Library Preparation for Mtb Tn-Seq (@author: Yancheng Liu)

The aim of this experiment is to identify transposon insertion sites by sequencing flanking regions, and determine the relative abundance of each mutant in the whole transposon mutant pool by Illumina Hi-Seq single-end 100bp sequencing.

Workflow for library prep:

1. Isolate >1ug genomic DNA from drug/no-drug treated samples.

2. Fragment DNA by sonication using a Covaris machine. Optimize condition to maximize the yields between 100~300bp.

3. Perform end repairing, ‘A’ addition, adapter ligation, ‘U’ excision and purification using NEBNext Ultra DNA Library Prep Kit for Illumina (NEB #E7370).

4. PCR to enrich transposon-containing fragments.  Optimize cycling condition and cycle number (6 – 15) to ensure both sufficient and proportional amplification.

The primers for the PCR will be:

Tn-P1L\_v2 (with increased matching Tn ITR length):

AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAGACCGGGGACTTATCAGCCAACCTG

Primer 2: NEBNext index primers set1 (NEB #E7335) – complimentary to Illumina adapter II and incorporating barcode for multiplexing

5. After clean-up, submit the PCR products for Hi-Seq sequencing using a custom primer annealing to the very end of the transposon sequence.

Primer for sequencing: CCGGGGACTTATCAGCCAACC

Alternatively, Illumina PE sequencing primer 1 can also be used for sequencing (need to use Tn-P1L in step 4).

Alternative primer for sequencing: ACACTCTTTCCCTACACGACGCTCTTCCGATCT