**Supplemental Materials for Teachers**

**Investigating the Molecular Mechanism of Evolution: Mutation and Natural Selection**

**Tina Hubler, Patti Adams and Jonathan Scammell**

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**I. PCR and Electrophoresis (background information and student activity)**

**PCR**

PCR is a powerful tool that allows researchers to produce millions of copies of selected regions of DNA. This quantity of DNA is required for downstream applications such as DNA fingerprinting and DNA sequencing. The *in vitro* copying of DNA in the laboratory follows the same basic steps that occur *in vivo* in the cell each time DNA is replicated prior to cell division. However there are some important differences. First, denaturing (separating) DNA is accomplished by heating the sample rather than by enzymatic activity (helicase), as it is accomplished in the cell. Second DNA primers are used to initiate new DNA synthesis, rather than RNA primers that are synthesized by primase in cells. Third, Okazaki fragments are not created because the DNA is completely denatured by heat. Fourth, as a consequence of these differences between *in vitro* and *in vivo* DNA synthesis, a thermostable DNA polymerase is the only enzyme required for synthesis of new DNA strands. The most commonly used thermostable polymerase originated from the thermophilic bacterium *Thermus aquaticus* and is abbreviated as *Taq* DNA polymerase.

The reagents needed for PCR are: (1) a template, the DNA to be copied, (2) nucleotide triphosphates, the nucleotide subunits of DNA, (3) thermostable DNA polymerase, (4) DNA primers complementary to the ends of the DNA region to be copied, (5) buffer, to maintain pH and ionic strength of the reaction mixture and (6) nuclease-free water. Supplies and equipment needed for PCR are: (1) a PCR cycler with a programmable heat block that allows one to change the temperature of the reaction mixture, (2) PCR tubes to hold the reaction mixture, and (3) pipettors and sterile pipet tips.

After the reagents are added to the PCR tube, reaction mixtures are placed into a PCR cycler and the stages of PCR are initiated. The stages are: (1) denaturation at 92-95 ⁰C, (2) annealing of primers to the template DNA at approximately 45-65 ⁰C and (3) synthesis of new DNA at approximately 68-72 ⁰C. Denaturation separates the DNA strands so that each may be used as a template for new DNA synthesis. Annealing requires lower temperatures to allow nucleic acids to associate by complementary base pairing. The primers are usually 15-25 nucleotides in length and are complementary to the ends of the region of DNA to be copied. The primer length and composition (percentage of A, T, C, and G) largely determine the annealing temperature. For example, longer primers with higher percentages of C and G are used with higher annealing temperatures. Synthesis of DNA requires the enzyme DNA Polymerase. As is the case for all enzymes, its optimal function depends on pH, ionic strength and temperature. The manufacturer of the polymerase will recommend the optimal conditions and the time needed for the synthesis.

The above three steps represent one PCR cycle that produces two DNA molecules from each template molecule. In the next cycle, the two DNA molecules serve as template and four DNA molecules are produced. The amount of DNA doubles with each cycle (i.e. 1, 2, 4, 8, 16, 32, etc.). This exponential amplification can produce more than 1 million copies of DNA after 20 to 30 cycles.

A typical PCR setup and conditions for a 1 kb template are listed below:

**Setup:**

10X reaction buffer (with MgCl2) 5 ul   
dNTP mix (10 mM of each dNTP) 1 ul   
Taq DNA Polymerase (5 U/ul) 0.25ul   
downstream primer (50 pmol/ul) 1 ul   
upstream primer (50 pmol/ul) 1 ul   
template DNA (10-100 ng/ul) 1-2 ul   
nuclease-free water (adjust to a final 50 ul)

**Cycle conditions:**

95 ⁰C 5 min initial denature step  
25-35 cycles:  
 95 ⁰C 30 sec denature step  
 55 ⁰C - 65 ⁰C 30 - 60 sec annealing primer   
 72 ⁰C 90 - 120 sec extension step

72 ⁰C 7 min final extension step

Additional technical information may be found at websites maintained by Promega Corp. and Integrated DNA Technologies, Inc.

