National Institute of Standards and Technology
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Recovery of DNA From Aged Bloodstains

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Mini-Symposium: Dry-State Storage of DNA
March 29, 2005 SAIC-Frederick, Inc. Bldg 549 Auditorium

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?

Armed Forces DNA Repository

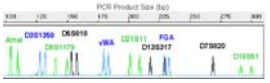
>4.5 million blood cards on file from members of U.S. military

Being used to identify remains in case of combat casualties (e.g., Operation Iraqi Freedom)

Located off Gaither Rd. in Gaithersburg, MD

World Trade Center Towers
(Sept 11, 2001)

DNA typing was used as only possible method to identify over 2,700 victims of this tragedy



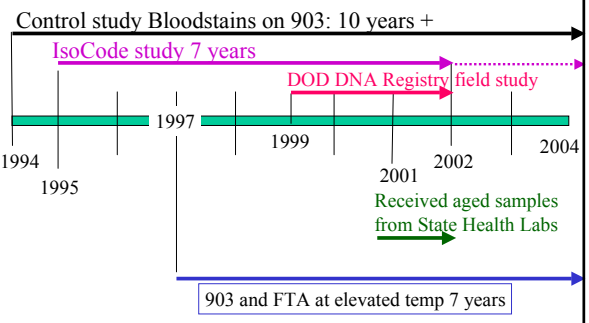
Highly degraded DNA; ~20,000 samples and ~3 years to complete…

Wreckage at Ground Zero

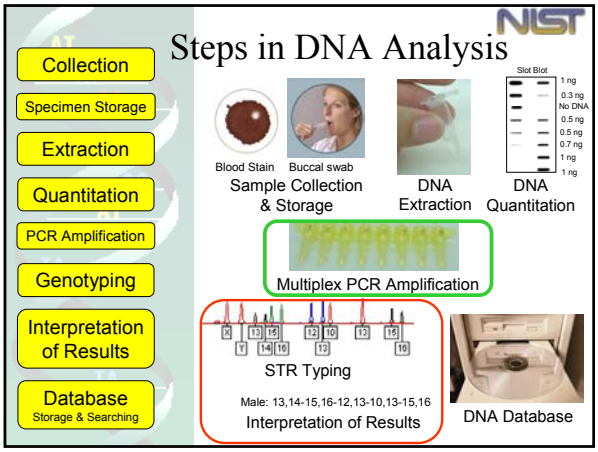
Outline of the studies

- Controlled bloodstains on 903 paper (94)
- Interlaboratory study with one sample shipped on Isocode, remaining samples tested later (95)
  Kline et al., JFS 1997; 42(5):897-906.
- Papers at elevated temperature (97)
  - Samples vacuum sealed (903, FTA)
- DOD DNA Registry field study 4 archival media (99)
  Kline et al., Anal Chem. 2002 Apr 15;74(8):1863-9.
- Aged bloodstains from State Health Labs (01)
  - Samples were ambient stored up to15 years
  - All samples on 903 paper

