INQUIRY & INVESTIGATION

Laboratory Activities to Support Student Understanding of the Molecular Mechanisms of Mutation & Natural Selection

TINA HUBLER, PATTI ADAMS, JONATHAN SCAMMELL

Abstract

The molecular basis of evolution is an important and challenging concept for students to understand. In a previous article, we provided some of the scientific background necessary to teach this topic. This article features a series of laboratory activities demonstrating that molecular events can alter the genomes of organisms. These activities are most appropriate for undergraduate students in Honors Biology, Genetics, or Molecular Biology courses. Student laboratory instructions are included to allow students to conduct the activities, make observations, interpret the results, and draw conclusions.

Key Words: *Molecular evolution; mutation; natural selection; conserved DNA sequences.*

The exercises provided here are designed to help students visualize how molecular evolution occurs by acquainting students with the basic principles of variable and conserved DNA sequences. These activities also provide living examples of organisms that illustrate the roles that mutation and selection have played in their evolution. Prerequisites for the students are a basic understanding of DNA structure, replication, transcription, translation, and mutation. A recommended classroom discussion and lab activity sequence are shown in Table 1, student instruction sheets are included in Appendix 1, and Appendix 2 is the teachers' key for student instructions.

> *The molecular basis of evolution is an important and challenging concept for students to understand.*

○ TASK 1

Classroom Discussion of Molecular Evolution Concepts

The activities described in this article should be prefaced with a classroom discussion of basic molecular evolution concepts. In a previous article, we provided some of the scientific background necessary to discuss the classification of genomic sequences, mutations, and the effects of selective pressure on DNA sequence variability. Discussing these topics will help students understand the basis for the activity results they will observe.

○ TASK 2

Lab Preparation: The Principles of PCR & Electrophoresis

Because polymerase chain reaction (PCR) and electrophoresis are powerful tools that allow researchers to produce copies of selected regions of DNA and visualize them, instructors should discuss the basic components and steps involved (Table 2). Background information for discussing these techniques, as well as simple activities to enhance student understanding, are available in a file of Supplemental Materials at http://www.buildingthepride.com/faculty/trhubler/.

○ TASK 3 (Activity 1)

Variability of Nonfunctional DNA Sequences

Data from the Human Genome Project and similar sequencing projects have allowed researchers to compare the genomes of a variety of organisms. When DNA sequences of organisms are compared, the sequences located in nonfunctional regions of the genome tend to exhibit considerable variability among organisms. Activity 1 demonstrates the tendency for nonfunctional DNA sequences to exhibit variation, even in closely related organisms. In this activity, students first use PCR to amplify a nonfunctional DNA sequence from two closely related primate species and gel electrophoresis to visualize the PCR products. Next, students utilize the BLAST tool available from the National Center for Biotechnology website (http://www.ncbi.nlm.nih.gov) to investigate sequence variability between the two species and to identify a mobile genetic element that introduces changes in primate DNA sequences.

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Table 1. Summary of activities.

Task Order[a]	Task	Topics	Time Allotment[a] & Description
1	Class discussion	Genome organization, mutations, selection	Discuss: sequence classification (noncoding, coding, nonfunctional, functional), mutations, selection, nonfunctional sequence variability, functional sequence conservation.
2	Lab preparation	Principles of PCR and electrophoresis	Lab (2–3 hours). Discuss PCR and electrophoresis. Pour agarose gels and set up PCR reactions for Lab Activities 1 and 2. Completed PCR cycling reactions and agarose gels may be refrigerated until the next lab period.
3	Activity 1	Variability of nonfunctional DNA sequences	Lab (2–3 hours). Discuss Alu elements and the BLAST tool while performing electrophoresis. Review variable nonfunctional sequences. Stain gels, interpret results. Perform BLAST analyses. Students complete Lab Worksheet.
4	Activity 2	Conservation of functional DNA sequences	Lab (2–3 hours). Discuss New World and Old World primate evolutionary relationships during electrophoresis. Review conserved functional sequences. Stain gels, interpret results. Perform BLAST analyses. Students complete Lab Worksheet.
5	Reinforcement	Icefish as an example of molecular evolution	View the video about icefish evolution. Assign the recommended article about icefish evolution for reading.