<http://nld.promega.com/resources/product-guides-and-selectors/protocols-and-applications->

guide/pcr-amplification/#title6

http://cdn.idtdna.com/Support/Technical/TechnicalBulletinPDF/A\_Basic\_PCR\_Protocol.pdf

Textbook references for PCR figures:

iGenetics, A Molecular Approach, Third Edition, Russell, ISBN 0321569768, Pearson Education Inc. (Figure 9.3)

Essentials of Genetics, Seventh Edition, Klug,ISBN 0321618696, Pearson Education Inc. (Figure 17.10)

Biology Today and Tomorrow, 4th Edition, Starr, ISBN 9781133364450, Brookes/Cole Cengage Learning (Figure 10.5)

Biology , 9th Edition, Campbell, ISBN 9780321558237, Pearson Education Inc. (Figure 20.8)

**PCR student activity**

As an introductory activity, students will graph the exponential growth of the number of DNA molecules accumulated during PCR cycling. Given the data below, ask students to prepare a graphical representation of the number of DNA molecules that would accumulate during PCR cycling if you start with 1 molecule. This activity may also be used as a review of graphical representation of data. Important points to discuss are: choosing and labeling axes, plotting points and drawing conclusions.

|  |  |
| --- | --- |
| cycles | number of molecules |
| 1 | 2 |
| 2 | 4 |
| 3 | 8 |
| 5 | 32 |
| 10 | 1024 |
| 15 | 32768 |
| 20 | 1048576 |

**Electrophoresis**

Electrophoresis is a common technique used in molecular laboratories to separate mixtures of charged molecules. In our activities the molecules are separated according to size. An agarose gel may be used as the supporting medium to separate nucleic acids. The gels are made by dissolving solid agarose in boiling buffer, pouring the partially cooled solution into a casting tray, and allowing it to solidify by cooling to room temperature. The gel casting tray includes a comb that forms sample wells as the gel solidifies. Agarose gels are a porous matrix, with the pore size depending on the concentration of agarose used. Higher concentrations of agarose yield gels with smaller pores. 1.2 - 1.5 % agarose gels are used to separate DNA molecules from 200 to 10000 base pairs in size.

The gel is submerged in an electrophoretic chamber containing buffer (usually Tris Acetate-EDTA, pH=8). Samples are loaded into the sample wells and current is applied to the chamber. At pH=8, nucleic acids are negatively charged and travel under the influence of the electric field toward the opposite end of the gel (the positive electrode). Smaller molecules travel through the pores more quickly and migrate further in the gel.

Electrophoresis buffer is used to control pH and ionic strength of the gel and chamber. In contrast, sample buffer containing 10-20% glycerol is sometimes added to samples to cause samples to settle to the bottom of the sample well before electrophoresis begins.

After electrophoresis, sample components are usually not visible unless stained. Agarose gels are commonly stained with either methylene blue or ethidium bromide. Both stains have affinity for nucleic acids. Ethidium bromide is more sensitive and allows one to visualize smaller quantities of DNA. However, it is a mutagen and requires ultraviolet light for visualization. If sufficient quantities of nucleic acids are present, methylene blue is a safer alternative for visualization.

**Electrophoresis student activity**

To ensure that students understand the principle of electrophoretic separation according to size, ask students to predict the migration of molecules from a mixture of DNA. Assume a DNA ladder is added to one well in a gel and the following mixture of molecules is added to the second well: 1500 bp, 1000 bp, and 500 bp of DNA. Students can predict how far the individual molecules from the mixture will migrate and draw the positions on the gel below.

