Mitochondrial DNA Analysis

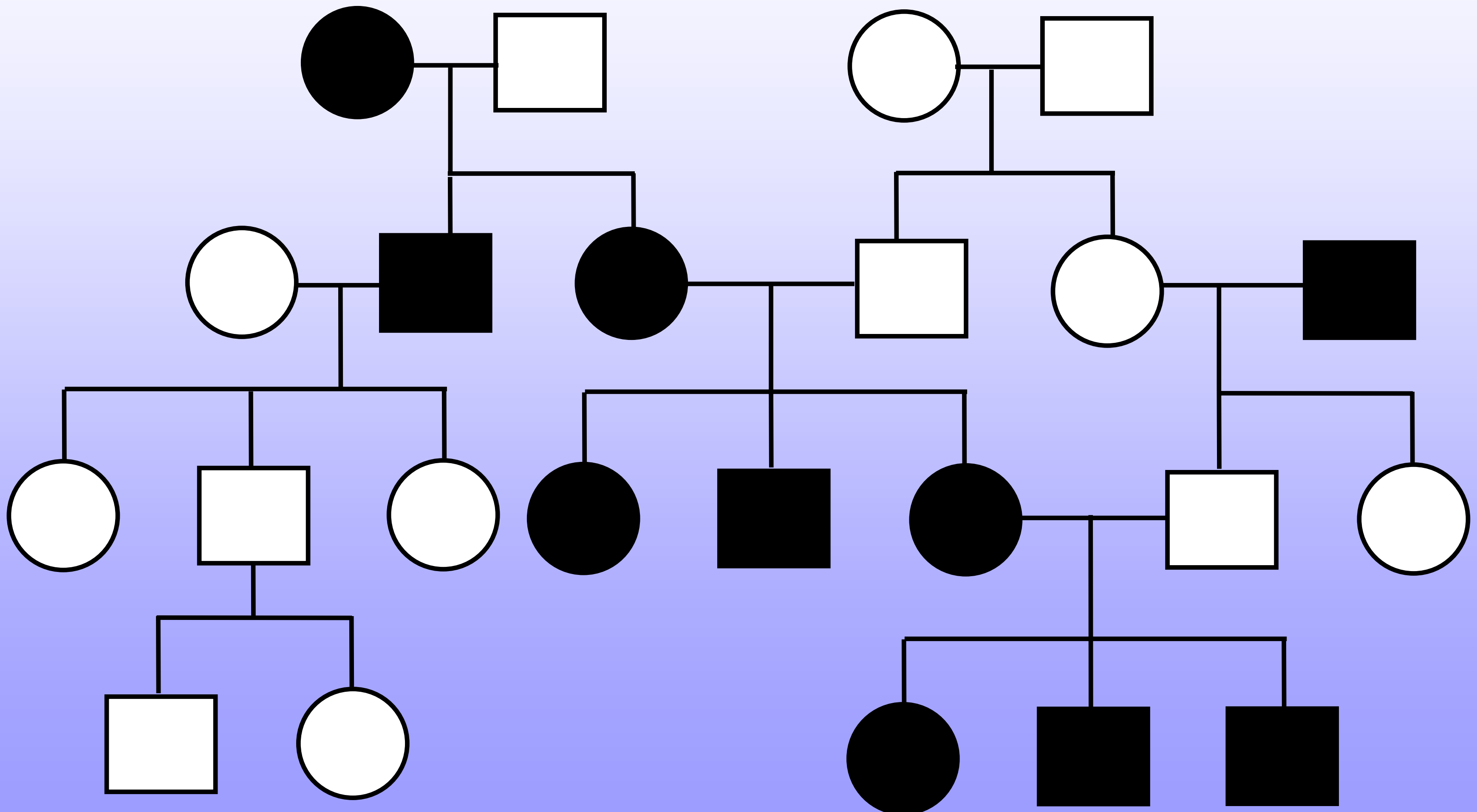
Lineage Markers

- Lineage markers are passed down from generation to generation without changing
  - Except for rare mutation events
- They can help determine the lineage (family tree) of an individual
- Y Chromosome Markers
  - Determine Paternal Lineage
- Mitochondrial Markers
  - Determine Maternal Lineage

Maternal Inheritance of mtDNA

- Mitochondria are in cytoplasm – providing energy for the cell
- All cytoplasm comes from the egg or ovum
- Sperm donates only nucleus to zygote
- Therefore, all mitochondria are inherited from mother only
  - No recombination
  - No paternal contribution

Maternal Lineage



Lineage Markers

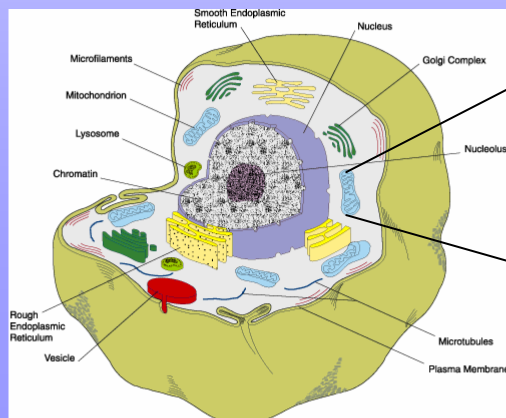
- Great for genealogy or tracing evolution
- However, the fact that these markers do not recombine is a disadvantage for Forensics
- Cannot use the product rule when determine the probability of an ID match
- Cannot separate direct relatives apart:
  - mtDNA Profile could be anyone in family who shares a maternal relative

