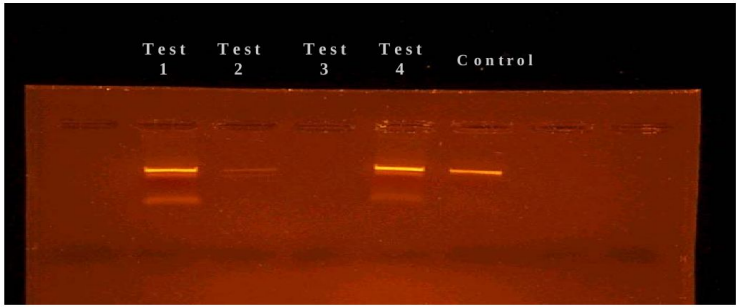
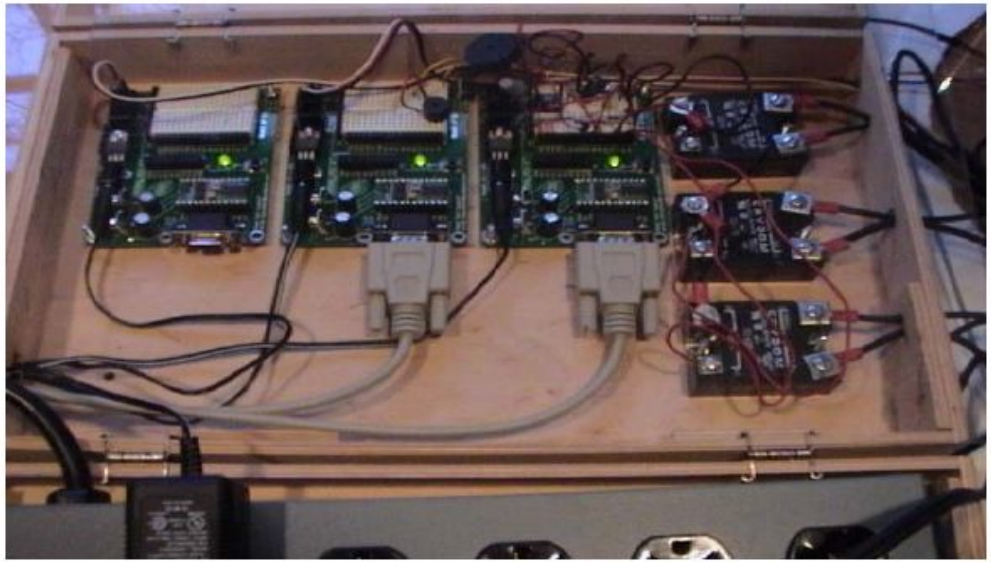
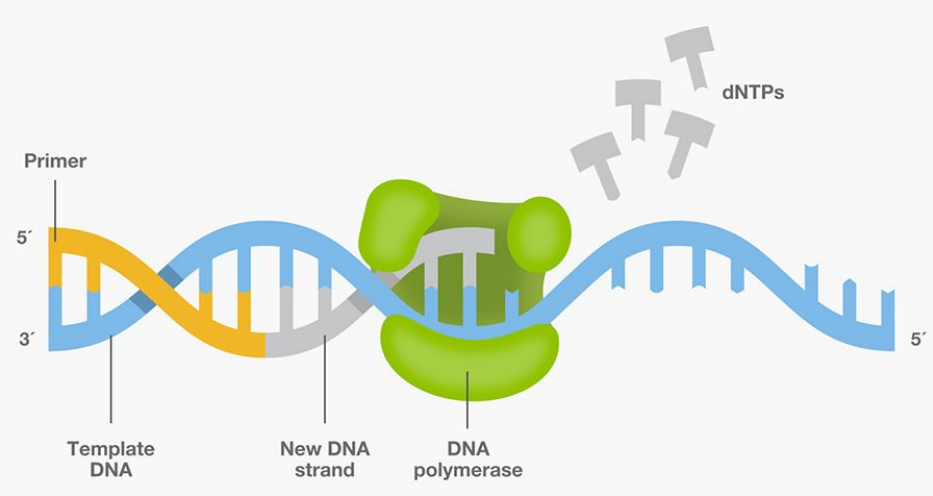
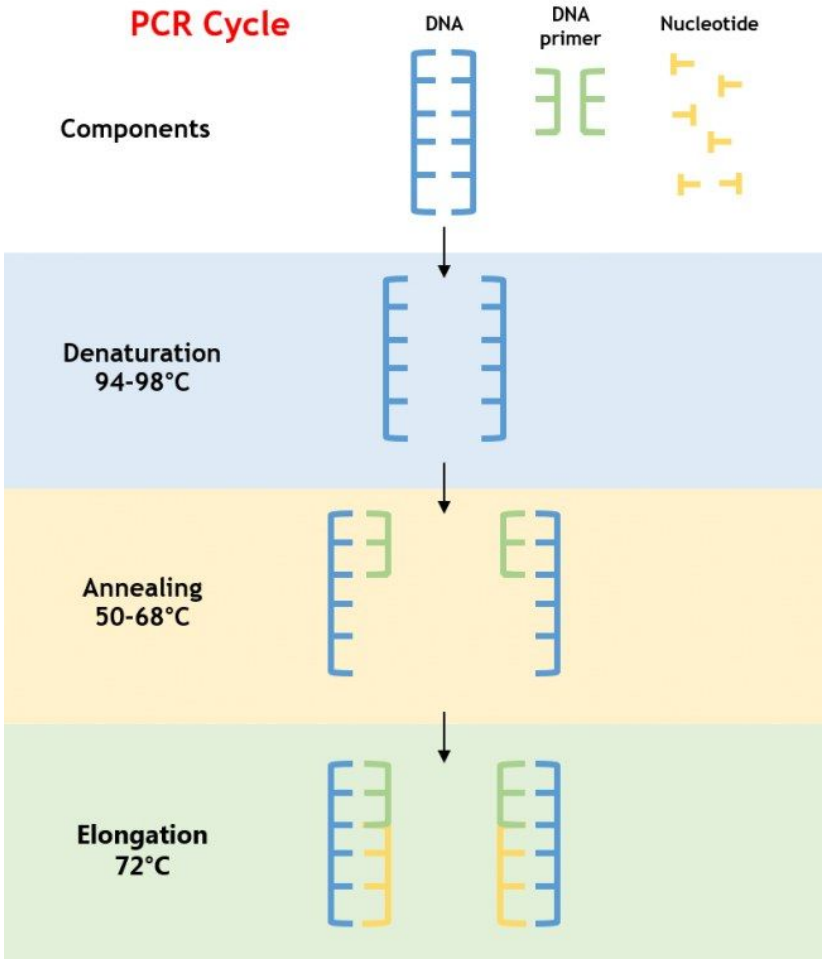
My Kitchen Table PCR Sophomore Year of High School





