M2D6: Characterizing Your Cph8 Mutant

10/30/12

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**

Today:

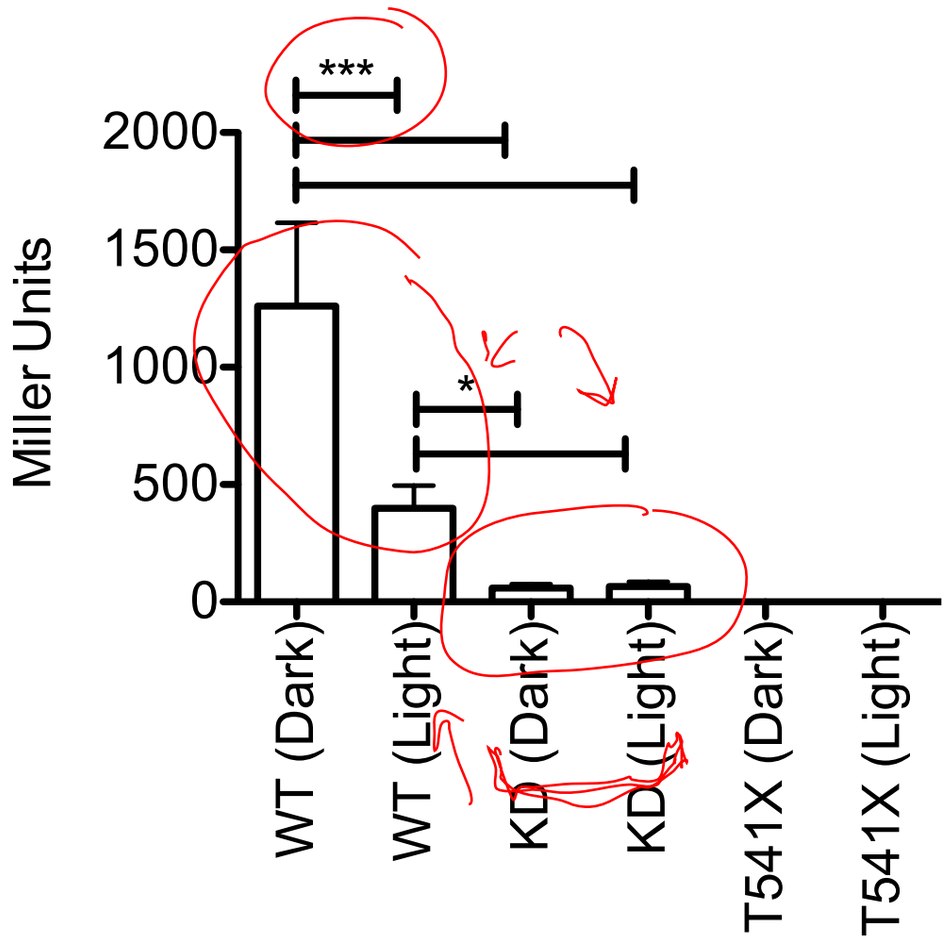
1. Western Blot
2. Sequencing Results
3. Photograph

