Y-Chromosome and Mitochondrial DNA Analysis

mitochondrial DNA

NEAFS 2006 Workshop
Rye Brook, NY
November 1, 2006

Northeastern Association of Forensic Scientists

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Goals and Objectives

- Overview and theory behind mtDNA analysis
- The science behind mtDNA sequencing.
- Forensic casework applications of mtDNA.
- Tools for mtDNA screening – Linear Arrays.
- Emerging mtDNA technologies – mtDNA genome sequencing for increased discrimination, mtDNA micro-chip technology.
- Summary and **Questions**

June 26, 2000

"A day for the ages"

Associated Press

April 09, 1981

Mitochondrial DNA – The OTHER Human Genome

Fred Sanger standing in front of the Sanger Institute

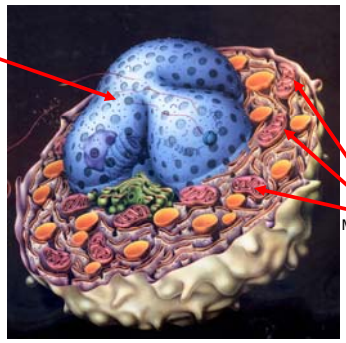
http://www.wellcome.ac.uk/

The Human Genome

Nuclear DNA

3 billion bp

High Power
Of Discrimination

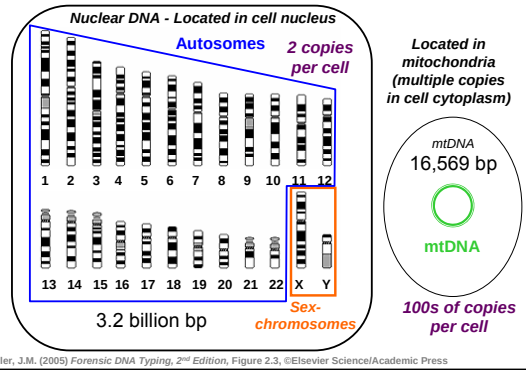


Mitochondrial DNA

16.5 Kbp

High Copy
Number

The Human Genome



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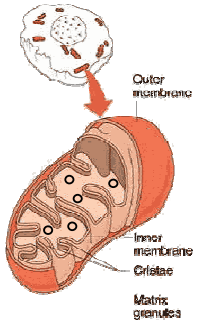
You Say Tomato…

- Cowdry (1918) review of what microscopists called "mitochondria"

Blepharoblasts	Fadenkorper	
Chondriokonts	Mitogel	
Chondriomites	Parabasal bodies	*mitos* = thread
Chondrioplasts	Plasmabioblasts	*chondros* = granule
Chondriosomes	Plastochondria	
Chondriospheres	Plastosomes	
Filia	Vermicules	
Fuchsinophilic Granules	Sarcosomes	
Korner	Interstitial bodies	
	Bioblasts	

Mitochondrial Morphology



Cytoplasmic organelle

Double membrane

Outer membrane – porin proteins for the transportation of materials.

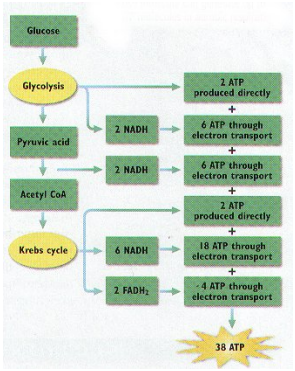
Inner membrane – highly folded (increased surface area) and highly impermeable.

Inner Matrix – several copies of mtDNA

Mitochondrial Functions

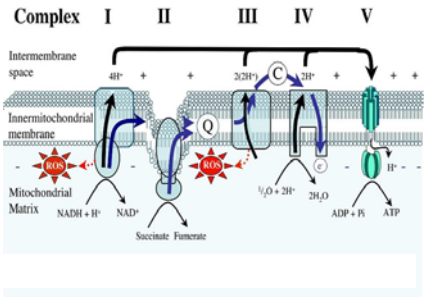
Cellular Respiration – ATP production via oxidative-phosphorylation (OX-PHOS).

Apoptosis – programmed cell death
Steroid synthesis
Elongation of fatty acids
Oxidation of epinephrine (adrenaline)
Degradation of tryptophan
Heme synthesis
Heat production



http://sps.k12.ar.us/massengale/cell_respiration_bi.htm

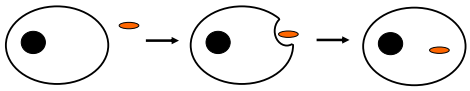
Ox-Phos



http://myweb.uiowa.edu/bballard/Research%20Figures/OXPHOS.jpg

Mitochondrial Evolution

- Endosymbiotic Theory – Ivan Wallin (1920s) and Lynn Margulis (1981).
- Proto-Eukaryotic cell incorporated a proto-bacterial cell and formed a symbiotic relationship.



