FORENSICS @ NIST
November 28-30, 2012 • #NISTForensics

Stability Studies for DNA in Bloodstains and as Extracted Material

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Forensics@NIST 2012 Meeting
Gaithersburg, MD
November 28, 2012

Why do we care about the stability of DNA?

There's two sides of that coin

Find the guilty

Protect the innocent

NIST
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Cold-case unit: **DNA solves** 1991 homicide : American Canyon Eagle
napavalleyregister.com/.../**cold-case**...**dna-solves**.../article_8eb2e0d0-...
Jun 29, 2012 – **DNA** evidence has **solved** a "**cold**" **case** — a 1991 homicide in which **...** Lantz was identified **by DNA** found on the handle of the knife used to kill **...**

DNA solves 13-year-old **cold case** murder mystery | ksl.com

www.ksl.com/?nid=148&sid=18133538
Nov 17, 2011
DNA solves 13-year-old **cold case** murder mystery. **By** Sandra Yi. November 17th , 2011 @ 10:07pm. This **...**

More videos for **cold cases solved through dna** »

Cold Case Investigations and Forensic **DNA** | National Institute of **...**
nij.gov/topics/forensics/investigations/**cold-case**/
Jul 16, 2012 – Overview of **Using DNA** to **Solve Cold Cases**. Experience has shown that **cold case** programs can **solve** a substantial number of violent crime **...**

40 Years Later, LBPD **Solves** City's **Oldest Cold Case** Homicide
www.lbpost.com › News

by Sarah Bennett - More by Sarah Bennett
Aug 28, 2012 – Police said that the 40 year-old murder is the Department's **oldest cold case** homicide **solved** to date. Using modern DNA-testing methods paid **...**

55 years

1957 **cold case solved**, man convicted for murder of 7-year-old
www.heraldextra.com › News › Latest National News
Sep 15, 2012 – 1957 **cold case solved**, man convicted for murder of 7-year-old **...** him in one of the **oldest** unsolved crimes to make it to trial in the U.S. **...**

DNA Frees Innocent Man, But What About Eyewitnesses ...
news.discovery.com › Human News
May 7, 2010 – An **innocent man** was recently **freed** by **DNA** testing; but what about the people who saw him do it?

DNA evidence **frees innocent man** 24 years later - New York Daily ...
articles.nydailynews.com/.../33406831_1_nina-morrison-**dna**-eviden...
Aug 26, 2012 – A Texas **man** who spent half his life behind bars for a crime he did not commit is finally **free**. David Lee Wiggins, 48, was released from prison ...

New **DNA** testing **frees** convicted Colorado rapist, killer - U.S. News
usnews.nbcnews.com/_.../11466476-new-**dna**-testing-**frees**-convicted...
Apr 30, 2012 – Update: A **man** sentenced to life in prison for the rape and killing of a Colorado woman was **freed** on Monday based on advanced **DNA** testing ...

Innocent man accused of rape **freed** by **DNA** after 20 years | NOLA ...
www.nola.com › ... › Breaking News
Apr 27, 2012 – It was the first exoneration under the Orleans Parish Post-Conviction **DNA** Evidence Project in its two-year existence.

DNA frees innocent man after 30 years | News24
www.news24.com/.../**DNA-frees-innocent-man**-after-30-years-20110...
Jan 4, 2011 – Dallas - Prosecutors declared a Texas **man innocent** on Monday of a rape and robbery that put him in prison for 30 years, more than any other ...

Innocent Man Free After 35 Years - **DNA** evidence clears him of rape ...
www.newser.com/story/76448/**innocent-man-free**-after-35-years.html
Dec 17, 2009 – (Newser) – James Bain left prison today after serving 35 years for a crime he didn 't commit. The 54-year-old Florida **man**, cleared by **DNA** ...

Michael Morton Goes **Free** After Nearly 25 Years in Prison ...
abcnews.go.com › US
Oct 4, 2011 – **Man** Shot in the Head Awakens From Coma. ... Michael Morton Set **Free** After Spending Nearly 25 Years in Prison, Exonerated by **DNA** Evidence for His ... The

Considerations for DNA Stability studies?

- What is the form of your DNA?
  - Bloodstain or Buccal swab
  - Extracted liquid in a tube
  - Extracted and dried in a tube or as a stain
  - Stabilized with a matrix/additive

Considerations for DNA stability studies?

- What are the best conditions for storage?
  - Room temperature?
  - Refrigerated?
  - Frozen?
- How long you can keep a sample under specified storage conditions?
  - Days?
  - Months?
  - Years?

Considerations for DNA stability studies?

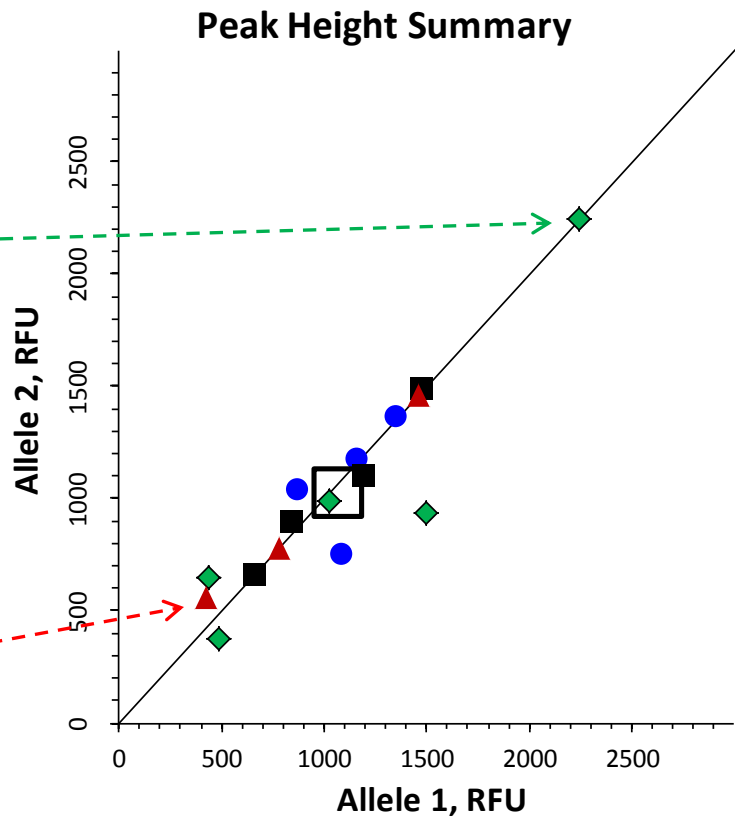
- What environmental factors influence stability?
  - Temperature excursions
    - Power failures
    - Shipping/ Transporting the samples
- Does the sample need to stay pristine, or can you tolerate some degradation?
- What are you measuring and how do you rate the results, DNA Quality/Quantity or both?

So what metric are you going to use?

Since our bottom line is can you get an STR profile ?

We can take the electropherogram and plot allele1 vs allele 2 peak heights

Or the results of matrix 1 vs matrix 2



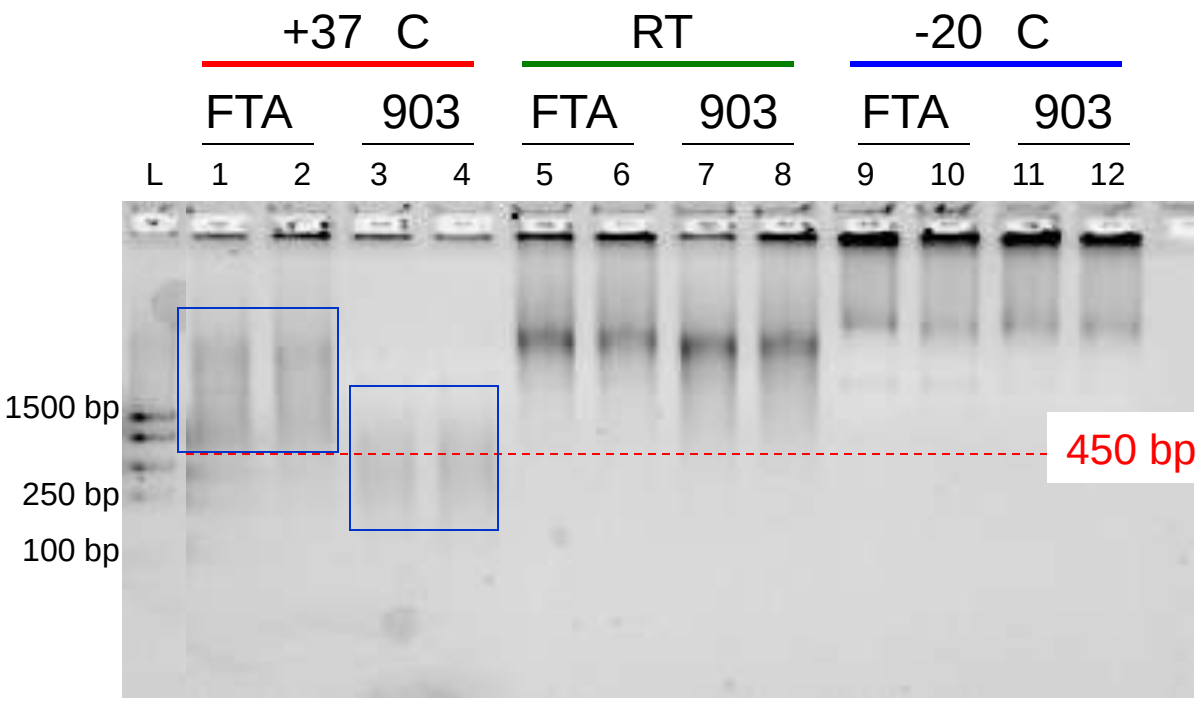
Dried Bloodstains

- Blood from 2 different donors
- Storage Media
  - ✓FTA paper
  - ✓903 paper
- Environmental factors
  - ✓37 C (some places this is ambient)
  - ✓Laboratory Room Temperature
  - ✓-20 C.

