Effects of Storage Temperature and Humidity Control on the Recovery of DNA From Aged Bloodstains

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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?

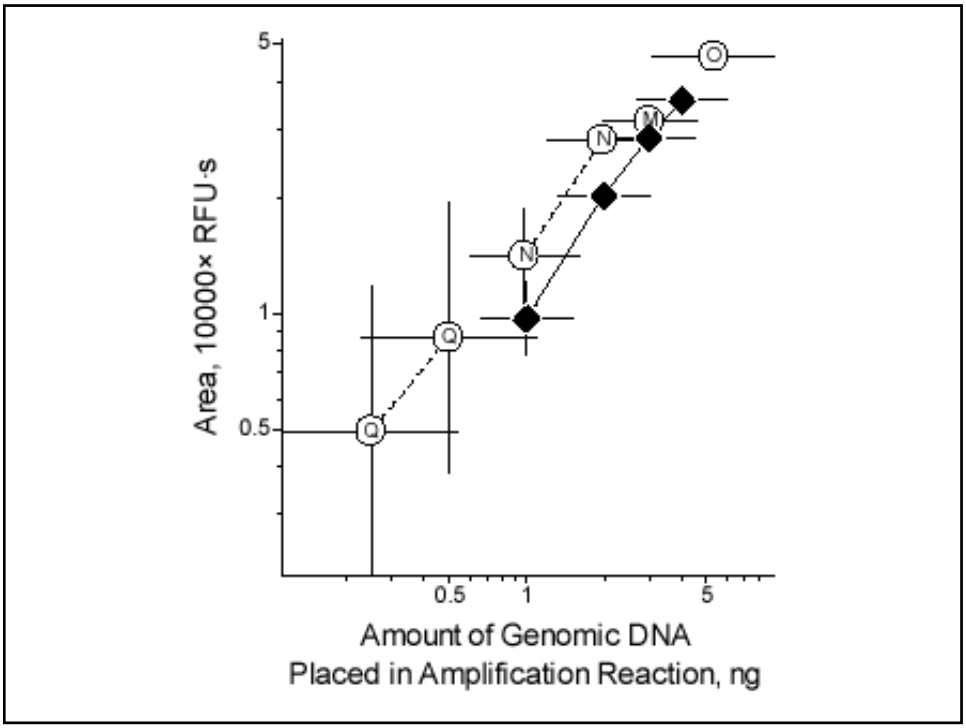
Short Term Study
in conjunction with AFDIL

- 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.

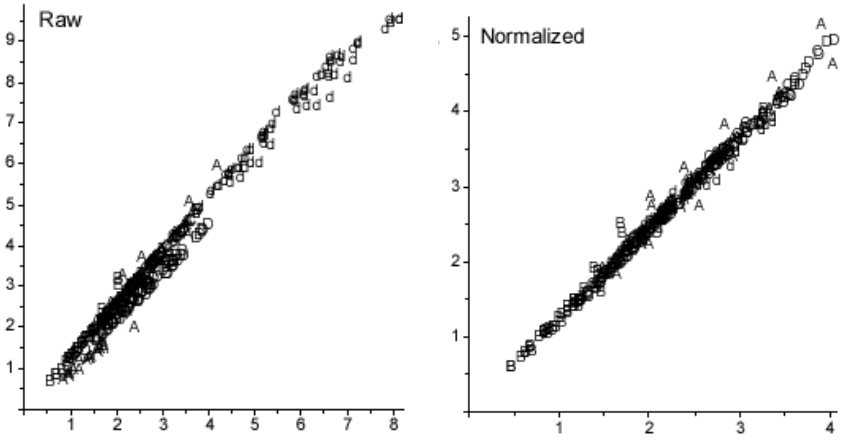
Results of the Short Term Study

- 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.