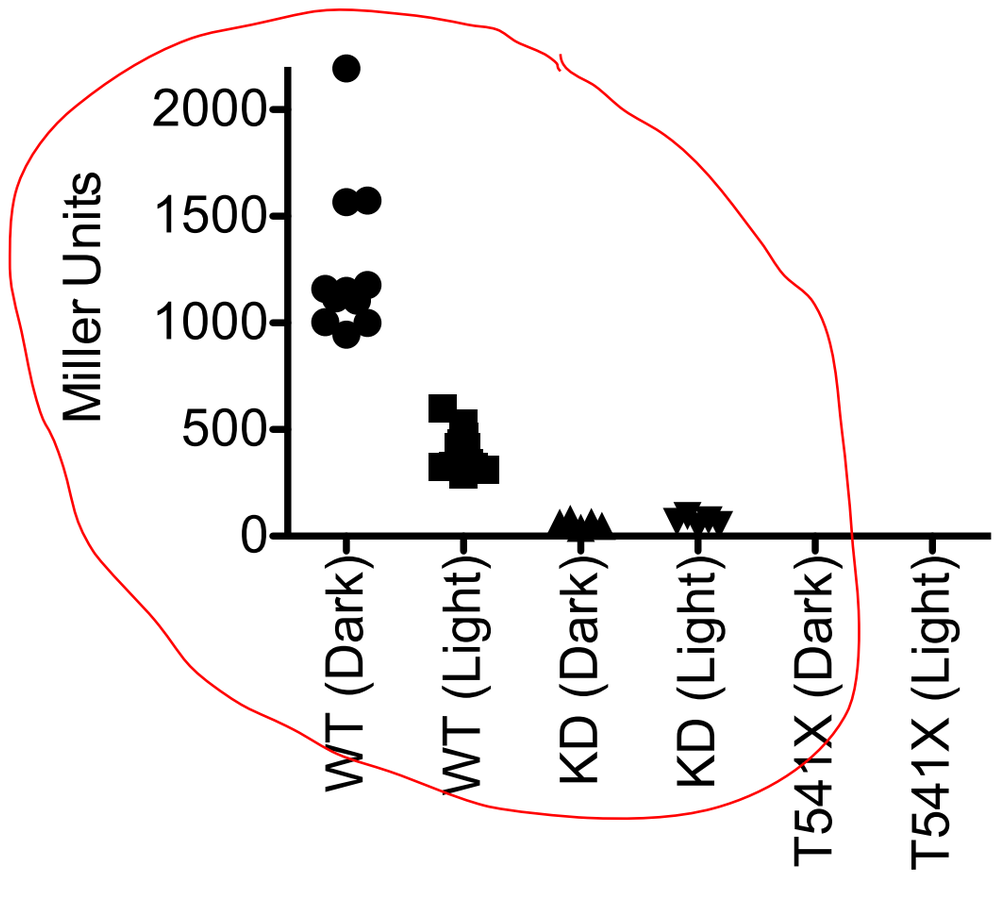
Talk:20.109(F12): Mod 2 Day 5 Assessing re-tuned system

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		1268.2	1106.4		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										
Green	1287.79	531.26		749.18	390.12		11331.42	3597.32	523.80	497.65
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





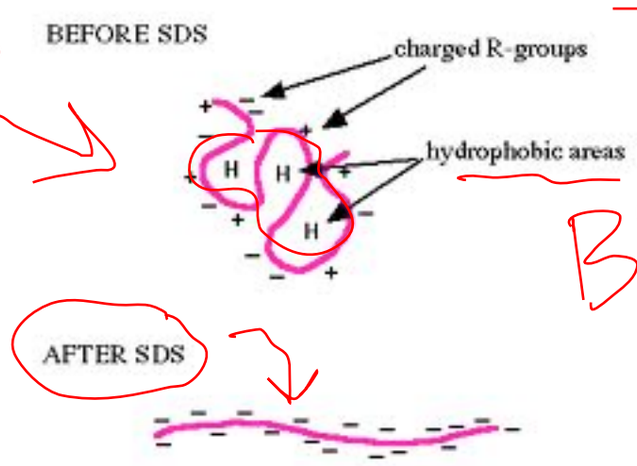
Western Blot analysis of Cph8 Mutant:

1. Measure OD600 of 1:10 dilution of mutants and WT
2. Calculate volume for 2OD:

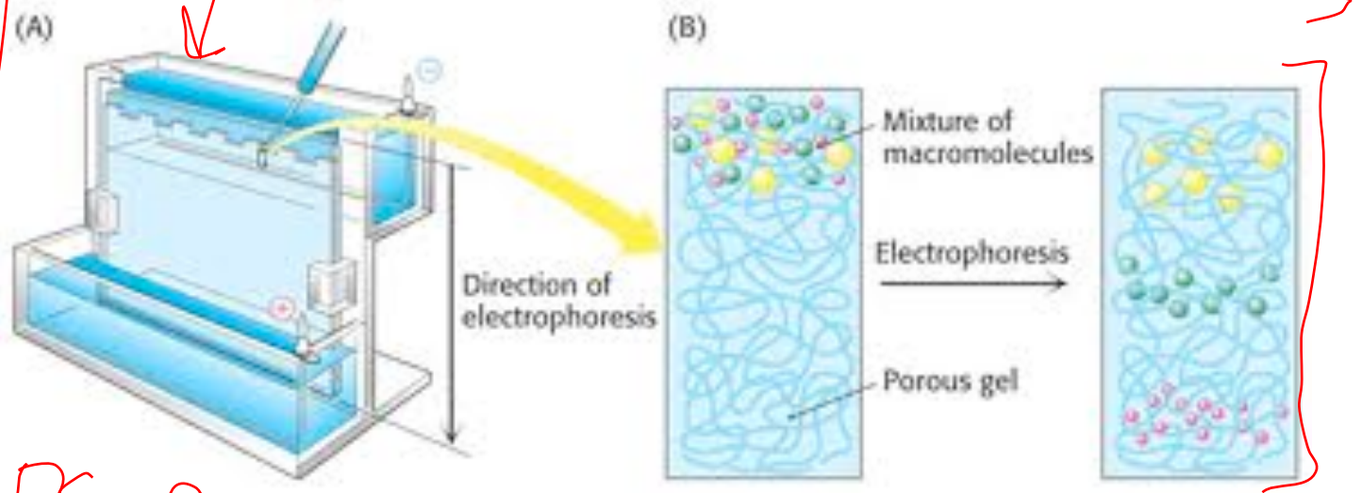
   e.g. if OD = 0.5; need 4 mL of 1:10 or 0.4 mL of undiluted

3. Lyse protein with Epicentre reagents
4. Add sample buffer and boil
5. Separate proteins with SDS-PAGE
6. Transfer proteins to nitrocellulose membrane for Western Blot

Western Blot analysis of Cph8 Mutant:



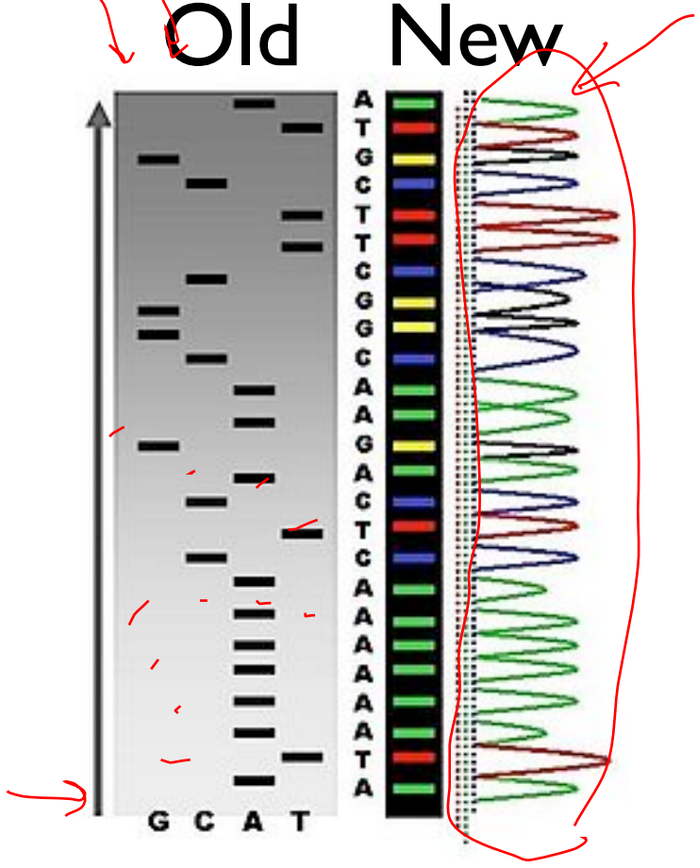
Lane	Sample	Volume to load
1	"Kaleidoscope" protein molecular weight standards	10 ul
2	H6-EnvZ positive control protein	40 ul
3	wild type light sensor	40 ul
4	mutant candidate 1	40 ul
5	mutant candidate 2	40 ul
6	"Kaleidoscope" protein molecular weight standards	10 ul
7	H6-EnvZ positive control protein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