DNA/Bloodstain Storage Papers

903	FTA
• high-purity cotton linter pulp	• high-purity cotton linter pulp
• no chemicals added	• chemically treated with several compounds designed to kill pathogens and resist bacterial growth and DNA degradation Tris, EDTA, SDS, and uric acid
• **DNA not bound to paper**	• **DNA binds to paper**

Quality of the Extracted DNA



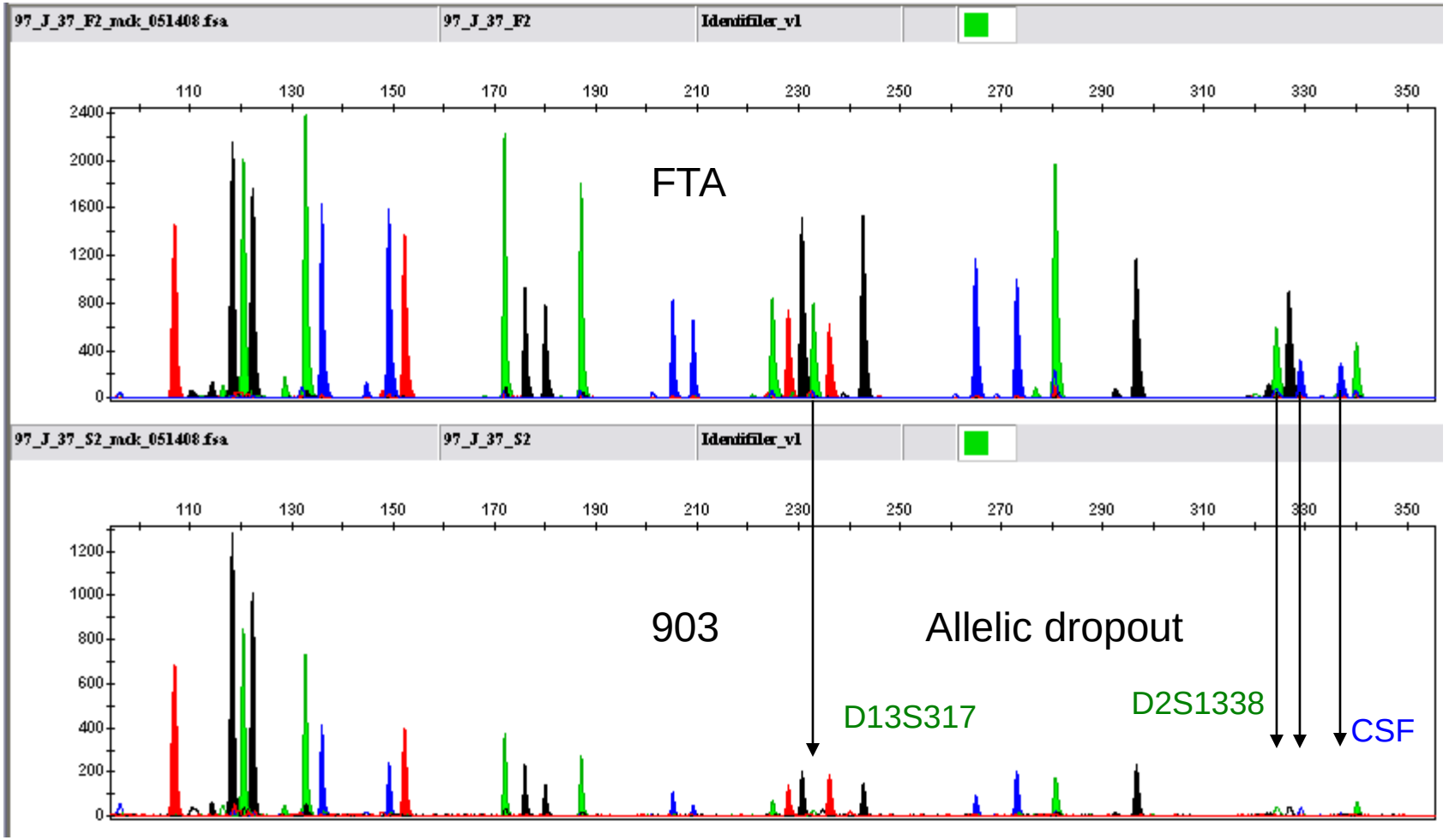
Most STR typing kits have products that are less than 450 bp

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.

