**Lambda-red recombineering**

Day 1

1. Start an overnight culture (37°C) by inoculating LB medium from a single colony.

Day 2

1. Prepare competent cells following your favourite protocol.
2. Transform *E. coli* cells with plasmid pKD46 and plate the cells at 30°C in LB/Carbe

Day 3

1. Start an overnight culture at 30°C in LB/Amp from a single colony.

Day 4

1. Next morning refresh the culture with LB/Amp and grow the cells until OD600 reaches 0.1.
2. Add arabinose to a final concentration of 0.7% and grow the cells to an OD600 =0.6 (recombination proteins are being expressed at this point).
3. Cells are then frosted on ice for 20 minutes and electrocompetent cells are then prepared by washing bacteria with ice-cold 10% glycerol after spinning aliquots 10 min at 5000 rpm in a 4°C centrifuge.
4. After 2 washes, cells are resuspended in the residual glycerol and electroporated with 400 ng barcode DNA with a Gene Pulser (25μF, 200ohms at 1.8kV).
5. After zapping cells, 950μl fresh LB without antibiotics is quickly added to samples and resuspended cultures are grown for 2h at 37°C.
6. Then, 100μL bacterial culture is spread onto nutrient agar (NA) plates supplemented with 34μg/mL chloramphenicol.

Day 5

1. Single colonies are restreaked both on NA/Cam (to check the cassette insertion) and NA/Amp (to check helper plasmid loss) plates and grown overnight at 37°C.

Day 6

1. Perform PCR and sequencing experiments to check the proper insertion has occurred.
2. To remove the antibiotic cassette, the pCP20 plasmid is transformed. Prepare liquid culture of cells containing the barcoding cassette in LB/Cam.

Day 7

1. Prepare competent cells and transform pCP20 at 30°C.
2. Plate in NA/Cam/Amp

Day 8

1. After pCP20 transformation single colony is inoculated in LB/Amp/Cam and grown overnight at 30°C.

Day 9

1. Next morning cells are diluted in LB and grown at 30°C until OD600 reached 0.1
2. Bacteria is swapped to 42°C incubator and grown until OD600 reaches 0.9.
3. 30μL are spotted in NA plate and streaked over the plate and incubated at 37°C.

Day 10

1. Barcode presence is checked again by PCR and sequencing.
2. Single colonies are restreaked in three different plates (LB, LB/Cam and LB/Amp).