**Learning Goals/Objectives**

**Introduction to DNA Barcoding (Prezi)**

The students will be able to:

1.     define Linnaean taxonomy and explain the  progress made with this process over the past. 250 years.

2.     describe the importance of DNA barcoding by listing several applications.

  3.     describe the goal of the iBOL project.

  4.     Explain eBOL and navigate the website

  5.     explain the BOLD

  6.     give an outline of the process necessary for DNA barcoding of rockfish

7.      explain the difference between nuclear and mitochondrial DNA.

**Beyond the Barcode Metaphor**

  1.     describe the DNA barcode metaphor

  2.     describe how proteins are formed and what they are composed of

3.     be aware of amino acids categorizations

  4.     draw a model to show the basic structure on a nucleotide

5.     explain the bonds between consecutive nucleotides and the bonds between the bases, and explain their relative strengths.

            6.     state the central Dogma of Molecular Biology and understand when transcription and translation occur

  7.     generate a complementary strand when given a DNA source

  8.     define transcription and briefly explain the role of RNA polymerase.

  9.     name at least 2 differences between DNA and RNA?

  10.  generate a complementary strand of RNA when given the template strand of DNA

  11.  define translation with reference to the roles of mRNA and tRNA.

  12.  translate a sequence of mRNA

13.  explain the vital role the COI gene plays in electron transport chain

14.  understand the degeneracy of the genetic code and the outcome of nucleotide changes at various positions

  15.  calculate the expected rate of nucleotide differences in a segment of DNA

**The COI Barcoding Gene**

  1.     give 3 reasons why the COI gene selected

2.     explain the differences between nuclear DNA and mitochondrial DNA

3.     understand why a mitochondrial gene was selected over a nuclear one

4.     list what the mitochondrial genome encodes

**Isolating Total DNA from Specimen Tissue**

  1.     give the two locations DNA resides

2.     define genomic DNA (gDNA) or total DNA

3.     describe what is happening in a digestion with each of these reagents:

  a.     Proteinase K

b.     RNase

4.     list the other macromolecules present within your sample

5.     explain how to separate DNA from other macromolecules

6.     Define the terms binding, elute, lysis, wash and provide the order they occur to isolate the genomic DNA.

**Examining gDNA using gel electrophoresis**

  1. Describe the basic principal of gel electrophoresis.

2. Describe the list of tools below.

- Electrophoresis chamber

- Gel casting tray

- Sample combs

- Power supply

3. Explain the purpose of gel loading buffer and list the contents of the buffer

4. Explain how the DNA visualized

5. describe the purpose of running gel with gDNA

**Targeted Amplification of the COI Barcode Region**

1.     give the process is PCR based on

2.     list what you need to copy DNA in a test tube

3.     Describe the 3 steps of PCR and the approximate temperatures

4.     State how Taq polymerase is able to remain stable at high temperatures

**Spin-Column Purification of COI Amplicons**

   1.     Explain how to separate the COI DNA from the other reagents

2.     State what happens to the DNA template

**Examining COI amplicons using gel electrophoresis**

  1.     Explain the purpose of this particular gel

2.     Explain what DNA ladder is and why is it necessary to run a 1KB DNA ladder

**Dye Terminator Cycle Sequencing**

  1.     List what goes into your PCR reaction tubes when doing automated sequencing

2.     Describe the process of automated DNA sequencing.

3.     List at least two unique qualities about the ddNTPS that make them useful in DNA sequencing

4.     Understand the four color electropherogram produced by DNA sequencing (each peak, color, quality score, etc.)

  5.     Explain the basic steps of the editing process (trim ends, look for ambiguous nucleotides, etc.)

  6.     Explain how to manipulate the sequence of the bottom strand and splicing it to the sequence of the top strand

**Assembling COI Contigs in BOLD-SDP**

1.     Define Bioinformatics.

  2.     Explain what the forward & reverse sequencing reaction generate

  3.     Define a contig

**Identifying STOP Codons in COI Contigs**

1.     Explain what a stop codon indicates in a protein coding gene

2.     Understand why the stop codons in vertebrate mitochondrial protein-coding genes different than the stop codons found nuclear RNA

  3.     Explain why it is necessary to translate all three reading frames of the COI amplicon when looking for stop codons

  4.     Understand the following steps:

  a.     <trim primers>

b.     <check for contaminant>