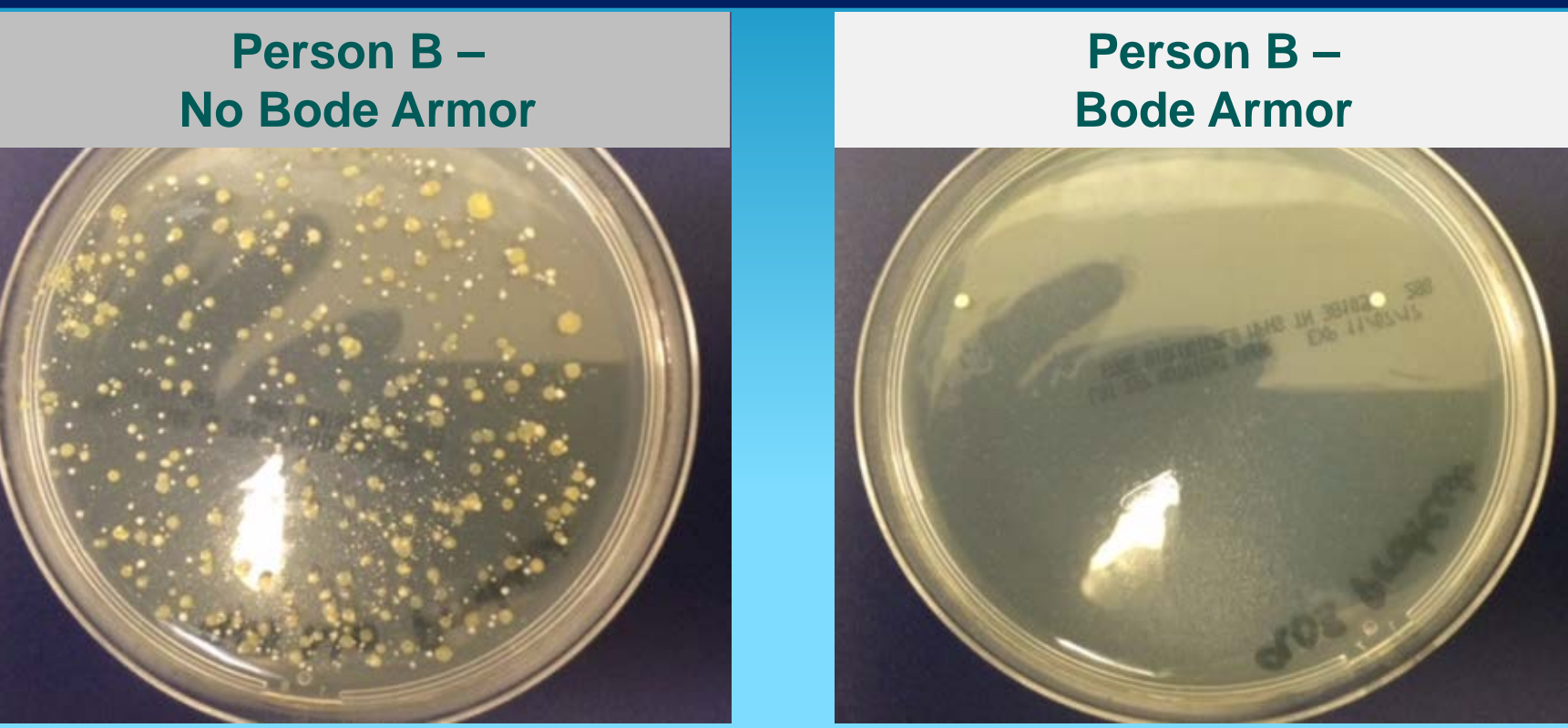
PowerPlex is a registered trademark of Promega Corporation. Quantifiler is a registered trademark of Applied Biosystems. Investigator is a registered trademark of QIAGEN Group.