[a]Instructors may need to adapt this to their available class and laboratory time.

Table 2. Materials needed for Tasks 3 and 4.

Plasmids: May be requested from corresponding author[a]
Primers[a]
Variable forward: 5′-AGTTCCTCTCTACCTTGTACC-3′
Variable reverse: 5′-GCCCTACTCTTGCATTAATGC-3′
CG forward: 5′-GCACCAAGGATGGAGATG-3′
CG reverse: 5′-GCGGATTGAGAAGCCTTTA-3′
DNA ladder[a]
GoTaq Green PCR Master Mix[a]
Nuclease-free water[a]
PCR tubes,[a] appropriate for thermal cycler
Pipettors and tips[a]
Agarose gels (1% agarose in pH = 8 Tris acetate/EDTA electrophoresis buffer)[a,b]
Electrophoresis system: Power supply, electrophoresis cell, gel tray, comb, and gel caster[c]
Thermal cycler[d]
Methylene blue staining solution[a]

The following safety precautions should be observed:
[a]Wear gloves.
[b]Wear eye protection when pouring hot solutions.
[c]High voltage; disconnect power before opening chambers.
[d]Thermal cycler components reach high temperatures during PCR cycling.
Suggested vendors are listed in a file of Supplemental Materials at http://www.buildingthepride.com/faculty/trhubler/.

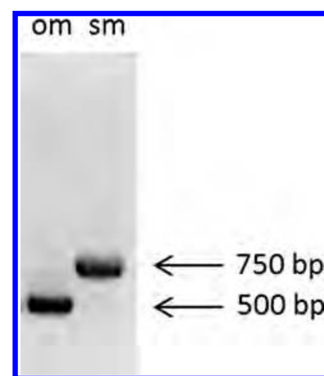

Figure 1. Results of PCR amplification of a variable region from two species of New World primates: owl monkey (om, *Aotus trivirgatus*) and squirrel monkey (sm, *Saimiri boliviensis*).

For each of the PCR reactions in Activity 1, the template DNA is provided in the form of plasmid DNA containing an intergenic region from owl monkey (om) or squirrel monkey (sm), two species of Platyrrhines, or New World monkeys. These plasmids were developed in our research laboratories, specifically for use in Activity 1. Instructions for setting up the PCR reactions are included in Appendix 1. Figure 1 shows that PCR, gel electrophoresis, and staining reveal a 500-bp product from the owl monkey sample, whereas the squirrel monkey sample contains a 750-bp product. These data demonstrate that nonfunctional regions contain sequences that can differ, even among closely related organisms (two New World monkeys).

Next, students will use the BLAST tool to determine the reason for the difference in size of the PCR products. DNA sequences for the PCR products were either generated in our labs (owl monkey) or obtained from the Roos lab (squirrel monkey) (Osterholz et al., 2008). These DNA sequences are provided below and are available electronically in Supplemental Materials. When students compare the nucleotide sequences of these nonfunctional DNA regions, they should observe that the size difference in the PCR products results from an insertion into the squirrel monkey sequence.

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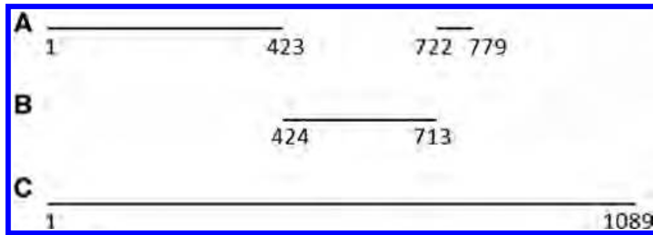

Figure 2. Schematic of the results of BLAST alignment of DNA regions from squirrel monkey (sm, *Saimiri boliviensis*), owl monkey (om, *Aotus trivirgatus*), and human (h, *Homo sapiens*). (A) BLAST alignment of the sm and om variable regions. (B) BLAST alignment of the sm variable region and an Alu element database. (C) BLAST alignment of the sm, om, and human chorionic gonadotropin (CG) genes. The numbers below each alignment indicate the position in the sm DNA sequence.