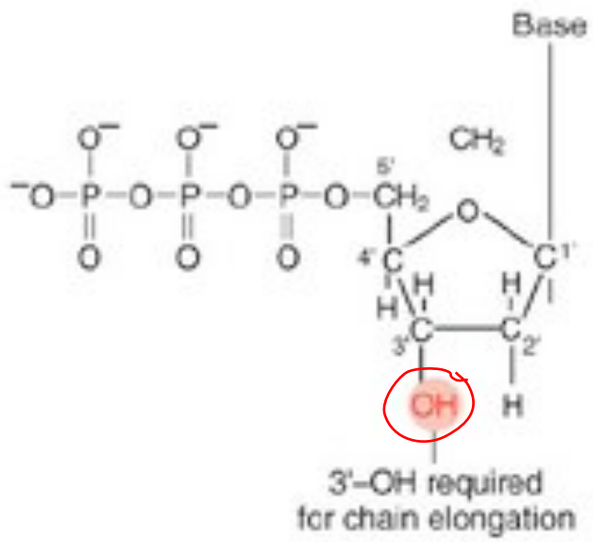


What is the mutation? DNA Sequencing

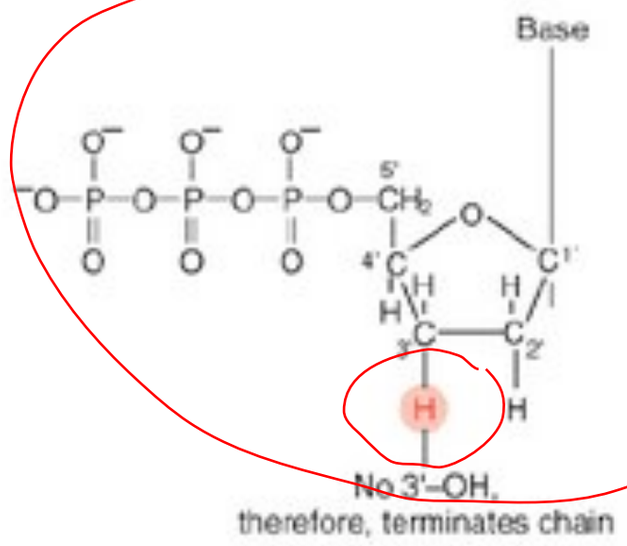
Four dye labeled dideoxynucleotides added to each reaction

Sanger Seq.





ddT



'Chain terminating reaction'

Today you will determine your Cph8 mutant sequence

What is the mutation? DNA Sequencing

Wild Type Cph8 Sequence

~1000bp

ATGGCCACCACCGTACAACTCAGCGACCAATCCCTCCGTCAGCTAG
AAACCCTCGCCATCC..... (1200 bp)

