MINNESOTA iGEM TEAM 2014

ECORI SQUAD: SYNTHETIC BIOLOGY CURRICULUM

Science for everyone! | ECORI Squad

Preface

The ECORI (**E**ducating **C**lassrooms **O**n **R**esearch **I**nnovation) Squad from the University of Minnesota 2013 iGEM team developed a **curriculum on synthetic biology** that **can be tailored to any age group**. After much research, discussion, and revision, we've developed the first edition of our curriculum to include hands-on activities to help students at any age understand tools in biotechnology such as recombinant DNA, microorganisms, and synthetic biology toolkits.

The ECORI squad launched its **pilot program** Salk Middle School in Elk River, Minnesota, where the team taught synthetic biology to **over 150 7th grade students** in five different sessions. This curriculum was developed for class sizes around 30, and implemented within two 50-minute time spans. All the materials and procedures are designed in accordance to safety measures, so students can learn and enjoy doing science in the safest environment.

The pilot program was a **success** at Salk Middle School, and the team has **been invited back** in the spring. We will also be pairing up to work with Salk's "Girls in STEM" group to help promote women in STEM fields. Excitement is brimming around The ECORI Squad, and we are quickly lining up more schools who wish to run the two-day course! We are actively **seeking more collaboration** opportunities, not only to improve this curriculum but also to spread our **love and excitement for synthetic biology**!

As always, the University of Minnesota 2013 iGEM team and the ECORI Squad **promote open-access science**! Therefore, we **hold NO copyright and encourage use of this material**, as it has proven successful in our first pilot school. That being said, we would love a shout-out and to hear any comments or feedback when you use this curriculum in your communities or schools.

Sample 2-Day Lesson Timeline

Day 1

1. Introduction to Synthetic Biology (10 min)
2. Strawberry DNA Extraction Exercise (15min)
3. Draw "Bacterial Art" (15 min)
4. Show glow-in-the-dark bacteria: *Vibrio harveyi* (5 min)

Day 2

1. Recap & Introduction of restriction enzymes (10 min)
2. Activity: Restriction enzyme using yarn (15min)
3. View bacteria art (15 min)
4. Q & A/Ethics (10 min)

Safety Information

An important part of studying biology is to observe living organisms through experiments. It is important to realize that your safety is the foremost priority when performing experiments. Most of the laboratory activities are quite safe; nevertheless, the equipment and materials can be dangerous if handled improperly. In this section, you will learn how to work safely and prevent accidents.

General Practices:

- No running during demonstrations
- No eating or drinking is permitted
- Wear gloves at all time when handling biological materials including microorganisms
- Immediately report any accident - no matter how small - to your instructor.

Physical Hazard:

Gloves: Some individuals develop allergic symptoms when in contact with certain types of gloves. Latex-free gloves should be provided.

Chemical Hazards:

Rubbing alcohol/ isopropyl alcohol: Hazardous in case of eye contact (irritant), of ingestion, of inhalation. Check for and remove any contact lenses. In case of eye contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention.
In case of skin contact, wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops. Cold water may be used. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.
http://www.sciencelab.com/msds.php?msdsId=9924412

Biohazard:

Biohazards refer to the biological agents such as organisms, viruses or biological materials pose potentially risk to human health. The other category could be defined to include blood samples, DNA, RNA, proteins, enzymes, prions or anything that is produced by biological systems. Biohazard waste generators are responsible for ensuring waste is treated correctly and labeled properly. This series of laboratory exercises complies with standard microbiological practices. Organisms used in this curriculum should be autoclaved before disposal.

Escherichia coli (BL21) and *Vibrio harveyi* are the primary biohazards used in this exercise. These two species of bacteria are derived from laboratory-safe strains and are considered Biosafety Level 1 (BSL1), which is the designation for microbes that are not known to consistently cause disease in healthy adults and present minimal potential hazard to students and the environment. Work associated with BSL1 microbes can be performed on an open table or bench without posing risk to human health. However, we will treat everything contaminated with living bacteria as biohazard and collect in biohazard bags.
http://www.cdc.gov/training/QuickLearns/biosafety/

Exercise 1: Extracting DNA from Strawberries

Objectives:

Demonstrate how the blueprint of life, **deoxyribonucleic acid (DNA)**, can be extracted from strawberries using everyday household materials. This procedure, if followed precisely, will produce a large amount of macroscopic, visible DNA strands which carry the information directing all actions of a cell and the physical attributes of an organism. You will create a detergent mixture that will disrupt the strawberry cells and enable extraction of DNA from the solution. The DNA will then be precipitated out by adding rubbing alcohol.

You Will Need

- Strawberries
- 2 Plastic cups
- 1 Resealable plastic bag
- 2 tablespoons of detergent (dish soap)
- 1 tablespoon of salt
- 1/2 cup of water
- 1/3 cup of cold rubbing alcohol
- 1 Coffee Filter
- 1 Funnel

Procedure

1. Chill the rubbing alcohol.
2. Place a strawberry into the plastic bag, seal it and gently squeeze and crush the strawberry completely.
3. In a plastic cup, mix 2 tablespoons of detergent, and a tablespoon of salt with ½ a cup of water.
4. Add 2 tablespoons of your mixture to the bag with the strawberry and gently crush it.
5. Place the coffee filter in a funnel on the other plastic cup.
6. Pour the liquid strawberry into the filter.
7. Lift the filter and gently squeeze the remaining liquid into the cup.
8. Pour 1/3 a cup of cold rubbing alcohol. Cloudy white DNA strings should appear at the top of the solution within a few seconds.