DNA

ladder

Mixture

2.0 kb

1.0 kb

1.5 kb

0.5 kb

0.3 kb

0.8 kb

Direction of migration of molecules

**II. Sequences**

**Squirrel monkey variable region (Alu element bold and underlined)**

agttcctctc taccttgtac ctgttccaga cccccggcct aggcctggac

actaaggaaa ttcttactaa acaaatgctt gcccagctca tccgtccctc

actcttctct acctctcacc ttgattcccc agaggaggag gggaagtgat

aagagaactg tcgagaacag ctgtcattta cccgggactt gctatgggcc

agggacttta cagacagcat cttgtctaag tttgacatca tcccatgaag

tggatcttac tattatcccc atttaacaaa tgagaaatct gaggcatggg

aaagttaagt gacttgtcca agctcacata atgaagtagt ggtaccaggc

agaactggct atataatctg tgggacccag tgcaaaatga aaatgtgggg

cctctgttaa aaaactatta atc**ggccggg cgcggtggct caagcctgta**

**atcccagcac tttgggaggc cgaggtgggt ggatcacaag gtcgagagat**

**cgagaccatc ctggtcaaca tggtgaaacc ccgtctctac taaaaataca**

**aaaagttagc tgggcgtggt ggtgcatgcc tgtaatccca gctactcagg**

**aggctgaggc aggagaattg cctgagccca ggaggcggag gttgcggtga**

**gccgagatcg cgccattgca ctccagcctg ggtaacaaga gcgaaactcc**

**gtctcaaaaa a**aaaaaaaaa aaaaaaacta ttaatcattt caagaccagg

acagaagagc attaatgcaa gagtagggc

**Owl monkey variable region**

tagttcctct ctaccttgta cctgtcccag acccccggcc taggcctgga cactgaggag

attcttacta aacaaatgct tgcccagctc atcctcccct cactcttctc tacctctcac

cttgattccc cagaggagga gggaaagggg gaggggaggg gaagtggnnn gagaattgac

gagaacagct gtcatttagc cgggacttgc tatgggccag ggactttann nacagcgtct

tgtctaagct tgacatcacc ccatgaagtg gatcttactg ttatccccat ttaacaaatg

agaaatctga ggcatgggaa agttaagtga cttgtccaag ctcacataac caagtagtgt

accaggcaga actggctata taatttgtgg gacccagcgc aaaatgaaaa tgtggggcct

ctgttaaaaa accattaatc atttcaagac caggacaaag agcattaatg caagagtagg

gcta

**Human CG** Accession no. NC\_000019.9 GI:224589810

atggagatg ttccaggtaa gactgcaggg cccctgggca ccttccacct

ccttccaggc aatcactggc atgagaaggg gcagaccagt gtgagctgtg gaaggaggcc

tctttctgga ggagcgtgac ccccagtaag cttcaggtgg ggcagttcct aagggtgggg

atctgaaatg ttggggcatc tcaggtcctc tgggctgtgg ggtggactct gaaaggcagg

tgtccgggtg gtgggtcctg aataggagat gccgggaagg gtctctgggt ctttgtgggt

ggtgtgccac gtgggatggg aaggccgggg ctcggggctg cggtctcaga cccgggtgaa

gcagtgtcct tgtcccaggg gctgctgctg ttgctgctgc tgagcatggg cgggacatgg

gcatccaagg agccgcttcg gccacggtgc cgccccatca atgccaccct ggctgtggag