Specifically, the two sequences align except in a region toward one end of the squirrel monkey sequence. This gap in alignment represents a region that is present in the squirrel monkey sequence but absent in the owl monkey sequence (Figure 2A).

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

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

Finally, students will use the BLAST tool to compare the squirrel monkey sequence to a database of currently known "Alu sequences," a type of mobile genetic element. As diagrammed in Figure 2B, this will allow them to identify the inserted region in the squirrel monkey DNA as an Alu element. Mobile genetic elements are DNA sequences that can be inserted into new locations in the genome through the action of specific enzymes. Alu elements are a common type of mobile genetic element found in the human genome (Batzer & Deininger, 2002). Insertion of Alu sequences has several impacts on genomes. Not only do they produce genetic variation, they may also disrupt regulatory regions and coding regions (Schmitz, 2012). These data demonstrate that one source of genomic variability is the insertion of DNA sequences via mobile genetic elements.

"Student Instructions for Activity 1: Variability of Nonfunctional DNA Sequences" in Appendix 1 provides guidance for students to perform the activity. BLAST results showing actual sequence alignments and additional information about Alu elements as important mechanisms of molecular evolution are provided in a file of Supplemental Materials at http://www.buildingthepride.com/faculty/trhubler/. Some important points to discuss with students are that Alu elements (1) are mobile, (2) are inserted >1 million times in the human genome, (3) contribute to a dynamic expansion of primate genomes, and (4) are known to cause human diseases such as hemophilia and breast cancer, as a result of disrupting gene coding sequences (Batzer & Deininger, 2002; Kramerov & Vassetzky, 2005).

○ TASK 4 (Activity 2)

Conservation of Functional DNA Sequences

The sequencing and comparison of genomes from a variety of organisms have revealed that although genomes are constantly changing, some regions exhibit remarkable similarity among organisms. As a result of comparative genomics, it is now understood that DNA sequences that perform important cellular functions tend to be similar, or conserved. Lab Activity 2 is intended to help students visualize conservation of functional DNA sequences. PCR is used to demonstrate that the DNA sequence of an important gene is conserved among two groups of primates: Catarrhines and Platyrrhines.

Catarrhines include Old World monkeys such as macaques and baboons, as well as chimpanzees, apes, and humans; they are found primarily in Africa and southern Asia, with the exception of humans, which are widespread. Platyrrhines include the New World monkeys, such as squirrel monkeys and owl monkeys, that inhabit Central and South America. Catarrhines and Platyrrhines diverged from a common ancestor >35 million years ago (Goodman et al., 1998; Schrago & Russo, 2003; Perelman et al., 2011). Although Catarrhines and Platyrrhines have evolved genetic and physiological differences over millions of years in geographic isolation (Müller et al., 2004; Westberry et al., 2006; Ward & Vallender, 2012), many functional DNA sequences, including genes coding for peptide hormones, have been conserved.

In Activity 2, students will (1) use PCR to amplify the chorionic gonadotropin (CG) gene from three primate species, (2) analyze the PCR products by electrophoresis, and (3) perform sequence comparisons to demonstrate that functional DNA sequences are conserved. CG is a peptide hormone made in primates by placental cells

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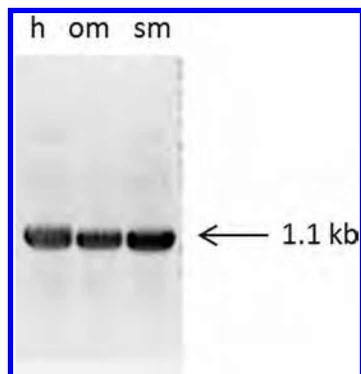

Figure 3. Results of PCR amplification of the chorionic gonadotropin gene from human (h, *Homo sapiens*), owl monkey (om, *Aotus trivirgatus*), and squirrel monkey (sm, *Saimiri boliviensis*).

