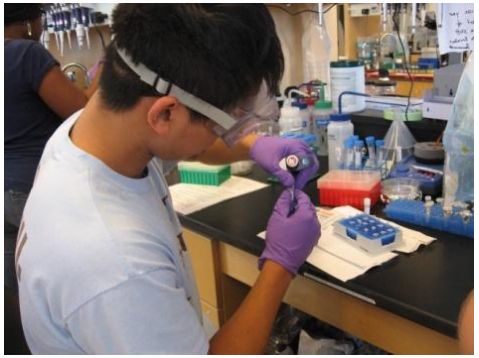
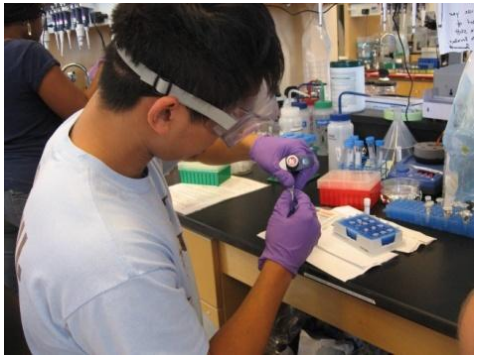


Research as a Goal of Education and Education as a Goal of Research

David R. Walt - HHMI Professor

Meredith Knight – Program Director





Research as a goal for education

Education as a goal for research

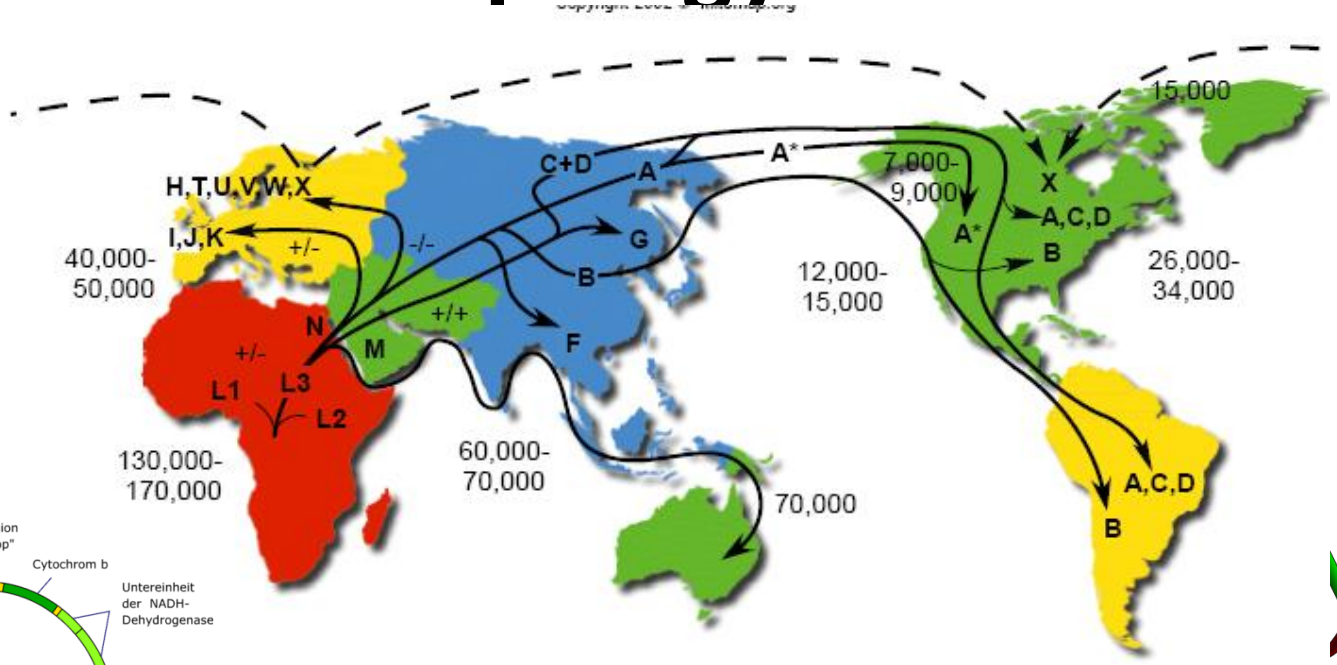
Outline

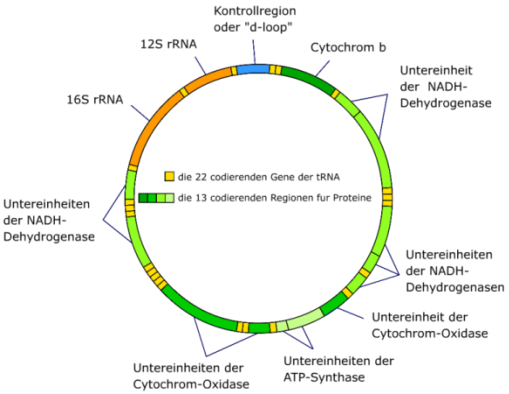
- DNA Analysis for High School students
- From the Big Bang to Humankind

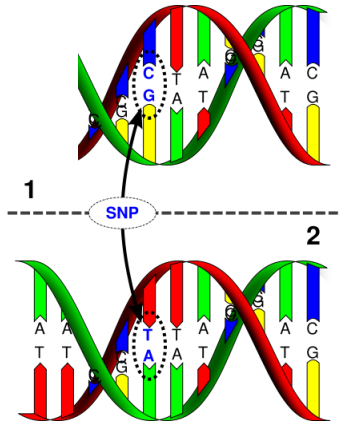
Outline

- **DNA Analysis for High School students**
- From the Big Bang to Humankind

Mitochondrial DNA: Where Chemistry, Biology and Anthropology Meet





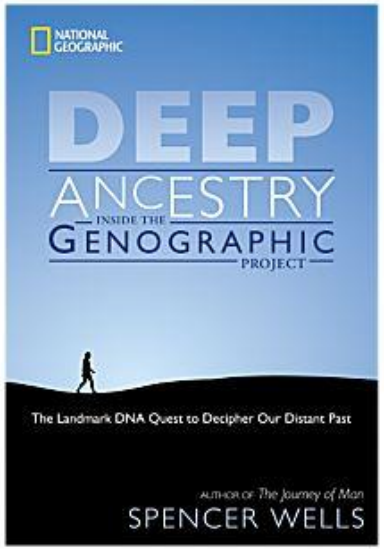


Concept – “From spit to SNP”

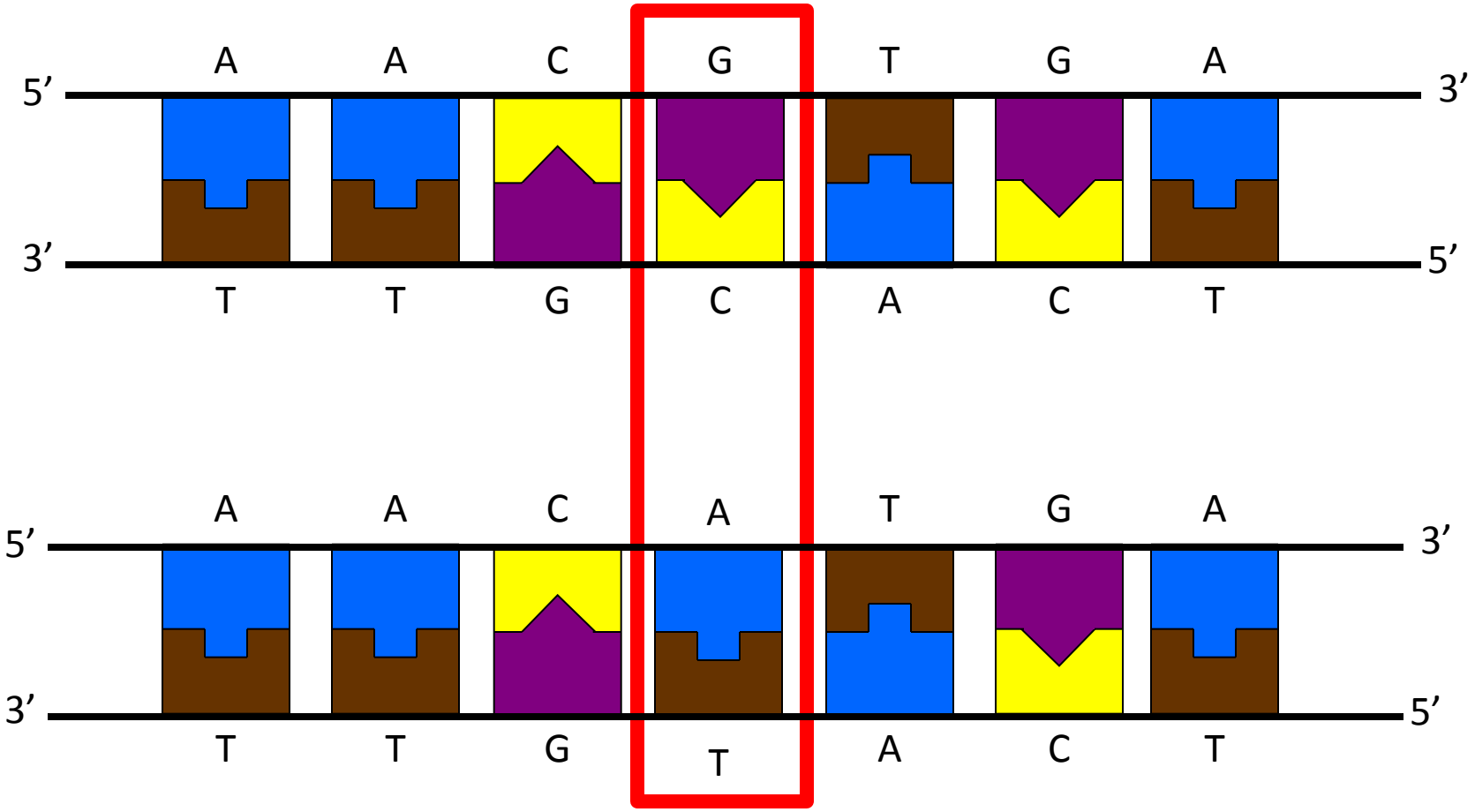
- Develop an experiment to allow high school and college students to determine their “deep ancestry” using the mtDNA in their saliva.

