Growth lab book

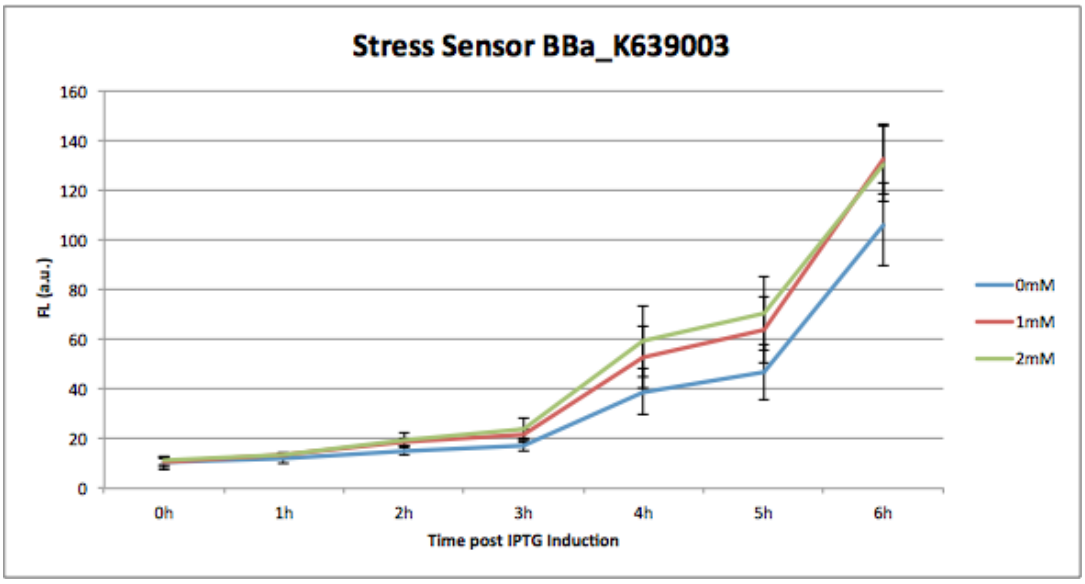
Date **October 3, 2013**

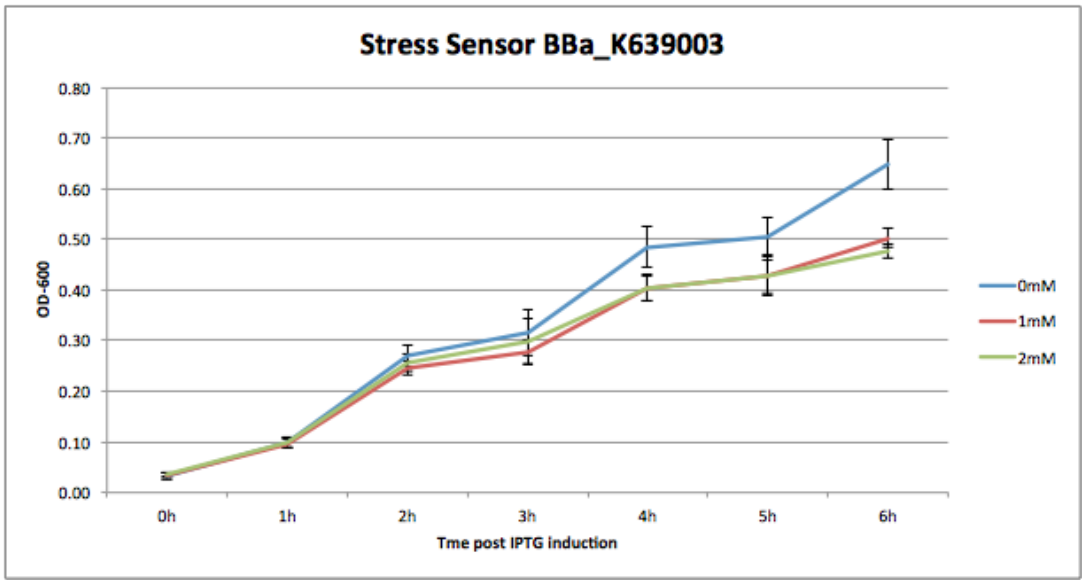
Contents

13/8/13 Growth curves OD and FL (RFP) of Stress sensor BBa_K639003.

Margarita grew a 3ml LB O/N culture from a BBa_K639003 colony (See 10/8/13 for transformation details). The colony's on the plate were MG1655 transformed.

