**P1 transduction assay**

The recombination efficiency of the *E. coli* strains carrying wild-type RecA and RecA mutant proteins were assayed by P1 transduction as described\(^1\).

The assay measures the efficiency of the wild-type RecA or its variants, to recombine the selectable genetic marker (GFP-Cm gene) into their chromosome, using P1 phage mediated transduction. P1 lysate was prepared by growing the donor bacterial strain (CH458≡MG1655 lacA::GFPCm) overnight in LB medium with chloramphenicol antibiotic.

**Preparation of P-1 lysate:**

1) The overnight culture was diluted 1:4 in fresh LB+ 5 mM CaCl\(_2\) and 0.2% glucose and allowed to stand for 30 min at RT.

2) Then wildtype P1 phage lysate was added to the diluted overnight culture, incubated with shaking @ 37\(^\circ\)C for 20 min followed by plating them on LB plates with 5 mM CaCl\(_2\) and 0.2% glucose.

3) Next day after overnight incubation of the plates, the top layer of lysed cells were scrapped-off into sterile centrifuge tubes, and ~300\(\mu\)l of chloroform added to the lysate, vortexed and allowed to stand for 30 min at RT with intermittent vortexing followed by centrifugation @ 10000 rpm for 10 min to collect the supernatant P1 lysate.

4) The P1 phage lysate was subsequently titred against *E. coli* strain SMR6765 containing wild-type RecA on pGE591 plasmid (see protocol for P1 titration)

5) The viability of wild-type RecA and RecA mutant strains was also assayed, so that approximately 1 phage for every 100 viable cells was used in the P1 transduction assay (see protocol for viability assay).

**P1 assay:**

1) During the assay, the recipient bacterial strains (wild-type RecA and the RecA-mutant strains) were grown overnight and subcultured next day till the OD\(_{600}\) reached 0.5.

2) P1 lysate was added to the cultures in such a way that the ratio of phage to viable cell counts as ~ 1:100, vortexed, and incubated with shaking @ 37\(^\circ\)C for 18 min followed by centrifugation for 2 min at 7000 rpm to pellet the cells.

3) The cells were resuspended in LB medium with 100mM sodium citrate and plated on LB-citrate plates with chloramphenicol, incubated overnight at 37\(^\circ\)C.

4) Next day, the number of transductant colonies in each strain was counted.

5) The transduction or recombination efficiency of the wild-type RecA and mutant RecA strains were calculated by the number of transductants relative to the phage titer.
6) The assay was repeated at least 3 times for all the wild-type RecA or RecA mutant strains and the mean ± S.E. values for recombination efficiency were used for graphical representation.

Bibliography