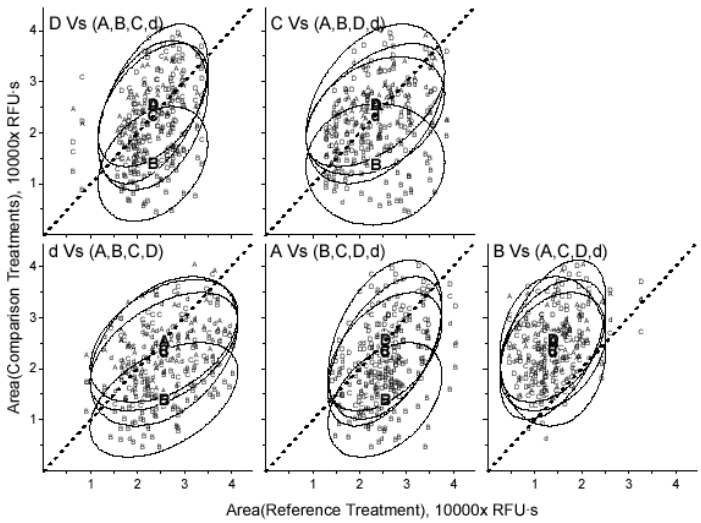
Manuscript describing the details of this study has been accepted for publication in *Analytical Chemistry*



Peak Ht 1000x RFU Vs Peak Area 10000x RFU



Bivariate Distributions



Untreated Paper Studies, S&S 903

Control samples

- NIST prepared
- Dried vacuum desiccator
- Sealed in tubes
- Stored at -198 ºC, -80 ºC, -20 ºC, and lab ambient
- Stored for 7.5 years
- 6 reps of 4 temperatures

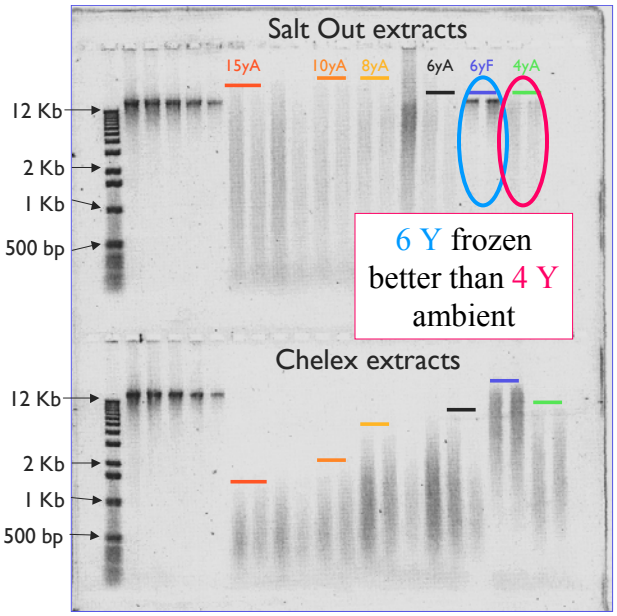
Field samples

- State Health Labs
- Dried ???
- Sealed ????
- Most stored at warehouse ambient, one set at -20 ºC for 6 years
- Stored for 2 to 15 years
- 1 rep of 318 samples

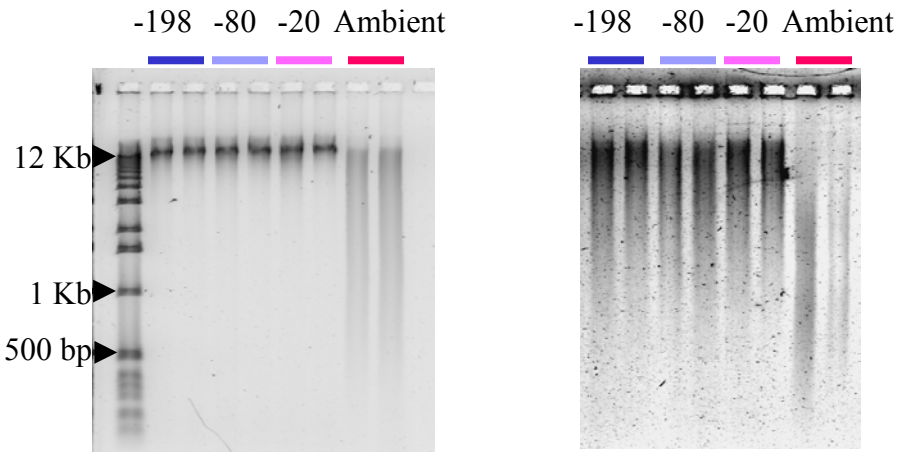
Field Samples Evaluated

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)	-20°C	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



