Supplemental Materials for
Who Scared the Cat? A Molecular Crime Scene Investigation Laboratory Exercise

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APPENDIX 1: LECTURE MATERIALS
LECTURE 1

DNA structure, agarose gel electrophoresis, and quantification of DNA concentration and purity

NOTE: Students will need clickers
DNA: The ultimate instruction manual

• 3,080,000,000 characters (750 MB data or 5000 textbooks)
  – Only 4 letters make up the code

• Human possess approx. 29,000 protein-coding genes on 23 chromosomes
  – Approximately 1000 genes per chromosome

• All of this information is in each and every one of our 60-90 trillion

http://askabiologist.asu.edu/explore/dna-abcs
The structure of deoxyribonucleic acid (DNA)

Nucleus

Sugar phosphate backbone

Base (nucleotide)

Hydrogen bond

Adapted from: http://campus.udayton.edu/~hume/DNA/DNA.htm
CLICKER QUESTION 1

What is the directionality of DNA?

A. 5’ to 3’
B. 3’ to 5’
C. 4’ to 2’
The double helix

Adapted from www.genome.gov

www.wikipedia.org
Phosphodiester Bond

Adapted from: http://www.web-books.com/MoBio/Free/Ch3A5.htm
Which nucleotide base pairs with guanine?

A. Adenine
B. Thymine
C. Cytosine
D. Guanine
The phosphodiester bond forms between the 5’ PO₄ and ________

A. 1’ OH
B. 2’ OH
C. 3’ OH
D. 4’ OH
DNA Replication

DNA Replication Video!

http://universe-review.ca/F11-monocell.htm
CLICKER QUESTION 4

What is the purpose of helicase in DNA replication?

A. Unwinds the dsDNA into single strands
B. Adds a short single stranded oligo to initiate DNA synthesis
C. Forms phosphodiester bonds
DNA Agarose Gel Electrophoresis

DNA is negatively charged, so it moves toward the anode. The agarose gel matrix retards large molecules more than small, so small DNA fragments move a further distance through the gel.

REMEMBER: ALWAYS RUN TO RED!!!
DNA Agarose Gel Electrophoresis

Ethidium bromide (EtBr) is typically used to allow for visualization of the DNA under UV light.

- EtBr binds specifically to DNA and is excited by UV light; therefore expose gel to UV light and visualize DNA (wear eye protection).
- EtBr is a carcinogen!
- Alternative is GelRed (Phenix Research Products)
  - Detects DNA in an analogous way
  - Safer b/c it’s unable to enter cell membranes

DNA loading buffer (also called “loading dye” or “sample buffer” is added to DNA sample before loading the gel.

- Contains glycerol which is heavier than TBE or TAE buffers
- Contains one or more dyes in it, e.g., bromophenol blue
Agarose Gel Electrophoresis

Figure from Recombinant DNA Genes and Genomes- a Short Course book by Watson, Caudy, Myers and Witkowski (2007). http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html
What are we looking at?

Large DNA

Small DNA

A molecular weight marker (or DNA ladder)
CLICKER QUESTION 5

Refer to the agarose gel below. Which band is larger, A or B?

A. A
B. B
CLICKER QUESTION 6

Referring to the 1kb ladder diagram below, what size is band B?

A. 500 bp  
B. 1000 bp  
C. 2000 bp  
D. I don’t know
DNA Quantification

Most experiments require that a specific concentration of DNA is used

Concentration = mass/volume (ng/µL)
DNA Quantification

To quantify the amount of DNA, measure the absorbance at 260 nm and 280 nm.

One $A_{260}$ unit of double stranded DNA corresponds to 50 ng/µL.

To calculate DNA concentration, use the equation:

$\text{(A}_{260}\text{)} \times (50 \text{ ng/µL}) \times \text{(dilution factor)} = \text{DNA (ng/µL)}$
Dilution Factors

Often times, samples (including DNA) must be diluted for accurate measurement

• If too concentrated, machine will not be able to actively measure

To dilute a sample, add 1 part sample to multiple (X) parts buffer. This dilution factor would be denoted as \(1: (X+1)\).

For example: you diluted your sample by adding 1 µL DNA and 9 µL water. Thus, you would be performing a 1:10 dilution and your dilution factor would be 10.
You added 1 µL DNA to 3 µL water. What is your dilution factor?

A. 0.3
B. 3
C. 4
CLICKER QUESTION 8

You measure the absorbance of an undiluted sample and obtain an $A_{260}$ value of 0.1. What is the concentration of your DNA sample?

A. 5 ng/µL
B. 50 ng/µL
C. 500 ng/µL
DNA Purity

Absorbance ratios for pure samples:

DNA: \( \frac{A_{260}}{A_{280}} = 1.8 \)

RNA: \( \frac{A_{260}}{A_{280}} = 2.0 \)

Protein: \( \frac{A_{260}}{A_{280}} = 0.8 \)

Figure adapted from: http://www.laboratory-journal.com/applications/laborbedarf-laborgeraete/measure-your-purity
You measure the $A_{260/280}$ of your sample to be 1.24. What is true about your sample?

A. It’s pure DNA
B. It’s contaminated with mRNA
C. It’s contaminated with protein
LECTURE 2

PCR and Sanger sequencing

NOTE: Students will need clickers
Review of DNA Replication

http://universe-review.ca/F11-monocell.htm
Components of a PCR reaction

- Exponential, in vitro amplification of DNA
- Invented by Kary Mullis (1993 Nobel Prize)

Reaction components
- Template DNA
- DNA Primers
- Nucleotides
- Buffer
- DNA Polymerase (taq)
Template DNA and Primers

The antisense strand is used as a template to create a copy of the sense strand, and the sense strand is used as a template to create a copy of the antisense strand.
CLICKER QUESTION 10

The forward primer binds to:
A. Sense strand
B. Anti-sense strand
CLICKER QUESTION 11

When extended by *Taq* polymerase, the reverse primer makes a copy of which strand?
A. Sense strand
B. Anti-sense strand
## Steps of a PCR reaction

<table>
<thead>
<tr>
<th>STEP</th>
<th>TEMPERATURE</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denature</td>
<td>94°</td>
<td>1 min</td>
</tr>
<tr>
<td>Anneal</td>
<td>Variable*</td>
<td>1 min</td>
</tr>
<tr>
<td>Extend</td>
<td>72°</td>
<td>Variable**</td>
</tr>
</tbody>
</table>

**Repeat Denature, Anneal, and Extend approximately 30 times**

* For maximum stringency, annealing temperature should be $4(G+C)+2(A+T)$

**Rule of thumb: 1 minute per kilobase for extension time**
CLICKER QUESTION 12

What is the purpose of the annealing temperature of PCR?

A. Allows primers to bind to complimentary regions of template DNA
B. Separates the two strands of DNA
C. Optimal temperature for Taq polymerase to synthesize new strands of DNA
You want to amplify a 2 kb gene. What should the extension time be?

A. 0.5 minutes
B. 1 minute
C. 2 minutes
D. 4 minutes
PCR is exponential!

1. DNA is heated to 90°–100°C to separate the two strands.
2. The DNA is quickly cooled to 30°–65°C to allow short single-strand primers to anneal to their complementary sequences.
3. The solution is heated to 60°–70°C; DNA polymerase synthesizes new DNA strands, creating two new, double-stranded DNA molecules.

The entire cycle is repeated. Each time the cycle is repeated, the amount of target DNA doubles.

http://openwetware.org/wiki/BME100_f2013:W900_Group17_L4
Applications of PCR

• Make DNA for cloning or sequencing
• Genotyping - screening DNA to “see” if a specific sequence is present
• DNA fingerprinting (forensics/paternity testing)
You can determine the nucleotide sequence, one strand of DNA at a time, by using template DNA, DNA polymerase and one primer in a reaction along with the four dNTPs and one special nucleotide (ddNTP).

The special nucleotide (dideoxynucleoside triphosphate, ddNTP) is a **chain terminator** because it has a H instead of OH in the 3’ position. Thus, there is no “end” to use in order to form a phosphodiester bond and strand elongation is halted.

https://www.ocf.berkeley.edu/~edy/genome/sequencing.html
Sanger Sequencing

You set up four reactions (one for each ddNTP): add 4 “normal” dNTPs along with limiting quantities of one of the ddNTP.

DNA polymerase will use the template and the one primer to synthesize the complementary strand.

Most of the time the “normal” dNTP (e.g., dC) will be added.

However, at some point (occurs at random), a ddNTP (e.g., ddC instead of dC) will be incorporated and elongation stops.

Run reactions on a polyacrylamide gel and “read” sequence from bottom of film. Either the primer or the ddNTPs in the reactions are radiolabeled to be able to visualize bands on X-ray film.

DNA Sequencing – NOW!

Same general idea as before, but new technology available:
1. Fluorescently labeled ddNTPs (1 rxn, not 4)
2. Capillary electrophoresis (no more gels!)
3. Robots!
DNA Sequencing
How many primers are included in a single sequencing reaction?

A. 0
B. 1
C. 2
D. 3
CLICKER QUESTION 15

A chain terminator (ddNTP) used in sequencing lacks a 5’ PO$_4$.

A. True
B. False
LECTURE 3

in silico PCR and BLAST

NOTE: Students will need computers with Internet and Word processing capabilities
**In silico PCR**

Bioinformatics tool to predict the PCR products (or amplicons) generated based on the primer sequence and template DNA used.

- Provides information on:
  - DNA sequence
  - DNA size
  - DNA chromosomal location
  - The primer annealing temperature
Let’s do a search for the PCR products that will be obtained from the following dog primers:

**Primer Sequences:**
F: 5’CATTTTCTTTTCTCTGCTG 3’
R: 5’AACATAGCACTACCTCTTT 3’

http://genome.ucsc.edu/index.html
### in silico PCR Practice!

<table>
<thead>
<tr>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Species</th>
<th>PCR product size</th>
<th>Primer melting temp (Tm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGGGCAGCTTC</td>
<td>GGGAGAATTGA</td>
<td>Horse (<em>Equus caballus</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAAGAC</td>
<td>AGCTGGATGC</td>
<td></td>
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</tr>
<tr>
<td>ATGACTCGAGCT</td>
<td>ATTGCACGTGTG</td>
<td>Dog (<em>Canis familiaris</em>)</td>
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<tr>
<td>CAGAGGGGT</td>
<td>GCAAGTTC</td>
<td></td>
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<tr>
<td>TTTCTCCCACAG</td>
<td>ATGAGCACATC</td>
<td>Human (<em>Homo sapiens</em>)</td>
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<tr>
<td>AGCACTCG</td>
<td>GCTGAAGGGT</td>
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<td>ATGGGTGGCCCA</td>
<td>TTACTCGGCTGC</td>
<td>Cow (<em>Bos taurus</em>)</td>
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<tr>
<td>GTTCTCCAA</td>
<td>CTCCGCTGG</td>
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</table>

[http://genome.ucsc.edu/index.html](http://genome.ucsc.edu/index.html)
National Center for Biotechnology Information

- A series of databases relevant to biotechnology and biomedicine
  - Nucleotide sequences (GenBank)
  - Journal articles (Pubmed)
- Bioinformatics tools
  - Basic local alignment search tool (BLAST)
- Maintained by the National Institutes of Health
  - Division of the National Library of Medicine

Basic Local Alignment Search Tool


- Used to compare your favorite nucleotide or protein sequence to a database of known sequences
- Why do we need sequence similarity searching?
<table>
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<tr>
<th>Types of BLAST searches</th>
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</thead>
<tbody>
<tr>
<td><strong>nucleotide blast</strong></td>
</tr>
<tr>
<td>Search a <strong>nucleotide</strong> database using a <strong>nucleotide</strong> query</td>
</tr>
<tr>
<td><em>Algorithms:</em> blastn, megablast, discontiguous megablast</td>
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<tr>
<td><strong>protein blast</strong></td>
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<tr>
<td>Search <strong>protein</strong> database using a <strong>protein</strong> query</td>
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<tr>
<td><em>Algorithms:</em> blastp, psi-blast, phi-blast</td>
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<td><strong>blastx</strong></td>
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<tr>
<td>Search <strong>protein</strong> database using a <strong>translated nucleotide</strong> query</td>
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<td><strong>tblastn</strong></td>
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<tr>
<td>Search <strong>translated nucleotide</strong> database using a <strong>protein</strong> query</td>
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<td><strong>tblastx</strong></td>
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<tr>
<td>Search <strong>translated nucleotide</strong> database using a <strong>translated nucleotide</strong> query</td>
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</table>

Let’s BLAST our dog *in silico* PCR data

> Doginsilicoresults
CATTTTTCTTTCTCTGCTGgggcccctttctttgacactctctcactcggtggtcctctgt
cccctcaaccccatctgtggctgcgtctttcagaaacttcaacctctcttccctacc
ctcatctgcaactccatcattgacccctttcatctacgcctttccgagccagga
gtccgaaagactctccAAGAGGTAGTGCTATGTT
Your Turn!

BLAST the predicted PCR product sequences from your *in silico* PCR (should be in Word file) and fill out the following table for the top hit.

- Use nucleotide BLAST (BLASTn)
- Database should be set to “Other”


<table>
<thead>
<tr>
<th>PCR Product</th>
<th>Accession Number</th>
<th>Gene</th>
<th>E-value</th>
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KEY FOR CLICKER QUESTIONS AND CLASSROOM ACTIVITIES
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</tbody>
</table>
**In Silico PCR**

Let’s do a search for the PCR products that will be obtained from the following dog primers:

**Primer Sequences:**

F: 5’CATTTTCTTTTCTCTGCTG  (49 C)
R: 5’AACATAGCACTACCTCTTT  (43.9 C)

PCR PRODUCT = 203 BP

[http://genome.ucsc.edu/index.html](http://genome.ucsc.edu/index.html)
# in silico PCR Practice!

