Forensics @ NIST
December 8, 2010 – Gaithersburg, MD

DNA Stability Studies:
FTA vs 903

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Overview

- History of DNA storage studies at NIST
- Stability at different temperatures and different papers
- Review Anal Chem 2002 paper
- Review other studies
- Impact on AFDIL and the repository freezers
- Biomatrica and GenVault

Talk Topics

- Differences between FTA and 903 paper
- Long term Stability of Bloodstains
- Extraction issues
- Direct amplification
- New technology

Why Study DNA Storage Conditions?

- DNA databanks exist.
  Can you recover typeable DNA from them?
- Refrigerating samples is expensive.
  Is it necessary?
- Different DNA storage media are used.
  Are they equivalent?

The Initial 903 Experimental Design

- Initiated July 1994 at the request of the Armed Forces DNA Identification Laboratory (AFDIL)
- 20 µL whole blood, spotted on 903 paper, dried overnight in a vacuum desiccator at ambient temperature
- 6.3 mm punches from dried bloodstains into cryogenic vials
- Vials stored at: ambient, 4 °C, -20 °C, -80 °C and Liquid Nitrogen (-198 °C).

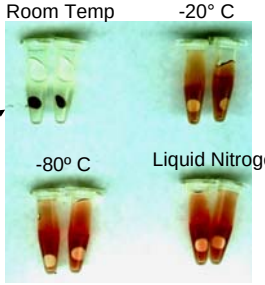
Measurement Criteria: Typeable DNA

- In 1994 “Typeable DNA” meant you could successfully PCR amplify the D1S80 locus, target size range of 300 bp to 700 bp
- In 1998 switching to PCR amplification of Short Tandem Repeat (STR) loci reduced the size range of 80 bp to 450 bp

DNA Stability Study
6 year time point

S&S 903 paper

During Chelex extraction RT sample during water washes remains colored.



Short Term Study
in conjunction with AFDIL

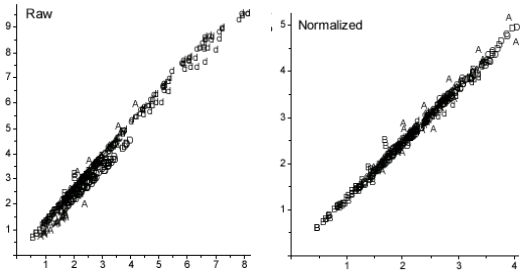
- 70 individuals' blood spotted on four different storage media
  - S&S 903™ & IsoCode® papers
  - FITZCO, Inc Whatman BFC 180 & FTA papers
- Blood stains and desiccant vacuum sealed
- Stored for 19 months at lab ambient temperature

Results of the Short Term Study

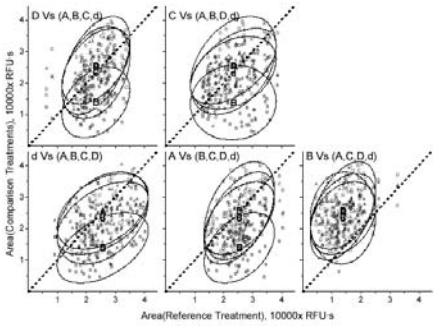
- All four storage media provided fully typeable (qualitatively identical) samples
- The average normalized among-locus Relative Fluorescent Unit (RFU) signal provided a metric for determining the relative amounts of amplifiable DNA recovered

Kline *et al*, Anal Chem 2002;74:1863

Peak Ht 1000x RFU Vs Peak Area 10000x RFU



Bivariate Distributions



FTA - 903 Characteristics

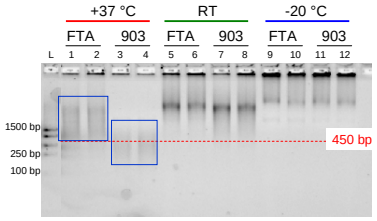
FTA	903
• high-purity cotton linter pulp • chemically treated with several compounds designed to kill pathogens and resist bacterial growth and DNA degradation Tris, EDTA, SDS, and uric acid • lysis cells on contact high molecular weight DNA to become entangled in the fibers of the paper • DNA binds to paper	• high-purity cotton linter pulp • no chemicals added • used in newborn screening programs. • used to screen for HIV, HCV, hemoglobin A1c, and other epidemiological applications • support media • DNA not bound to paper