Exercise 2: Restriction Enzyme Game

Objectives:

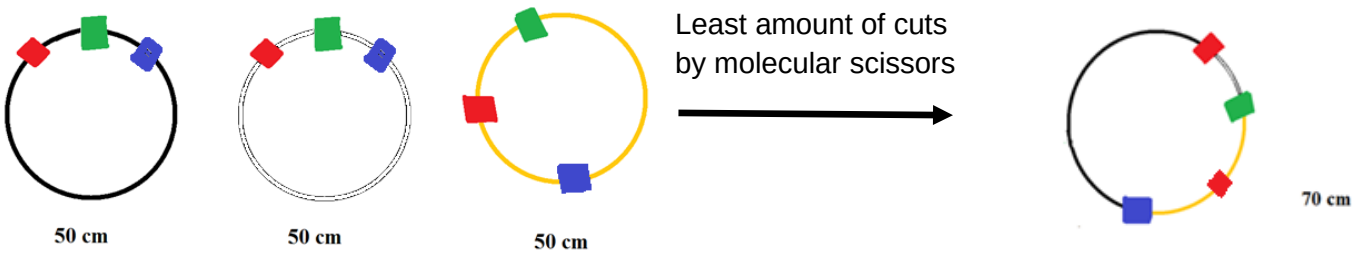
Restriction enzymes are the protein machines that cut DNA at specific sites and are often referred to as "molecular scissors." Restriction enzymes are one of the most important tools in biotechnology as the DNA fragments they generate can be rearranged, moved to different organisms, and code for new DNA devices. In this activity, you will learn how "molecular scissors" work. You will be given three different colored yarn ropes that symbolize plasmids (circularized DNA), each with one unique function. By cutting the DNA fragments with "molecular scissors," you will be able to combine two new functions (yellow and gray) into your main plasmid (black) (see below). After the activity, have the students brainstorm and discuss the two novel functions they want to incorporate into their microorganism.

You Will Need:

- Three circularized yarns of different color (black, white, yellow)
- One target plasmid
- A pair of scissors

The three plasmids

Goal plasmid



Please refer to **Appendix** to see preparation for circularized yarns with restriction enzyme.

Goal and Rules

Our goal is to use least amount of cuts to generate DNA fragments from different plasmids so we can create the plasmid we want. Here are the rules of the game:

1. Only the ends with the same color can be joined together
2. Re-joined the ends with the clear tape of the same color

Discussion Questions

What components or functions do you want to design in your biobricked plasmid?

Where should we draw the line concerning manipulating human genetics?

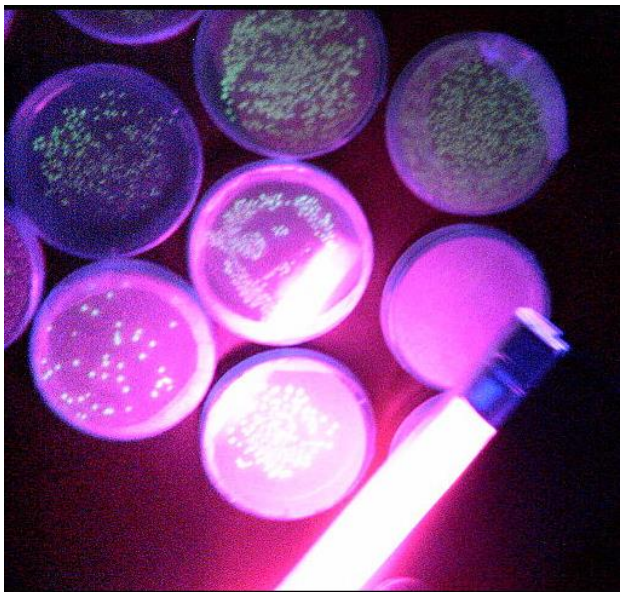
How would modified organisms be responsibly controlled?

What would be the impact of introducing genetically altered organisms into a preexisting ecosystem?

Exercise 3: Bacterial Art Plates

Objectives:

In this activity, you will be creating "bacterial art" plates with bioluminescent and fluorescent strains that would only be visible in the dark or under black light, respectively.



Green Fluorescent Protein (GFP) is a protein produced by a jellyfish *Aequorea victoria*; which produces glowing points of light around the margin of its umbrella. By transferring and incorporating this gene into a bacterial strain, we are able to make bacteria fluoresce in the presence of a handheld black light.

Escherichia coli is a very common bacteria that lives in gastrointestine tracts and part of the common gut flora (Yes, there are actually millions of bacteria in your gut!). These small ordinary bacteria play a significant role in biotechnology. The features that draw scientists attention to *E. coli* are its simple and completely sequenced genome, rapid rate of growth, and easiness to cultivate and handle. Compared to humans which have 21,000 genes, *E. coli* has only 4,400 genes.

