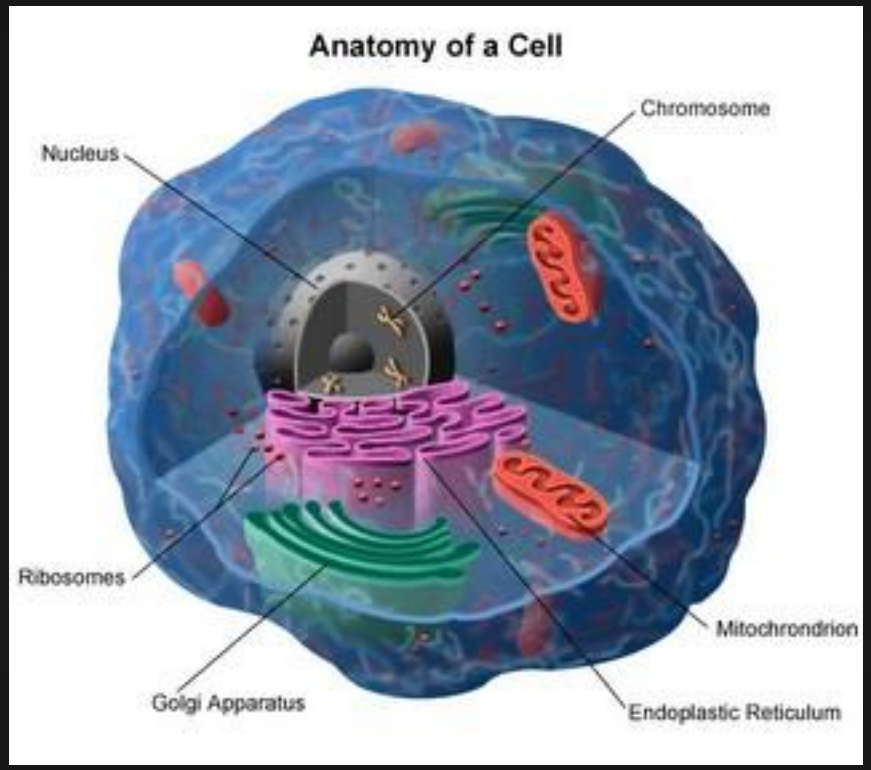
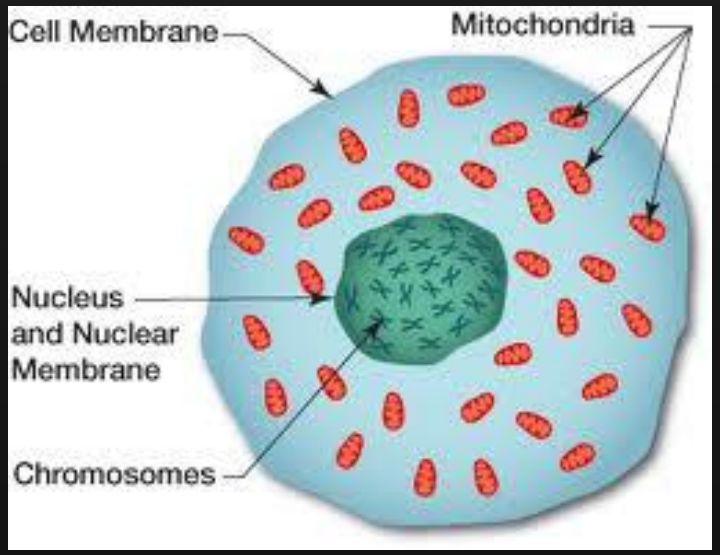