Time line of studies



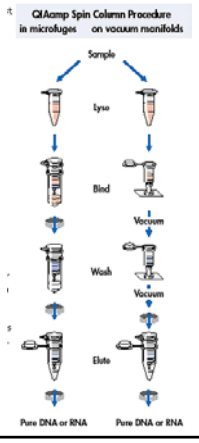
Steps in DNA Analysis



Extraction Methods

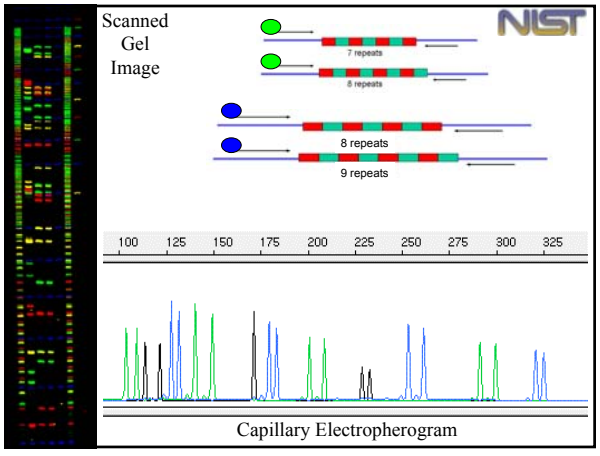
Chelex Extraction - Sample is heated (98-100ºC) in the presence of a Chelex resin. The heating breaks open the cells, releasing the DNA. The Chelex binds other cellular components that might interfere with subsequent analysis. The Chelex resin is removed by centrifugation, leaving the DNA in the supernatant. DNA extracted this way is single-stranded and is therefore unsuitable for RFLP analysis. However, it is suitable for PCR amplification.

Magnetic Affinity Resin (Promega - DNA IQ™ System) - A magnetic Resin is used to capture a consistent amount of DNA. The Resin has a defined DNA capacity in the presence of excess DNA and will only bind a specific amount of DNA. This property is used to isolate approximately 100ng of DNA from a range of liquid blood, stains or swabs. The resin with the DNA bound to it is held in a microcentrifuge tube by placing the tube in a stand containing a small magnet. The resin bound DNA can then be washed several times to remove other substances. Finally, the DNA is eluted into 100µl of Elution Buffer to give a DNA concentration of approximately 1ng/µl. -. DNA extracted this way is single-stranded and is therefore unsuitable for RFLP analysis. However, it is suitable for PCR amplification. It also removes all contaminants that could interfere with subsequent PCR amplification and should, therefore, offer an advantage over either Chelex or NaOH extraction methods.



QIAmp Mini blood extraction

Optimized buffers lyse samples, stabilize nucleic acids, and enhance selective DNA adsorption to the QIAamp membrane. Alcohol is added and lysates loaded onto the QIAamp spin column. Wash buffers are used to remove impurities and pure, ready-to-use DNA is then eluted in water or low-salt buffer. The entire process requires only 20 minutes of handling time (lysis times differ according to the sample source).



Types of Storage Media Studied

- **903 -** high-purity cotton linter pulp paper
- **IsoCode** – 903 designed to tightly bind non-DNA blood components, enabling fast selective extraction of DNA from the matrix.
- **BFC180 -** high-purity cotton linter pulp paper
- **FTA** - high-purity cotton linter pulp paper treated with a coating designed to tightly bind DNA and RNA, enabling selective removal of PCR inhibitors leaving the DNA bound to the storage matrix.

The Initial Experimental Design

- A long term study on the stability of DNA recovered from a dried bloodstain stored on 903 paper was initiated July 1994 at the request of the DOD DNA Registry.
- Whole blood, as 20 µL aliquots, were spotted on 903 paper, and dried overnight in a vacuum desiccator at ambient temperature.

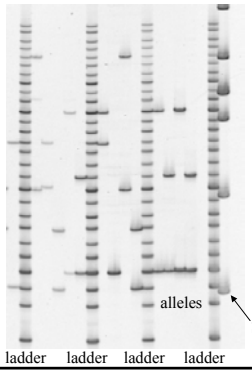
Experimental design continued

- The six dried bloodstains were punched into each cryogenic vial using a 6.3 mm hole punch.
- Sixteen vials were secured in each of four storage boxes.
- Boxes were stored at either ambient, -20° C, -80° C or Liquid Nitrogen (-150° C).
- One vial was removed from each box, extracted, amplified and analyzed at a variety of time periods over 10 years.

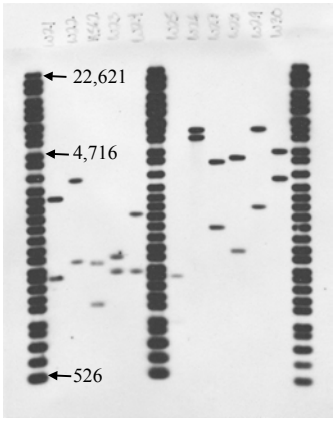
Measurement Criteria: Typeable DNA

- In 1994 when this study was started typeable DNA to the forensic DNA human identity community meant you could successfully PCR amplify the D1S80 locus that ranged from ~300 bp to 800 bp. Or your could get results by RFLP which required DNA up to 20 kb.
- By 1997 the size range for PCR products had been reduced to 100 bp to 350 bp with the adoption of STR marker systems..