Vibrio harveyi is a species of marine bacteria that can be found in free-living planktonic state. Its close relative, *Vibrio fischeri* colonizes the specialized light organ and emits bioluminescence to eliminate shadows caused by moonlight and thus protect the luminescent animal from predators. Through a process called bioluminescence, *V. harveyi* and *V. fischeri* are capable of emitting light via a chemical reaction that originates within the organism. Similarly bioluminescent organisms include fireflies, anglerfish, glowworms, and jellyfish.

Materials:

- Gloves
- Incubator
- Luria-Bertani (LB) agar plates
- Goggles
- Disposable wooden applicator sticks
- Lab coat
- Hand-held blacklight

Procedure:

- Using wooden applicator sticks, gently streak bacteria on agar media to create your desired image. It might be helpful to draw it on a piece of paper which would be placed beneath the plate and traced.
- The plates will be incubated at 37C overnight so the bacteria will grow to become visible to naked eyes.
- View bioluminescent bacteria in a dark area and fluorescent bacteria by shining black light.

The lab strains of bacteria and media recipe are available upon request. Please contact Aunica Kane if you have any questions regarding this particular activity: ***skog0122@umn.edu***

Exercise 4: DNA Base-Pair Matching

Objectives:

DNA is genetic code, which can be thought of as a language that is made up of 4 characters to tell the information about how living organisms look like and function. The 4 characters are: A, T, C and G. And, A always pairs with T and C always pairs with G. This activity is designed to teach the students to learn about the properties of DNA code.

You Will Need:

- Base Templates (Refer to **Appendix II**)
    - Adenine on blue cardstock
    - Thymine on yellow cardstock
    - Cytosine on red cardstock
    - Guanine on green cardstock
- Assignments of template printouts that have DNA sequences of different genes, *LuxR*, eGFP, *mCherry*, and *LacZ*. (Refer to **Appendix II**)

Procedure:

1. The instructor first ask the students these following questions:
    - Do you know what is DNA?
    - How does DNA carry genetic information? (or, how is DNA written?)
2. Next, the instructor teaches about base-pairing rules: A always pairs with T, and C always pair with G.
3. Now, show four assignments of template printouts and let the student choose which DNA sequence they would like to practice DNA base-pairing. The instructor gives one strand of DNA sequence and have the students match up the other strand correctly
4. (Optional) The students can proceed to ***Exercise 6: DNA Bracelet Activity*** and make sequence bracelet according to the sequence of their choice.

Exercise 5: DNA Name Activity

Objectives:

The central dogma of biology is fundamental knowledge for any hopeful synthetic biologist, starting with the knowledge of the four DNA bases: A, T, C, and G. This activity is a fun introduction to thinking about the coding concepts of DNA and RNA into protein products. The end result is translating your name into a DNA sequence and finding an organism with a gene corresponding to your unique sequence.

You Will Need:

- 1 sheet of paper and a writing utensil
- A computer with online access to the European Bioinformatics Institute web-site, OR a codon table with the 23 amino acids and corresponding codon sequences and access to another gene database
- Optional: bracelet material and beads decorated with the letters A, T, C, and G

Procedure:

1. Write your first name on the sheet of paper. For the database to provide results, your name will need to have at least 6 letters (if necessary, make your name longer by combining your first name with your middle or last names).

2. Next, using the codon table, below your name write a codon sequence corresponding to each letter of your name if it were the abbreviation for an amino acid. (For J, O, and U, which are not abbreviations for codons, use the codon "NNN".) This gives you your DNA name. After your name is translated, use a database to find organisms that have your particular sequence.

3. For easiest results, go to the database page at http://www.ebi.ac.uk/cgi-bin/decode/decode.cgi, type your name into the white field, and click "Decode". Let the database search, and...presto! The top hit for the organism having your name's DNA sequence should appear on the page. To see cool photos of your organism, type its scientific name into Google Images or another search engine.

4. As a fun, wearable way to remember your sequence, use the bracelet cord and beads make a bracelet with the letters of your unique DNA name sequence.

Exercise 6: DNA Bracelet

Objectives:

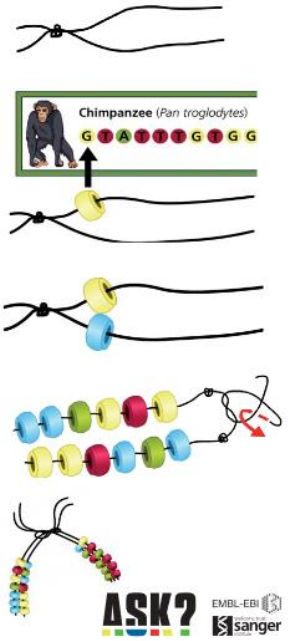
This craft based activity reinforces the principle of base pairing of DNA taught in ***Exercise 4*** and ***Exercise 5***. The students make a DNA sequence bracelet from four different color beads that correspond to the genetic code of an organism such as wolf, Escherichia coli, trout or squid.