<u>Advantages</u>
Engaging for students
Real world application
Genetics

<u>Challenges</u>
Keeping cost low
Limited time
Limited equipment

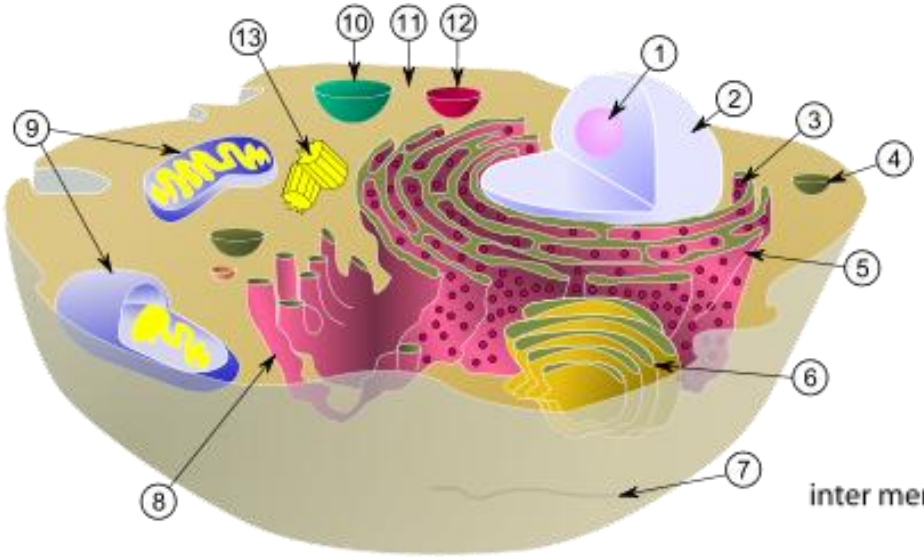


Single Nucleotide Polymorphsim



SNP

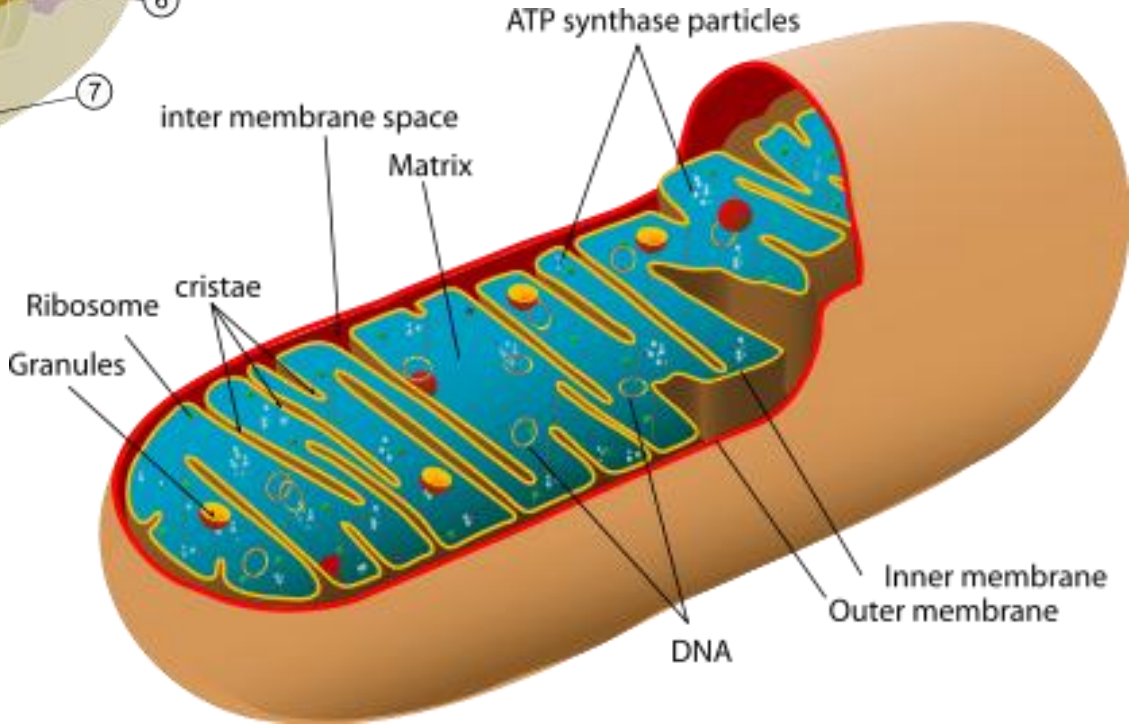
Science Background



Wikipedia - File:Biological_cell.svg

Mitochondria are in all cells

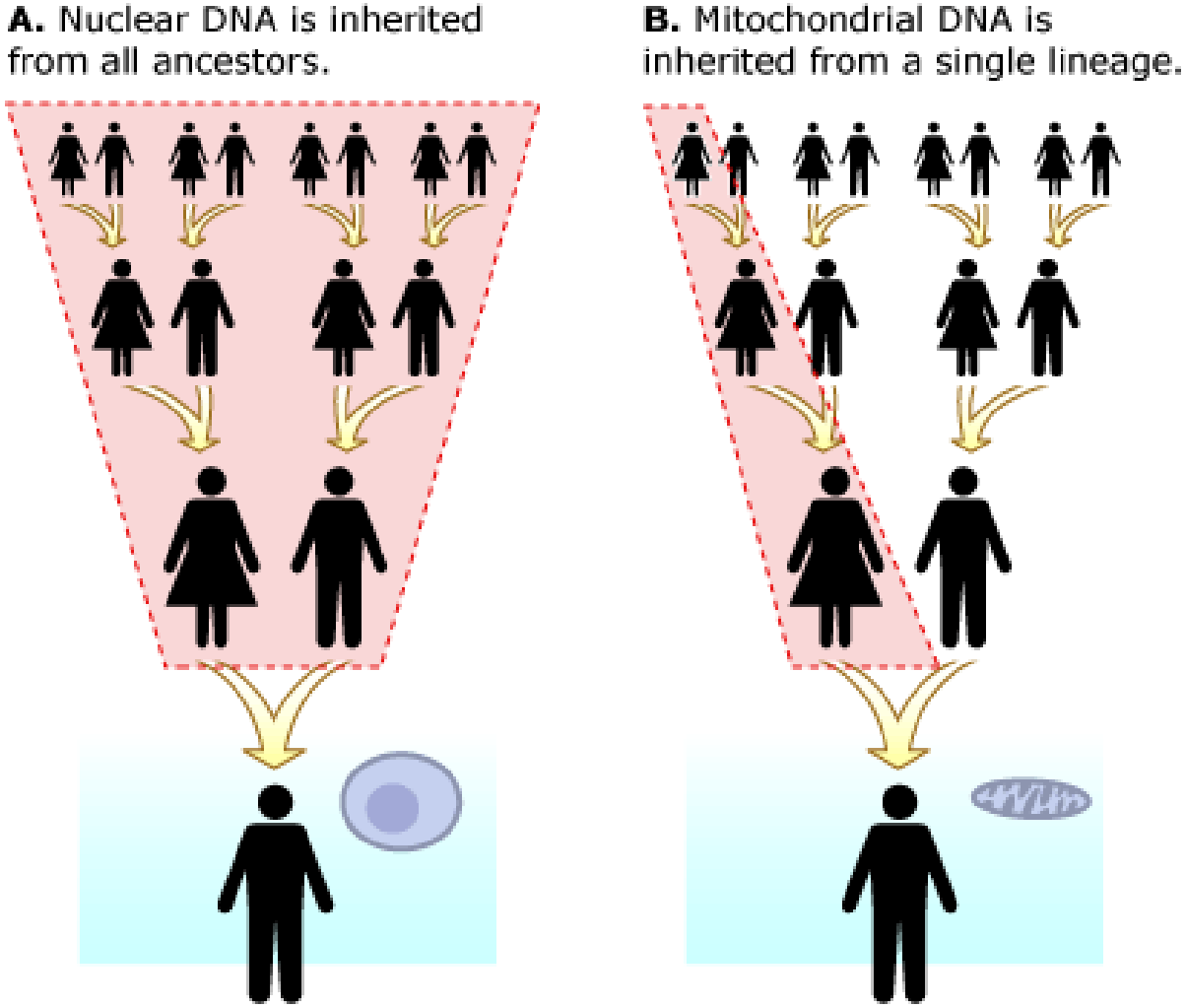
Mitochondria contain DNA



Wikipedia - Animal_mitochondrion_diagram_en_(edit).svg

Science Background

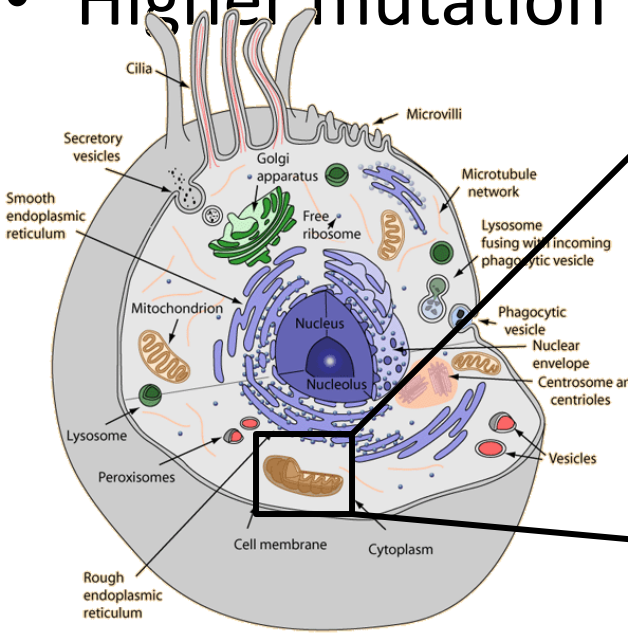
Mitochondrial DNA is only inherited from

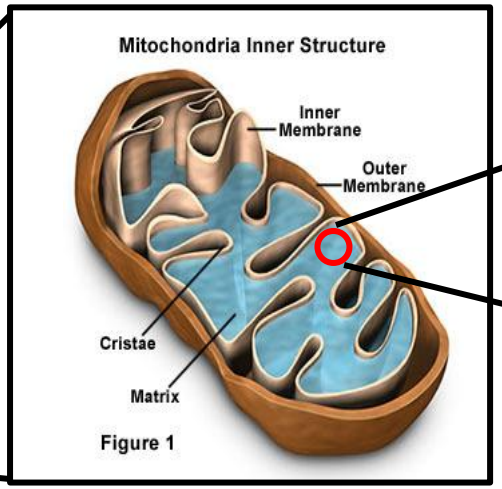


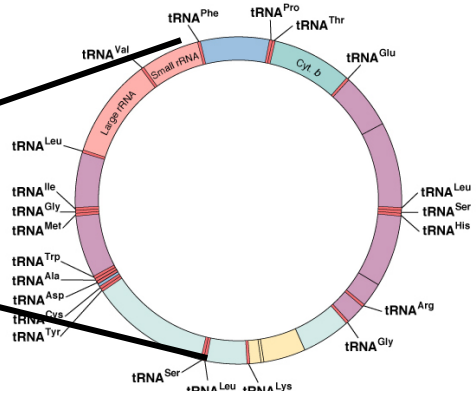
Mitochondrial DNA

- Maternally inherited
- Several mitochondria per cell
- 16,600 bp
  - Shorter than genomic DNA
- No recombination
- Higher mutation rate