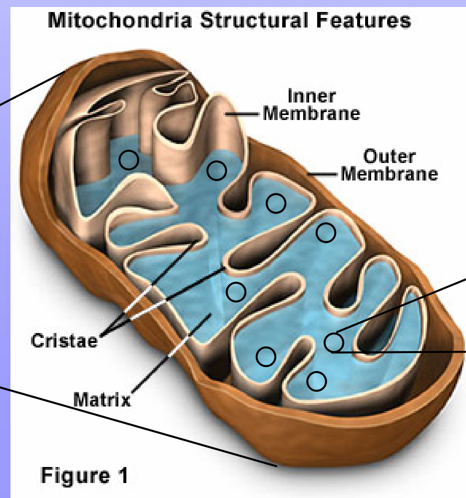
Why mtDNA?

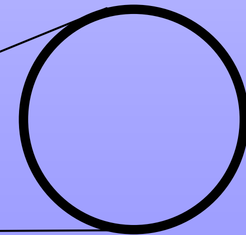
- Although nuclear DNA is more powerful
- mtDNA is more stable over time/conditions
- Why is that?
  - mtDNA is present in many copies
  - mtDNA exists within a double membrane organelle
- Can get more DNA – if sample is limited
- Can get DNA from highly degraded source

What is Mitochondria?

- Energy Power Plant of the Cell
- Organelle – vital for Cellular Respiration
- Many copies within one cell
- Only organelle with it's own DNA
  - Many copies of mtDNA genome within one organelle







Circular mtDNA

Mitochondrial DNA

- Contains 37 genes
- Two main regions:
  – Non-coding region – control's mtDNA
  – Coding region – contains 37 genes
- Gene categories:
  – 22 tRNAs (translation RNA)
  – 2 rRNAs (ribosomal RNA – translation)
  – 13 genes used in cellular energy production

Mitochondrial DNA

- Heavy vs. Light strands
  - Heavy greater number of guanines
  - Contains most of genes (28 out of 37)
- Tightly packed genome
  - No introns
  - Only 55 nucleotides not being used
- Two hypervariable regions
  - Within D-loop
  - HV1 and HV2

mtDNA vs. Nuclear DNA

Nuclear

- 3.2 Billion bps
- 2 copies per cell
- Linear
- Inherited from father and mother
- Diploid genome
- Recombination
- Unique

mtDNA

- 17,000 bps
- Hundred's copies
- Circular
- Inherited from mother only
- Haploid genome
- No recombination
- Shared by everyone within maternal lineage

