

# Test of detection system in *Saccharomyces cerevisiae*

## Extraction of h<sup>+</sup> pheromones from *S. pombe*:

*S. pombe* was grown in YPD media at 30 °C. The pheromones were extracted by filtration. To increase the concentration of the h<sup>+</sup> pheromones the solution was run in a concentration with cold trap for 5,5 h. The concentration was increased by 20.

## Test of detection system:

1. A colony of *S. cerevisiae* was inoculated in 5 ml YPD media over night.
2. The OD was measured and the cells were centrifuged at 1100 rcf for 5 min.
3. The pellet was dissolved in 5 ml YPD media and transferred to a shake flask. The solution was diluted with YPD to a OD of 0,4. The cells were inoculated at 30 °C for 2h.
4. In four eppendorf tubes, 1 ml of the cell mixture was added. 0, 50, 115 and 230µl of the concentrated pheromone solution was added to each tube respectively. The cell was once again incubated at 30 °C for 2h.
5. The cells were washed with dH<sub>2</sub>O and centrifuged for 5 min at 1100 rcf. The pellet was dissolved in 70µl dH<sub>2</sub>O.
6. 3µl of the solution was used for studying of the detection system with a fluorescence microscope.