M2D7: Characterizing Your Cph8 Mutant Part II

11/1/12

1 Finish WB ← 1°Ab
2°Ab

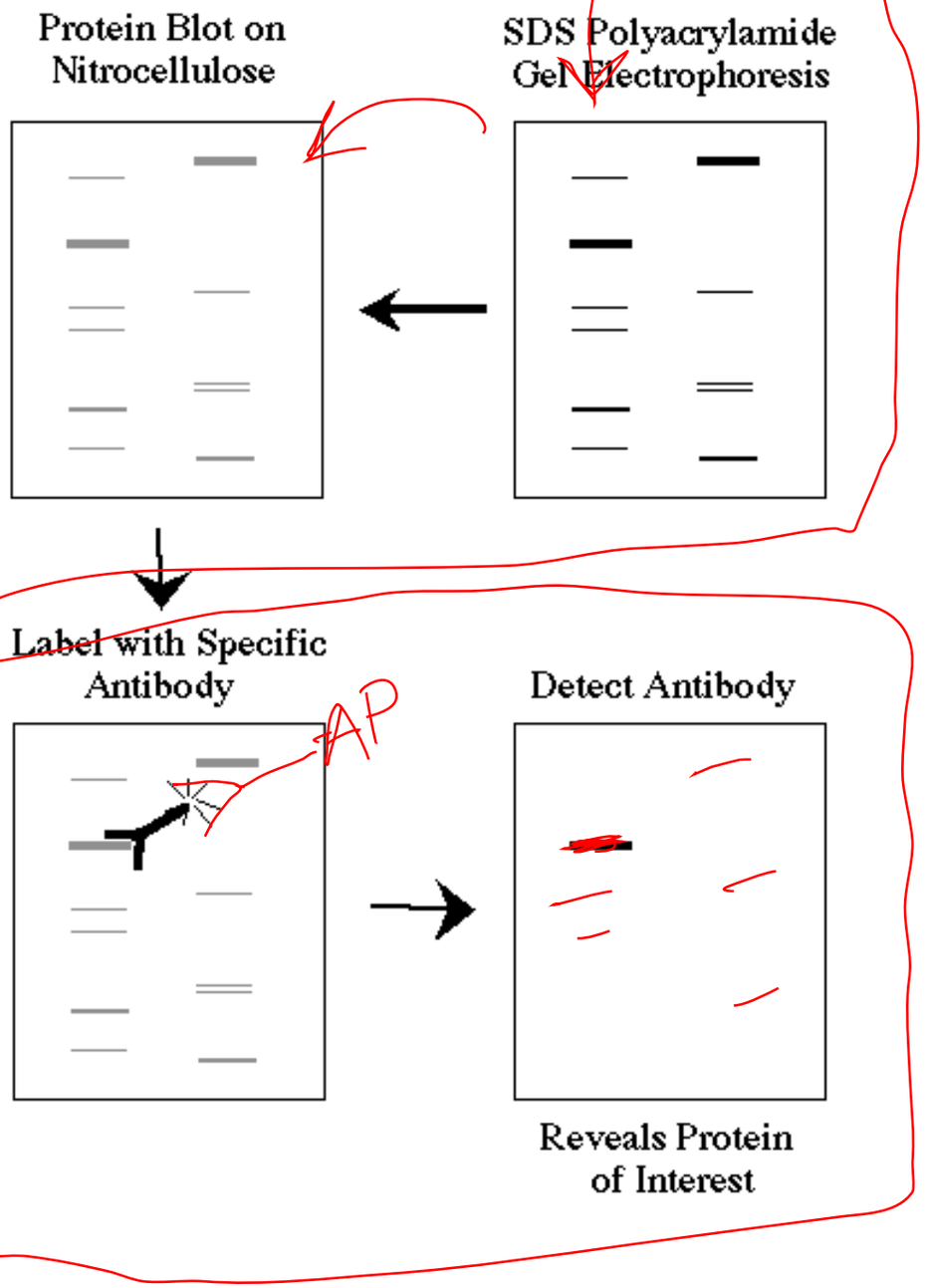
vis results

2. Your experiments!

Western Blot analysis of Cph8 Mutant:

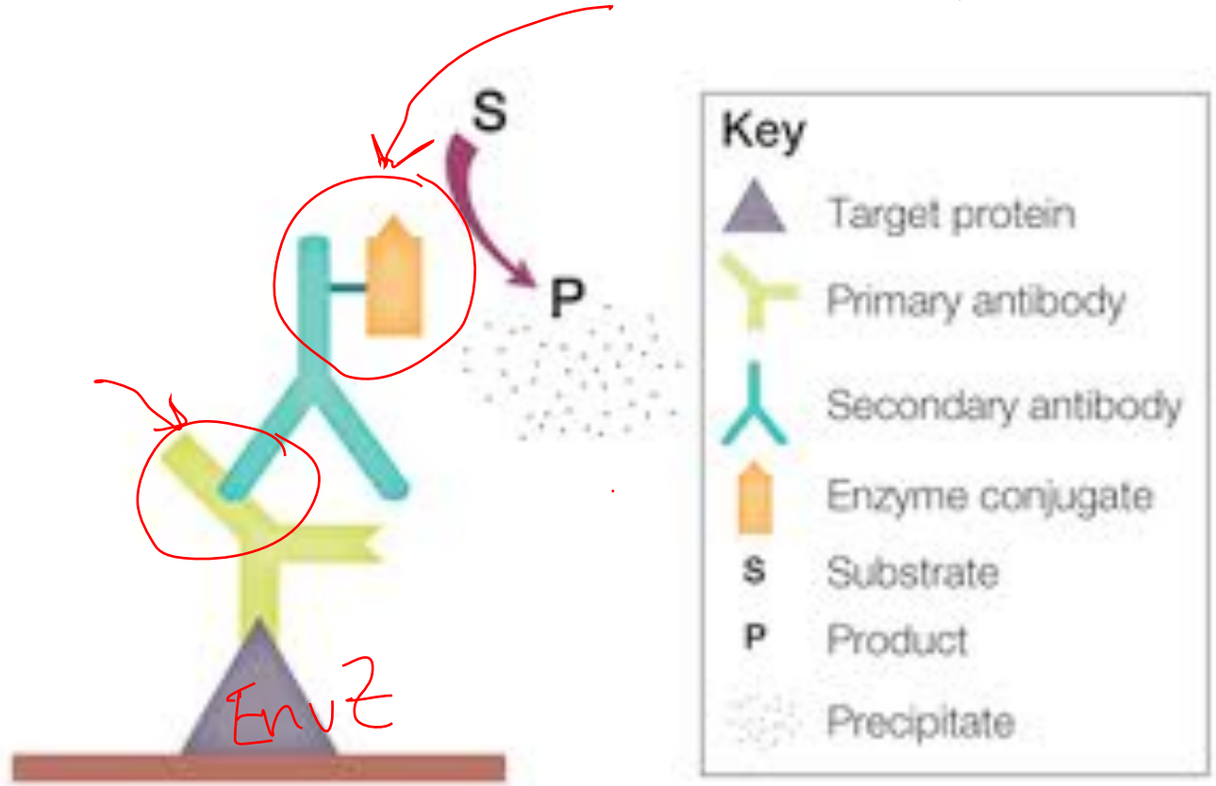
5% Milk

2OD



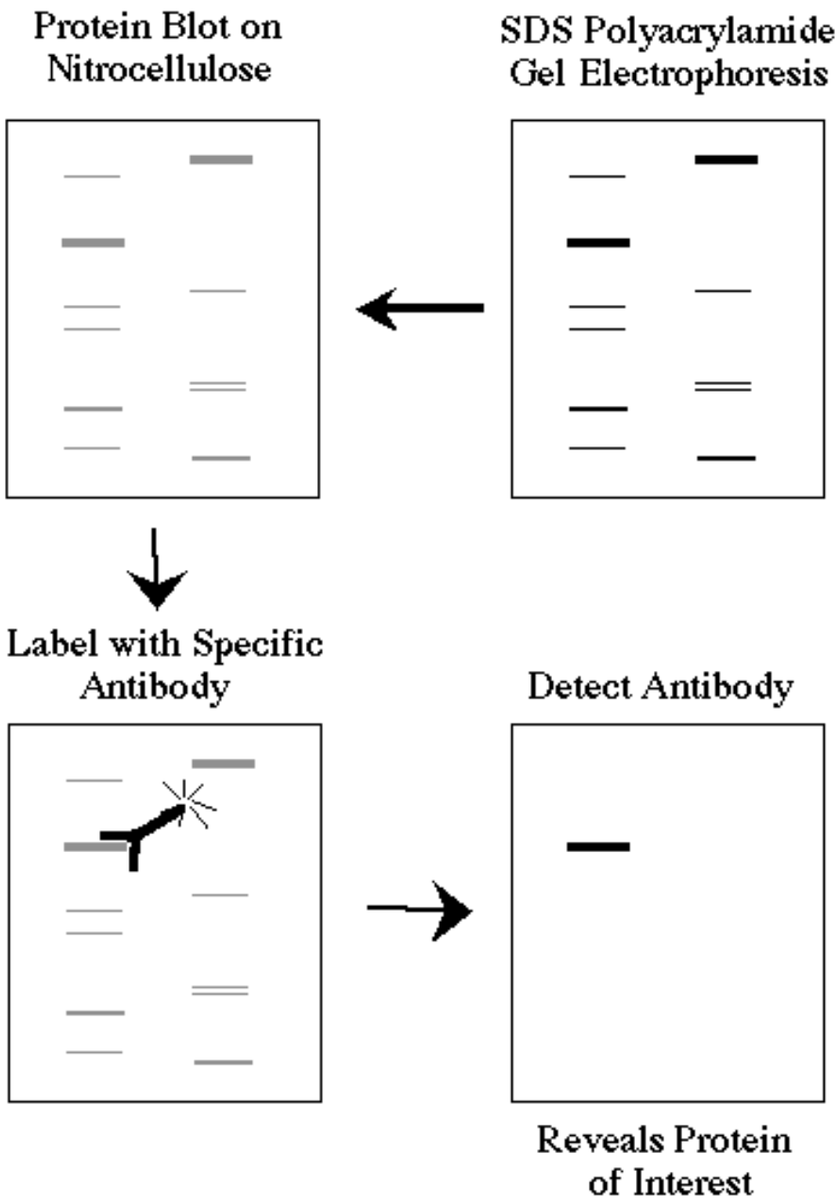
Lane	Sample	Volume to load
1	"Kaleidoscope" protein molecular weight standards	10 ul
2	H6-EnvZ positive control protein	40 ul
3	wild type light sensor	40 ul
4	mutant candidate 1	40 ul
5	mutant candidate 2	40 ul
6	"Kaleidoscope" protein molecular weight standards	10 ul
7	H6-EnvZ positive control protein	40 ul
8	wild type light sensor	40 ul
9	mutant candidate 1	40 ul
10	mutant candidate 2	40 ul

2°Ab Alkaline Phosphatase



bio-rad.com

Western Blot analysis of Cph8 Mutant:



Cph8

MATTVQLSDQSLRQLETLAIHTAHLIQPHGLVVVLQEP
DLTISQISANCTGILGRSPEDLLGRTLGEVFDSFQIDP
IQSRLTAGQISSLNPSKLWARVMGDDFVIFDGVFHRNS
DGLLVCELEPAYTSDNLPFLGFYHMANAALNRLRQQAN
LRDFYDVIVEEVRRMTGFDRVMLYRFDENNHGDVIAED
KRDDMEPYLGLHYPESDIPQPARRLFIHNPIRVIPDVY
GVAVPLTPAVNPSTNRAVDLTESILRSAYHCHLTYLKN
MGVGASLTISLIKDGHLWGLIACHHQTPKVIPFELRKA
CEFFGRVVFSNISAQEDTETFDYRVQLAEHEAVLLDKM
TTAADFVEGLTNHPDRLLGLTGSQGAAICFGEKLILVG
ETPDEKAVQYLLQWLENREVQDVFFTSSLSQIYPDAVN
FKSVASGLLAIPIARHNFLLWFRPEVLQTVNWGGDPNH
AYEATQEDGKIELHPRQSFDLWKEIVRLQSLPWQSVEI
QSALALKKAIVNLILRQAEELH

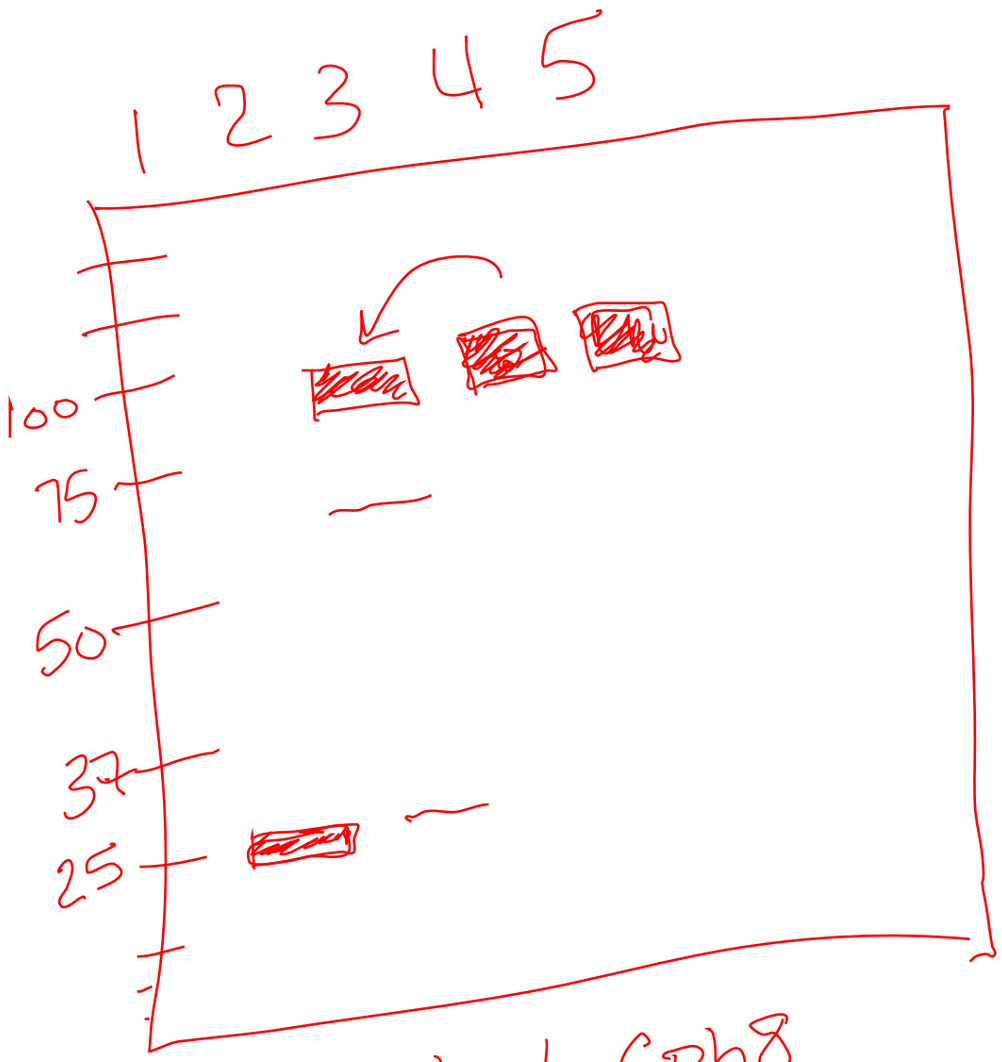
Cph8

229 aa = 25 kDa

MAAGVKQLADDRTLLMAGVSHDLRTPLTRIRLATEMMS
EQDGYLAESINKDIEECNAIIEQFIDYLRTGQEMPMEM