Support for the Endosymbiotic Theory

- Mitochondria have double membranes – and the inner membrane is rich in cardiolipin.
- Mitochondria have their own genome, which is circular like bacteria (no histones), and use a genetic code for amino acids different that the nuclear DNA.
- New mitochondria are formed by a process similar to binary fission.
- Mitochondrial ribosomes are very similar to bacterial ribosomes (affected by antibiotics such as linezolid).

Lucky Guess or Clairvoyant?

- 1890 – R. Altman writes that “bioplasts” (mitochondria) are, “autonomous, elemental living units, forming bacteria-like colonies in the cytoplasm of the host cell.”

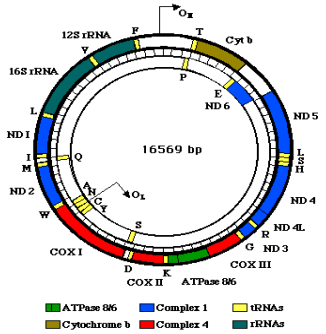
Immo Scheffler, Mitochondria (1999)

Mitochondrial Evolution

Complex	I	II	III	IV	V
Enzyme	NADH-CoQ Reductase	Succinate-CoQ Reductase	CoQ-Cytochrome C Reductase	Cytochrome C Oxidase	ATP Synthase
Inhibitor	Rotenone Amytal	TTFA malonate	Antimycin A	Cyanide Carbon Monoxide Azide	Oligomycin
Nuclear DNA Subunits	~43	4	10	10	~14
mtDNA Subunits	7 ND1-6, ND4L	0	1 Cytochrome b	3 COX I, II, III	2 ATPase 6 ATPase 8

~81 subunits encoded by the nuclear genome

mtDNA Genome



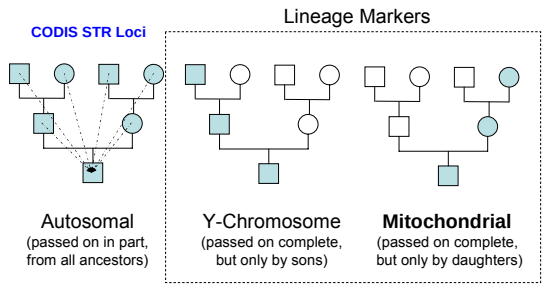
Coding Region
13 polypeptides
2 rRNAs
13 tRNAs

All necessary for OXPHOS

Highly compact (few intergenic spaces)

http://www.mitomap.org/

Different Inheritance Patterns



Autosomal
(passed on in part, from all ancestors)

Y-Chromosome
(passed on complete, but only by sons)

Mitochondrial
(passed on complete, but only by daughters)

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Location and Copy Number of mtDNA

- Found within the mitochondria in the cellular cytoplasm.
- On average 4-5 copies of mtDNA molecules per mitochondria (range of 1-15 mtDNA copies).
- Number of mitochondria vary by cell type (e.g., muscles have more…).
- Generally, hundreds of mitochondria per cell.

Forensic Focus

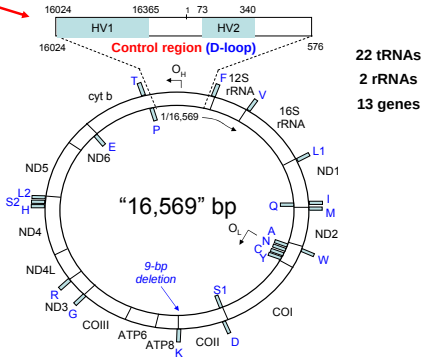


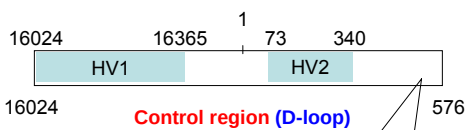
Figure 10.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

mtDNA Is Not Always 16,569 bp …

- Dinucleotide repeat at positions 514-524 (near end of control region)
  - Usually ACACACACAC or $(AC)_5$ in most individuals
  - Can vary from $(AC)_3$ to $(AC)_7$
- Other insertions and deletions may occur
  - 9 bp deletion (positions 8277 to 8285) in some individuals from Asia and Pacific Islands (haplogroup B) and Africans (haplogroup L).

Control Region (16024-576)

1,122 nucleotide positions



Forensic Focus

Typically only **610 bases examined**

- (HVI: 16024-16365; HVII: 73-340)

$(AC)_3$
$(AC)_4$
$(AC)_5$
$(AC)_6$
$(AC)_7$

"Heavy" vs. "Light" Strand

- The two strands ("inner" and "outer" loops) of mtDNA can be separated with an alkaline CsCl gradient.
- Heavy or H-strand contains a greater number of guanine nucleotides (largest molecular weight of the four nucleotides) – purine rich.
- Light or L-strand contains more C and T nucleotides and is thus physically lighter (pyrimidine rich).
- H-strand codes for 28 gene products while the L-strand is used to transcribe 8 tRNAs and the ND6 protein product.

