M2D5:
Assessing re-tuned system
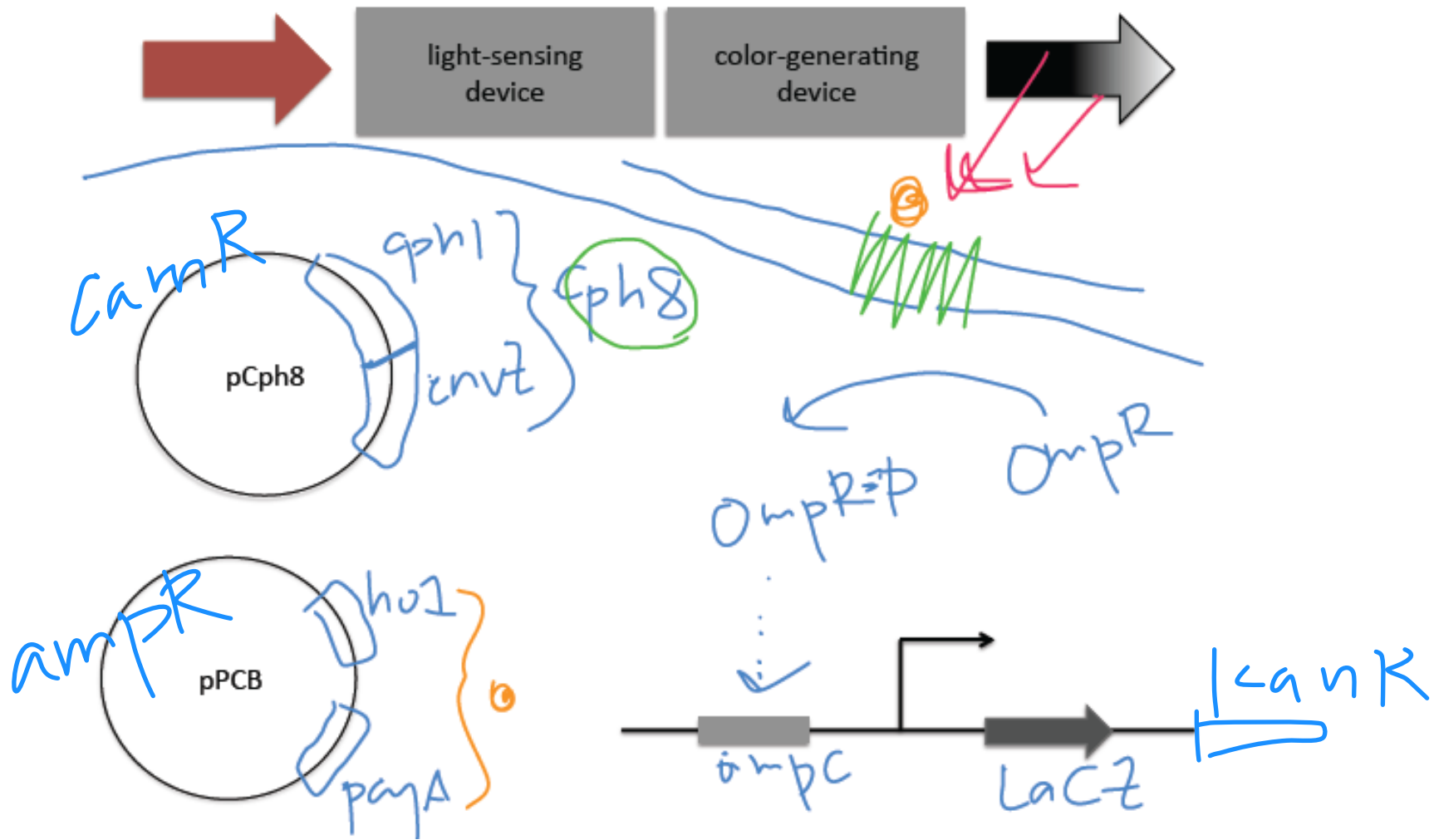
10/23/14

