Protein production (expression)

Introduction

Cultivation of the Expression-strain (with plasmid) and expression of the gene for IPTG-activated plasmids.

Materials

- LB-Medium (50ml for ONC, 800ml for each expression-culture)
- Antibiotics
- 40% Glycerol

Procedure

Day 1

1. Prepare LB-medium in erlenmeyer flasks, each flask contains 800ml. Autoclave the flasks and let them cool
2. Prepare a liquid ONC of the desired culture with LB-medium and the same antibiotics as will be used later

Day 2

1. **Make a stock**
   - Take 1ml of the ONC with 1ml of 40% glycerol. Freeze at -80°C.
2. **Add the desired antibiotics** to the medium in the expression-flasks.
3. **Add 12ml of the ONC** to each 800ml-flask
   - You can measure the 12ml with a 15ml-falcon-tube
   - The solution has now an OD600 between 0.05 and 0.1
4. **Shake the flasks** for 2 hours at 37°C with 120-140 rpm
   - In the first 1.5 hours not much will happen, as the bacteria are in the lag-phase. The OD600 after this time is expected to be around 0.2 and 0.3

5. **Measure the OD600 (Checkpoint 1)**

- Then calculate the time which is necessary for the cultures to reach OD600 between 0.5 and 0. 6 with the doubling time of the bacteria (they should have reached the log phase), the OD600 is now roughly linear. (For E.coli OD 0.25 equals 30min, OD 0.2 equals 45min, OD 0.3 equals 20min
- Keep the flasks shaking on 37°C until the OD600 should theoretically be between 0.5 and 0.6

6. **Measure the OD600 (Checkpoint 2)**

- If it is between 0.5 and 0.6, reduce the temperature to 20°C, keep shaking for 20 minutes
- If it is lower than 0.5, keep it shaking on 37°C until the OD600 should be between 0.5 and 0.6, calculate this time as in step 7, repeat the measurement after that time
- If it is between 0.6 and 1, immediately take a sample (step 11) and add IPTG (step 12) and reduce the temperature to 20°C, incubate over night with 20°C and 120-140rpm

7. Cool flasks down

- Reduce the temperature in the shaker to 20°C and keep them shaking on 120-140 rpm for around 20 min (less, if the OD is very high or if there are only few flasks).

8. Take negative sample

- Take 1ml of the culture and freeze it as a negative sample bevor you add IPTG to load it as negative probe onto the SDS-gel (desired protein should only be expressed strongly in the IPTG-activated probe)

9. Add IPTG

- The final concentration should be 0.1mM

10. Keep shaking for 16+ hours (20h)

- on 20°C with 120-140 rpm

Day 3

1. Harvest the cells by centrifuging the culture at 5000 rpm in 400ml-beakers
2. Resuspend the pellets in 0.9% NaCl
3. Transfer to one 50ml-tube, centrifuge at 4500 rpm for 45 minutes, discard the supernatant, freeze the pellet at -20°C