

October 7, 2014

Continue testing sensor

- Transform plasmids into Top10 Cells
- Overnight cultures - dilute 100 μ l in 2 ml
 - grow at 37 °C for 1.5 hours
 - Add 1 μ l of beta-estradiol in ethanol (10 mg/ml)
 - cultures treated with ~20 μ M of estrogen
- Incubate at 30 °C
- Time points:
- Grow overnight

October 8, 2014

Continue testing sensor

- Set up cultures of WT sensor, DEAD Sensor, 115 YFP, 115 YFP and T7RFP term, T7RNAP YFP and T7RFP term, and Top10 cells only at 37 °C, 30 °C and 20 °C to grow overnight

Transformation of Killer Red Codon Optimized and Supernova Codon Optimized into Top10 Cells

- 100 µl of cells for remaining DNA (about 1 - 3 µl)
- Plate 400 µl transformants on LB + Cam plates

iGEM Transformation Protocol: <http://parts.igem.org/Help:Protocols/Transformation>

October 9, 2014

Continue testing sensor

- Cells at 20 °C grew too slowly to be read
- Cells at 30 °C and 37 °C read in TECAN at yellow and red wavelengths

Table 1. TECAN Parameters used to Read Samples

Fluorescent Protein	Wavelength (excitation)	Wavelength (emission)	Gain (if not optimal)
YFP	514	527	90
RFP	584	607	175

- Set up cultures of 115 YFP, 115 YFP and T7RFP term, T7RNAP YFP and T7RFP term in triplicate at 37 °C, 30 °C and 20 °C to grow overnight

October 15, 2014

Photobleaching

- Collect final data, plate counts

Sensor

- Test for growth of MACH and Top10 Cells with the sensor
- Time points: 1 hour, 3.5 hours, overnight