PCR Primer


dNTPs
Primer
5´
3´
5´
Template DNA
New DNA strand
DNA polymerase


PCR Cycle
DNA
DNA primer
Nucleotide
Components
Denaturation 94-98°C
Annealing 50-68°C
Elongation 72°C

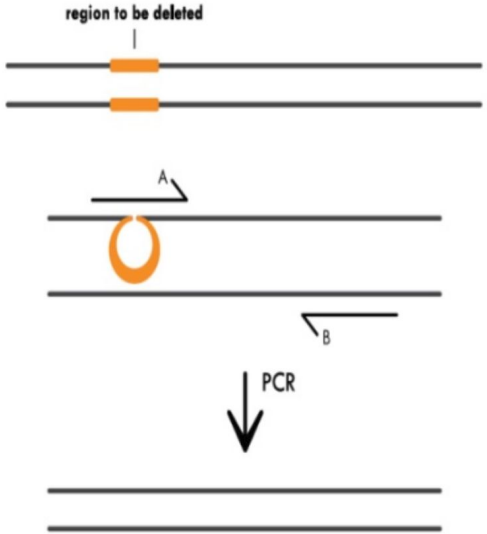
Primer-Defined Changes to the PCR Copies

\-

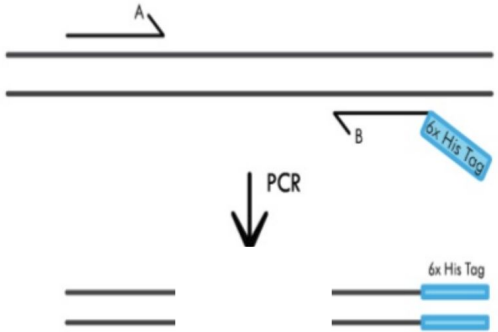
\+

Δ

PCR for Deletions



PCR for Terminal Additions



PCR for Base Substitutions.