aaggagggct gccccgtgtg catcaccgtc aacaccacca tctgtgccgg ctactgcccc

accatggtga gctgcccggg gccggggcag gtgctgccac ctcagggcca gacccacaga

ggcagcgggg gaggaagggt ggtctgcctc tctggtcagg ggctgcggaa tggggtgtgg

gagggcagga acagagggct tcccggaccc ctgagtctga gacctgtggg ggcaactggg

gagctcagct gaggcgctgg cccaggcaca tgctcattcc cccactcaca cggcttccag

acccgcgtgc tgcagggggt cctgccggcc ctgcctcagg tggtgtgcaa ctaccgcgat

gtgcgcttcg agtccatccg gctccctggc tgcccgcgcg gcgtgaaccc cgtggtctcc

tacgccgtgg ctctcagctg tcaatgtgca ctctgccgcc gcagcaccac tgactgcggg

ggtcccaagg accacccctt gacctgtgat gacccccgct tccaggactc ctcttcctca

aaggcccctc cccccagcct tccaagtcca tcccgactcc cggggccctc ggacaccccg

atcctcccac aataa

**Squirrel monkey CG** Accession no. GU117708.1

atg gagatgctcc

aggtaagact gcagggcccc tgggtacctt ccaccgccct ccaggccatc actggcatga

agaggggcag agtcgtgtga gctggggaag gaggcctttt tctggagggg tgtgactctg

cagtaagctt caggtggaga agtccctgag ggtggagaac tgaaatgttg ggctgggggt

gggctctgaa aggcaggtgt ctgggtggca ggtcctgaat aagacatgcc aggcagggtc

cctgggtcct tgagggtggt atacccctgg ggatgggcca gggctcaggg cttcagtctc

aggctcggct gaagcaccgg tcttgtccca gggactgctg ctgtgtctgc tgctgagcac

aggtggggca tgggcatcca aggagccact tcggccgccg tgccgcccca ccaatgtcat

cctggctgtt gagaaggagg gctgccctgt ttgcgttccc ttcaacacca ccatctgcgc

cggctactgc tccagcatgg tgagctgccc gggaccgggg gcaggtgctg ccacctcagg

gcggggccca cagaggcact ggggaagggt gtctggctct ctgggcaggg gctgggaaat

ggggctggag ggcaggaaca gatggcttcc tggacatgag tctgggacct gtggaggggg

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ccttaggtac gagtgatgca gaccttgccg cccttacccc agacggtgtg caactaccac

gagctgcgct tcacctccgt ccggctccct ggctgtcggc gcggcgtgga tcccgtggtc

tacatgccca tggctgtcag ctgtcgctgt gcactctgcc gccgaagcta ttctgactgt

gggagtttca ggaacgagtc cctgggctgt gactacgcca cctcccagga ctcttcctct

aatgtccctc ccagcaacct tacaagtcca tcccaactcc tggagccagc agtcactcca

ttagtcccac aataa

**Owl monkey CG** Accession no. JN613228 GI:372126675

atgg agatgctcca ggtaagactg cagggcccct gggtaccttc

cacctccctc caggccatca ctgaaatgaa gaggggcaga gtcatgtgag ctggggaagg

aggccttttt ctggaggggt gtgaccccgc agtaagcttc aggtgaagtc cctgagggtg

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aggagggctg ccccgtgtgc gtcgccttca acaccaccat ctgtgccggc tactgctcca

