Day 2 - SynBio Tool Kit

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Authors: Jessica Laury
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The problems we face on earth; food, clean water, clean air, shelter, fuel, medicine, diagnostic tools will all be present on Mars, but we cannot use conventional science to meet those needs. This is why we have a need for synthetic biology for long term space travel.

Techniques that we use in SynBio

1. CRISPR-Cas9
2. Transformation - Insertion of a Plasmid into competent cells
3. Genetic Circuits
4. PCR

Things not included
Genetic sequencing - getting more cost effective
Artificial gene synthesis

1.. CRISPR-Cas9
Restriction endonuclease called Cas 9 is an enzyme that cuts DNA at or near a target sequence. Cas 9 is guided by a guide RNA (gRNA) which is used to recognize sequences (roughly 20 bp long recognized by complementary base pairing) that indicate a target sequence (in the natural system this is often a viral genome). The Cas 9 then cuts that sequence. **The enzyme screens the full DNA sequence but only cuts at the target site.** The break can be used to disrupt a coding sequence. The cell responds to the DNA cut by trying to repair it. Scientists can use manipulate the repair machinery in the cell to change the target gene through non-homologous end joining. During the repair process scientists make linear DNA available to the DNA repair machinery this DNA can be incorporated into the genome at the previously disrupted cut site. If scientists simply want to disrupt the gene, repeated cycles of cut and repair eventually lead to a mutation, and this error leaves the undesired gene ineffective.

2. Transformations - Used for cloning and for placing circuits in E. Coli
Competent bacteria cells are capable of taking up foreign DNA. Competent cells have altered cell membrane which leaves them capable of taking up extracellular DNA, (this alteration can be done with salt washes, temperature changes or electroporation to create pores). Plasmids carry the desired DNA sequence and the sequence which codes for antibiotic resistance, this resistance gene will be important in screening for successfully transformed cells later.
Transformations are conducted on ice and competent cells and desired plasmid are combined in a tube. In the chilled conditions cells and plasmid are in close proximity. The cells are then taken from ice and put into a heat block where they are heat shocked at 45-50* C, during this heat shock step plasmids are allowed to enter the cell through the pores in the plasma membrane. The cells are then put back on ice to chill and recover, the plasma membrane will heal securing the plasmid.
To select for successfully transformed cells, the cells are plated on selective media with an antibiotic. Cells that have taken up the plasmid will have both the gene of interest and the anti-biotic resistance gene which will allow them to grow on the selective media. Untransformed bacteria will not grow.

3. PCR and Gel Electrophoresis

PCR (polymerase chain reaction) is used to amplify DNA samples. It utilizes Taq polymerase (a heat tolerant polymerase) and changing temperatures. In a small tube a DNA sample, taq polymerase, forward and reverse primers (short nucleotide sequences which are complementary to the sequences found on the forward and reverse ends of the target DNA), and free nucleotides are combined. The tube is then placed in a thermocycler. The temperature of the tube is increased (above 68* C typically) until the hydrogen bonds holding the two strands of DNA together are broken causing the DNA to separate into two complementary single stranded DNA pieces. The temperature is then dropped to the annealing temperature (usually at least 5* lower than the Tm), the forward and reverse primers then bind to their complementary strands of DNA. Taq polymerase is able to bind to this strip of double strand DNA, it moves down the DNA and repairs the single stranded DNA by attaching complementary base pairs pulling from the pool of loose nucleotides in the PCR tube. After sufficient time has passed for the polymerase to work its way down the length of the DNA strand the process is repeated with a heating step to divide the now 2x strands of dsDNA.
Typically we complete 25 or 30 cycles on the thermocycler. After this we run our results on a gel using gel electrophoresis to confirm our DNA has been replicated and is the correct length. The gel for gel electrophoresis is a red algae derivative and works a little like a ball pit or a series of webbing. It provides resistance to DNA strands trying to move down the gel. Smaller strands are more agile and can move down the gel more quickly (Think mouse in ball pit), where are larger pieces are slower to progress (think large child jumping into ball pit). The push/pull down the gel is provided by charged ions. DNA is negatively charged and loaded in wells at the top of the gel. Negative charge is channeled at the top of the gel, pushing the DNA, positive charge is channeled at the bottom of the gel, pulling the DNA like a magnet.

4. Genetic Circuits - Visuals are helpful here
A circuit needs a promoter, ribosome binding site, coding sequence and terminator.
Promoter - where polymerase binds, which transcribes the DNA into mRNA
Ribosome binding site - binds the ribosome which takes the transcription from the polymerase and translates it into protein
Coding Sequence - holds our genetic code for our desired protein
Terminator - triggers the release of the mRNA and ends transcription.

