DDM Update

4-26-12

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Cloning: Moving genes into an appropriate vector for physiological experiments

- 121 of 130 cloned
- 9 clones remain

PhoH story: Determine its function

PhoA assay: To investigate whether PhoH induction plays a role in PhoA's regulation

Previous (4-19-12)

- Unsuccessful with the assay so far
  - Should occur by 10-20 minutes, not seeing any change until next day
- Trouble shooting plans (for upcoming week)
  - Will increase size of sample, may not have enough enzyme
  - Will test the assay by using purified alkaline phosphatase
  - Use a freshly prepared substrate (PNPP) solution on the day of the experiment

4-26-12

- Assay is now working!
  - Likely issue being the preparation of the substrate
    - Needed to be made fresh in a buffer (1M Tris pH8.0), and stored in the dark at 4C for up to a week

Future work:

- Reclone the remaining 9 of 130 clones
- Scale up the phoA assay
  - 1st Run – (In triplicates)
    - Strains: empty vector, pEMB11-phoH , ΔuhpT / pEMB11-phoH
    - Media:
      - 0.2% G-6-P + 0.01% Glucose
      - PO_4 – 200 μM
      - +/- Arabinose
    - Time points: (Based off of below growth curve)
      1. O/N (or time zero),
      2. Early time point (4hrs)
      3. Late time point (16-24hrs)
  - 2nd Run – To be determined after evaluating 1st run