

# System Engineering M2D5

10.27.11

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# 20.109(F11): Laboratory Fundamentals of Biological Engineering

Today is a day when you'll start to collect information about the mutant candidates you've chosen. Over the next 3 lab sessions, you will look at

1. the expression level of the mutant Cph8 proteins relative to the wildtype light sensor
2. the DNA sequence of the region around the K-P+ hotspot
3. the  $\beta$ -gal activity of the mutant photography strains grown in the light and dark
4. the resulting bacterial photograph taken with your mutant strain

The data should be informative, though preliminary.

TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
JC I		b-gal (light/dk) Send to seq ONs for protein	
Protein Gel/blot Bact Photo Analyze seq		Western  Dealer's choice	
JC II		No lecture No lab	Draft of article due 11.11 at 11:11

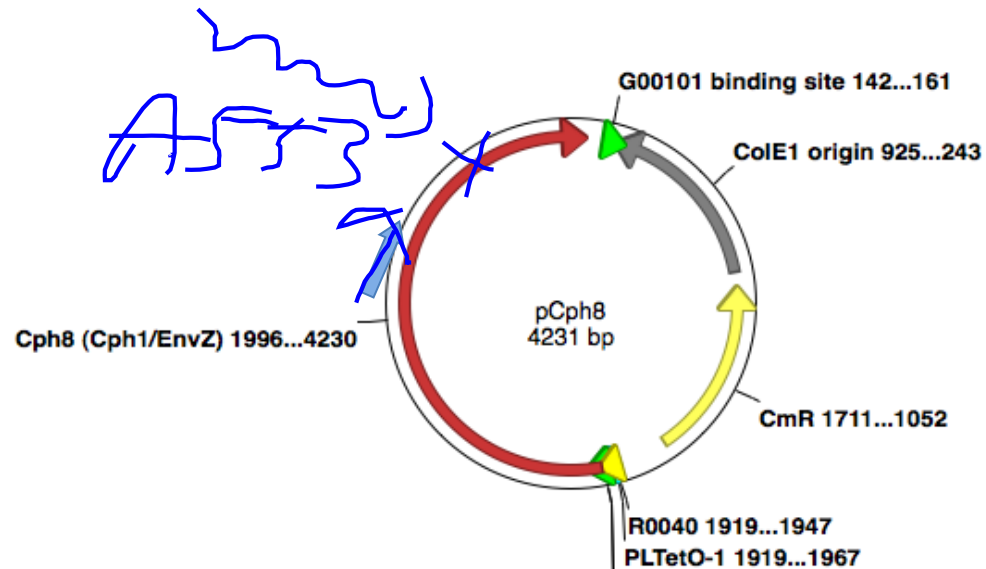
# Sequencing K-P+ candidates

Miniprep DNA as you did in Module 1  
(Soln I, Soln II, Soln III, EtOH, wash, dry)

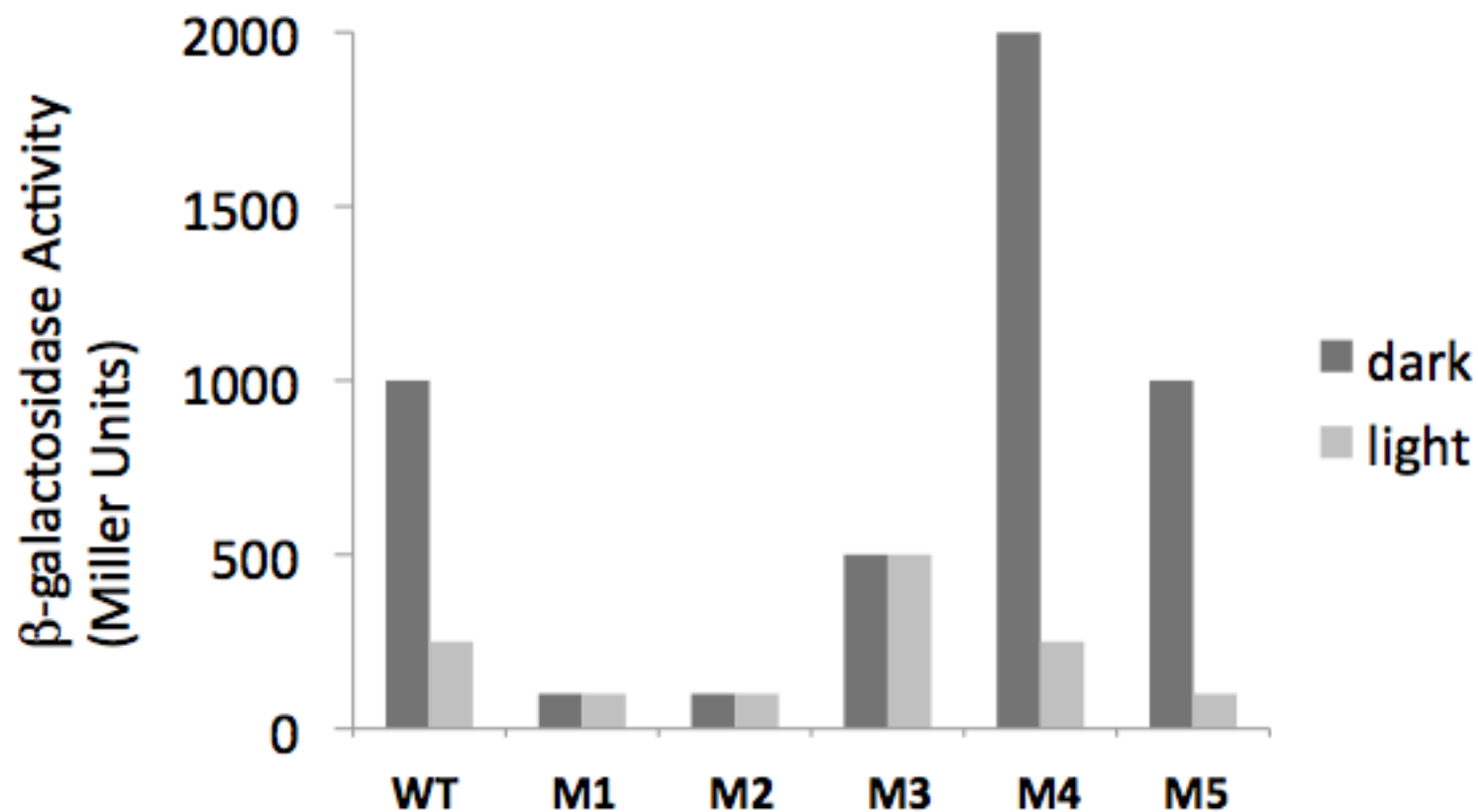
Resuspend pellets in 40 ul of water

For sequencing, mix:

- 4 ul plasmid DNA
- 10 ul of a 1:20 dilution of the primer NO296
- 16 ul sterile water



## $\beta$ -gal assay of K-P+ candidates



Cph1/EnvZ	A553	G554	V555	S556	H557
EnvZ	A239T	G240E	V241G	S242D	H243A

Today/next week in lab....