Original Reference Sequence

- Human mtDNA was first sequenced in 1981 in Frederick Sanger's lab located in Cambridge, England.
- Authors for this paper (Nature 1981, 290:457-465) were listed in alphabetical order so Stan Anderson was the first author.
- This sequence has come to be referred to as the **"Anderson" sequence** (GenBank accession: M63933).
- This first sequence is sometimes called the **Cambridge Reference Sequence (CRS).**

Re-Sequencing of CRS

- The 1981 sequence was derived primarily from a placenta of an individual with European ancestry; however, some HeLa and bovine sequence was used to fill in gaps due to early sequencing procedures performed.
- Re-analysis of original placental material by Andrews et al. (1999) found 11 nucleotides that differed from Anderson et al. (1981) sequence.
- This **revised Cambridge Reference Sequence (rCRS)** is now the accepted standard for comparison.

Evaluation of Sequence Differences

Between CRS (Anderson et al. 1981) and rCRS (Andrews et al. 1999)

Nucleotide Position	Region of mtGenome	Original CRS	Revised CRS	Remarks
3106–3107	16S rRNA	CC	C	Error
3423	ND1	G	T	Error
4985	ND2	G	A	Error
9559	COIII	G	C	Error
11335	ND4	T	C	Error
13702	ND5	G	C	Error
14199	ND6	G	T	Error
14272	ND6	G	C	Error (bovine sequence inserted)
14365	ND6	G	C	Error (bovine sequence inserted)
14368	ND6	G	C	Error
14766	cyt b	T	C	Error (HeLa sequence inserted)

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Further Comparison of CRS and rCRS

- No differences seen between CRS and rCRS within the mtDNA control region.
- **The original CRS contained a "CC" at positions 3106-3107 but rCRS was found to possess only a single "C"**

3100 3106 3108
TATCTACCTT Original CRS
TATCTAC - TT Revised CRS

- Thus, rCRS is only 16,568 bp!

Maternal Inheritance of mtDNA

- Fertilizing sperm contributes only nuclear DNA.
- Cellular components including the mitochondria in the cytoplasm come from the mother's ovum.
- Any sperm mitochondria that may enter a fertilized egg are selectively destroyed due to a ubiquitin tag added during spermatogenesis.
- Barring mutation, a mother passes her mtDNA type on to her children.

Maternal Inheritance of mtDNA

Note that mtDNA is not unique to an individual

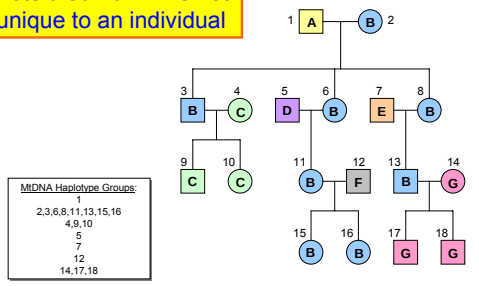

Figure 10.2, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Summary – mtDNA Characteristics

- High copy number of mtDNA.
- Maternal inheritance of mtDNA.
- Lack of recombination.
- High mutation rate compared to single copy nucDNA.

Methods for Measuring mtDNA Variation

- Low-resolution RFLP (1980s)
- High-resolution RFLP (1990s)
- Sequence analysis of HV1 and HV2 within control region (1991-present)
- Sequence analysis of complete mtDNA genome (2000-present)

Mitochondrial DNA Sequencing in Forensic Casework

Issues and Examples

Role of mtDNA

Compared to Autosomal STRs

- **Autosomal STRs provide a higher power of discrimination and <u>are the preferred method</u> whenever possible**
- **Due to high copy number**, mitochondrial DNA (**mtDNA**) may be the only source of surviving DNA in highly degraded specimens or low quantity samples such as hair shafts
- A mtDNA result is better than no result at all…

Comparison of Human nucDNA and mtDNA

Characteristics	Nuclear DNA (nucDNA)	Mitochondrial DNA (mtDNA)
Size of genome	~3.2 billion bp	~16 569 bp
Copies per cell	2 (1 allele from each parent)	Can be > 1000
Percent of total DNA content per cell	99.75%	0.25%
Structure	Linear; packaged in chromosomes	Circular
Inherited from	Father and Mother	Mother
Chromosomal pairing	Diploid	Haploid
Generational recombination	Yes	No
Replication repair	Yes	No
Unique	Unique to individual (except identical twins)	Not unique to individual (same as maternal relatives)
Mutation rate	Low	At least 5–10 times nucDNA
Reference sequence	Described in 2001 by the Human Genome Project	Described in 1981 by Anderson and co-workers

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Candidates for mtDNA Testing

- Shed hairs lacking root bulb or attached tissue
- Fragments of hair shafts.
- Aged bones or teeth that have been subjected to long periods of exposure.
- Crime scene stains or swabs that were unsuccessful for nuclear DNA testing.
- Tissues (muscle, organ, skin) that were unsuccessful for nuclear DNA testing.

