SEED Academy, Spring 2009 Synthetic Biology Module

Pre-Lab #5 Due April 3, 2009

1) Read the background and protocol for Saturday's experiments: http://openwetware.org/wiki/Beta-Galactosidase Assay (A better Miller)

$$1 \text{ Miller Unit} = \frac{1000*\frac{(Abs_{420} - (1.75*Abs_{550}))}{(t*v*Abs_{600})}}{(t*v*Abs_{600})}$$

i. Explain the purpose of each term in the equation above (not just what the variable stands for but why it is in the equation). Include: Miller Unit, Abs_{420} , Abs_{550} , Abs_{600} , t, and v.

- ii. What chemical is responsible for the color in the Miller assay? How does this work?
- iii. Why is it important to keep careful track of the time?
- iv. Complete the following table & indicate the rank of the various cultures in the per cell Beta-galactosidase activity (1 having the most activity per cell).

| Sample | | | | | | | Activity |
|--------|--------|--------|--------|---------|--------|--------------|----------|
| # | Abs420 | Abs550 | Abs600 | t (min) | v (uL) | Miller Units | Rank |
| 1 | 0.5 | 0.1 | 0.2 | 50 | 0.02 | | |
| 2 | 0.75 | 0.1 | 0.2 | 50 | 0.02 | | |
| 3 | 0.75 | 0.1 | 1 | 50 | 0.02 | | |
| 4 | 1 | 0.2 | 0.1 | 10 | 0.02 | | |
| 5 | 1 | 0.2 | 0.1 | 50 | 0.02 | | |
| 6 | 1.5 | 0.5 | 1 | 100 | 0.02 | | |
| 7 | 1.5 | 0.2 | 1 | 100 | 0.04 | | |
| 8 | 1.75 | 1 | 1 | 20 | 0.04 | | |