<table>
<thead>
<tr>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Species</th>
<th>PCR product size</th>
<th>Primer melting temp (Tm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGGGCAGCTTC</td>
<td>GGGAGAATTGA</td>
<td>Horse (Equus caballus)</td>
<td>66 bp</td>
<td>F: 56.3</td>
</tr>
<tr>
<td>CAAGAC</td>
<td>AGCTGGATGC</td>
<td></td>
<td></td>
<td>R: 63.2</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ATGACTCGAGCT</td>
<td>ATTCAGCACGTGTG</td>
<td>Dog (Canis familiaris)</td>
<td>197 bp</td>
<td>F: 59.0</td>
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<tr>
<td>CAGAGGGGT</td>
<td>GCAAGTTTC</td>
<td></td>
<td></td>
<td>R: 62.1</td>
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<tr>
<td>TTTCTCCCAACAG</td>
<td>ATGAGCACATC</td>
<td>Human (Homo sapiens)</td>
<td>169 bp</td>
<td>F: 61.5</td>
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<tr>
<td>AGCCTCG</td>
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<td>TTACTCGGCTGC</td>
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<td>F: 65</td>
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<tr>
<td>GTTCTCCAA</td>
<td>CTCCGCTGG</td>
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<td>R: 70.9</td>
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</tbody>
</table>

[http://genome.ucsc.edu/index.html](http://genome.ucsc.edu/index.html)
Horse *in silico* PCR results

AGGGCAGCTTCCAAGACctggacctcagctccatgggcgatggggGCATC
CAGCTTCAATTCTCCC
Dog *in silico* PCR results

ATGACTCGAGCTCAGAGGGTgcgggctgctatgttcccctgagacattaga
tgaaggcatgcagatccccatctacacagttcgatgctgctcatcctacta
atgtccagcgtttggctgaaccatcacagatgctgaacacatgcagttgta
aatttgattaactatcaagatgatgcaGAACTTGCCACACGTGCAAT
Human *in silico* PCR results

TTTCTCCCACAGAGCACTCGgccgcagccaaagacgggccggtgtgctttcct
gccggagccccaggtacgcctggagagtggggcgctgtggccttgtcagc
cgtgaggccgggagaccgttgtgtgctggccatggggagagatgggagcccccA
CCTTCAGCGATGTGCTCAT
Cow in silico PCR results

ATGGGTGCCAGTTCCTCCAGAgaccgcgcggaagggagaagccaccgcggga
gagtaaatctactcatagtgtttgctttttatatttgggtgcttttttcatag
agagaagacgtgacgcagatttgcgaagatggagacagttagtgggttcgctgctccgagacgagcattcggcgc
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ttcaggattttctgtgtgattgtatgttgctgtgagttaggtgggtgctctttgcggaaggagcggagaggttcgcttgccgtgctcgtttcgaataagcc
agttaaatgctgtttttttctctttgagttgacgctgctcttgagcttttacag
tggctgtttttttctctttttttctttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
Let’s BLAST our dog *in silico* PCR data

`>Doginsilicoreresults
CATTTTCTTTTCTCTGCTGgggcccctttcttcttgacactctcactcgtggtcctctgccctcaacaccccatctgtggctgcgtctttcagaacttcaacctcttcctcaccctcatactgcaactccatcattgaccccttcatctacgctttccgcagccagga
gtccgaaagactctccAAGAGGTAGTGCTATGTT

ACCESSION NUMBER: KJ363163.1
GENE: CANIS LUPIS FAMILIARIS MELANOCORTIN RECEPTOR 1 (MC1R) GENE, MC1R-EM ALLELE
E-VALUE: 1E-100

**NOTE** BLAST results may change as new sequences are deposited to NCBI.
Your Turn!

BLAST the predicted PCR product sequences from your *in silico* PCR (should be in Word file) and fill out the following table for the top hit.

- Use nucleotide BLAST (BLASTn)
- Database should be set to “Other”

**NOTE** BLAST results may change as new sequences are deposited to NCBI.

---

<table>
<thead>
<tr>
<th>PCR Product</th>
<th>Accession Number</th>
<th>Gene</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – HORSE PCR PRODUCT</td>
<td>NM_001317261.1</td>
<td>Equus caballus interleukin 1 beta (IL1B), mRNA</td>
<td>6e-26</td>
</tr>
<tr>
<td>2 – DOG PCR PRODUCT</td>
<td>XM_014106480.1</td>
<td>PREDICTED: <em>Canis lupus</em> familiaris catenin (cadherin-associated protein), beta 1, 88 kDa</td>
<td>3e-97</td>
</tr>
<tr>
<td>3 – HUMAN PCR PRODUCT</td>
<td>NG_016741.1</td>
<td>Homo sapiens indian hedgehog (IHH), RefSeq Hene on chromosome 2</td>
<td>8e-82</td>
</tr>
<tr>
<td>4 - COW PCR PRODUCT</td>
<td>M24638.1</td>
<td>Bovine 80-87 kd myristoylated alanine-rich protein kinase C substrate, mRNA</td>
<td>0.0</td>
</tr>
</tbody>
</table>
APPENDIX 2: INSTRUCTOR MATERIALS
Summary of Laboratory Exercise

This crime scene activity is designed to expose students to introductory molecular biology and bioinformatics. This activity is designed for introductory undergraduate life science majors or non-majors. It is recommended that students work either individually or in pairs on this activity. There are five parts of this activity and it is recommended that the activity spans three (3-4 hour) lab sessions, although the laboratory exercise can be adjusted to fit course constraints. Refer to Figure 1 in the manuscript for a flow chart and recommended scheduling of the lab activity.

In this crime scene laboratory, students are asked to solve a fictional crime involving the scarring of a cat, Savannah. Savannah’s owner comes home to find her normally friendly cat very scared. Savannah’s owner finds blood and rushes Savannah to a veterinarian, who determines that the blood must be from the individual who scared the cat. Savannah’s owner immediately suspects two culprits who live in her neighborhood: Lady (a human) and Kona (a dog). Savannah’s owner calls a pet detective, who is able to obtain DNA evidence from the crime scene as well as the two suspects. Students serve as molecular biology experts in this activity and use bioinformatics and molecular biology to solve the crime.

To start the lab, students are provided two sets of primer pairs: one for human and one for dog. Students use *in silico* PCR and BLAST (Basic Local Alignment Search Tool) to determine that both primers amplify a genomic fragment of the human or dog cardiac actin gene.

Students are then given three samples: a sample from the crime scene, a sample from Lady (the human suspect) and a sample from Kona (the dog suspect). Students will use DNA spectrophotometry to determine the concentration and purity of the DNA samples and prepare dilutions for use in polymerase chain reaction (PCR). After the students perform the PCR, they will analyze results using gel agarose electrophoresis to obtain preliminary results identifying the suspect. Students confirm their PCR by analyzing Sanger sequencing results and ultimately solve the crime.

A student handout for this activity, which includes a summary of the crime, background information on the techniques used in the lab, experimental protocol, and student discussion questions, can be found in Appendix 3. The discussion questions are designed to get students to think critically about the procedure, their data, and any sources of error that may contribute to their experiment. Students should write answers to the discussion questions at the conclusion of each component of the lab. Instructors should review student answers and discuss the questions in a classroom discussion format at the conclusion of the laboratory exercise.

Safety Issues
This laboratory activity is designed for a biosafety level 1 (BSL-1) lab. Personal protective equipment (PPE) should be worn during the wet lab portion of the activity: part 2 (measurement of DNA quantification and purity), part 3 (PCR setup), and part 4 (analysis of PCR by agarose gel electrophoresis). PPE includes:

- Gloves
- Lab coat
- Eye goggles
- Closed-toe shoes.

Students should not need to wear PPE during the computer portions (parts 1 and 5) of this activity, assuming that all wet lab reagents have been properly stored and/or put away. Instructors may want to have students complete the portions of the activity that are computer based (part 1 and 5) in a classroom, if possible.

While microbes are not used in this laboratory activity, refer to the ASM Biosafety Guidelines\(^a\) for a description of BSL-1 guidelines for teaching laboratories.

Three safety precautions that Instructors should be aware of during the agarose gel electrophoresis (part 4):

1. When imaging the agarose gel electrophoresis results, UV protection should be worn. Most gel imagers contain built in UV protection, but having UV face shields available is good practice.

2. Gel Red is used in this activity to visualize DNA in the agarose gel electrophoresis instead of the classically used carcinogen, ethidium bromide. Gel Red, like ethidium bromide, is a dye that intercalates into double stranded DNA and fluoresces when exposed to UV light, allowing for DNA visualization in agarose gels. Gel Red is not considered a carcinogen, as it has not been shown to cross cell membranes. Regardless, caution should used with this chemical and students should be reminded of the importance of wearing gloves when doing all aspects of agarose gel electrophoresis.

3. When preparing the agarose solution by microwaving 1% agarose in TBE buffer, students should be reminded to not swirl the solution near their face. The solution will be very hot and may boil over, potentially burning students’ skin.

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**Equipment, Supplies, and Reagents**

The following equipment is required for this activity:

- Computers with internet access and word processing software (e.g., Microsoft Word or Google Docs) – 1 per student or student pair
- Calculators – 1 per student or student pair
- Spectrophotometer capable of DNA detection, with appropriate cuvettes if required (Nanodrop 2000 UV-Vis Spectrophotometer is recommended, although any UV spectrophotometer is sufficient) – 1 to 2 per class
- PCR thermocycler – 1 per class
- Agarose gel electrophoresis gel boxes with casting trays – 1 per student or student pair
- Power supplies – 1 per 1-2 students or student pairs
- Gel imager capable of ultra violet or agarose gel electrophoresis detection – 1 per class
- Balance – 1 to 2 per class
- Microwave (not used for food consumption) – 1 per class

The following standard laboratory chemicals and materials are required for this activity:

- Non-latex gloves (all sizes; one box per class or enough for each student to have multiple pairs)
- Eye protection – 1 per student
- Lab coat – 1 per student
- Ice buckets with ice – 1 per student or student pair
- 1.5 mL eppendorf tubes – approximately 25 per student or student pair
- 1.5 mL eppendorf tube racks – 1 per student or student pair
- PCR tubes – 4 per student or student pair
- PCR tube racks – 1 per student
- Micropipettes (p10, p20, p100, and p1000 recommended) and tips – 1 set per student or student pair
- Weigh boats and spatulas – 2-3 per class
- KimWipes – 2-3 boxes per class
- Non-biohazard waste receptacles – 1 per student or student pair (plus extras)
- 500 mL glass bottles or flasks – 1 per student or student pair
- 1L graduated cylinders – 2-3 per class
- 50 mL conical tubes – 1 per student or student pair
- Hot hands or oven mits – 1 per student or student pair
- Fine point, permanent markers – 1 per student or student pair
- Sterile (autoclaved) water – 10 mL per student or student pair
- Aluminum foil – 1 roll per class
- Freezer boxes – 1 to 2 per class
- Glycerol
- EDTA
- Bromophenol blue
- Tris
- Boric acid
- EDTA
The following reagents are required for this activity (quantity provided is based on a class size of 8 students or student pairs):

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Recommended Vendor</th>
<th>Catalog #</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog genomic DNA</td>
<td>Zyagen</td>
<td>GD-150F</td>
<td>1</td>
</tr>
<tr>
<td>Human genomic DNA</td>
<td>Zyagen</td>
<td>GH-180F</td>
<td>1</td>
</tr>
<tr>
<td>Primers</td>
<td>Integrated DNA technologies</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>2X PCR master mix</td>
<td>ThermoFisher Scientific</td>
<td>K0171</td>
<td>1</td>
</tr>
<tr>
<td>Agarose</td>
<td>Fisher Scientific</td>
<td>BP2410100</td>
<td>1</td>
</tr>
<tr>
<td>Low DNA Mass Ladder</td>
<td>ThermoFisher Scientific</td>
<td>10068-013</td>
<td>1</td>
</tr>
<tr>
<td>GelRed</td>
<td>Phenix Research Products</td>
<td>RGB-4103</td>
<td>1</td>
</tr>
</tbody>
</table>

The following primer pairs should be ordered (IDT is recommended vendor)\(^{b}\):

- Dog forward primer: 5’ – AGCACTGTAGAGACACCTG
- Dog reverse primer: 5’ – CGGATGACACGTTGTTGGCA

- Human forward primer: 5’ – CTGCAGTGTTCTTATAGGG
- Human reverse primer: 5’ – GAATACCAAGACTTGCTCG

\(^{b}\) Primers used in this activity are modified from: Chiou, et al. (2005). Discovery of Epstein-Barr virus (EBV) encoded RNA signal and EBV nuclear antigen leader protein DNA sequence in pet dogs. *J of General Virology* 86: 899-905.
Instructor Advance Preparation

Prior to the start of this laboratory activity, the items below should be prepared and/or aliquot ted. Instructors are encouraged to have multiple back ups of each reagent in case students need to repeat any aspect of the experiment.

☐ **5X Tris-Borate-EDTA (TBE) Buffer** should be prepared by mixing (stored at room temperature):

- 54 g Tris
- 27.5 g Boric acid
- 4.15 g EDTA
- Bring to 1 L volume with dH₂O

Dilute 5X TBE Buffer to 1X (200 mL 5X TBE + 800 mL dH₂O). 2-3 mL 1X TBE Buffer should be sufficient for 8 students or student pairs.

**10X DNA Loading Buffer** (25% glycerol, 0.1 M EDTA, and 0.25% bromophenol blue) should be prepared by mixing the following reagents below (store at room temperature):

- 12.5 mL glycerol
- 10 mL of 0.5M EDTA
- 0.125 g bromophenol blue.

Aliquot 0.5 mL of 10X DNA Loading Buffer in 1.5 mL eppendorf tubes; store at room temperature (each student or student pair should receive one aliquot).

Lyophilized **primers** should be reconstituted in sterile water at a stock concentration of 100 pmol/µL. This stock should be stored at -20°C.

**Primer working stocks** should be diluted to 10 pmol/µL and stored at -20°C.

**Gel Red** (10 µL per student or student pair/group) should be aliquoted into 1.5 mL eppendorf tubes. GelRed is sensitive to light; thus it is recommended that the aliquots be wrapped in aluminum foil and stored in the dark (i.e., freezer box) at room temperature. Remember to wear gloves as a precaution when handling.

Prepare the **Low DNA Mass Ladder** as outlined in manufacturer’s protocol. Aliquot 15 µL of the Low DNA Mass Ladder for each student or student pair; store at -20°C.

**Verify the concentration of the human and dog genomic DNA** via DNA spectrophotometetry.

Aliquot **Lady (human) control DNA** and **Kona (dog) control DNA**. Each student or student pair should receive approximately 10 µL of a 500 ng/µL solution, each. Instructors may want to slightly vary the concentration of DNA given to each student (recommended concentration range of 400-600 ng/µL). DNA should be stored at -20°C until use.

For the **crime scene DNA**, **instructors should choose if Kona (dog) or Lady (human) committed the crime**. Instructors may want to vary who committed the crime within the class, such that Lady is the culprit for some students and Kona is the culprit for others (instructors are encouraged to prepare a key for verification of results if they choose to vary who committed the crime within a class). Each student

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*a* Recipe for 0.5M EDTA can be found at: [http://chemistry.about.com/od/labrecipes/a/Edta-Solution.htm](http://chemistry.about.com/od/labrecipes/a/Edta-Solution.htm) (last accessed 01/05/16)

*b* The Integrated DNA Technologies Resuspension Calculator is a useful tool for resuspending lyophilized primers. [https://www.idtdna.com/Calc/resuspension/](https://www.idtdna.com/Calc/resuspension/) (last accessed 01/05/16)
or student pair/group should receive approximately 10 µL of a 500 ng/µL solution. Instructors may want to slightly vary the concentration of DNA given to each student (recommended concentration range of 400-600 ng/µL). DNA should be stored at -20°C until use.