.....GTGGAAATCCAAAGTGCCCTGGCCCTGAAAAAGGCGA***TCGTC***
AACCTCATTTTGCGCCAGGCAGAAGAATTGCATATGGCGGCTGGT
GTTAAGCAACTGGCGGATGACCGCACGCTGCTGATGGCGGGGGTA
AGT***CACGACTTGCGCACGCCG***CTGACGCGTATTCGCCTGGCGACT
GAGATGATGAGCGAGCAGGATGGCTATCTGGCAGAATCGATCAAT
AAAGATATCGAAGAGTGCAACGCCATCATTGAGCAGTTTATCGAC
TACCTGCGCACCGGGCAGGAGATGCCGATGGAAATGGCGGATCTT
AATGCAGTACTCGGTGAGGTGATTGCTGCCGAAAGTGGCTATGAG
CGGGAAATTGAAACCGCGCTTTACCCCGGCAGCATTGAAGTGAAA
ATGCACCCGCTGTCGATCAAACGCGCGGTGGCGAATATGGTGGTC
AACGCCGCCCGTTATGGCAATGGCTGGGTCAAAGTCAGCAGCGGA
ACGGAGCCGAATCGCGCCTGGTTCCAGGTGGAAGATGACGGTCCG
GGAATTGCGCCGGAACAACGTAAGCACCTGTTCCAGCCGTTTGTC
CGCGGCGACAGTGCGCGCACCATTAGCGGCACGGGATTAGGGCTG
GCAATTGTGCAGCGTATCGTGGATAACCATAACGGGATGCTGGAGC
TTGGCACCAGCGAGCGGGGCGGGCTTTCCATTCGCGCCTGGCTGC
CAGTGCCGGTAACGCGGGCGCAGGGCATGACAAAAGAAGGGTAA

primer (~30 bp)

