Transformation:
- One "shot" of the Rosetta cells were thawed on ice. 
- 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, 7 autoclave 15 min.
  [150 mL dH2O]
  [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

- A decent # of colonies were present when checked in the morning.
- In Falcon tubes, 4 mL of LB w/ 4 mL carbenicillin and chloramphenicol stocks were prepared. 15 colonies were selected and grown overnight shaking at 37°C.
Pilot Expression Continued:

- All 7 colonies selected grew up nicely in the 4 mL of LB media.
- 1 mL was taken for a glycerol stock (1 mL 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 0.1 M IPTG and both the uninduced and induced samples were grown overnight. (These were in a water bath supposedly at 37°C, but possibly

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- 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 was loaded onto a 10% gel and electrophoresed. Both a coomassie and a western (developed w/ mouse anti-human paxillin as the primary antibody) were run.

Rosetta Cells- pilot expression