Supplemental Materials for Teachers, ABT Materials

I. PCR and Electrophoresis

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 ^{o}C, (2) annealing of primers to the template DNA at approximately 45-65 ^{o}C and (3) synthesis of new DNA at approximately 68-72 ^{o}C. 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 ^{o}C	5 min	initial denature step
25-35 cycles:		
	95 ^{o}C	30 sec denature step
	55 ^{o}C - 65 ^{o}C	30 - 60 sec annealing primer
	72 ^{o}C	90 - 120 sec extension step
72 ^{o}C	7 min	final extension step

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

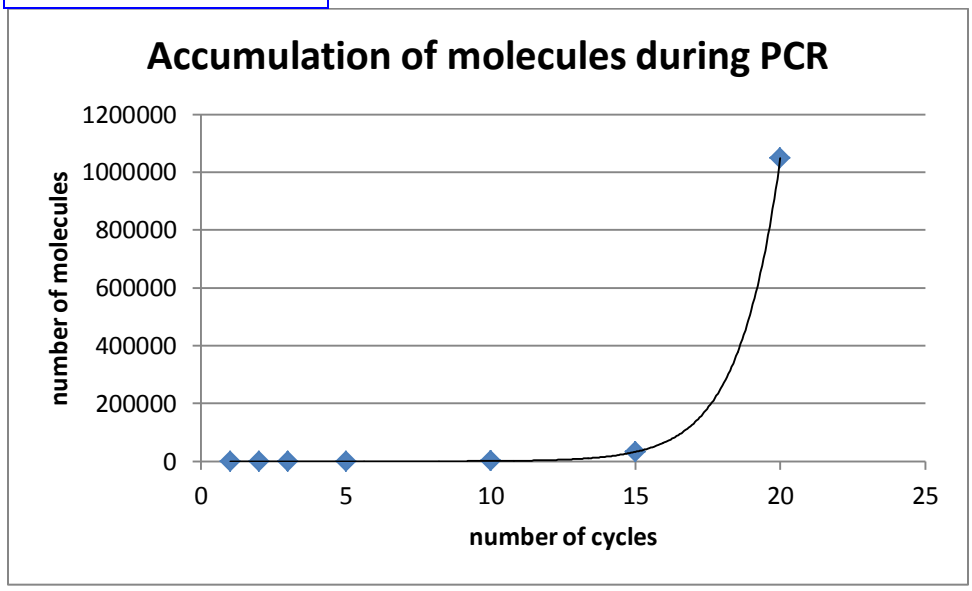
http://nld.promega.com/resources/product-guides-and-selectors/protocols-and-applications-guide/pcr-amplification/#title6

http://cdn.idtdna.com/Support/Technical/TechnicalBulletinPDF/A_Basic_PCR_Protocol.pdf

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.