- Obtain mtDNA from cheek cells through saliva collection







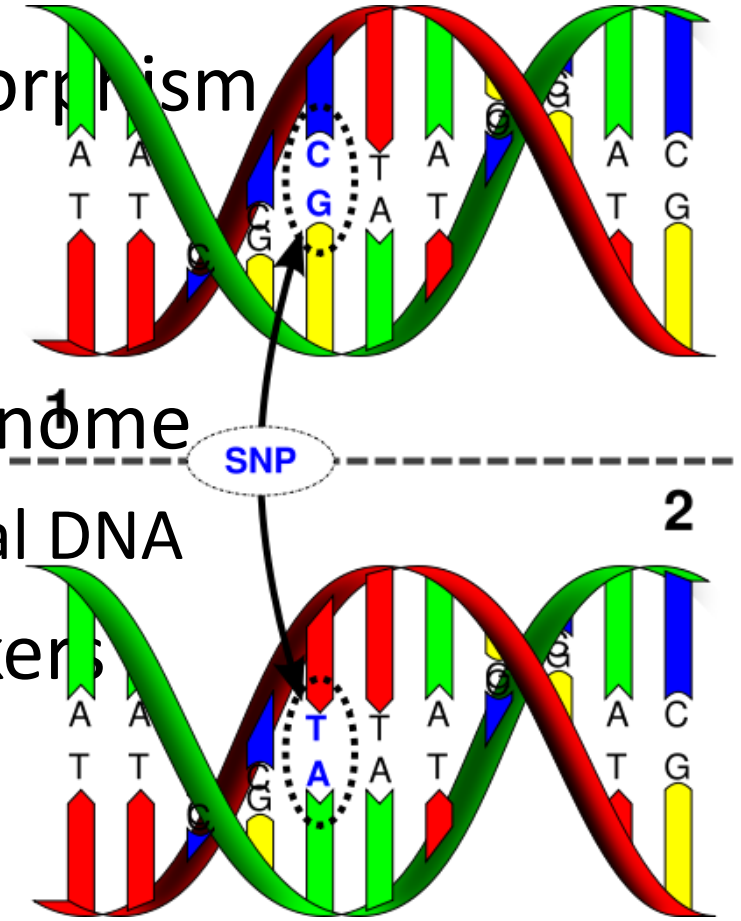
http://hyperphysics.phy-astr.gsu.edu/hbase/biology/cell.html

http://micro.magnet.fsu.edu/cells/mitochondria/mitochondria.html

Greentiger.com

Science Background - SNPs

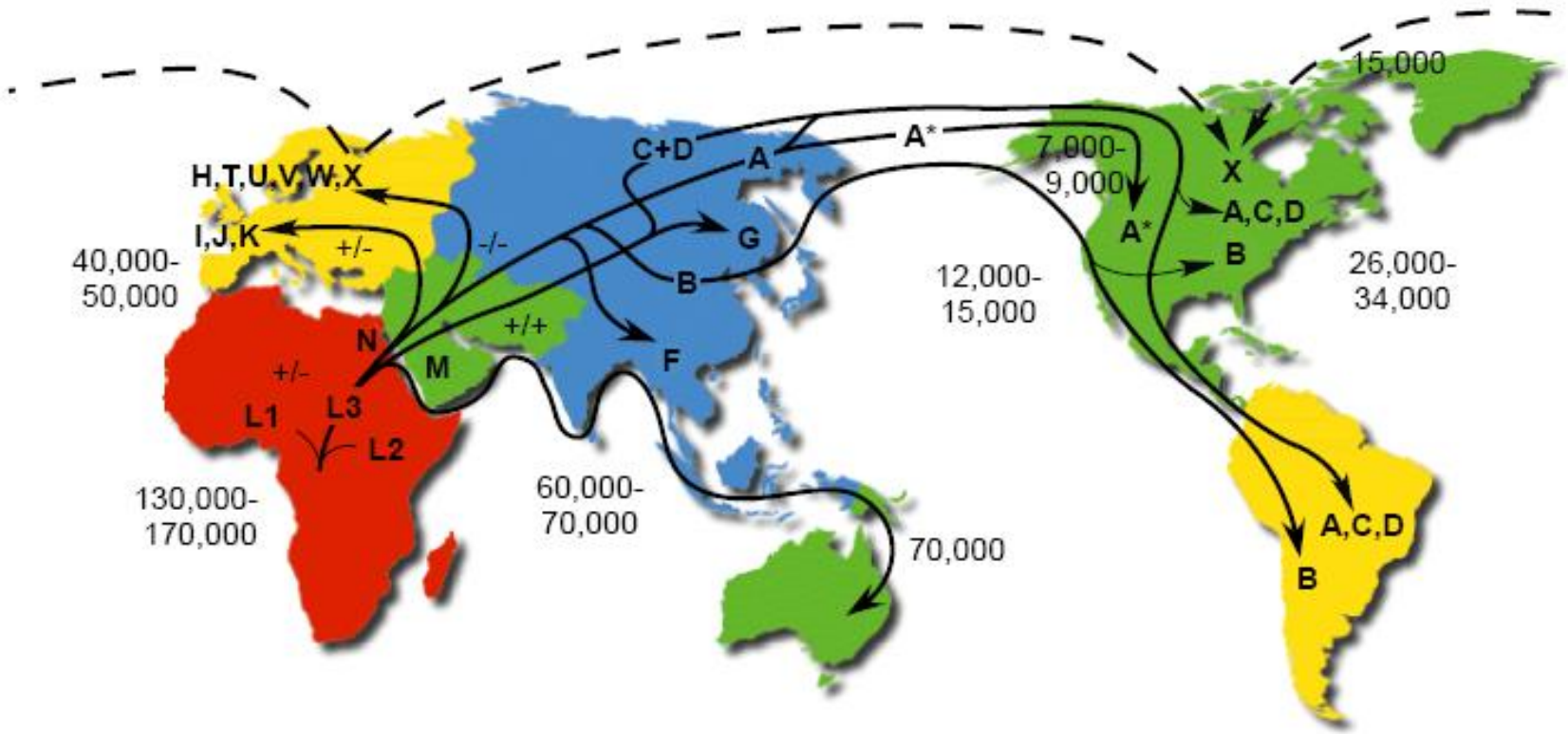
- Single Nucleotide Polymorphism
  - ACCTTGCGCGCTATA
  - ACCTTGGGCGCTATA
- Occur throughout the genome
  - Nuclear and mitochondrial DNA
- Are used as genetic markers



Wikipedia - Dna-SNP.svg

Human mtDNA Migrations

http://www.mitomap.org/mitomap/WorldMigrations.pdf



+/-, +/+, or -/- = Dde I 10394 / Alu I 10397

* = Rsa I 16329

Mutation rate = 2.2 - 2.9 % / MYR

Time estimates are YBP

L3

Positive For A (OUT of L3)

Positive For G (IN L3)

Positive For C (OUT of M)

L0 L1 L2-6

L5 L2'3'4'6

L2 L3'4'6

L6 L3'4

M

Positive For T (IN M)

D D4 M9 E M8 Z C M1 M7 M12'G

N*

Positive For C (OUT of N)

You are in Haplogroup L3

Positive For T (IN N)

A

Positive For G (IN A)

A3 A4 A5 A7 A9

Positive For A (OUT of A)

U

Positive For A (OUT of U)

Positive For G (IN U)

U1 U5 U6 U2'3'4'7'8'9

U3 U8 U7 U4'9

K

U4 U9

R0

Positive For A (OUT of RO)

Positive For G (IN RO)

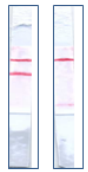
HV

HV0 H

V

At this point, you may fall under one of several Haplogroup categories: N1, N2, W, N9, R, S, X, P(PT), B, F, or JT

These correspond to Asia, Australia, North and South America, and certain parts of Europe



Positive Result on Left

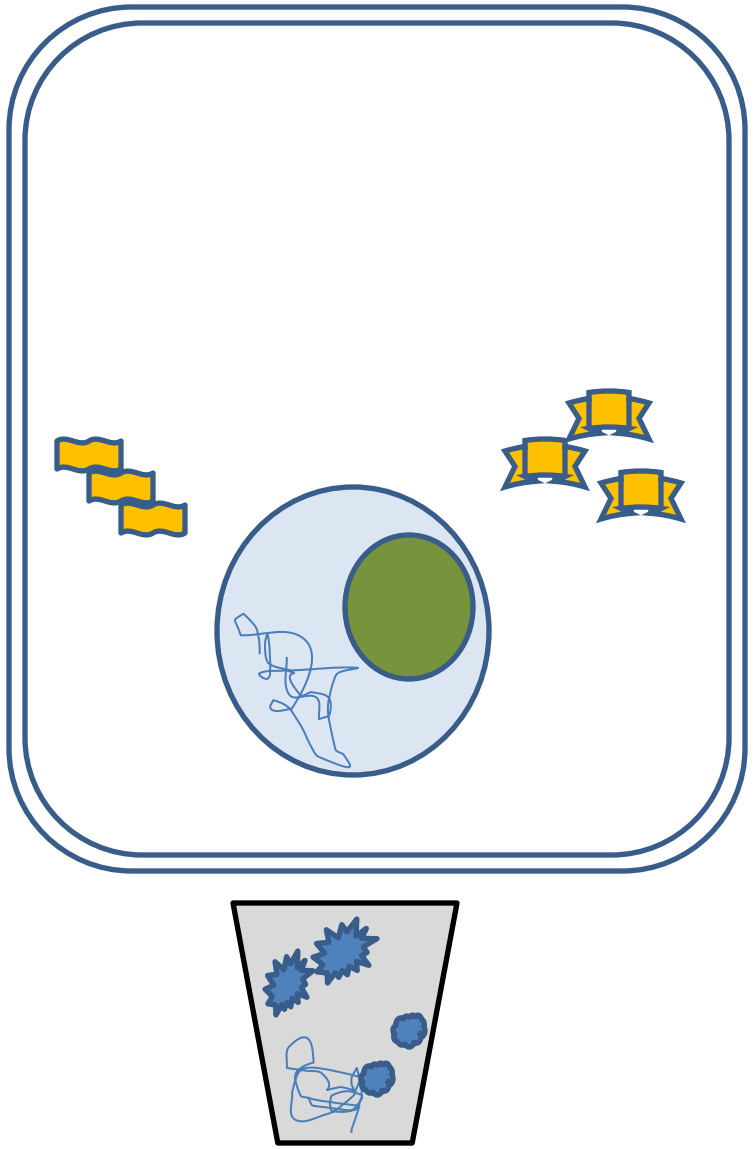
Negative Result on Right

*Note: If you have positive or negative results for both biosensors at this point, and there are further signals present, you may disregard your results for the N haplogroup and continue.