Human Genome

23 Pairs of Chromosomes + mtDNA

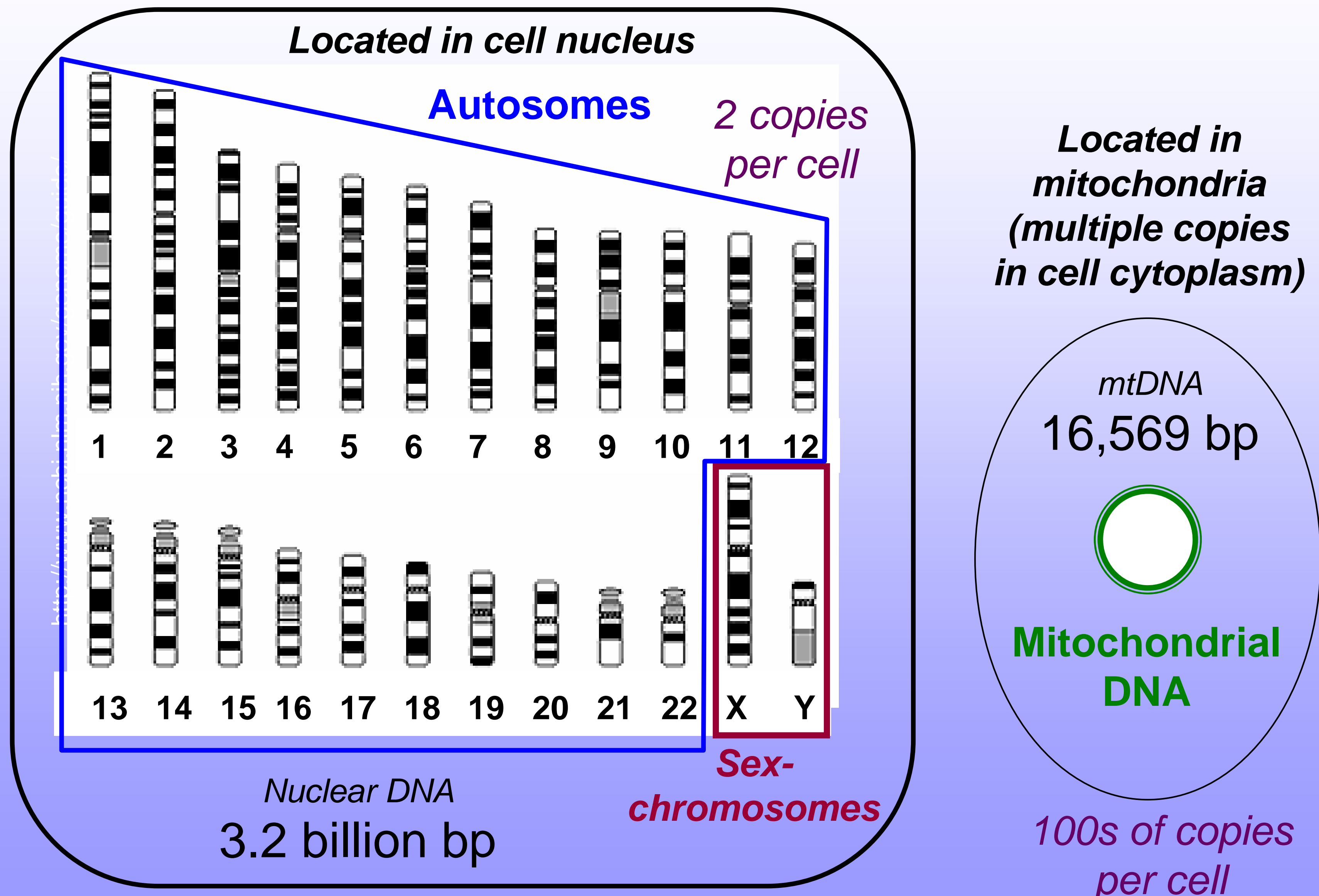


Figure 2.3, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Reference Sequence

- Human mtDNA has been entirely sequenced:
  - First in 1981 (Anderson et al)
  - Then confirmed in 1999 (Andrews et al)
- Cambridge Reference Sequence (CRS)
- Now use the revised version (rCRS)
- All other mtDNA is compared to the reference sequence

Differences from Nuclear DNA

- mtDNA has different Genetic Code
  - Which codons encode which amino acids
  - Small number of differences
- Fewer repair mechanisms
  - mtDNA has much higher mutation rate (10 X)
- Circular genome
  - Part of why mtDNA is more stable over time
- Increased number of genomes
  - 10's of mito./cell and 100's of genomes/mito.

Applications of mtDNA

- Forensics of severely degraded samples
- Medical studies of mitochondrial diseases
- Evolutionary studies of humans
- Genealogical studies
- Real life examples:
  - Tsar Nicholas II's remains ID'd
  - Princess Anastasia proved to be false claim
  - Lacy Peterson's body ID'd

Genotyping mtDNA

First:

- Used restriction enzymes to ID RFLP differences
- Began with 6 – eventually 12 enzymes

Now

- Sequence hypervariable regions and examine sequence differences
- Agreed upon regions are compared

Hypervariable Regions

- D - loop or Control Region
- Contains two regions with a lot of variation among different individuals
- These regions are amplified with PCR and then sequenced
- HV1 – 342 bps
- HV2 – 268 bps
- Profile is determined by differences in sequence from the CRS

Genotyping by Sequencing

- Rather than genotyping STRs or SNPs
- mtDNA profile is determined by sequencing both hypervariable regions
- mtDNA is a haploid genome
- Determining the mitochondria's haplotype