Table 3. Comparison of the nucleotide similarity of selected genes

Genes compared[a]	Percent Identical Nucleotides
h CG vs om CG	83
h CG vs. sm CG	80
sm CG vs. om CG	92
h CG vs. h Growth Hormone	No similarity
h CG vs. h Oxytocin	No similarity

[a]Abbreviations: h = human, om = owl monkey, sm = squirrel monkey, and CG = chorionic gonadotropin.

during the first 10 weeks of pregnancy. It supports the implantation and development of the fetus (Hanson et al., 1971). CG therefore represents a critical gene for reproductive success in primates.

Plasmids containing the CG gene from humans, owl monkeys, or squirrel monkeys were developed in our research laboratories for use as the template for PCR. Instructions for setting up the PCR reactions are included in Appendix 1. Figure 3 shows that PCR, electrophoresis, and staining reveal a 1.1-kb PCR product in each sample lane.

Next, students will determine the similarity between Catarrhine and Platyrrhine CG gene sequences using the BLAST tool. The DNA sequences for BLAST analyses are available electronically in a file of Supplemental Materials at http://www.buildingthepride.com/faculty/trhubler/ or can be obtained from NCBI (http://www.ncbi.nlm.nih.gov) using the following accession numbers: human CG (X00265.1), squirrel monkey CG (GU117708.1), owl monkey CG (JN613228), human growth hormone (E00140.1), and human oxytocin (M11186.1). The squirrel monkey and owl monkey CG sequences were generated in our research labs (Vasauskas et al., 2010). DNA sequences for growth hormone and oxytocin are employed as examples of unrelated genes. Figure 2C shows graphically that the squirrel monkey, owl monkey, and human DNA sequences align throughout the entire length of the PCR products. The BLAST results will also display the percent identical nucleotides and should agree with the information in Table 3. These data (1) provide students with a method for quantitative assessment of sequence similarity, (2) demonstrate that functional DNA sequences are similar among organisms, (3) reveal higher sequence conservation among Platyrrhines than between Catarrhines and Platyrrhines, and (4) indicate the degree of similarity that is observed in conserved DNA sequences. We do not expect 100% identity, because neutral mutations contribute to variability. “Student Instructions for Activity 2: Conservation of Functional DNA Sequences” in Appendix 1 provides guidance for students in performing the activity.

○ TASK 5 (Reinforcement)

Icefish as an Example of Molecular Evolution

After observing examples of variable and conserved sequences, we suggest that teachers emphasize the mutually important roles of mutation and selection by providing a living example of the effect of beneficial mutations on organisms. Icefish are believed to have evolved from a population of temperate-environment fish. The fish were exposed, over time, to a significant drop in water temperature due to changes brought about by continental drift. Because of mutations, some fish produced a protein that defended them against freezing temperatures. Fish with this increased hardiness survived and passed the new trait and the gene that controlled it to their offspring. This is a classic example of natural selection.

To reinforce how molecular changes contribute to survival and diversity, we recommend a short video about icefish that is found on the DVD titled *The Making of the Fittest*. It is available at no charge from the Howard Hughes Medical Institute (http://www.hhmi.org) and contains high-quality videos on natural selection in fish, rock pocket mice, and humans. Additionally, we recommend the story “In Cold Blood: The Tale of the Icefish” for students with some knowledge of genetics (Carroll, 2009). In the story, Sean Carroll provides a vivid historical account of the discovery of the icefish and its evolutionary implications. A question sheet to help students read the article is available in a file of Supplemental Materials (http://www.buildingthepride.com/faculty/trhubler/). These examples elucidate a definitive relationship between mutations, selection, and evolution.

○ Summary

To conceptualize the process of molecular evolution, students need to understand mechanisms that contribute to the dynamic nature of genomes and the effect that natural selection has on sequence conservation. Our instructional series includes classroom discussion of basic molecular-evolution concepts followed by two lab activities. The activities use analyses of variable and conserved DNA sequences to demonstrate how selective pressure affects the persistence of mutations in populations. For reinforcement of the process by which evolution occurs, the molecular evidence recorded in the genome of the icefish is used to explain how mutation followed by natural selection produces changes in organisms.

○ Acknowledgments

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Appendix 1

Student Instructions for Activity 1: Variability of Nonfunctional DNA Sequences

PCR of Nonfunctional DNA Sequences

This activity will be used to illustrate that nonfunctional DNA sequences are variable, even in closely related organisms. PCR will be performed to show that an intergenic nonfunctional region exhibits variability in two species of New World monkeys: squirrel monkeys and owl monkeys. DNA sequences for the variable region will be used to identify the nature of the variation.