gcatggtgag ctgcccggga ccaggggcag gtgctgccac ctcagggcgg ggcccacaga

ggcagtgggg aagggtggtc tggctctctg ggcaggggct gggaaatggg gctggagggc

aggaacagag gtcttcctgg atatctgagt ctgggacctg tggagggagc tggggtgctc

agctgaggtg ctggccccca gacacatgcc cactccccca cccacatggc cttaggtacg

ggtgctgcag accgtcatgc cgcccttacc ccagttggtg tgcaactacc acgagctgcg

cttcacctct gtccggctcc ctggctgtcg gcgcggcgtg aatcccgtgg tctactttcc

cgtggctgtc agctgtcgct gtgcactctg ccgccgaagc tattctgact gcgggaatct

caagagcgag cccctgggct gtgactacca cacctcccag gactcttcct ctaaggaccc

tccccgcaac cttacaagtc catcccaact cccggagcca gcagacgctc cattagtccc

acaataa

**Human growth hormone** Accession no. E00140.1 GI:224589808

gaattcagca ctgaatcatg cccagaaccc ccgcaatcta ttggctgtgc tttggcccct

tttcccaaca cacacattct gtctggtggg tggaggggaa acatgcgggg aggaggaaag

gaataggata gagagtggga tggggtcgct aggggtctca aggactggcc tatcctgaca

tccttctccg cgttcaggtt ggccaccatg gcctgctgcc agagggcacc cacgtgaccc

ttaaagagag gacaagttgg gtggtatctc tggctgacat tctgtgcaca accctcacaa

cgctggtgat ggtgggaagg gaaagatgac aagtcagggg gcatgatccc agcatgtgtg

ggaggagctt ctaaattatc cattagcaca agcccgtcag tggccccagg cctaaacatg

cagagaaaca ggtgaggaga agcagcgaga gagaaggggc caggtataaa aagggcccac

aagagaccag ctcaaggatc ccaaggccca actccccgaa ccactcaggg tcctgtggac

agctcactag cggcaatggc tgcaggtaag cgcccctaaa atccctttgg cacaatgtgt

cctgagggga gaggcggcgt cctgtagatg ggacgggggc actaaccctc aggtttgggg

cttatgaatg ttagctatcg ccatctaagc ccagtatttg gccaatctct gaatgttcct

ggtccctgga ggaggcagag agagagagag agaaaaaaaa aacccagctc ctggaacagg

gagagcgctg gcctcttgct ctccagctcc ctctgttgcc tccggtttct ccccaggctc

ccggacgtcc ctgctcctgg cttttggcct gctctgcctg tcctggcttc aagagggcag

tgccttccca accattccct tatccaggct ttttgacaac gctatgctcc gcgcccgtcg

cctgtaccag ctggcatatg acacctatca ggagtttgta agctcttggg taatgggtgc

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aggagctcag ggttgttttc tgaagtgaaa atgcaggcag atgagcatac gctgagtgag