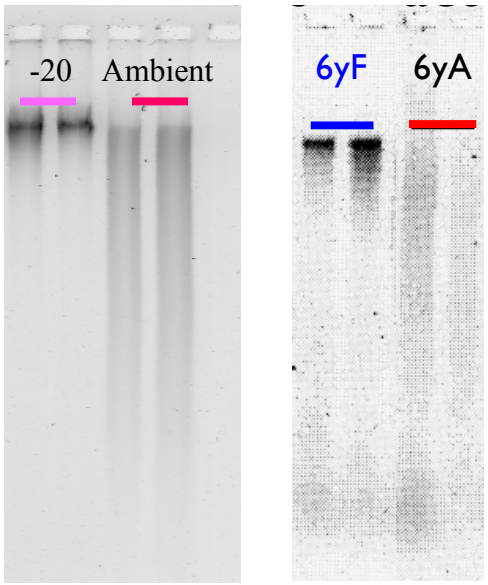
Quality of the DNA Extracted from the 7.5 y Control Samples



Organic Extracts Chelex Extracts

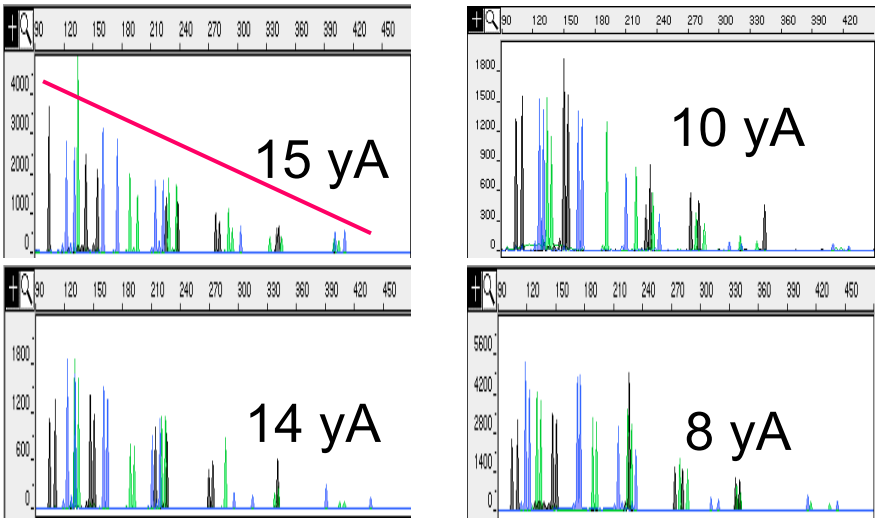
Quality of the Extracted DNA

7.5 Y Controls

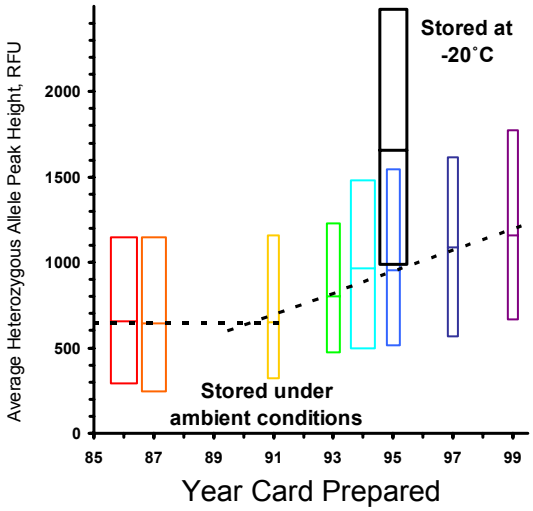


6 Y Field samples

Ambient Stored Field Samples



Peak Heights Vs Years Stored
Chelex Extracts

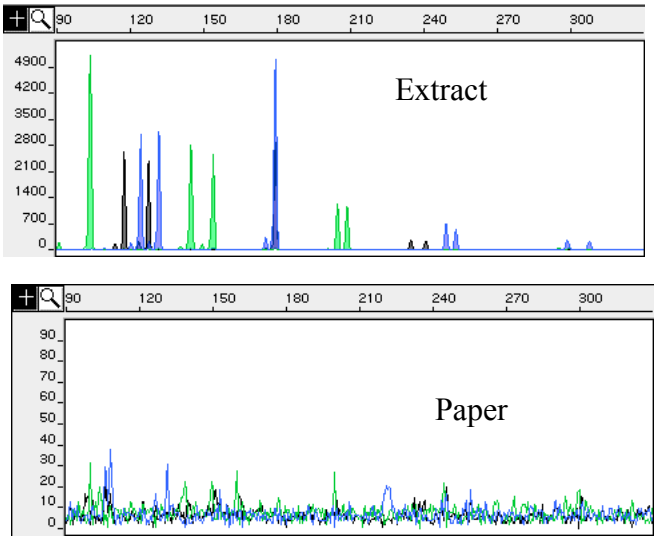


Is the DNA still on the Paper?