You Will Need:

- Four types of colored beads – red, blue, green, yellow. ((Red corresponds to cytosine, blue corresponds to adenine, green corresponds to guanine, and yellow corresponds to thymine.)
- Elastic threads

Procedure:

1. Choose a DNA sequence to make. (Refer to **Appendix II** for DNA sequence options)
2. Cut two pieces of elastic threads each about 30 cm long
3. Tie together the threads and leave about 5 cm at one ends
4. Look at the first letter of the sequence and find the corresponding colored bead to thread
5. Thread the bead onto string 1 and thread the bead for the matching base onto string 2.
6. Repeat step 4 and step 5 until you've complete threading all the letters and matching bases.
7. Tie a knot at the end of each thread, and then tie the two knots together.



This activity is adapted from Wellcome Trust Sanger Institute- yourgenome.org "sequence bracelet" activity plan. The diagram is adapted from that activity plan.

Exercise 7: Water Filtration Activity

Objectives:

Water contamination poses a serious health concern in areas around the world which are affected by natural geological processes such as volcanic eruptions as well as unnatural, industrial processes such as mining, manufacturing, and hydraulic fracturing. It is necessary to develop reliable ways to remove contaminants from water in order to reclaim contaminated water sources. This activity encourages students to consider this issue and at-tempt their own solutions to it on a comprehensible scale by creating a filter for removing household "contaminants" from water.

You Will Need:

- 1 2-liter bottle, without cap and cut in half along the circumference (sand or cover jagged edges)
- 1 Funnel
- Filter materials: cotton balls, gravel, sand, paper, etc.
- 1-2 cups of dirty water, made by adding food coloring, vegetable oil, confetti paper, etc. to tap water

Goals and Rules:

Use the bottle top as the filter and the bottom half as a reservoir to catch the filtered wa-ter. Set the bottle top on the bottom half, cap-hole side down Add filter materials through the broad opening of the top half until the cap hole is plugged. Make as many filters as you like, testing different filter materials alone and in combination. The only thing not to make is a mess!

Discussion Questions:

- What changed after the water went through your filter? What didn't change?
- Which filter materials removed most of the water contaminants?
- What other possible materials or ways of removing contaminants from water can you think of?

Appendix I

This section will help you to prepare the materials needed for **Exercise 2: Restriction enzyme game.**

Figure 1. The materials and three circularized yarns for Exercise 2. The green, red and blue blocks represent the restriction sites, where the molecular scissors recognize and cut in the middle. The restriction sites can be created using color tapesWe suggested to use 50 cm or 10 inches of yarns to make circularized DNA.

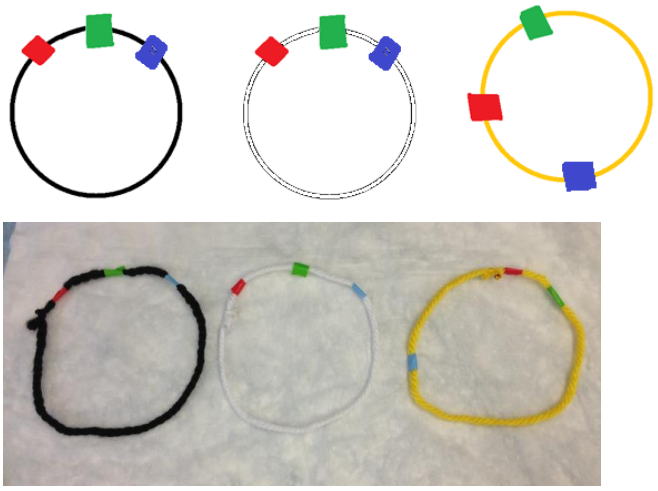
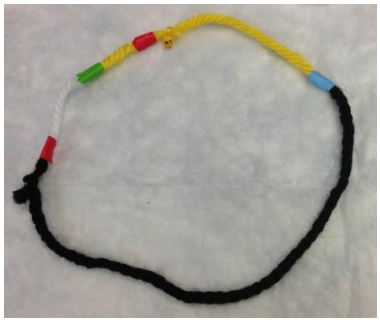
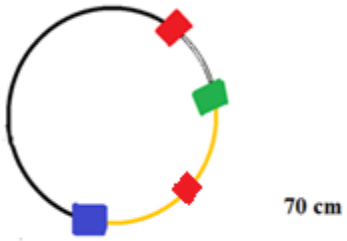


Figure 2. The goal plasmid.