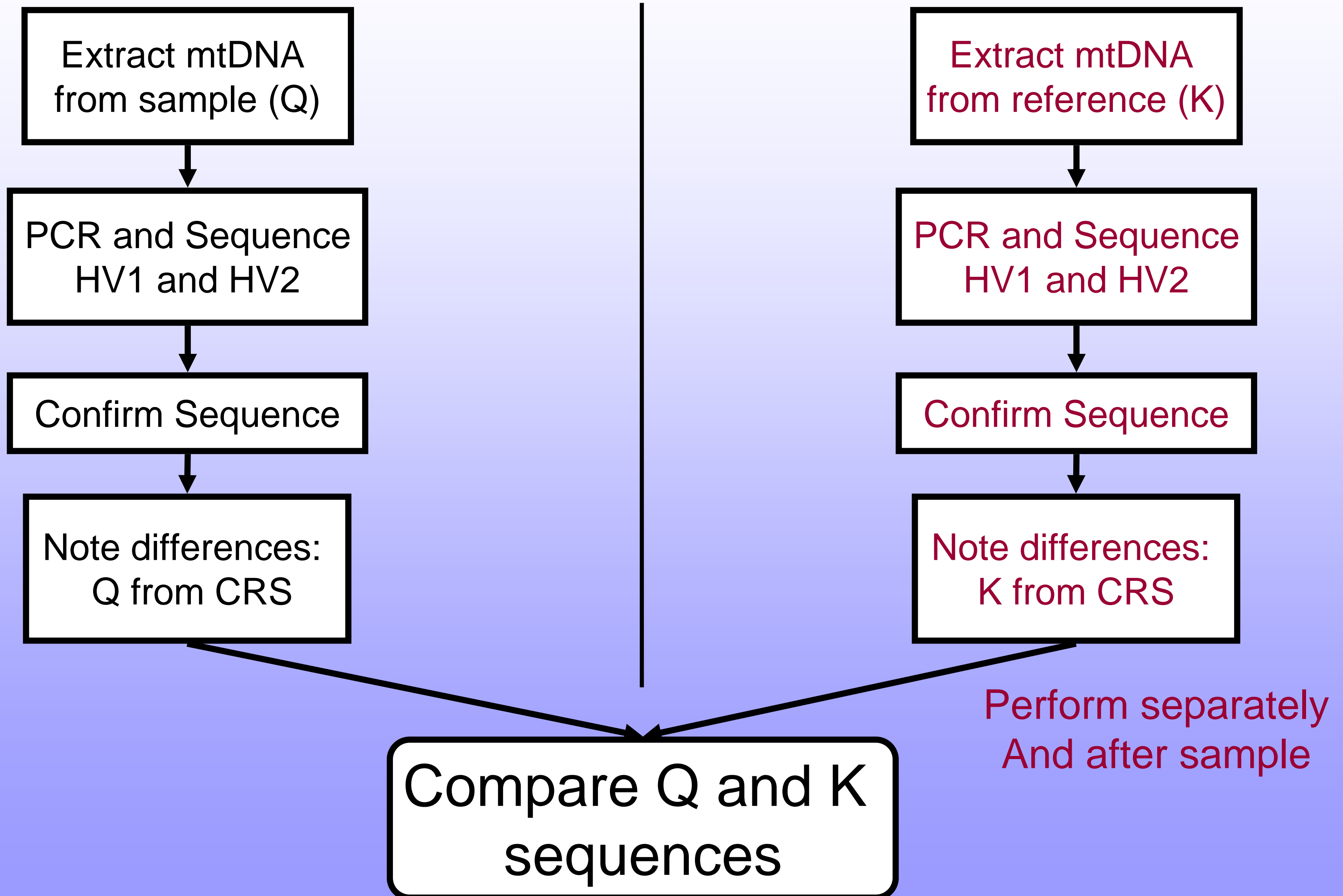
Screening Genotypes

- Before time is taken to sequence both regions entirely
- Many labs begin with a few screening genotypes first
- Screening:
  - Sequence specific oligo probes
  - Mini-sequencing
  - Cut with restriction enzymes
  - Others

Clean Laboratory

- PCR is done with more cycles
- mtDNA is already amplified due to multiple copies per cell
- Sample is already heavily degraded and rare (otherwise wouldn’t be using mtDNA)
- Therefore it is extra important that all procedures are done in very clean lab
- Limit or stop contamination

Sequencing Methods



Sample Extraction

- Typically dealing with materials with little DNA:
  - Teeth, hair, dry bone, etc
- Sample is extremely degraded
  - Very fragile
- Must be handled with extreme caution
- Usually tissue is ground up and then DNA extracted via Organic Extraction
- Also worry about PCR inhibitors

Microscopic Comparisons of Hair

- Must be done before DNA is extracted
- Hair (and fibers) can be compared under microscope
- Look for similarities between samples
  - Color, shape, texture, internal components
- May be able to identify whether hair is from suspect, victim or someone unrelated to crime scene

mtDNA Quantity

- mtDNA must be quantified just like nuclear DNA – before PCR can begin
- Use same methods as nuclear DNA:
  - Real time PCR
  - Blots, etc
- Some laboratories will quantify the amount of nuclear DNA and then use a rough formula to estimate amount of mtDNA that should be expected

PCR Protocol for mtDNA

- Normal nuclear DNA PCR uses around 30 cycles
- mtDNA usually uses 34 to 40 cycles
  - Or even more!
- Excessive Taq Polymerase might be added
- More of PCR product may be used for sequence than normal protocols

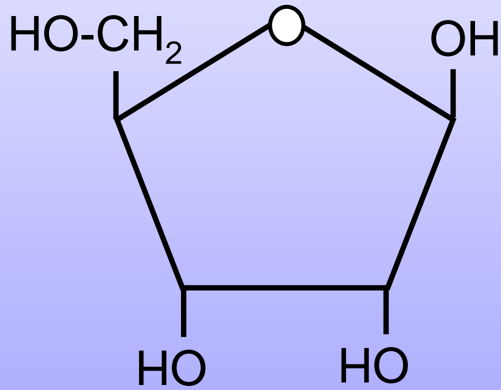
Sanger Sequencing Method

1977

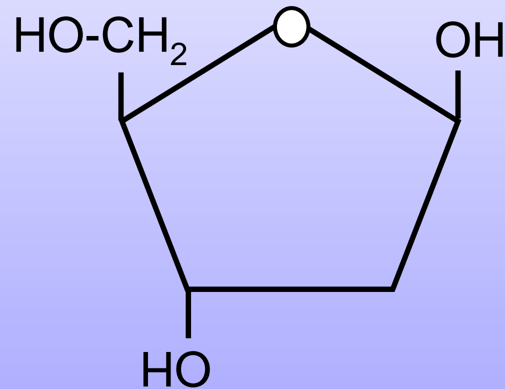
- Fredrick Sanger
- Dideoxy sequencing
- Dideoxys are nucleotides that contain no free oxygen at all
  - These nucleotides cannot form chains
  - Polymerase stops copying DNA's sequence when it adds a dideoxy base
  - Make each nucleotide with dideoxy sugar