11 year FTA - 903 Study

Results as of 13 August 2008

- Bloodstains prepared 09/97 on FTA and 903 stain cards
- Stains dried and vacuum sealed in Mylar bags
- Stains stored at -20 °C, RT, and +37 °C
- Duplicate stains extracted with DNA IQ
- Extract analyzed on a Flashgel to check degradation
- Extracts quantified with Quantifiler Human qPCR kit
- Quantified extracts amplified with Identifiler
- If allele dropout observed, samples amplified with Minifiler

Quality of the Extracted DNA

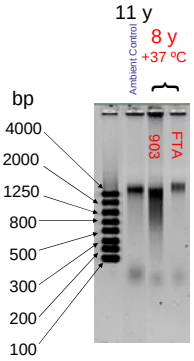


Most STR typing kits have products that are less than 450 bp

L: ladder with 250 bp, 400 bp, 800 bp and 1500 bp bands visible
Lanes 1, 2: + 37 °C FTA; Lanes 3, 4: + 37 °C 903;
Lanes 5, 6: RT FTA; Lanes 7, 8: RT 903;
Lanes 9, 10: -20 °C FTA; Lanes 11, 12: -20 °C 903;

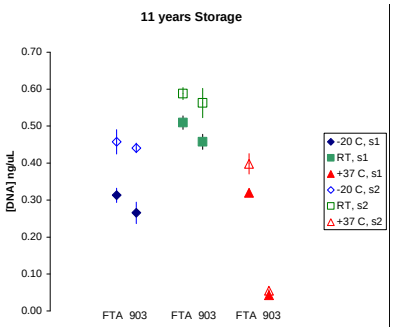
After 11 years of storage at 37 °C both FTA and 903 show signs of degradation, the FTA samples exhibit DNA with slightly higher molecular weight than the 903 samples.

DNA Extracts of Aged Bloodstains

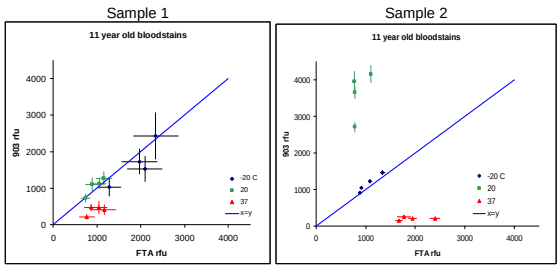


After 8 years at +37 °C the 903 DNA extract was just starting to show signs of degradation starting to occur when compared to the FTA DNA extract

Extracted DNA Quantitation

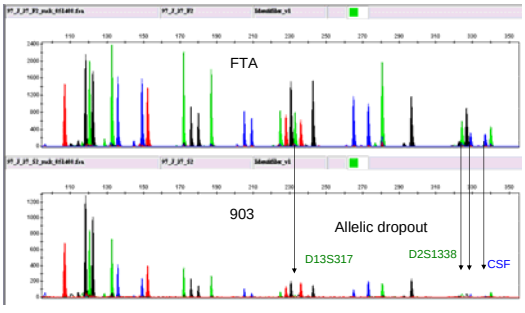


Comparison of Idfiler Peak Hts FTA vs 903

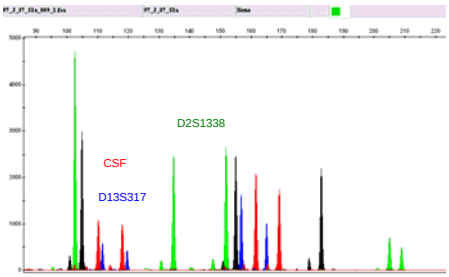


10 µL Idfiler reaction volume with 0.2 ng to 0.6 ng extracted DNA
Allelic dropout seen in CSF, D16 and D2 in some of the +37 °C stored samples; these alleles recovered with Minifiler

FTA – 903 +37 °C Storage Idfiler



903 +37 °C Storage Minifiler amp



Alleles dropping out with Idfiler amplification are recovered with Minifiler

FTA-903 Controlled Study Summary

- After 11 years at 37 °C, the FTA samples yield a full profile with Identifiler while the 903 samples have allelic dropout due to the DNA degradation. However, the loci lost with Identifiler can be recovered with the use of miniSTRs.
- There is no difference in DNA recovery between ambient and -20 °C storage.