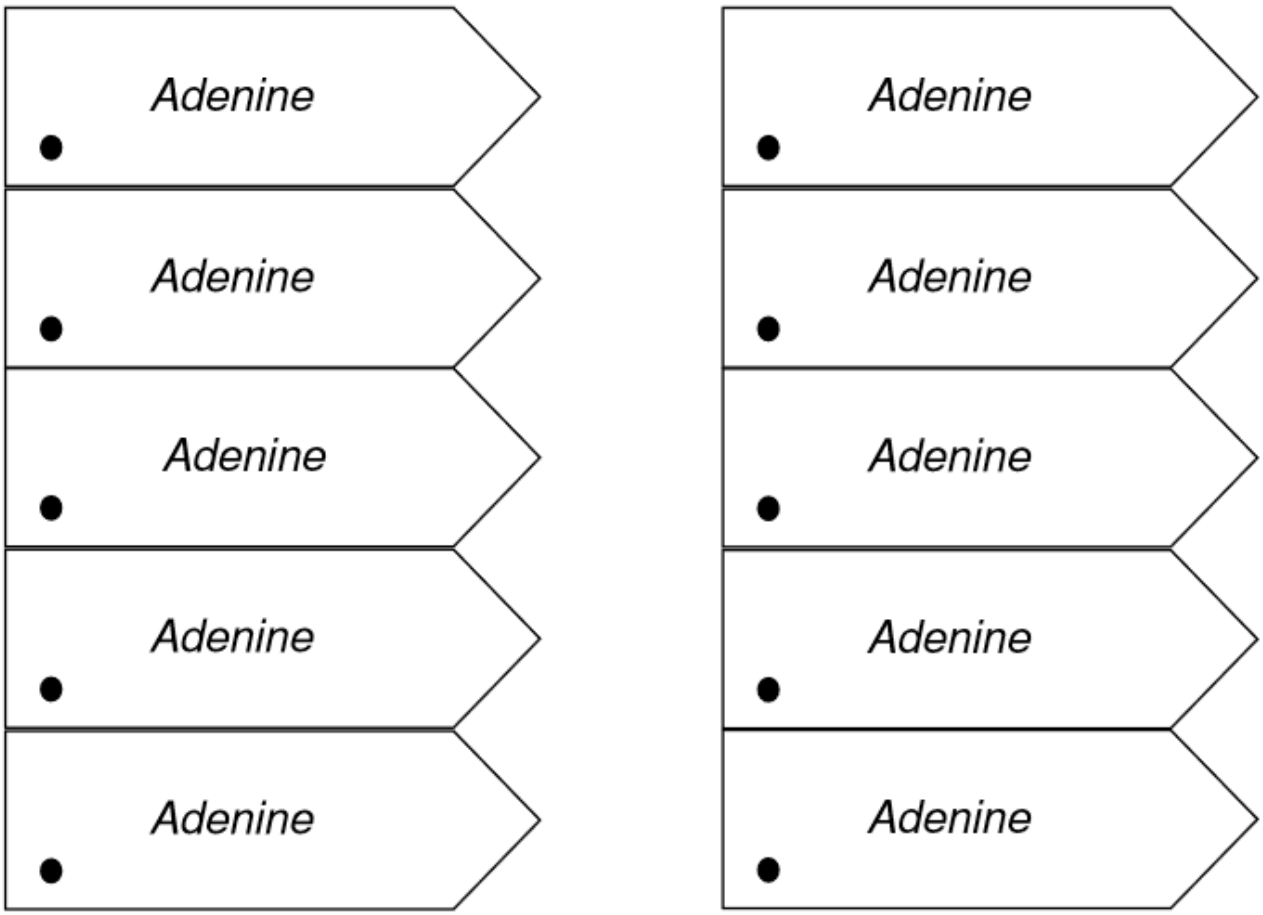




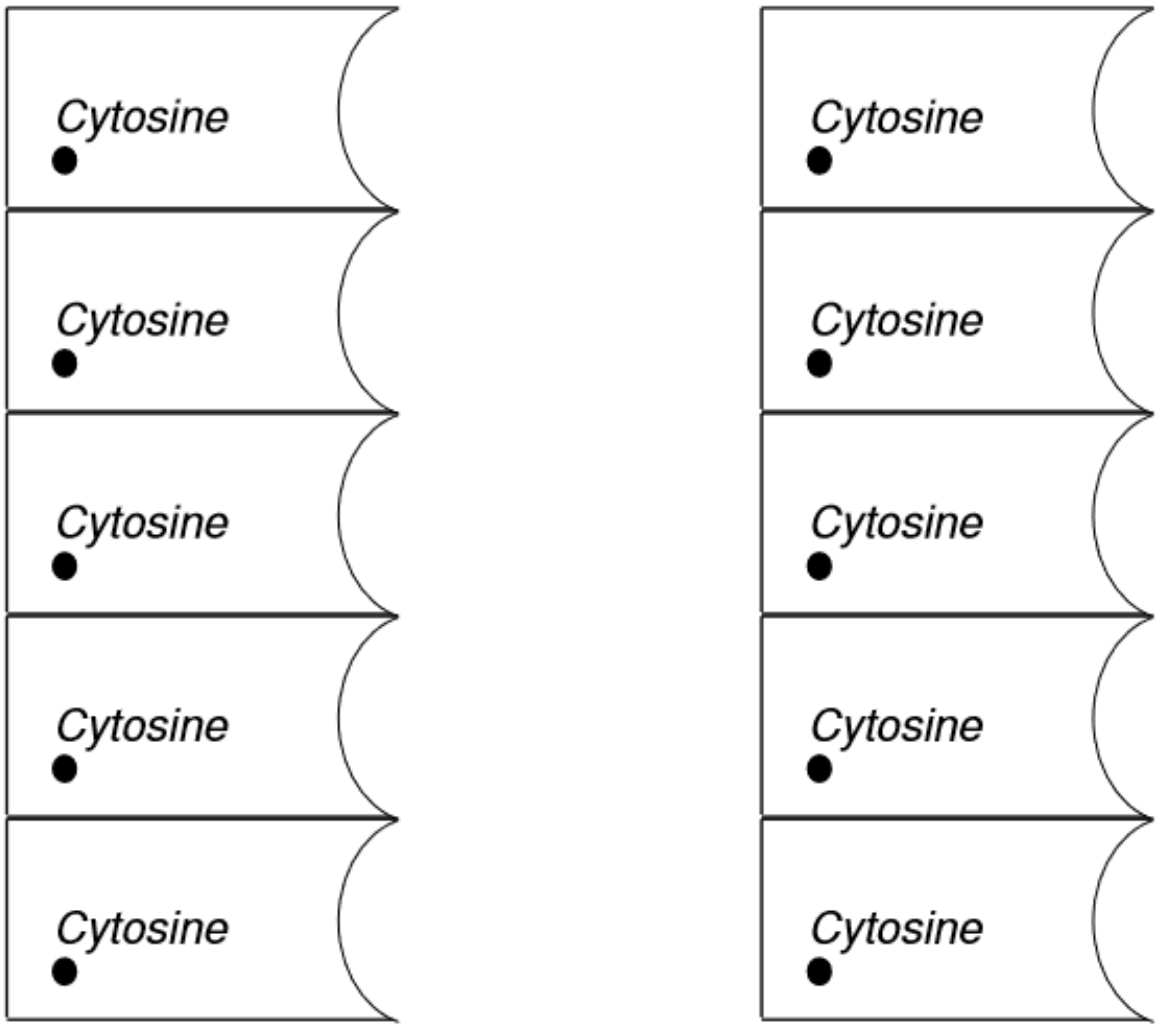
Appendix II

This section will help you to prepare the materials