Plasmid DNA containing the variable region from owl monkeys or squirrel monkeys is used as the template for PCR. The components needed for a 25-μL reaction in a 200-μL PCR tube and the PCR conditions are listed below (Table A1). Following PCR, samples are loaded onto 1% agarose gels and electrophoresed at 100 V for 55–60 minutes. A DNA ladder should be loaded into a separate well to estimate PCR product size. The gels are stained with methylene blue gel stain according to the manufacturer's instructions. PCR products are visualized by placing the gel on a white illuminated surface.

Investigating the Reason for the Difference in Size of the PCR Products

The DNA sequence for the squirrel monkey PCR product will be provided electronically. The insertion of a DNA sequence into the squirrel monkey variable region can be detected by accessing the BLAST page (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and following these steps. Select "Align" under Specialized BLAST. Copy and paste the squirrel monkey sequence into the query box and the owl monkey sequence into the subject box. Select "somewhat similar sequences" under Program Selection. At the bottom of the page select "BLAST." Next, the insertion can be identified as an Alu element, a type of mobile DNA sequence, by following these steps at the BLAST page. Select "nucleotide blast." Copy and paste the squirrel monkey sequence into the query box. In the second box, select the database "Human Alu repeat elements" from the dropdown list. At the bottom of the page select "BLAST."

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Table A1. PCR setup.

PCR Components	
Component	**Volume (µL)**
DNA template (dilute to 10 ng/µL)	1.0
Forward primer (10 µM) 5′-AGTTCCTCTCTACCTTGTACC-3′	1.0
Reverse primer (10 µM) 5′-GCCCTACTCTTGCATTAATGC-3′	1.0
GoTaq Green Master Mix	12.5
Nuclease-free water	9.5

PCR Conditions		Record of PCR Observations	
Stage	**Conditions**	**Sample**	**PCR Product Size**
Initial denaturation	94°C, 1 minute	1. Owl monkey	
		2. Squirrel monkey	
Cycling (30 times): Denaturation Annealing Elongation	 94°C, 1 minute 54°C, 30 seconds 72°C, 90 seconds		
Final extension	72°C, 5 minutes		

BLAST Interpretations

1. Sketch the results of the BLAST of sm and om variable regions. Approximately how many nucleotides are inserted into the squirrel monkey region? Indicate this on your sketch.
2. Sketch the results of the BLAST of the sm variable region and the database of Alu sequences. How does this compare to the sketch above?

Concluding Questions

1. Owl monkeys and squirrel monkeys are closely related primates of the parvorder Platyrrhini (New World monkeys). What do your observations of PCR products tell you about DNA sequences in nonfunctional regions of closely related organisms?
2. In this example, what is the form of genetic variability (substitution, insertion, or deletion) that occurs in a nonfunctional region of the genome?
3. Based on lecture discussions of mutation and natural selection, describe in your own words why mutations in nonfunctional regions may persist over many generations and lead to high variability in these regions.

Student Instructions for Activity 2: Conservation of Functional DNA Sequences

Students who complete this activity will use PCR to begin to understand conservation of functional DNA sequences among two groups of primates: Catarrhines and Platyrrhines. Catarrhines include Old World monkeys such as baboons and rhesus macaques as well as chimpanzees, apes, and humans. Catarrhines primarily inhabit Africa and southern Asia, with the exception of humans, whose distribution is widespread. By contrast, Platyrrhines such as squirrel monkeys, marmosets, and owl monkeys inhabit Central and South America. Catarrhines and Platyrrhines evolved independently from a common ancestor >35 mya. Still, functional DNA sequences have been conserved. The sequences of the PCR products will be provided electronically for evaluation of the similarity in the DNA sequences.

In this activity, the size and DNA sequence of the primate chorionic gonadotropin (CG) gene will be compared. CG is a peptide hormone made in primates by placental cells during the first 10 weeks of pregnancy. It supports the implantation and development of the fetus. CG therefore represents a critical gene for reproductive success in primates.

