**Lambda-red recombineering. Adapted from:** K. A. Datsenko, B. L. Wanner, and J. Beckwith, “One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 97, no. 12, pp. 6640–6645, 2000. Available: http://www.pnas.org/content/pnas/97/12/6640.full.pdf.

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 supplemented with 100 µg/mL ampicillin (or carbenicillin).

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 30 mM and grow the cells to an OD600 =0.5 (recombination proteins are being expressed at this point).
3. Frost cells on ice for 20 minutes and prepare electrocompetent cells by washing bacteria with ice-cold milli-Q water after spinning aliquots 10 min at 5000 rpm in a 4°C centrifuge.
4. After 2 washes, resuspend cells in the residual water and electroporate with 500 ng barcode DNA cassette (coming from the amplification of pEC-Red2-BC) with a Gene Pulser (25μF, 200ohms at 1.8kV).
5. After electroporating the cells, add 950μl of fresh LB without antibiotics to samples and resuspended cultures are grown for 2h at 37°C.
6. Plate cells in LB supplemented with chloramphenicol 25 µg/mL.

Day 5:

1. Restreak colonies on LB/CM and grow 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 LB/Cam/Amp

Day 8:

1. After pCP20 transformation, inoculate single colonies in LB/Amp/Cam and grow overnight at 30°C.

Day 9:

1. Next morning, dilute cells in LB and grow at 30°C until OD600 reaches 0.1
2. Swap cells to 42°C incubator and grow until OD600 reaches 0.9.
3. Spot 30 μL in LB plate, streak over the plate and incubated at 37°C.

Day 10:

1. Barcode presence is checked again by PCR and sequencing.
2. Restreak single colonies in three different plates (LB, Cam and Amp) to check resistances loss.