Existing sequence

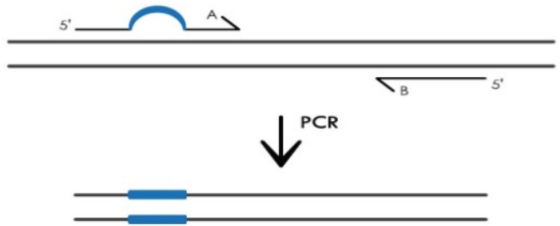
5′ggacgcaagc----------aggacattga 3′

3′cctgcgttcgac---------- tcctgtaact 5’

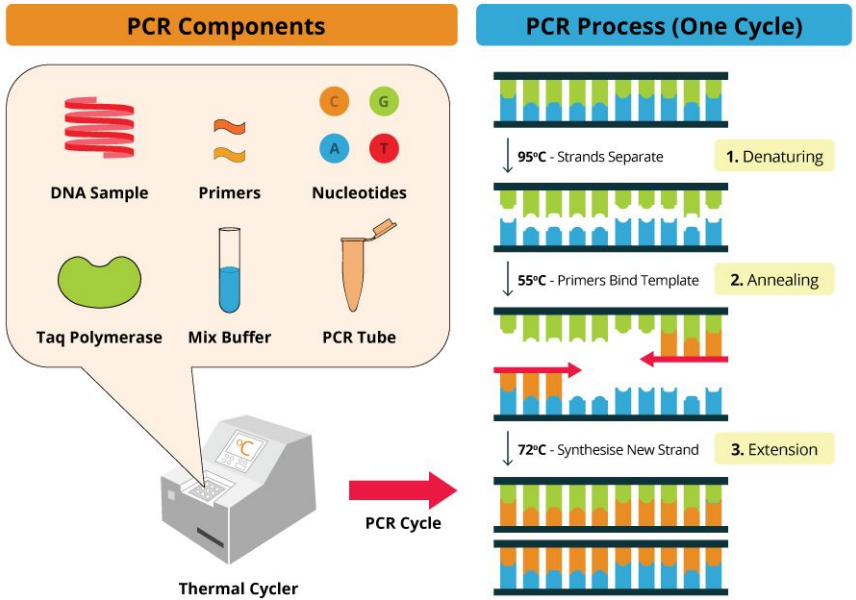
Desired sequence

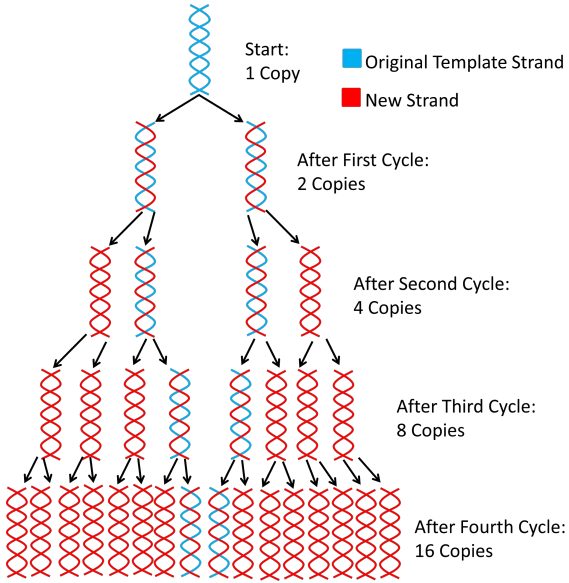
5′ggaTCcaagc----------aggacattga 3′

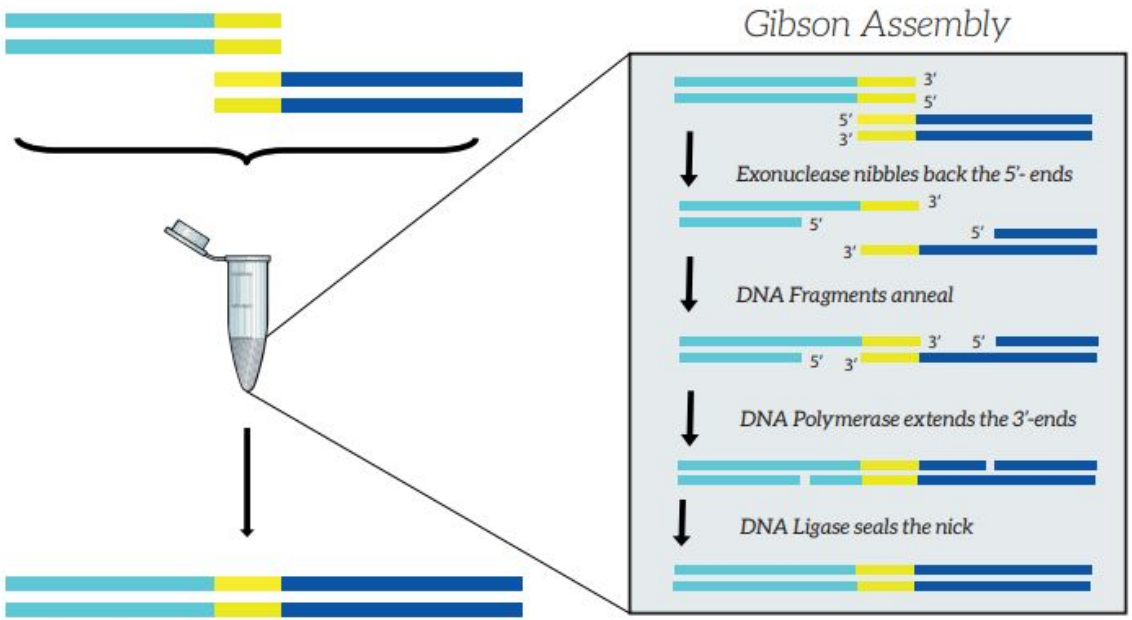
3′cctAGgttcgac---------- tcctgtaact 5′



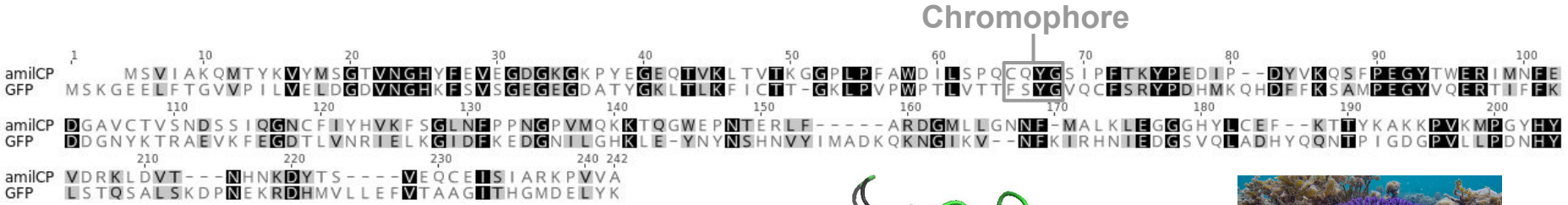
Copying DNA with Polymerase Chain Reaction