Step 1: Add lysis buffer

Key ingredients:

- SDS – breaks cell walls
- EDTA – deactivates enzymes in the cell
- Proteinase K –digests nucleases which would degrade DNA
- Spin – keep liquid



Step 2: Isolate the DNA

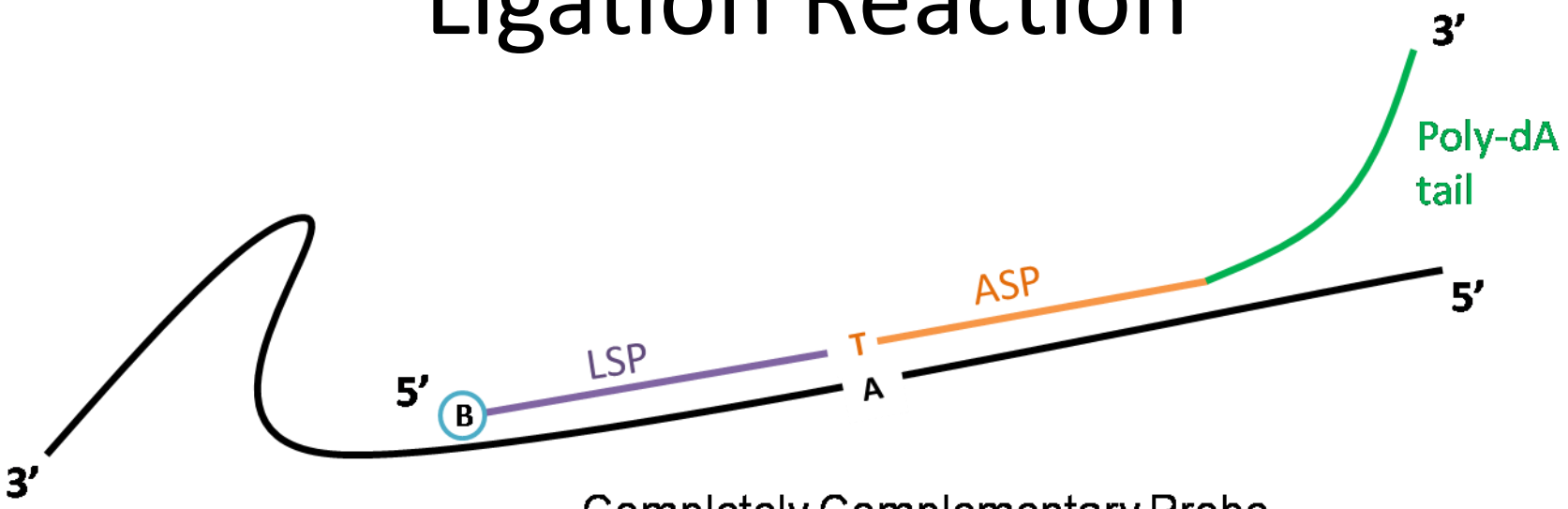
- NaCl (salt) neutralizes charge on DNA
- Add cold ethanol
- Centrifuge (spin)
- DNA forms white solid

Step 3: Make many copies - PCR

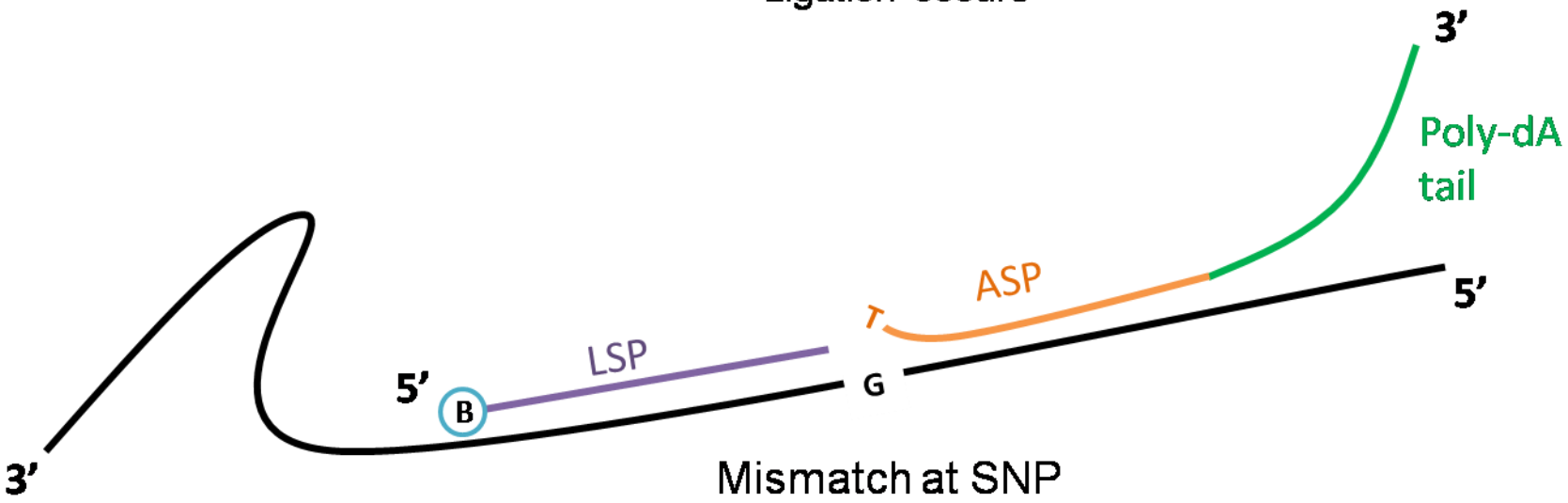
- Animation of PCR

  http://www.dnalc.org/view/15924-Making-many-copies-of-DNA.html

Ligation Reaction



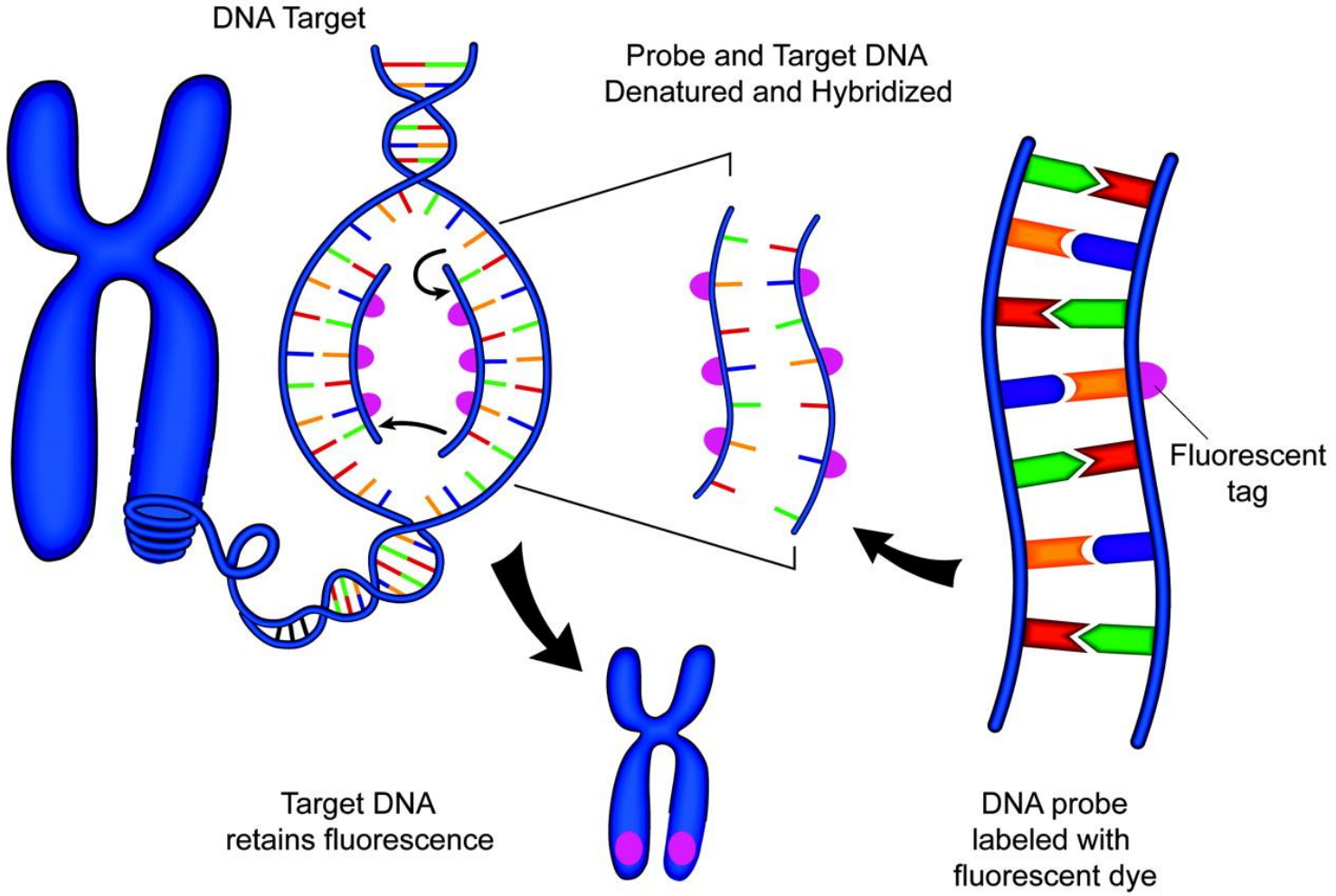
Completely Complementary Probe
Ligation occurs



