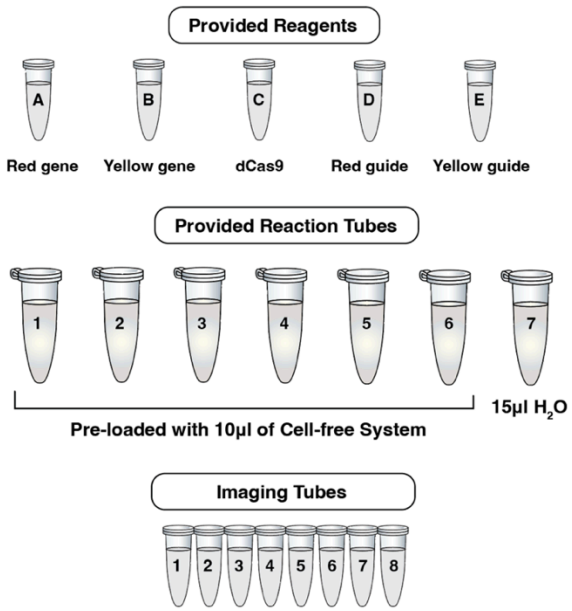
Protocol for the CRISPRkit (Day 1) - Dual Color Kit

DAY 1:

Before you start the experiment, we recommend that you go through the Overview slides and Key Concepts page to get familiar with CRISPR and CRISPRkit. In addition to this protocol sheet, we also recommend that you follow along to our Day 1 Experiment Video while doing the experiment.

If you are a teacher or would like to learn more about how our kits can fit into school curricula, please visit this page on our website.

Each kit contains a 3D-printed tube holder, 5 small tubes containing reagents, and 7 big tubes for reactions. The small tubes are labeled A-E, while the big tubes are labeled 1-7, preloaded with the cell-free system (#1-6) or H_2O (#7). The kit should look like below:



The question is: **Can you perform a successful gene editing experiment with high efficiency and precision?**
To do this, we'll guide you step-by-step through the procedure using the CRISPR kit:

A table for adding components into reaction tubes:

Reaction Tube #	Tube A: R Gene	Tube B: Y Gene	Tube C: dCas9	Tube D: R guide	Tube E: Y guide
1 – R Pos Control	✓				
2 – Y Pos Control		✓			
3 – R+Y Pos Control	✓	✓			
4 – R Editing	✓	✓	✓	✓	
5 – Y Editing	✓	✓	✓		✓
6 – R+Y Editing	✓	✓	✓	✓	✓
7 – Neg Control					

1. Each kit contains a pack of inoculation loops, which will be used for liquid transfer. Use one inoculation loop for transferring each reagent of A-E into their corresponding reaction tubes 1-6. When performing the liquid transfer be sure to do the following:
    - Submerge the loop fully in both the reagent and reaction solution

- Spin the loop several times to ensure complete fluid transfer
- (**Important**) After removing the loop from the reagent tube, check the center of the loop to make sure that there is fluid present
- Use a new inoculation loop after each reagent
- Add reagents in the order A, B, C, D, E to their respective reaction tubes

2. Seal the caps of the big tubes **tightly** to prevent evaporation
3. Return all tubes to the box
4. Allow the reactions to proceed for more than 16 hours. Any time between 16-48 hours is fine

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Appendix 1: Contents of the CRISPR Kit

The kit includes:

- Chromoprotein genes: Red and Yellow
- dCas9 protein
- Two plasmids each encoding guide RNAs for targeting Red and Yellow
- 5x Reagent Tubes (small)
- 7x Reaction Tubes (big)
- 8x Imaging Tubes (small)
- Cell-Free System (gene transcription-translation) – Preloaded to reaction tubes
- 20 inoculation loops
- A white paper