Colony PCR Protocol (Using GE Ready-to-go PCR beads)

The dehydrated bead visible in each PCR tube contains buffer components, dNTPs, and high-fidelity DNA polymerase. We will add PCR-grade water to rehydrate the components, primers to amplify just the RFP gene, and a DNA template. For PCR from bacteria, the DNA template comes directly from a bacterial colony sample. The high temperatures of the thermal cycler will burst the cells, which exposes the pET-15b plasmids containing the mutant RFP of interest.

Our experimental design involves these samples: 5 colonies of interest and your ancestral (un-mutated) RFP plasmid. You'll want to set up one reaction tube for each sample and also one as a negative control, a PCR tube that has all the PCR reagents *but no* DNA sample.

1. Obtain Ready-to-go PCR tubes and lids from your TA and label them according to the 2-digit PCR tube code you were assigned. Put the label on the side of the tube: with your group, section, and sample information. Check that the white bead is located at the bottom of the tube before opening. PCR tubes are small and thin-walled, so handle these carefully. They are easiest to open if securely lodged in a PCR tube rack.

2. To each tube, add 25 µl of primer mix. This contains the same primers we used previously for mutagenic PCR:

   22.5 PCR grade water
   1.25 µl RFP Forward primer, 10 µM
   1.25 µl RFP Reverse primer, 10 µM

   RFP Forward: 5'-CTGGTCGGCCATATGGCGTCTTCTGAAGACGTTATC-3'
   RFP Reverse: 5'-CGGATCCTCGAGCTATTACGCACCGGTAGAGTG-3'

3. Keep all tubes on ice and allow the white bead to fully dissolve, up to a minute.

4. Obtain a sterile pipette tip and barely touch it to the labeled bacterial colony. There should not be any visible bacteria on the tip; transferring too many cells can cause problems with the reaction.

5. Immerse the pipette tip in the PCR tube solution and roll it back and forth several times along the inner wall of the PCR tube to dislodge the cells while avoiding any fragments of the white bead.

6. Spin the tubes for a few seconds in the PCR centrifuge to bring all reagents together in the bottom. Keep on ice until time to place the tubes into the thermal cycler in your assigned column of PCR wells, and carefully note on the PCR map each of your tube IDs. Keep a copy for your records.

7. Once the entire class has loaded their PCR tubes in the thermalcycler, initiate the polymerase chain reaction with the following conditions:

Initial Denaturation:	95˚C for 2 minutes	
Denaturation	95˚C for 30 seconds	} Repeat 29 times
Annealing	59˚C for 30 seconds	
Extension	72˚C for 60 seconds	
Final Extension:	72˚C for 10 minutes	

8. After lab, we will send the amplified PCR product for sequencing in the forward and reverse directions by Genewiz (requires 20 ul of PCR project). We will compare the DNA sequence of the original ancestral strain of *RFP* to the derived (mutant) colonies exhibiting potentially new fluorescence patterns.