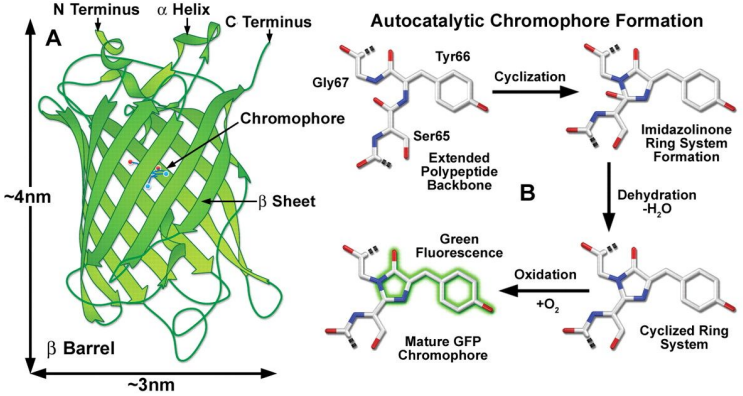


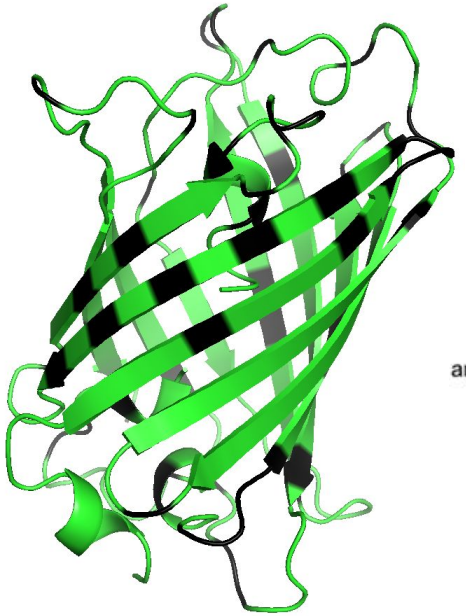

Gibson Assembly
3'
5'
5'
3'
Exonuclease nibbles back the 5'- ends
3'
5'
5'
3'
DNA Fragments anneal
3'
5'
5'
3'
DNA Polymerase extends the 3'-ends
DNA Ligase seals the nick