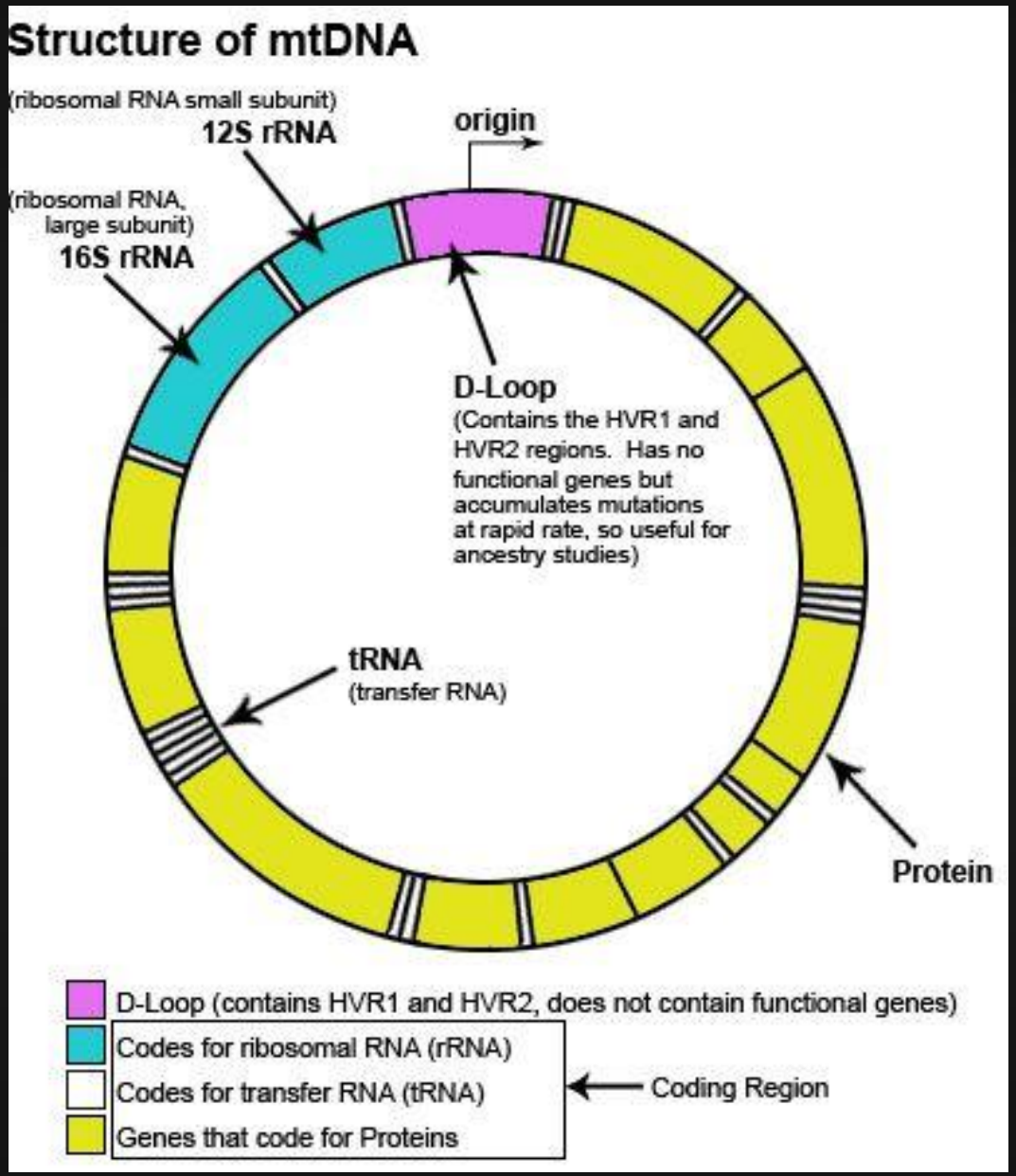
Lab Activity - PCR of Mitochondrial DNA