D1S80 allelic ladders and samples



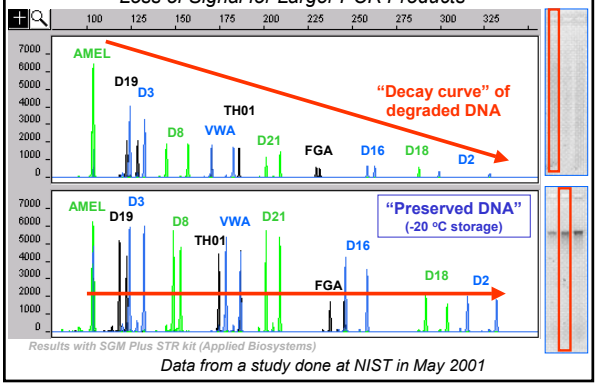
D1S80 VNTR locus with a repeat of 16 bp



1990 Single Locus RFLP

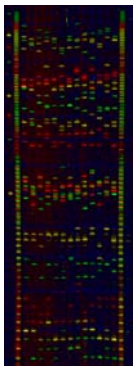
Degraded DNA

Loss of Signal for Larger PCR Products

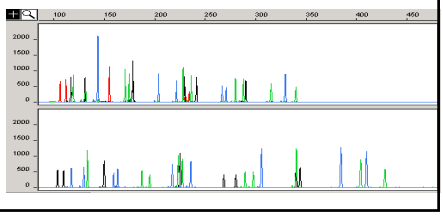


Data from a study done at NIST in May 2001

2003: SRM 2391b



- 22 STR Loci,
- D1S80, and DQa1/PM
- Many labs using 16-plexes
- 0.5 – 1 ng DNA



Initial Observations

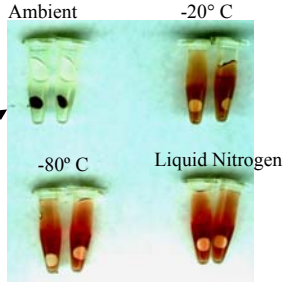
- Visual observation of the samples:
  - all remained "red" as they were at the beginning except the ambient samples.
  - Ambient samples were turning a darker "brown" through the years.
- Chelex extractions performed on the samples indicated that the "heme" bound to the paper of ambient samples.

DNA Stability Study 6 year time point

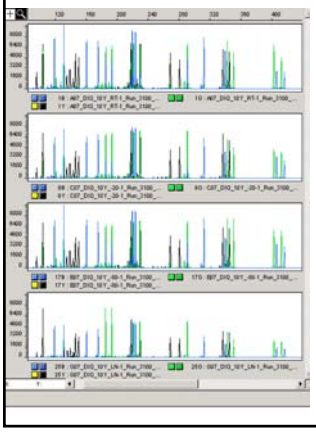
S&S 903 paper

Ambient -20 -80 LN

During Chelex extraction RT sample during water washes remains colored.

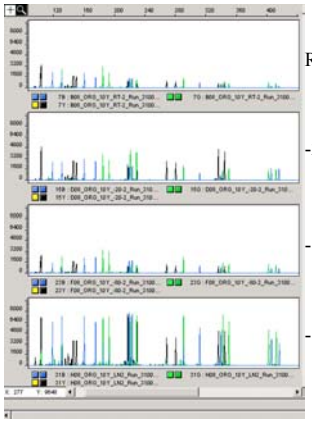


DNA IQ Extracts Controlled 10y Study



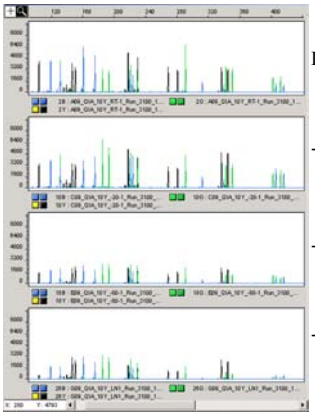
All loci present and equally amplified with PP16. No effort was made to balance the amount of DNA amplified

