

System Engineering

20.109(F14)
M2D6 lecture
10.28.14

Possible mutant "phenotypes"

PCB ⁻	EnvZ HK domain only	K ⁺ P ⁻
K ⁻ P ⁺	Kinase Dead	Cph8 ⁻
Possible Mutants		

M1 =
M2 =
M3 =
M4 =

Looking at real data...

M2D2									
Sample	OD600 of 1:10	OD600	Time elapsed	OD420	OD550	Units	Average	Fold	
466 Dark1	0.25	2.5	1.33	0.545	0.145	876			
466 Dark2	0.25	2.5	1.33	0.516	0.143	799	838		
466 Light1	0.14	1.4	5	0.277	0	396			
466 Light2	0.14	1.4	5	0.288	0	411	404	2.1	

Looking at real data...

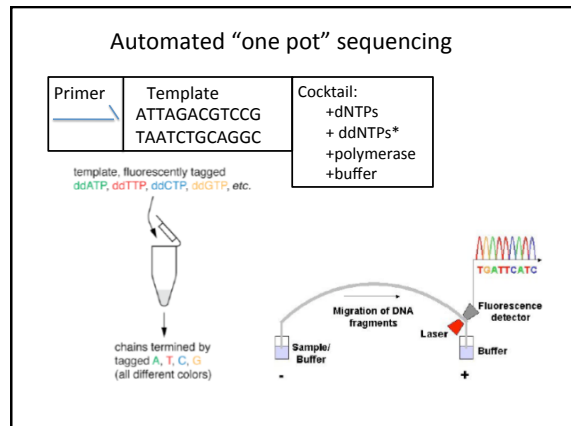
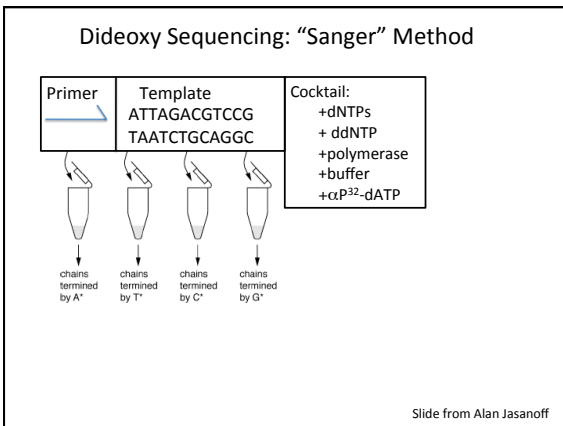
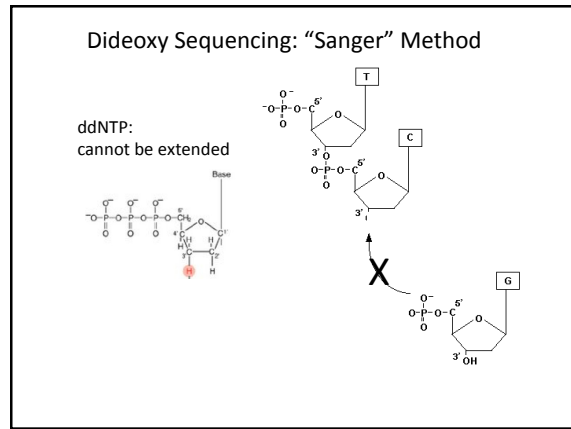
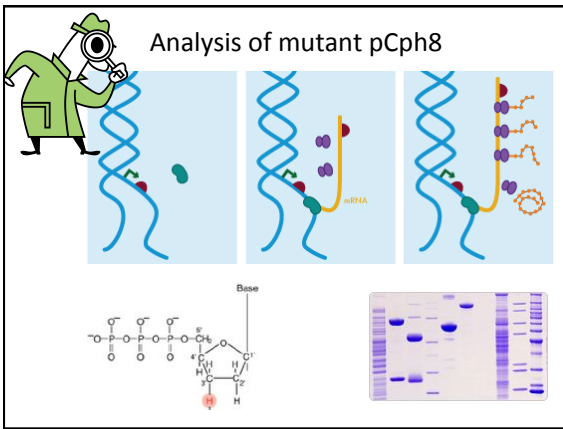
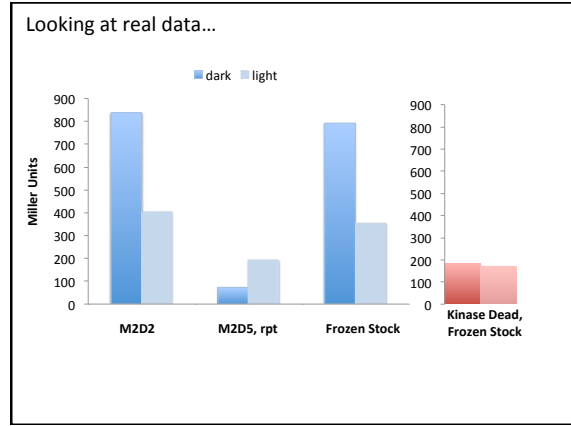
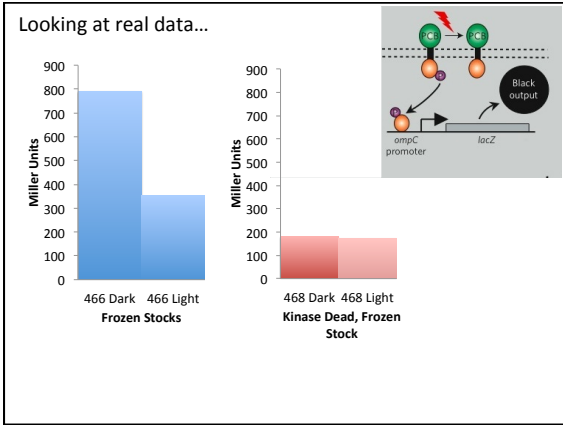
M2D5, rpt									
Sample	OD600 of 1:10	OD600	Time elapsed	OD420	OD550	Units	Average	Fold	
466 Dark1	0.16	1.6	10	0.2006	0.0474	74			
466 Dark2	0.16	1.6	10	0.1934	0.0444	72	73		
466 Light1	0.2119	2.119	4	0.24	0.0445	191			
466 Light2	0.2119	2.119	4	0.2432	0.0448	194	193	0.4	

