# Cultivation of *P.Pastoris*

Separation of the clones:

- Four clones of each *Pichia pastoris* plate are chosen and plated on a new YPD-Agar plates containing the antibiotic nourseothricin using the fractionated smear method and incubated for 72 hours.
- This procedure is repeated to ensure the purity of the selected clones.

Pre culture:

- 10mL of liquid YPG-Medium containing nourseothricin are inoculated with two purified clones of each sample and incubated at 28 degrees Celsius for 24 hours shaking.
- A wild-type control is also inoculated in YPG-Medium not containing any antibiotics.

Main culture:

- 20mL of BM-Medium containing the antibiotic nourseothricin are inoculated to an OD600 of 0,1, set to a glucose-concentration of 2% and incubated for 48 hours at 28 degrees Celsius shaking.
- After 12, 24, 36 and 48 hours 2mL samples are taking of each culture and the glucose-concentration is set to 1%.
- 1mL of the sample is used for the OD-measurement to control the cell growth, the other mL is centrifuged at 16,000xg for 5min and used for the protein conformation.