

Transforming chemically competent bacteria

- 1) Warm LB/antibiotic plates to RT in the fume hood with lids partially open to partially dry plates.
- 2) Thaw chemically competent cells on the surface of ice for 10-15 minutes.
- 3) Place 1 – 10 ul plasmid DNA or ligation at the bottom of a 14ml polyprop. Culture tube.
- 4) Add 50 ul competent cells and mix gently by pipeting up and down once or twice.
- 5) Incubate tube on ice 15 minutes.
- 6) Heat shock at 42C for 45 sec.
- 7) Place tubes, without disturbing cells on ice 2-3 minutes.
- 8) Add 250 ul SOC at RT.
- 9) Place in 37C shaker 30-60 minutes for AmpR and 60 min for KanR.
- 10) Plate 10-250ul bacterial mix on plate, making sure all liquid is absorbed.
- 11) Incubate plates upside down overnight (for retroviruses incubate at 30C; for mammalian expression constructs, incubate at 37C).