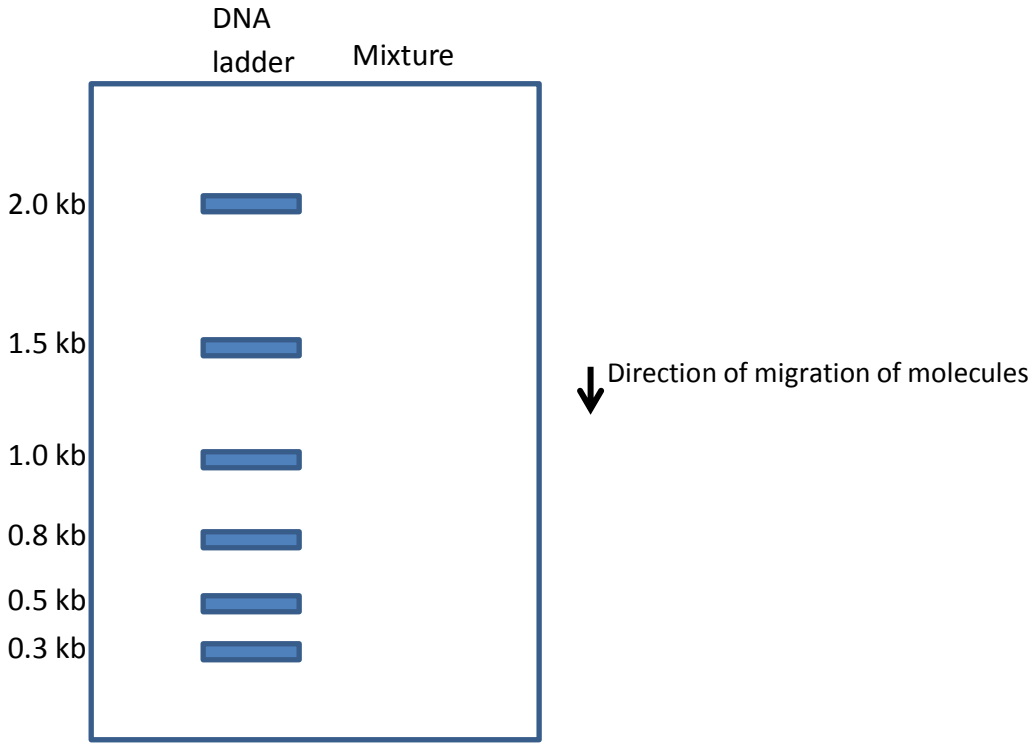
The gel is submerged in an electrophoretic chamber containing buffer (usually Tris Acetate-EDTA, pH=8). Samples are loaded into the sample wells and current is applied to the chamber. At pH=8, nucleic acids are negatively charged and travel under the influence of the electric field toward the opposite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



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 aaaaaaaaaa aaaaaaacta ttaatcattt caagaccagg
acagaagagc attaatgcaa gagtagggc

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 ataccctgg 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
ctggggtgct cagctgaggt gctggccccc agacacatgc ccactctccc acccacatgg
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
gggaactgaa atgttgggac atctcaggtc ctctgggctg tggggtgggc tctgaaaggc
aggtgtctgg gtggcaggtc ctgaataaga catgtcaggc agggtccctg ggtccttgag
ggtggtgtac ccctggggat gggccagggc tcagggcttc agtctcaggc tcggctgaag
caccggtctt gtcccagggg ctgctgctgt gtctgctgct cagcacgggt ggggcatggg
catccaatga gccacttcgg ccactgtgcc gccccaccca tgccatcctg gctgctgaga
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
gcttcagagg tggcaggaag gggtgaattt cccccgctgg gaagtaatgg gaggagacta
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
agtagatgct tgttgaattc

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
ccgccccggc tcccgctcac cccgcccgtc cccgcagtgc ctcccctgcg gccccggggg
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

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

1. Lander, E.S., et al., *Initial sequencing and analysis of the human genome.* Nature, 2001. **409**(6822): p. 860-921.
2. Cordaux, R. and M.A. Batzer, *The impact of retrotransposons on human genome evolution.* Nat Rev Genet, 2009. **10**(10): p. 691-703.
3. van Hoek, A.H., et al., *Acquired antibiotic resistance genes: an overview.* Front Microbiol, 2011. **2**: p. 203.

4. Schmitz, J., *SINEs as driving forces in genome evolution.* Genome Dyn, 2012. **7**: p. 92-107.
5. Dombroski, B.A., et al., *An in vivo assay for the reverse transcriptase of human retrotransposon L1 in Saccharomyces cerevisiae.* Mol Cell Biol, 1994. **14**(7): p. 4485-92.
6. Yadav, V.P., et al., *Characterization of the restriction enzyme-like endonuclease encoded by the Entamoeba histolytica non-long terminal repeat retrotransposon EhLINE1.* FEBS J, 2009. **276**(23): p. 7070-82.
7. Ohshima, K. and N. Okada, *SINEs and LINEs: symbionts of eukaryotic genomes with a common tail.* Cytogenet Genome Res, 2005. **110**(1-4): p. 475-90.
8. Singer, M.F., *SINEs and LINEs: highly repeated short and long interspersed sequences in mammalian genomes.* Cell, 1982. **28**(3): p. 433-4.
9. Batzer, M.A. and P.L. Deininger, *Alu repeats and human genomic diversity.* Nat Rev Genet, 2002. **3**(5): p. 370-9.
10. Deininger, P., *Alu elements: know the SINEs.* Genome Biol, 2011. **12**(12): p. 236.
11. Jurka, J. and T. Smith, *A fundamental division in the Alu family of repeated sequences.* Proc Natl Acad Sci U S A, 1988. **85**(13): p. 4775-8.
12. Schmitz, J., *SINEs as driving forces in genome evolution.* Genome Dyn. **7**: p. 92-107.
13. Shen, S., et al., *Widespread establishment and regulatory impact of Alu exons in human genes.* Proc Natl Acad Sci U S A. **108**(7): p. 2837-42.

