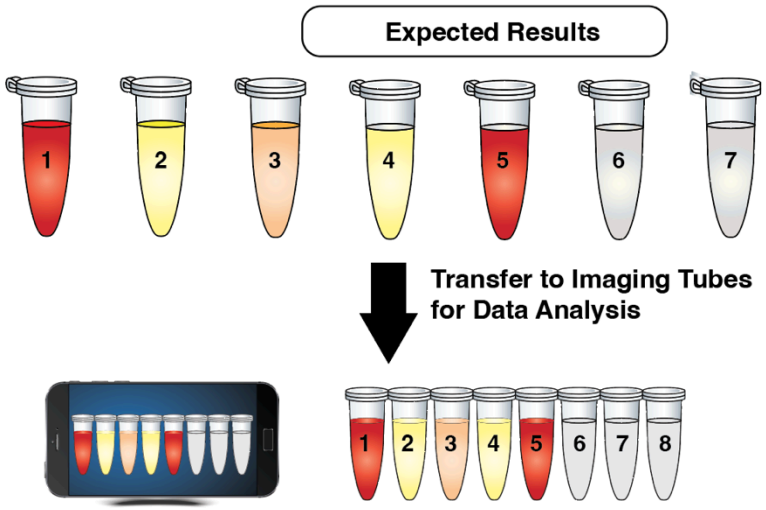
Protocol for the CRISPRkit (Day 2) - Dual Color Kit

Day 2: In addition to this protocol sheet, watch this video for Day 2 Results Analysis

On Day 2, you will analyze the results of the reactions. Your reactions should look like this (Try to explain to yourself why, based on what you added to each tube):

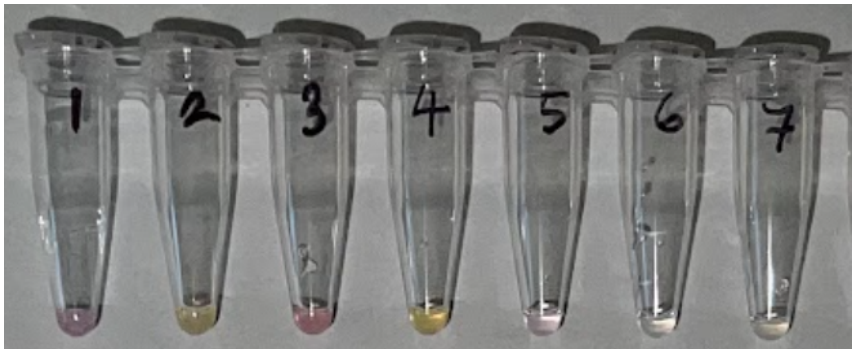


If you have a pipette (transfer reactions to imaging tubes):

1. For better imaging analysis, use the pipette and tips to transfer the reactions (transfer around ~5uL) in each tube to the corresponding Imaging Tubes (small)
    - Refer to **Appendix 2** for how to use a pipette
    - Use a different pipette tip after transferring each reagent

If you don't have a pipette (use original reaction tubes):

1. Use the original reaction tubes you conducted the experiment with for imaging
2. Carefully lay the tubes on a piece of white paper. Take a picture with flash on. Airdrop/send it to your laptop. (**Important**) be sure to minimize glare and shadows! ***Example (This is with the imaging tubes – with pipette transfer)****:*



What you should have now:

You will now analyze your results using CRISPectra. Learn more about the algorithm here and how it can quantify and approximate gene expression for each reaction. To access the CRISPectra GUI to conduct analysis, click here.

3. Go to the CRISPectra page and follow the instructions on the video to conduct detailed analysis on your experiment.

Appendix 2: How to use a pipette, step by step:

A pipette is a lab tool commonly used in biology, chemistry, and medical tests to transport measured volumes of liquid. Here's a step-by-step guide on how to use a pipette:

1. **Check Pipette's Volume Range:** Before starting, make sure the pipette is designed to measure the volume you need. Over- or under-loading can cause inaccuracies and damage the instrument.
2. **Attach Pipette Tip:** Pipettes typically require disposable tips that fit onto the end. Press the shaft of the pipette into a new tip located in a tip box, making sure it's attached securely, but avoid touching the tip with your hands to prevent contamination.
3. **Adjust the Volume:** Pipettes usually have a dial or button that allows you to set the desired volume. Make sure to adjust this to the correct measurement.
4. **Pipetting Liquid:**
   - Depress the plunger button to the first stop. (Pipettes usually have two stops when you press down. The first stop is for measuring the liquid, and the second is for expelling it completely.)
   - Dip the tip into the liquid (usually 1-3mm), ensuring not to touch the sides of the container and not to immerse the tip too deeply.
   - Slowly release the plunger to draw up the liquid. Wait a moment to ensure all liquid is drawn up.
   - Withdraw the pipette from the liquid while keeping it vertical to prevent liquid from adhering to the outside of the tip.
5. **Dispensing Liquid:**
   - Touch the pipette tip to the side of the receiving vessel.
   - Slowly depress the plunger to the first stop to dispense the measured volume.
   - Without lifting the tip from the side of the container, press to the second stop to expel any remaining liquid.
   - Hold the plunger down and then remove the pipette from the vessel to avoid drawing any liquid back in.
6. **Eject the Pipette Tip:** Most pipettes have a button or mechanism that allows you to eject the tip into a waste container without touching it, ensuring the pipette remains uncontaminated for future uses.

Remember, accuracy in pipetting requires practice and precision. Also, always wear appropriate personal protection equipment (PPE) when handling liquids.