903 Bloodstain Study

Stains obtained from outside sources

"Uncontrolled Bloodstain Stability"

Examined over 300 anonymous bloodstains:

Stored on untreated 903 paper 2 - 15 years at ambient temperature, no humidity control

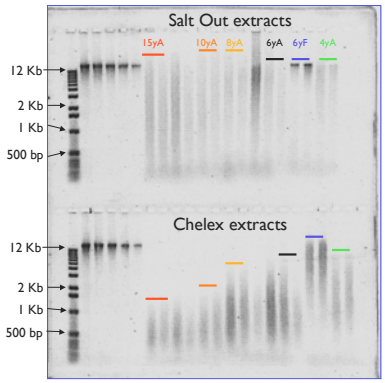
Control samples stored at -20 °C for 6 years

Different methods of extraction:

Chelex and Salt Out

Evaluation of the quality of the recovered DNA:

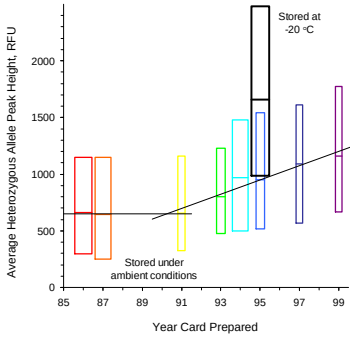
Yield gel and STR-typeability



DNA in frozen samples (6yF) basically intact (size >12 kb)

DNA from all samples stored at ambient appear as smears (fragment sizes range from 12 kb to ≈100 bp)

STR analysis results: Average Heterozygousity Peak Heights



Median allele height as a function of storage time.

Each "box" denotes the central 50% of each height distribution (25%, median, 75%). The box width is proportional to the number of alleles. The lines suggest a possible "plateau" in height loss after about a decade's storage at ambient temperatures.

Study conducted in 2001

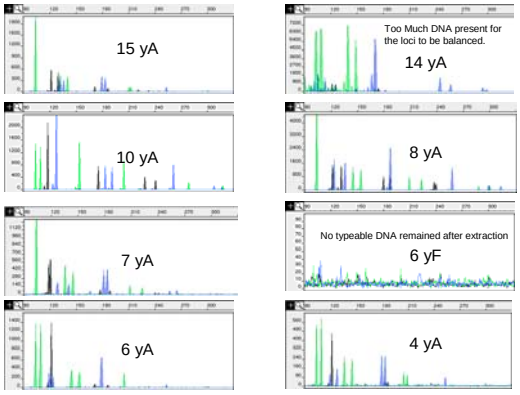
But how efficient is the extraction?

- Observations
  - The longer the bloodstain is stored at room temperature the harder it is to wash the heme away
  - Heme washes away easily from stains stored at -20 °C
- Theory:
  - If protein (i.e. heme) sticks to the paper, what stops DNA from sticking?

What's left on the paper?

- Take bloodstain that has been Chelex-extracted
- Wash with a tris buffer
- Take a 1.2 mm punch of the washed stain
- Place the washed 1.2 mm punch in a PCR tube and amplify

Amplification of the 903 spot after Chelex extraction. Sample Size: 1.2 mm



Summary "Uncontrolled" Bloodstain Stability

- All samples have typeable DNA
- Loss of some larger STR loci in older, more degraded samples
- Chelex extracts typed as well as the "salt out" extracts
- DNA is more tightly bound to 903 paper the longer it is stored at ambient; the number of "dropout" alleles increases with storage time
- More DNA available from stains stored for 6 years at -20 °C than from those stored for 2+ years at ambient
- DNA from the -20 °C stored samples readily Chelex extracted from the 903 paper
- Bound DNA can be amplified directly from 903 paper stored at ambient temperature after Chelex extraction

New Amplification Technologies Direct PCR

- Investigating the use of direct PCR kits
  - Identifiler Direct
  - PowerPlex 16 HS
  - Identifiler Plus

Direct amplification of STRs from blood or buccal cell samples Wang et al., FSI Genetics Supplement Series 2: 113-114

- Identifiler Direct was specifically developed for direct PCR, but the other kits can be adapted for this purpose

Direct Amplification from Buccal and Blood Samples Preserved on Cards Using the PowerPlex® 16 HS System

http://www.promega.com/profiles/1202/1202_01.htm

Samples

- 50 anonymous blood samples were obtained from Interstate Blood Bank
  - Prepare a set of reference blood cards for Direct PCR testing
    - 4 Caucasian
    - 46 African American

- 4 mL blood received
  - 2 mL spotted
    - Whatman 903 Cards (3 cards for each sample)
    - FTA Cards (2 cards for each sample)
  - 2 mL extracted
    - Three extraction protocols

Currently the blood cards are being stored at room temperature

Direct PCR Protocol

- 1.2 mm punch added to Master Mix
  - Whatman 903 Cards
  - FTA Cards
- All samples amplified with a 25 µL reaction