Anti-Microbial Property

Bode Armor Significantly Inhibits Bacterial Growth

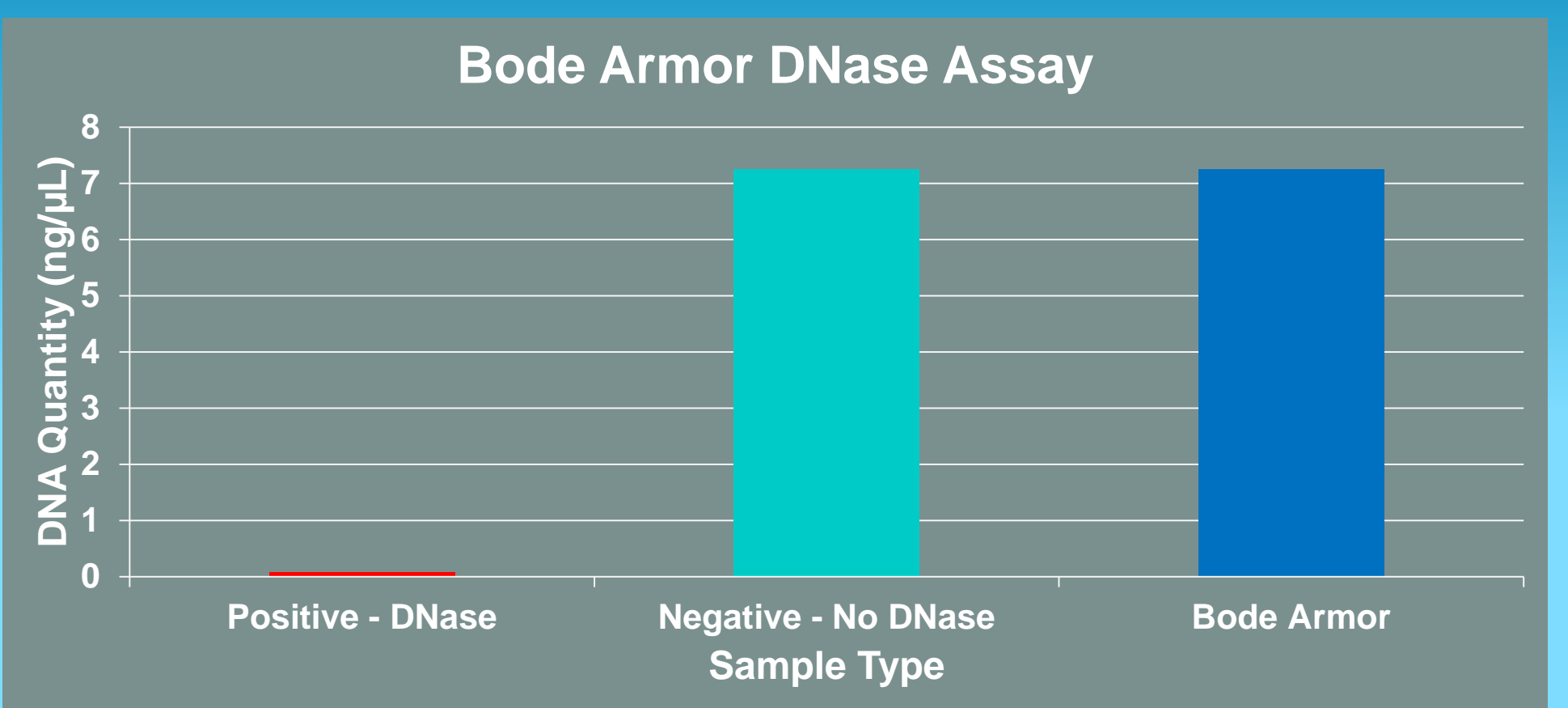


Anti-Microbial Property



Number of Colonies Per Plate	No Bode Armor	Bode Armor	% Reduction
Person A	184	0	>99.46%
Person B	>700	2	>99.82%
Average (n=10)	154	1.9	98.7%