Terry Melton – International Symposium on the Application of DNA Technologies in Analytical Sciences

mtDNA Testing on Hairs

- Human hair shafts contain very little DNA but because mtDNA is in higher copy number it can often be recovered and successfully analyzed
- Melanin found in hair is a PCR inhibitor

Important Publications:

- **Wilson, M.R., *et al.* (1995) Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts. Biotechniques 18(4): 662-669.**
  - **Tissue grinding method described by FBI Lab**
- **Melton *et al.* (2005) Forensic mitochondrial DNA analysis of 691 casework hairs. J. Forensic Sci. 50(1): 73-80.**
  - **Obtained a full or partial mtDNA profile for >92% of hairs tested**

The Mitotyping Experience

Terry Melton,[1] Ph.D.; Gloria Dimick,[1] M.S.; Bonnie Higgins,[1] M.S.; Lynn Lindstrom,[1,2] B.S.; and Kimberlyn Nelson,[1] Ph.D.

Forensic Mitochondrial DNA Analysis of 691 Casework Hairs*

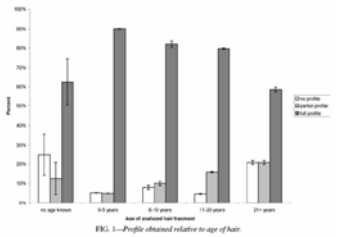


FIG. 1—*Profile obtained relative to age of hair.*

Journal of Forensic Science (2005) **50(1):** 73-80.

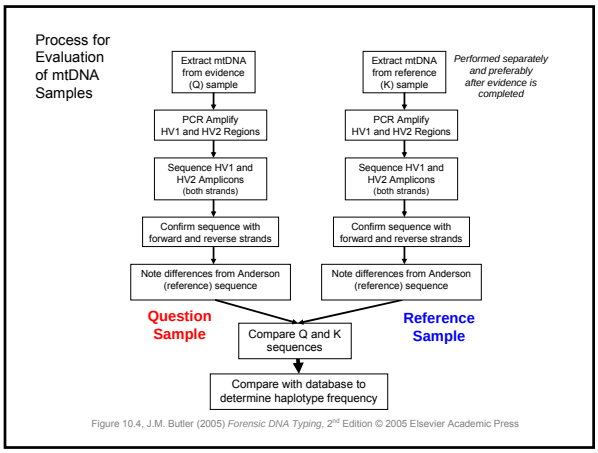


Figure 10.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Mitochondrial DNA as a Means of Identification

When do you need it and why?

Why go to mtDNA?

- Disadvantages
  - mtDNA is not a positive form of identification
    (You have many maternal relatives!!)
  - Easily contaminated with modern DNA

Contamination

- Modern DNA can easily be introduced and overwhelm target DNA from the sample.
  - Due to the sensitivity of the reaction
    - Increased cycle number
    - Increased Taq
- Appropriate controls must be implemented to assure that the mtDNA sequence being reported is authentic.
- Laboratories need to be designed to lessen the chances of contamination.

Why go to mtDNA?

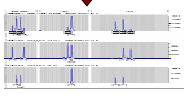
- Disadvantages
  - mtDNA is not a positive form of identification
    (You have many maternal relatives!!)
  - Easily contaminated with modern DNA
  - Time-consuming and costly

Nuclear DNA Analysis

Sample Collection

Laboratory

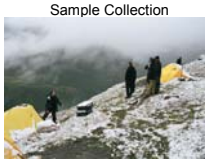
- 24-36 hours
- ~$100 per sample
- Use commercially available kits for processing



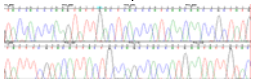
Profile generation

MtDNA Analysis

Sample Collection



Laboratory



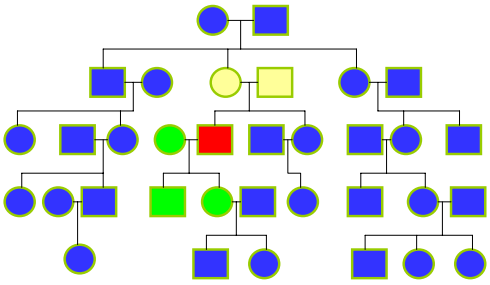
Profile generation

- 1-6 weeks post-submission to the laboratory
- ~$1,000 per sample
- Custom designed primers

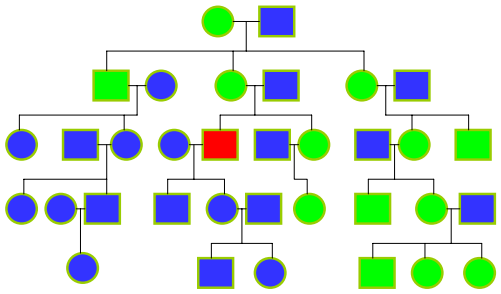
Why go to mtDNA?