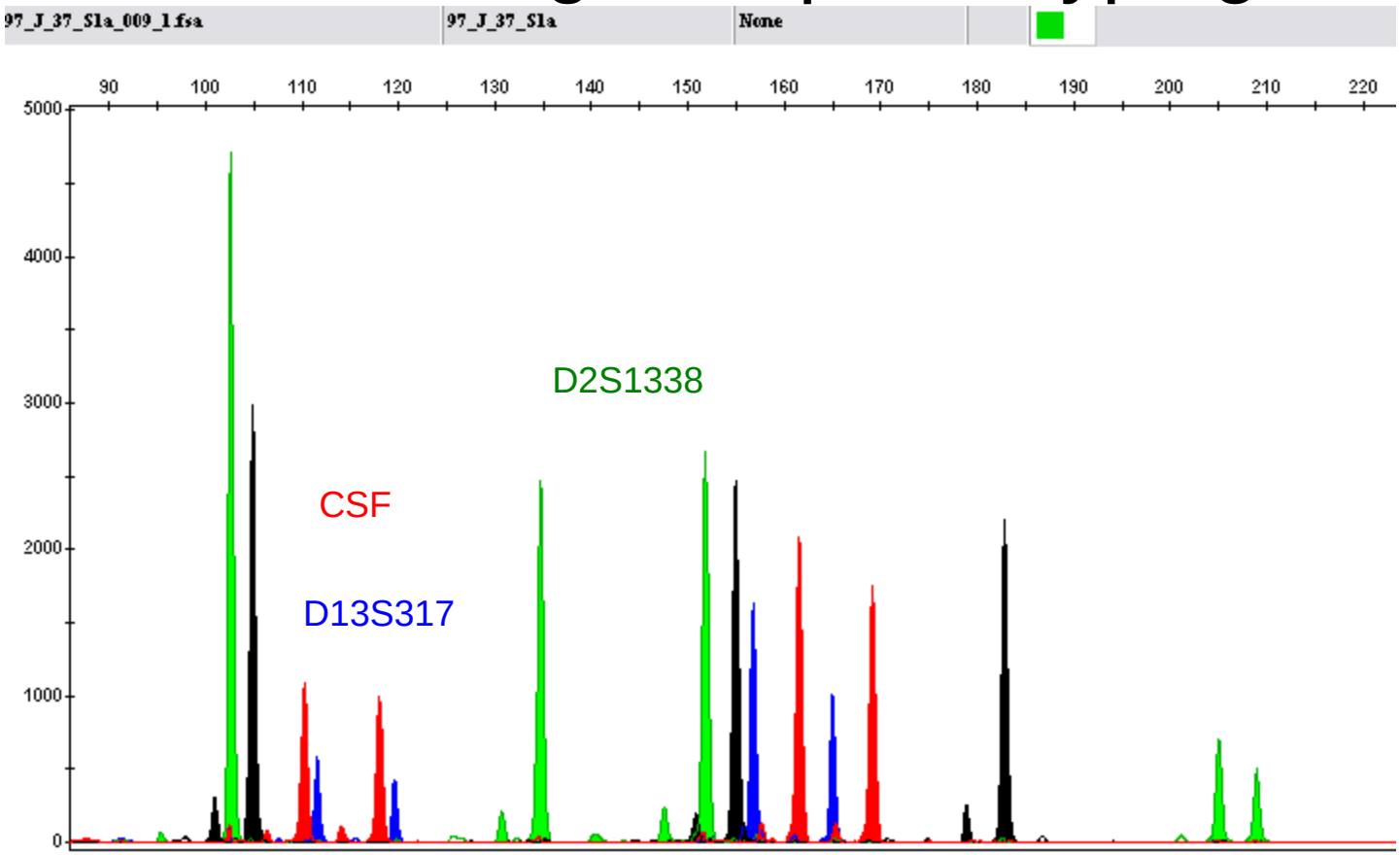
FTA: 903 +37 C Storage

Normal length amplicon typing kit



903 +37 C Storage

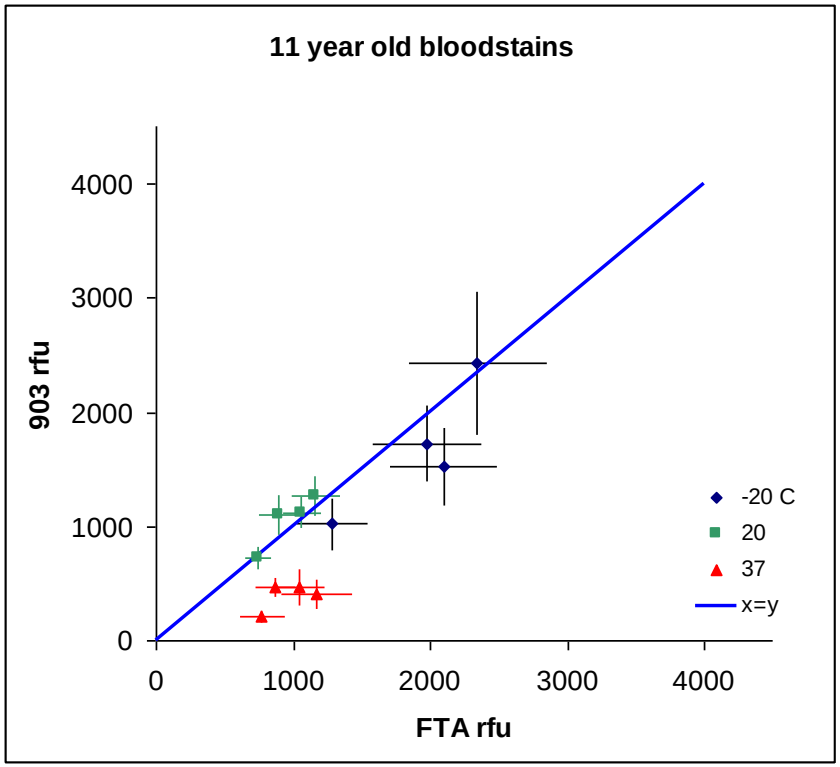
Minimum length amplicon typing kit



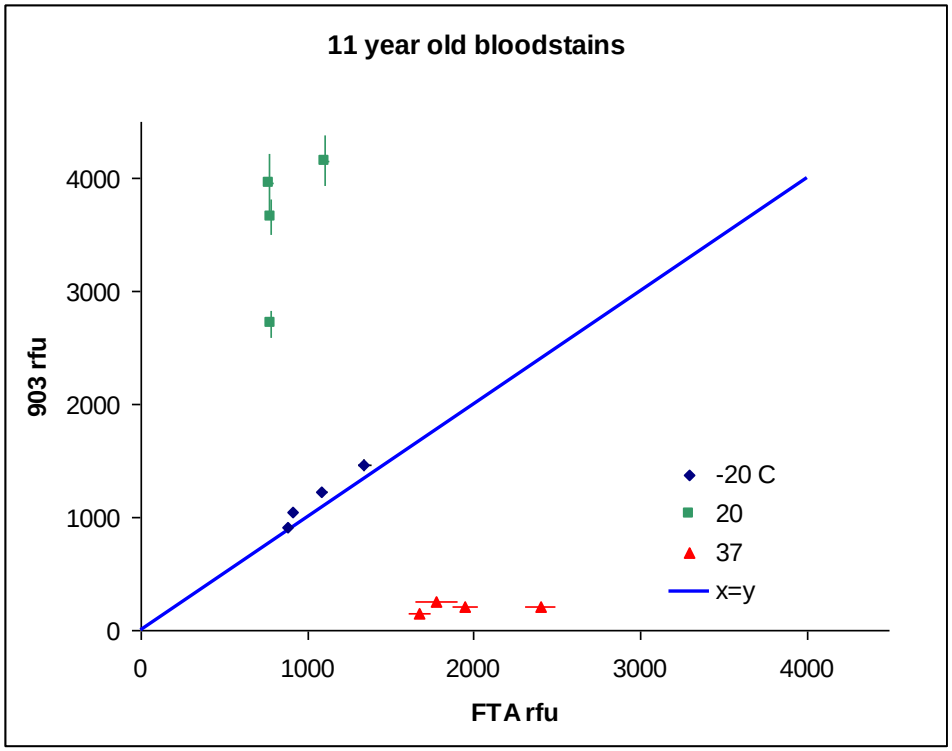
Alleles dropping out before are recovered.

Comparison of the Peak Heights FTA vs 903

Sample 1



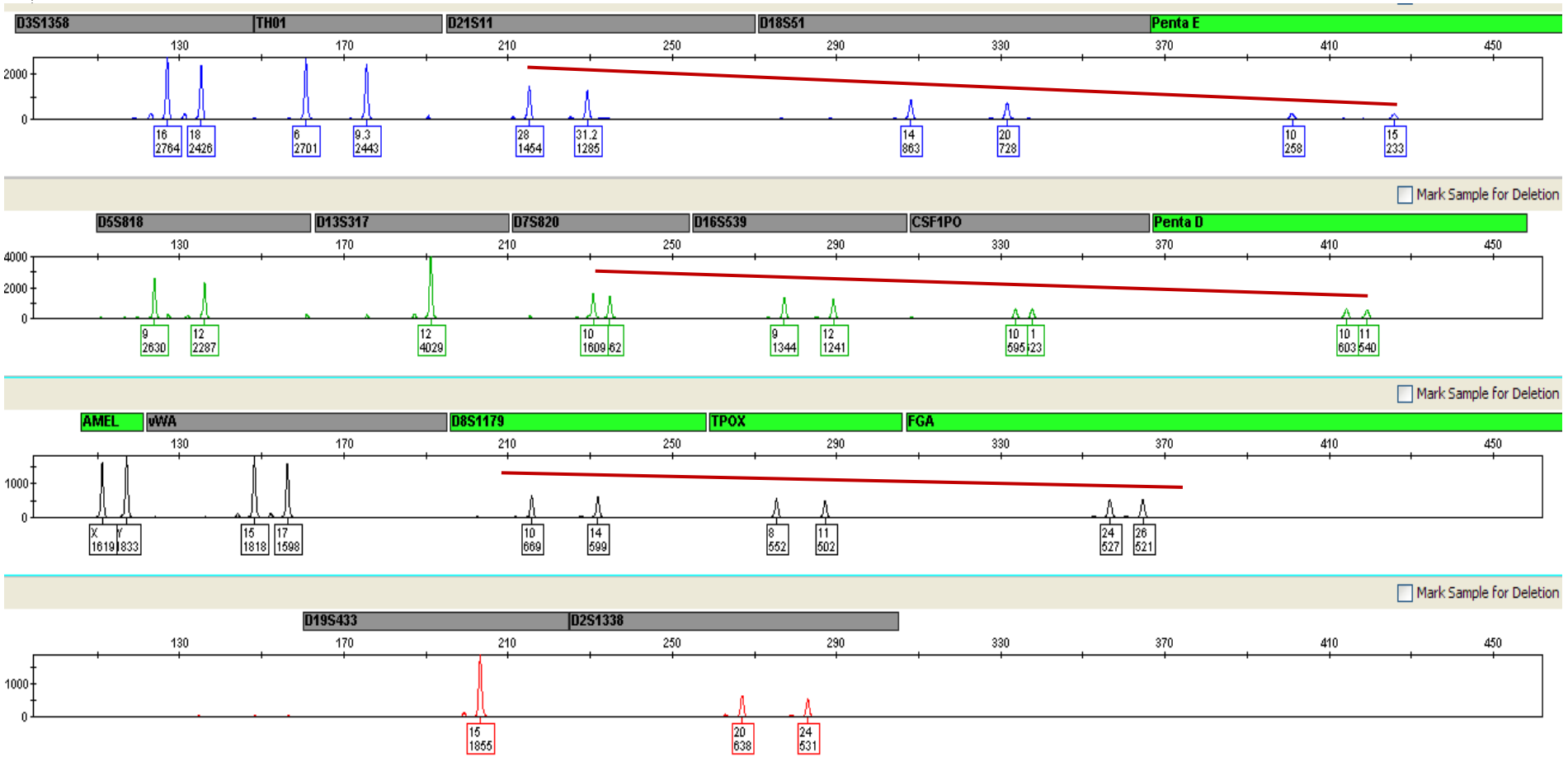
Sample 2



Allelic dropout seen in some of the +37 C stored samples.
Those alleles were recovered with minimum length amplicons.