Creating the Chromophore Colorwheel

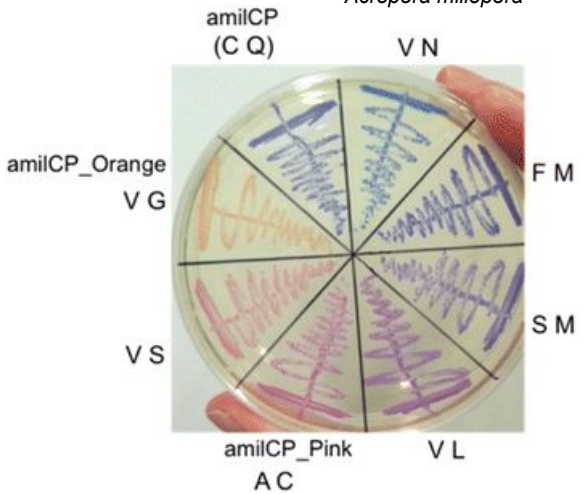


Conserved Similar Divergent

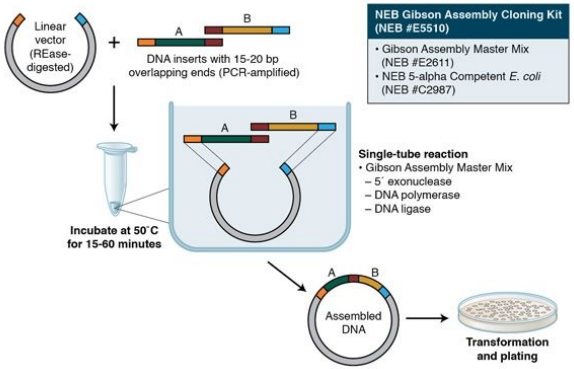


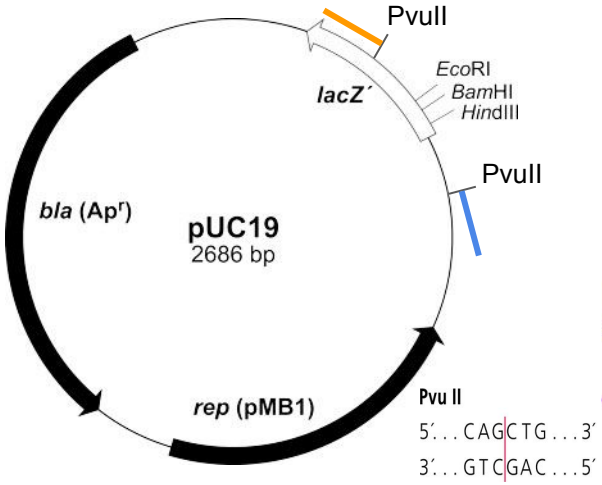


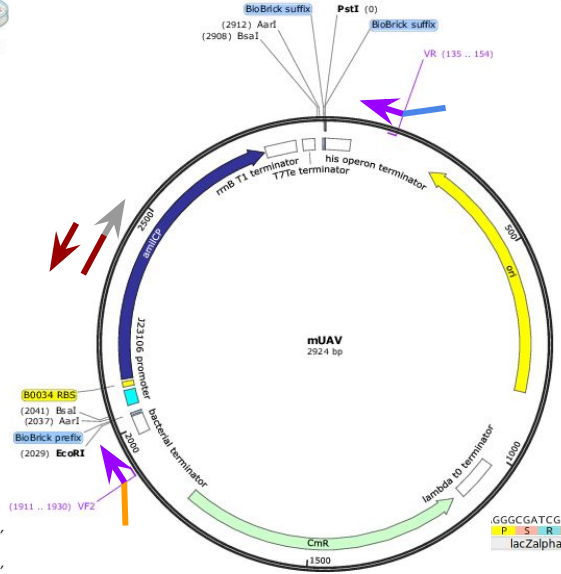