Organic Extracts Controlled 10y Study



All loci present and equally amplified with PP16. No effort was made to balance the amount of DNA amplified

QIA Extracts Controlled 10y Study



All loci present and equally amplified with PP16. No effort was made to balance the amount of DNA amplified

Control Studies with Treated Papers

- Prepared at NIST
- Dried 3 h in laminar flow hood
- S&S 903 paper & IsoCode paper
- Stored -20 ºC & Lab Ambient Respectively
- Ziplock bags
- 1995 start date

- Prepared at NIST
- Dried 3 h in laminar flow hood
- S&S 903 paper & FTA paper
- All media stored at +37 ºC, Lab Ambient, & -20 ºC
- Vacuum sealed bags
- 1997 start date

IsoCode Study 1995

Fresh whole blood was :

1) collected in EDTA tubes

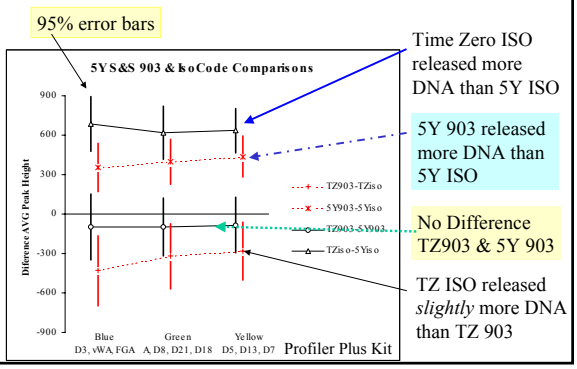
2) spotted onto IsoCode Cards, and 903

3) dried in a laminar flow hood for 3 h

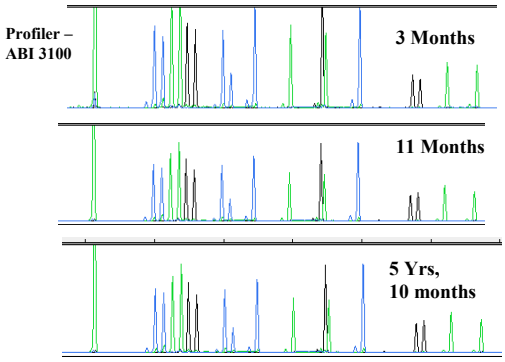
4) IsoCode sealed in a plastic bag.

5) stored at room temperature.