- 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…

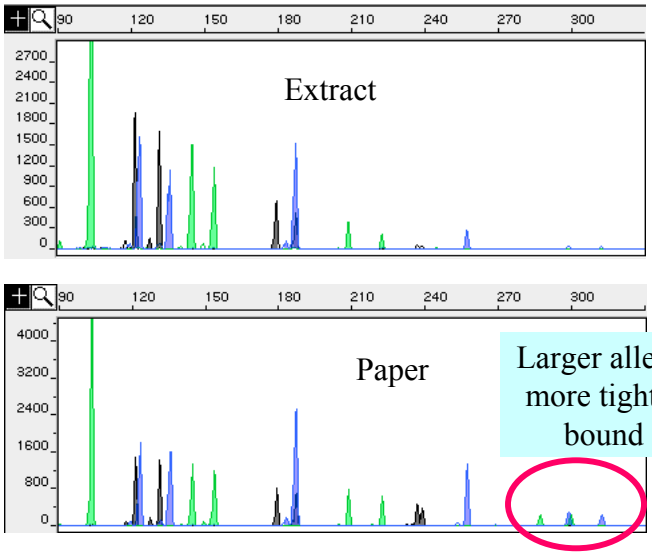
What's Left on the 6 Y Paper after Chelex Extraction?

Field Samples Stored At –20 ºC



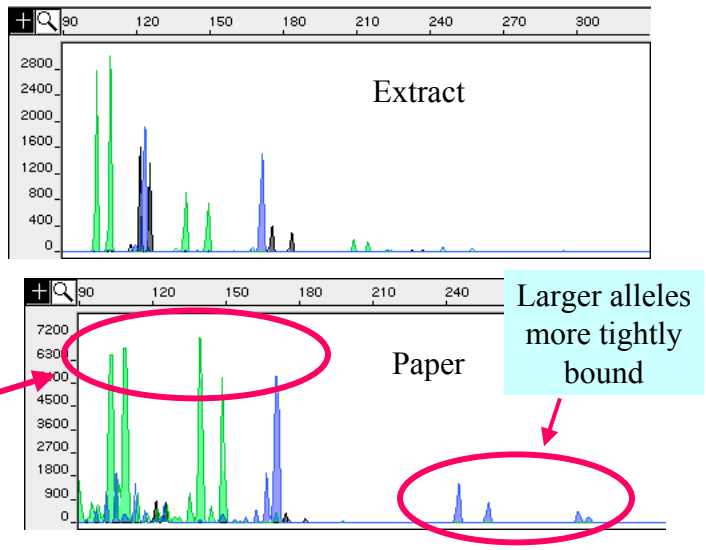
What's Left on the 8 Y Paper after Chelex Extraction?

Field Samples Stored Ambient

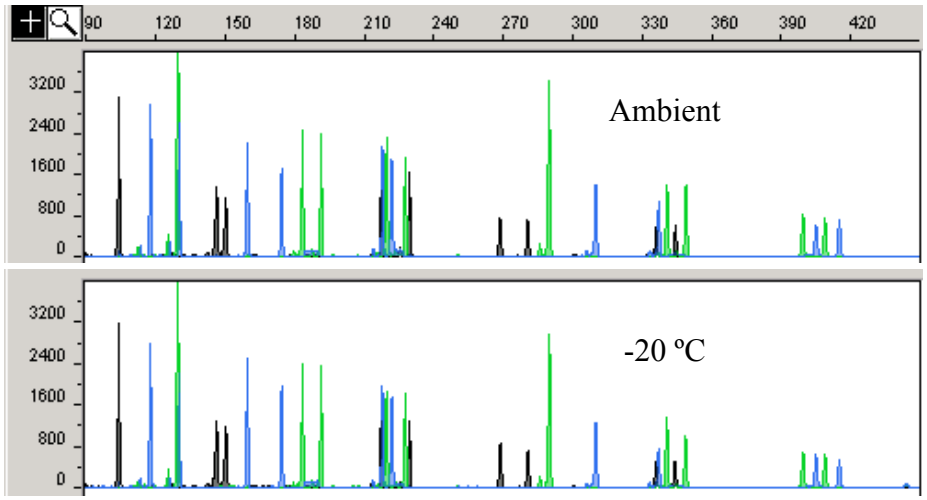


What's Left on the 14 Y Paper after Chelex Extraction?