Direct PCR Master Mix Recipe
1.Reaction Mix
2.Primer Mix
3.Water to Bring to Volume

Add to PCR tubes containing DNA punch and amplify

Thermal Cycling Protocols

- Following manufacturers' protocol

Identifiler Direct (**27 cycles**)

Initial incubation step	Cycle (27 cycles)			Final extension	Final hold
	Denature	Anneal	Extend		
HOLD	CYCLE			HOLD	HOLD
95 °C 11 min	94 °C 20 sec	59 °C 2 min	72 °C 1 min	60 °C 25 min	4 °C ∞

Identifiler Plus (**28 cycles**)

Initial incubation step	Cycle (28 or 29 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95 °C 11 min	94 °C 20 sec	59 °C 3 min	60 °C 10 min	4 °C ∞

2-step cycling

PowerPlex 16 HS (**32 Cycles**)

Protocol for the GeneAmp® PCR System 9700 Thermal Cycler[1]

96°C for 2 minutes, then:

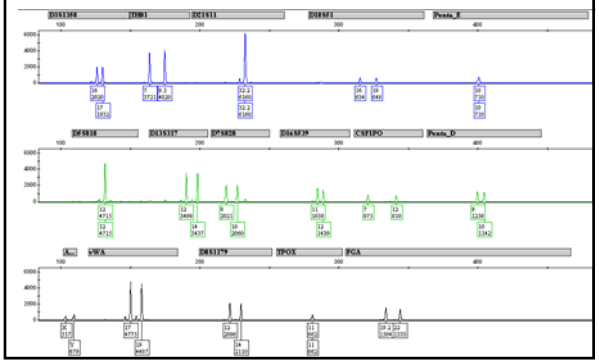
ramp 100% to 94°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 10 cycles, then:

ramp 100% to 90°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 22 cycles, then:

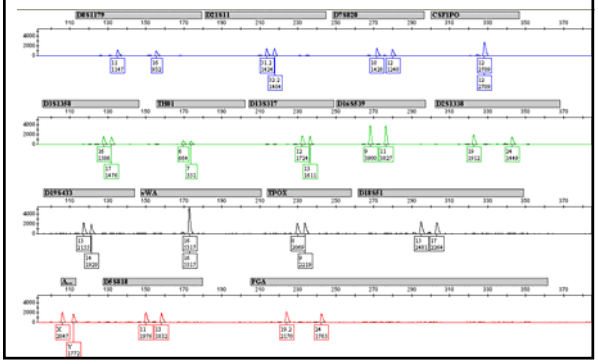
60°C for 30 minutes

4°C soak

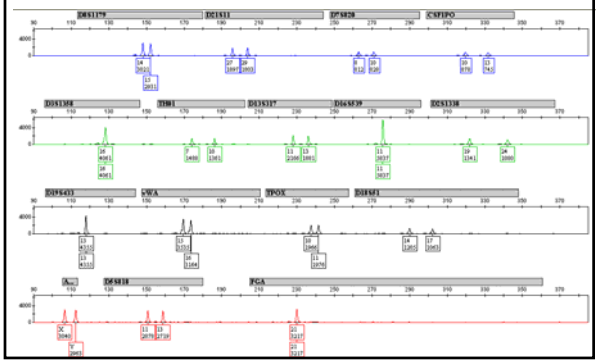
PP16HS (903)



Identifiler Direct (FTA)



Identifiler Plus (FTA)



New Amplification technologies with Aged Samples

- **PowerPlex 16HS** and direct PCR
  - Samples from 1986, 1987, 1991, 1993, 1994
  - One 1.2 mm punch of a 903 stain placed into each of two PCR tubes for each sample
  - 8 sample tubes per year amplified
  - No extraction of these 16 to 24 year-old, ambient-stored samples

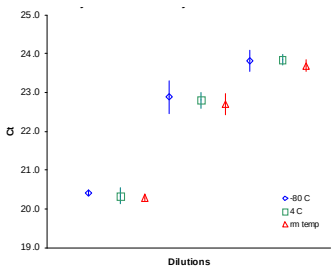
Results of the Direct amplification

- One of the duplicate samples from 1987 lost one allele (Penta E locus)
- All other samples yielded full STR Profiles
- Another direct amplification kit (AB Identifiler direct) has not been tested

Stability of Extracted DNA

Liquid extract or dry storage

Extracted DNA Stability in PFA (Teflon) Tubes



Highly purified DNA

Storage temperatures
-80 °C
+4 °C
Room temperature

Three DNA concentrations

Storage time 6 years

qPCR Assay:
Quantifiler human
Triplicates analyzed.

