

Bacteria transformation

- spin down → 1 μ l
- add 9 μ l H₂O
- thaw chemo-competent E.coli SURE cells (Stratagene) on ice
- take 40 μ l cells and add 1 μ l of the dilution
- incubate 30 min on ice
- heat shock 90 sec at 42°C
- recover on ice for 5 min
- add 1 ml 2xTY-Medium
- incubate at 37 °C for 30 min
- spin for 4 min at 4000 rpm
- remove supernatant and resuspend the pellet in 50 μ l 2xTY-Medium
- plate out (LB+Amp-plate)
- incubate at 37 °C o/n
- pick 6 colonies, incubate in 4 ml 2xTY- Medium + Amp. (not all colonies will be positive for the IRs)
- incubate at 37 °C (shaker) for 16 hrs
- spin down
- use QIAprep Miniprep kit to extract the plasmid DNA
- control digest
4 μ l DNA with 0.5 μ l XbaI in a 20 μ l reaction for 1hr 30' (depending on the construct we used EcoRI and BglII too)
- load on 2 % agarose gel
- control: product length = 2 * sequence length