Activity Flow of Events

1. Prior to the laboratory activity, students should be introduced to the concepts of DNA structure, spectrophotometry of DNA to determine concentration and purity, polymerase chain reaction (PCR), agarose gel electrophoresis, Sanger sequencing and the bioinformatics tools in silico PCR and BLAST search via lecture and/or classroom activities. Lecture materials (which include clicker questions and classroom activities) for disseminating this information can be found in Appendix 1. It is recommended that dissemination of this material be broken into 3, 30-60 minute class periods. A key for the clicker questions and classroom activities are found in the final slides of the lecture materials (Appendix 1).

2. Students should be asked to read the Student Handout, particularly pages 1-5. These pages describe the crime and provide background information on the various techniques used in the lab (this is a brief description, designed to supplement classroom learning above).

3. Students should be reminded to wear personal protective equipment (PPE) during parts 2, 3, and 4 of this laboratory. This includes: gloves, goggles, lab coats, and closed-toe shoes.

Activity, part 1:

4. Students should start with the in silico PCR and BLAST portions of the activity, which can be found on pages 6-8 of the Student Handout. Students (individually or in pairs) will need computers with Internet access and word processing software (e.g., Microsoft Word or Google Docs) to complete this portion of the activity. Students should be encouraged to fill out Tables 1 and 2 in the student handout with their results on a printed version of the protocol and/or copy these tables into lab notebooks (refer to Table 1 below for a summary of in silico PCR and BLAST results). Discussion Question 1 (page 13) of the Student Handout is relevant to this portion of the activity – students should write an answer to this question to be reviewed by the instructor and discussed as a class at the conclusion of the laboratory exercise.

**Table 1**: Summary of expected in silico PCR and BLAST results from part 1 of the lab activity.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Dog primers</th>
<th>Human primers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F: 5’– AGCAGCTGGTAGAGACACCTG</td>
<td>F: 5’– CTGCACTGTGTTCTTAGGC</td>
</tr>
<tr>
<td></td>
<td>R: 5’– CCGATAGCAGTGTGGGCA</td>
<td>R: 5’– GAATACCAAGACTTGCTGCTG</td>
</tr>
<tr>
<td>Expected PCR Product Size</td>
<td>275 bp</td>
<td>397 bp</td>
</tr>
<tr>
<td>Primer Melting Temperature (°C)</td>
<td>F: 52.9</td>
<td>F: 53.4</td>
</tr>
<tr>
<td></td>
<td>R: 65.3</td>
<td>R: 57.4</td>
</tr>
<tr>
<td>Chromosomal location</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Gene</td>
<td><em>Canis lupus</em> familiaris cardiac actin (ACTC) gene</td>
<td><em>Homo sapiens</em> actin, alpha, cardiac muscle 1 (ACTC1)</td>
</tr>
<tr>
<td>Accession Number¹</td>
<td>AH010031.2</td>
<td>NG_007553.1</td>
</tr>
</tbody>
</table>
Activity, part 2 (student PPE required):

5. At the start of part 2, instructors should set up the DNA spectrophotometer. This should include turning on and calibrating the equipment as well as setting up the area (place KimWipes, set of micropipettes with tips, sterile water, and waste receptacle near the spectrophotometer). It should be noted that the instructions provided in the Student Handout (page 9) are specific to the Nanodrop 2000 UV-Vis spectrophotometer. If a different spectrophotometer is used, the appropriate procedure should be provided. Also, depending on the spectrophotometer used, students may need to use greater volumes of DNA and/or dilute DNA prior to measuring DNA on the spectrophotometer.

6. Students should each receive an aliquot of the Lady (human) control DNA, Kona (dog) control DNA, and crime scene DNA that has been prepared by the instructor in advance as well as an aliquot of sterile (autoclaved) water. If instructors vary who committed the crime (Lady or Kona), instructors should generate a key detailing which student or student pair received which crime scene sample. Students will determine the concentration and purity of the DNA using DNA spectrophotometry. Table 3 in the Student Handout is provided for students to record their DNA spectrophotometry results. Instructors should remind students to bring a pen and a printed version of the Student Handout or a lab notebook to the spectrophotometer to record results. It should be noted that discussion question 2 on page 13 of the student handout is relevant to this portion of the protocol – students should write an answer to this question to be reviewed by the instructor and discussed as a class at the conclusion of the laboratory exercise.

7. Students should use the concentration values obtained from the DNA spectrophotometer to calculate how to dilute each sample to a concentration of 200 ng/µL. Students should use Table 5 of Student Handout to help with these calculations and should be encouraged to have their math verified by the instructor prior to preparing the dilutions. Dilutions should be made in labeled 1.5 mL eppendorf tubes with sterile water.

Activity, part 3 (student PPE required):

8. Prior to the PCR portion of the activity, the instructor should program the PCR parameters in the PCR thermocycler (Table 2).

Table 2: PCR thermocycling conditions for crime scene lab.

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time (min)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94°C</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>0.5</td>
<td>Repeat 35X</td>
</tr>
<tr>
<td>Annealing</td>
<td>61°C</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td>Infinity</td>
<td></td>
</tr>
</tbody>
</table>
9. The instructor should refer to Table 2 below with the recipe for the **Dog and Human PCR master mixes**. Instructors should prepare the master mixes from reagents thawed on ice immediately before starting part 3 of the activity and should aliquot the total volume listed (120 µL) for each student or student pair (aliquoted master mixes should be stored on ice). Students will aliquot 48 µL of the master mix plus 2 µL of template DNA per reaction (refer to Table 4 below for a description of the PCR reactions). *Note, the volumes of reagents listed in Table 3 should be multiplied by the number of students or student pairs in the class.*

| Table 3: PCR master mixes to be prepared for a single student or student pair. |
|-----------------------------|------------------|------------------|
| Reagent                    | Dog master mix   | Human master mix |
| Forward Primer (10 pmol/µL)| 5 µL             | 5 µL             |
| Reverse Primer (10 pmol/µL)| 5 µL             | 5 µL             |
| 2X PCR master mix           | 62.5 µL          | 62.5 µL          |
| Sterile water              | 47.5 µL          | 47.5 µL          |
| TOTAL VOLUME                | 120 µL           | 120 µL           |

| Table 4: PCR reactions in this activity. |
|-----------------------------|------------------|
| Reaction | DNA source | PCR master mix | Primers                   |
| 1 | Kona | Dog | Dog Forward: 5’ – AGCAGTGTAGAGACACCTG<br>Dog Reverse: 5’ – CCGATAGCACGTGTGCCGCA |
| 2 | Lady | Human | Human Forward: 5’ – CTGCAGTGTGTCTTATAGGG<br>Human Reverse: 5’ – GAATACCAAGACTTGCCG |
| 3 | Crime scene | Dog | Dog Forward: 5’ – AGCAGTGTAGAGACACCTG<br>Dog Reverse: 5’ – CCGATAGCACGTGTGCCGCA |
| 4 | Crime scene | Human | Human Forward: 5’ – CTGCAGTGTGTCTTATAGGG<br>Human Reverse: 5’ – GAATACCAAGACTTGCCG |

10. Students should receive 4 PCR tubes in a PCR rack and label the tubes appropriately with their name(s) and contents (sample and master mix) on the side and lid of the tubes (refer to Table 4 above, which is also Table 4 in the Student Handout). Students should aliquot the dog or human master mix into two tubes each. Students should then add 2 µL of the appropriately diluted (200 ng/µL) DNA - Kona control, Lady control, or crime scene – into the appropriate tube. Again, students should be encouraged to pay close attention to Table 4 in the Student Handout when aliquoting reagents (master mixes and template DNA) for the PCR reaction. Once students have prepared their PCR reactions, students can place their PCR reactions on ice until all students in the class are ready to put their reactions in the PCR thermocycler.

11. Prior to starting the PCR reaction in the thermocycler, the instructor should take note of the location of each student or student pair’s PCRs in the thermocycler for reference. Students should be reminded to label the tubes on the side as well as on the lid with their name and the contents. Once the PCR reaction for the class is running, students will be done with the activity for at least 2.5 hours (if not the day). *Instructors should remember to remove the PCR reactions*
WHO SCARED THE CAT? INSTRUCTOR’S MATERIALS

from the thermocycler and store in a PCR rack at -20°C until the next lab meeting. It should be
noted that discussion question 3 on page 13 of the student handout is relevant to this portion of
the protocol – students should write an answer to this question to be reviewed by the instructor
and discussed as a class at the conclusion of the laboratory exercise.

Activity, part 4 (student PPE and UV protection required):

12. Prior to part 4 of the activity, the instructor should set out the agarose gel electrophoresis
casting tray and comb, power supplies, graduated cylinders, 1X TBE buffer, balance, agarose,
weigh boats, and spatulas. Each student or student pair should receive a 500 mL glass bottle
or flask, hot hands or oven mit, aliquot of GelRed, 50 mL conical tube, aliquot of 10X DNA
loading dye, and aliquot of low DNA mass ladder. The instructor should also turn on the gel
imaging system and allow it to warm up. UV protection should be placed near the gel
imaging system.

13. If students haven’t loaded an agarose gel before, instructors may want to have a couple
agarose gels prepared in advance for students to practice loading with 10X DNA loading dye.
Have enough gels such that each student can practice loading 1-2 lanes. Practice can occur
during the gel solidifying incubation (#15 below).

14. At the start of part 2, students first prepare and pour their agarose gels for agarose gel
 electrophoresis. Students should use caution when microwaving the agarose solution to avoid
being burned. Students should also be reminded to add GelRed to the melted agarose solution.
It should be noted that the gel casting directions listed in the student handout are for gel casting
trays that fit 30 mL of melted agarose. If larger or smaller gel casting systems are used, the
protocol on page 12 of the student handout should be modified and provided to students.

15. While the gels are solidifying (minimum 30 minutes), students should prepare their DNA
samples by adding 10X DNA loading dye directly to the PCR tubes. Students can also practice
loading 10X DNA loading dye into practice agarose gels gels (as described in #13 above) while
gels are solidifying.

16. Once the gels are set, students should remove the comb and add 1X TBE (enough to cover the
gel). Instructors should verify that the combs are on the correct side of the gel box (towards the
black cathode – remember, DNA runs to red!) before students load their samples. Students
should load the low DNA mass ladder and all four PCR samples onto the gel and should be
reminded to write down the order that the samples were loaded into the gel. Typically, 1-2 gels
can be run on the same power supply. Note, the running specifications (voltage and time) for
the agarose gel electrophoresis may need to be adjusted based on the specific equipment used.
Gels should be run for approximately 1 hour, or until the dye front has traveled at least half the
distance of the gel. While gels are running, students can work on other class related activities.

17. When the gels are finished, students should image their gel and take a picture of their
results. Students should expect to see a band at approximately 275 bp for the dog PCR
product and 397 bp for the human PCR product. If instructors varied who committed the crime
(Lady or Kona), instructors should refer to the student sample key made during part 2 to verify
results. A printed and/or digital copy of the gel image may be needed depending on if students
will record their data in a lab notebook and/or write a lab report on their results. Thus, students
may need to be reminded to bring a USB drive to the gel imager to save electronic data.
GelRed containing gels can be discarded in the general trash. It should be noted that
discussion questions 4-6 on page 13 of the Student Handout are relevant to this portion of the
protocol – students should write answers to these questions to be reviewed by the instructor
and discussed as a class at the conclusion of the laboratory exercise.
Activity, part 5:

18. In the final part of the activity, students will again need computers with Internet access to analyze sequencing results. Two sets of sequencing results are available in Appendix 3: one where Lady (human) committed the crime and the other where Kona (dog) committed the crime. Each sequencing result consists of the Kona and Lady control sample as well as the crime scene sample. Sequencing results should be shared with students electronically (e.g., email or class website) prior to the start of part 5. Again, if instructors varied who committed the crime (Lady or Kona), instructors should refer to the student sample key made during part 2 when distributing sequencing results. Students will use BLAST to analyze the sequencing results and should respond to discussion questions 7 and 8 found on page 13 of the Student Handout – students should write answers to these questions to be reviewed by the instructor and discussed as a class at the conclusion of the laboratory exercise.

Discussion Question Key

The discussion questions are found at the end of the Student Handout and are designed to get students to think critically about the procedure, their data, and any experimental error that may have occurred. At the conclusion of each component of the lab exercise, students should write answers to the discussion questions. The instructor should then lead a class discussion of the discussion questions at the completion of the laboratory exercise. This will serve as a way to formatively assess student critical thinking and the SLOs of the activity. If students write lab reports for this exercise, instructors may want students to include answers to discussion questions in lab reports (refer to Appendix 5).

Refer to a key below with anticipated answers to discussion questions:

Part 1

1. What similarities did you observe about the expected PCR product for dog and human based on the in silico PCR and BLAST search? What differences did you observe between the PCR products?

   Similarities include that both the dog and human PCRs should amplify the cardiac actin gene. Differences include size (dog is 275 bp, human is 397 bp) and chromosomal location (dog chromosome 30, human chromosome 15).

Part 2

2. What were the A260/A280 values for your DNA samples? Do the A260/A280 values that were measured indicate that the DNA was contaminated? If so, what was it contaminated with?

   Pure DNA has an A260/280 value of 1.8. If A260/280 is below 1.8, the sample is contaminated with protein. If it is above 1.8, it implies that there may be RNA in the sample. Protein is the likely contaminant.

Part 3

3. In the PCR reaction, why was the extension time only 30 seconds? Could a longer or shorter extension time have been set? Explain.

   The PCR products were less than 500 bp, so only 30 seconds were needed. The general rule is that 1 minute per kb is used for the extension time. A longer time could have been set, but
may result in off target products. A shorter time would likely result in no PCR product because there isn’t enough time to amplify the DNA of interest.

Part 4

4. What size PCR products did you observe in the Kona and Lady control samples? Were these PCR products expected?

Kona was 275 bp, Lady was 397 bp. Both were expected based on in silico PCR results.

5. Did you observe any PCR products that were not expected? If so, what could they represent?

If unexpected PCR products are observed, it is likely due to off-target affects. This could be due to primers annealing to inappropriate areas and/or samples being contaminated with DNA that is amplified by the primer.

6. Based on your PCR results, who committed the crime? Explain.

Will depend on which sample (Lady-human or Kona-dog) was given as the crime scene sample. Students should provide rationale for argument (such as amplicon presence and size in crime scene sample)

Part 5

7. According to your BLAST search, which gene was amplified from the Kona DNA sample? What gene was amplified from the Lady DNA sample? Does this support of your in silico PCR results?