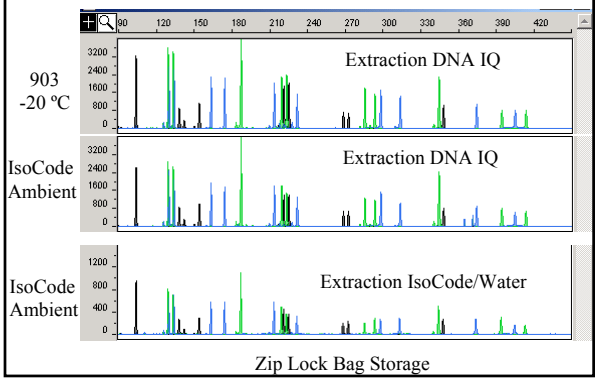
5Y 903 & IsoCode Comparisons



IsoCode Stored at Ambient Temperature



6 Y Samples -20 ºC Vs Ambient



IsoCode

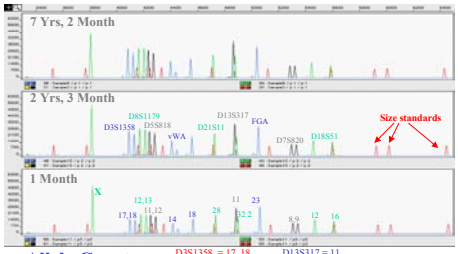
Shelf Life Data – Blood Spots

- Samples represent blood dried and stored on IsoCode for 1 month, 2 years/3 months, and 7 years/ 2 months.
- Fresh whole blood was collected in EDTA tubes and subsequently spotted onto IsoCode Cards, dried in a laminar flow hood for 3 hours, sealed in a plastic bag and stored at room temperature.
- DNA was eluted from 3 mm punches in 100 ul water after heating to 95°C and pulse vortexing for 1 minute.
- 1 ng of DNA was used to type the STR's using ABI's AmpFLSTR Cofiler/Profiler Plus System and Promega's PowerPlex system.
- The data shows that DNA stored on IsoCode for up to 7 years, 2 months generates the same STR profile as blood collected from the same source stored for 1 month. No allele drop-out observed.

IsoCode Shelf Life Data

DNA extracted from Dried blood spots on IsoCode and typed with ABI's AmpFLSTR Cofiler System

Profiler Plus – ABI Prism 310



Allele Genotype

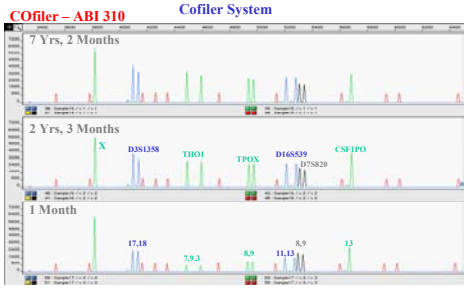
9 of 13 CODIS STR's

D3S1358 = 17, 18
D8S1179 = 12, 13
D5S818 = 11, 12
vWA = 14, 18
D21S11 = 28, 32.2
D13S317 = 11
FGA = 23
D7S820 = 8, 9
D18S51 = 12, 16

IsoCode Shelf Life Data

DNA extracted from Dried blood spots on IsoCode and typed with ABI's AmpFLSTR Cofiler System

COfiler – ABI 310



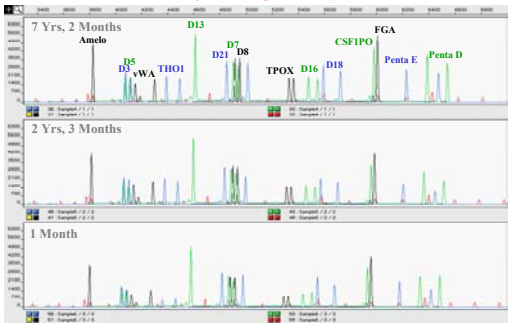
Allele Genotype

6 of 13 CODIS STR's

D3S1358 = 17, 18
THO1 = 7, 9.3
D16S539 = 11, 39
D7S820 = 8, 9
TPOX = 8, 9
CSF1PO = 13

IsoCode Shelf Life Data

DNA extracted from dried blood spots on IsoCode and typed with Promega's Powerplex® 16 System

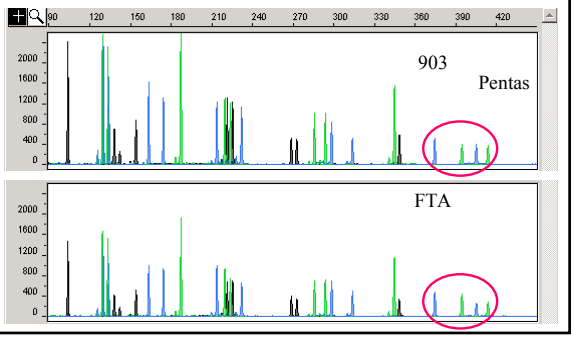


ABI 310 POP-4, 47 cm x 50 um Cap
Run time: 3 sec inj, 30 min @ 60C

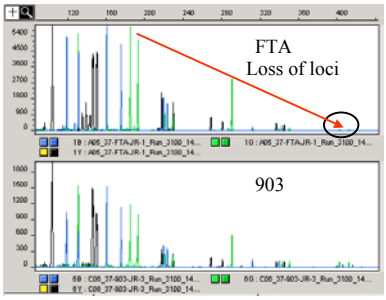
Papers at elevated temperature Start date (1997)