Sanger Method

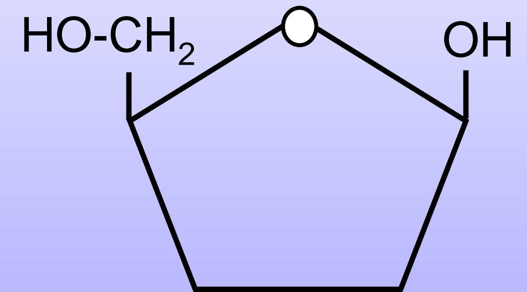
- Dideoxy nucleotides:



ribose



deoxyribose



dideoxyribose

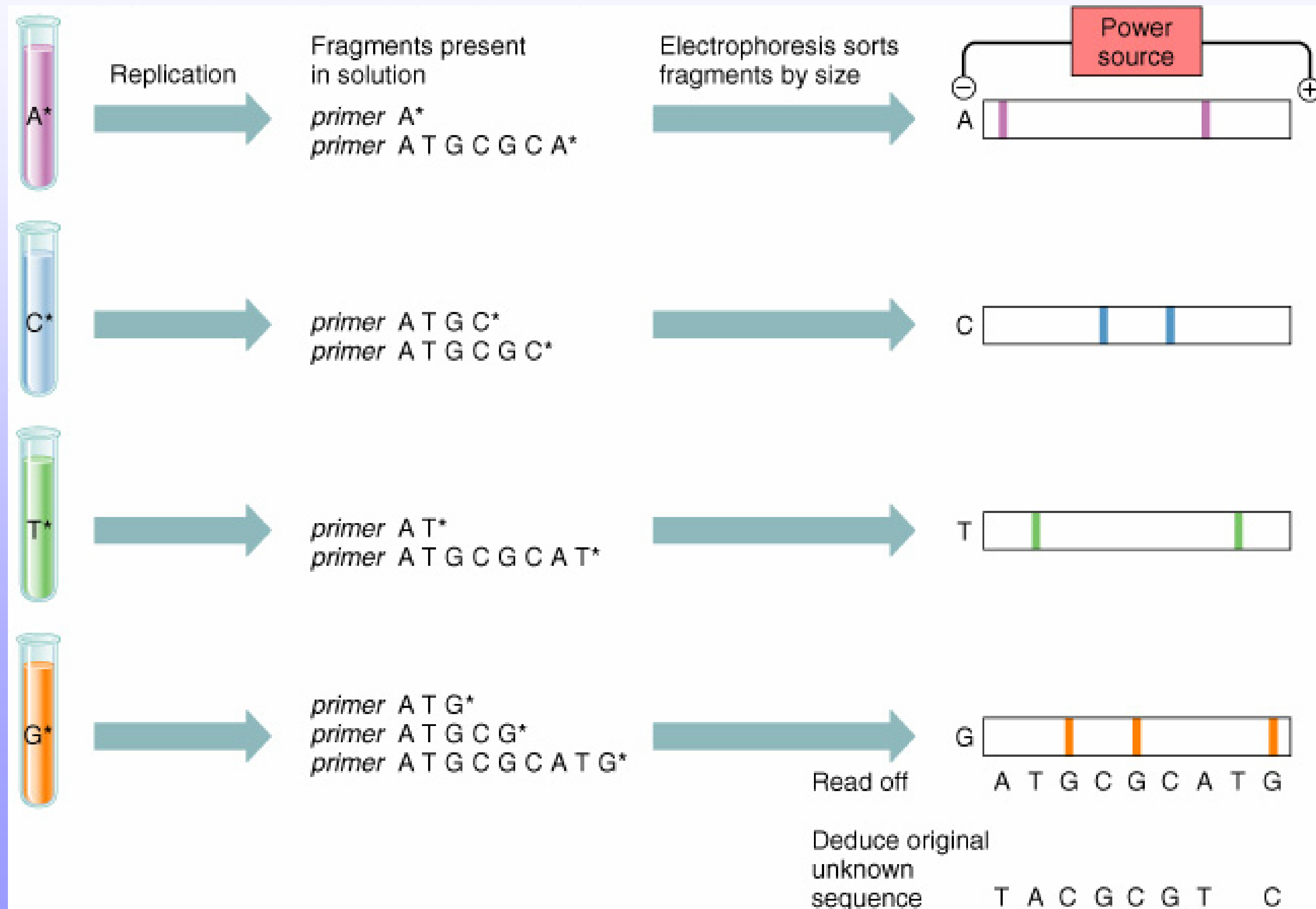
Sanger Method

- In four tubes add:
  - DNA of unknown sequence
  - Everything necessary for DNA replication
  - One of each dideoxy nucleotide
- Each tube has a different dideoxy nucleotide in it (A, C, G or T)
- DNA polymerase will stop working once it adds a dideoxy base
- Therefore, get different lengths of copied DNA

Sanger Method

- Run each separate tube in it’s own lane on a gel
  - “A” lane, the “T” lane, etc
- Important – some regular nucleotide is also added so that sequence can continue past some of the time
- Fragments of different lengths
- Read the four lanes to determine sequence of complete DNA fragment

