Genome editing via the CRISPR-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 electrocompetent *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 non-selectively.

From: iGEM Bielefeld-CeBiTec