Mismatch at SNP
No ligation occurs

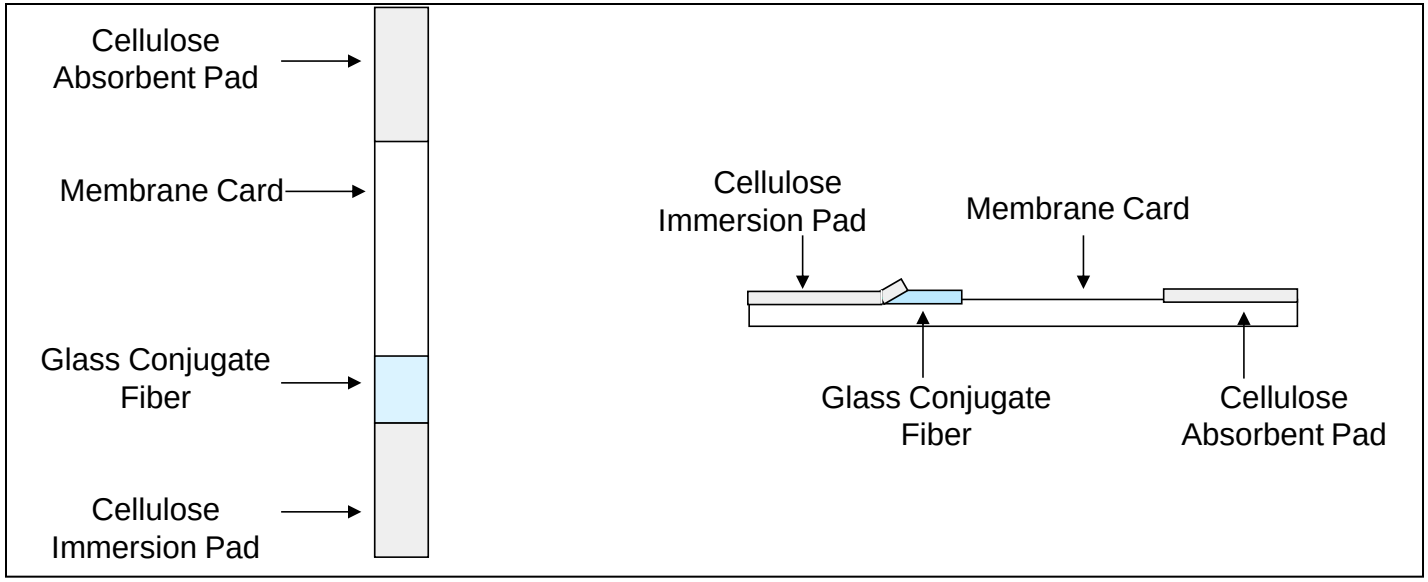
Step 4: Tag DNA with probes

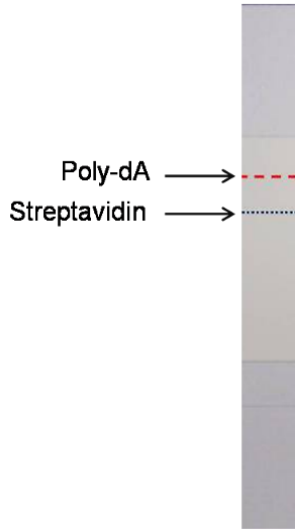
- Probes let you “see” what DNA you have



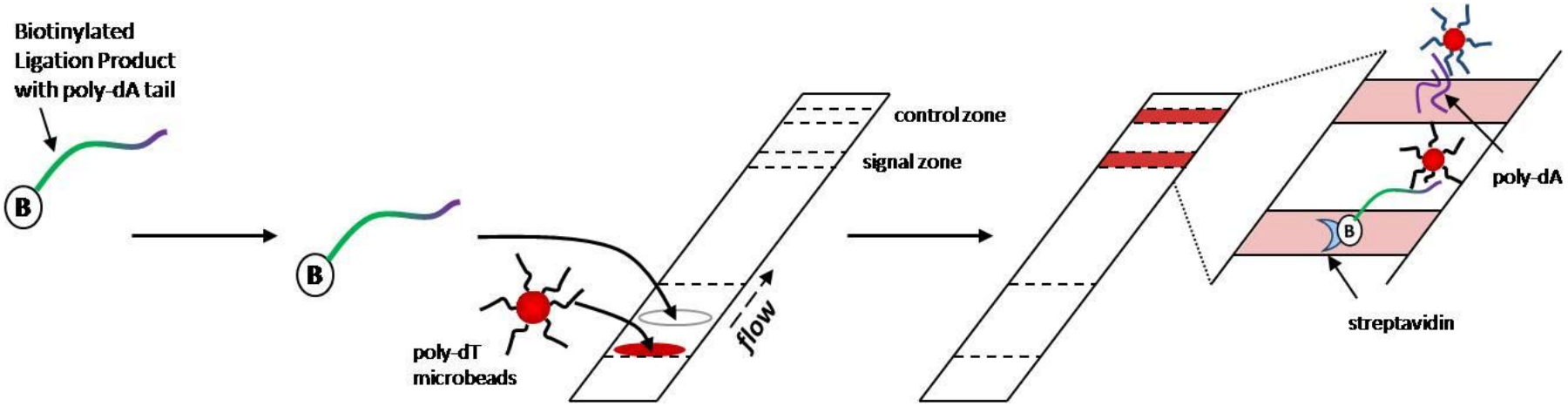
Lateral Flow Biosensor Creation

- Nitrocellulose Membrane- immobilized test zones
- Cellulose Immersion Pad- absorb running buffer
- Glass Conjugate Fiber- collect sample solution
- Cellulose Absorbent Pad- ensure complete flow of running buffer

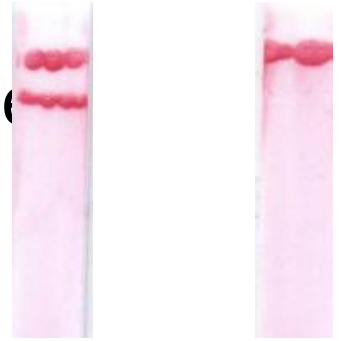




Colorimetric Readout



- Poly-T beads bind to Poly-A tailed probe
- Biotin binds to spotted streptavidin
- Beads produce colorimetric read-out
- Excess Poly-T beads bind to Poly-A control line