- Advantages
  - Maternally inherited
    - The pool of potential references is greatly increased.

Limited references available for nDNA



Maternal inheritance



Why go to mtDNA?

- Advantages
  - Maternally inherited
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  - Numerous copies of the mitochondrial DNA genome in each cell.

Why go to mtDNA?

- Advantages
  - Maternally inherited
    - The pool of potential references is greatly increased.
  - Numerous copies of the mitochondrial DNA genome in each cell.
  - Small genome size and multiple copies increase chances of recovering DNA from degraded samples.

Laboratory Design

Organizing Your Space,
People, and Samples

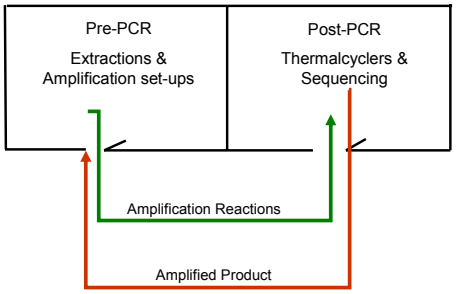
Laboratory Design

- All laboratories should be designed to be separated by use.
- At AFDIL, pre-PCR labs are physically separated from post-PCR by magnetically sealed doors and airlocks

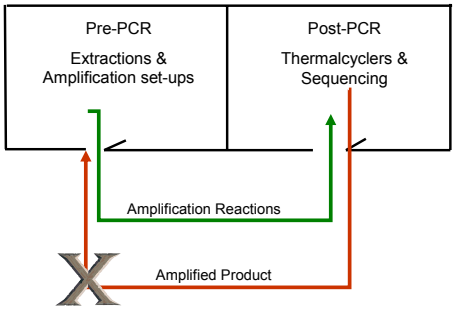
Pre- and post-PCR Separation

- Separation of pre- and post-PCR areas prevents contamination.
  - Amplified product needs to be kept away from low quantity DNA areas.
  - Personnel flow from pre- and post-PCR areas needs to be controlled.

Pre- and post-PCR Separation



Pre- and post-PCR Separation



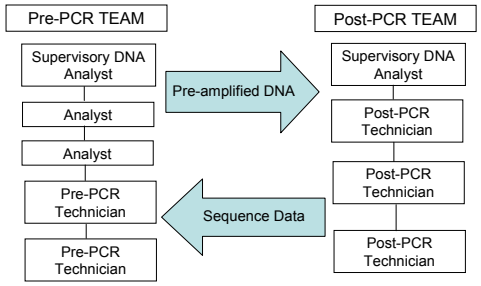
Pre-PCR Organization

- To further minimize contamination:
  - Pre-PCR lab space can be dedicated to teams of individuals.

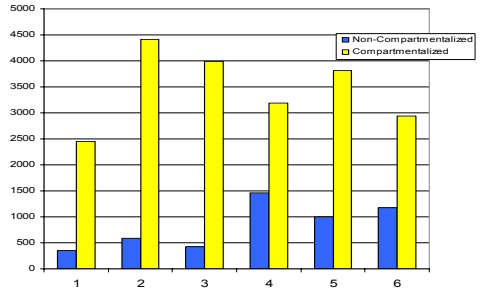
Staff Organization

- Now that the spaces are separated, what do you do with the people?
  - Staff organization can not only reduce contamination but increase efficiency.

Division of Labor



Evaluation of the Number of Sequencing Reactions Per Month in a Six Month Time Period



Sample and Contamination Tracking

- With this type of sample volume comes the additional issues of tracking your samples and contamination.
  - Even if the lab is fairly small, chain of custody issues and overall processing need to be tracked efficiently.
  - Contamination needs to be tracked, found and eradicated before it becomes an issue.

LIMS System

- An automated computer system is the most efficient method for accomplishing these goals.
- Many laboratory information management systems are available commercially.
- The name of our system is Laboratory Information Systems Application or LISA.

Case Accessioning

- Names and identifies each piece of evidence that is received.
- Assigns a sequential case number.

- Controls who has access to which samples based on set of 'privileges'.
- Tracks Chain of Custody.
- Every step requires a password even once you are in the system.

Sample Storage

- Samples need to be stored at the appropriate temperatures.
  - Heat or large temperature fluctuations can cause further degradation of the DNA.
  - Bone material can be stored at ≤-20°C
  - Blood should be dried and stored at -20°C

Lab Processing

- Requires passwords throughout.
- Links all the forms and protocols used at AFDIL together.
- Procedures predicated on the completion of a step are not allowed until that step is finished.
- Designed to be compatible with sequencing equipment.