Area of interest

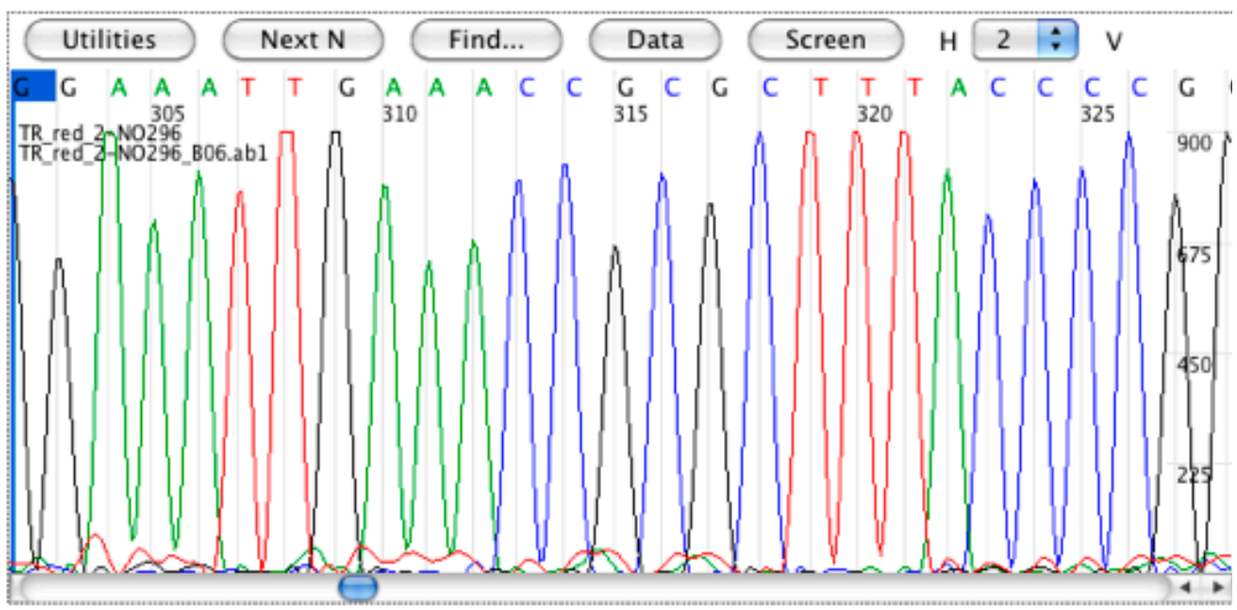
NNN

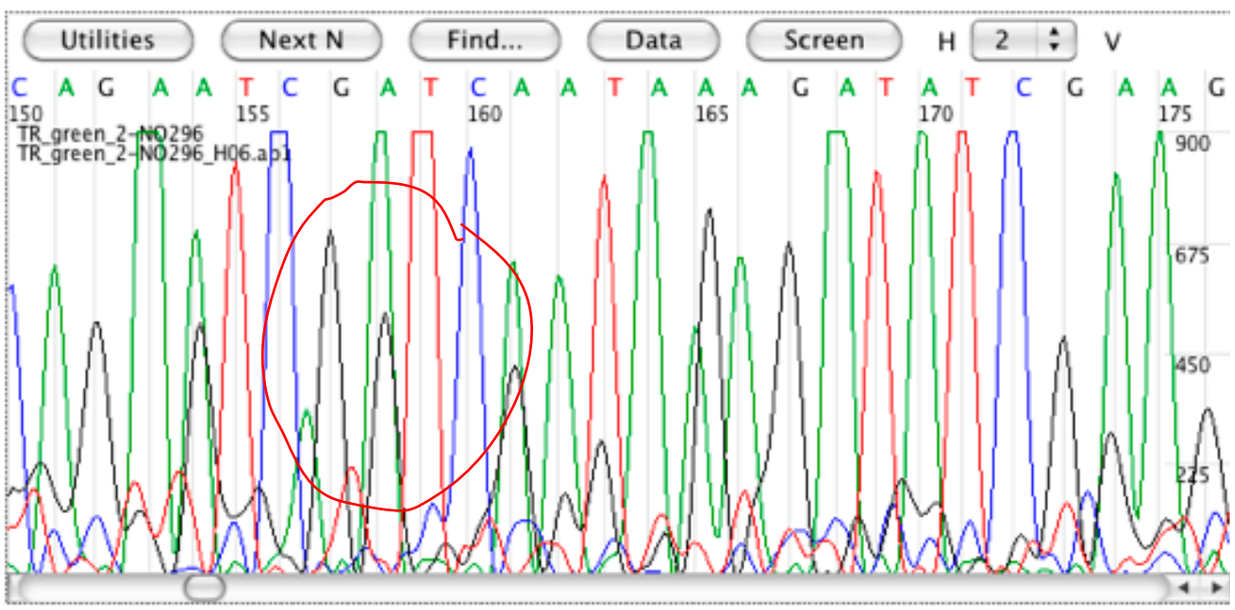
1. Retrieve your sequences from Genewiz (see wiki)
2. Download your sequence files
3. Compare to the WT (NB466) sequence

BLAST

```
 Score =  1243 bits (673),  Expect = 0.0
 Identities = 679/682 (99%), Gaps = 0/682 (0%)
 Strand=Plus/Plus

Query  1554  GGCTGGTGTTAAGCAACTGGCGGATGACCGCACGCTGCTGATGGCGGGGGTAAGTCACGA  1613
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  23    GGCTGGTGTTAAGCAACTGGCGGATGACCGCACGCTGCTGATGGCGGGGGTAAGTCACGA  82

Query  1614  CTTGCGCACGCCGCTGACGCGTATTCGCCTGGCGACTGAGATGATGAGCGAGCAGGATGG  1673
              |||||||   ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  83    CTTGCGCCTTCCGCTGACGCGTATTCGCCTGGCGACTGAGATGATGAGCGAGCAGGATGG  142

Query  1674  CTATCTGGCAGAATCGATCAATAAAGATATCGAAGAGTGCAACGCCATCATTGAGCAGTT  1733
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  143   CTATCTGGCAGAATCGATCAATAAAGATATCGAAGAGTGCAACGCCATCATTGAGCAGTT  202

Query  1734  TATCGACTACCTGCGCACCGGGCAGGAGATGCCGATGGAAATGGCGGATCTTAATGCAGT  1793
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  203   TATCGACTACCTGCGCACCGGGCAGGAGATGCCGATGGAAATGGCGGATCTTAATGCAGT  262
```

99%





Plans for today:

1. Lab 

2. Start your WB

3. Analyze sequencing data during downtime

4. If you have a mutant, pour a new photograph plate

What are you going to do next?