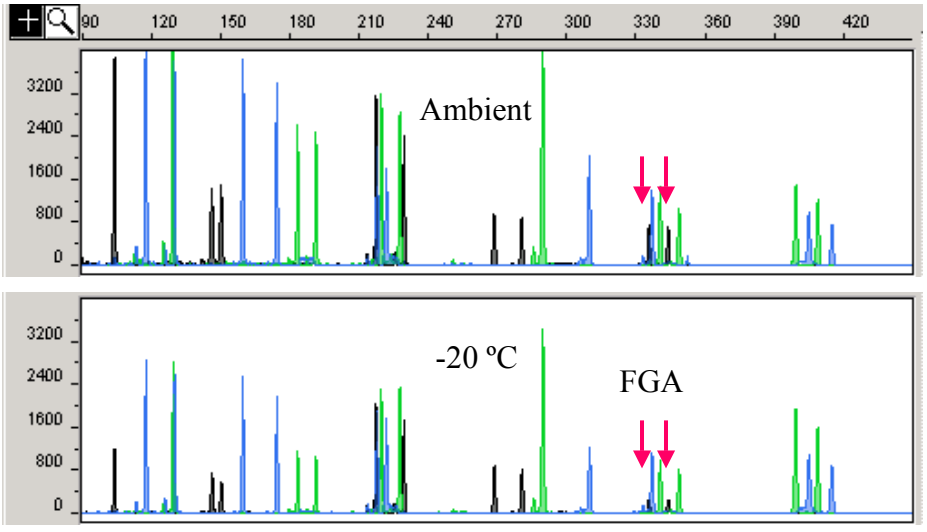
Field Samples Stored Ambient



Chelex Extracts of 7.5 Control Samples

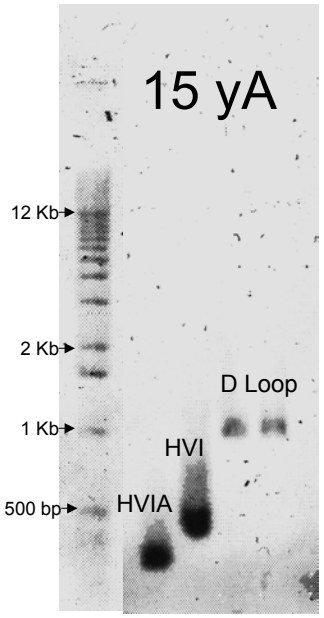


DNA IQ Extracts of 7.5 Y Control Samples



Amplification of Field Sample Mitochondrial DNA

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

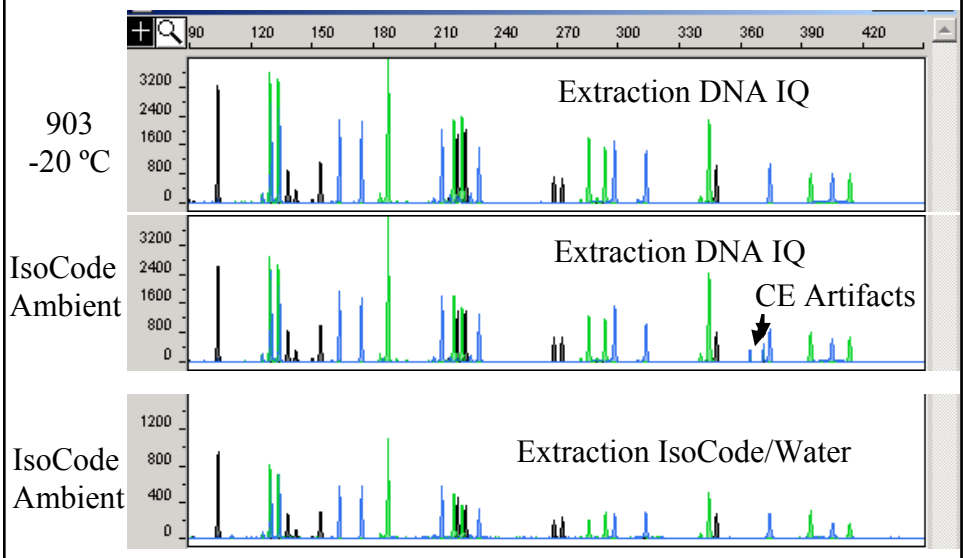


Control Studies with Treated Papers

- Prepared at NIST
- Dried 2 h in laminar flow hood
- S&S 903 paper & IsoCode paper
- Stored -20 ºC & Lab Ambient Respectively
- Ziplock bags
- 6 years

