Repeat of Transformation (w/ new TOP 10F' cells) of → Tuesday, March 4, 2003

Purified PCR product w/ 3'-A overhang addition

- The PCR product cloned into the TOPO vector was transformed into the One shot® E. coli cells following the TOPO vector instructions. (Saturday, 3/1/03)

- The transformation product was plated in two different amounts (10 µL and 50 µL) onto LB plates w/ carbanecillin. 50 µL of SOC media was added to aid in spreading.

- (Sunday 3/2/03) A decent number of colonies had grown on the 50 µL plate and a few on the 10 µL plate. 10 colonies (#1-10) were selected and grown up in 3mL of LB media w/ carbenicillin overnight, shaking at 37° C.

- 6 minipreps were done for #1-6 (ran out of cent. matrixes from eppindorf, so only did 6 (not 10) preps.

- EcoRI digest of miniprep #1-6 (done as on p. 3-78)

1 2 3 4 5 6 7 8 9 10

1 500 bp marker
2 EcoRI digest #4
3 #4
4 EcoRI digest #3
5 #3
6 EcoRI digest #2
7 #2
8 EcoRI digest #1
9 #1
10 500 bp ladder

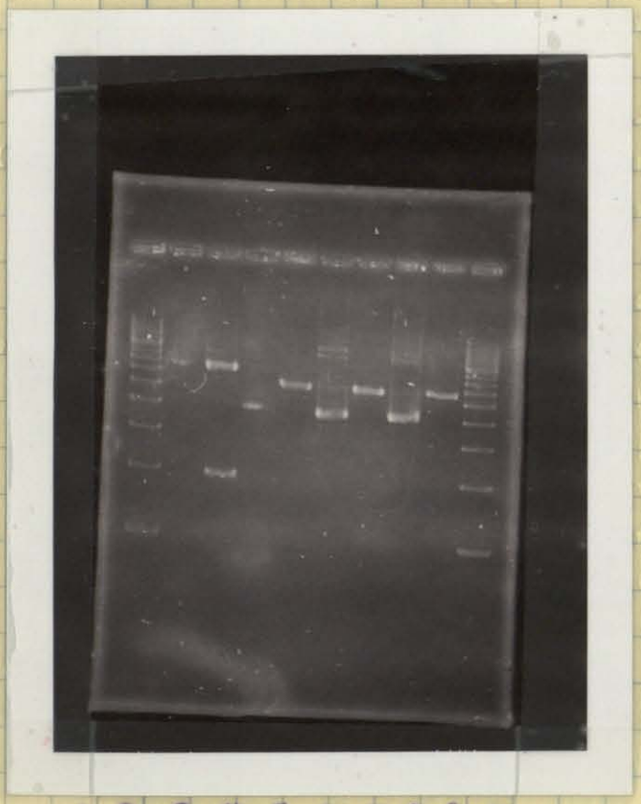
- #2-#6 look like the TOPO vector closed in on itself (2,872 bp)

- #1 looks promising... (vector + insert should be 4,450 bp. Will do a BamH I digest ~ see p. 3-75 for drawing)

BamHI digest (continued from p. 82) Wednesday, March 5, 2003

Bam HI digestion (on #1-6)

		stock (for immediate use)
DNA from miniprep	8 μL	
BamHI 10× rest. buffer	2 μL	30 μL
water	9 μL	15 μL
200 μg/mL BSA soln.	1 μL	135 μL
BamHI enzyme (added last and always ~~kept on ice~~)	0.25 μL	3.75 μL
	20 μL	added 12.25 μL to each DNA sample



1 2 3 4 5 6 7 8 9 10

ln 1	500 bp ladder
2	#1
3	BamHI digestion #1
4	#2
5	BamHI digestion #2
← 6	#3
7	BamHI digestion #3
8	#4
9	BamHI digestion #4
10	500 bp ladder

1 2 3 4 5 6 7 8 9 10

ln 1	500 bp ladder	
* 2	#1	#1 sent for sequencing
* 3	EcoRI digestion #1	
* 4	BamHI digestion #1	
5	#5	
6	BamHI digestion #5	
7	#6	
8	BamHI digestion #6	
9	purified PCR product (p. 3-81)	
10	purified PCR w/ A-overhangs added (p. 3-80).	