ADLNAVLGEVIAAESGYEREIETALYPGSIEVKMHPLS
IKRAVANMVVNAARYGNGWVKVSSGTEPNRAWFQVEDD
GPGIAPEQRKHLFQPFVRGDSARTISGTGLGLAIVQRI
VDNHNGMLELGTSERGGLSIRAWLPVPVTRAQGMTKEG
-

744 amino acids

~ 83,000 = 83 kDa



mutant Cph8 "normalized" to WT

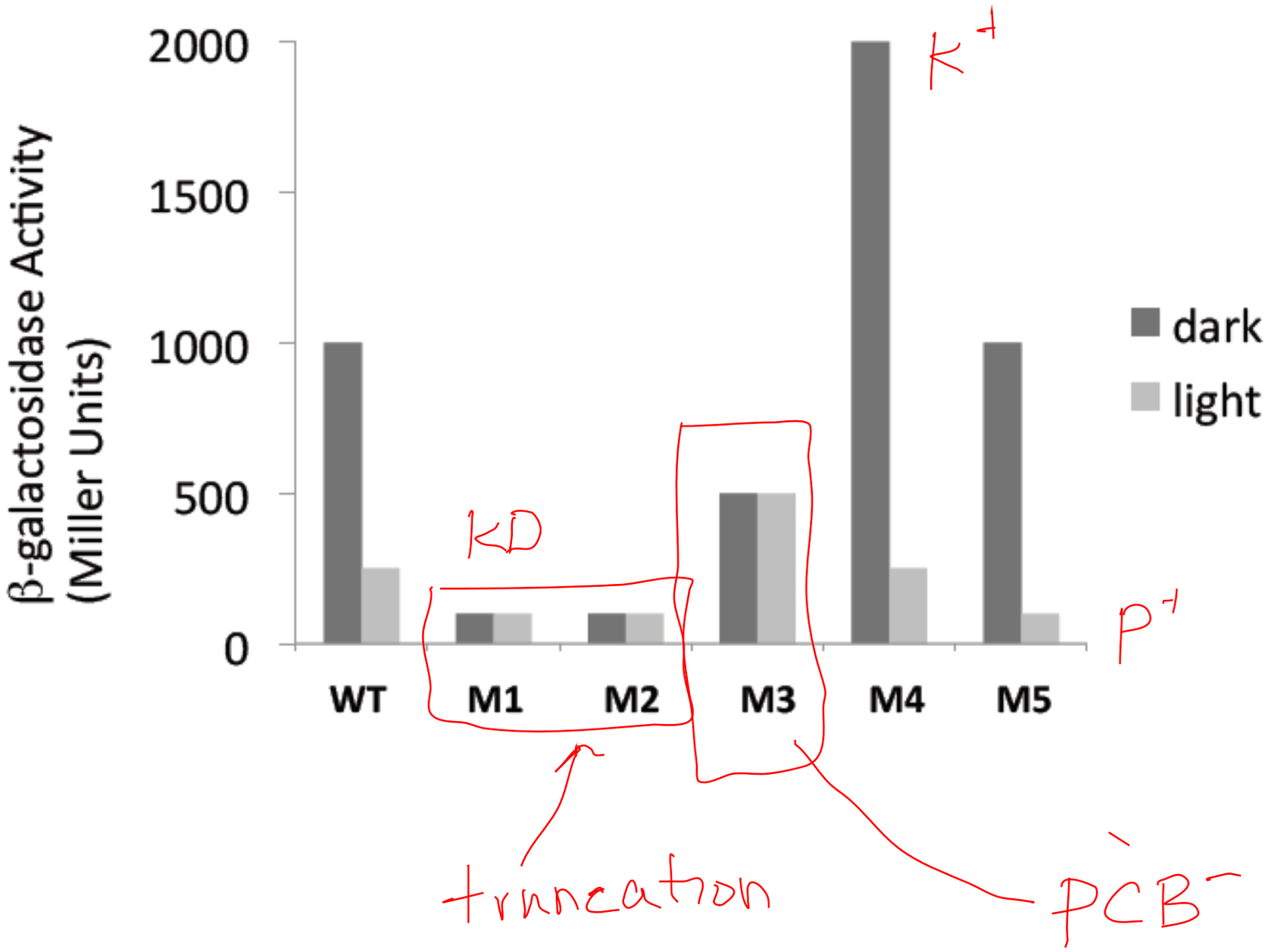
Lane	Sample	Volume to load
1	"Kaleidoscope" protein molecular weight standards	10 ul
2	H6-EnvZ positive control protein	40 ul
3	wild type light sensor	40 ul
4	mutant candidate 1	40 ul
5	mutant candidate 2	40 ul
6	"Kaleidoscope" protein molecular weight standards	10 ul
7	H6-EnvZ positive control protein	40 ul
8	wild type light sensor	40 ul
9	mutant candidate 1	40 ul
10	mutant candidate 2	40 ul