gttcccagaa aagtaacaat gggagcaggt ctccagcata gaccttggtg ggcggtcctt

ctcctaggaa gaagcctata tcctgaagga gcagaagtat tcattcctgc agaaccccca

gacctccctc tgcttctcag agtctattcc aacaccttcc aacagggtga aaacgcagca

gaaatctgtg agtggatgcc ttctccccag gtgggatggg gtagacctgt ggtcagaccc

cccgggcagc acacccactg ccggtccttc ccctgcagaa cctagagctg ctccgcatct

ccctgctgct catccagtca tggctggagc ccgtgcagct cctcaggagc gtcttcgcca

acagcctggt gtatggcgcc tcggacagca acgtctatcg ccacctgaag gacctagagg

aaggcatcca aacgctgatg tgggtgaggg tggcaccagg atccaatcct ggggccccac

tggcttccag ggactgggga gagaaacact gctgccctct ttttagcagt caggcgctga

cccaagagaa ctcaccgtat tcttcatttc ccctcgtgaa tcctccaggc ctttctctac

aacctggagg ggagggagga aaatggatga atgagagagg gagggaacag tgcccaagcg

cttggcctct ccttctcttc cttcactttg cagaggctgg aagatggcag cccccggact

gggcagatct tcaatcagtc ctacagcaag tttgacacaa aatcgcacaa cgatgacgca

ctgctcaaga actacgggct gctctactgc ttcaggaagg acatggacaa ggtcgagaca

ttcctgcgca tcgtgcagtg ccgctctgtg gagggcagct gtggcttcta gctgcccggg

tggcatccct gtgacccctc cccagtgcct ctcctggtcg tggaaggtgc tactccagtg

cccaccagcc ttgtcctaat aaaattaagt tgcatcattt tgtttgacta ggtgtccttg

tataatatta tggggtggag gcgggtggta tggagcaagg ggccaggttg ggaagacaac

ctgtagggcc ttcagggtct attcgggaac caggctggag tgcagtggca gtcttggctc

gctgcaatct ccgcctcctg ggttcaagcg attctcctgc ctcagtctcc cgaatagttg

ggattccagg catgcaagac caggctcagc taatttttgt atttttggta gagacggggt

ttcaccatat tggccagtct ggtctccatc tcctgacctc aggtaatccg cccgcctcgg

cctcccaaat tgctgggatt acaggtatga gccactgggc ccttccctgt cctgtgattt

taaaataatt ataccagcag aaggacgtcc agacacagca tgggctacct ggccatgccc

agccagttgg acatttgagt tgtttgcttg gcactgtcct ctcatgcatt gggtccactc

**Human oxytocin** Accession no. NC\_000020.10 GI: 224589812

tgtctgctcg gcctcctggc gctgacctcc gcctgctaca tccagaactg ccccctggga

ggcaagaggg ccgcgccgga cctcgacgtg cgcaaggtga gtccccagcc ctggtcccgc

ggcgctccgg ggagggaggg acccgcagcc acaggggcgc gccccgctcc ggcctcgcct

gagaactcca ggagctgagc ggattttgac gccccgccct tgaccgcggt cgaggccccc

acggcgcccc agcgcgtctc agccccgctg tcccgcccga actccgaacc ccggacccca

gcatccttgc ccggcgcacc ccggccggcc tcgcagggtc ctccgagcga gtccccagcg

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caaaggccgc tgcttcgggc ccaatatctg ctgcgcggaa gagctgggct gcttcgtggg

caccgccgaa gcgctgcgct gccaggagga gaactacctg ccgtcgccct gccagtccgg

ccagaaggcg tgcgggagcg ggggccgctg cgcggtcttg ggcctctgct gcagcccggg

tgagcggggc aaggcgctcc ggggccaggg ggaggcgggc gggggtgcgg ccgggattcc

cctgactcca cctcttcctc cagacggctg ccacgccgac cctgcctgcg acgcggaagc

caccttctcc cagcgctgaa acttgatggc tccgaacacc ctcgaagcgc gccactcgct

tcccccatag ccaccccaga aatggtgaaa ataaaataaa gcaggttttt ctcctcta

**BLAST results : sm vs. om variable regions**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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| |  |  | | --- | --- | | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/score.gif |  |  | | --- | | http://blast.ncbi.nlm.nih.gov/images/query_no_scale.gif |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/1.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/1.gif | http://blast.ncbi.nlm.nih.gov/images/5.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/3.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/4.gif | http://blast.ncbi.nlm.nih.gov/images/5.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/6.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/7.gif | http://blast.ncbi.nlm.nih.gov/images/5.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif |  |  |  |  |  | | --- | --- | --- | --- | | **Sbjct**http://blast.ncbi.nlm.nih.gov/images/white.gif | [score 618](http://blast.ncbi.nlm.nih.gov/Blast.cgi#42833) | http://blast.ncbi.nlm.nih.gov/images/grey.gif | [score 93](http://blast.ncbi.nlm.nih.gov/Blast.cgi#42833) |  |  | | --- | | http://blast.ncbi.nlm.nih.gov/images/white.gif | |

Query = squirrel monkey variable region

Sbjct = owl monkey variable region

Query 1 AGTTCCTCTCTACCTTGTACCTGTTCCAGACCCCCGGCCTAGGCCTGGACACTAAGGAAA 60

|||||||||||||||||||||||| |||||||||||||||||||||||||||| |||| |

Sbjct 2 AGTTCCTCTCTACCTTGTACCTGTCCCAGACCCCCGGCCTAGGCCTGGACACTGAGGAGA 61

Query 61 TTCTTACTAAACAAATGCTTGCCCAGCTCATCCGTCCCTCACTCTTCTCTACCTCTCACC 120

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Sbjct 62 TTCTTACTAAACAAATGCTTGCCCAGCTCATCCTCCCCTCACTCTTCTCTACCTCTCACC 121

Query 121 TTGATTCCCCAGAGGAGGAGGG-----------------GAAGTGATAAGAGAACTGTCG 163

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Sbjct 122 TTGATTCCCCAGAGGAGGAGGGAAAGGGGGAGGGGAGGGGAAGTGGNNNGAGAATTGACG 181

Query 164 AGAACAGCTGTCATTTACCCGGGACTTGCTATGGGCCAGGGACTTTACAGACAGCATCTT 223

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Sbjct 182 AGAACAGCTGTCATTTAGCCGGGACTTGCTATGGGCCAGGGACTTTANNNACAGCGTCTT 241

Query 224 GTCTAAGTTTGACATCATCCCATGAAGTGGATCTTACTATTATCCCCATTTAACAAATGA 283

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Sbjct 242 GTCTAAGCTTGACATCACCCCATGAAGTGGATCTTACTGTTATCCCCATTTAACAAATGA 301