Error bars are 2 sd

No differences between storage temperatures regardless of DNA concentration.
(But PFA tubes are not cheap!)

Biomatrica - GenVault

- Biomatrica's DNA SampleMatrix :
- based on a glass polymer that "shrink-wraps" and protects DNA from heat and UV light through a mechanism similar to that used by extremophiles, small organisms that can survive in dry environments for up to 120 years (Crowe et al., 1998).
- GenVault's GenTegra DNA:
- is an inorganic mineral matrix with oxidation protection and antimicrobial activity for storage of purified DNA at room temperature.

Crowe *et al*. The role of vitrification in anhydrobiosis. Annu Rev Physiol 1998;60:73

Biomatrica SampleGard Plate experiments 208 days of Shipping/Stressing

S.B. Lee, C.A. Crouse, and M.C. Kline. Optimizing Storage and Handling of DNA Extracts. Forensic Science Review 2010;22:132

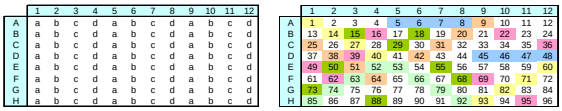
Study Design

- Well characterized DNA (highly purified) of known concentration was diluted in PFA (Teflon) containers to target concentrations of:
  - 1.0 ng/µL (a)
  - 0.25 ng/µL (b)
  - 0.05 ng/µL (c)
  - TE^{-4} buffer was used as the fourth sample set. (d)
- 20 µL of prepared solutions were aliquoted in 4 different Biomatrica SampleGard 96 well plates.
- DNA solutions remaining in PFA containers were stored at 4 °C as a cold storage control.

Study Design Continued

- SampleGard plates air dried in a laminar flow hood overnight
- Plates labeled A, B, C, and D
- Plates A and C stayed at NIST
- Plates B and D shipped/stressed
- Temperature and humidity dataloggers stored with the plates

Biomatrica Plate set-up



Sample wells highlighted in colors have been tested
Each color represents a different analysis time point

Four Biomatrica SampleGard plates were prepared using a well - characterized and purified genomic DNA solution listed as follows:

a = 20 µL of a DNA solution at 1 ng/µL
b = 20 µL of a DNA solution at 0.25 ng/µL
c = 20 µL of a DNA solution at 0.05 ng/µL
d = 20 µL of TE^{-4} to serve as a negative control

Two prepared plates were stored at lab ambient temperatures, while the remaining two plates were "shipped" cross-country and exposed to harsh environmental conditions prior storage at lab ambient temperatures.

Study Data analysis

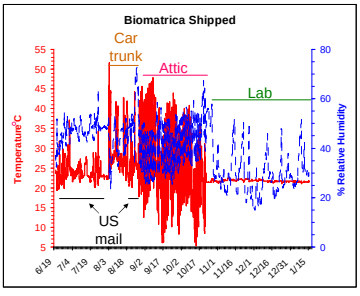
- 2 wells of each DNA concentration, one from a "shipped/stressed" plate and one a lab-ambient control plate, re-hydrated with 20 µL of DI water
- Plates containing the re-hydrated wells were slowly rotated for 45 min
- Immediately following rotation, samples quantified with a Quantifiler Human qPCR kit along with 4 °C PFA-stored DNA solutions.

Study Data analysis con't

- Re-hydrated materials and PFA controls amplified with Identifiler.
- Volumes used for amplification based on the [DNA] applied.
  - 12.5 µL total reaction volume

[DNA] ng/µL	µL used for Idfiler	ng DNA amplified
1	1	1
0.25	2	0.5
0.05	5	0.25
TE^{-4}	5	0

"Shipped/Stressed" Temperature & % Relative Humidity Profile, 208 days



Two Biomatrica SampleGard plates were "shipped" back and forth between MD and CA during the Summer of 2007

After 6 cross country trips the plates were placed in a car trunk for 14 days

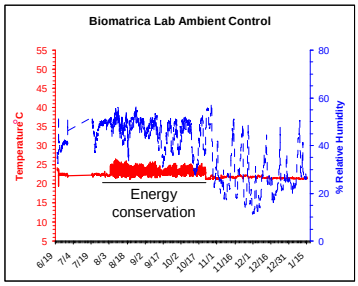
Two more cross country trips

Exposed to ambient attic temperatures for 56 days

Finally plates were placed at lab ambient conditions

Max: 51.6 °C, 73 % RH Median: 22.1 °C, 40 % RH
Min: 5.3 °C, 15 % RH Avg: 23.6 °C, 39 % RH

Lab Ambient Temperature & % Relative Humidity Profile, 208 days



Two Biomatrica SampleGard plates stored in a office/lab during the Summer of 2007. Materials were transferred to the lab when it became apparent that summer energy conservation efforts were measurable.