Activities

K-5 -
"My Friend CRISPR"
Talk to students about DNA being the code for life and how all living things have DNA in their cells. Explain that we can now edit DNA to change how things look and act. Tell the students about our helper bacteria that produce insulin (or any of your favorite friendly bacteria), explain that we can edit bacteria and turn it into an amazing machine.

- Let the students brainstorm what kind of machines they could build with friendly bacteria and "Their friend CRISPR". The following activity can be done with just paper and drawing tools, or you can supply your students with pip cleaners, string (can be awesome for webbing), colored yarn, cotton balls and pompoms, googly eyes, beads, popsicle sticks etc. Let the students create a creature and explain what they want their engineered organism to do.

Circuit Creation
Students are given Circular pieces of paper, there are different shapes cut out at their tables, one representing promoters, one representing ribosome binding sites, one representing coding sequences and one representing a terminator. Students must put the pieces together in order to create a functioning circuit.

DNA Extraction & Gel Electrophoresis are also super fun with this age group to just let students see DNA, and to explain how scientists can visualize DNA.

6-8 -
"Monster Genome" + "My Friend CRISPR"
https://askabiologist.asu.edu/body-depot/monster-manual_teachers **Jessica will have to edit this to make the CRISPR part work for each sheet**

Students are each given a worksheet and have to "decode(transcribe)" and "translate" (draw) their monster. Once students have drawn their monster, pass out 2 "Restriction enzymes" with a corresponding coding sequence (It is best if this corresponds with two letters or 6 base pairs & it works well if one is a space and one letter). Students should then cut their "code" where the restriction enzymes have a match and swap their cut piece of DNA with another student, this swapped sequence should only be one or two traits. Students then can draw another version of their monster with their edited DNA.

PCR Beads

Students are given:

- Two chains of beads (representing complementary strands of DNA one 5'-3' one 3'-5'). They are not completely attached but should be pushed together on the desk so they are touching representing a double stranded piece of DNA
- A bowl of free beads representing loose nucleotides
- 14 "primer" strings, which have several beads already attached to them. 7 of these strands should have beads which match the first 5 basepairs on the 5' to 3' strand, the other 7 should have beads that match the first 5 basepairs of the 3'-5' string.
- Note - normally primers are closer to 20+ base pairs long but we don't want to have huge strings full of hundreds of beads.
- Note - It can be helpful both for teachers and students to have the 5'-3' string and the 3'-5' string be different colors.

In this activity, students are going to be our Taq polymerase, the classroom will be our PCR tube. At the front of the room the teacher (after explaining PCR and the function of taq polymerase) will control our thermocycler (or our temperature). S/he will increase the temperature up up up, and as s/he does, students will separate their two strands of beads. As the teacher brings the temperature back down, students will match up the correct primers to their now single stranded DNA. After lining their primer sequence up against their template DNA strands students will build off this primer like Taq polymerase, pulling new base pairs from their bowl of nucleotides and adding them to their string. Once they have matched up all of the nucleotides for the complete sequence of their DNA strand they will tie a knot in the end and push the two strands snuggly together. All students should now have 2 strands of identical DNA. This is repeated two more times until students have 8 strands of identical dsDNA.

Circuit Creation - Electrical click together circuits - talk to Saha about that

Transformation - Activities booklet on page 39.

DNA Extraction & Gel Electrophoresis are also super fun with this age group to just let students see DNA, and to explain how scientists can visualize DNA.

9-12

Manual PCR found on page 16 of activities booklet.

Have students think about how many copies of DNA they will have at the end of 30 cycles if they started with X copies of DNA
You can use the bead activity from 6-8 as a conceptual example.
Completing an actual PCR would be wonderful if the school has the resources, NOTE a gel would be necessary after this, and detailed explanations as students fill there PCR tubes would be required for this to be an opportunity to develop understanding. Students mindlessly working through a protocol are not learning!

Circuit Creation - Electrical click together circuits - talk to Saha about that

Transformation - Activities booklet on page 39.

Talking about competent cells is going to have to be added, really explain the use of selective media and its connection to a specific gene on the plasmid.
Trying to figure out a way to let them do this in class
We can always give them bacteria that we have edited and they can draw with it on plates, another good chance to talk about streaking for isolated colonies.

Gel electrophoresis
https://www.addgene.org/protocols/bacterial-transformation/