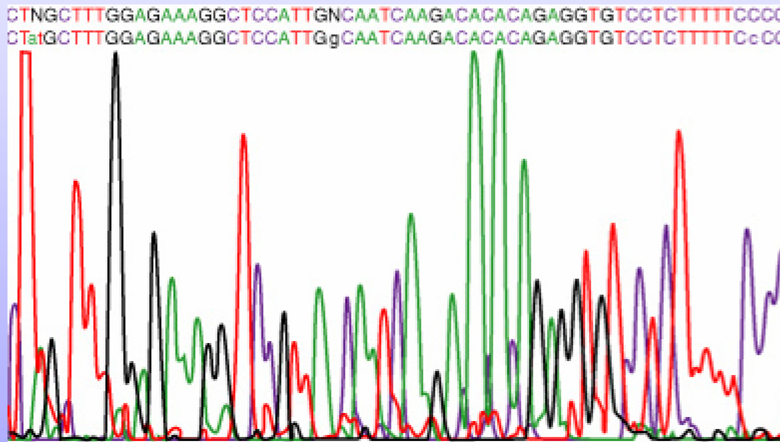
Sanger Method

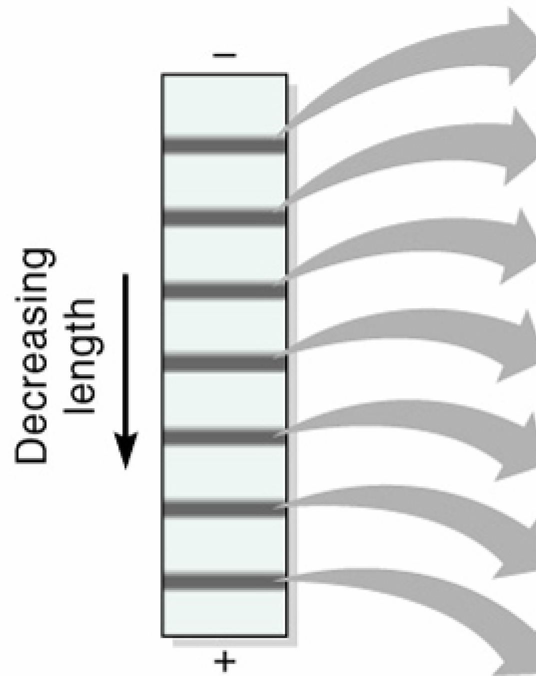


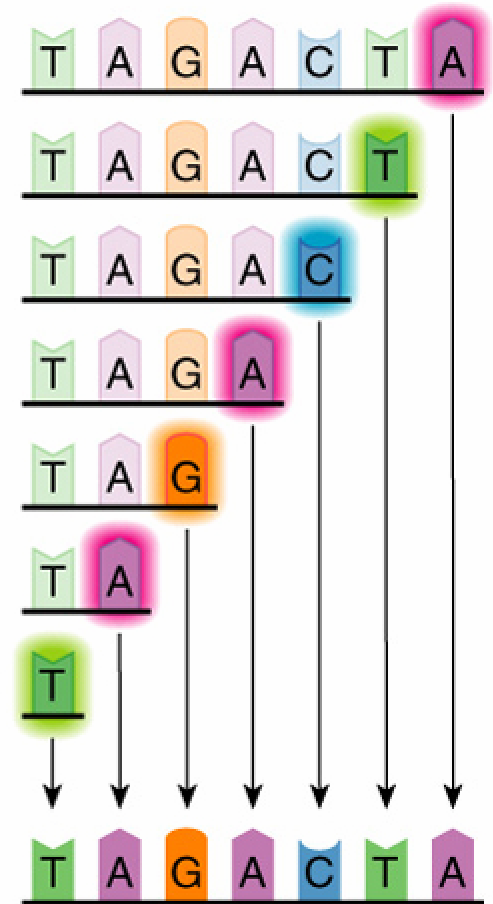
Modern Sequencing

- Added florescent dyes:
  - A is red
  - C is blue
  - T is green
  - G is yellow
- Now can run all four tubes in same lane
- Automate entire process:
  - Invented an automated sequencer and a computer program to read the gels