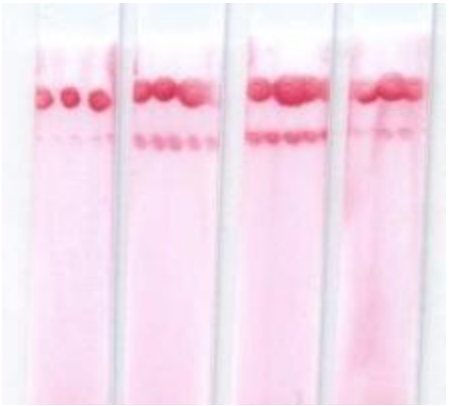


Positive Negative

Image Courtesy of Dr. Ryan Belfour Hayman

Example of Successful Biosensor

C-A13263G

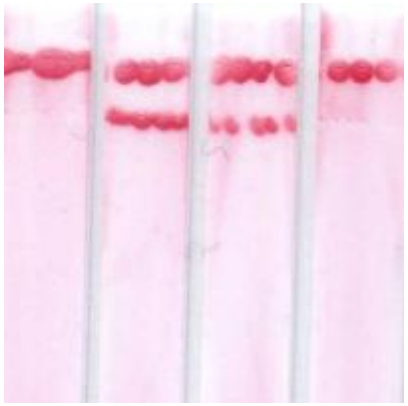


A G A G

68 46

IN NOT

M-C10400T

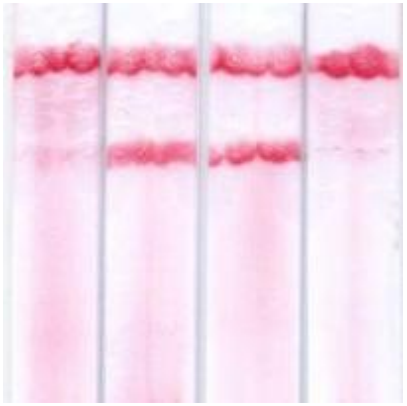


C T C T

21 2

IN NOT

X-A13966G

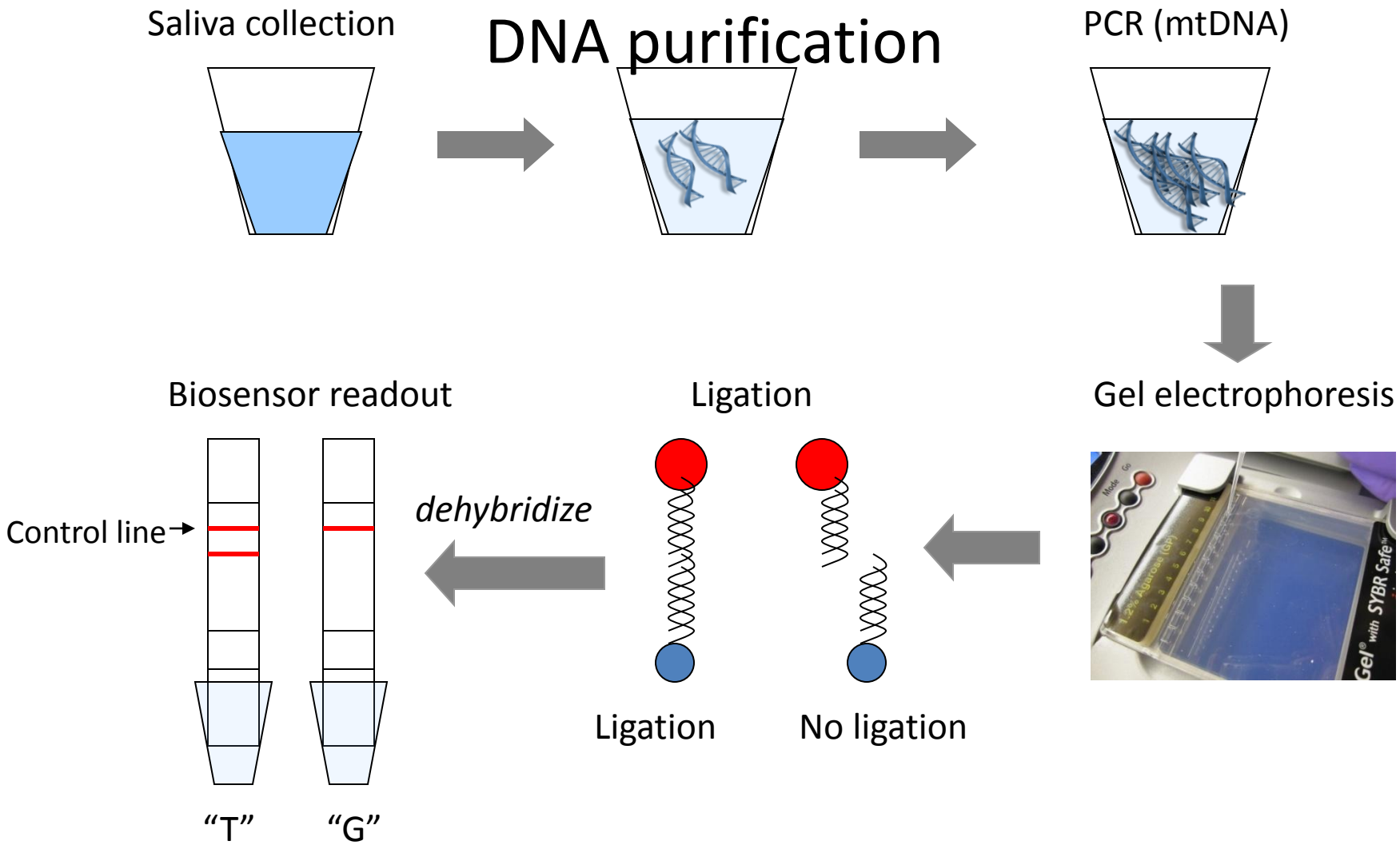


A G A G

17 54

IN NOT

Overview of Experiment

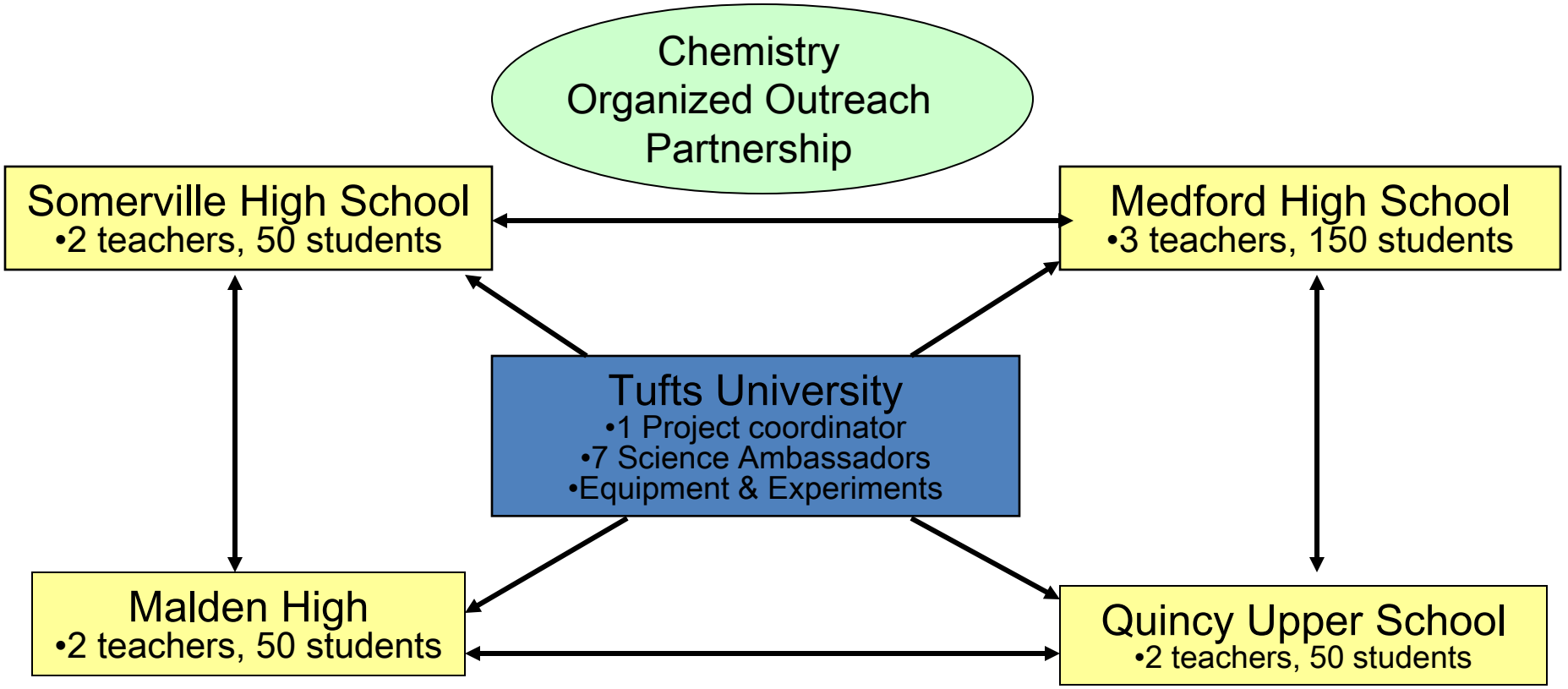


Mentoring

Mentoring

Outreach Implementation

- Maternal Ancestry
- GM Foods



300 students in 1 year
3-5 day experiments

Science Knowledge as a tool

	Mean	StDev
Before experiment	15.68	2.33**
After experiment	18.41	3.47**

- $t(171)=9.5$, $p<.0005$
- Eta squared statistic (.35) = large effect size
- 4 items in scale – applying science

Content Knowledge

- Maternal ancestry - 3 of 7 items sig p<.001

	N	Mean pre	Mean post	Effect size
In a cell, which of the following organelles contains its own DNA?	61	.51	.97**	large
Anthropologists can use the information from an individual to determine maternal ancestry. They do so by…..	61	.18	.69**	large
Polymerase Chain Reaction, or PCR, is…	61	.28	.88**	large

**p<.001, response 0=incorrect, 1=correct

Laboratory experience

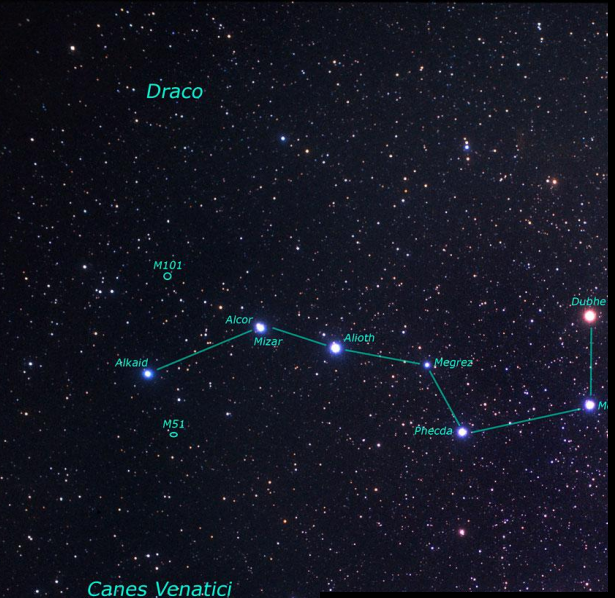
- Maternal ancestry and GM food

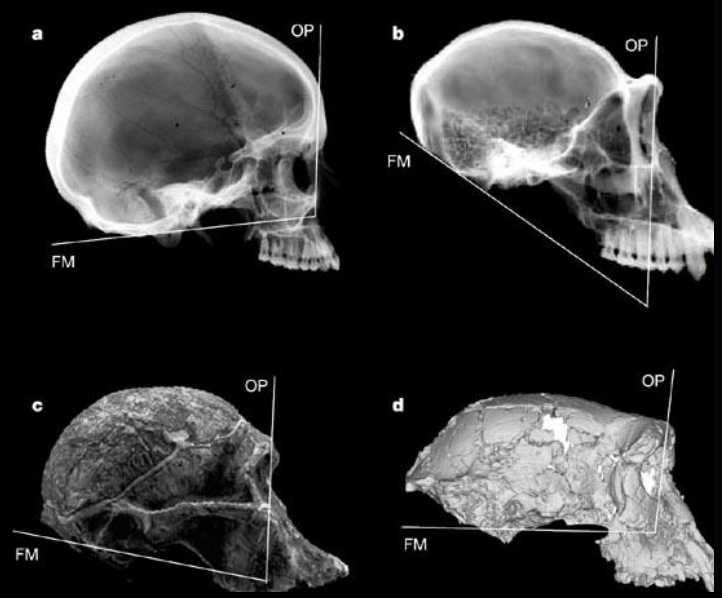
Statements	N - GM	Mean post	N- MA	Mean post
This lab was more interesting than other labs	183	4.9**	59	2.5**
This lab is something I would talk about even after class	181	4.1**	59	4**
I felt like I was doing real science during this lab	181	4.7**	59	3.5**

**p<.001, scale for GM 6 points 1=strongly disagree, 6 = strongly agree
Scale for MA 5 points 1= strongly disagree, 5 = strongly agree