Inhibit DNase I Activity



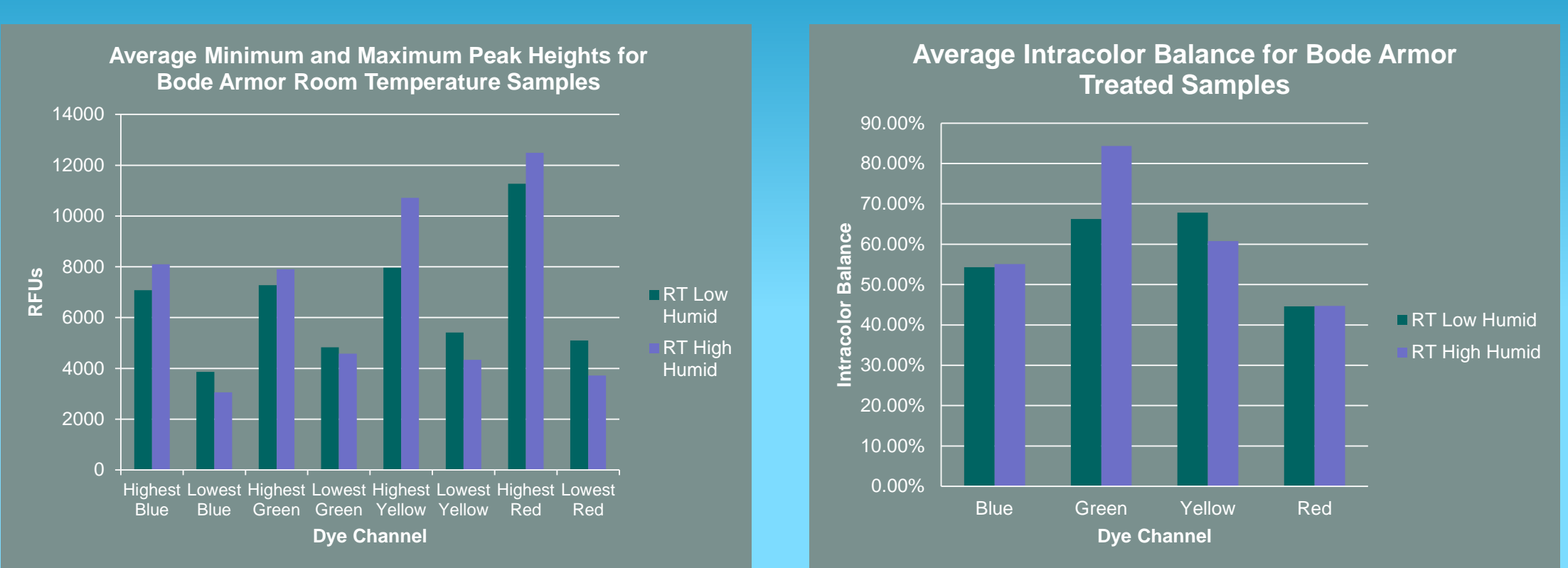
- Bode Armor inhibited 100% of 0.1 units of DNase I from degrading 350 ng of template DNA degrade.
- 0.1 units DNase (DNase Positive control) degrades 100% of 350 ng DNA.

Bode Armor DNA Stability Study

Sample Age (Years)	Storage Temperature	Humidity	Average Small Autosomal Target (ng/µl)	Average Large Autosomal Target (ng/µl)	Degradation Index
2.8	Room Temp	Low	7.28	8.53	0.86
2.8	Room Temp	High	4.99	4.18	1.33
30 (simulated)	56°C	Low	2.64	0.81	3.77