25 year old Bloodstain(1986)

Direct amp kit (903 paper)



Single 1.2 mm punch stored at room temperature

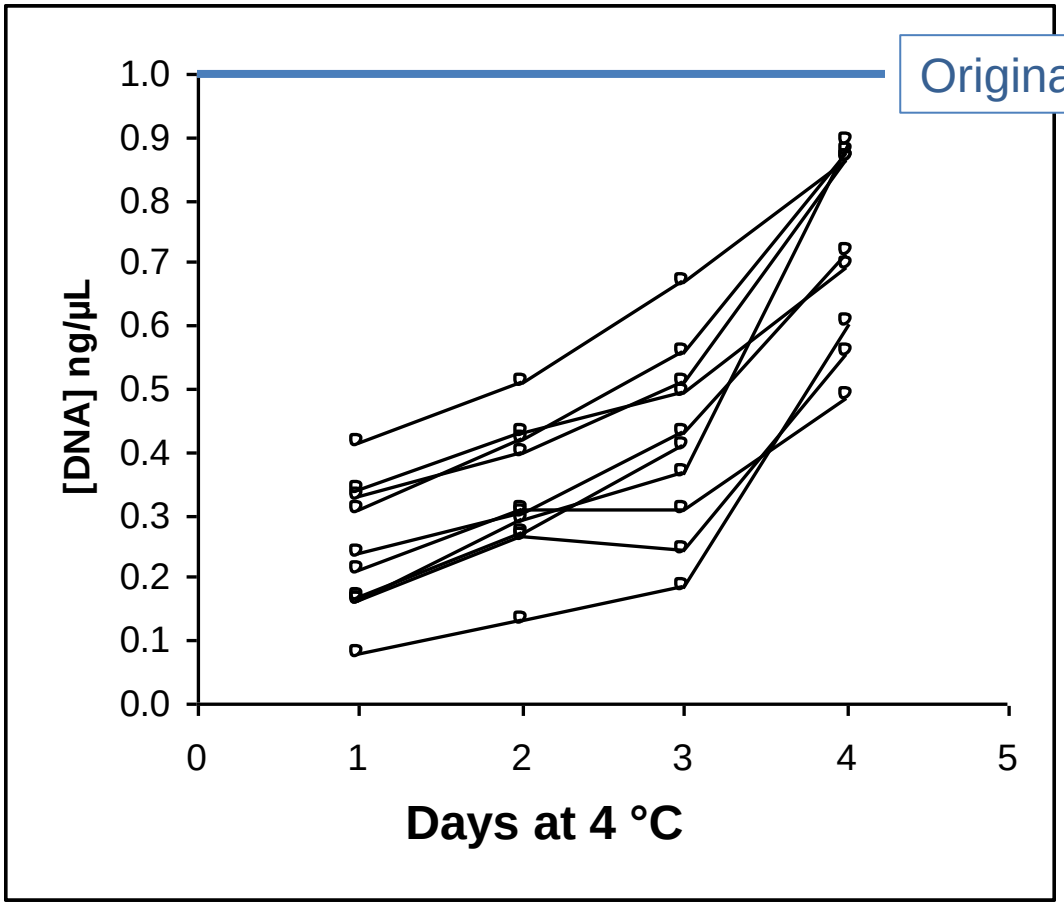
No Extraction

Data from Pete Vallone and Erica Butts

Extracted DNA

- Factors:
  - Liquid or Dried
    - Storage Media
      - Tube type (liquid)
      - Storage Paper-Matrix (dried)
    - Storage temperature
    - Environmental excursions (planned or unplanned)

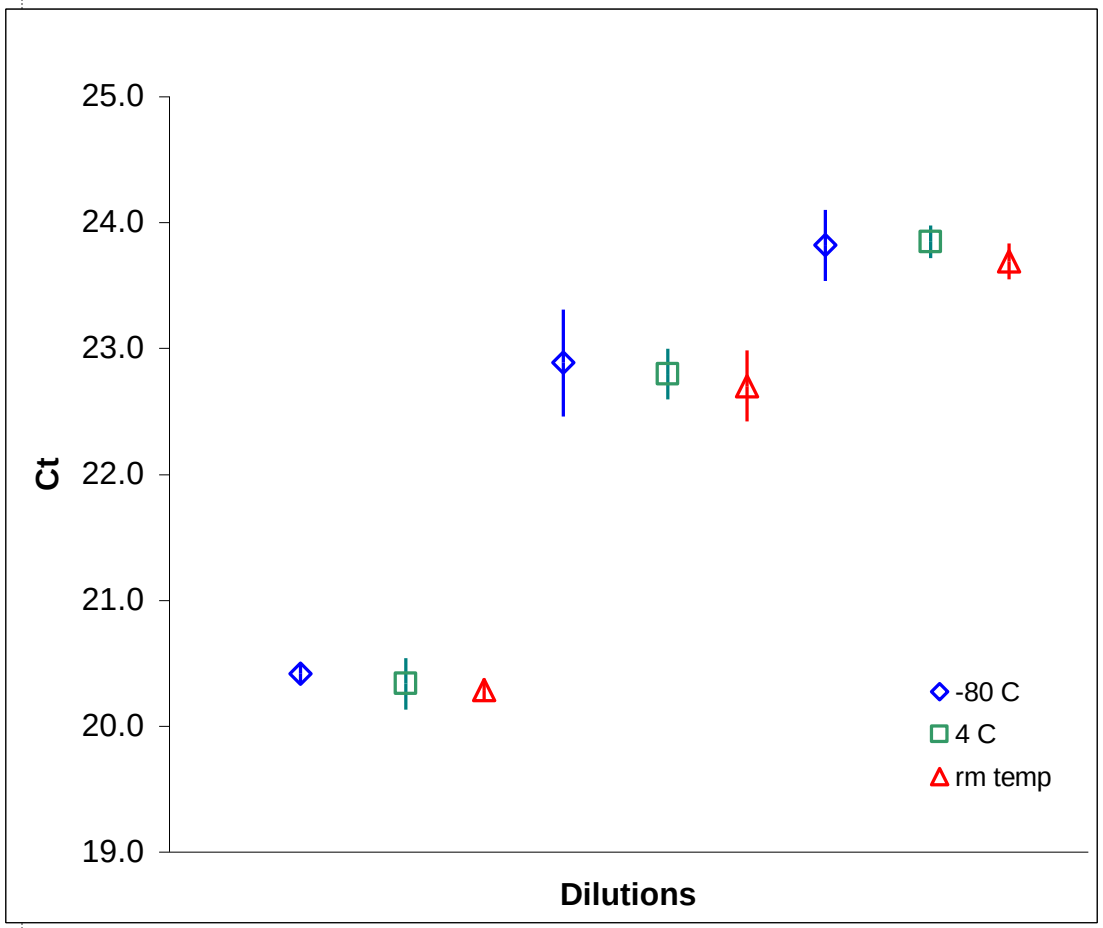
DNA sticking and releasing from tube walls



DNA stored in tubes for 5 years at -20 °C then moved to 4 °C

Each line represents an SRM 2391b component and the points along the lines (open circles) correspond to DNA concentrations obtained in this analysis. Several components (2, 8, 9,and 10) were approaching the original nominal DNA concentration of 1 ng/µL at the last time point of 4 days.

6 year Extracted DNA Stability in PFA Tubes



Data from DNA extracts stored in PFA tubes at -80 °C, 4 °C and room temperature for 6 years.

Each storage temperature had had 3 DNA concentrations neat, 1→5, 1→10 dilutions.

Results are the qPCR Cts. Error bars representing 2 sd.

There is no difference as a result of temperature storage after 6 years.

