Genome editing via the CRISP-Cas9 system

Genome Editing

- Generating E. coli BL21 DE3 harboring pCas
  - Do a heatshock transformation of pCas with *E. coli* BL21 DE3
  - Grow the culture overnight in LB media and kanamycin at 30 °C.
  - Generate elektrocompetent *E. coli* BL21 DE3 cells harboring pCas.
    - Add Arabinose (10 mM) to the culture for lambda-Red induction.
- Transformation via electroporation of pTarget
  - Thaw 50 µL electrocompetent *E. coli* cells on ice.
  - Add 100 ng pTarget and 400 ng of the donor DNA (PCR fragment).
  - Store cells on ice for 1 minute.
  - Electroporate at U = 2.5 kV, C = 25 µF, R = 400 Ω
  - Recover cells at 30 °C for 1 hour.
  - Plate on selective LB agar containing kanamycin (50 mg L^{-1}) and spectinomycin (50 mg L^{-1}).
  - Incubate overnight at 30 °C.
  - Verify positive transformants by colony PCR and DNA sequencing.

Plasmid curing

- Inoculate a positive colony, harboring pCas and pTarget in 2 mL LB media containing kanamycin (50 mg L^{-1}) and IPTG (0.5 mM).
- Incubate at 30 °C for 8 to 16 hours.
- Plate on selective LB agar containing kanamycin (50 mg L^{-1})
- To cure pCas, grow the cells overnight at 37 °C nonselectively.

Reference

Jiang, Y., Chen, B., Duan, C., Sun, B., Yang, J., and Yang, S. (2015). Multigene editing in the Escherichia coli genome via the CRISPR-Cas9 system. Appl. Environ. Microbiol. 81: 2506–2514.