- Prepared at NIST
- Whole blood collected in EDTA tube
- Spotted on S&S 903 paper & FTA paper
- Dried 3 h in laminar flow hood
- Vacuum sealed bags used for storage
- All media stored at +37 ºC, Lab Ambient, & -20 ºC

4 Y at +37 ºC in Vacuum Sealed Bags Chelex Extracts

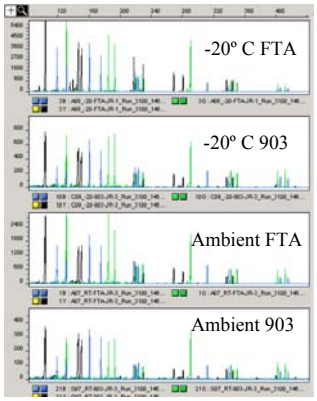


37º C FTA and 903 papers at 7 years



Over amplification of the smaller loci with loss of the larger loci with the FTA paper

903 large loci weak but still typeable. Smaller loci are not "over amplified.



-20º C FTA & 903
7 years

All loci present and equally amplified with PP16

Short Term Study

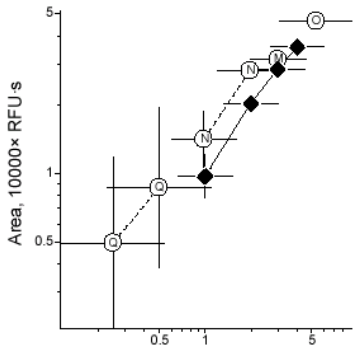
in conjunction with DOD DNA Registry

- 70 individuals' blood was spotted on 4 different storage media
  - S&S 903™ & IsoCode® papers
  - FITZCO, Inc Whatman BFC 180 & FTA papers
- The blood stains were vacuum sealed with desiccant.
- Storage was 19 months at lab ambient temperature.

Results of the Short Term Study

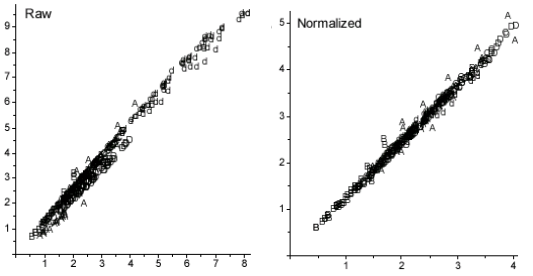
- All four storage media provided fully typeable (qualitatively identical) samples.
- After standardization, the average among-locus (peak ht /peak area) fluorescent signal provided a metric for determining the relative amounts of amplifiable DNA recovered.

Kline et al., Anal Chem. 2002 Apr 15;74(8):1863-9.
Pdf available at www.cstl.nist.gov/biotech/strbase/NISTpub.htm



Kline et al., Anal Chem. 2002 Apr 15;74(8):1863-9.

Peak Ht 1000x RFU Vs Peak Area 10000x RFU



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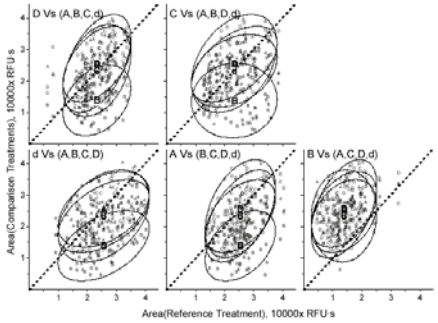
Bivariate Distributions



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Aged bloodstains from
State Health Labs (2001)

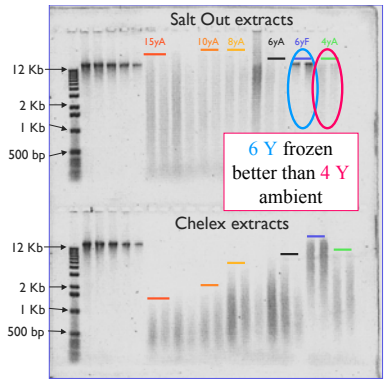
Untreated Paper Studies, S&S 903

Control samples	Field samples
• NIST prepared	• State Health Labs
• Dried vacuum desiccator	• Dried ???
• Sealed in tubes	• Sealed ????
• Stored at -150 ºC, -80 ºC, -20 ºC, and lab ambient	• Most stored at warehouse ambient, one set at -20 ºC for 6 years
• Stored for 7.5 years	• Stored for 2 to 15 years
• 6 reps of 4 temperatures	• 1 rep of 318 samples