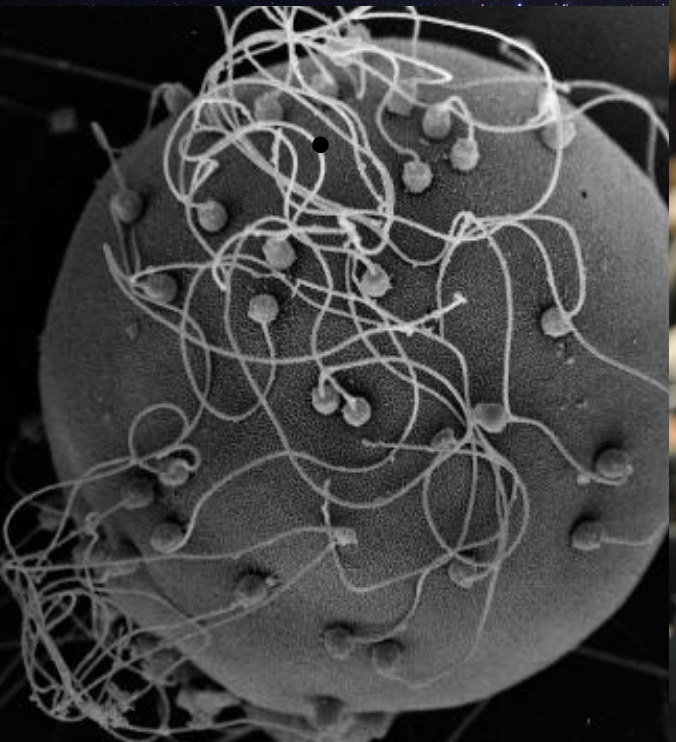
Outline

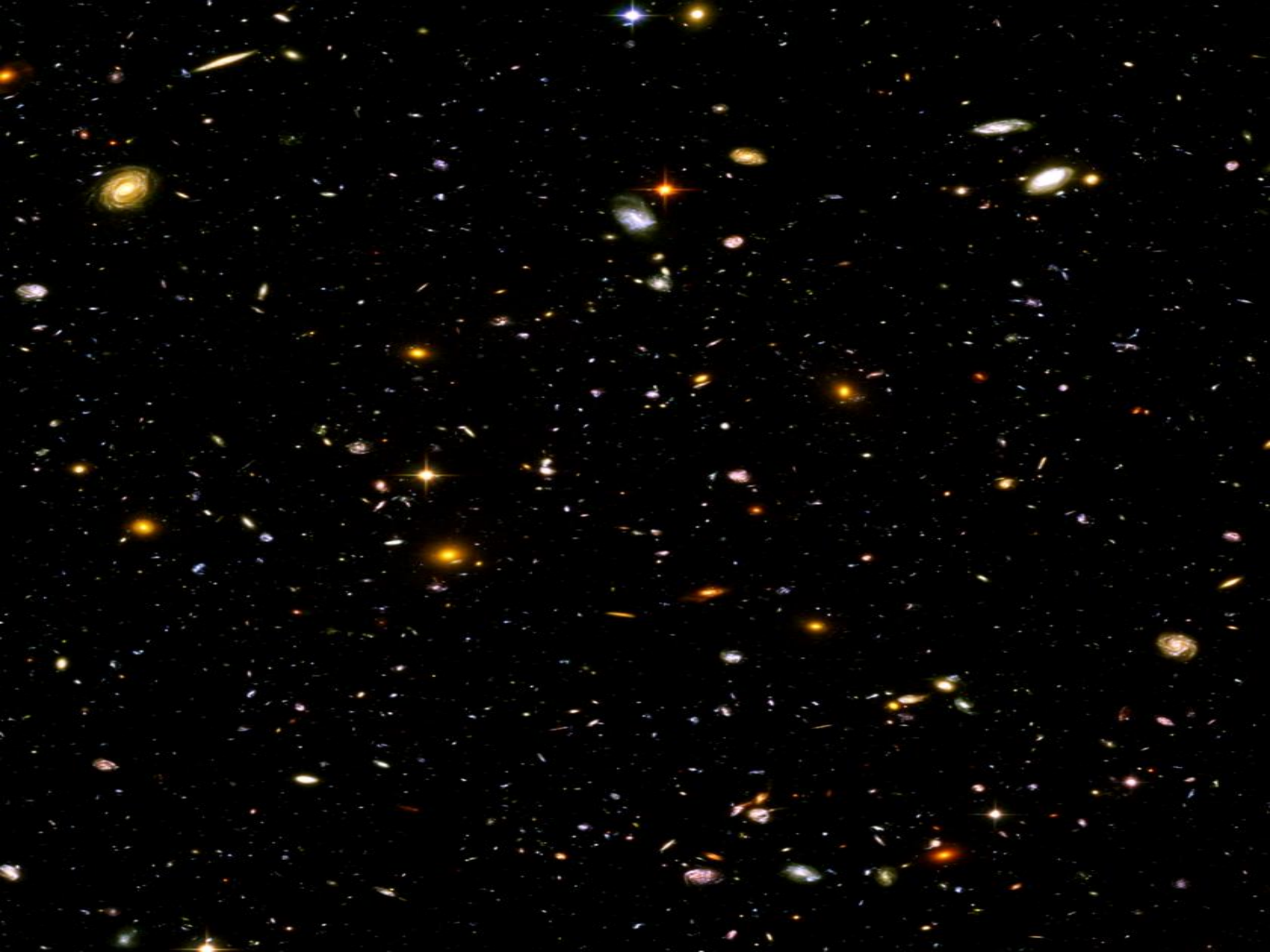
- DNA Analysis for High School students
- **From the Big Bang to Humankind**



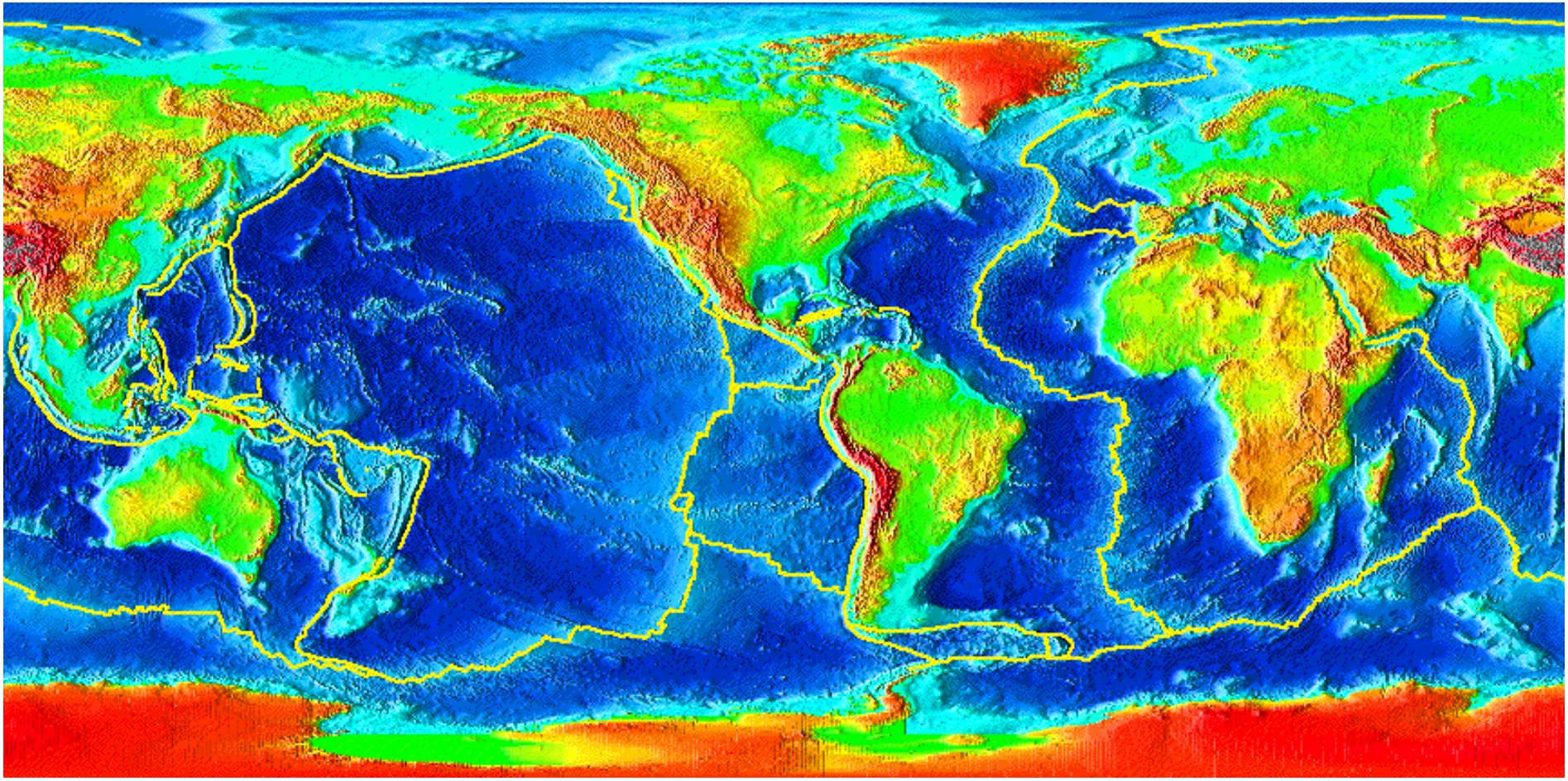


Teaching – From the Big Bang to Humankind

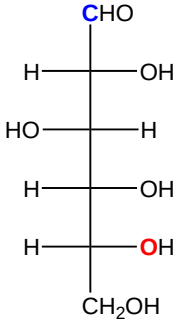


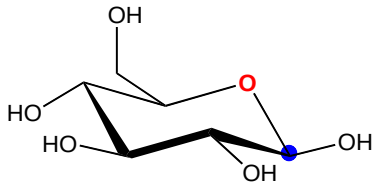


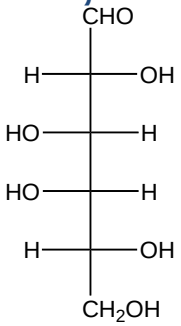
Geology - The Solid Earth and Plate Tectonics



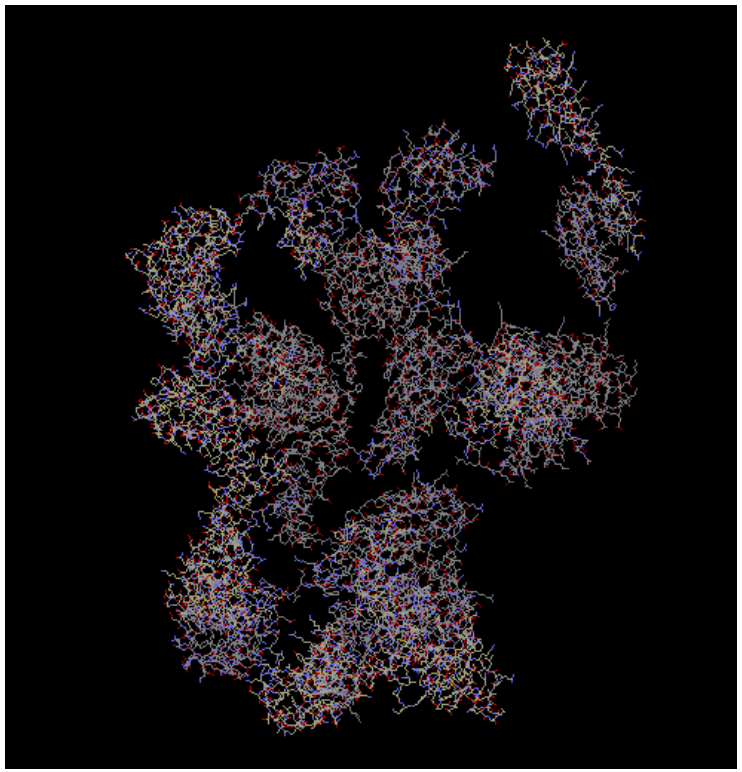
Chemistry – carbon, origin of life, central dogma