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.

Question sheet for *Into the Jungle* Sean B. Carroll, In Cold blood: the Tale of the Icefish

A Bloodless Fish?

1. The bloodless fish was discovered accidentally during commercial whaling expeditions. One of the researchers, Rudd, noted "it was impossible because…all vertebrates share…presence of O_2-binding hemoglobin in red blood cells". What does this tell you about the nature of current knowledge (dogma)?

2. When blood was centrifuged what was absent in the bloodless icefish?

3. How does O_2 capacity of icefish compare to red-blooded relatives?

A Matter of Degrees

4. Arctic waters are on average -1.9 ^oC. The freezing point of water is 0 ^oC. Why don't organisms freeze? Remember freezing point depression from chemistry and what causes it.

5. What geologic changes may have occurred around Antarctica ~ 33-34 MYA (million years ago)?

6. What happened to water temperatures ~ 15 MYA, about the same time that icefish fossils appear in fossil records?

7. What type of molecule is AFGP? What does glyco mean?

8. The AFCP are composed of repeats of what 3 amino acids?

9. Draw the structure of threonine and show where you can add sugar groups to an amino acid.

10. How does AFGP prevent ice crystals forming in the icefish circulation?

11. The AFGP DNA in icefish is extraordinarily similar to the trypsinogen gene. What regions of the AFGP gene are found in the trypsinogen gene?

12. What does this degree of similarity suggest?

13. In what part of the trypsinogen gene is a repetitive sequence found?

14. How is this region (in #13) of a gene normally used?

15. The relationship of AFGP to trypsinogen suggests a mechanism for genetic (molecular) evolution. It is:
    a) tinkering with materials (DNA) already available
    b) inventing new DNA

<u>Fossil Genes</u>

16. Hemoglobin protein is made from α-globin and β-globin genes. What is the status of these in icefish?
    α-globin is_____ β-globin is _____

17. A nonfunctional remnant of a gene is a _____ (a molecular fossil).

18. What features of the icefish cardiovascular system allow the icefish to survive without hemoglobin?
a) b) c)

19. What happened to the myoglobin gene in icefish that caused it to become a molecular fossil?

The DNA Record of Evolution

20. Paleontologist Simpson states "species evolve …as if they are adapting as best they could to a changing world, and not at all as if they were moving toward a set goal".
Below is a summary of the proposed evolution of icefish based on geological and molecular evidence. Fill in the blanks.

Time, MYA	geologic conditions	Antarctic fish types
> 40	warm water	red-blooded fish (sharks, rays, catfish)
33-34	antarctic separated	
15-25	water temperatures dropped to below freezing	fish with AFGPs (e.g. notothenoids)
14	thick sea ice formed	
2-8		icefish with loss of _____gene function
<2 and still occurring		icefish with changes in _____ genes

21. Evolution is not necessarily progressive i.e. developing new traits. Sometimes genes and functions are _____.

22. What may happen to icefish if waters warm again?
_____ or _____

V. Vendors for supplies (catalog numbers in parentheses)

Author Tina Hubler, 256-765-4761, trhubler@una.edu

Plasmids containing h, om or sm CG genes or variable regions

BioRad 1-800-424-6723

PCR reagents

PCR tubes (TFI-0201 or TBI-0501)

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)

Eurofins MWG Operon 1-800-688-2248

Primers for PCR

Integrated DNA Technologies 1-800-328-2661

Primers for PCR

Promega 1-800-356-9526

DNA ladder (G5711)

GoTaq PCR Master Mix (M7112)

Nuclease free water (DW0991)

Carolina Biologicals (1-800-334-5551)

Methylene blue stain (875911)