Mitochondria



- Organelles responsible for supplying energy to cell
- Have their own DNA, separate from that in the cell nucleus





D-Loop

- Also known as the control region
- Origin of replication
- Non-coding so accumulates mutations at a rapid rate

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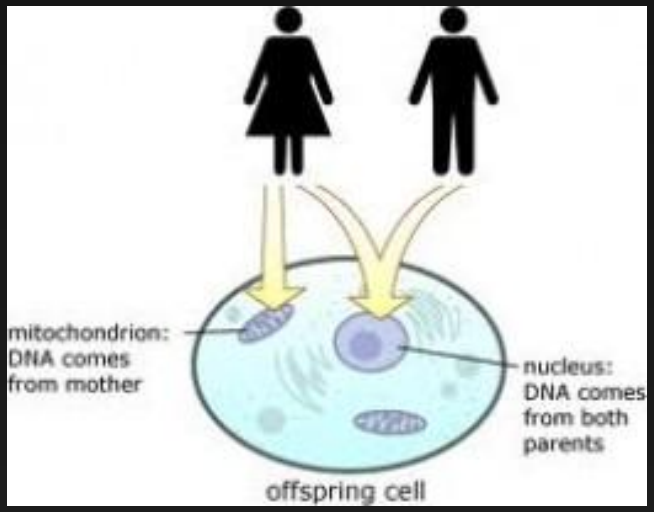
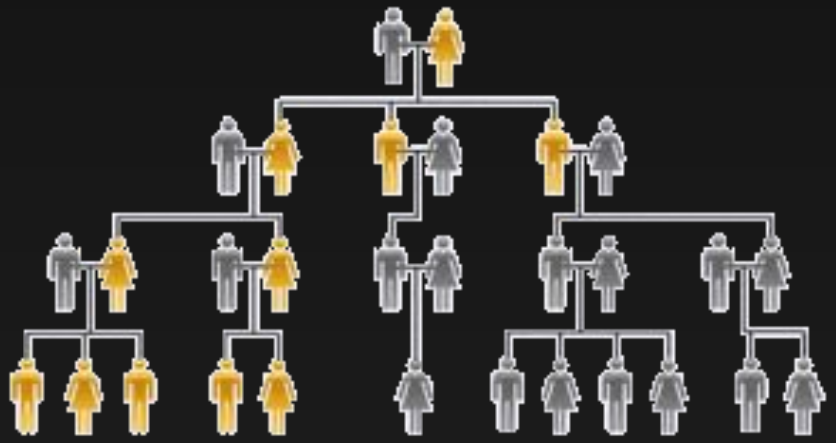
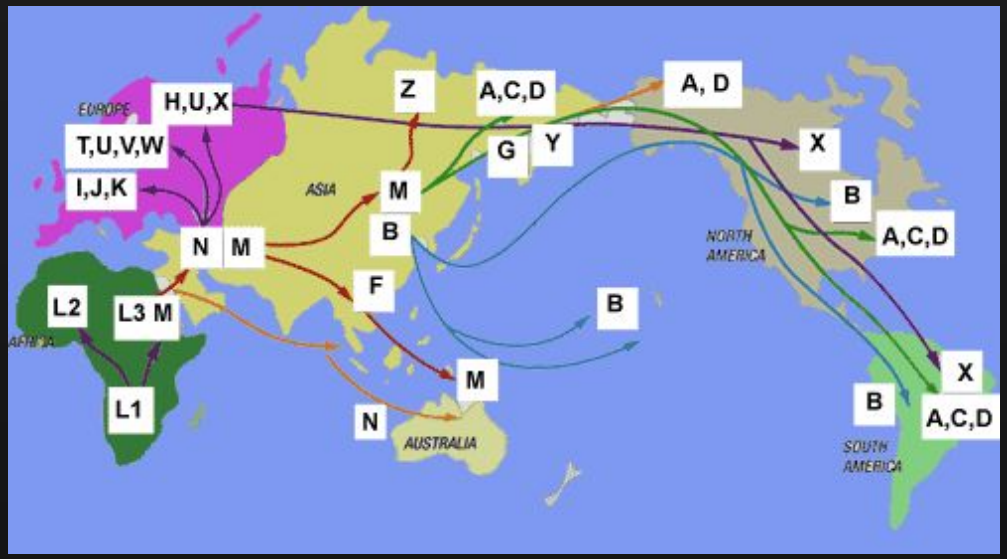
when a region is non-coding, mutations often have no effect on phenotype so they are not favored or selected against

