**Media and growth conditions:**

All fitness assays are performed in DM25 (Davis Minimal Media). The following recipe makes one 1000-ml batch. Note also that E. coli cannot use citrate to support growth; it serves only as a chelating agent in this medium.

· Potassium phosphate dibasic trihydrate (Sigma P5504)- 7 g

· Potassium phosphate monobasic anhydrous (Sigma P5379)- 2 g

· Ammonium sulfate (EMD AX1385) - 1 g

· Sodium citrate (Sigma C7254) - 0.5 g

· Water - 1000 ml

Immediately after autoclaving, add the following from sterile stock solutions (kept in 100-ml bottles covered in foil)

· 10% Glucose - 250 µL (Stock solution: 10 g glucose (Sigma G8270)in 100 mL water autoclaved and covered in foil)

· 10% Magnesium sulfate - 1000 µL (Stock solution: 10 g MgSO4 (Sigma M7506) in 100 mL water autoclaved and covered in foil)

· 0.2% Thiamine/Vitamin B1) - 1000 µL (0.2 g thiamine hydrochloride (Sigma T4625) in 100 mL water filter sterilized; do NOT autoclave)

All fitness assays are performed in 15mm glass test tubes, and incubated at 37C and 220rpm

*NOTE*: While this is different from the traditionally used Erlenmeyer Flasks, from personal communication with Tanush Jagdish (Murray and Desai Labs at Harvard), any well-mixed glass container is a good substitute. We decided to use glass tubes for the convenience of being able to do many fitness assays in parallel.

**Daily Transfers:**

1. For each transposon library, estimate the density of cells (CFU/mL in the frozen library)
2. Extract DNA from at least 5x10E8 CFU/mL thawed transposon library. This serves as time-point -1.
3. Seed 5 glass test tubes with 10mL of DM25 with ~5x10E6 cells from the transposon library for one fitness assay
4. Incubate and 37C at 220 rpm for 24 hours.
5. Pool the five cultures corresponding to the same fitness assay into a 50mL falcon tube. Mix thoroughly by vortexing.
6. Transfer 0.5mL of the pooled cultures into 49.5mL of DM25 and mix well by vortexing
7. Pellet the remaining overnight culture by centrifuging at 4300 rpm for 30 mins, removing most of the supernatant, suspending in 1ml of DM25, and centrifuging at 16,000 rpm for 2 mins.
8. Store the cell pellets at -20C. Call this time-point 0.
9. Pipette 10mL of the diluted cultures into labelled glass test tubes and incubate again at 37C and 220 rpm for 24 hours
10. Repeat steps 3-8 until you reach time-point 3

**Rationale for having multiple glass tubes for fitness assay:** By having 5 cultures per fitness assay, we increase the population size by a factor of 5, reducing noise from the daily dilution of cells into fresh media. We still continue to grow the cultures in 10mL volumes to replicate the conditions of LTEE as closely as possible.