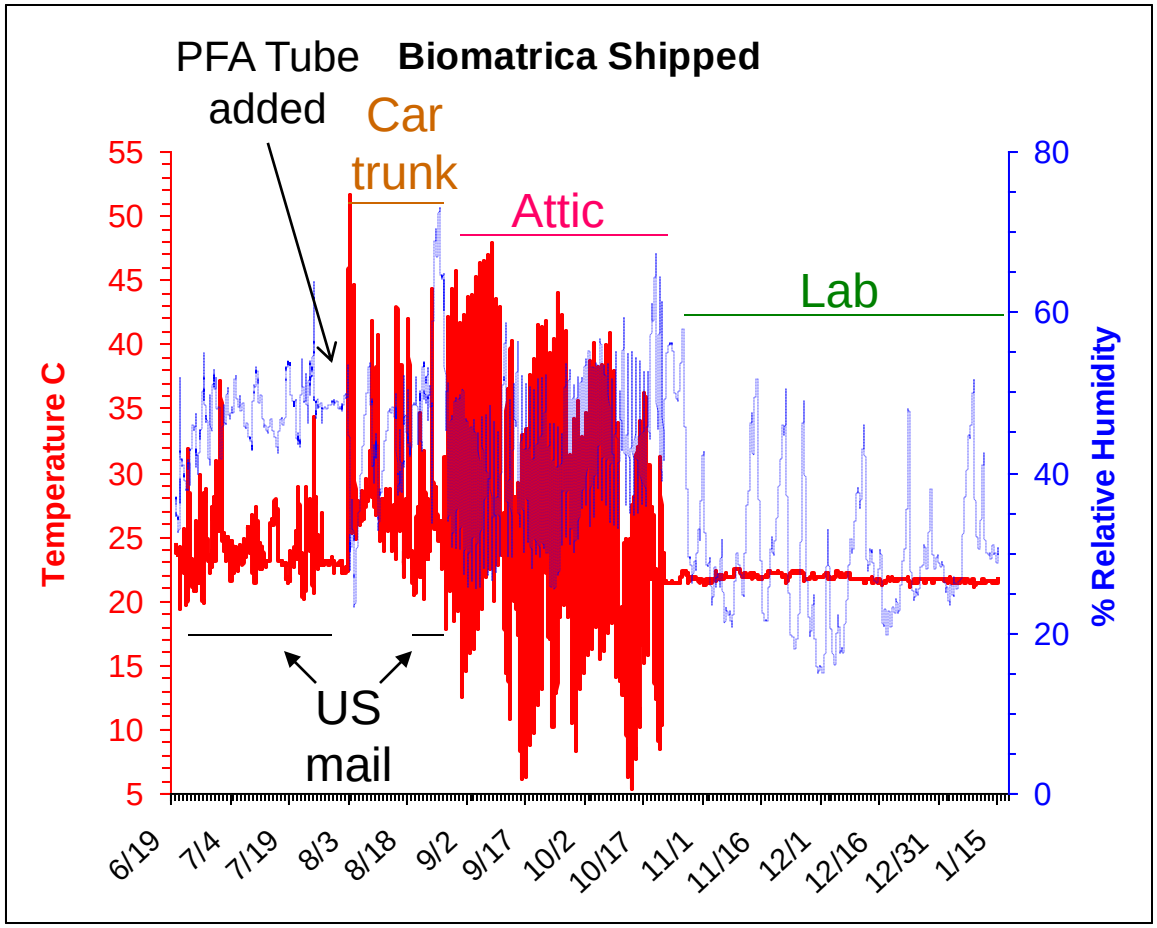
Study Design with Additive

- Stored DNA extracts with preservation additive. Four 96 well plates prepared
  - 3 different concentrations
  - 1 blank (no DNA)

    - Plates were labeled A, B, C, and D.
    - Plates A and C stay at NIST
    - Plates B and D are shipped/stressed .
    - Temperature and Humidity dataloggers are stored with the plates.

- Control DNA without additive stored in the PFA containers at 4 C.

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



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

Two plates with additive 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.

Followed by exposure to ambient attic temperatures for 56 days.

Finally plates were placed at lab ambient conditions.

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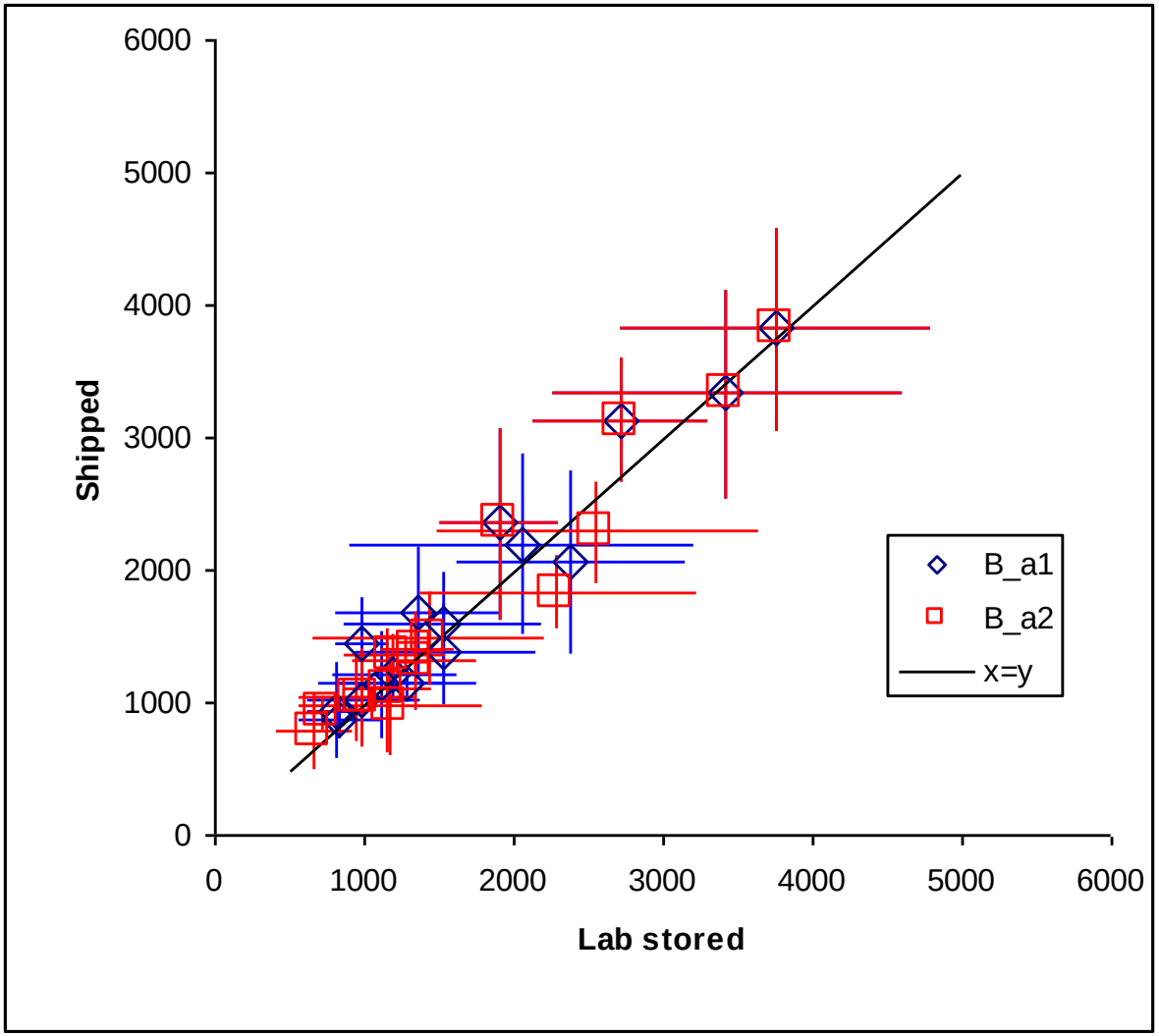
208 Day Quantitation Results

	[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

The decrease in the DNA concentration of the materials added to the plates with additive had been noted earlier, based on the genotyping results obtained the quantification data **may be** influenced by the color present in the additive.

PFA Stored at 4 C versus PFA Shipped



Blue symbols are from 4 C.

Red Symbols from harsh “ambient” conditions.

So you want harsher conditions?

Or DNA extracts dried on paper?

OK

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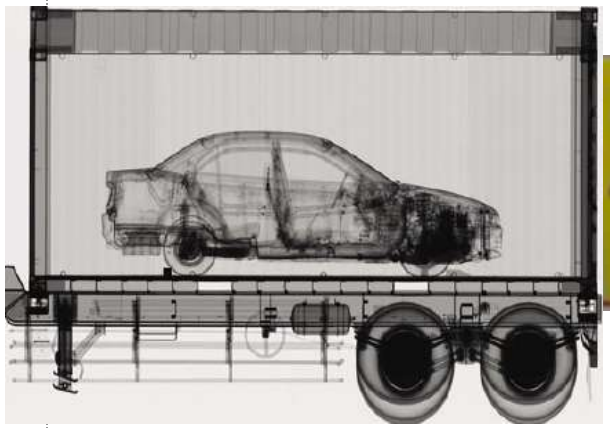
X Ray screening of DNA Samples?



X Ray Package screening machines are found in more places than airport security lines these days.

