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.

Advantages
Engaging for students
Real world application
Genetics

Challenges
Keeping cost low
Limited time
Limited equipment
Single Nucleotide Polymorphism

SNP
Science Background

Mitochondria are in all cells

Mitochondria contain DNA

Wikipedia - File:Biological_cell.svg

Wikipedia - Animal_mitochondrion_diagram_en_(edit).svg
Science Background

Mitochondrial DNA is only inherited from

A. Nuclear DNA is inherited from all ancestors.
B. Mitochondrial DNA is inherited from a single lineage.
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**
  - ACCTTGC\textcolor{red}{G}CGCTATA
  - ACCTTGG\textcolor{red}{G}CGCTATA

- Occur throughout the genome
  - Nuclear and mitochondrial DNA

- Are used as genetic markers
Human mtDNA Migrations

Copyright 2002 © Mitomap.org

+/-, +/+ or -/- = Dde I 10394 / Alu I 10397
* = Rsa I 16329

Mutation rate = 2.2 - 2.9 % / MYR
Time estimates are YBP
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.

*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
  
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

Image Courtesy of Dr. Ryan Belfour Hayman
Example of Successful Biosensor

C-A13263G

M-C10400T

X-A13966G

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<tbody>
<tr>
<td>A</td>
<td>G</td>
<td>A</td>
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<tr>
<td>68</td>
<td>46</td>
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<tr>
<td>IN</td>
<td>NOT</td>
<td>IN</td>
<td>NOT</td>
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<tr>
<td>C</td>
<td>T</td>
<td>C</td>
<td>T</td>
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<tr>
<td>21</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>IN</td>
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<tr>
<td>17</td>
<td>54</td>
<td></td>
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</tr>
<tr>
<td>IN</td>
<td>NOT</td>
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</table>
Overview of Experiment

Saliva collection → DNA purification → PCR (mtDNA)

Biosensor readout

Control line
“T” → “G”

dehybridize

Ligation → No ligation

Gel electrophoresis
Mentoring
Mentoring
Outreach Implementation

• Maternal Ancestry
• GM Foods
Organized Outreach Partnership

Somerville High School
- 2 teachers, 50 students

Tufts University
- 1 Project coordinator
- 7 Science Ambassadors
- Equipment & Experiments

Malden High
- 2 teachers, 50 students

Medford High School
- 3 teachers, 150 students

Quincy Upper School
- 2 teachers, 50 students

300 students in 1 year
3-5 day experiments
Science Knowledge as a tool

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before experiment</td>
<td>15.68</td>
<td>2.33**</td>
</tr>
<tr>
<td>After experiment</td>
<td>18.41</td>
<td>3.47**</td>
</tr>
</tbody>
</table>

- $t(171)=9.5$, $p<.0005$
- Eta squared statistic (.35) = large effect size
- 4 items in scale – applying science
• Maternal ancestry - 3 of 7 items sig p<.001

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

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

• Maternal ancestry and GM food

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

**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

[Diagram of a cell with labeled parts: nucleus, mitochondrion, ribosome, microtubules, cytoplasm, lumen, smooth endoplasmic reticulum, free ribosome, centriole, rough endoplasmic reticulum, plasma membrane, lysosome, nucleus, nucleolus, chromatin, nuclear pore, nuclear envelope, Golgi complex.]

[Diagram of DNA helix and base pairs.]
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

<table>
<thead>
<tr>
<th>Question topic</th>
<th>Kappa</th>
<th>95% CI</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropology</td>
<td>0.87**</td>
<td>(0.97, 0.77)</td>
<td>Almost perfect agreement</td>
</tr>
<tr>
<td>Astrophysics</td>
<td>0.86**</td>
<td>(0.96, 0.75)</td>
<td>Almost perfect agreement</td>
</tr>
<tr>
<td>Geology</td>
<td>0.78**</td>
<td>(0.90, 0.66)</td>
<td>Substantial agreement</td>
</tr>
<tr>
<td>Chemistry</td>
<td>0.73**</td>
<td>(0.87, 0.60)</td>
<td>Substantial agreement</td>
</tr>
<tr>
<td>Biology</td>
<td>0.65**</td>
<td>(0.84, 0.46)</td>
<td>Moderate agreement</td>
</tr>
</tbody>
</table>

**Significance level at p<.001
# Results: Scoring of responses

<table>
<thead>
<tr>
<th>Topic</th>
<th>Mean post-pre</th>
<th>STDEV of post-pre</th>
<th>Cohen’s d effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrophysics</td>
<td>0.69**</td>
<td>0.27</td>
<td>large</td>
</tr>
<tr>
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<td>0.68**</td>
<td>0.33</td>
<td>large</td>
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<tr>
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<td>0.59**</td>
<td>0.25</td>
<td>large</td>
</tr>
<tr>
<td>Biology</td>
<td>0.58**</td>
<td>0.36</td>
<td>large</td>
</tr>
<tr>
<td>Anthropology</td>
<td>0.42**</td>
<td>0.29</td>
<td>large</td>
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**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.

<table>
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<tr>
<th>Pre test response, Student 136 male</th>
<th>Post test response, Student 136 male</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
<td>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.</td>
</tr>
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</table>
Anthropology

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

<table>
<thead>
<tr>
<th>Pre test response, Student 40 male</th>
<th>Post test response, Student 40 male</th>
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<tbody>
<tr>
<td>The evolution of human beings is said to be from apes and the evolution skeletons proving this were found in Africa.</td>
<td>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.</td>
</tr>
</tbody>
</table>
• Chemistry
• Question: What is one possible theory for the origin of life on Earth? Give one piece of evidence to support that theory.

<table>
<thead>
<tr>
<th>Pre test response, #116 female</th>
<th>Post test response, # 116 female</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>This study that was conducted where chemicals (present on earth before humans) were &quot;shocked&quot; with electricity to create the fundamental building blocks for DNA</em></td>
<td><em>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.</em></td>
</tr>
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