Contamination Tracking

- Contamination is a huge challenge in 'ancient' DNA laboratories.
- Must be able to guarantee that the sequence being generated is authentic and not modern.

How to control for contamination?

- Laboratory design
- Staff databases
- Contamination tracking via LISA
- Amplification controls

Control Databases

- All members of the staff, laboratory and administrative, at AFDIL have been profiled for both mitochondrial and nuclear DNA.
- The case management module of LISA has a separate database specifically for the sequences generated for contaminants

Contamination Tracking

- Contamination can be tracked through the processing steps.
- LISA has a separate database specifically for the sequences generated for contaminants.
- Reports can be generated in LISA per primer pair and scientist to pinpoint a specific issue.

Degraded Skeletal Remains

What to choose and how to generate a full mtDNA profile.

Degraded Skeletal Remains

- Sample Selection
- Extraction Methods
- Amplification Strategies
- Sequencing Strategies

Degraded Specimens

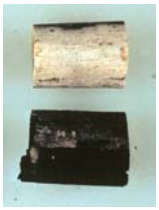
- In general terms all skeletal remains are degraded.
- Some are more degraded then others due to environmental stressors.
- Prudent sample selection will increase the rate of success.

Environment

- Recovery sites vary
  - Extreme conditions
    - Salt-water marshes
    - Glaciers
  - High/Low temperatures
  - Repeated freezing and thawing
  - High/Low pH
  - High water levels
  - Salt or brackish water

Environment

- Remains may be
  - On the surface
  - Buried in soil or other substrates
  - Highly fragmented
  - Subjected to burning or high heat
  - Exposed to fuel or other chemicals
  - Disturbed or moved by humans or animals
  - Animal destruction (feeding)

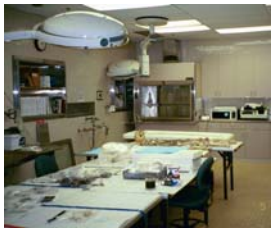


Storage Effects

- Handling of Remains
- Temperature
- Humidity
- Storage Container

Sample Selection

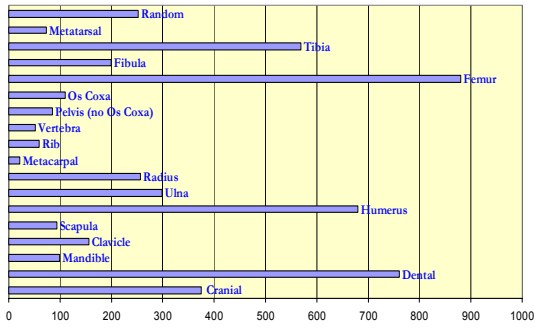
- Unknown skeletal remains
  - Remains are examined and samples selected by anthropologists or medical examiners



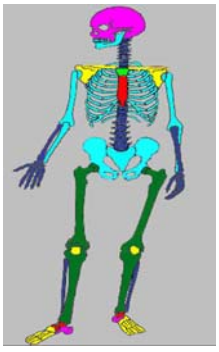
Selecting samples for analysis

- What are the best skeletal elements to use for analysis?

Bones Submitted for Analysis



Skeletal Success Rate

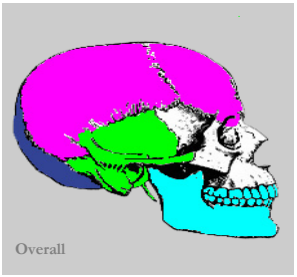


- 90 – 100%
- 80 – 89.9%
- 70 – 79.9%
- 60 – 69.9%
- 50 – 59.9%
- 40 – 49.9%
- 30 – 39.9%
- 0 – 20%

Bone Structure

- Bones with dense cortical structure tend to have a greater success rate.
  - Compact bone may inherently afford greater protection for it's deeper layers.
  - Trabecular bone and elements composed of thin cortical bone have a greater surface area
- Cranial fragments vary in success
  - Formed of a layer of trabecular bone sandwiched between two layers of cortical bone
  - Temporal and occipital tend to have denser cortical bone

Cranial Success



90 – 100%
80 – 89.9%
70 – 79.9%
60 – 69.9%
50 – 59.9%
40 – 49.9%
30 – 39.9%
0 – 20%

Dentition

- Dental remains provide a particular challenge
  - The enamel gives a greater protection to the dentin from which the DNA is extracted.
  - Anecdotally shown to provide copious quantities of DNA from even medieval era remains.
  - Require a lot of handling.

Extraction Methods

- Cleaning the samples – how much is too much?
- What protocols give the greatest yield of DNA?
- What method is right for you?
- Trouble-shooting the extraction.

