# M2D5: Assessing re-tuned system

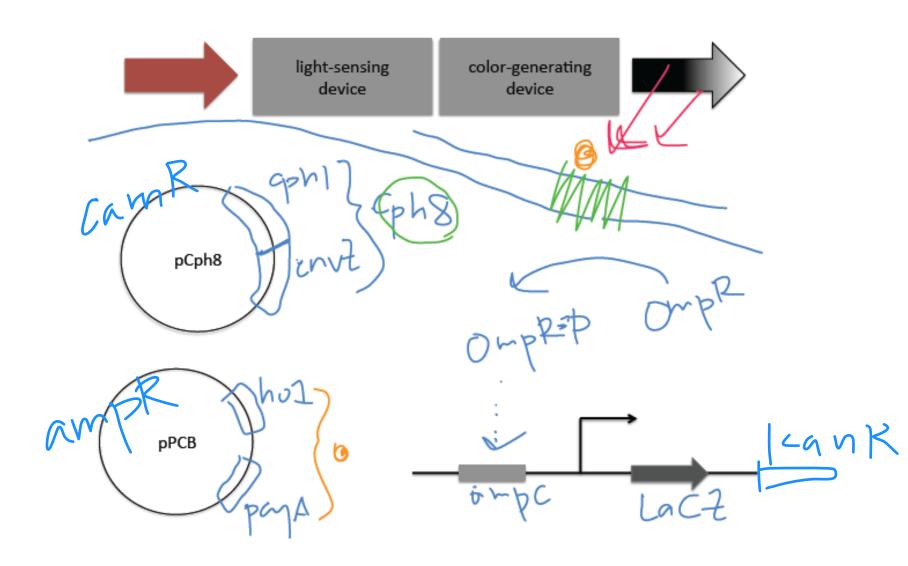
10/23/14

#### Lab business

For next week...

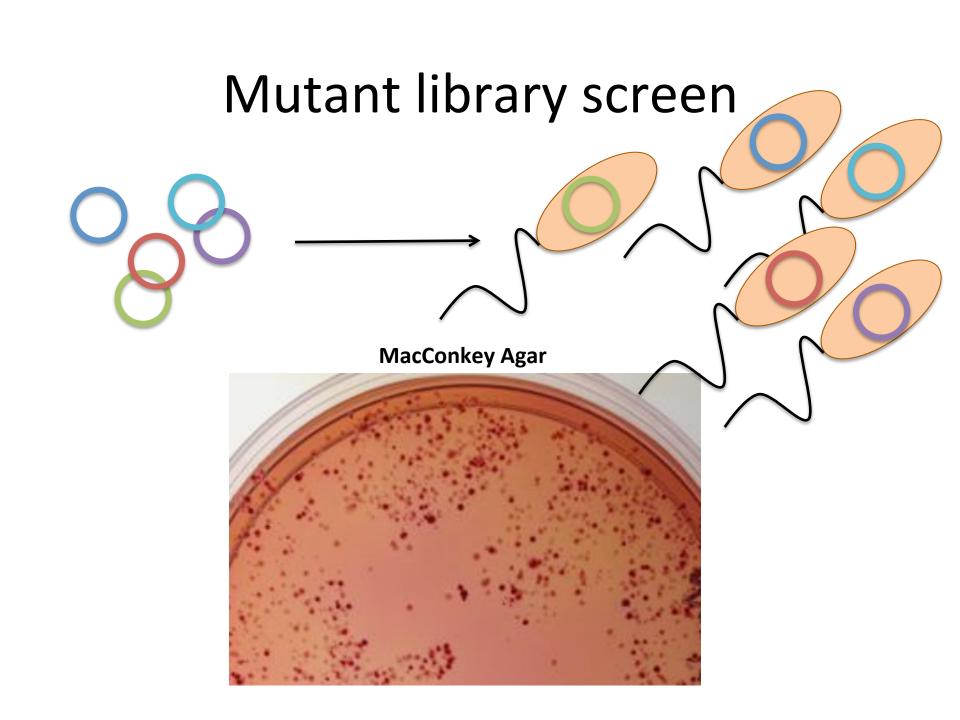
- October 30
- Calculate length of Cph8
- Draft of Materials and Methods for Mod 2 report
- Reserve your article for M2D7 Journal Club
  - Upload slides to Stellar (.ppt)
  - Presentations will start at 11a in 6-104
  - Lunch will be provided
- Thank you again to our presenters and to the audience!

#### Bacterial photography system details



## Mutant library construction

	H243	D244			L245				
vt seq CAC		GAC	GAC		TTG			Thr = ACG	CCG
	H537	D538	D538		L539		)	T541	P542
	Kinase Dead mutant							NNY mutagen	esis
	GCC = Ala							CTY = Leu	
								CCY = Pro	
	ΙU	C	A		G				
U	Phe	Ser	Tvr		Cvs	U			
_			-		_	-			
			-			-	1	•	
						-			
	Leu	Ser	STO	<u>P</u>	Trp	G			
С	Leu	Pro	His		Arg	U			
	Leu	Pro	His		Ara	С	1		again
					_			GTY = Val	
								GCY = Ala	
_						_			
Α	Ile	Thr	Asn		Ser	-		GGY = Gly	
	lle	Thr	Asn		Ser	C			
	lle	Thr	Lvs		Ara	Α		N CATC	
	Met		-		_	G			
G						_	1	15 possible amino acids	
	Val				_			No stops	
	Val	Ala	Glu		-	Α	1		
	Val	Ala	Glu		Gly	G	1		
	C	Kinase De GCC = Ala  U U Phe Phe Leu Leu Leu Leu Leu Leu C Ile Ile Ile Met Val	Kinase Dead mutar GCC = Ala  U C U Phe Ser Phe Ser Leu Ser Leu Ser C Leu Pro C Leu Pro Leu Pro Leu Pro Leu Pro Leu Pro Leu Pro Leu Ala Val Ala Val Ala	CAC GAC  H537 D538  Kinase Dead mutant GCC = Ala  U C A  U Phe Ser Tyr Phe Ser Tyr Leu Ser STO Leu Ser STO Leu Pro His Leu Pro His Leu Pro Gln Leu Pro Gln Leu Pro Gln Leu Pro Gln Leu Pro Gln Leu Pro Gln Leu Pro Gln A Ile Thr Asn Ile Thr Lys Met Thr Lys Met Thr Lys Val Ala Asp Val Ala Asp	Name   Dead   Dead	H537  D538  Kinase Dead mutant GCC = Ala  U  Phe Ser Tyr Cys Phe Ser Tyr Cys Leu Ser STOP STOP Leu Ser STOP Trp  C Leu Pro His Arg Leu Pro His Arg Leu Pro Gln Arg Leu Pro Gln Arg Leu Pro Gln Arg Leu Pro Gln Arg Leu Pro His Arg Leu Pro Gln Arg  A Ile Thr Asn Ser Ile Thr Lys Arg Met Thr Lys Arg  G Val Ala Asp Gly Val Ala Asp Gly	Name   Dead mutant   Cys   C	CAC   GAC   TTG   CGC     H537	NAY mutageneral   Section   Sectio



#### Assessing Cph8 mutants

How can we identify the Cph8 mutation?

 How do we know that mutation is altering activity and not protein production?

### Today

- 1. Prepare mutant plasmids for sequencing
- 2. Complete β-galactosidase assay using:
  - Your Cph8 mutants (grown in light and dark)
  - NB466 (grown in light and dark)
  - Kinase dead H537 mutant (grown in light and dark)
- 3. Inoculate for protein analysis
- 4. Visit from the writing faculty at 4p