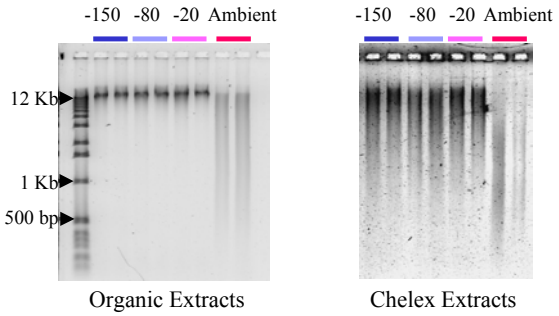
Field Samples Evaluated

Year Spotted (Code)	Storage Conditions	# Samples Received
1986 (15 yA)	ambient	51
1987 (14 yA)	ambient	51
1991 (10 yA)	ambient	25
1993 (8 yA)	ambient	26
1994 (7 yA)	ambient	50
1995 (6 yF)	-20ºC	50
1995 (6 yA)	ambient	25
1997 (4 yA)	ambient	20
1999 (2 yA)	ambient	20
	Total	318

Quality of the DNA Extracted from the Field Samples

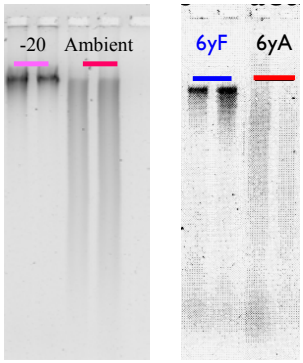


Quality of the DNA Extracted from the 7.5 y Control Samples



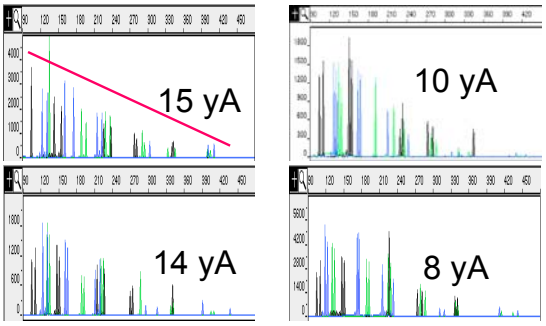
Quality of the Extracted DNA

7.5 Y Controls

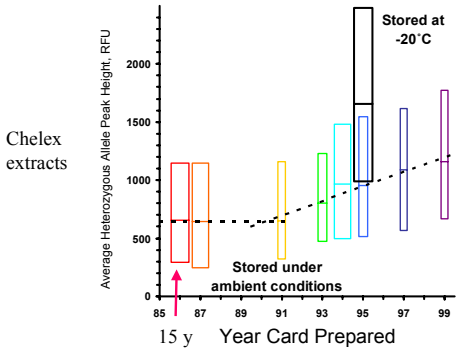


6 Y Field samples

Ambient Stored Field Samples



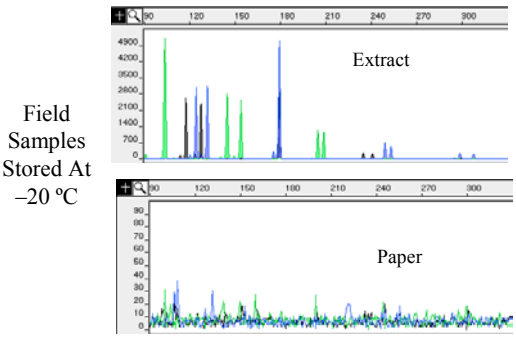
Peak Heights Vs Years Stored



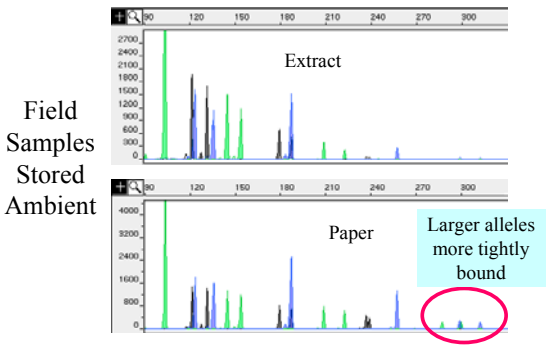
Is the DNA still on the Paper?

- Take bloodstain "spots" that have gone through the Chelex extraction Process
- Wash the Extracted Spots with 10 mM Tris buffer.
- Air dry the spots and take 1.2 mm sub-samples.