Modern Sequencing

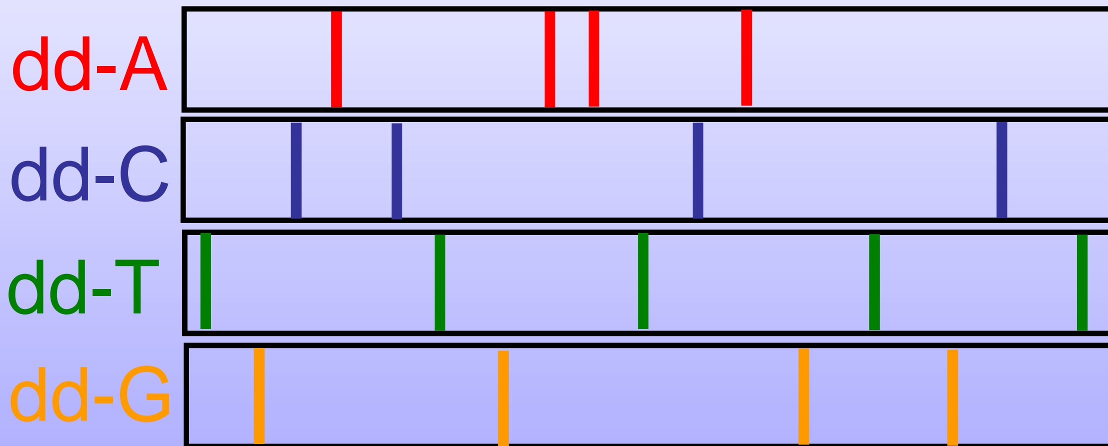






Reading Sequence off Gel

- Determine the sequence from this gel:



TGCACTGAATCAGTGCT Direct Read

ACGTGACTTAGTCACGA Actual Sequence

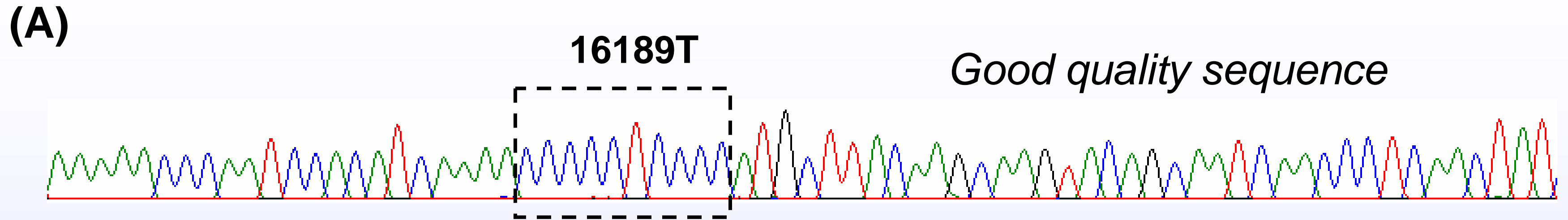
Building sequences

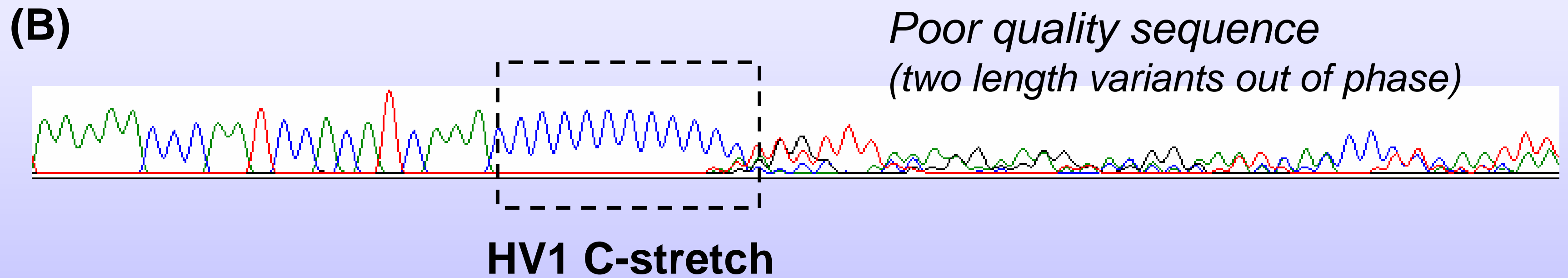
- Based on overlapping fragments

FEELING WE'RE NOT
TOTO, I HAVE A FEE
ELING WE'RE NOT IN KANSAS ANYMORE
A FEELING
AVE A FEELING WE'RE NOT IN KANSAS A
ING WE'R
'RE NOT IN KANSAS ANYMORE
ELING WE'RE NOT IN KANS
I HAVE A FEELING WE
FEELING WE'R
NG WE'RE NOT IN KANSAS ANYMO
TOTO, I HAVE A FEELING WE'RE NOT IN KANSAS ANYMORE

C – Stretches

- String of cytosine’s in a row
  - 8 to 14, or more
- Exist in both hypervariable regions
- Sequencing after the C-Stretch is very difficult
- Causes strand slippage for DNA polymerase
- Becomes “out of phase” between two strands – sequences cannot be aligned
- Need to re-sequence this region