BLAST results should confirm in silico PCR results – both the Lady and Kona control should depict the cardiac actin gene as the first hit on BLAST.

8. Based on all of your data, who committed the crime?

Will depend on which sample (Lady-human or Kona-dog) was given as the crime scene sample. Students should relate back to sequencing results confirming the PCR results.

Table 5: Representative student responses on activity discussion questions

<table>
<thead>
<tr>
<th>Discussion Question(s)</th>
<th>Representative Student Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. What similarities did you observe about the expected PCR product for dog and human</td>
<td>“The primers used for PCR separated a piece of DNA that coded for Cardiac Actin (ACTC) for the dog and Actin Alpha Cardiac Muscle (ACTC 1) for the human according to both in silico PCR and BLAST results of primer sequence. The ACTC gene is 275 base pairs, whereas the ACTC 1 is 397 base pairs.”</td>
</tr>
<tr>
<td>2. What were the A260/A280 values for your DNA samples? Do the A260/A280 values that were measured indicate that the DNA was contaminated by protein.”</td>
<td>“The DNA sample purities from the NanoDrop test for Kona, the old Lady, and the Crime Scene were 1.33, 1.46, and 1.49, respectively. Because all these values were below 1.8, it demonstrates that the samples were contaminated by protein.”</td>
</tr>
</tbody>
</table>
### Question 3
In the PCR reaction, why was the extension time only 30 seconds? Could a longer or shorter extension time have been set? Explain.

#### Answer
“While running the PCR, we used the rule of extension of one kilobase/minute. Using this ideology, a 30 second extension time was enough time to replicate the sequences of 275 base pairs and 397 base pairs. Using a longer extension time will initially result in longer extension while a shorter time may not allow enough extension.”

### Question 4
What size PCR products did you observe in the Kona and Lady control samples? Were these PCR products expected?

#### Answer
“The expected product size for Kona was 275 base pairs and 397 base pairs for the old lady. After running the gel, lanes 3 and 5 had similar band patterns around 400 base pairs. Lane 3 contained the old lady DNA and human master mix, and lane [5] contained crime scene DNA and human master mix. Thus, in order to get the same band pattern around the 400 base pair mark, the crime scene DNA must belong to the old lady. Also, lane 4 contained dog master mix and crime scene DNA, and no bands were observed. This indicates that PCR was not able to isolate a gene, which makes sense because the human master mix contains the human primers that will not be able to bind to the dog/Kona’s DNA.”

### Question 5
Did you observe any PCR products that were not expected? If so, what could they represent?

#### Answer
“Even though our Dog/Kona mixture and the human/crime mixture did not show up, because the Lady PCR and the crime scene PCR did not line up and the crime scene PCR is close to 275 (the expected PCR size for Kona), Kona scared the cat. Despite this, there are some sources of error in our experiment since we are not positive the Kona sample would have matched even though we assume it would. Error in the experiment could have resulted when loading DNA during PCR set up or if the wells during the gel electrophoresis were loaded badly.”

### Question 6
Based on your PCR results, who committed the crime? Explain.

### Question 8
Based on all of your data, who committed the crime?

#### Answer
“After running the agarose gel electrophoresis it was determined that the old lady scared Savannah the cat, and was later confirmed the BLAST of sequenced PCR DNA…This was confirmed with a nucleotide Basic BLAST of the PCR DNA after sequencing. The results of this showed that the crime scene and old lady DNA coded for the same gene and had the same accession number, which is unique.”

### Question 7
According to your BLAST search, which gene was amplified from the Kona DNA sample? What gene was amplified from the Lady DNA sample? Does this support of your in silico PCR

#### Answer
“Based on the in silico PCR data as well as the BLAST search, we concluded that the gene isolated by the primers in both the dog and the human DNA sequence was the gene for ACTC…This data, however, was reaffirmed through the BLAST search of the..."
| results? | sequenced DNA which matched the Cardiac Actin (ACTC) gene in dogs. Kona committed the crime of scaring Savannah. |
APPENDIX 3: STUDENT HANDOUT
Your friend Jack is a local Pet Detective and has just been assigned a case to determine who scared a cat named Savannah. Given your expertise in molecular biology, Jack has recruited you to help him solve the case.

On the night of May 5, 2013 at approximately 6:15 pm, Julie came home from a long day at work and was looking forward to cuddling on the couch with her beloved cat, Savannah. Savannah is an indoor/outdoor Calico that has been trained to stay in Julie’s yard and greets her in the driveway every night after work. On this particular evening, however, when Julie arrived home Savannah was nowhere to be found. Assuming that Savannah was likely napping inside, she went to look for her cat in the guest bedroom, Savannah’s favorite place to nap. To Julie’s surprise, Savannah was not in the guest bedroom and she started looking for her. Julie found a trail of blood on the back deck and immediately started to fear the worst. After 20 minutes of searching, she found Savannah under a bush in the back yard, shivering; it was obvious that an intruder had scared Savannah.

Since Julie noticed a trail of blood, she rushed Savannah to her veterinarian, Dr. Lou, fearing that Savannah may have been injured. Upon a thorough examination, Dr. Lou concluded that the blood most likely came from the perpetrator, as there wasn’t a single scratch on Savannah. Dr. Lou suspected that Savannah likely scratched the perpetrator in self-defense. Julie immediately thought of two potential suspects: Kona, a golden retriever mix that lives three houses down and likes to wander into neighboring yards, and the old Lady who lives across the street, who has publically voiced her distaste for cats. Julie immediately called Jack to investigate whether Kona or the Lady scared Savannah in hopes of preventing this from happening again.

Jack arrived at Julie’s house at approximately 9:00 pm that evening to interview Julie and Savannah and survey the scene. He collected a sample of the blood found on the back deck, which he termed the crime scene sample. The next day, he set up interviews with both Kona’s owner and the Lady across the street. Both denied any involvement in scaring Savannah although Jack did notice suspicious scratches on Kona’s face and the Lady’s left arm, both of which appeared to be recent. Both suspects agreed to donate DNA samples to prove their innocence.

Jack was able to isolate genomic DNA from the crime scene as well as the samples obtained from Kona and Lady. Now he needs your help to solve the crime and he has asked you to perform polymerase chain reaction (PCR) and use bioinformatics tools to help him determine who scared the cat.
Quantifying and visualizing DNA

When performing molecular biology experiments, a specific concentration of DNA is used in the reaction. To determine both the concentration and purity of a DNA sample, a spectrophotometer is used and the absorbance at 260 and 280 nm is measured. The following equation is used to calculate the concentration of DNA:

\[
\text{DNA concentration (ng/µL)} = \text{OD}_{260} \times 50 \text{ ng/µL} \times \text{dilution factor}
\]

In the equation above, \(\text{OD}_{260}\) is the absorbance reading at 260 nm and the dilution factor is the level of which the sample was diluted (if applicable). For example, if you diluted 1 µL of DNA into 9 µL of sterile water, the dilution factor would be 10 because you have 1 part DNA in 10 parts total (denoted 1:10 dilution). Most spectrophotometers, to include the NanoDrop, will calculate the concentration of the DNA sample for you (although you will have to tell the machine if you diluted your sample). To determine the purity of a DNA sample, the ratio of the absorbance at 260 nm (\(\text{A}_{260}\)) over the absorbance at 280 nm (\(\text{A}_{280}\)) is calculated. Pure DNA will have an \(\text{A}_{260}/\text{A}_{280}\) ratio of 1.8, while pure RNA and protein will have \(\text{A}_{260}/\text{A}_{280}\) ratios of 2.0 and 0.8, respectively. Thus, if a DNA sample has an \(\text{A}_{260}/\text{A}_{280}\) ratio greater than 1.8, the sample is likely contaminated with RNA. Conversely, if a DNA sample has an \(\text{A}_{260}/\text{A}_{280}\) ratio less than 1.8, the sample is likely contaminated with protein. Once the concentration and purity of the DNA sample are determined, the DNA is then diluted to a specific concentration for the molecular biology technique (i.e. PCR, see below).

Many molecular biology experiments analyze and visualize DNA using **agarose gel electrophoresis**. This is a technique that allows for separation of DNA fragments based on size. Since DNA is negatively charged, loading samples into wells of an agarose gel positioned near the cathode (negative end) and applying an electrical current will result in negatively charged DNA migrating towards the positively charged anode. The agarose gels have pores of varying size that DNA can migrate through, with smaller DNA fragments migrating farther than larger DNA fragments. Running a standard of known DNA sizes (DNA ladder or molecular weight ladder) along with samples on the gel will allow for estimation of DNA fragment size. To visualize the DNA, a fluorescent dye (such as ethidium bromide or GelRed) is added to the agarose gel. These dyes intercalate into double stranded DNA and will fluoresce upon exposure to UV light. It should be noted that ethidium bromide is a carcinogen that is capable of crossing cell membranes. Thus, it is imperative that gloves are always worn when working with ethidium bromide to avoid exposure. GelRed, on the other hand, has been shown to be incapable of penetrating cell membranes and is thus not considered a carcinogen. However, it is good practice to always wear gloves when running agarose gel electrophoresis just to be on the safe side!

It should be noted that before samples are loaded into an agarose gel, DNA loading buffer is added to the DNA samples. This dye contains two essential components: glycerol and bromophenol blue. Glycerol is added to keep the DNA samples in the wells and bromophenol blue is added to visualize loading of DNA into wells as well as to visualize how far the DNA has traveled through the gel.

**Polymerase-chain reaction (PCR), in silico PCR, and BLAST**

**Polymerase chain reaction** (PCR) is a technique to amplify specific DNA sequences in a relatively short amount of time (approximately 1-3 hours). During PCR, a process called thermocycling occurs, where there are repeated cycles (15-40) of heating and cooling the DNA in the presence of a polymerase enzyme, nucleotides and primers that result in amplification of DNA. The DNA amplification in PCR is exponential, as each new strand of DNA that is synthesized becomes template DNA in the next cycle (i.e. one DNA strand becomes 2, 2 DNA strands become 4, etc.).
For a PCR to work properly, the following reagents are required:

- **Template DNA**, containing the DNA sequence to be amplified.
- Two **primers** (forward and reverse), which will bind to complimentary sequences on the template DNA and guide the location of DNA amplification.
- **Taq DNA polymerase**, the thermostable enzyme that synthesizes new strands of DNA during PCR.
- **Deoxynucleotide triphosphates** (dNTPs), which are the source of adenine (A), guanine (G), thymine (T) and cytosine (C).
- **Buffer**, with optimal salts for Taq polymerase to function properly.
- **Magnesium chloride** (MgCl₂), which is required for the activity of Taq polymerase.

In this laboratory, all of the above reagents except the template DNA will be mixed before the PCR reaction and will be called the master mix. Template DNA will then be added to the master mix prior to starting the PCR reaction.

Once reagents have been added to a PCR tube, tubes are placed into a PCR machine (also called a thermocycler) and thermocycling will begin. The thermocycling conditions of PCR are as follows:

1. **Denaturation** – heating of the DNA to 92-95°C for 30 seconds to separate the two strands of DNA into single strands.
2. **Annealing** – cooling the reaction to approximately 60°C for 30 seconds. This allows the primers to anneal (i.e., bind) to their complimentary DNA sequences. The annealing temperature of primers is based on the melting temperature, which is based on the length and sequence of the primer sequence and is the temperature in which half of the DNA is single stranded.
3. **Extension** – increasing the temperature to 72°C. This is the optimal temperature for Taq DNA polymerase to synthesize new DNA. The amount of time assigned to this step is dependent on the size of the DNA to be amplified. In general, Taq DNA polymerase needs 1 minute of extension time for a 1kb (1000 bp) DNA fragment. Thus, if your template DNA were 0.5kb then 30 seconds of extension time would be needed.

After a PCR reaction is complete, an agarose gel is run to analyze the results. If the PCR worked correctly, a single band the size of the expected DNA that was amplified (called the amplicon) should appear.

Before running a PCR reaction, it is a good idea to test primer specificity to ensure that non-specific amplification of DNA will not occur. A great tool to check for primer specificity of genomic DNA targets is **in silico PCR**. In silico PCR predicts the amplified PCR product based on the primer sequences. Using software available through the University of Santa Cruz Genome Bioinformatics Group (www.genome.ucsc.edu), scientists can enter their primer sequences and the source of the DNA (i.e. human or dog). The software will then display the sequence and size of the anticipated PCR product(s), the melting temperatures of primers, and the chromosomal location of the DNA sequence. Using in silico PCR, investigators can confirm that the PCR primers that they are using will amplify the correct gene by performing a BLAST search. BLAST stands for Basic Local Alignment Search Tool, which is available from NCBI or the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST). BLAST performs a search of all sequences submitted to NCBI and finds sequences that are similar to a sequence that an investigator inputs into the software (called a query). BLAST will then create an alignment between the two sequences and provide an E-value (or expect value), which is a measurement of the accuracy of the alignments, with a lower E-value corresponding to a greater significance in the alignment. Note that all NCBI submissions are given an id number, called an accession number, and the BLAST results will include this for future reference of
those data files. There are a few types of BLAST searches, with a BLASTn (or nucleotide BLAST) search being most relevant when comparing DNA sequence of a query to DNA sequences in the NCBI database.

**DNA Sequencing**

*DNA sequencing* is the technique used to determine the DNA sequence of a sample. DNA sequencing is a modified version of traditional PCR with two major differences. First, DNA sequencing does not result in exponential amplification of DNA because it relies on a single primer instead of two. Second, *dideoxynucleotide triphosphates (ddNTPs)* are added in limited quantity to the reaction in addition to dNTPs. In standard PCR, phosphodiester bonds are formed between dNTPs by covalently joining the 5’ phosphate of one dNTP to the 3’ OH of another dNTP. During sequencing, however, a ddNTP will be randomly inserted into the newly synthesized DNA strand. When this occurs, a phosphodiester bond cannot be formed with an adjacent nucleotide when incorporated because ddNTPs lack a 3’ OH (Figure 1). For this reason, ddNTPs are called chain terminators because *Taq* polymerase cannot add a new dNTP on to the DNA strand when a ddNTP has been incorporated.

The ddNTPs used during sequencing are tagged with a fluorescent molecule with each base (A, T, C, G) having its own unique color. After the sequencing reaction has finished, the products are run through capillary tubes that have lasers that can detect the fluorescence emitted by each ddNTPs. The lasers send the signals to a computer, which generates a chromatogram for the DNA sequence that was measured.

