**Transformation of Yeast with CRISPR Plasmids**

To perform CRISPR mutagenesis, we require three components:

* The Cas9 protein, which cuts both strands of the DNA double helix
* The guide RNA, which targets Cas9 to place in the genome that we wish to mutate
* The crRNA that will serve as the template to create the mutation that we desire

These components will be provided in the following ways:

* The Cas9 protein, which cuts both strands of the DNA double helix
  + This is already present in the yeast cells that we will be starting with
* The guide RNA, which targets Cas9 to place in the genome that we wish to mutate
  + **We will introduce the gene for the guide RNA on the plasmid pRS426 gRNA CAN1.Y**
* The crRNA that will serve as the template to create the mutation that we desire
  + **We will introduce the gene for the crRNA as a short double-stranded piece of DNA**.

**Prepare the double-stranded DNA containing the gene for the crRNA:**

1. Obtain the two single-stranded oligonucleotides, CAN1.Y FOR and CAN1.Y REV

2. Combine 5 ul of each oligo (1 nmol each) in a PCR tube.

3. Place the PCR tube in the PCR machine. The program will heat the mixture at 100°C for 5 min to make sure the DNA is fully single-stranded to begin. It then will cool to 25°C (with a ramp of 0.1°C per second) to allow those single-stranded DNA pieces to come together to form a double-stranded DNA.

**Yeast transformation:**

1. Prepare a yeast transformation master mix: You have a tube containing 1920 ul of 50% polyethylene glycol (PEG). To this tube add 288 ul of 1.0M lithium acetate (LiAc), and 80 ul of single-stranded herring sperm DNA. Mix well by vortexing for 10 seconds. Keep the mixture on ice after mixing.
2. Obtain two tubes of yeast cells. Label these tubes “CAN1” and “NC”.
3. Centrifuge the yeast cells at full speed for 1 minute at room temperature. Remove the supernatant (liquid) as completely as possible by using a P1000 pipet.
4. Resuspend each tube of cells in 1000 ul of water, pipetting up and down to mix until the mixture is smooth without any visible chunks.
5. Repeat step 3.
6. Resuspend each tube of cells in 1000 ul of 0.1M LiAc, pipetting up and down to mix until the mixture is smooth without any visible chunks.
7. Repeat step 3.
8. Resuspend each tube of cells in 100 ul of 0.1M LiAc, pipetting up and down to mix until the mixture is smooth without any visible chunks.
9. Aliquot 572 uL of yeast transformation mix from step 1 into each of the two microcentrifuge tubes containing the yeast cells. Pipet up and down to mix the yeast cells.
10. To your “CAN1” tube, add 10 ul of the CAN1.Y oligos and 10 ul (500 ng) of pRS426 gRNA CAN1.Y plasmid
11. To the “NC” tube, add 20 ul of water.
12. Vortex the tubes for 10 seconds to thoroughly mix the DNA, transformation mix and yeast cells.
13. Incubate in a 42°C water bath for 30 minutes. During this time, obtain 2 SC-Ura-Trp plates and 2 YPD plates. Label one of each type of plate “CAN1”, one of each type of plate “NC”.
14. After the heat shock, centrifuge the two tubes at top speed for 1 minute.
15. Remove the supernatant with a P1000 pipette set to 1000 ul and discard the liquid.
16. Add 250 μL of sterile water to each tube. Gently pipette to resuspend the pellet.
17. From each tube, transfer 250 ul of the cells onto your appropriately labeled SC-Ura-Trp plates and spread to distribute evenly.

**Optional steps to calculate your efficiency of transformation:**

1. For the remainder of your cells in the CAN1 tube, perform serial dilutions: Obtain two new microcentrifuge tubes and fill them with 990 ul of water. Label one tube 10-2 and the other 10-4.
2. Pipette 10 ul of your cells and add them to the tube labeled 10-2. Vortex this tube well.
3. Pipette 10 ul from the tube labeled 10-2 and transfer this liquid to the tube labeled 10-4. Vortex well.
4. Transfer 100 ul from the tube labeled 10-4 onto the YPD plate labeled CAN1. Spread the cells to distribute evenly.
5. Repeat steps 18-21 for the remainder of your cells in the NC tube.
6. Once the plates are no longer wet, incubate the plates (upside down) at 30°C for 2 days.