Matrilineal Ancestry





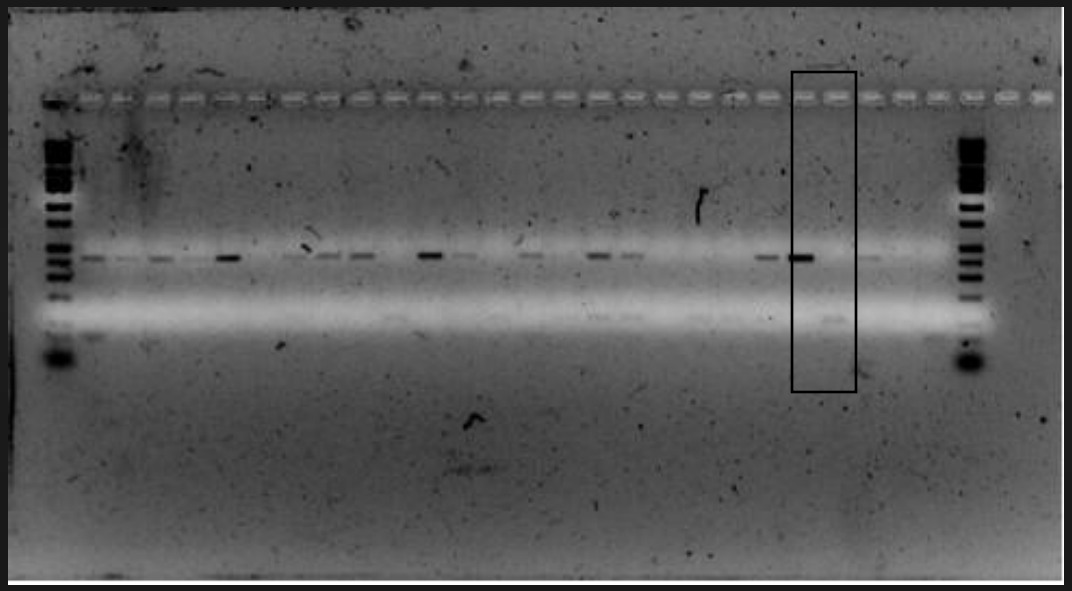
- Mother contributes egg cell, along with mitochondria



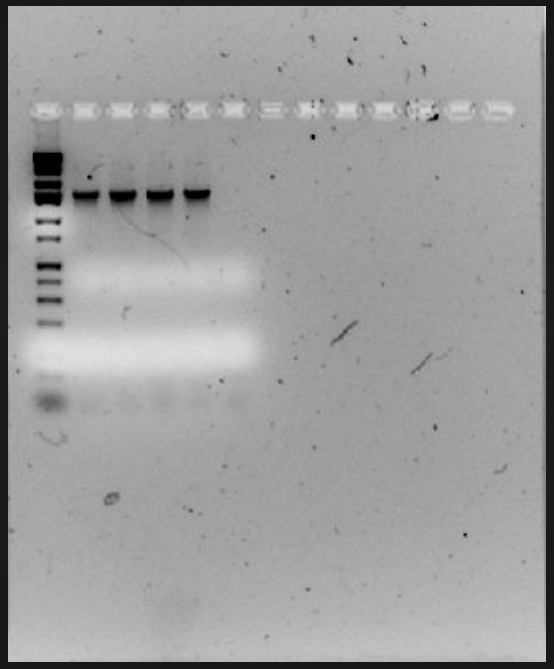
- Mono-allelic inheritance pattern and the fact that it accumulates mutations rapidly makes it less complicated to use in some cases for tracing migration and geographical patterns

Analysis of D-loop Data

- D-loop roughly the same size in all individuals, but sequence can be different…compare sequence to understand relationships



- Nuclear DNA (TPA-25) - one allele inherited from mom, one from dad, so look at genotype (can distinguish alleles based on size differences)



- Mitochondrial DNA - one "allele" only from mom, no size differences (most of the time)

Making a PCR Master Mix

1. Determine the number of reactions you will need in your Master Mix --> number of reactions you need to run, plus 2 extra

2 people in my group + 1 negative + 2 extra = 5

2. Use $C_1V_1 = C_2V_2$ to calculate volume of each reagent needed for ONE PCR reaction, with a final volume of 25 micro liters

C_1 = stock concentration

V_1 = unknown

C_2 = Final concentration per reaction

V_2 = 25 micro liters (volume of one PCR reaction

Making a PCR Master Mix

- Example:

Buffer --> $(5X)(V_1) = (1X)(25 \text{ micro liters})$

3. Add up all the volumes and add the amount of DNA you are going to be using (2 micro liters) --> subtract this total from 25 to get the volume of water in ONE PCR reaction

Buffer

$MgCl_2$

dNTPs

F primer

R primer

DNA volume (2 micro liters)

Add together and subtract from 25 (answer is volume of water)

Making a PCR Master Mix

4. You never run just ONE PCR reaction --> multiply the volumes of each reagent by the number of reactions you will be running

Example:

	Vol. in 1 reaction	Vol. in 5 reactions
Buffer	5 microliters	25 micro liters

* Check your math --> Add volume of all reagents in your Master Mix (after multiplication) and divide by the total number of reactions in your Master Mix