Looking at real data...

Kinase Dead, rpt									
Sample	OD600 of 1:10	OD600	Time elapsed	OD420	OD550	Units	Average	Fold	
466 Dark1	0.26	2.6	2.5	0.58	0.039	787	792		
466 Dark2	0.26	2.6	2.5	0.597	0.045	797	792		
466 Light1	0.299	2.99	5	0.568	0.03	345			
466 Light2	0.299	2.99	5	0.588	0.026	363	354	2.2	

Looking at real data...

Frozen Stocks									
Sample	OD600 of 1:10	OD600	Time elapsed	OD420	OD550	Units	Average	Fold	
466 Dark1	0.26	2.6	2.5	0.58	0.039	787	792		
466 Dark2	0.26	2.6	2.5	0.597	0.045	797	792		
466 Light1	0.299	2.99	5	0.568	0.03	345			
466 Light2	0.299	2.99	5	0.588	0.026	363	354	2.2	



Analysis of mutant pCph8

protein activity?
protein stability?
cross-talk with other systems?

How protein stability might affect β -gal activity?

	Autophos events	Kinasing events	P-tase events	Amount OmpR+P
Wild type	5 "units"	3 "units"	2 "units"	1

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Loading dye has glycerol, SDS, reducing agent
Samples boiled before loading

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

http://www.bme.gatech.edu/vcl/SDS_PAGE/Background/Introduction.htm

Detection through Western Analysis

http://www.genscript.com/product_001/western_application/grp_id/60065/op/detail/Uvrag_Antibody_Analysis.html

Summary

Bacterial Photography K⁺P⁻

Critical analysis of data

Analysis
DNA sequencing
Protein expression

2005 Florida enacted its 'Stand Your Ground' law