needed for **Exercise 4: DNA Base-Pair Matching.**

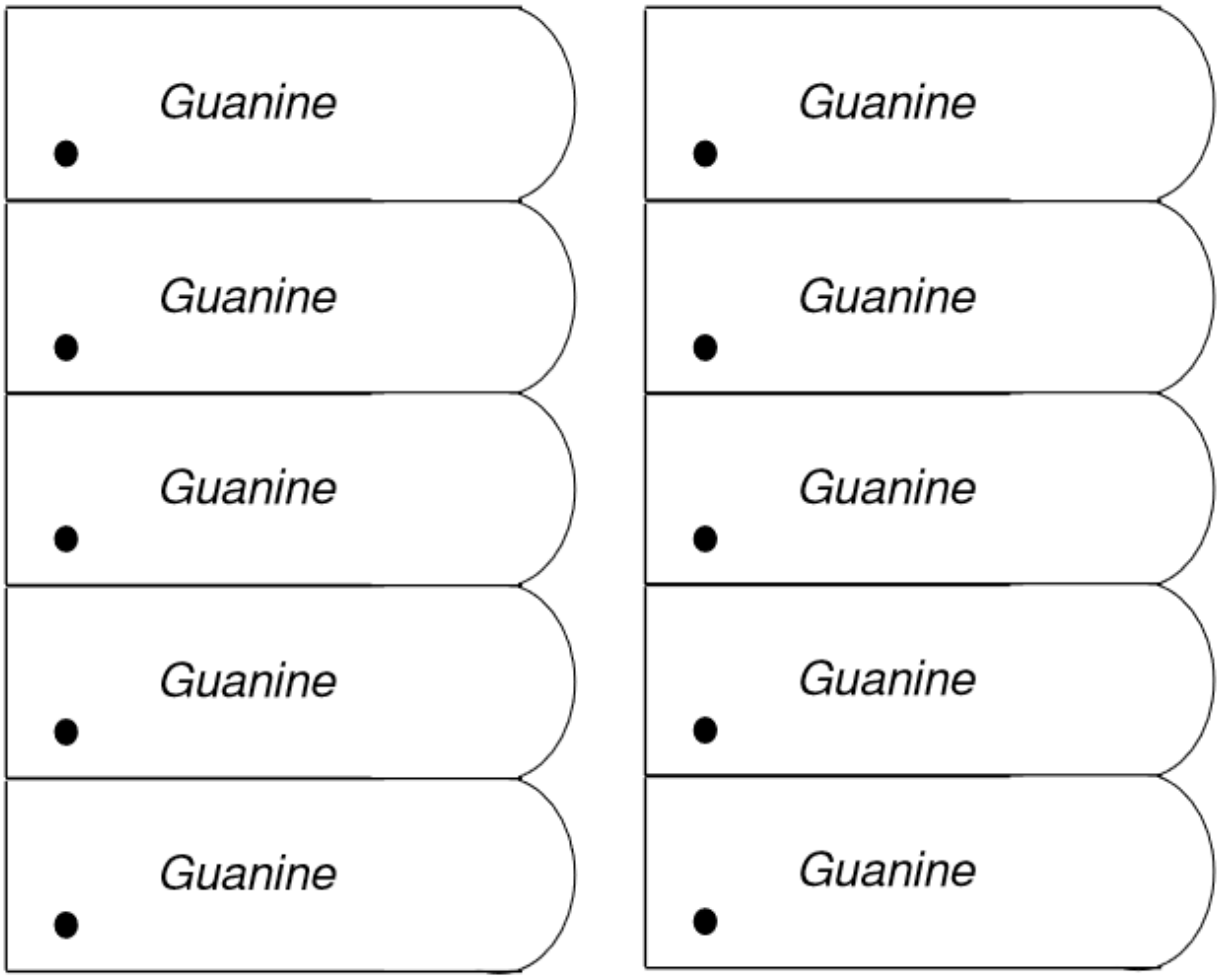
Please print on blue cardstock and cut out each piece.