It should be noted that most labs do not perform their own sequencing and send it to one of many companies that perform the sequencing for a small fee instead. The investigator sends the company the DNA to be sequenced in the appropriate concentration and volume as well as the primer to be used in the sequencing reaction. Sequencing reactions are fairly inexpensive (less than $10 per reaction) and the results are available quickly, often the next day. The sequencing companies upload the chromatogram (.abi file) and a text file of the DNA sequence for the investigator to download and analyze. It should be noted that there are often a string of N’s at the beginning and end of the sequences. An N stands for any nucleotide and occurs when the sequencing software has a difficult time accurately determining the nucleotide that was incorporated. Very few, if any, N’s should be found within the middle of the sequence. Investigators analyze their sequencing results using various methods. The most common is to BLAST sequencing results to compare the DNA that was sequenced to known sequences in the NCBI database.

![Figure 1: Molecular structure of deoxynucleotide triphosphate (dNTP) and dideoxynucleotide triphosphate (ddNTP).](https://www.ocf.berkeley.edu/~edy/genome/sequencing.html)
Experimental Procedure

PART 1:

*In silico* PCR

Whitney, a colleague of yours who has performed a similar experiment, provided you with DNA primers to amplify dog and human genomic DNA. However, she can’t remember the sequences that the primers amplify or the size of the expected PCR products. Thus, you will need to perform *in silico* PCR and BLAST to determine the primer melting temperature, PCR product sequence, expected size, and chromosomal location for both the dog and human primers.

For dog DNA, the following primers will be used:

- Forward primer: 5’ – AGCACTGTTAGAGACACCTG
- Reverse primer: 5’ – CGGATAGCAGCGTTGTTGCA

For human DNA, the following primers will be used:

- Forward primer: 5’ – CTGCAGTGTGTCTTATAGGG
- Reverse primer: 5’ – GAATACCAAGACTTGCGCTCG

1. Go to [www.genome.ucsc.edu](http://www.genome.ucsc.edu), click “tools” in the top blue menu and then “in-silico PCR” from the drop down menu.

2. Select dog under the genome setting.

3. Enter in the forward and reverse dog primers from above into the appropriate boxes.

4. Click submit.

5. Enter the size of the PCR product, primer melting temperature, and chromosomal location of the PCR product in Table 1.

6. Copy the DNA sequence to a Word document. To do this, highlight the sequence in its entirety, right click and then select copy. Then open Word, right click and select paste.

7. Repeat steps 1-6 for the human primers. Remember to change the genome to human and enter in the human PCR primers before clicking submit. Save the expected human PCR product sequence in the same Word document as the dog.
Table 1: Results of *in silico* PCR

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Expected PCR product size</th>
<th>Primer melting temperature</th>
<th>Chromosomal location of PCR product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog primers</td>
<td></td>
<td>F:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:</td>
<td></td>
</tr>
<tr>
<td>Human primers</td>
<td></td>
<td>F:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:</td>
<td></td>
</tr>
</tbody>
</table>


9. Select nucleotide BLAST under Basic BLAST

10. Copy the dog *in silico* PCR results and paste into the dialog box under “Enter Query Sequence”.

11. Under “Choose Search Set”, select the “Other” Database and make sure that “Nucleotide collection (nr/nt)” is selected from the drop down menu. Enter dog for organism (Refer to Figure 2).

12. Click BLAST

Figure 2: Nucleotide BLAST search page. (A) Is where the sequence to be analyzed is entered, (B) is to select the database and organism to search and (C) is the BLAST button to select to initiate the search.

13. When the results are displayed, locate the name of the gene with the highest homology to the query along with the accession number and E-value (Refer to Figure 3). Write this information in Table 2.

14. Repeat steps 8-13 with the Human PCR expected in silico PCR results. Make sure to select human for organism under “Choose Search Set”.

Table 2: BLAST of in silico PCR results

<table>
<thead>
<tr>
<th></th>
<th>Gene name</th>
<th>Accession number</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog primers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human primers</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PART 2

DNA quantification and purity

Obtain a crime scene DNA sample as well as control DNA samples from Kona and Lady.

A NanoDrop will be used to determine DNA concentration and purity, as it is easy to use and only requires a small volume of DNA. If a NanoDrop is not available, follow the directions of the spectrophotometer that is available.

1. Turn on the computer that is connected to the NanoDrop.
2. Open the NanoDrop “ND-1000” software.
3. Select “Nucleic Acid”
4. Using a kimwipe, wipe the top and bottom of the sample pedestal.
5. Pipette 1 µL of sterile water onto the bottom part of the sample pedestal. The water will form a bead.
6. Close the sample pedestal and click “OK” to initiate the machine.
7. Wipe the sample pedestal with kimwipes again and load 1 µL of sterile water. Close the sample pedestal.
8. Click “BLANK”, which is found in the upper left corner of the software.
9. Wipe the sample pedestal again with a kimwipe and load 1 µL of Kona control DNA. Close the sample pedestal and click “Measure”.
10. Write the concentration and A260/280 value of this sample in Table 3 (found on next page).
11. Wipe the sample pedestal (top and bottom) with a kimwipe and repeat steps 9-10 for both the Lady control DNA and crime scene samples.

Table 3: Concentration and A260/280 values of control and crime scene DNA samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ng/µL)</th>
<th>A260/280</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kona</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lady</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crime scene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PART 3

PCR

You will now set up four PCR reactions, which are outlined in Table 4. Make sure to fill out the final column, which is the size of the expected PCR product (refer to in silico PCR results).

Table 4: PCR reactions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>DNA source</th>
<th>PCR master mix</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kona</td>
<td>Dog</td>
<td>Dog Forward: 5’ – AGCACTGTTAGAGACACCTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dog Reverse: 5’ – CGGATAGCACGTGGTGGCA</td>
</tr>
<tr>
<td>2</td>
<td>Lady</td>
<td>Human</td>
<td>Human Forward: 5’ – CTGCAGTGTGTCTTAGGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Human Reverse: 5’ – GAATACCAAGACCTGCCTCG</td>
</tr>
<tr>
<td>3</td>
<td>Crime scene</td>
<td>Dog</td>
<td>Dog Forward: 5’ – AGCACTGTTAGAGACACCTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dog Reverse: 5’ – CGGATAGCACGTGGTGGCA</td>
</tr>
<tr>
<td>4</td>
<td>Crime scene</td>
<td>Human</td>
<td>Human Forward: 5’ – CTGCAGTGTGTCTTAGGG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Human Reverse: 5’ – GAATACCAAGACCTGCCTCG</td>
</tr>
</tbody>
</table>

1. Calculate the amount of DNA and water needed to dilute DNA to a concentration of 200 ng/µL in a final volume of 10 µL. Use the DNA concentrations measured by nanodrop (Table 3). To do this, use the equation below and solve for volume-have (this will be your DNA volume):

   $[\text{Concentration-have}] \times [\text{volume-have}] = [\text{concentration-wanted}] \times [\text{volume-wanted}]$

   Subtract the calculated volume from the equation above from 10 to calculate the volume of sterile water to add to each tube, which should have a total volume of 10 µL. Enter the calculated volumes of DNA and water into Table 5 and ask the instructor to double-check your work.

Table 5: Volume of DNA and water to generate 200 ng/µL stocks (in 10 µL final volume)

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA concentration (ng/µL)</th>
<th>Volume of DNA (µL)</th>
<th>Volume of sterile water (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kona</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lady</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crime Scene</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. Prepare the dilutions calculated in Table 5 above in fresh 1.5 mL Eppendorf tubes.

3. The instructor will generate a master mix of the PCR reagents for you (refer to Table 6). A commercially available 2X master mix from Thermo scientific will be used, which contains Taq DNA polymerase, MgCl₂, and dNTPs. All that needs to be added are specific forward and reverse primers, sterile water, and template DNA.

Table 6: PCR master mixes for amplifying dog and human DNA

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Dog master mix</th>
<th>Human master mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Primer (10 pmol/µL)</td>
<td>2 µL</td>
<td>2 µL</td>
</tr>
<tr>
<td>Reverse Primer (10 pmol/µL)</td>
<td>2 µL</td>
<td>2 µL</td>
</tr>
<tr>
<td>2X PCR master mix</td>
<td>25 µL</td>
<td>25 µL</td>
</tr>
<tr>
<td>Sterile water</td>
<td>19 µL</td>
<td>19 µL</td>
</tr>
<tr>
<td><strong>TOTAL VOLUME</strong></td>
<td><strong>48 µL</strong></td>
<td><strong>48 µL</strong></td>
</tr>
</tbody>
</table>

4. Label 4 PCR tubes with your name (initials) and reaction number (refer to table 4) and place on ice. For example, if your name is John Doe, label your tube “LD-2” for John Doe, reaction 2.

5. Aliquot 48 µL of the appropriate master mix into pre-labeled PCR tubes. Make sure to keep the PCR tubes on ice during this step.

6. Add 2 µL of the 200 ng/µL DNA stock to the appropriate PCR tube (Refer to Table 4 for which DNA sample goes into each tube). This will result in a total of 400 ng per PCR reaction.

7. Make sure that the lids of the PCR tubes are tightly closed and store on ice until the instructor is ready to load the reactions into the PCR machine.

8. When the instructor is ready, load your PCR tubes into the PCR machine (also called a thermocycler). The instructor will start the thermocycler and will remove samples from the thermocycler when complete. Samples will be stored at -20°C until the next session. The following PCR parameters or thermocycling conditions will be run (Table 7):

Figure 7: Thermocycling conditions to amplify dog and human DNA

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time (min)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94°C</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>0.5</td>
<td>Repeat 35X</td>
</tr>
<tr>
<td>Annealing</td>
<td>61°C</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td>Infinity</td>
<td></td>
</tr>
</tbody>
</table>
PART 4

Agarose Gel Electrophoresis

1. Prepare a 1% agarose gel by adding 1 g of agarose to 100 mL of 1X TBE buffer in a glass bottle. Microwave this solution for 30-second intervals with the cap on loosely. Swirl between each interval and repeat until the agarose is completely dissolved (never leave the agarose solution unattended when using the microwave).

CAUTION: The agarose solution will be very hot, so be sure to wear “hot hands” to handle the bottle and never swirl the bottle close to your face in case the liquid boils over.

2. Pour 30 mL of melted agarose into a disposable 50 mL conical tube.

3. Add 3 µL of GelRed to the melted agarose and gently invert the tube 2-3 times to mix (be gentle when mixing to avoid generating bubbles).

4. Assemble the gel apparatus, to include placing the comb.

5. Gently pour the melted agarose containing GelRed into the casting tray and remove any bubbles with a p10 pipette tip. Allow the agarose gel to solidify at room temperature (about 20 minutes).

6. Once the gel has completely solidified, gently remove the dams and comb and add 160 mL of 1X TBE over the gel.

7. Obtain your PCR samples from the instructor.

8. In pre-labeled fresh Eppendorf tubes, add 3 µL of 10X DNA loading dye and 27 µL of DNA.

9. Load the low mass ladder (see Figure 4) and samples onto the gel. Load 12 µL of ladder or sample and remember to write down the order you loaded your gel.

10. Close the lid and hook up the electrical leads (cables should go “red to red, black to black”). Run the gel at 100 volts for at least one hour (run until the dye front runs at least ¾ the length of the gel).

11. Image the gel, print a picture for your laboratory notebook, and save a jpeg copy of the gel image to a USB drive to incorporate into your lab report. Remember to label which specimen is in each lane and to label the bands of the ladder (refer to Figure 4).
Your co-worker, Chad, prepared the PCR samples for sequencing and sent them off to the DNA sequencing company for you. The data has just come in and Chad has shared the data with you so that you can analyze it to confirm who committed the crime.

**BLAST search**

1. Download the sequencing results that are posted to Moodle.

2. Trim off any N’s that are at the beginning or end of the sequence by simply deleting them.


4. Select nucleotide BLAST under Basic BLAST

5. Copy the Kona sequencing data (with N’s trimmed) and paste into the dialog box under “Enter Query Sequence”.

6. Under “Choose Search Set”, select the “Other” Database and make sure that “Nucleotide collection (nr/nt)” is selected from the drop down menu.

7. Click BLAST

8. When the results are displayed, write the name of the gene with the highest homology to the query along with the accession number and E-value in Table 12.

9. Repeat steps 3-8 with the Lady sequencing data.

10. Repeat steps 3-8 with the crime scene sequencing data.
DISCUSSION QUESTIONS

Part 1

1. What similarities did you observe about the expected PCR product for dog and human based on the *in silico* PCR and BLAST search? What differences did you observe between the PCR products?

Part 2

2. What were the A260/A280 values for your DNA samples? Do the A260/A280 values that were measured indicate that the DNA was contaminated? If so, what was it contaminated with?

Part 3

3. In the PCR reaction, why was the extension time only 30 seconds? Could a longer or shorter extension time have been set? Explain.

Part 4

4. What size PCR products did you observe in the Kona and Lady control samples?

5. Did you observe any PCR products that were not expected? If so, what could they represent?

6. Based on your PCR results, who committed the crime? Explain.

Part 5

7. According to your BLAST search, which gene was amplified from the Kona DNA sample? What gene was amplified from the Lady DNA sample? Does this support of your *in silico* PCR and BLAST results from part 1?

8. Based on all of your data, who committed the crime?
APPENDIX 4: SEQUENCING RESULTS
Sequencing Results – Lady (human) committed the crime

>Kona
ATTAGCAGTGTAGAGACACCTGTGACACACACAAACGCCTTGCACTATCTCTAAAGAGAAGAATATGTTTTCAGGAGAATGGTGACAGCTCCCACACAAAGAAATGGTGACAGCTCCCCACACAAAGAAGAAATGGTGACAGCTCCCCACACAAAGAAATGGTGACAGCTCCCCACACAAAGAAGAAATGGTGACAGCTCCCCACACAAAGAAGAA

>Lady
ATTCTGCACTGTTCCACACCTGTGACAGCTCCCACACAAAGAAATGGTGACAGCTCCCACACAAAGAAATGGTGACAGCTCCCACACAAAGAAATGGTGACAGCTCCCACACAAAGAAGAAATGGTGACAGCTCCCACACAAAGAAATGGTGACAGCTCCCACACAAAGAAGAA

>crimescene
ATTCTGCACTGTTCCACACCTGTGACAGCTCCCACACAAAGAAATGGTGACAGCTCCCACACAAAGAAGAAATGGTGACAGCTCCCACACAAAGAAGAAATGGTGACAGCTCCCACACAAAGAAGAA
Sequencing Results – Kona (dog) committed the crime

>Kona
ATTAGCAGTGTAGAGACACCTGTCCACAAACGCCCTTGAGATCTTAAAGAAGAATGATTGGTACGC
AGACAAATGGTGACAGTTATCAACAAAAAGGATCTCTTTTTTTCTCCTTCCTCCATCCTGAGGACCT
GAGTGCACTGTGATTTTTTTTTCAAAACTTTTTCTAGGTATGGGAATTCTGCTGGGATCCATGAACAAACATT
TAATAGCAGTATGGAGATGTGACATTTGACATCCGCAAGGACCTTTTATGCCAAACGTGCTATCCGA

>Lady
ATTCTGCAGTGTCTTTATAGGGGAACATATGTTTCAGAGAAGAATGTGACAGCTCCCCACACAAAGAA
GTTCTGTTCTCTTCCCTCTACCTTGAGCCCTGAGATCCTGATGTTGATGTTGATGTTGATGTTGATGTT
GTTGATGTTGACTGTTCCAGGTATGGAAATCTGCTGGCATTGAAACACTTACATGACACATG
AGTGTGACATTGATATCCGCAAGGACCTTCTTTATCTGAGGCACTTCATTTGACCCTTTTCT
GATTGATGATGTAGATACGAAAGAGGAAAGCGAGTCCCTCCAGGACCTTTATG

>crimescene
GTTAGCAGTGGATAGAGACACCTGTCCACAAACGCCCTTGAGATCTTAAAGAAGAACATATGGTTTCAG
AGACAAATGGTGACAGTTATCAACAAAAAGGATCTCTTTTTCTCCTTCCTCCATCCTGAGGACCT
GAGTGCACTGTGATTTTTTTTTCAAAACTTTTTCTAGGTATGGGAATTCTGCTGGGATCCATGAACAAACATT
TAATAGCAGTATGGAGATGTGACATTTGACATCCGCAAGGACCTTTTATGCCAAACGTGCTATCCGA
APPENDIX 5: LAB REPORT GUIDELINES, RUBRIC, AND SAMPLE LAB REPORT
Lab Report Guidelines

1. Lab reports are due at the beginning of class on the day they are due. No late lab reports will be accepted; you will receive a zero for a lab report that is turned in late.