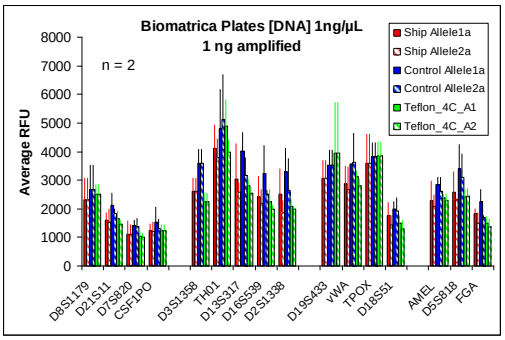
Max: 26.4 °C, 58 % RH
Min: 19.4 °C, 11 % RH
Median: 22.2 °C, 41 % RH
Avg: 22.4 °C, 38 % RH

208 Day Quantitation Results

n=4	[DNA] ng/µL	Shipped Stressed	Lab ambient	4 °C Teflon
a	1	0.65 ± 0.06	0.69 ± 0.03	1.01 ± 0.02
b	0.25	0.18 ± 0.03	0.20 ± 0.01	0.30 ± 0.01
c	0.05	0.04 ± 0.00	0.04 ± 0.06	0.05 ± 0.00

QFiler results run on a 7900 HT qPCR instrument. SRM 2372 Component A used to establish the calibration curve. The decrease in the DNA concentration of the materials added to the Biomatrica SampleGard plates had been noted earlier, based on genotyping results obtained from Identifiler. This quantification data **may be** influenced by the color present in the Biomatrica samples.

Identifiler results [DNA] 1 ng/µL after 208 days storage



Identifiler results

- At 208 days
  - Data from lab-ambient control SampleGard plate agrees with data from the 4 °C PFA controls
  - Data from "shipped/stressed" SampleGard plate has slightly lower RFUs than the data from the Lab ambient control SampleGard plate
  - All samples, including those at 250 pg, gave full 200^{+} RFU profiles

Additional information PFA stored extracts

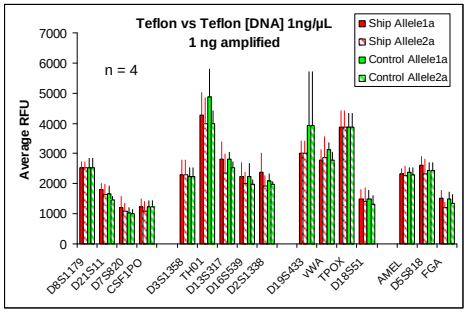
- On August 26, 2007, 100 µL aliquots from the Control 4 °C PFA containers were removed and placed in sterile labeled PFA vials
- These vials stored with the "shipped/stressed" Biomatrica SampleGard plates.
- The "shipped/stress" box placed in an attic for 8 weeks then moved to Lab ambient temperature.
- At analysis time the PFA vials were stressed for 147 days out of the total 208 days.

147 Day Quantitation Results

	[DNA] ng/µL	Shipped Stressed	Lab ambient	Shipped PFA (147 days)	4 °C PFA
a	1	0.65 ± 0.06	0.69 ± 0.03	1.00 ± 0.02	1.01 ± 0.02
b	0.25	0.18 ± 0.03	0.20 ± 0.01	0.25 ± 0.02	0.30 ± 0.01
c	0.05	0.04 ± 0.00	0.04 ± 0.06	0.04 ± 0.01	0.05 ± 0.00

QFiler results from a 7900 HT qPCR instrument
SRM 2372 Component A used to establish the calibration curve

Identifiler results [DNA] 1 ng/µL after 147 days storage.



PFA vs PFA results

- The “shipped/stressed” DNA in the PFA vials after 147 days quantified and amplified the same as the 4 °C PFA containers that were stored for 208 days
- Highly purified DNA is extremely stable

NIST
Applied Genetics

Thank You for Your Attention…

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Thanks to:

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John Butler
Becky Hill
Erica Butts
Pete Vallone

For their assistance in collecting and analyzing some of the data