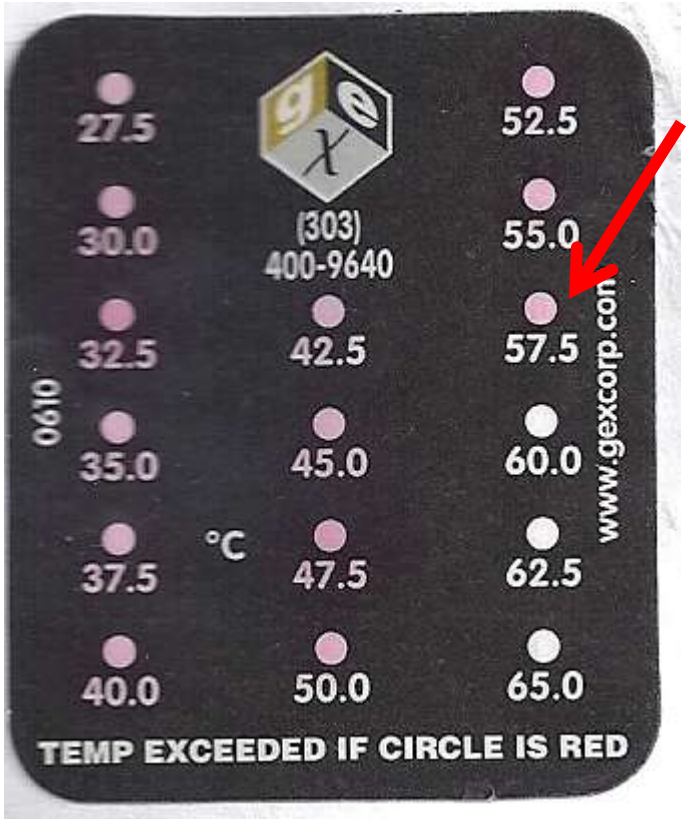
It's "rumored" that shipping companies such as UPS and FedEx randomly screen shipped packages.

Could shipping DNA /evidence to other laboratories be a problem?

When we are conducting interlaboratory studies are the results bias due to shipping conditions?

Study High Dose Irradiation (Sterilization)

- 5 MeV industrial x-ray beam
- Alanine dosimeter
  - (NIST reference class)
- Magazine Container
  - Dose (91 ±4) kGy
  - Max temp (57.5 ±1) °C
- Letter Mail Container
  - Dose (87 ±4) kGy
  - Max temp (62.5 ±1) °C



Sample Packages were at these temperatures for 20 to 30 minutes.

Experiment Parameters

- DNA extracts at 2 concentrations
  - ✓ with and without stabilizer
- Stored in 3 different Tubes types
  - ✓ with and without stabilizer
  - ✓ Stored as liquid or dried
- Stored as “Stains” on paper:
  - ✓ FTA:
  - ✓ 903: with and without stabilizer

One set of Irradiation Study samples

Treatments

- Control : Stored 4 °C
- Lab Ambient:
  - Stayed in the lab until processed
- Shipped:
  - Shipped UPS, returned UPS (?diagnostic x-rayed)
  - 15 days in transit to and from (Washington State Patrol)
- X Rayed:
  - Shipped UPS, X-rayed when received, returned UPS
  - 7 days in transit (Evidence Control Unit FBI, VA)
- High-Dose Sterilization X Ray:
  - U.S. Postal Service Government Mail

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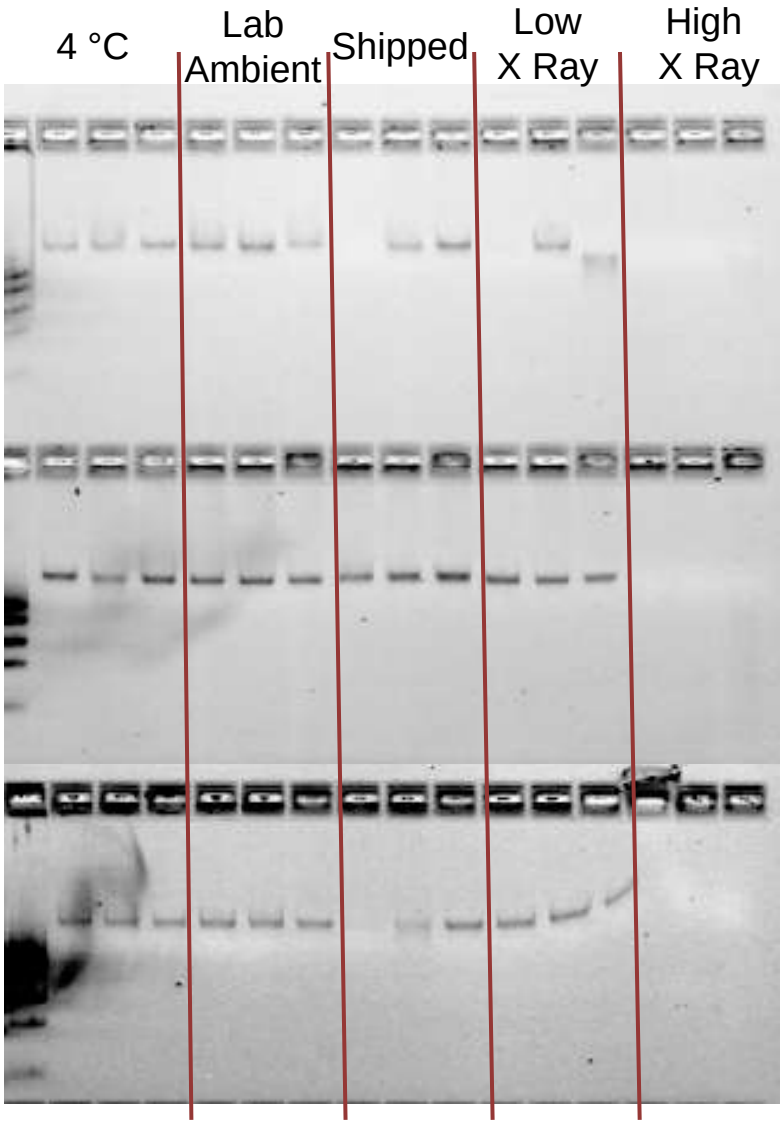
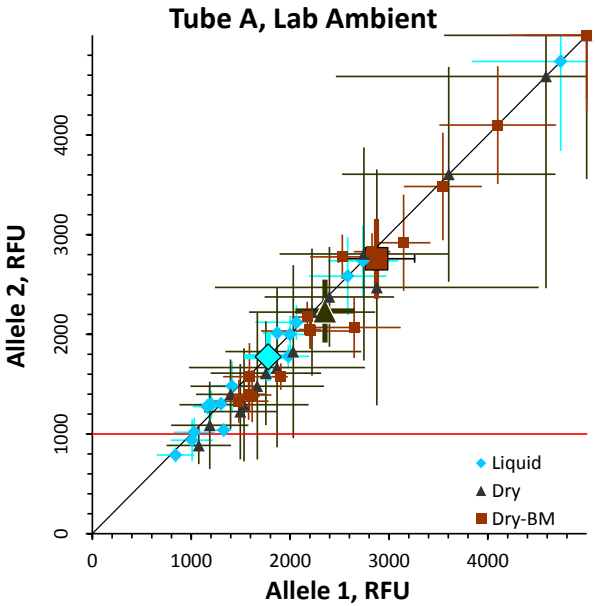
Flash Gel Results
Post Treatment

