Transformation and Expression of Pax into Rosetta Cells Thursday, April 10, 2003

Transformation:

- One "shot" of the Rosetta cells were thawed on ice (20 μL cells)
- 3 μL of my miniprep #1 (from p. 3-86) were added and gently stirred.
- The tube was incubated on ice 5 min.
- The tube was heat shocked at 42°C for exactly 30 sec. then placed on ice 2 min.
- 250 μL of SOC (rt) were added to each tube
- A 100 μL and 150 μL plate was prepared on an LB plate containing both 34 μg/mL chloramphenicol and 50 μg/mL carbenicillin. Grown overnight.

- Rosetta(DE3) singles from Novagen - 34 μg/mL chloramphenicol resistance. Expresses rare tRNAs; facilitates the expression of genes encoding rare E. coli codons.

- Recipe for the LB plates used above (for 4 plates)
  - 2.5 g LB } autoclave 15 min.
  - 100 mL dH_2O }
  - 100 μL of 50 mg/mL carbenicillin } added once
  - 100 μL of 34 mg/mL chloramphenicol } LB is <60°C
  - 1.5 g bacto agar

Friday, April 11, 2003

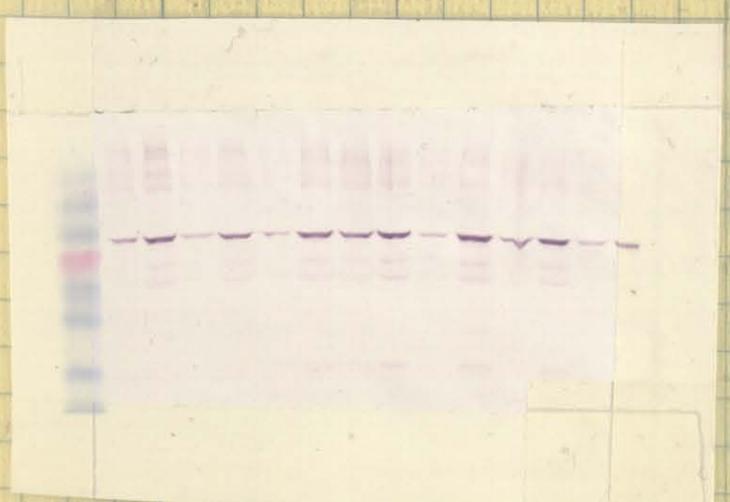
- A decent # of colonies were present when checked in the morning.
- In Falcon tubes, 4 mL of LB w/ 4 μL carbenicillin and chloramphenicol stocks were prepared. 1-57 colonies we selected and grown overnight shaking at 37°C.

Pilot Expression Continued: Saturday, April 12, 2003

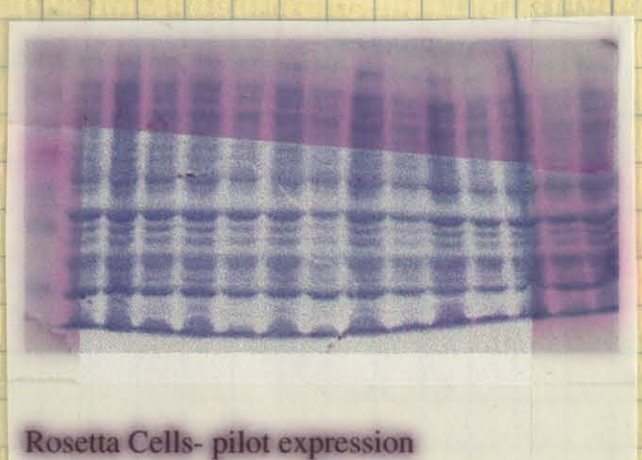
- All 7 colonies selected grew up nicely in the 4 mL of LB media.
- 1 mL was taken for a glycerol stock (1mL culture in 350 µL of 60% glycerol) and stored at -80° C for each of the 7 samples. (Note: after calling Novagen I was told that plasmid instability was found when more than a final concentration of 10% glycerol was used.)
- 1 mL of each was placed in a new tube (#1-7) as the uninduced.
- Each of the 7 2-mL samples was induced with of 1M IPTG and both the uninduced and induced samples were grown overnight. (These were in a water bath supposedly at 37°C, but possibly

Sunday, April 13, 2003

- 500 µL aliquots of each sample were removed. Each was centrifuged for 3 sec. at max speed and the supernatant was decanted and discarded.
- Each cell pellet was resuspended by vortexing in 40 µL water and 40 µL 2x SDS page reducing sample buffer.
- 10 µL of each sample were loaded onto a 10% gel and electrophoresed. Both a coomasie and a Western (developed w/ mouse anti-human paxillin as the primary antibody) were run.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15



Rosetta Cells- pilot expression

1 protein ladder
2 #1 uninduced
3 #1 induced
4 #2 uninduced
5 #2 induced
6 #3 uninduced
7 #3 induced
8... etc.