Cleaning the Sample

- The exterior of the bone fragment needs to be cleaned of any possible contaminants:
  - Dirt
  - Plant material
  - Extraneous DNA
  - Dried Tissue

Cleaning

- An easy way to clean the surface is using a sanding bit in a Dremel tool.

How far to clean?

- Everything on the surface needs to come off, along with the spongy bone.
- But, you'll hit a point where there is no solid bone left.

Other Cleaning Methods

- Bleaching
  - Bones can be subjected to a bleach sonication to remove external contaminants.
  - A fresh water sonication should follow to get rid of the bleach or DNA can be lost.
- "DNA Off" or other DNA removal products

Too much?

- Aggressive cleaning can remove or otherwise damage available DNA.

Extraction Methods

- Numerous extraction methods available.
- Involve different methods of –
  - pulverizing the samples
  - removing the DNA from the samples
- Different starting quantities of bone can also be used.

Pulverization Methods

- Freezer Mill
  - Uses liquid nitrogen and a magnet to pulverize the bone into a very fine powder.
  - Disadvantage:
    - Requires storage and handling of liquid nitrogen.
    - Grinders and sample vials are reused – potential contamination.

Pulverization Method

- Waring Blender Cup
  - Also grinds bone to a relatively fine powder
  - Disadvantage: Cups are reused, so there is a possibility of contamination.

"Freeing" the DNA

- Samples may be subjected to a decalcification step.
  - Demineralizes the bone matrix.
- Other chemical/physical treatments are commercially available to more easily acquire the DNA.
  - Silica gel
  - Charge Switch™
  - DNA IQ™

Extraction of Skeletal Remains

- The powdered bone is extracted with
  - 20mg/ml Proteinase K and extraction buffer
  - Overnight at 56°C
- DNA is removed from the extraction buffer with
  - a series of washes with Phenol/ Chloroform/ Isoamyl alcohol
  - Purification of product with filters.

There's DNA, now what?

- Quantification –
  - At AFDIL, we do not quantify prior to amplification.
  - Can quantify using a 1% Agarose gel and ethidium bromide.
  - CalDOJ has a quantitation method for both nuclear and mtDNA using qPCR.
    - Timken, et al. (2005), A duplex real-time qPCR Assay for the quantification of human nuclear and mitochondrial DNA in forensic samples: Implications for quantifying DNA in degraded samples. J. of Fors. Sci. 50(5): 1044-60.

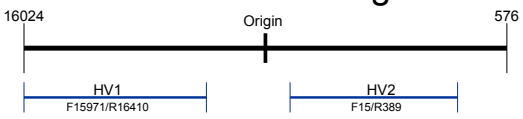
Amplification

- A standard program for amplification is used for the 9700's.
- The basic program is modified based on the primer pair used (Gabriel, et al. 2001)
  - 10-minute soak at 96.0°C
  - Followed by 38 cycles of
    - 20s at 94.0°C
    - 20s at 56.0°C
    - 30s at 72.0°C
  - Final hold at 4°C

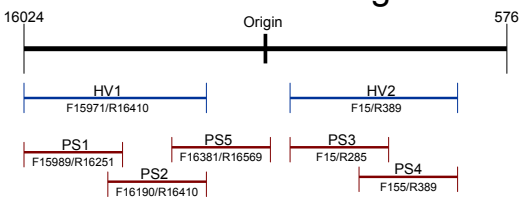
PCR Amplification of mtDNA

- Usually performed with 34-38 cycles
- Some protocols may go to 42 cycles for highly degraded specimens

Primer Design



Primer Design



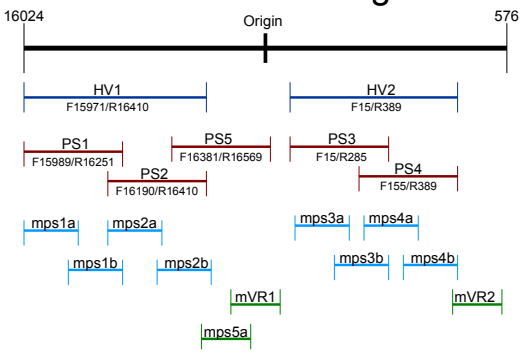
miniPrimer Sets for HV1/HV2

Matthew N. Gabriel,[1] M.F.S.; Edwin F. Huffine,[2] M.S.; John H. Ryan,[1] Ph.D.; Mitchell M. Holland,[1] Ph.D.; and Thomas J. Parsons,[1] Ph.D.