2. There are some days in lab where we are performing more than one experiment. A lab report is on a particular topic (as indicated in the syllabus), NOT on the experiments performed on a particular day. Thus, your lab report may cover experiments performed on more than one day.

3. If you have a question concerning your grade or feel that you have been graded unfairly, please feel free to discuss your lab report grade with your Instructor.

4. You are allowed and encouraged to discuss the results of the lab with other Students and Instructors. However, each student must turn in their own, original work, which they have written in their own words.

5. Points will be taken off for multiple grammar and spelling mistakes.

6. Lab reports should be written clearly and succinctly. Points will be deducted for including unnecessary or irrelevant information in your lab report.

7. Imagine that the audience reading your lab report is upper-level biology majors.

8. Write in first person, passive tense.

9. All lab reports must be typed using single spacing (Arial, Times New Roman or Cambria, size 11-12 font).

Lab reports must contain the following sections:

**Title:** should be representative of lab work completed and should be in your own words.

**Purpose:** briefly state the goal or question of the experiment and any relevant background information that may have led you to ask this question. Make sure to cite any relevant background information (i.e. protocol, course notes or other outside sources).

**Hypothesis:** clearly state your hypothesis, as done for the “My Proposed Experiment” worksheet.

**Materials and Methods:** summarize the procedure(s) used in the experiment in your own words. This should build on the minimal details that are outlined in the protocol as well as the “My Proposed Experiment” worksheet. For this section, you must demonstrate your understanding of the protocol (i.e. why you did what you did) and it should be written with enough detail that a classmate could repeat the procedure using your lab report as a guide. Make sure that you indicate any changes to the provided lab protocol (i.e. mistakes/errors or changes suggested by the Instructor) and include any calculations that may have been used while performing the experiment. The lab protocol and any additional sources used should be cited.
**Results:** This section must contain two parts: (1) presentation of the physical data in the form of figures and tables and (2) textual description of the data. Tables and figures may be helpful in summarizing the results, but should **NOT** constitute the entire Results section. Tables should be labeled as tables and anything else as a figure. **Figures and tables should include a title and figure should also have a brief legend included** (refer to the example figure and table at the end of these guidelines). Do not describe the results from the figure in the figure legend; description of the figure results should be found in the textual portion of the Results section. The **textual portion should be a paragraph-structured narration of the data.** Images of gels, blots or other physical data generated during lab must be included in the report and all lanes and molecular weight ladders must be labeled (labeling of gels can be neatly hand written). Refer to the end of these guidelines for samples figures and tables.

**Discussion:** This section should include a **brief summary** of your results and a **statement concerning whether or not your hypothesis was supported.** You should discuss **why the results were as expected or not** and any errors that you and/or your partner made and how these errors could have affected your results and their consequences. Keep in mind that mentioning student error (i.e. pipetting error) does not eliminate the need to describe other potential reasons why the results were not expected. Think about the biological implication of your results and where things could go awry. **Be sure to address the questions that are found in the Discussion section of the lab protocol;** do not answer these line by line but instead incorporate the answers into the Discussion section in paragraph format. You should also discuss the meaning of the results and explain what is the biological significance(s) of a “positive” and “negative” result. **In this section, you must demonstrate that you understand your lab results and the implications of any deviations from these results.**

**Reflections and Opinions:** this section should describe your **opinions of the subject and if they have changed upon completion of the laboratory exercise.** This can include changes to how you view science and/or the world around you, any opinions of the lab exercise or something interesting that you learned while performing the lab. **Students will not be deducted points for expressing negative opinions towards the laboratory exercise.**

**References:** A list of references that were used to write the lab report should be found, including the lab report. Remember to denote these citations within the text of the lab report.

Refer to the rubric below for section values.
<table>
<thead>
<tr>
<th>SECTION</th>
<th>GUIDE QUESTION</th>
<th>EXCELLENT</th>
<th>DEVELOPING</th>
<th>INADEQUATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title (3)</td>
<td>How could I summarize this lab in one sentence?</td>
<td>3 – Title clearly and accurately describes the topic of the experiment</td>
<td>1.5 – Description of the topic is missing more than one important element</td>
<td>0 – Title is missing or is not in own words.</td>
</tr>
<tr>
<td></td>
<td>Points: 3</td>
<td>5 – Clearly defined question based on background information</td>
<td>2.5 – Question is somewhat defined</td>
<td>0 – missing</td>
</tr>
<tr>
<td>Purpose (12)</td>
<td>What is my question or goal?</td>
<td>3 – Detailed description of relevant background information</td>
<td>1.5 – Lack of relevant background information or inclusion of irrelevant information</td>
<td>0 – missing</td>
</tr>
<tr>
<td></td>
<td>Points: 5</td>
<td>4 – Name all techniques used in the lab and briefly describe purpose</td>
<td>2 – Lack of relevant information or inclusion of irrelevant information regarding techniques used</td>
<td>0 – missing</td>
</tr>
<tr>
<td></td>
<td>What background information leads me to ask this question?</td>
<td>5 – Clearly defined hypothesis in the correct format</td>
<td>2.5 – lacking a clearly defined hypothesis and/or incorrect format</td>
<td>0 – missing</td>
</tr>
<tr>
<td>Hypothesis (5)</td>
<td>What is my hypothesis?</td>
<td>15 – detailed narrative of experimental approach in your own words, including any calculations performed</td>
<td>7.5 – lack of key experimental steps performed or inclusion of unnecessary information</td>
<td>0 – procedure missing or not in own words</td>
</tr>
<tr>
<td>Materials and Methods (15)</td>
<td>What experimental steps did I perform?</td>
<td>20 – comprehensive descriptions of my results in narrative form. This should include comparing experimental and control samples</td>
<td>10 – brief and/or incomprehensive description of results or inclusion of unnecessary information</td>
<td>0 – missing</td>
</tr>
<tr>
<td>Results (35)</td>
<td>What were the details of my observations?</td>
<td>15 – clearly labeled physical evidence, in the form of figures and/or tables, with appropriate titles and legends</td>
<td>7.5 – physical evidence lacks clear titles and/or appropriate legends.</td>
<td>0 – missing</td>
</tr>
<tr>
<td></td>
<td>Based on my data, what am I claiming?</td>
<td>Points: 10</td>
<td>Detailed description of how my question is answered by the collected data and its biological meaning</td>
<td>5 – lack of detail or inclusion of unnecessary information</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------------------</td>
<td>------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Discussion (25)</strong></td>
<td>How does my data support this claim?</td>
<td>Points: 15</td>
<td>15 – Detailed statement of whether data supports or refutes my hypothesis and why. Discussion questions are appropriately addressed.</td>
<td>7.5 – lack of detail and/or explanation as to why hypothesis is supported or refuted or inclusion of unnecessary information. Discussion questions are somewhat addressed.</td>
</tr>
<tr>
<td>Reflections and opinions (3)</td>
<td>Have my conceptions or opinions of this subject changed?</td>
<td>Points: 3</td>
<td>3 – complete</td>
<td>0 - missing</td>
</tr>
<tr>
<td><strong>Citations (2)</strong></td>
<td>Have I appropriately cited the lab protocol and other sources?</td>
<td>Points: 2</td>
<td>2 – complete list of references with citations properly denoted in the text</td>
<td>0 - missing</td>
</tr>
</tbody>
</table>

NAME ____________________________ GRADE _____________/100
Table 1: DNA concentration and A260/A280 values from dog samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ng/µL)</th>
<th>A260/A280</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>281.2</td>
<td>1.67</td>
</tr>
<tr>
<td>Dog 2</td>
<td>347.9</td>
<td>1.59</td>
</tr>
<tr>
<td>Dog 3</td>
<td>334.1</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Figure 1: DNA agarose gel results from restriction digestion of pBIT with BamHI and EcoRI. Lane 1, molecular weight ladder with sizes denoted; lane 2, uncut pBIT; lane 3, pBIT digested with EcoRI only; lane 4, pBIT digested with BamHI and EcoRI.
Instructor notes:
Students should have access to the lab report guidelines, rubric, and sample figures above when preparing lab reports.

Hypothesis

• There is not a hypothesis for this particular activity – thus all students should receive 5 “free” points. Students should be reminded to not complete this part of the activity and should not be penalized if they attempt to formulate a hypothesis.

Materials and Methods:

• Should provide detailed narrative of the experimental steps used in the procedure in their own words. This should include any calculations used during the experiment.
  o Do not penalize if calculations (such as Table 5 in the Student Handout) are presented in the results section instead of materials and methods.

Results:

• Students should describe their results in narrative form in this section. They should relate the crime scene results back to the control samples – Lady (human) or Kona (dog). The following results should be discussed:
  o In silico PCR and BLAST results (part 1)
  o DNA quantification and purity results from DNA spectrophotometer (part 2)
    ▪ Students may include calculations for diluting the DNA in this section
  o PCR results, as analyzed by agarose gel electrophoresis (part 4)
  o Sanger Sequencing and BLAST results (part 5)
• Students should display the following figures and/or tables:
  o In silico PCR and BLAST results (part 1)
    ▪ Student Handout table 1 (appropriate title in own words)
    ▪ Student Handout table 2 (appropriate title in own words)
  o DNA quantification and purity (part 2)
    ▪ Student Handout table 3 (appropriate title in own words)
    ▪ Student Handout table 5 (appropriate title in own words) – do not penalize if presented in the methods section.
  o Agarose gel results from PCR (part 4)
    ▪ Figure should be presented with appropriate title and legend. It should be clear what sample and master mix were used in each lane and the bands of the molecular weight ladder should be appropriately labeled.

Discussion:

• Students should clearly state who committed the crime. This should include a statement describing why they came to the conclusion made (discussion questions 6 and 8 guide students to include this statement).
• Students should answer the discussion questions (DQs) found in the student handout. During the laboratory activity, students should be encouraged to write answers to appropriate DQs in their lab notebook or on the Student Handout and have them reviewed by the instructor at the conclusion of each component of the lab exercise. These DQs are designed to get students to think critically about their data (DQs 1, 4, 6, 8) the experimental procedure (DQs 3, 7), and how
error may have affected their results (DQ 2, 5, 7). Refer to the Discussion question key below for anticipated answers and grading details.

Reflection and opinions:

- In this section, students should simply describe their thoughts on the experiment. If the thoughts are negative, students should not be penalized. This is simply a way for students to reflect on the laboratory experience, positive or negative.

Citations:

- Students should have a reference section at the end of the lab report, with consistently formatted in-text citations. The lab protocol at a minimum should be referenced, as well as any outside sources (lecture materials, materials students found on their own, etc.).

Discussion Questions and Key - Discussion questions should be graded based on completion and accuracy and worth 2 points each. One point should be deducted from each inaccurate discussion question response and two points should be deducted for each omitted discussion question.

Part 1

1. What similarities did you observe about the expected PCR product for dog and human based on the in silico PCR and BLAST search? What differences did you observe between the PCR products?

   Similarities include that both the dog and human PCRs should amplify the cardiac actin gene. Differences include size (dog is 275 bp, human is 397 bp) and chromosomal location (dog chromosome 30, human chromosome 15).

Part 2

2. What were the A260/A280 values for your DNA samples? Do the A260/A280 values that were measured indicate that the DNA was contaminated? If so, what was it contaminated with?

   Pure DNA has an A260/280 value of 1.8. If A260/280 is below 1.8, the sample is contaminated with protein.

Part 3

3. In the PCR reaction, why was the extension time only 30 seconds? Could a longer or shorter extension time have been set? Explain.

   The PCR products were less than 500 bp, so only 30 seconds were needed. The general rule is that 1 minute per kb is used for the extension time. A longer time could have been set, but
may result in off target products. A shorter time would likely result in no PCR product because there isn’t enough time to amplify the DNA of interest.

Part 4

4. What size PCR products did you observe in the Kona and Lady control samples? Were these PCR products expected?

Kona was 275 bp, Lady was 397 bp. Both were expected based on in silico PCR results.

5. Did you observe any PCR products that were not expected? If so, what could they represent?

If unexpected PCR products are observed, it is likely due to off-target affects. This could be due to primers annealing to inappropriate areas and/or samples being contaminated with DNA that is amplified by the primers.

6. Based on your PCR results, who committed the crime? Explain.

Will depend on which sample (Lady-human or Kona-dog) was given as the crime scene sample. Students should provide rationale for argument (such as amplicon presence and size in crime scene sample)

Part 5

7. According to your BLAST search, which gene was amplified from the Kona DNA sample? What gene was amplified from the Lady DNA sample? Does this support of your in silico PCR and BLAST results from part 1?

BLAST results should confirm in silico PCR results – both the Lady and Kona control should depict the cardiac actin gene as the first hit on BLAST.

