

EPL w/ Y31 Pax thioester:

Monday, May 2, 2005

Since the cleavage (p.109) did not appear to go to completion an additional 20  $\mu$ L of mTEVp was added to each 1 mL reaction and incubated 3.5 h. at 30°C.

(10<sup>9</sup> v/v Tris, pH 8 500 mM added)

The reactions were concentrated from 4 mL total to less than 1000  $\mu$ L. If they TEV cleavage went to completion, this would be: ( $\uparrow$  note: Millipore 500  $\mu$ L 50 MWCO filters used for conc.)

2.6 mg Cys-Pax (38-557)-FLAG in  $\sim$  1000  $\mu$ L (2.6 mg/mL)

3 EPL reactions were run simultaneously in 0.65 mL eppendorf tubes incubated at rt overnight:

rxn a + b: (based on 54 kDa, 520  $\mu$ g = 9.63 nmol)= 520  $\mu$ g Cys-Pax-FL in 200  $\mu$ L (0.048 mM)800  $\mu$ g Y31 thioester (lyophilized)

(4 mg/mL, 0.83 mM)

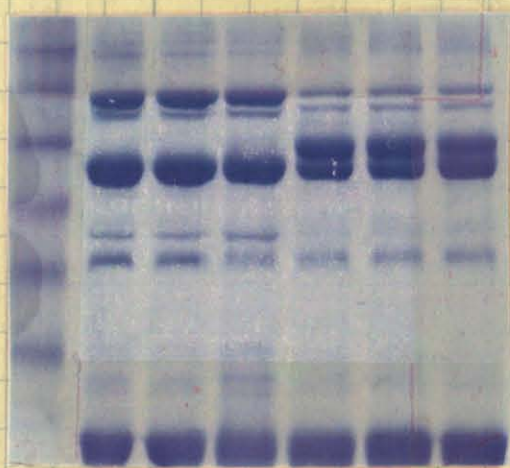
100 mM MESNA (10  $\mu$ L of a 2M soln.)

rxn c:

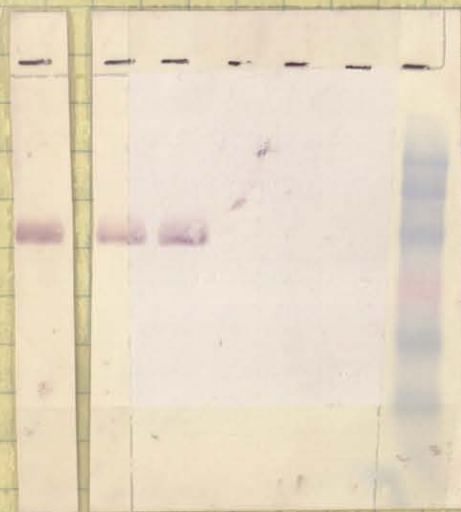
= 520  $\mu$ g Cys-Pax-FL in 200  $\mu$ L300  $\mu$ g thioester

(1.5 mg/mL, 0.311 mM)

100 mM MESNA



1 2 3 4 5 6



6 5 4 3 2 1

mouse  
anti-His 6 1° abab from Amersham  
(5 mL in 15 mL)  
for rows 1-5ab from Novagen  
(2.5 mL in 15 mL)  
for lane 6

cat # 70796-4

Kit lot # N57182

In 1 ~~original TEV cleavage (as on p.109)~~2  $\uparrow$  TEV after additional 20  $\mu$ L mTEVp3  $\uparrow$  TEV after additional 20  $\mu$ L mTEVp

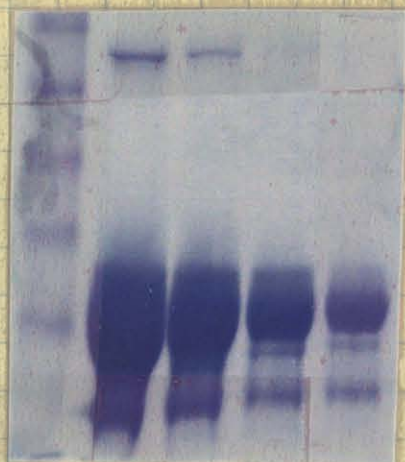
3 TEV cleavages concentrated to 2.6 mg/mL

4 EPL a: for 2 gels (1 coomassie, 1 west) 20  $\mu$ L protein + 20  $\mu$ L 2x SDS5 EPL b so that's 26  $\mu$ g/gel w/ complete conversion6 EPL c (w/out accounting for the extra 4.8 Da)  
or 0.48 nmol/gel (from 10  $\mu$ L of soln.)

(For Western blots, the nitrocellulose was blocked 1 hour w/ milk in TBST. Prior to this it was kept moist in TBS to avoid overblocking)



## Expression of GST-PTP-PEST



PTP-PEST ( ) was expressed as a GST fusion in Codon Plus RIL cells.

The protein pellet (= 4 g) was lysed w/ 40 mL of lysis buffer, as prev. described and purified w/ 1.5 mL of fresh GST affinity resin (Amersham)

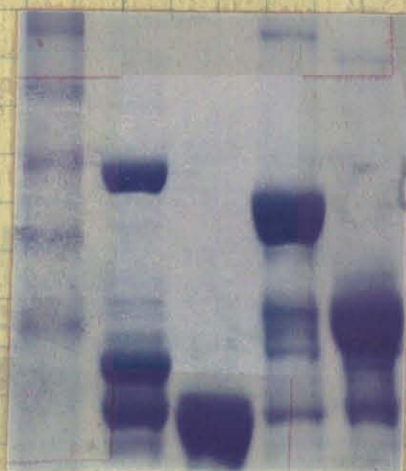
Coomasie shows elutions 1-4 (7  $\mu$ L loaded [3.5 soln., 3.5 loading buffer] per well.) 4  $\times$  1.5 mL elutions were collected.

Dialysis into PBS (to get rid of the glutathione):  
GST (only), GST-FAK, GST-GIT, and GST were dialyzed in 3  $\times$  2L PBS in the cold room overnight.

Biorad quant.

standards: b1 = 0.325, 2.5 = 0.472, 5 = 0.673, 7.5 = 0.808, 10 = 0.911

		Abs <sub>595</sub>	dilution	dilution	
GST (only)	(3 mL)	0.612	0.777	1.8 mg/mL	
GST-FAK	(3 mL)	0.594	0.820	2.0 mg/mL	
GST-GIT	(6 mL)	0.623	0.782	1.8 mg/mL	
GST-PEST	(6 mL)	0.658	0.964	2.6 mg/mL	



In 1 GST-FAK  
2 GST (only)  
3 GST-GIT  
4 GST-PEST