Query 284 GAAATCTGAGGCATGGGAAAGTTAAGTGACTTGTCCAAGCTCACATAATGAAGTAGTGGT 343

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Sbjct 302 GAAATCTGAGGCATGGGAAAGTTAAGTGACTTGTCCAAGCTCACATAACCAAGTAGT-GT 360

Query 344 ACCAGGCAGAACTGGCTATATAATCTGTGGGACCCAGTGCAAAATGAAAATGTGGGGCCT 403

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Sbjct 361 ACCAGGCAGAACTGGCTATATAATTTGTGGGACCCAGCGCAAAATGAAAATGTGGGGCCT 420

Query 404 CTGTTAAAAAACTATTAATC **423**

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Sbjct 421 CTGTTAAAAAACCATTAATC 440

**Note gap in sequence from Alu insertion in sm sequence**

Query **722** aaaaaaCTATTAATCATTTCAAGACCAGGACAGAAGAGCATTAATGCAAGAGTAGGGC 779

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Sbjct 426 AAAAAACCATTAATCATTTCAAGACCAGGACA-AAGAGCATTAATGCAAGAGTAGGGC 482

**BLAST results : identification of an Alu sequence in sm variable region**

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| http://blast.ncbi.nlm.nih.gov/images/score.gif |

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| http://blast.ncbi.nlm.nih.gov/images/query_no_scale.gif |

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| http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/scale.gif |

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| http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/1.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/1.gif | http://blast.ncbi.nlm.nih.gov/images/5.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/3.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/4.gif | http://blast.ncbi.nlm.nih.gov/images/5.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/6.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/7.gif | http://blast.ncbi.nlm.nih.gov/images/5.gif | http://blast.ncbi.nlm.nih.gov/images/0.gif |

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| **Sbjct** http://blast.ncbi.nlm.nih.gov/images/white.gif | http://blast.ncbi.nlm.nih.gov/images/white.gif | [score 420](http://blast.ncbi.nlm.nih.gov/Blast.cgi#alu:HSU14572) |

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Query = squirrel monkey variable region

Sbjct = Alu sequence

Query 424 GGCCGGGCGCGGTGGCTCAAGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGTGGGTGGA 483

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Sbjct 1 GGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGGA 60

Query 484 TCACAAGGTCGAGAGATCGAGACCATCCTGGTCAACATGGTGAAACCCCGTCTCTACTAA 543

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Sbjct 61 TCACGAGGTCAAGAGATCGAGACCATCCTGGCCAACATGGTGAAACCCCGTCTCTACTAA 120

Query 544 AAATACAAAAAGTTAGCTGGGCGTGGTGGTGCATGCCTGTAATCCCAGCTACTCAGGAGG 603

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Sbjct 121 AAATACAAAAA-TTAGCTGGGCGTGGTGGCGCGCGCCTGTAGTCCCAGCTACTCGGGAGG 179

Query 604 CTGAGGCAGGAGAATTGCCTGAGCCCAGGAGGCGGAGGTTGCGGTGAGCCGAGATCGCGC 663

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Sbjct 180 CTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCGGAGGTTGCAGTGAGCCGAGATCGCGC 239

Query 664 CATTGCACTCCAGCCTGGGTAACAAGAGCGAAACTCCGTCTCaaaaaaaa 713

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Sbjct 240 CACTGCACTCCAGCCTGGCGA-C-AGAGCGAGACTCCGTCTCAAAAAAAA 287

**III. Mobile genetic elements**

**Transposons**

Over half of the human genome can be traced to mobile sequences. Transposons are mobile genetic elements whose DNA sequence can be copied and inserted into new locations within the genome [1, 2]. The DNA sequence included in transposons often encodes a unique set of genes, some of which are involved in transposition and some of which may provide a benefit to the organism (e.g. antibiotic resistance genes) [3]. If an inserted gene confers a biological advantage in the organism (e.g. positive selection), the frequency of the gene may increase in the population. The insertion of mobile genetic elements into various sites of a genome may also have deleterious effects on the organism [4].

