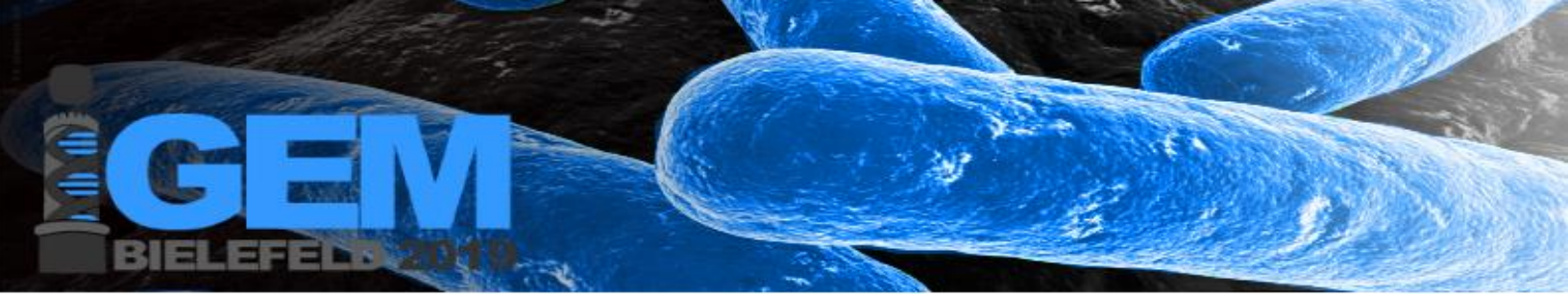

Endocytosis Test: Fluorescence Measurement in Culture Supernatant

- Inoculate an overnight culture of *S. cerevisae* in full medium.
- On the following day, inoculate a new culture from the overnight culture with start OD: 0.2 in a minimal medium.
- Let grow until OD 0.5 – 0.8.
- For every tested protein: take and cultivate 5 mL of the inoculated culture (plus 1x negative control).
- As comparation/control: cultivate 5 mL of minimal medium without yeast cells
- Add 1 µL protein of choice to the culture and to the control culture. Cultivate at 30°C, 180 rpm, and in the dark.
- Take 500 µL of samples from the culture at the time point of 0 min, 15 min, 30 min, 45 min, 60 min. Centrifuge the samples at 12,000 rpm for 1 minute.
- Measure the fluorescence in the supernatant with TECAN-reader in 4 x 100 µL volumes. Initiation at 570 nm and emission at 610 nm. Standardization to 2,5 µM Texas Red.

Fluorescence intensity of the proteins:

- Perform a serial dilution of Texas Red in the following order: 0; 0.1; 0.25; 0.5; 1; 2.5 µM
- Also perform the following serial dilutions of the protein: 0.01; 0.025; 0.05; 0.1; 0.25; 0.5 µM
- Measure the fluorescence with TECAN-reader in 4 x 100 µL volumes. Initiation at 570 nm and emission at 610 nm. Standardization to 2,5 µM Texas Red.
- Determination of the fluorescence from the gradient of the resulted line.

From: iGEM Bielefeld-CeBiTec

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