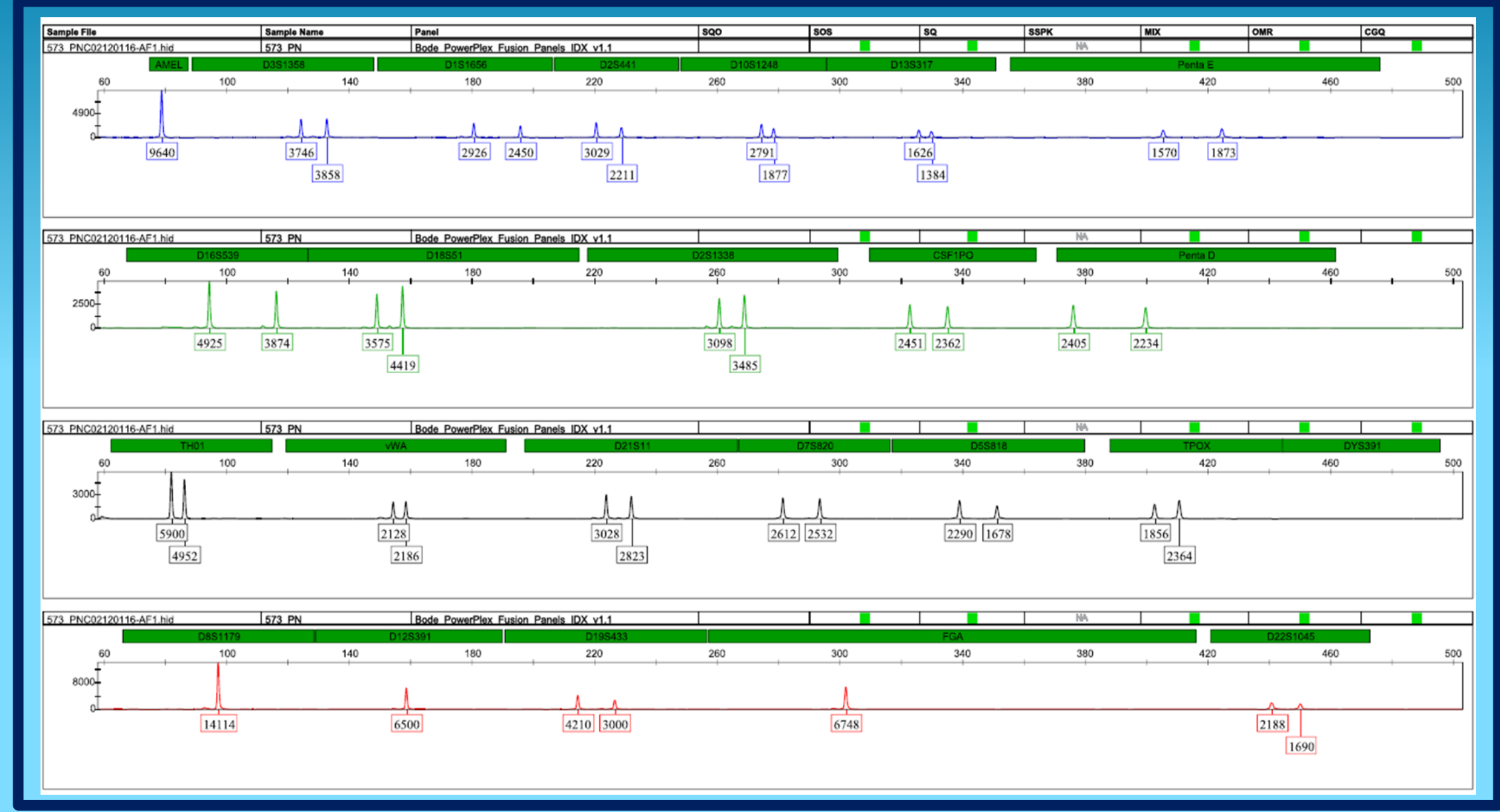
- Degradation index= DNA quantity of large target / DNA quantity of small target
- Degradation index 1=Little to no degradation
- Degradation index 2-4=Some degradation has occurred
- Degradation index 5+= Sample is degrading

DNA Stability: 2.8 Year Room Temperature Bode Armor Peak Heights and Intracolor Balance