8. Based on all of your data, who committed the crime?

Will depend on which sample (Lady-human or Kona-dog) was given as the crime scene sample. Students should relate back to sequencing results confirming the PCR results.
Utilization of polymerase chain reaction and bioinformatics tools for DNA comparison to solve a crime

Purpose:
On May 5, 2013 Julie came home to a trail of blood that lead to her frightened cat, Savannah. Due to the blood, Savannah was taken to the vet, where she was examined and found to have no injuries or scratches; meaning the blood came from the perpetrator who scared Julie’s cat. In an effort to solve who scared Savannah, a blood sample was taken from the crime scene, and the next day interviews were conducted with the possible culprits: Kona and the old Lady. Both Kona, a golden retriever mix, and the old Lady, who is known to hate cats, lived nearby and had fresh wound patterns that could have been inflicted by Savannah. After the interview, Kona and the old Lady agreed to give a blood sample, and blood samples were then taken. All three samples, Kona’s, Lady’s, and the crime scene, were given to a pet detective who isolated genomic DNA from the samples.

Given the isolated genomic DNA, Savannah’s culprit could be named using polymerase chain reaction (PCR) and bioinformatics tools. PCR is a technique used to amplify a specific gene sequence by generating many copies of the target DNA. Gel electrophoresis is then run to create a visual representation by creating band patterns of the target DNA obtained through PCR. By comparing band sizes of the two suspects with the crime scene sample, Savannah’s attacker can be identified. Even though the same gene was isolated, the gene length varies between species, which is utilized by PCR and comparing genes. Bioinformatics tools can also be used to assist in PCR, as they give information on gene size, what the gene codes for, and location of the gene, which can be used to confirm results by comparing expected results with experimental results. Bioinformatics tools include in silico PCR and BLAST. As a final comparison and confirmation, DNA sequencing can be performed. This method is a modified version of PCR and results in the exact nucleotide sequence of DNA samples.

Hypothesis:
If the either the old lady or Kona scared the cat, then PCR, agarose gel electrophoresis, and bioinformatics tools can be used to identify who scared the cat. PCR amplifies the target DNA, gel electrophoresis allows DNA to be visualized by dying and separating DNA strands based on length, and bioinformatics tools can be used to set up PCR, identify the gene, and confirmation.

Materials and Methods:
Day 1
In order to understand PCR and the conditions to use for run time and temperature during the annealing and extension steps, which are based on the target DNA and primers, information about the gene and primers must first be obtained. The annealing temperature, in step two of PCR, is affected by the primer melting point. The extension time is based on target DNA length, and is given the conversion of 1Kb/minute; meaning for every 1000 base pairs it takes 1 minute to add the bases to the primers. An in silico PCR was used to obtain the necessary information about chromosome location, primer melting points, and expected PCR product size on www.genome.ucsc.edu on the following primers:
  Dog Forward Primer: 5' - AGCACTGTTAGAGACACCTG
  Dog Reverse Primer: 5' - CGGATAGCACGTTGTTGGCA
  Human Forward Primer: 5' - CTGCAGTGTGTCCTTATAGGG
  Human Reverse Primer: 5' - GAATACCAAGACTTGCCCTCG
A BLAST search was then run using the *in silico* PCR results to figure out the gene name, accession number, and E-value of the target DNA.

The three samples were then obtained of Kona, Lady, and the crime scene, and using a NanoDrop and “ND-1000” software the DNA purity and concentration were determined after blanking with 1 µL of sterile water. Each 1 µL sample was run separately, and the concentration and A260/280 value were recorded. The A260/280 value is used to access the purity of the samples, and in compared to the 1.8 ratio that is considered pure DNA. Samples with values below 1.8 indicate contamination with protein. Dilutions of 200ng/µL of 10 µL stock samples were then prepared using the concentrations found by the NanoDrop and the following calculation to find out the volume of sterile water and DNA for the dilution:

\[
[\text{Concentration-have}] \times [\text{volume}] = [\text{concentration-wanted}] \times [\text{volume-wanted}]
\]

The dilutions were created in labeled 1.5mL Eppendorf tubes. A master mix was then obtained which consisted of *Taq* DNA polymerase, MgCl₂, and dNTPs as described in table 1 (Ott 2015).

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Dog master mix</th>
<th>Human master mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Primer (10 pmol/µL)</td>
<td>2 µL</td>
<td>2 µL</td>
</tr>
<tr>
<td>Reverse Primer (10 pmol/µL)</td>
<td>2 µL</td>
<td>2 µL</td>
</tr>
<tr>
<td>2X PCR master mix</td>
<td>25 µL</td>
<td>25 µL</td>
</tr>
<tr>
<td>Sterile water</td>
<td>19 µL</td>
<td>19 µL</td>
</tr>
<tr>
<td>TOTAL VOLUME</td>
<td>48 µL</td>
<td>48 µL</td>
</tr>
</tbody>
</table>

Table 1: Reaction mix makeup of solutions used in PCR for both dog and human DNA (Ott 2015)

Then 4 PCR tubes were obtained, labeled and prepared following table 2. Using a micropipette, 48 µL of the dog master mix was placed in labeled PCR tubes 1 and 3, and 48 µL of the human master mix was placed in labeled PCR tubes 2 and 4. Next, 2 µL of Kona’s dilution sample was placed in PCR tube 1, 2 µL of Lady’s dilution sample in PCR tube 2, and 2 µL each of the crime scene dilution in PCR tubes 3 and 4.

<table>
<thead>
<tr>
<th>PCR Tube</th>
<th>Makeup</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48 µL dog master mix 2 µL Kona dilution</td>
</tr>
<tr>
<td>2</td>
<td>48 µL human master mix 2 µL Lady dilution</td>
</tr>
<tr>
<td>3</td>
<td>48 µL dog master mix 2 µL crime scene dilution</td>
</tr>
<tr>
<td>4</td>
<td>48 µL human master mix 2 µL crime scene dilution</td>
</tr>
</tbody>
</table>

Table 2: PCR tube makeup of solutions for PCR

PCR tubes were then loaded into the PCR machine which was run under the following conditions in table 3 (Ott 2015). Conditions vary depending on the three steps that PCR follows: denaturation, annealing, and extension. Denaturation unzips the DNA into single stands, this is closely followed by the annealing step where primers bind to single strands, and completed during extension where *Taq* polymerase synthesizes new DNA.

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time (min)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94°C</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>.5</td>
<td>Repeat 35X</td>
</tr>
<tr>
<td>Annealing</td>
<td>61°C</td>
<td>.5</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>.5</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>72°C</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td>Infinity</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: conditions run to amplify dog and human DNA samples in PCR tubes (Ott 2015)
Day 2
The next step in visualizing and comparing DNA samples was setting up and running an agarose gel electrophoresis. A 1% agarose gel was prepared using 1 gram of agarose and adding it to 100mL of 1X TBE buffer to a glass bottle. This solution was then heating in the microwave until agarose was completely dissolved. Then 30mL of melted agarose was measure into a 50mL conical test tube, and using a micropipette 3µL of GelRed was added. The tube was then slowly inverted 3 times, trying to prevent bubbles from forming while mixing the GelRed and melted agarose. The gel apparatus was then assembled by taping up the sides of glass plate, placing blocks and comb, and adding agarose GelRed mixture into the casting tray. Tape was added so that the DNA being run does not exit the plate and enter into solution. The agarose gel was allowed to solidify for 20 minutes before removing the blocks, comb and tape. Then 160mL of 1X TBE was poured over the gel in the apparatus.

The samples for the gel electrophoresis were then made by adding 3µL of 10X DNA loading dye and 27µL of PCR DNA samples into four fresh, labeled Eppendorf tubes. 12µL of the low mass ladder and each of the samples were added to the gel by carefully loading them into the wells created by the comb. Once the samples were loaded the lid was close and electrical leads were hooked up following the saying “red to red, black to black”. The red cable was opposite the wells because it creates a positive charge, and DNA is negatively charged, thus the DNA will run to the positive because opposites attract. The gel was run at 120 volts for approximately forty-five minutes. After the gel was run, a picture was taken under UV light to visualize the DNA and compare the samples so to identify who scared the cat.

Day 3
As a final conformation, the PCR samples were sequenced and given back to be blasted on www.ncbi.nlm.nih.gov/BLAST using a nucleotide Basic BLAST. The name of the gene, accession number, and E-value were recorded. Kona’s and Lady’s BLAST resulted were compared to the crime scene BLAST results.

Results:
Day 1 Results
Table 4 shows the expected PCR product sizes, melting temperature of primers, and chromosomal location of gene isolated by primers using in silico PCR. For the dog primers the expected PCR product size was 275 base pairs. The primer melting point was determined to be 52.9°C for the forward primer and 65.3°C for the reverse on chromosome 30. For the human primers the expected PCR product size was 397 base pairs on chromosome 15. The melting points for these primers were 53.4°C and 57.4°C for the forward and reverse respectively.

<table>
<thead>
<tr>
<th>Primer Pair</th>
<th>Expected PCR Product Size</th>
<th>Primer Melting Temperature (°C)</th>
<th>Chromosomal Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog Primers</td>
<td>275bp</td>
<td>F: 52.9 R: 65.3</td>
<td>30</td>
</tr>
<tr>
<td>Human Primers</td>
<td>397bp</td>
<td>F: 53.4 R: 57.4</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4: Results of in silico PCR
Table 5 depicts the results of Basic BLAST using the results found during in silico PCR. The results found out that the gene isolated by the dog primers was the ACTC, also known as the Cardiac Actin gene, which had an E-value of 2e-137 and accession number of AH010031.2. The results for the
human primers showed that they isolated the ACTC 1 gene, or the actin alpha cardiac muscle gene, which had an accession number of NG 007553.1 and 0.0 E-value.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Accession Number</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog Primers Cardiac Actin (ACTC)</td>
<td>AH010031.2</td>
<td>3e-137</td>
</tr>
<tr>
<td>Human Primers Actin Alpha Cardiac Muscle (ACTC 1)</td>
<td>NG 007553.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 5: Blast of in silico PCR

Table 6 shows the results after analyzing the purity and concentration of DNA samples using a NanoDrop. Kona's DNA had a concentration of 534.6 ng/µL and an A260/280 ratio of 1.76. Lady’s DNA had a concentration of 472.6 ng/µL and an A260/280 ratio of 1.75. The crime scene DNA had a concentration of 544.7 ng/µL and an A260/280 ratio of 1.76. Even though all values were below 1.8 the purity was within an accepted range.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA Concentration (ng/µL)</th>
<th>A260/280</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kona</td>
<td>534.6</td>
<td>1.76</td>
</tr>
<tr>
<td>Lady</td>
<td>472.6</td>
<td>1.75</td>
</tr>
<tr>
<td>Crime Scene</td>
<td>544.7</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Table 6: Results of NanoDrop for concentration and purity given in A260/280 value for DNA samples

Table 7 shows the calculated volumes necessary to make 10µL of 200ng/µL dilutions given the DNA concentration results from NanoDrop. To create this dilution with Kona’s DNA sample it would take 3.7µL of her DNA and 6.3µL of sterile water. For Lady’s DNA sample it would take 4.2µL of her DNA and 5.8µL sterile water. For the crime scene dilution it would take 3.7µL and 6.3µL of DNA and water respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA Concentration (ng/µL)</th>
<th>Volume of DNA (µL)</th>
<th>Volume of Sterile Water (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kona</td>
<td>534.6</td>
<td>3.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Lady</td>
<td>472.6</td>
<td>4.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Crime Scene</td>
<td>544.7</td>
<td>3.7</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Table 7: Calculated volumes for 200ng/µL dilution of 10µL of DNA samples given the DNA concentrations found using NanoDrop

Day 2 Results

Figure 8 shows the results of gel electrophoresis. Lane 1 is the DNA ladder which has a known number of base pairs and is used to compare and approximate the number of base pairs on experiment DNA. Lane 2 contains the DNA of Kona with dog master mix. Lane 3 contains the DNA of Lady with human master mix. Lane 4 contains the crime scene DNA with dog master mix. Lane 5 contains the crime scene DNA with human master mix. Lanes 3 and 5 had similar band patterns around the 400 base pair mark. Lane 2 had a band around 275 base pairs. Lane 4 did not have any bands.
Figure 8: Results of agarose gel electrophoresis on PCR samples and DNA ladder. Lane 1-DNA ladder; lane 2-Kona DNA and dog master mix; lane 3-Lady DNA and human master mix; lane 4-crime scene DNA and dog master mix; lane 5-crime scene DNA and human master mix

**Day 3 Results**

Table 9 depicts the nucleotide Basic BLAST results after the PCR samples were sequenced. The gene isolated during PCR for Kona was Cardiac Actin (ACTC), which had an accession number of AH010031.2 and E-value of 3e-135. The results for Lady and crime scene PCR DNA were the same; the gene isolated was actin alpha cardiac muscle (ACTC 1) with an accession number of NG 007553.1 and E-value of 0.0.

<table>
<thead>
<tr>
<th>PCR Sample</th>
<th>Gene Name</th>
<th>Accession Number</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kona</td>
<td>Cardiac Actin (ACTC)</td>
<td>AH010031.2</td>
<td>3e-135</td>
</tr>
<tr>
<td>Lady</td>
<td>Actin Alpha Cardiac Muscle (ACTC 1)</td>
<td>NG 007553.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Crime Scene</td>
<td>Actin Alpha Cardiac Muscle (ACTC 1)</td>
<td>NG 007553.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 9: Nucleotide Basic BLAST results on sequenced PCR DNA

**Discussion:**

The primers used for PCR separated a piece of DNA that coded for Cardiac Actin (ACTC) for the dog and Actin Alpha Cardiac Muscle (ACTC 1) for the human according to both *in silico* PCR and BLAST results of primer sequence. The ACTC gene is 275 base pairs, whereas the ACTC 1 is 397 base pairs. The extension time was thus chosen to be 30 seconds because of the PCR expected product sizes. Using the conversion 1 kilobases per minute, and the largest expected product was 397 base pairs, this
gives Taq polymerase enough time to completely synthesis target DNA. The A260/280 value indicated the samples purities and where determined using the NanoDrop. This value was 1.76 for Kona, 1.75 for Lady, and 1.76 for the crime scene. A pure DNA sample would have an A260/280 value of 1.8, and anything lower is considered to be contaminated with protein. Thus the values found of the samples were all slightly contaminated with protein; however the values were well within the range for error and deemed not significant.

After running the agarose gel electrophoresis it was determined that the old lady scared Savannah the cat, and was later confirmed the BLAST of sequenced PCR DNA. This supported my hypothesis, as there was both visual confirmation and confirmation through nucleotide BLAST that the old lady scared the cat. This conclusion was based on the band pattern and expect PCR product size found using in silico PCR. The expected product size for Kona was 275 base pairs and 397 base pairs for the old lady. After running the gel, lanes 3 and 5 had similar band patterns around 400 base pairs. Lane 3 contained the old lady DNA and human master mix, and lane 4 contained crime scene DNA and human master mix. Thus, in order to get the same band pattern around the 400 base pair mark, the crime scene DNA must belong to the old lady. Also, lane 4 contained dog master mix and crime scene DNA, and no bands were observed. This indicates that PCR was not able to isolate a gene, which makes sense because the human master mix contains the human primers that will not be able to bind to the dog/Kona’s DNA. This was confirmed with a nucleotide Basic BLAST of the PCR DNA after sequencing. The results of this showed that the crime scene and old lady DNA coded for the same gene and had the same accession number, which is unique.