(C) Primer strategies typically used with C-stretch containing samples

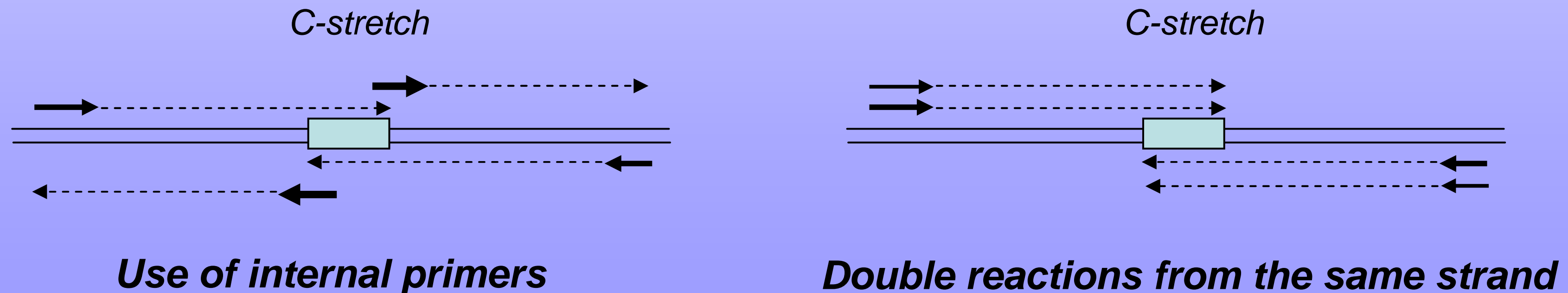


Figure 10.7, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Mini-primers

- For highly degraded mtDNA use mini-primers just like for nuclear DNA
- Amplify small portions of the HV1 and HV2
- Then sequence all pieces
- Try to align to get entire sequence
- This approach was used successfully to recover a DNA profile from Neanderthal bones that were hundreds of thousands of years old

Any Questions?

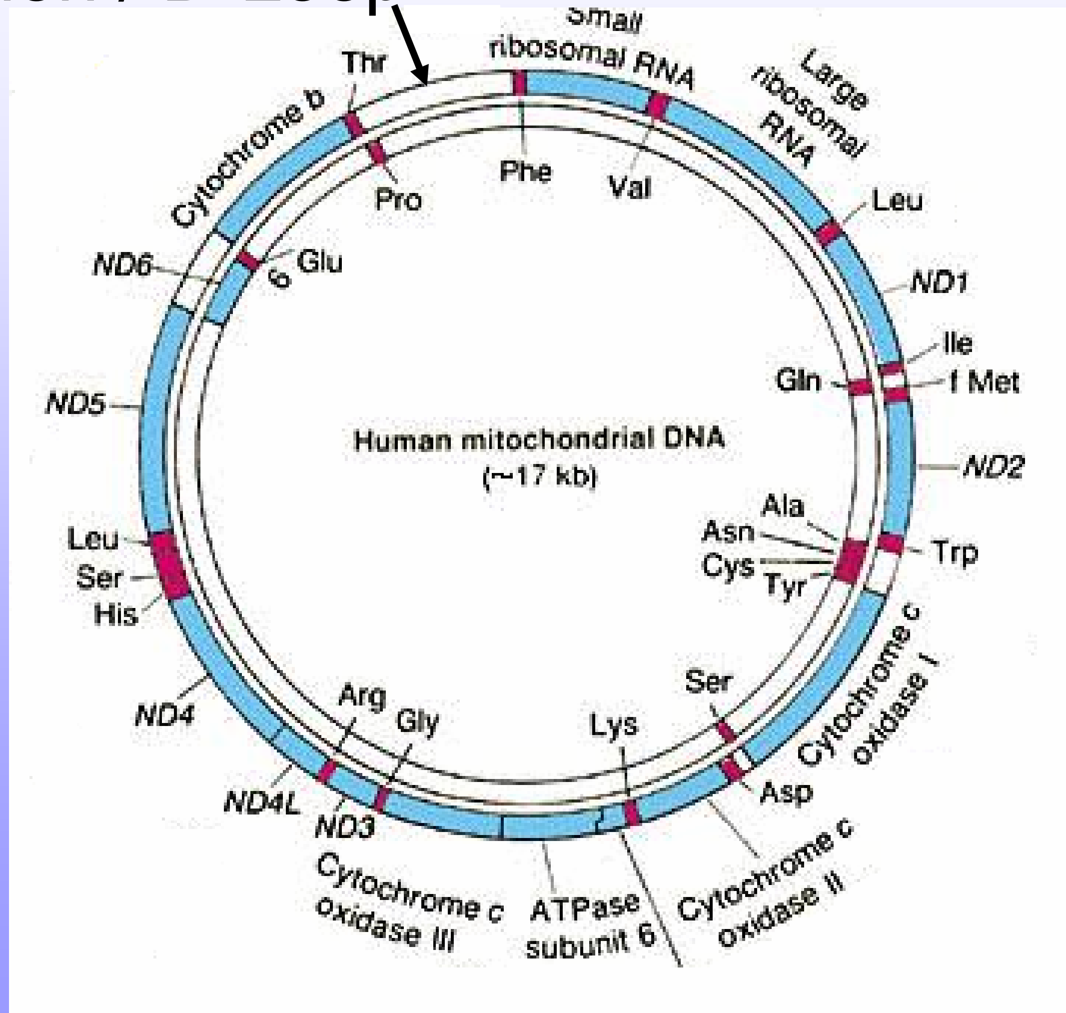
President's Day Monday

Finish mtDNA next class

Read Chapter 11

Structure of mtDNA

Control Region / D-Loop



Controls

- Negative Control
  - Same master mix but with water or buffer
  - No DNA sample
  - Proves PCR reagents are not contaminated
- Positive Control
  - Master mix plus DNA of known concentration
  - Proves PCR reaction/conditions work
- Extraction Blanks
  - Controls for DNA extraction reagents/methods