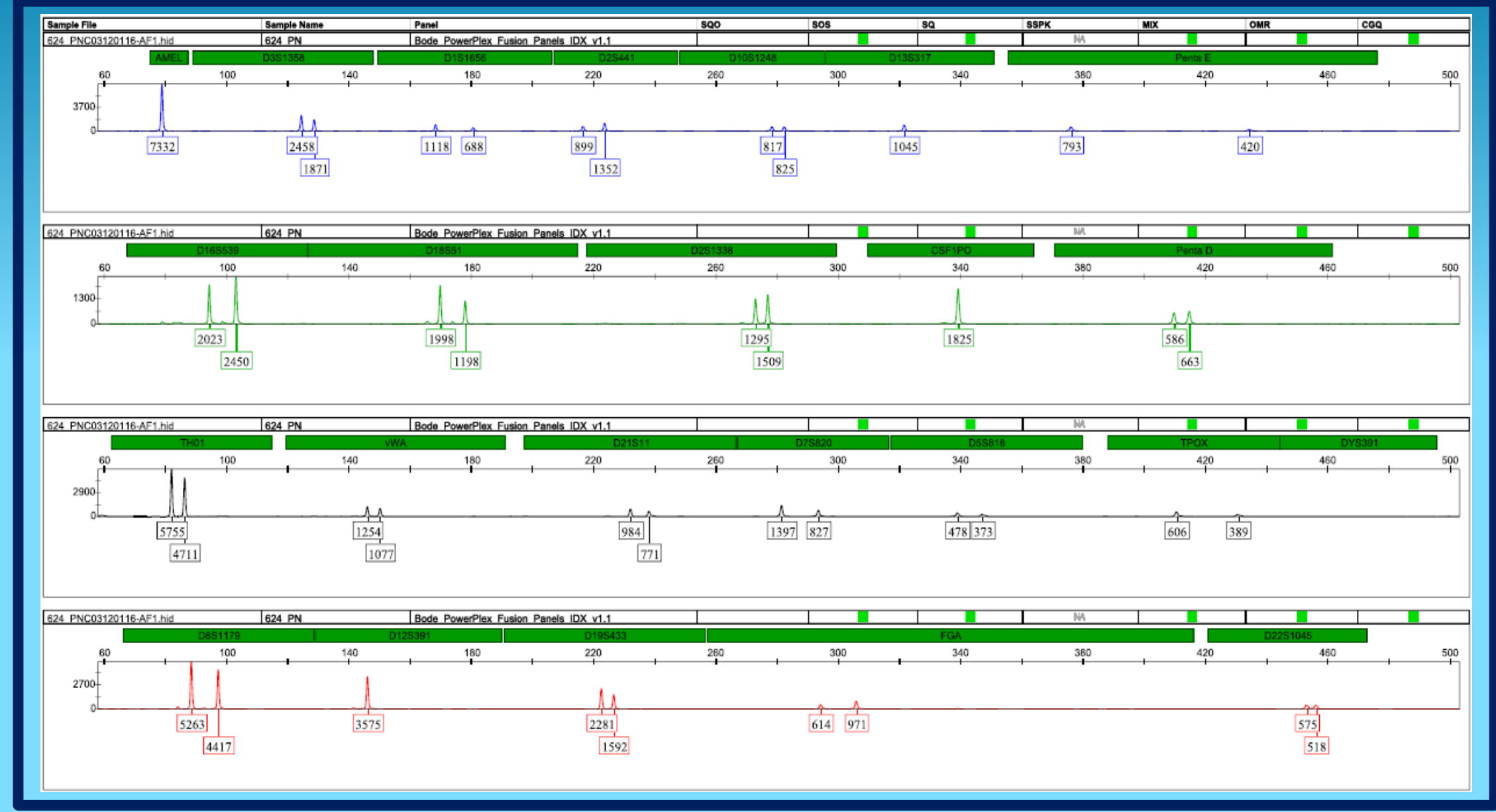


Bode Armor treated samples stored in the Bode Vault at 22°C for 2.8 years in high or low humidity.

2.8 Years Room Temperature High Humidity with Bode Armor



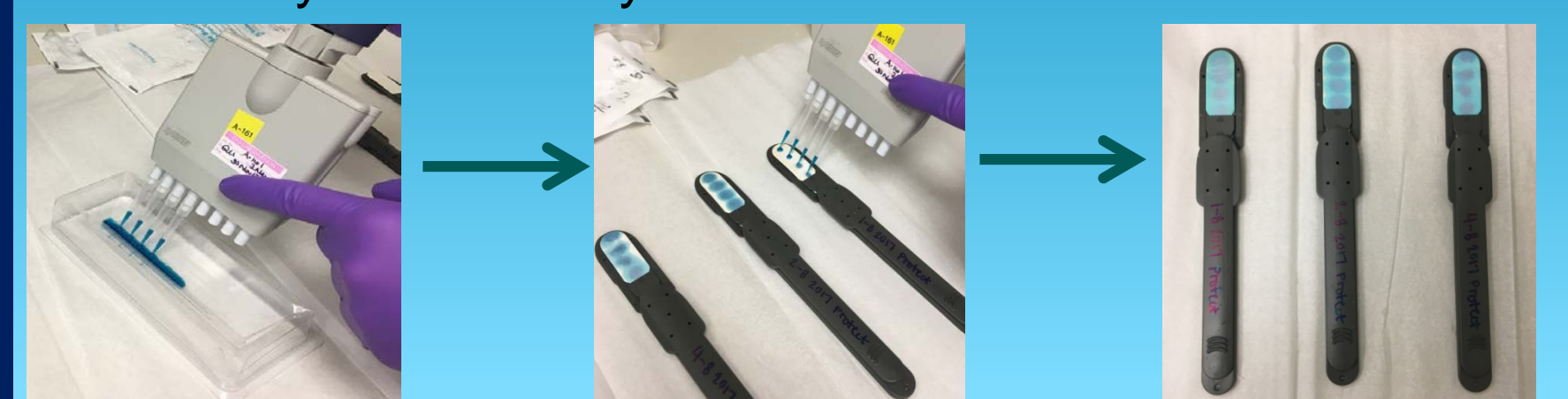
Thirty Year Accelerated Aging (2.8 Years at 56°C) with Bode Armor



Bode's Enhancements In Stability

Bode Armor

- Inhibits bacterial growth and enzymatic activity



Bode Vault

- Provides protection of the DNA from environmental effects such as uncontrolled humidity.



Conclusions

- Bode Armor enhances stability of Buccal samples.
 - Accelerated testing indicates stability at 30 years.
- Bode Vault and Bode Armor stabilizes DNA in a high humidity environment.
- Bode Armor prevents microbial growth.
- Bode Armor inhibits DNase activity.