Analysis of Cph8 mutant:

EnvZ	**H243**	D244	L245	R246	**T247R (K+P-)**	P248
wt seq	CAC	GAC	TTG	CGC	**Thr = ACG**	CCG
Cph8	**H537**	D538	L539	R540	**T541**	P542
	Kinase Dead mutant				**NNY mutagenesis**	
	GCC = Ala				CTY = Leu	
					CCY = Pro	
					CAY = His	
					CGY = Arg*	
					TTY = Phe	
					TCY= Ser	
					TAY = Tyr	
					TGY = Cys	
					ATY = Ile	
					ACY = Thr	
					AAY = Asn	
					AGY = Ser	again
					GTY = Val	
					GCY = Ala	
					GAY = Asp	
					GGY = Gly	
					N = G A T C	
					Y = C T	
					15 possible amino acids	
					No stops	

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	**U**
	Phe	Ser	Tyr	Cys	**C**
	Leu	Ser	STOP	STOP	**A**
	Leu	Ser	STOP	Trp	**G**
C	Leu	Pro	His	Arg	**U**
	Leu	Pro	His	Arg	**C**
	Leu	Pro	Gln	Arg	**A**
	Leu	Pro	Gln	Arg	**G**
A	Ile	Thr	Asn	Ser	**U**
	Ile	Thr	Asn	Ser	**C**
	Ile	Thr	Lys	Arg	**A**
	Met	Thr	Lys	Arg	**G**
G	Val	Ala	Asp	Gly	**U**
	Val	Ala	Asp	Gly	**C**
	Val	Ala	Glu	Gly	**A**
	Val	Ala	Glu	Gly	**G**

Possible mutant "phenotypes"

