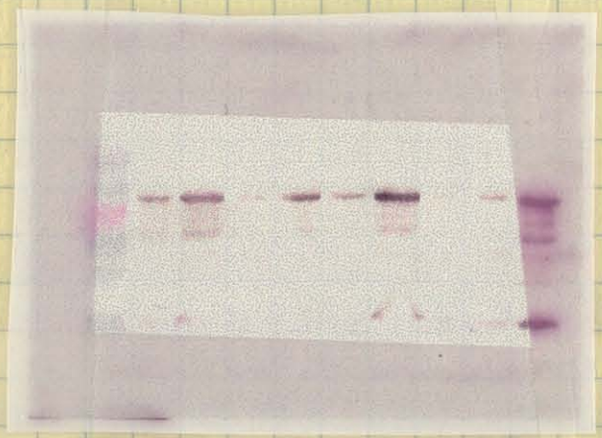
Transformation into BL(21)-codon Plus (RP) cells

- Same procedure as for transformation into the RIL cells, described on p. 3-109.
- 100 µL and 150 µL of the transformed cells were plated onto LB agar plates with 50 µg/mL carbenecillin and 50 µg/mL chloramphenicol (see p. 106 for plate recipe)

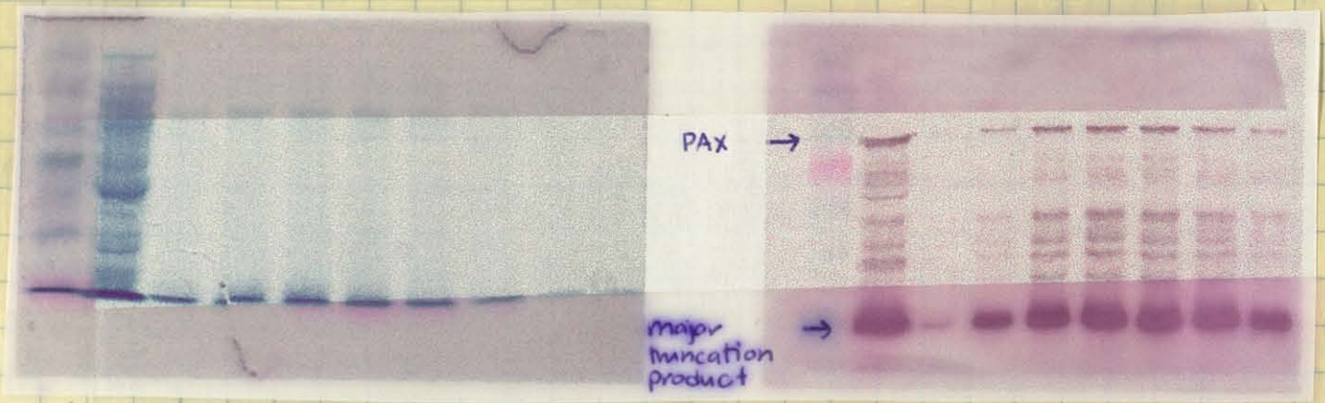
RP Pilot Expression:



in 1	protein ladder	
2	colony 1	uninduced (w/ 1 M IPTG)
3	colony 1	induced
4	" 2	uninduced
5	" 2	induced
6	" 3	uninduced
7	" 3	induced
8	" 4	uninduced
9	" 4	induced
10	" 5	induced

RP Cells Continued:

- 200 mL prep, followed by Ni purification (These were grown from frozen culture overnight in 5 mL. 5 mL were added to 1 L, grown at 37° C to 0.6 OD_{600} and induced w/ 1mM IPTG for 4 hr.



- each lane loaded w/ 20 µL of a mixture of sample (from 1.5 mL fractions off the Ni column) using 5X SDS loading buffer.

(from colony 3 p. 3-111)

- Prep 2: 1 L grown up to an OD_{600} = 0.5 at 37°C, then placed in a water bath shaker at 16°C for 15 min and induced w/ 1mM IPTG. The 1 L was shaken at 16°C overnight before the cells were spun down and frozen at -80°C.
- The cells were lysed and purified (all 1 L) on a Ni column.