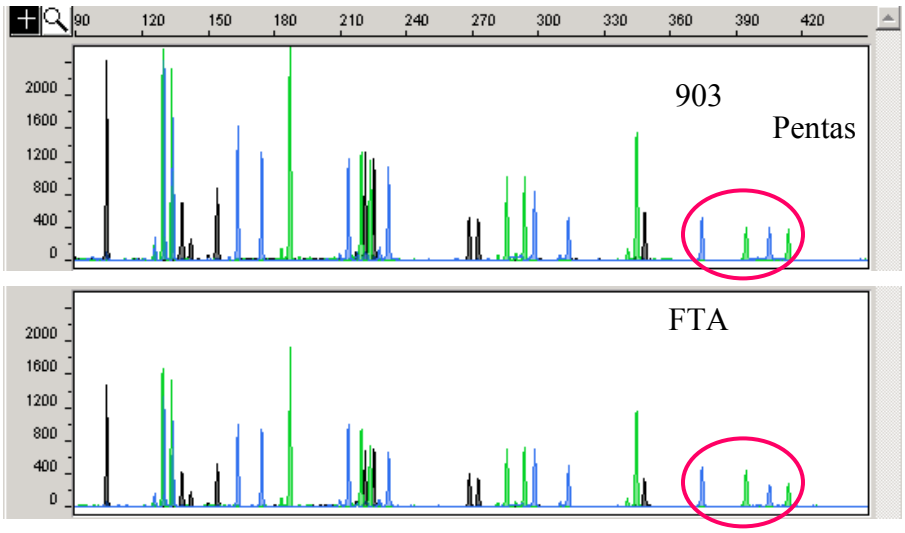
- Prepared at NIST
- Dried 2 h in laminar flow hood
- S&S 903 paper & FTA paper
- All media stored at +37 ºC, Lab Ambient, & -20 ºC
- Vacuum sealed bags
- 4 years

6 Y Samples -20 ºC Vs Ambient

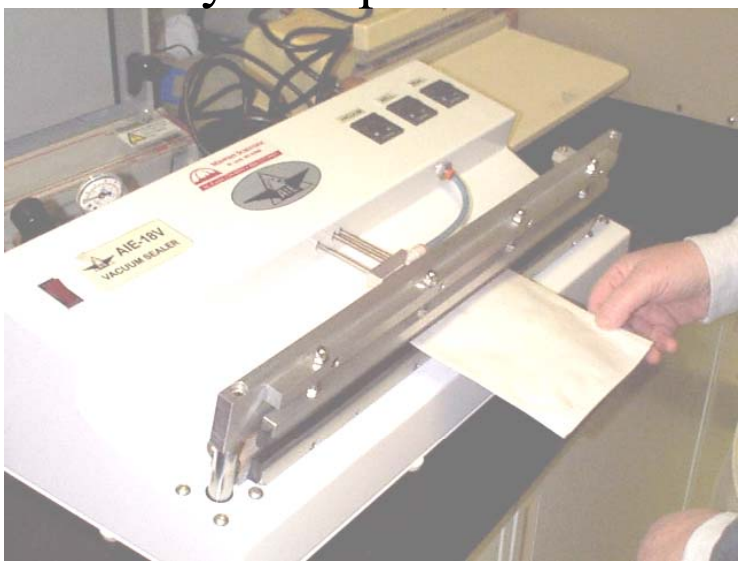


Zip Lock Bag Storage

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



Vacuum Sealer
Run by Compressed Air.



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 (Dloop 1000 bp) was amplified from all media types.

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