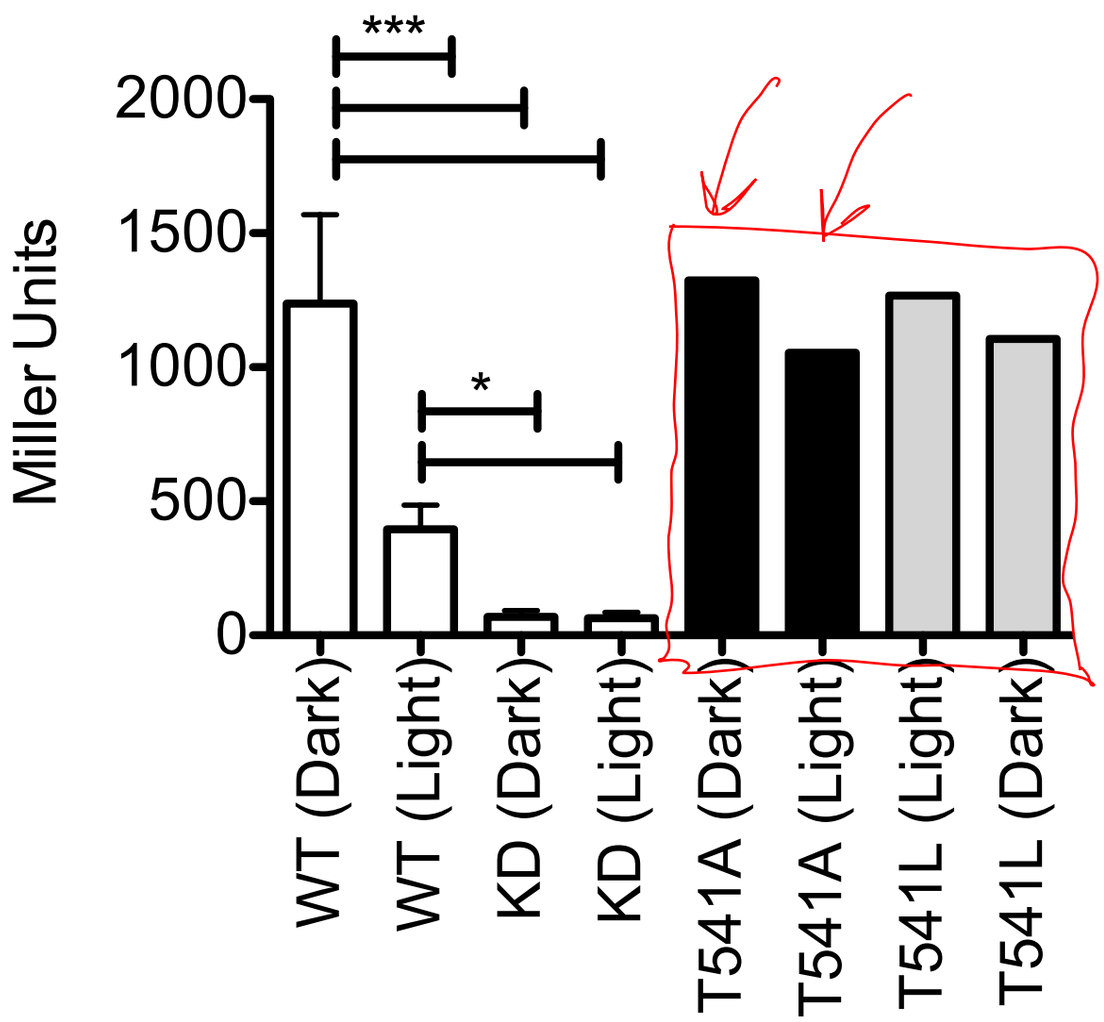


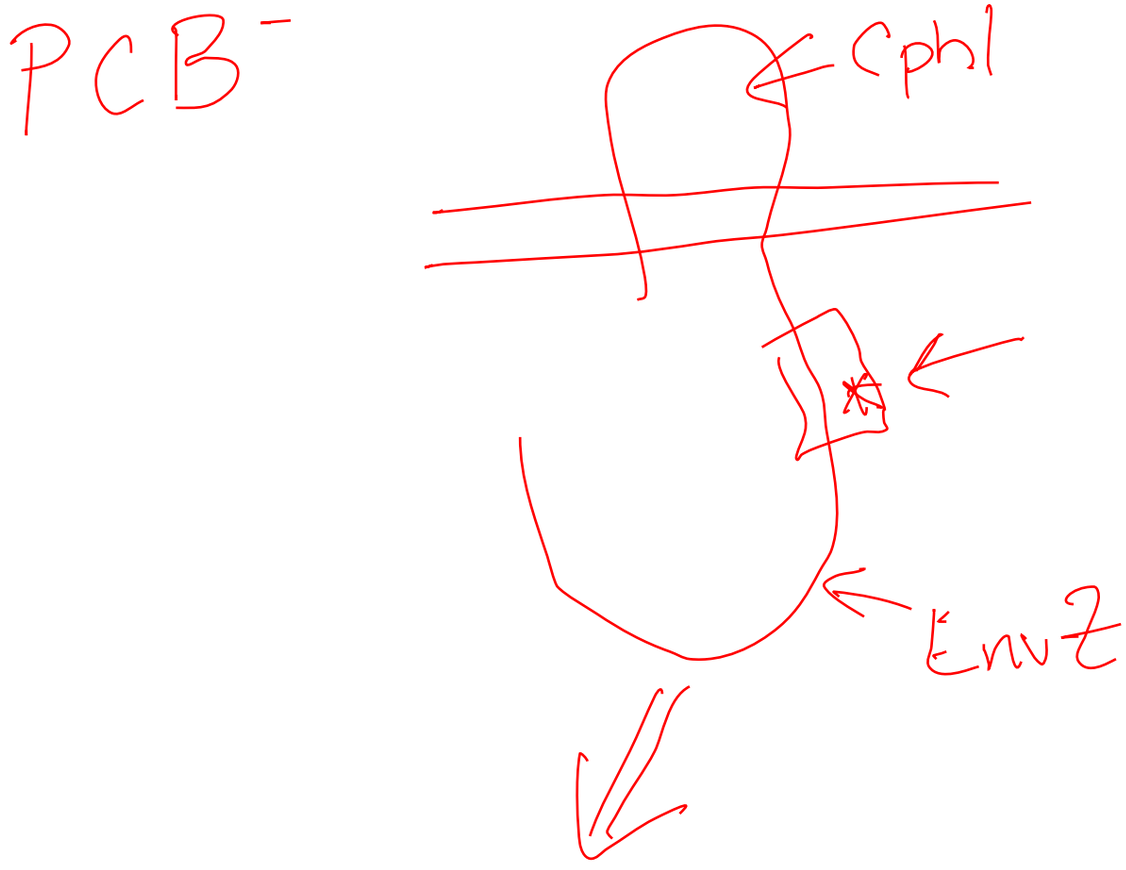
Talk:20.109(F12): Mod 2 Day 5 Assessing re-tuned system

T/R Mutant Miller Assay [edit]

Please enter the results of your B-gal assay and your mutation (after sequencing)

Team Color	Mutant 1 B-Gal Average (Dark)	Mutant 1 B-Gal Average (Light)	Mutant 1 T541X; X =	Mutant 2 B-Gal Average (Dark)	Mutant 2 B-Gal Average (Light)	Mutant 2 T541X; X =	Wild Type System B-Gal (Dark)	Wild Type System B-Gal (Light)	H243A Mutant B-Gal (Dark)	H243A Mutant B-Gal (Light)
Red	1324.65	1054.65	Leu	1268.2	1106.4	~~A~~/a	944.2	330.8	71.95	66
Orange	1077.76	351.56		1228.68	458.33		1105.34	418.94	65.42	63.91
Yellow	1552.5	494.95		1711.65	458.85		1065.9	389.2	107.9	102.0
Green	1287.79	531.26		749.18	390.12		1133.14	359.73	52.38	49.77
Blue	1149.27	244.80		1208.32	532.60		1573.52	537.10	91.79	80.99
Pink	1597.37	528.91		1037.31	237.98		1000.73	323.69	49.52	48.48
Purple	1208.35	1018.45		1328.9	190.95		2192.9	322.85	43.15	38.80





Plans for today:

1. Lab 

2. Second half of WB analysis

3. Your turn: ***MAKE A PLAN***

2.5 mL vol
(↑ minip vol. β-gal)

phenotypes

last

miniprepping-
Sequencing

β-gal ⇒ photograph
WT +
mutants

What's left for Mod2?

Tuesday (11/6): Synthetic Biology Journal Club

Thursday (11/8): No lecture or lab

Monday (11/12): Research Article / R&D Due at 11:12 am