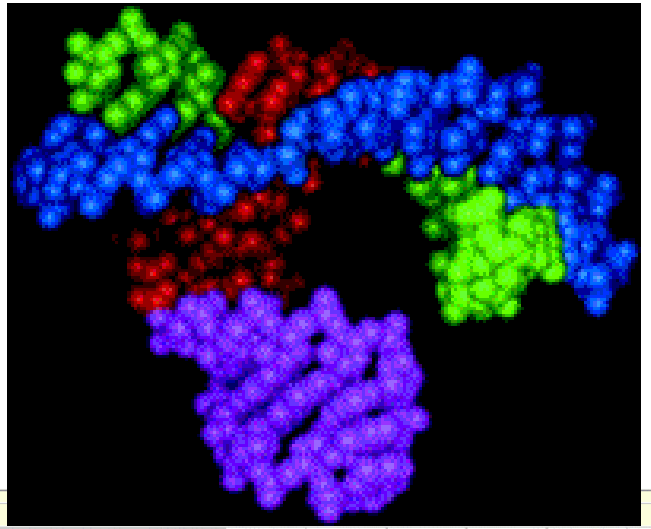


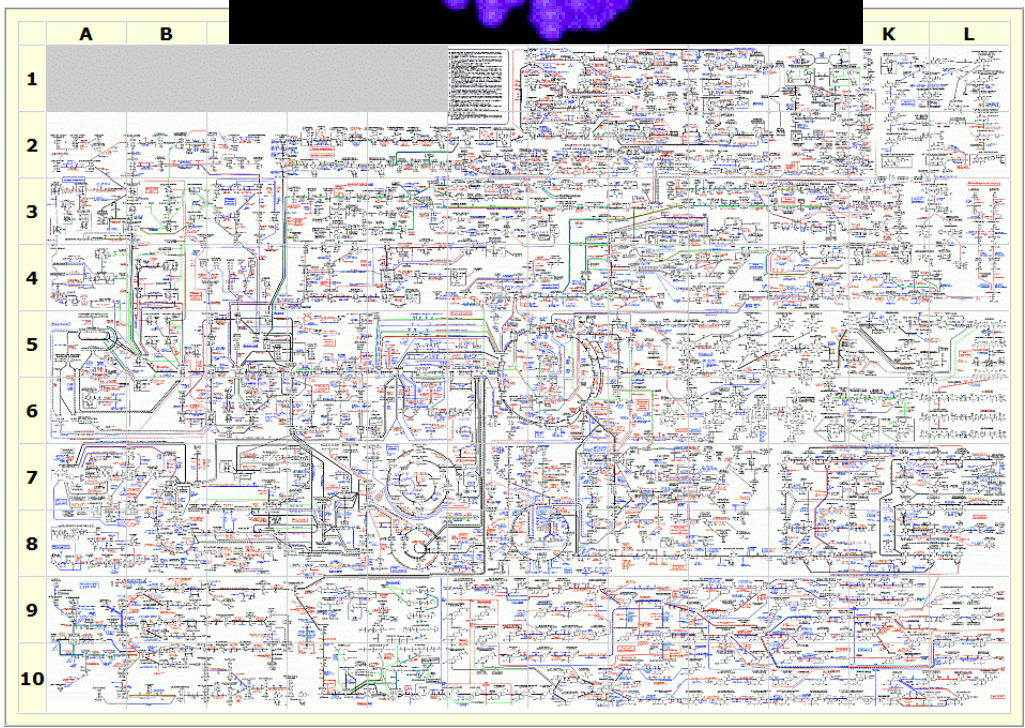




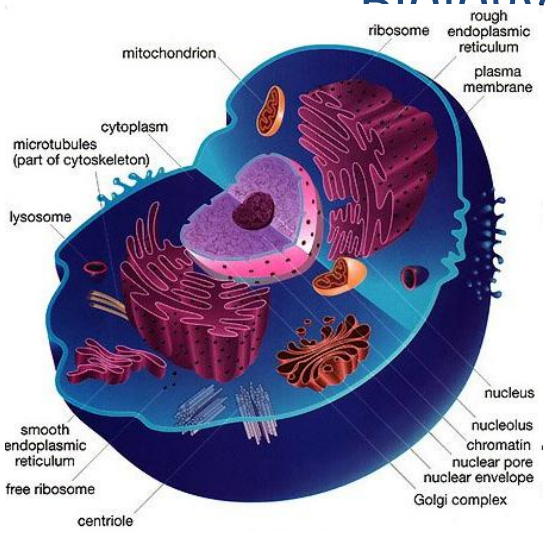
D-Galactose

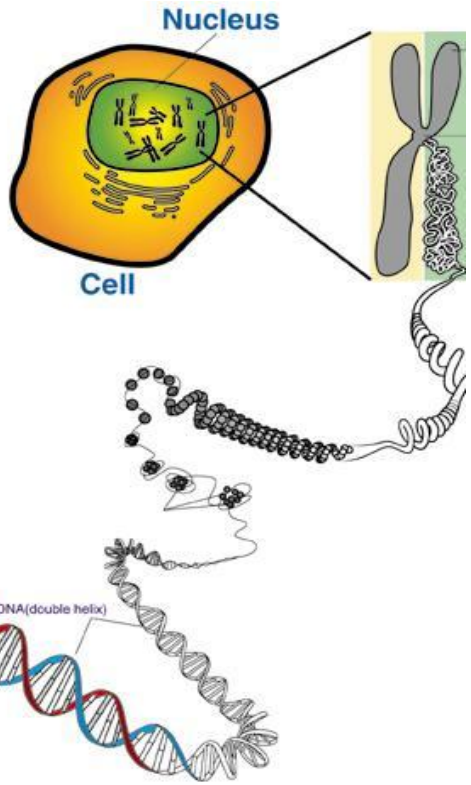


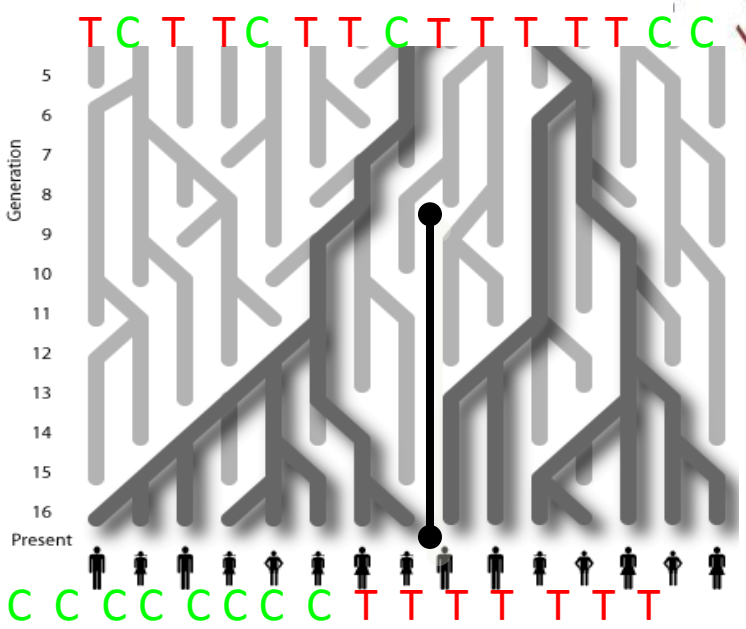




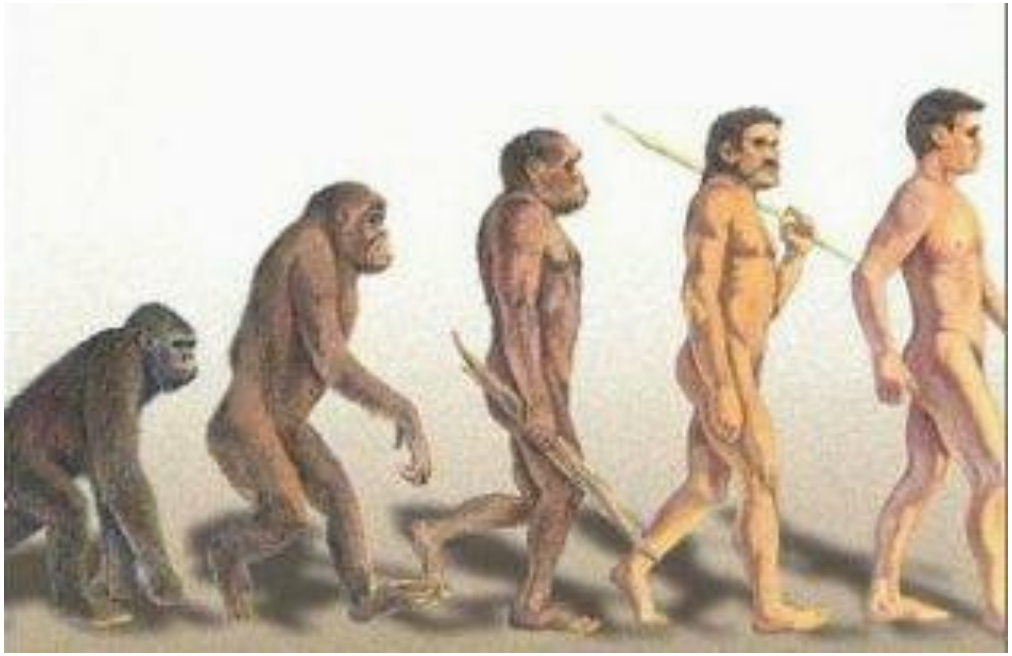
Biology – cells, genetics and evolution







Anthropology – evidence for human evolution



Five big questions about the universe

- Ast - evidence for Big Bang
- Geo - evidence that Earth is 4.5 by old
- Che - origin of life on Earth
- Bio - how genomes differ between individuals
- Ant - *Homo sapiens* started in Africa

Methods and Results: reliability

- Pre test - first day – coded
- Post test – final exam questions - graded

Question topic	Kappa	95% CI	Interpretation
Anthropology	0.87**	(0.97, 0.77)	Almost perfect agreement
Astrophysics	0.86**	(0.96, 0.75)	Almost perfect agreement
Geology	0.78**	(0.90, 0.66)	Substantial agreement
Chemistry	0.73**	(0.87, 0.60)	Substantial agreement
Biology	0.65**	(0.84, 0.46)	Moderate agreement

**Significance level at p<.001

Results: Scoring of responses

Topic	Mean post-pre	STDEV of post-pre	Cohen's d effect size
Astrophysics	0.69**	0.27	large
Chemistry	0.68**	0.33	large
Geology	0.59**	0.25	large
Biology	0.58**	0.36	large
Anthropology	0.42**	0.29	large

**Significance level at p<.001

- **Geology**
- Question: What is the evidence that Earth is 4.5 billion years old? Briefly explain how the evidence supports the age.

Pre test response, Student 136 male	Post test response, Student 136 male
Carbon dating tests the carbon samples in some of the earth's remains revealing approximately when the item was buried in the earth, fossilized and formed carbon.	*The evidence that the earth is 4.5 billion years old is the chondrite meteor whose age can be approximated through Uranium dating. Uranium is used to date this object because it has a particularly long half-life or decay rate. By using this we can look further back with more accuracy on objects like this.*

- **Anthropology**
- Question: What evidence do scientists use to support the theory that *Homo sapiens* started in Africa?

Pre test response, Student 40 male	Post test response, Student 40 male
The evolution of human beings is said to be from apes and the evolution skeletons proving this were found in Africa.	*The Great Rift Valley in Africa is home to the oldest known biped and supposed ancestors of the human race. There has also been DNA testing done that shows people in Africa are less similar to each other than people out of Africa. This shows that the hominid race spent more time evolving in Africa because when they left they exhibit far fewer genetic differences.*

- **Chemistry**
- Question: What is one possible theory for the origin of life on Earth? Give one piece of evidence to support that theory.

Pre test response, #116 female	Post test response, # 116 female
This study that was conducted where chemicals (present on earth before humans) were "shocked" with electricity to create the fundamental building blocks for DNA	*Miller–Urey experiment → "Primordial Soup". Methane, ammonia, and other compounds that were present in the early Earth, along with water were zapped with an electric arc discharge, imitating lightning, in order to recreate conditions of the early Earth. From this experiment, the fundamental building blocks of DNA were born, indicating that it was in fact possible for life to originate in such a matter on Earth.*

Teaching through research – Summer 2009