Pichia pastoris: transformation

- The completed plasmid was linearized, using the restriction enzyme AscI.
- 5-10µg of the linearized plasmid (the volume should not exceed 30µL) are added to 80µL of electrocompetent *P.pastoris*. As a negative control 30µL of dH_2O are used instead of the sample.
- The cell-mixture is then transferred to a chilled electroporation cuvette (2mm) and incubated on ice for 5min.
- The electroporation is performed by the "BioRad Gene Pulser" using 1,5kV for a period of 4ms.
- 1mL of ice-cold liquid YPD-media is added to the electroporated cells in the cuvette immediately after the electroporation. Then the mixture is transferred to a sterile reaction tube and incubated at 28 degrees Celsius for approximately 2 hours.
- 20µL, 200µL and the rest of each sample are plated on three YPD-Agar plates containing the antibiotic nourseothricin and incubated for approximately 72 hours at 28 degrees Celsius until distinctive cultures can be seen.