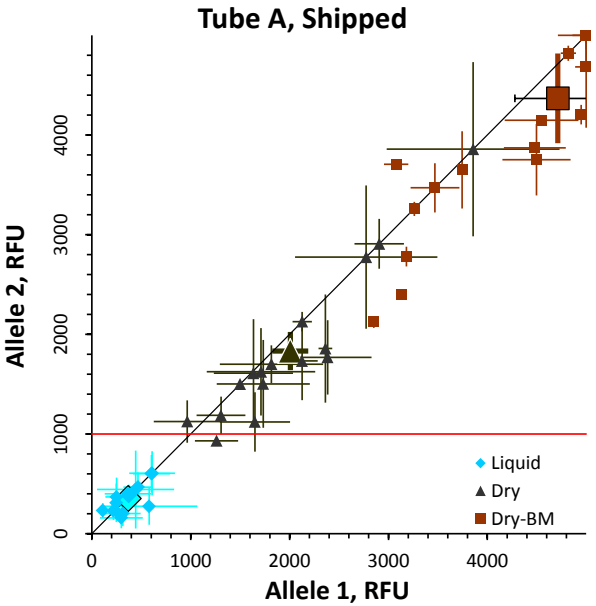


Tube A Sample Results

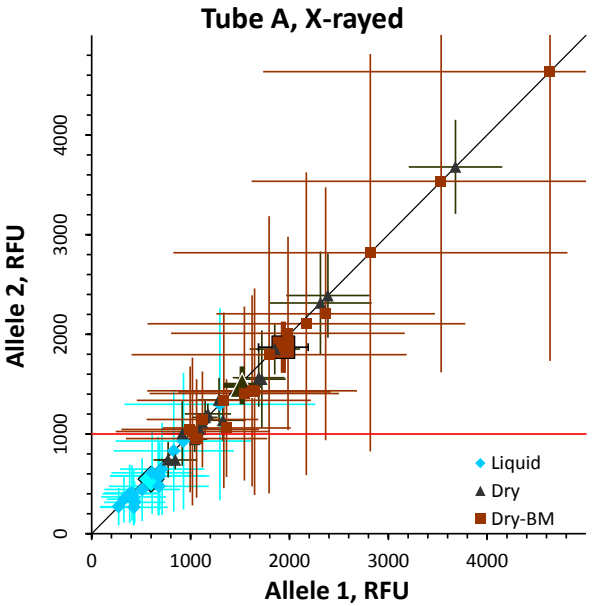
95 % volume loss



100 % volume loss



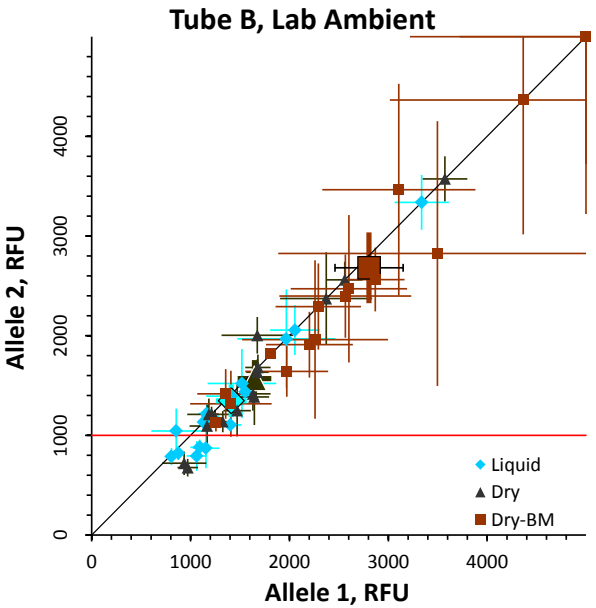
95 % volume loss



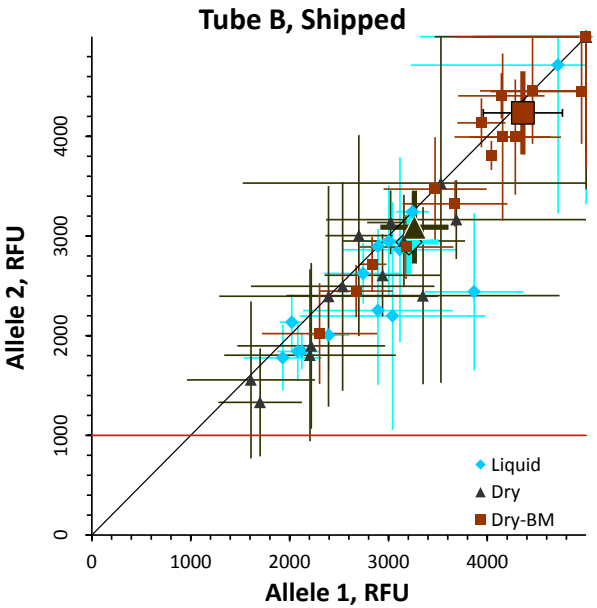
The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum
The large symbols represent the average over all loci

Tube B Sample Results

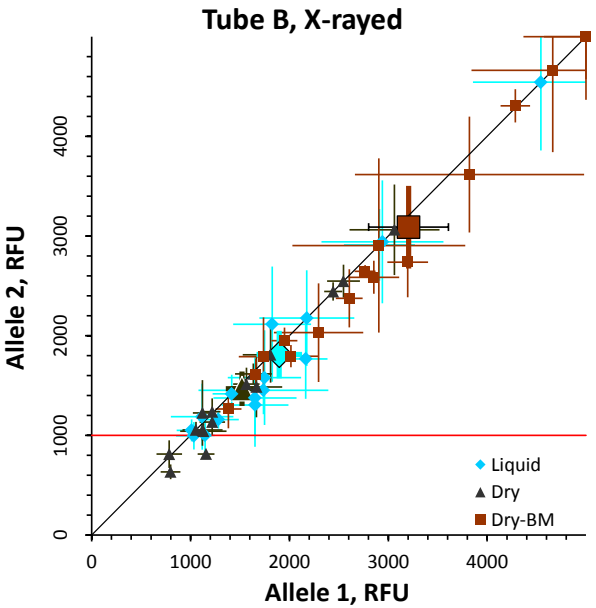
14 % volume loss



33 % volume loss



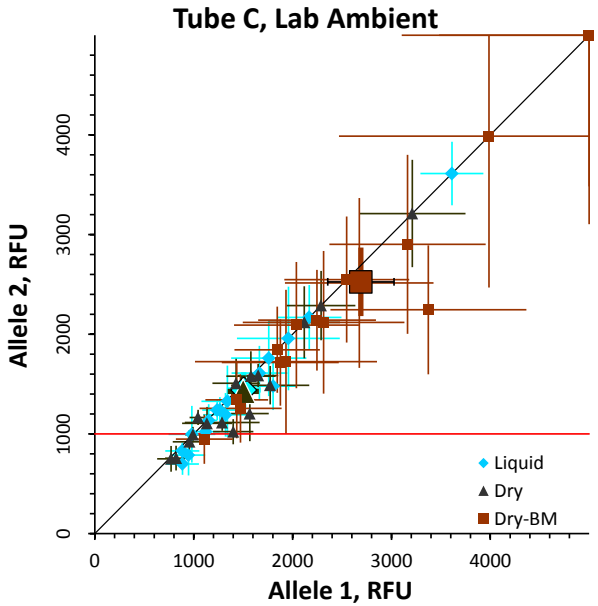
15 % volume loss



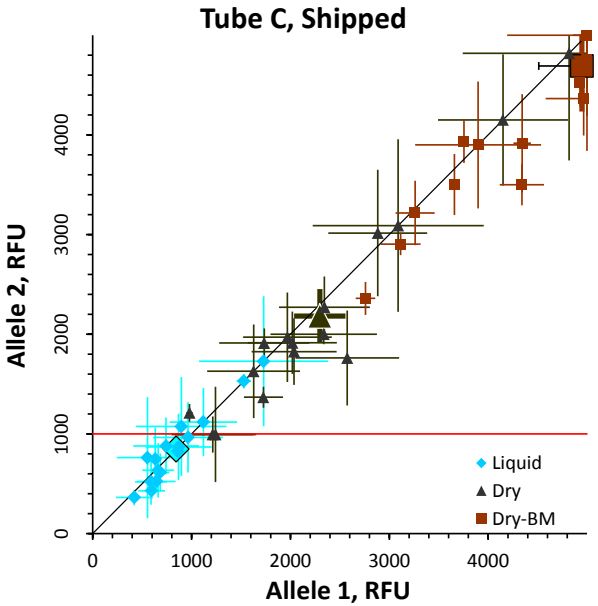
The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum
The large symbols represent the average over all loci

Tube C Sample Results

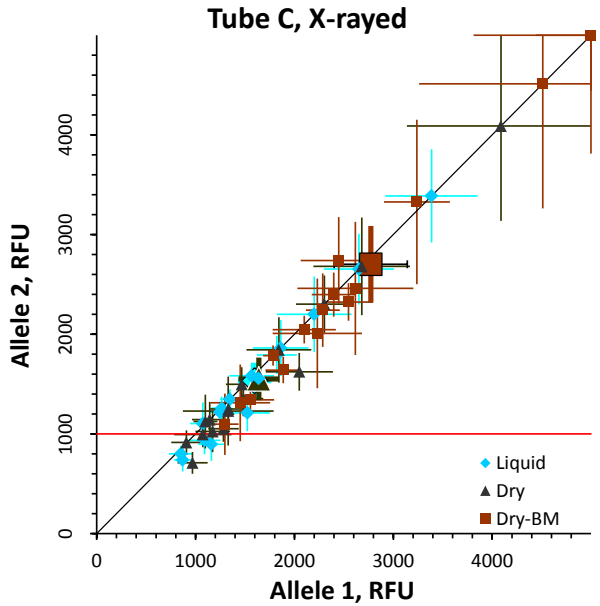
0 % volume loss



1 % volume loss



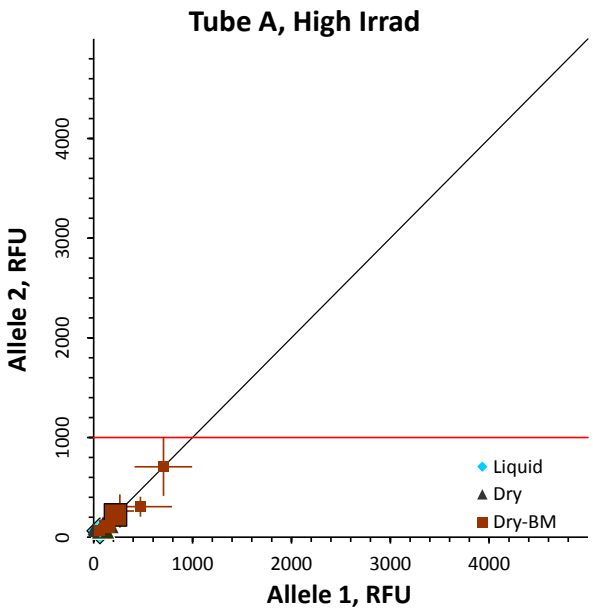
0 % volume loss



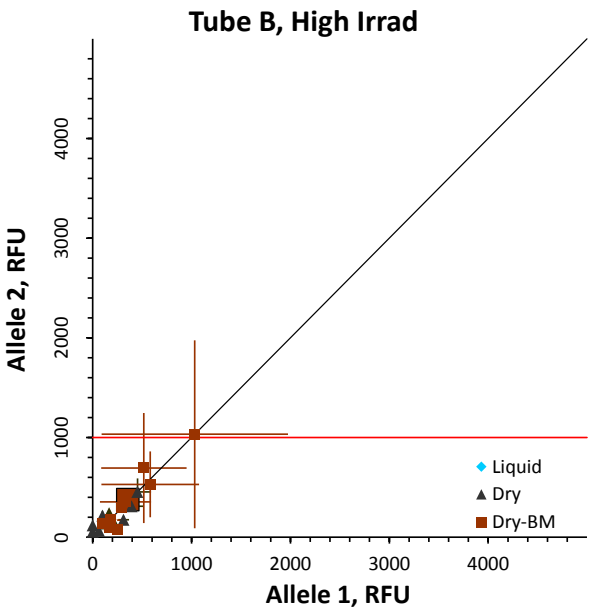
The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum
The large symbols represent the average over all loci