- Place the now dry sub-samples into PCR amplification tubes AND…

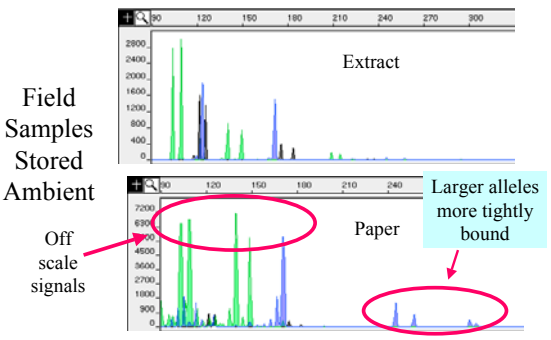
What's Left on the 6 Y Paper after Chelex Extraction?



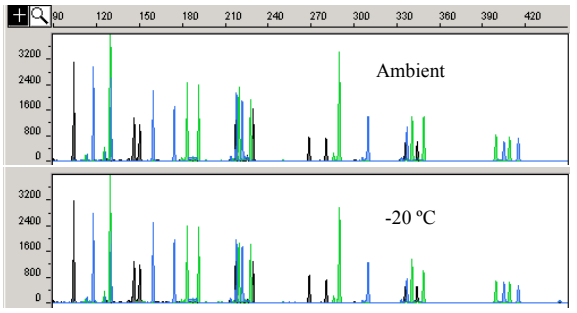
What's Left on the 8 Y Paper after Chelex Extraction?



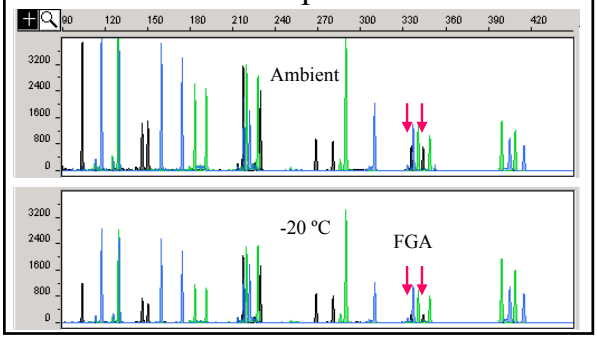
What's Left on the 14 Y Paper after Chelex Extraction?



Chelex Extracts of 7.5 Y Control Samples

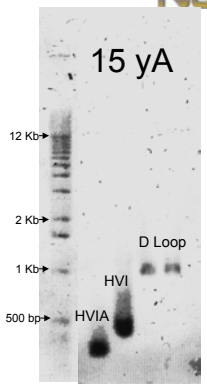


DNA IQ Extracts of 7.5 Y Control Samples



Amplification of Field Sample Mitochondrial DNA

Mitochondrial DNA was amplified using primer sets that amplified products up to1000 bp from the15 yA extracts.



Summary

- **Typeable DNA was recovered from all samples in all studies.**
- Some loss of the larger loci was seen in the oldest (15 Y) field samples.
- Samples stored at ≤ -20 ºC have intact high molecular weight DNA; ambient samples show signs of degradation.
- Mitochondrial DNA (D loop 1000 bp) was amplified from all media types.

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