

## Production of competent E-coli-cells

- Work always sterile and cold and speedy ☺ !
  - All volumes deal with the common cellline!
  - The cooling-centrifuge is in the tool shed (Geräteraum), cool down to 4°C early enough (Schalter hinten rechts), close the lid correctly!
  - Prepare early enough min. 55 Eppis (1,5µl) (per cellline) and cool down to -80°C before using
  - Use Milipore-filter for sterile CaCl<sub>2</sub>, keep cool!
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- prepare 15ml LB-Medium (or DYT) with the specific antibiotic (XL1-blue → Tet, BL21 → none!), inoculate and incubate over night
  - prepare 200ml LB-Medium (or DYT) with the specific antibiotic, inoculate with 2ml of the over-night-culture. Nurture the culture until OD<sub>600</sub> at 0,35 (0,2-0,5) (at too high OD the cell won't be competent)
  - keep cell suspension in sterile falcons (50ml) 20 min on ice, then centrifuge 20min; 4°C; 2500g
  - discard supernatant, **carefully** resuspend with 10ml CaCl<sub>2</sub>-solution (put a little of the 10ml in every falcon), pool every resuspended aliquot of one cell line and add 40ml CaCl<sub>2</sub>-solution (total volume 50ml). Keep 30 min on ice, then centrifuge 20min; 4°C; 2500g
  - discard supernatant, **carefully** resuspend pellet in 5,5ml CaCl<sub>2</sub>(80mM)/Glycerol (4:1), aliquot in Eppis á 100µl and store **immediately** at - 80 °C

CaCl<sub>2</sub>-production: mix 2,94g Calciumdichlorid (+ 2H<sub>2</sub>O) with 250ml Milipore-water (in kitchen)→ 80mM, filtrate with Milipore-filter in another 250ml-Schottbottle