High Irradiation Sample Results

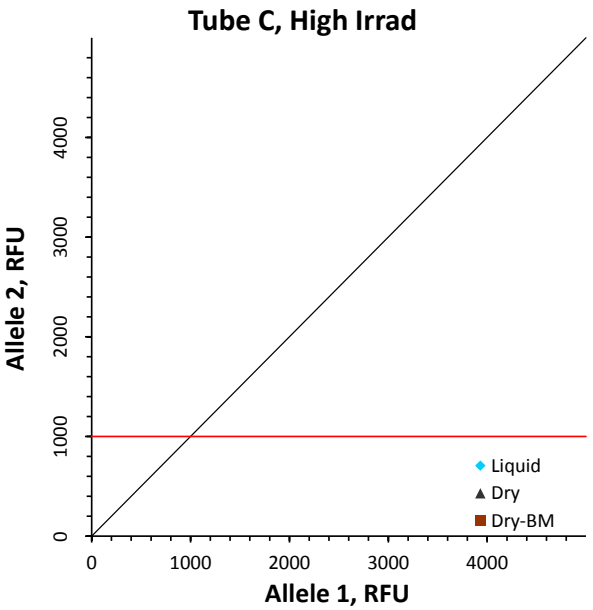
100 % volume loss



17 % volume loss



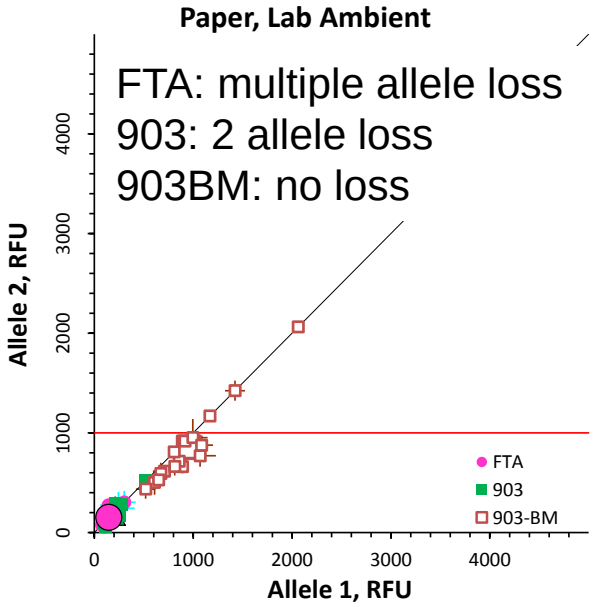
19 % volume loss

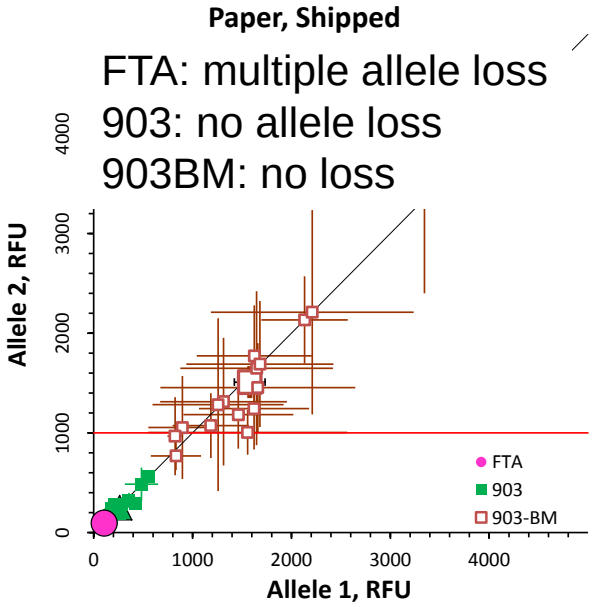


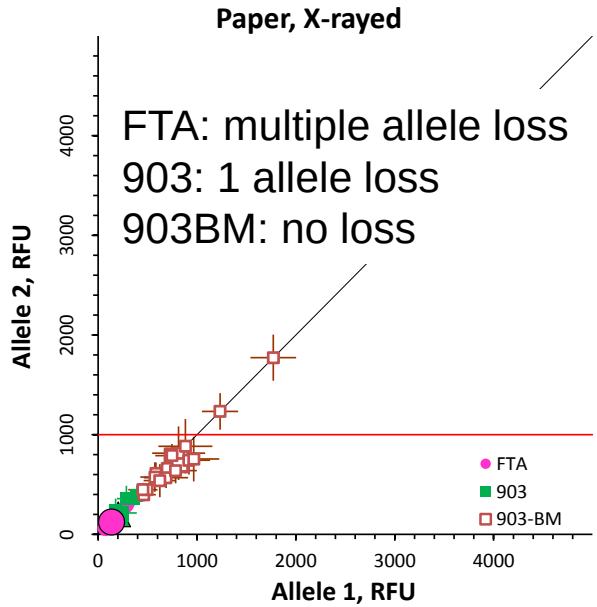
The peak heights of allele 1 are plotted on the X-axis
The peak heights of allele 2 are plotted on the Y-axis
Error bars span from the minimum to the maximum
The large symbols represent the average over all loci

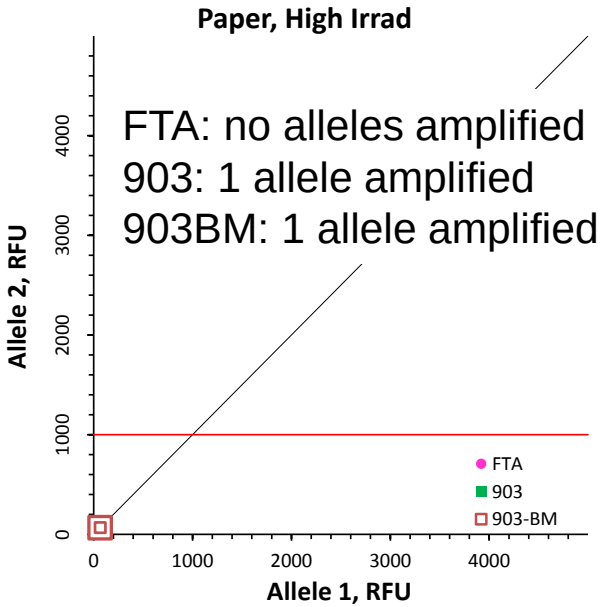
No full profiles were obtained - only a few scattered alleles amplified.

Extracted DNA on paper









FTA: 30 µL of 2 ng/µL extracted DNA spotted

903: 30 µL of 2 ng/µL extracted DNA spotted

903-BM: 30 µL of 2 ng/µL extracted DNA spotted with stabilizer added

2 mm punches (≈1.6 ng) amplified in 25 µL reaction volume.

Error bars span from the minimum to the maximum

The large symbols represent the average over all loci

Bottomline

- Tube packaging makes a difference
- Stabilizer doesn’t hurt, may help
- Getting a typing result from paper stain requires more DNA than you think
- Low dose X Ray doesn’t hurt
- High dose X Ray destroys

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Technical Working Group on Biological Evidence Preservation

A partnership between the **National Institute of Standards and Technology**, Law Enforcement Standards Office and the **National Institute of Justice**, Office of Investigative and Forensic Sciences

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Technical Working Group on Biological Evidence Preservation

The NIST/NIJ Technical Working Group on Biological Evidence Preservation (TWGBEP) is charged with: **creating best practices and guidance to ensure the integrity, prevent the loss, and reduce the premature destruction of biological evidence after collection through post-conviction proceedings.**

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Acknowledgments

NIST Team for This Work

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NIJ National Institute of Justice