Transformation into BL21-CodonPlus-RP Competent Cells Tuesday, 7/15/03

- two "shots" (100 µL each) of cells were thawed on ice. Two Falcon 15-mL 2059 polypropylene tubes were cooled on ice.

- The cells were mixed and 100 µL were transferred to each tube. (1 for PAX transformation and 1 for the control)

- 2.0 µL of a 1/10 dilution of the stratagene-provided β-mercapto-ethanol mix was added to each 100-µL aliquot. The contents were swirled, then incubated on ice 10 min, gently swirling every 2 min.

- 1 µL of the control puc18 plasmid was added to 1 tube and 2 µL of miniprep #2 (from p. 3-152) was added to the other tube (1-50 ng of expression plasmid DNA is recommended for the transformation) and each tube was gently swirled, then incubated on ice for 30 min. SOC medium was preheated to 42° C.

- Each transformation reaction was heat-pulsed for exactly 20 seconds at 42° C in a water bath, then immediately placed on ice for 2 min.

- 0.9 mL of 42° C-preheated SOC medium was added to each tube and the reactions were incubated in a 37° C shaker for 1 hr (actually 2 hrs).

- 100 µL of each reaction were spread onto LB plates containing both carbanecillin and chloramphenicol. The plates were incubated overnight at 37° C. (Procedure based on Stratagene BL-21 Codon Plus Instruction Manual.)

Wednesday 7/16/03

- Tons of colonies grew up on the plate containing the expression plasmid DNA (pGex-4T-2) containing the modified PAX insert. 50 (rather than 100) µL would have been more than enough cell culture to have spread on the LB plate. The control plate grew up a decent amount of colonies.

- 6 colonies were selected and grown up in 2 mL of LB with carbanecillin and chloramphenicol overnight shaking at 37°C.