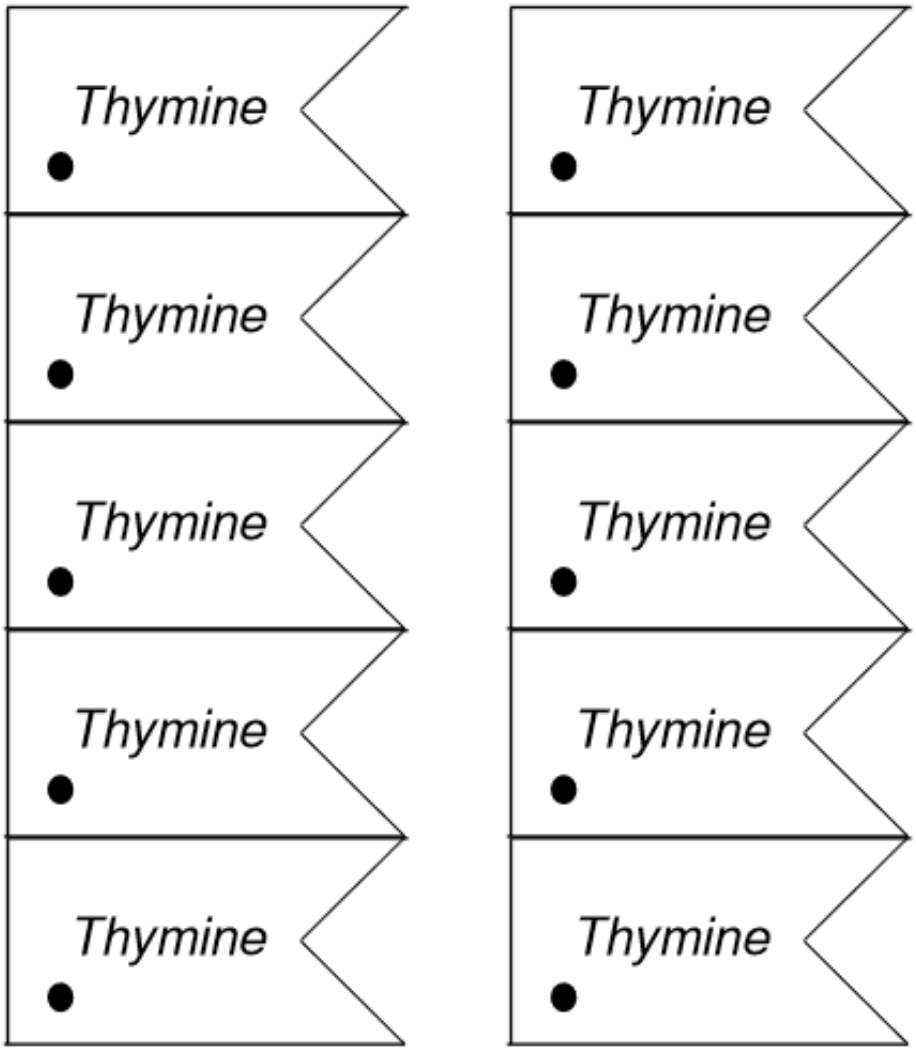
Please print on red cardstock *and cut out each piece*.



Please print on green cardstock *and cut out each piece*.



Please print on yellow cardstock *and cut out each piece*.

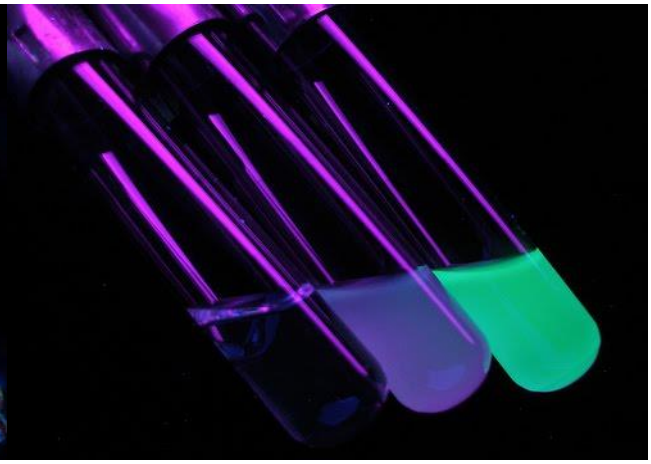


Assignment templates with corresponding gene names and sequences. Please print these out for ***Exercise 4, 5, or 6.***