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

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
GCC = Ala

NNY mutagenesis
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

		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

N = G A T C
Y = C T
15 possible amino acids
No stops

Mutant library screen

MacConkey Agar
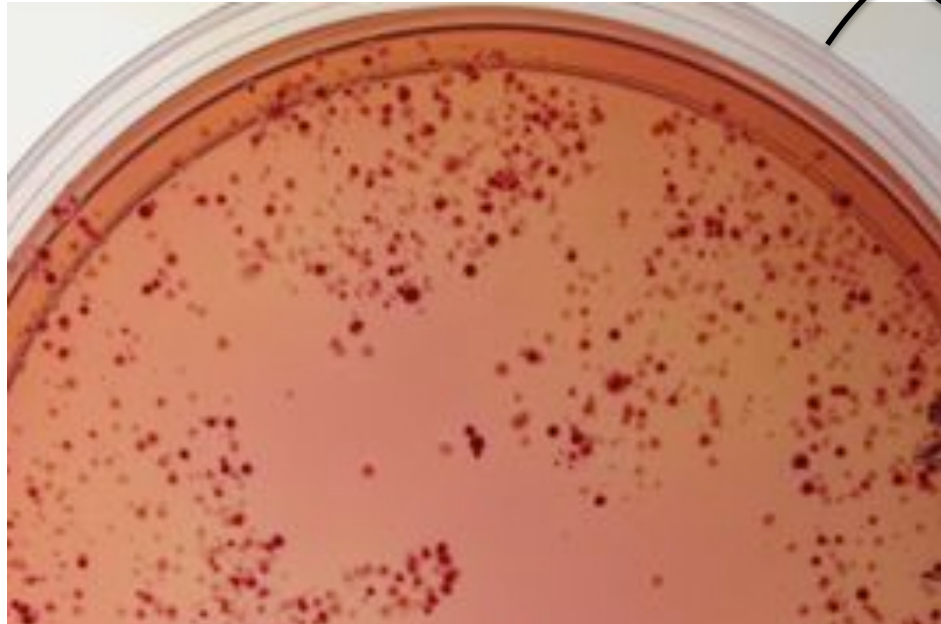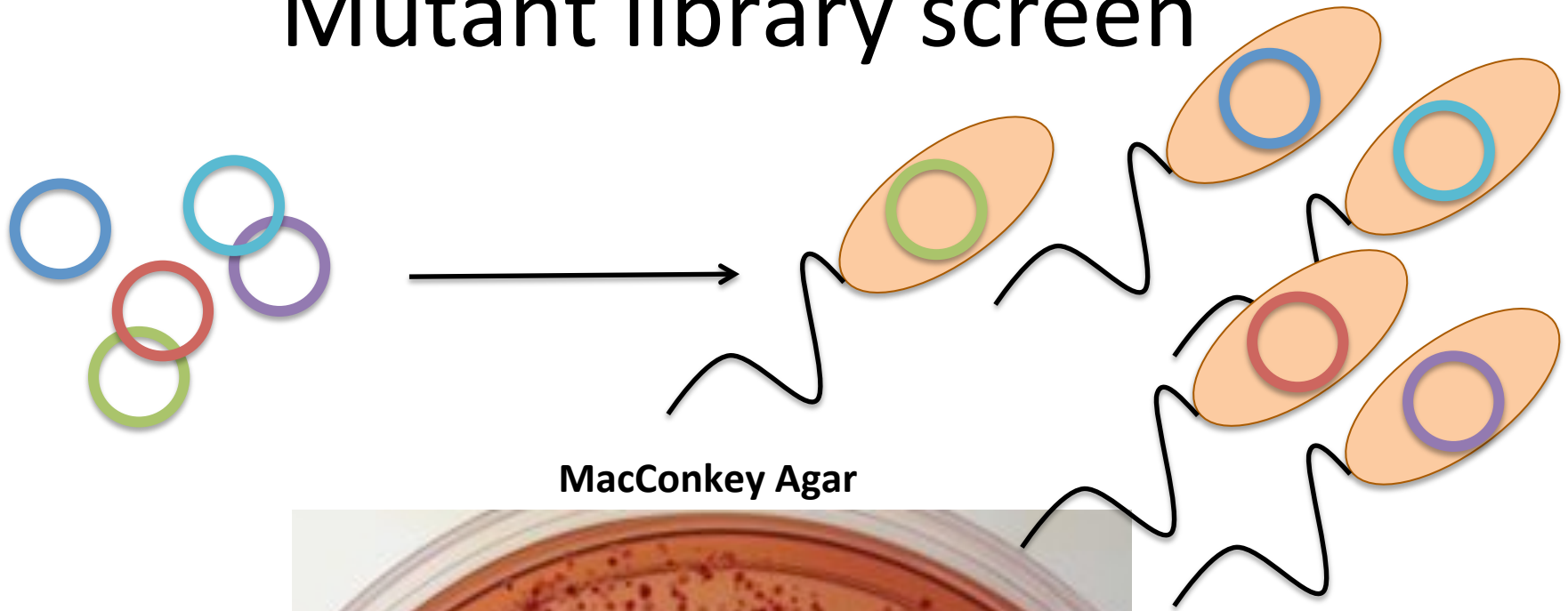
Assessing Cph8 mutants

- 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