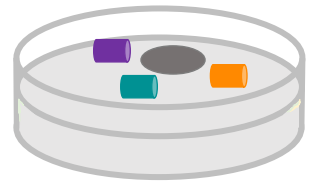
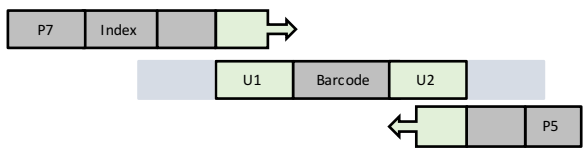
RB-TnSeq mutant library is grown in LB
(pool of 152,018 insertion mutants)

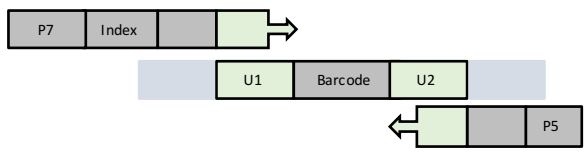


RB-TnSeq mutant library is grown in selective condition (alone or in the presence of other species (pairwise or community conditions))

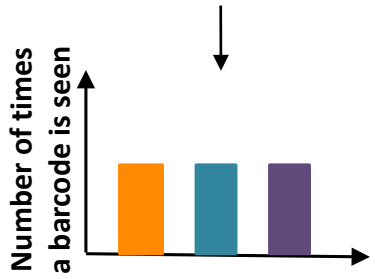
An aliquot of these cells is collected and DNA is extracted (T0 condition)

Cells are harvested from plate and DNA is extracted

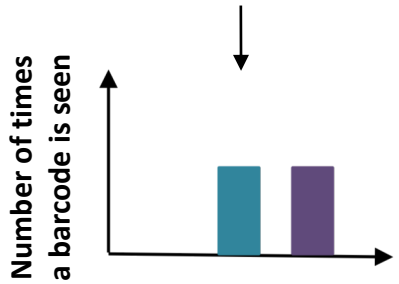




Unique barcodes are amplified with primers annealing to common U1 and U2 regions. These primers also add on Illumina adapter sequences and a unique index to facilitate pooled sequencing



Following amplicon sequencing, barcodes are counted



Following amplicon sequencing, barcodes are counted

Insertion mutant fitness $= f_s = \log_2(n_{after} + \sqrt{\varphi}) - \log_2(n_0 + \sqrt{\frac{1}{\varphi}})$

Unnormalized gene fitness $= f_u = \frac{\sum_i (w_i * f_s(i))}{\sum_i w_i}$

Normalized gene fitness: $f_{g1} = f_u - M$ then $f_g = f_{g1} - Md$

t-score $= \frac{f_g}{\sqrt{\sigma^2 + \max(V_n, V_e)}}$

n_{after} = abundance after growth
n_0 = abundance in T0
φ = pseudocount (avoid noise in low to null counts)
w_i = strain weight (inverse of the variance)
M = smoothed median of the unnormalized fitness
Md = Mode of the previously normalized gene fitness
σ = constant to represent uncertainty in the normalization of small fitness values
V_n = naïve gene fitness variance (based on counts)
V_e = estimated gene fitness variance (based on insertion mutant fitness)