- O/N culture (OD 600 Abs 0.364).
- Diluted 100ul of O/N culture (OD 600 Abs 0.036) into 4ml fresh LB.
- Induced IPTG (0mM, 1mM, 2mM), added 200ul multiple wells to greiner 96 well plate. Grew in shaker incubator OD600 and FL (used 590/640 for but 584/620 recommended RFP) read every hour from t=0 to t=6h. See Experimental data folder > 13.8.13





13/8/13 Setup waste conditioned media and O/N stress cultures for waste toxicity experiment.

Picked colonies A-C from BBa_K639003 plate (See 10/8/13 for transformation details).

O/N 4ml cultures plus Chloramphenicol (1:2000 dilution to 25ug/ml final).

Put 1g of pre-autoclaved Powerday mixed waste into 50ml of LB to generate waste conditioned media. I will grow the stress biosensor transformed cells within the mixed waste conditioned media. (I will filter sterilise it tomorrow).

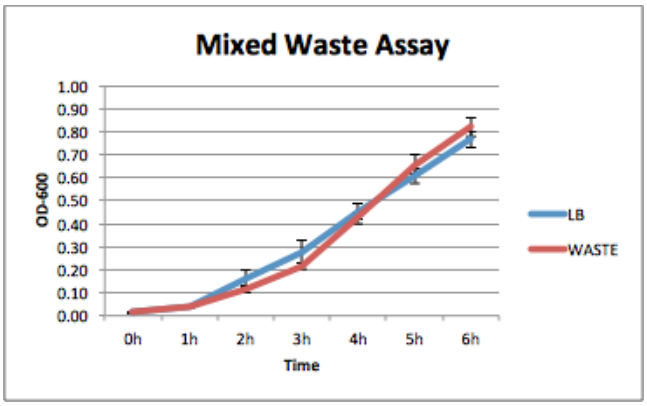
15/8/13 Waste Assay and L-Lactic assay second attempt

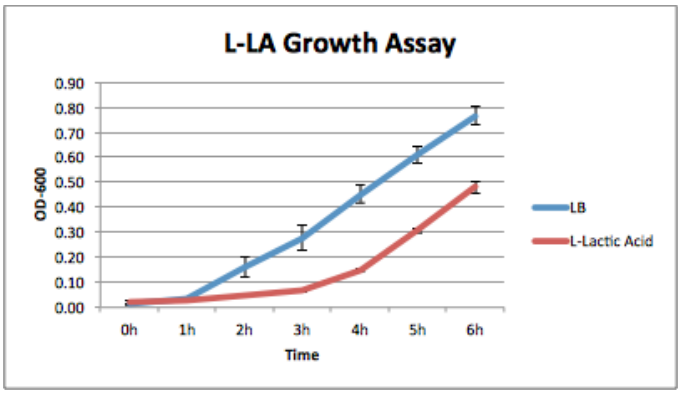
2 different assays run on the same plate.

- Waste assay is to see if cells can grow on mixed waste. Mixed waste conditioned media was generated through incubation of 1g (pre-autoclaved mixed waste) in 50ml of autoclaved LB. Cultured O/N 37oC. Then filter purified.
- L-Lactic acid assay is to see if cells can tolerate L-lactic acid (PLA degradation product). L-Lactic Acid from Alfa Aesar A17268 (FW 90.08).

O/N cultures of E+F colonies (as marked on transformation plate, see 14/8/13 WASTE EXP second attempt). were diluted 100ul of O/N culture into

- 4ml LB
- 4ml waste conditioned media
- 4ml LB+ 1ul/ml i.e. 4ul of L-Lactic acid neat from the bottle. Need to work out concentration
- 200ul of these in quadruplicate into 96 well plate see 15-8-13 experimental data folder for layout (+ LB only and waste conditioned media only lanes to remove background)





21/8/13 setup 3x colonies (X,Y,Z) from stress biosensor BBa_K639003 plate for EG Assay

(See 10/8/13 for transformation details).

O/N 4ml cultures plus Chloramphenicol (1:2000 dilution to 25ug/ml final).

22/8/13 Ethylene Glycol toxicity assay

Three colonies (X,Y,Z) from O/N cultures were diluted 100ul into 4ml LB + Chloramphenicol.

From these Each colony were plated 200ul/ well quadruplicate in to 96 well plate for 0,100mM, 200mM Ethylene Glycol. See EG 22-8-13 in experimental data folder on Google drive for full layout.

Grown at 30oC, time points every hour.

22/8/13 Plated WASTE EXPOSED CELLS JS and RK

On Tuesday Morning 1ml of stress biosensor expressing MG1655 were put into a duran with 100ml LB+~6g SRF. Grown for ~54h . On Thursday afternoon 7pm 100ul plated onto chloramphenical plate to see if cells are still alive. In other words whether cells can grow in direct contact with waste.