Plasmid DNA containing the CG gene from humans, owl monkeys, or squirrel monkeys will be used as the template for PCR. The PCR components and settings are indicated below (Table A2). PCR products are fractionated and visualized using methylene blue stain as in Lab Activity 1.

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Table A2. PCR setup.

PCR Components	
Component	**Volume (µL)**
DNA template (dilute to 10 ng/µL)	1.0
Forward primer (10 µM) 5′-GCACCAAGGATGGAGATG-3′	1.0
Reverse primer (10 µM) 5′-GCGGATTGAGAAGCCTTTA-3′	1.0
GoTaq Green Master Mix	12.5
Nuclease-free water	9.5

PCR Conditions		Record of PCR observations	
Stage	**Conditions**	**Sample**	**PCR Product Size**
Initial denaturation	94°C, 1 minute	1. Owl monkey	
		2. Squirrel monkey	
		3. Human	
Cycling (30 times): Denaturation Annealing Elongation	 94°C, 1 minute 54°C, 30 seconds 72°C, 90 seconds		
Final extension	72°C, 5 minutes		

Determining the Similarity between Catarrhine & Platyrrhine CG Gene Sequences

DNA sequences for the PCR products and for the unrelated genes, growth hormone and oxytocin, will be used for sequence comparisons. On the BLAST web page (http://blast.ncbi.nlm.nih.gov/Blast.cgi), select "align two sequences." Copy and paste the human sequence into the query box. Copy and paste the squirrel monkey sequence into the subject box. At the bottom of the page select "BLAST." Results will list the percent identical nucleotides. Repeat this to compare the other sequences.

BLAST Interpretations

Genes Compared	Percent Identical Nucleotides
1. hCG vs. om CG	
2. hCG vs. sm CG	
3. sm vs. om CG	
4. hCG vs. h growth hormone	
5. h CG vs. h oxytocin	

Concluding Questions

1. What do these results suggest about the similarity of functional DNA sequences?
2. Why do you think the sequence similarity is higher among Platyrrhines?
3. Why do you think 100% sequence similarity is not observed in the functional sequences?
4. Based on lecture discussions of mutation and natural selection, describe in your own words why conserved sequences persist in functional regions of genomes.

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APPENDIX 2: Teachers' Key for Student Instructions

Activity 1: Variability of Nonfunctional DNA Sequences

PCR Observations

1. 500 bp
2. 750 bp

BLAST Interpretation

1. The sketch should show that the sequences align, except that there is a gap toward one end of the sm sequence, representing a region that is NOT found in the om sequence (250 bp).
2. The sketch should show that the region missing in the sm sequence in the first sketch is the same region that aligns with an Alu sequence, thus identifying it as similar to an Alu sequence.

Conclusions

1. The sequences can be variable, even among closely related organisms.
2. Alu insertion
3. Mutations in nonfunctional regions have no effect on organism survival and are not selected out (organisms are not weakened or do not die).

Activity 2: Conservation of Functional DNA Sequences

PCR Observations

1. 1100 bp
2. 1100 bp
3. 1100 bp

BLAST Interpretation

1. 83%
2. 80%
3. 92%
4. No similarity
5. No similarity

Conclusions

1. Functional sequences remain similar among organisms.
2. Organismal relationships are based on morphological and physiological characteristics. These characteristics result from the use of DNA sequences that are used to produce proteins. Platyrrhines are more closely related to one another than Platyrrhines are to Catarrhines; thus, their DNA sequences are expected to be more similar.
3. Neutral and beneficial mutations persist because they either have no effect or provide an advantage to the organism, respectively.
4. Harmful mutations in functional sequences weaken organisms, reduce their reproductive capacity, and/or cause them to die. Therefore, harmful mutations are not likely to be passed to offspring. Neutral mutations in functional sequences contribute to some variability. Beneficial mutations in functional regions confer an advantage and may increase life span and/or reproduction. If reproduction is enhanced, more offspring harbor the beneficial mutation and it is passed to future generations. Therefore, as a result of removal of harmful mutations and transmission of beneficial mutations, functional sequences remain similar (conserved) from one generation to the next.

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