Improved MtDNA Sequence Analysis of Forensic Remains Using a "Mini-Primer Set" Amplification Strategy*

JFS (2001)

Primer Design

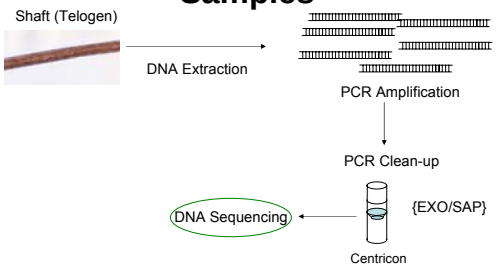


miniPrimer Sets for HV1/HV2

TABLE 2A—*Comparison of amplification success of most highly degraded remains.*

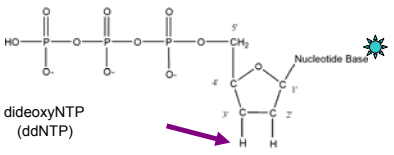
	Primer Set	Mini-Primer Set
Sample	PS1 (280bp)	MPS1A (170bp)
S1	–	–
S2	+	–
S3	–	–
S4	–	+
S5	–	+
S6	–	+
S7	–	+
S8	–	–
S9	–	+
S10	–	+
S11	–	–
S12	–	–
S13	–	+
S14	–	+
S15	–	+

Process for Evaluation of mtDNA Samples



Science of DNA Sequencing

- Fred Sanger – developed the dideoxy method of sequencing in the 1970s… still used today.



Sanger Sequencing

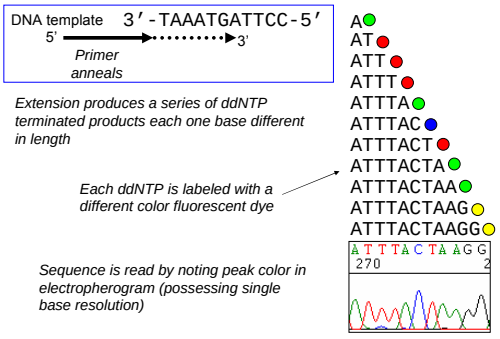
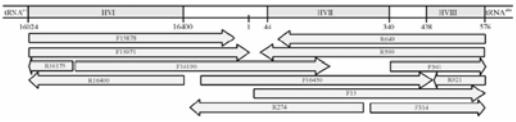
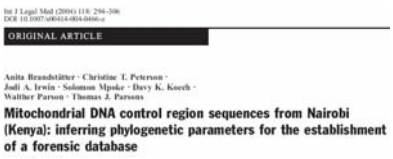


Figure 10.5, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Primers Used for Control Region Amplification and Sequencing



13 primers – 11 for routine sequencing



ORIGINAL ARTICLE

Anita Brandstätter · Christine T. Peterson · Jodi A. Irwin · Solomon Mpoke · Davy K. Koech · Walther Parson · Thomas J. Parsons

Mitochondrial DNA control region sequences from Nairobi (Kenya): inferring phylogenetic parameters for the establishment of a forensic database

Why the Redundancy?

- Homopolymeric stretches of Cytosines (C-stretches).

16189T

HV1 **AAAACCCCCTCCCCATG**

5 Cs 4 Cs

16189C

AAAACCCCCCCCCCATG → Strand slippage can create 11+ tandem Cs

10 Cs

Challenges with Sequencing Beyond the Polymeric C-Stretches in HV1 and HV2

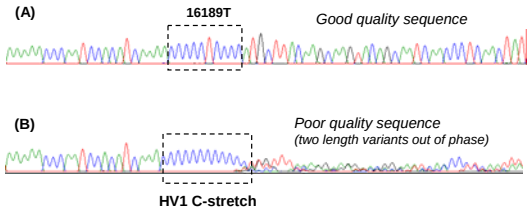


Figure 10.7, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Challenges with Sequencing Beyond the Polymeric C-Stretches in HV1 and HV2

Primer strategies typically used with C-stretch containing samples

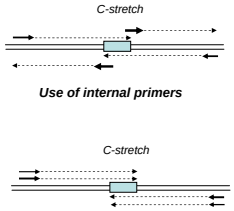
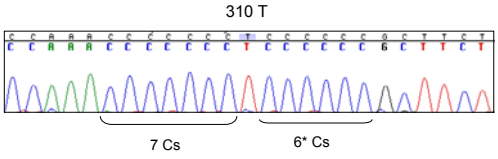


Figure 10.7, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

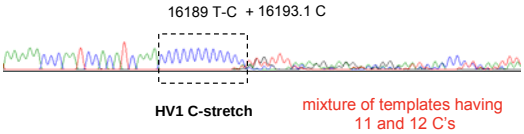
HV2 C-Stretch



*The rCRS has 5 Cs in this region

A word about SRMs…

- NIST mtDNA SRM 2392 (1999) – contains 2 apparently normal cell lines (CHR and GM09947a) and a cloned DNA from CHR for HV1.



The cloned DNA has only 11C's – so sequencing can continue without falling apart!

NIST SRM 2392-I

- (2003) Contains cell line HL-60 extract and documentation.
- SRM 2392-I complements SRM 2392 and was based on a suggestion from the FBI that this DNA would be particularly useful to the forensic community.

Process for Evaluation of mtDNA Samples