**Retrotransposons**

Retrotransposons are transposons that are copied and inserted into new locations in the genome via RNA intermediates. This involves transcribing a transposon DNA segment into RNA, followed by reverse transcribing the RNA into DNA. The reverse-transcribed DNA is then inserted into a new location within the genome by the action of enzymes (transposases) that are encoded by sequences contained in the the same or another transposon [5].

Retrotransposons are classified as either autonomous or nonautonomous. Autonomous retrotransposons consist of DNA sequences that encode the necessary enzymes for replication and insertion [6]. LINES, such as L1 are examples of autonomous retrotransposons [7, 8]. Nonautonomous retrotranposons are able to recruit the necessary enzymes, but these “trans” factors have been produced from other DNA sequences. SINES, such as Alu, are examples of nonautonomous retrotransposons that require other DNA sequences to achieve their transposition [8].

**Alu elements**

Alu elements are the largest family of retrotransposons in the human genome [9]. Alu elements are approximately 300 bp in length and are dispersed throughout the human genome over a million times [1]. The exact sequence of an Alu element varies as a consequence of changes incurred during copying and insertion [10]. Sequence variation has produced a number of Alu subfamilies that appear to be primate lineage-specific. For example, the Y subfamily is found primarily in old world monkeys and apes [10, 11].

Insertion of Alu sequences has several impacts on genomes. Not only do Alu insertions increase the size of genomes, they also disrupt regulatory regions and coding regions [12]. Alu insertions have been identified in a number of human disorders including hemophilia, immunodeficiency, cholinesterase deficiency, optic atrophy, hyperparathyroidism, hemolytic anemia, lipoprotein lipase deficiency, complement deficiency, breast cancer and neurofibromatosis [9, 10, 13]. Whether beneficial, neutral or harmful, the movement of mobile genetic elements inevitably increases genome size and produces genetic variation in organisms.

References

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**IV. Icefish materials**

DVD: *The Making of the Fittest: The Birth and Death of Genes.* <http://www.hhmi.org/biointeractive>, Howard Hughes Medical Institute, 2011.

Book: *Into the Jungle: Great Adventures in the Search for Evolution,* Sean Carroll, August 2008. p166-183. Benjamin Cummings ISBN: 0321556712.

**V. Assessment**

We suggest short quizzes on the material covered in each lab. Instructors should tailor this to their instruction. Additionally, we suggest a multiple choice quiz on the icefish article, after allowing students to complete the worksheet and ask questions. Finally, a comprehensive assessment should include all topics from lecture and lab. We suggest asking the students to contemplate and prepare answers to the essay question :

In your own words, explain how mutations and selection may result in the evolution of new species.

Advise students that their answers should address: 1) where mutations occur in the genome 2) how selective pressures act on a) neutral, b) harmful and c) beneficial mutations and 3) how the genome serves as a historical record of selection. Then the instructor may employ a rubric to score each section of the essay.

**VI. Vendors for supplies (catalog numbers in parentheses)**

Note: The authors have discussed with vendors the possibility of offering the reagents as a commercially available kit. We will update this information if that occurs. Until then, the following vendors are suggested.

**Materials Vendor\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Plasmids containing h, om or sm CG Author Tina Hubler, 256-765-4761, trhubler@una.edu

genes or variable regions

PCR tubes (TFI-0201 or TBI-0501) Bio-Rad 1-800-424-6723

Gel electrophoresis apparatus : Mini-Sub Cell

Gel System (164-0300 includes power

supply, electrophoresis cell, gel tray,

comb and gel caster)

Thermal cycler (PTC-1148C)

Agarose (161-3103)

Electrophoresis buffer, 50X Tris/Acetate/

EDTA (161-0743

Primers for PCR Eurofins MWG Operon 1-800-688-2248 or

Integrated DNA Technologies 1-800-328-2661

DNA ladder (G5711) Promega 1-800-356-9526

GoTaq PCR Master Mix (M7112)

Nuclease free water (DW0991)

Methylene blue stain (875911) Carolina Biologicals (1-800-334-5551)