Reflection and Opinions:
I found this lab and the application of PCR and bioinformatics tools very interesting. I very much enjoyed preforming this lab, as I was able to apply what I learned in lecture to the lab and gain a better understanding about how PCR and agarose gel electrophoresis is performed. It is a very satisfying feeling to be able to digest and apply knowledge to a lab like this. It also helped me retain the information about the PCR steps and about DNA purity. If I were to redo this experiment, I would be interesting about learning exacting how the different primers were picked and how the master mixes were created.

References:
“Who Scared the Cat” Lab Protocol, Dr. Ott. 2015 (posted on Moodle)
APPENDIX 6: PRE- AND POST-QUIZ WITH ANSWER KEY
PRE-/POST-ACTIVITY QUIZ

1. Using the nanodrop, you determine the A260/280 value of your DNA sample to be 1.6. Which of the following is true?
   a. Your DNA is pure
   b. Your DNA is contaminated with protein
   c. Your DNA is contaminated with RNA

2. If your 1:2 diluted sample has an A260 value of 0.5, what is the concentration of DNA in your sample?
   a. 25 ng/μL
   b. 50 ng/μL
   c. 100 ng/μL

3. If you pipette 1 μL of DNA into 4 μL of sterile water, what is the dilution factor of your sample?
   a. 1:5
   b. 1:4
   c. 1:3

4. What is the directionality of DNA?
   a. 3’ to 5’
   b. 5’ to 3’
   c. 2’ to 4’

5. DNA is _____________ charged.
   a. Negatively
   b. Positively
   c. Neutral

6. Refer to the picture of a DNA agarose gel on the right. Which of the following is true?
   a. The DNA in sample 1 is larger than sample 2
   b. The DNA in sample 2 is larger than sample 1
   c. The DNA in both samples are the same size

7. _____________ is an enzyme used to replicate DNA during PCR.
   a. Taq DNA polymerase
   b. DNA ligase
   c. Restriction endonuclease

8. In PCR, the forward primer anneals to which strand?
   a. The sense strand
   b. The anti-sense strand
   c. Both strands

9. If you wanted to amplify a 2 kb gene, what should your extension time be?
   a. 20 seconds
   b. 1 minute
   c. 2 minutes
10. PCR is able to make many copies of a single DNA molecule. Why?
   a. It takes a really long time (24 hours)
   b. Each strand of DNA is copied in a separate tube
   c. Each copy of DNA synthesized in the current cycle is used as template DNA in subsequent cycles

11. What is unique about the primers used in a Sanger sequencing reaction?
   a. A Sanger sequencing reaction uses 1 primer instead of 2 (like in PCR)
   b. Sequencing primers are double stranded
   c. The primers of a sequencing reaction are fluorescently labeled.

12. In Sanger sequencing, what is unique about the dideoxynucleotide triphosphate (ddNTP) that is included?
   a. It contains a hydrogen (H) instead of a hydroxyl group (OH) on the 2’ carbon
   b. It contains a hydrogen (H) instead of a hydroxyl group (OH) on the 3’ carbon
   c. It contains a hydrogen (H) instead of a hydroxyl group (OH) on the 5’ carbon

13. What is the purpose of performing in silico PCR?
   a. To replicate DNA inside a cell
   b. To predict PCR products (size and sequence) based on primer sequences
   c. To predict the primers that should be used to amplify DNA

14. You just sequenced a gene from a newly discovered animal, whose genome has not been sequenced. Which bioinformatics tool would be useful in predicting the potential function of this gene?
   a. In silico PCR
   b. BLAST
   c. Clustal W

**QUIZ KEY**

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td>8.</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td>9.</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td>10.</td>
<td>C</td>
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<tr>
<td>4.</td>
<td>B</td>
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<td></td>
<td>11.</td>
<td>A</td>
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<tr>
<td>5.</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td>12.</td>
<td>B</td>
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<td>6.</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td>13.</td>
<td>B</td>
<td></td>
</tr>
</tbody>
</table>
LEARNING GAINS ASSESSMENT (POST ACTIVITY)

By participating in this activity, I feel that I gained the ability to calculate the concentration of a DNA sample.
   1. Strongly disagree
   2. Disagree
   3. Neither agree nor disagree
   4. Agree
   5. Strongly agree

By participating in this activity, I feel that I gained the ability to determine the purity of a DNA sample.
   1. Strongly disagree
   2. Disagree
   3. Neither agree nor disagree
   4. Agree
   5. Strongly agree

By participating in this activity, I feel that I gained an understanding of DNA agarose gel electrophoresis.
   1. Strongly disagree
   2. Disagree
   3. Neither agree nor disagree
   4. Agree
   5. Strongly agree

By participating in this activity, I feel that I gained an understanding of the process of polymerase chain reaction (PCR)
   1. Strongly disagree
   2. Disagree
   3. Neither agree nor disagree
   4. Agree
   5. Strongly agree

By participating in this activity, I feel that I gained an understanding of DNA sequencing
   1. Strongly disagree
   2. Disagree
   3. Neither agree nor disagree
   4. Agree
   5. Strongly agree

By participating in this activity, I feel that I gained the ability to analyze PCR results
   1. Strongly disagree
   2. Disagree
   3. Neither agree nor disagree
   4. Agree
   5. Strongly agree
By participating in this activity, I feel that I gained the ability to use bioinformatics tools to predict genes to be amplified during PCR

1. Strongly disagree
2. Disagree
3. Neither agree nor disagree
4. Agree
5. Strongly agree

By participating in this activity, I feel that I gained the ability to use bioinformatics tools to analyze sequencing results

1. Strongly disagree
2. Disagree
3. Neither agree nor disagree
4. Agree
5. Strongly agree
APPENDIX 7: OTHER POSSIBLE ACTIVITY MODIFICATIONS
Possible Modifications to “Who Scared the Cat?”

An additional modification of this activity would be to highlight specific forensic PCR applications, such as short tandem repeat (STR) analysis. STR analysis is routinely performed in forensic investigations, as it is a method to investigate 13 core loci, with each loci having an established set of unique allele lengths. Given that the likelihood of two individuals having a similar STR profile is 1 in 1 billion, STR analysis is commonly used to identify individuals based on genetic samples.1,2 STR analysis is performed by a multiplex PCR, with fluorescently labeled primers, and data is analyzed using capillary electrophoresis. For this modification, which is adapted from previously described critical thinking scenarios, a discussion of STR analysis could be added at the completion of this activity through watching a video and a mini-lecture (see below). Students could then review a hypothetical STR analysis experimental design and data, where inappropriate conclusions are made. Students would then answer questions at the end of the activity, using a think-pair-share setup where students respond to questions individually, then form groups to review answers before debriefing as a class. The questions are designed to get students to think critically about the data and identify that inappropriate conclusions were made, the flaws in the experimental design, and that additional data or information is needed to confirm the culprit. The STR analysis critical thinking scenario, with a question answer key, can be found below. This activity, which should be performed at the conclusion of the laboratory component of the activity described above, would require students to apply their understanding of PCR to a new situation in the context of forensic investigations.

Additionally, the laboratory component of this activity could be modified to include more challenging technical skills. For example, students could prepare their own PCR master mix instead of using a previously prepared master mix, as performed in the current version of the activity. An additional modification would be for students to isolate genomic DNA themselves from a cheek swab or hair follicle instead of being provided purified DNA. The human suspect could be a consenting course instructor, teaching assistant, or student and the dog could be a family pet or lab animal. There are various commercial genomic DNA isolation kits available that instructors could employ for this modification, which should take approximately 2 hours and performed before part 2 of the existing laboratory component of the activity. If instructors choose to make this modification, the human and dog genomic DNA isolation protocols should be approved by the Institutional Review Board (IRB) and Institutional Animal Care and Use Committee (IACUC), respectively prior to implementation. This modification would not only advance students’ technical skills and content knowledge associated with DNA structure and isolation, but also provide a rich context for discussing various bioethical topics, such as the use of animals and humans in research and genetic testing. Along the same lines, discussing cases where individuals were imprisioned for committing crimes and later proven innocent through DNA testing would provide a nice interdisciplinary context for the ethical ramifications and technical limitations or advantages of molecular biology-based forensic testing. The Innocence Project (http://www.innocenceproject.org/) is a potential source of real cases involving exoneration of individuals due to DNA testing and could be used to help facilitate these discussions.

---

Short Tandem Repeats

https://www.youtube.com/watch?v=9bEAJYnVVBA&app=desktop

Video!

http://www.sciphun.com/ross/
<table>
<thead>
<tr>
<th>STR Loci</th>
<th>Chromosomal Location</th>
<th>Repeat Motif</th>
<th>Allele Range&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PCR Product Sizes in Identifier Kit (dye label)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>5q33.1</td>
<td>TAGA</td>
<td>6–15</td>
<td>305–342 bp (6-FAM)</td>
</tr>
<tr>
<td>FGA</td>
<td>4q31.3</td>
<td>CTTT</td>
<td>17–51.2</td>
<td>215–355 bp (PET)</td>
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<tr>
<td>TH01</td>
<td>11p15.5</td>
<td>TCAT</td>
<td>4–13.3</td>
<td>163–202 bp (VIC)</td>
</tr>
<tr>
<td>TPOX</td>
<td>2p25.3</td>
<td>GAAT</td>
<td>6–13</td>
<td>222–250 bp (NED)</td>
</tr>
<tr>
<td>VWA</td>
<td>12p13.31</td>
<td>[TCTG] [TCTA]</td>
<td>11–24</td>
<td>155–207 bp (NED)</td>
</tr>
<tr>
<td>D3S1358</td>
<td>3p21.31</td>
<td>[TCTG] [TCTA]</td>
<td>12–19</td>
<td>112–140 bp (VIC)</td>
</tr>
<tr>
<td>D5S818</td>
<td>5q23.2</td>
<td>AGAT</td>
<td>7–16</td>
<td>134–172 bp (PET)</td>
</tr>
<tr>
<td>D7S820</td>
<td>7q21.11</td>
<td>GATA</td>
<td>6–15</td>
<td>255–291 bp (6-FAM)</td>
</tr>
<tr>
<td>D8S1179</td>
<td>8q24.13</td>
<td>[TCTA] [TCTG]</td>
<td>8–19</td>
<td>123–170 bp (6-FAM)</td>
</tr>
<tr>
<td>D13S317</td>
<td>13q31.1</td>
<td>TATC</td>
<td>8–15</td>
<td>217–245 bp (VIC)</td>
</tr>
<tr>
<td>D16S539</td>
<td>16q24.1</td>
<td>GATA</td>
<td>5–15</td>
<td>252–292 bp (VIC)</td>
</tr>
<tr>
<td>D18S51</td>
<td>18q21.33</td>
<td>AGAA</td>
<td>7–27</td>
<td>262–345 bp (NED)</td>
</tr>
<tr>
<td>D21S11</td>
<td>21q21.1</td>
<td>[TCTA] [TCTG]</td>
<td>24–38</td>
<td>185–239 bp (6-FAM)</td>
</tr>
<tr>
<td>D2S1338</td>
<td>2q35</td>
<td>[TGCC] [TTCC]</td>
<td>15–28</td>
<td>307–359 bp (VIC)</td>
</tr>
<tr>
<td>D19S433</td>
<td>19q12</td>
<td>AAGG</td>
<td>9–17.2</td>
<td>102–135 bp (NED)</td>
</tr>
<tr>
<td>Amelogenin (sex-typing)</td>
<td>Xp22.22</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>X = 107 bp (PET)</td>
</tr>
<tr>
<td></td>
<td>Yp11.2</td>
<td></td>
<td></td>
<td>Y = 113 bp (PET)</td>
</tr>
</tbody>
</table>

The 13 core STR loci used for the U.S. national DNA database are shown in bold font. See [www.cstl.nist.gov/biotech/strbase/multiplex.htm](http://www.cstl.nist.gov/biotech/strbase/multiplex.htm) for information on other commercially available STR kits.

<sup>a</sup>Ranges are calculated from kit allelic ladders (see Figure 1) and do not represent the full range of alleles observed in world populations. A more complete allele listing of these short tandem repeat (STR) loci is available at [www.cstl.nist.gov/biotech/strbase/str_fact.htm](http://www.cstl.nist.gov/biotech/strbase/str_fact.htm).

Butler (2007). *BioTechniques*
STR PCR

- Multiplex PCR with multiple (usually 3) fluorescently labeled primer pairs in each reaction.
- Data analyzed with capillary electrophoresis (think Sanger sequencing)

Butler (2007). BioTechniques
You work in a crime scene laboratory and have just been approached by a coworker who is new to STR analysis. He asked you to review results of a recent STR analysis that he performed on samples collected from a crime scene and three potential suspects. Your colleague designed novel primers and developed his own multiplex PCR to three known STR loci (TH01, TPOX, and VWA). The PCR parameters included an annealing temperature of 54°C and an extension time of 1 minute.

Based on his results (next slide), he feels that he has sufficient evidence to convict suspect 3.
STR Activity

Review the data on the previous slide and answer the following questions:

1. Do you agree with your colleague’s conclusion that suspect 3 should be convicted? Explain.
2. Describe any problems you see with the experimental design and/or conclusions reached?
3. What further information would you need to determine if suspect 3 committed the crime (either known information or further experimentation that could be performed)?
STR Activity - Key

1. Do you agree with your colleague’s conclusion that suspect 3 should be convicted? Explain.

Students should come to the conclusion that the evidence is not strong enough to support suspect three. This should be based on subtle differences between the crime scene sample and sample 3 (to include an extra peak in sample 3 as well as a peak with less intensity) and the fact that only 3 of 13 known STRs are being analyzed.

2. Describe any problems you see with the experimental design and/or conclusions reached?

Problems with the experimental design include only investigating 3 of 13 known STRs and thus basing conviction on incomplete evidence. Further the extra peaks in the suspect 3 data could be the result of an inappropriately designed PCR (primer design, thermocycling conditions, etc.). Further, it is unclear how the DNA was isolated and if there is any contaminating DNA.

3. What further information would you need to determine if suspect 3 committed the crime (either known information or further experimentation that could be performed)?

Would want to review PCR design and procedure to ensure that the correct PCR was designed appropriately (students should mention using bioinformatics techniques to confirm specificity of primers). Further, the results from the other known STRs would be needed to assess whether suspect 3 is the culprit of the crime. Finally, a review of how the DNA was collected from each suspect and crime scene could reveal any contamination accounting for the differences in the crime scene and suspect 3 results.