Nucleus Protocols

Grow and Induce Expression Strains

- ☐ **Prep overnight cultures.**
  - ☐ Add 5 mL LB + Kanamycin (50 µg/mL) to 15 mL culture tubes and label.
  - ☐ Inoculate your tubes with your expression strain working stock using a pipette tip.
  - ☐ Incubate cultures overnight at 37 °C / 225 rpm for between 12 h and 16 h.
- ☐ **Perform bulk outgrowth.**
  - ☐ Back dilute overnight cultures 1:500 into fresh media (e.g., add 900 µL of overnight into 450 mL LB with Kanamycin in 2 L baffled flasks).
  - ☐ Incubate back diluted cultures at 37 °C / 225 rpm to mid-log phase (OD_{600} between 0.4 and 0.6, ~3.5 h).
- ☐ **Induce protein expression.**
  - ☐ At mid-log phase, induce your cultures with IPTG to 500 µM (e.g., add 450 µL of IPTG (0.5M) to a 450 mL culture).
  - ☐ Incubate induced cultures at 37 °C / 225 rpm / 4 h to allow cells to express proteins.
- ☐ **Centrifuge cells and freeze pellets.**
  - ☐ While incubating your induced cultures, pre-chill your centrifuge and rotor to 4 °C.
  - ☐ Fill 1 L centrifuge bottles with culture. Balance bottles and centrifuge at 16 000 rcf / 4 °C / 10 min.
  - ☐ Decant supernatant, add fresh culture, and repeat centrifugation until all culture is processed.
  - ☐ Weigh pellets to calculate your biomass yield (gDCM / L).
  - ☐ Store pellets at –80 °C and allow to freeze (at least overnight).