LuxR V. fischerii

ATGCTTAGCG

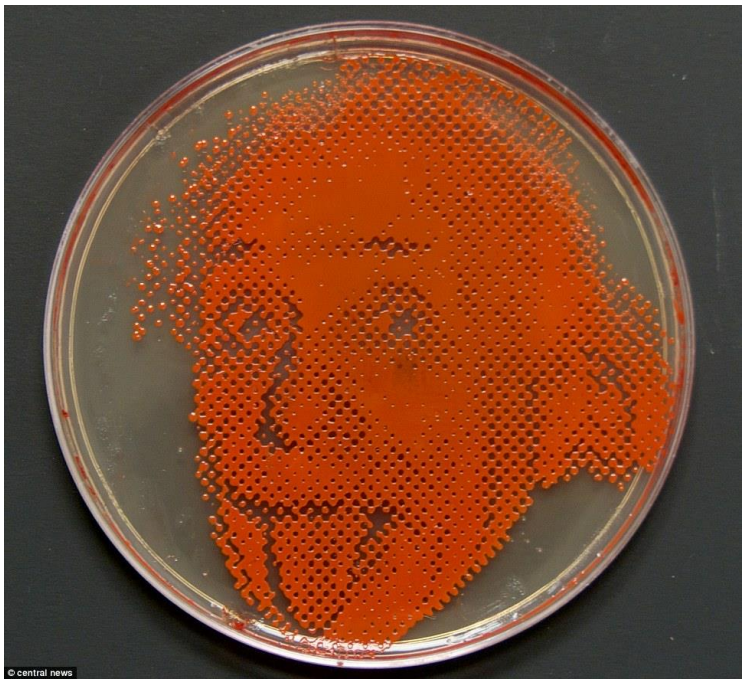
TACGAATCGC



mCherry E. coli

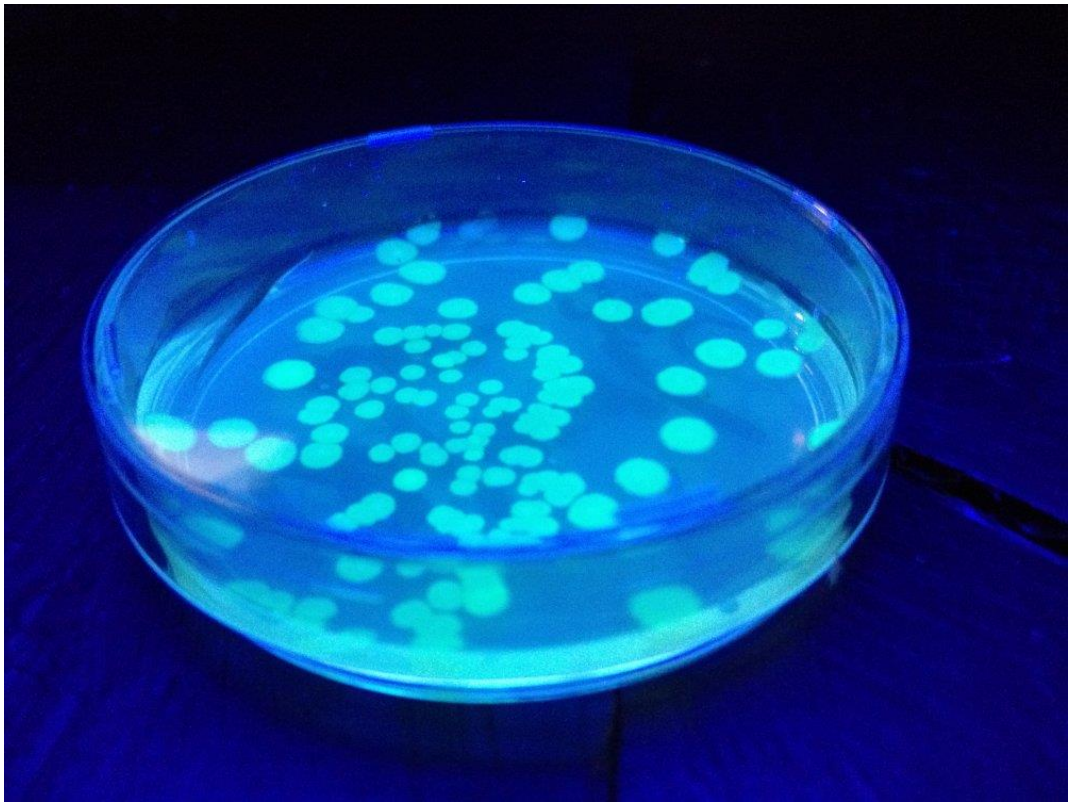
CTACGTGAAG

GATGCACTTC



GFP E. coli

TGTCACTACT ACAGTGATGA



LacZ E. coli

CAGCGCTGAC

GTCGCGACTG

X-gal identifies cells with beta-galactosidase