Making *E. coli* knockouts with the lambda red system

Part 1: Preparing your strain

Before you can knockout a gene in your strain you will first have to introduce a plasmid that contains all of the necessary proteins that make the knockout possible. This plasmid is called **pKD46. One very important thing to remember is that pKD46 is a temperature sensitive plasmid. This means that the plasmid is not stable above 30°C.** For this reason, all strains that contain **pKD46** should be grown at 30°C.

Day 1:

Step 1: Make competent cells from your strain.
Step 2: Transform with pKD46. Outgrow for 1 hour at 30°C.
Step 3: Plate on LB Amp or LB Carb. Place in 30°C incubator overnight.

Day 2:

Step 4: Pick a single colony from your overnight plate into 5mL of LB Amp or LB Carb.
Step 5: Grow in a shaking incubator overnight at 30°C

Day 3:

Step 6: It is usually a good idea to make a freezer stock from your overnight culture. This will save you from having to repeat Steps 1-4 if you wish to repeat this protocol in the future. Transfer 500uL of your overnight culture into a cryovial with 500uL of 50% glycerol. Store at -80°C.
Step 7: Proceed to Part 2

Part 2: Making your knockout

Day 3 (cont'd):

Growing up the Strain: ~3hours

Step 8: Prepare growth media by diluting Lambda Red Induction Solution 1:200 with LB Amp or LB Carb. In general, you will need 5mL of growth media for each transformation you want to do.

Lambda Red Induction Solution:	
$MgCl_2$	2M
L-Arabinose	200mM

Step 9: Dilute your overnight culture from Step 5 1:100 into the growth media. Grow in a shaking incubator at 30°C for three hours.
Step 10: After three hours of growth, your cells should be at O.D. ~0.4-0.6. Place the cells on ice for 30 minutes.

Preparing Competent Cells: ~1hour

IMPORTANT: KEEP CELLS ON ICE THROUGHOUT STEPS 11-16

Step 11: Spin the cells down at 5k rpm for 5 minutes.
Step 12: Discard the supernatant. Wash the cell pellet by gently resuspending with 1/5 volume of 10% ice cold glycerol (e.g. if you grew your cells in 25mL of growth media, use 5mL of 10% ice cold glycerol to wash the pellet).

Step 13: Repeat Steps 11 and 12. Spin the cells down one last time at 5k rpm for 5 minutes.

Step 14: Resuspend the pellet with 10% ice cold glycerol. Use 11uL of glycerol per mL of growth media (e.g. if you grew your cells in 25mL of growth media, use 275uL of 10% ice cold glycerol).

Electroporation and outgrowth: ~3.5hours

Step 15: For each transformation aliquot 1-5uL of gel purified antibiotic resistance cassette into an eppendorf tube. Add 50uL of competent cells from Step14. Mix by gently pipetting.

Step 16: Transfer each mixed transformation reaction to an electroporation cuvette.

Step 17: Electroporate (1.8kV). Immediately recover cells with 1mL of SOC. Transfer into 1.5mL eppendorf tube.

Step 18: Outgrow in a shaking incubator at **37^{o}C** for 3 hours.

Step 19: Plate 100uL of 1:10 and 1:1 dilutions on selective media plates. Incubate overnight at 37^{o}C. Store remaining outgrowth at 4^{o}C.