Test of detection system in *Saccharomyces cerevisiae*

Extraction of h+ pheromones from *S. pompe*:

S. pombe L972 +h was grown in YPD media at 30 ˚C for 3 weeks. The pheromones was extracted pelleting the cells and filtrating the supernatant through filter with 0.2 µM pore size. To increase the concentration of the h+ phermones the solution was run in a concentrator with cold trap for 5,5 h. The concentration was increased by approximately 20 times.

Test of detection system:

1. A colony of *S. cerevisiae* CEN.PK2 with integrated construct 4 was inoculated in 5 ml YPD media overnight. Wild type CEN.PK2 and a strain expressing RFP was used as negative and positive control.

2. The OD was measured after overnight preculture and the cells was 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 was inoculated at 30 ˚C for 2h.

4. 0, 50, 115 and 230 µL concentrated pheromone was added to 1 ml cell suspension of the negative control and the colony with C4. No pheromones was added to the positive control. The cell was once again incubated at 30 ˚C for 2h to allow expression of RFP.

5. The cells were pelleted and washed with once with dH_20. The pellet was dissolved in 70µl dH_20 and 3 µL of the solution was used for studying of the detection system with a fluorescence microscope.

6. Used 1 second of exposure time for RFP measurement. Used GFP exposure as a measure of inviable cells.