Acropora millepora

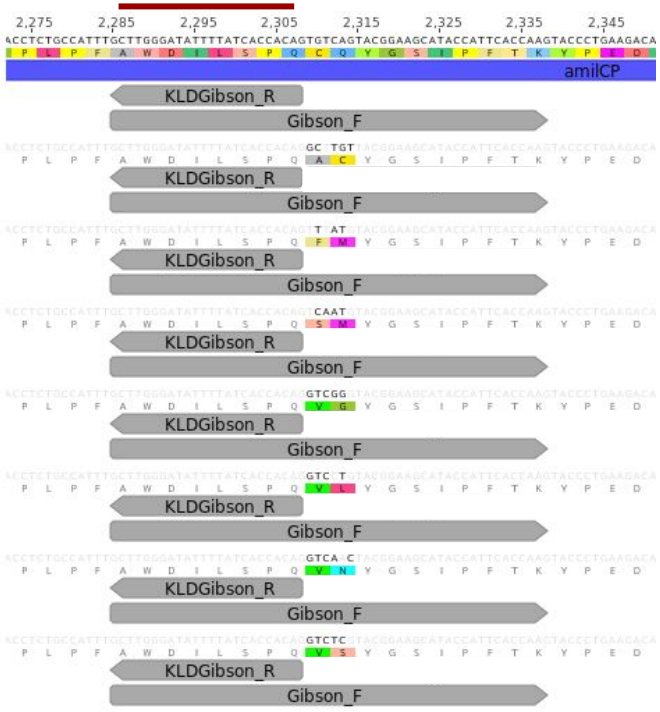


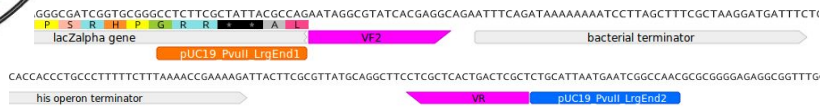
Gibson Assembly for Mutagenesis and Drug Selection Swap



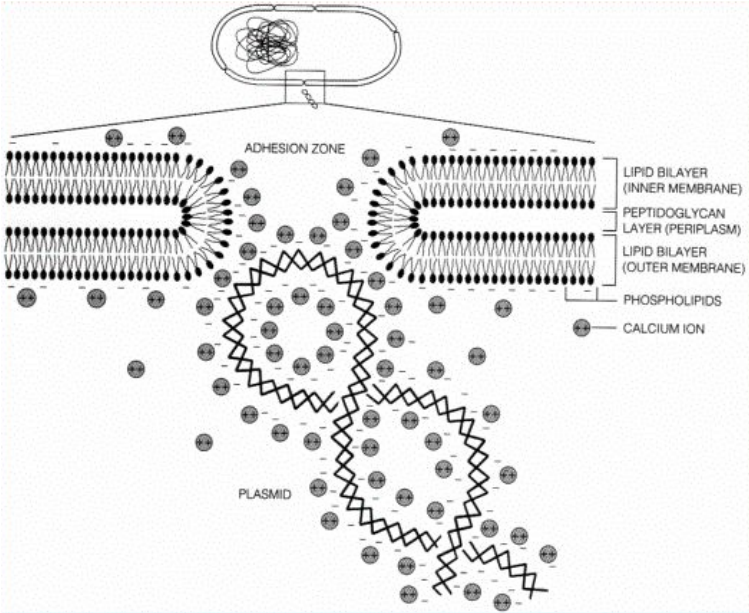


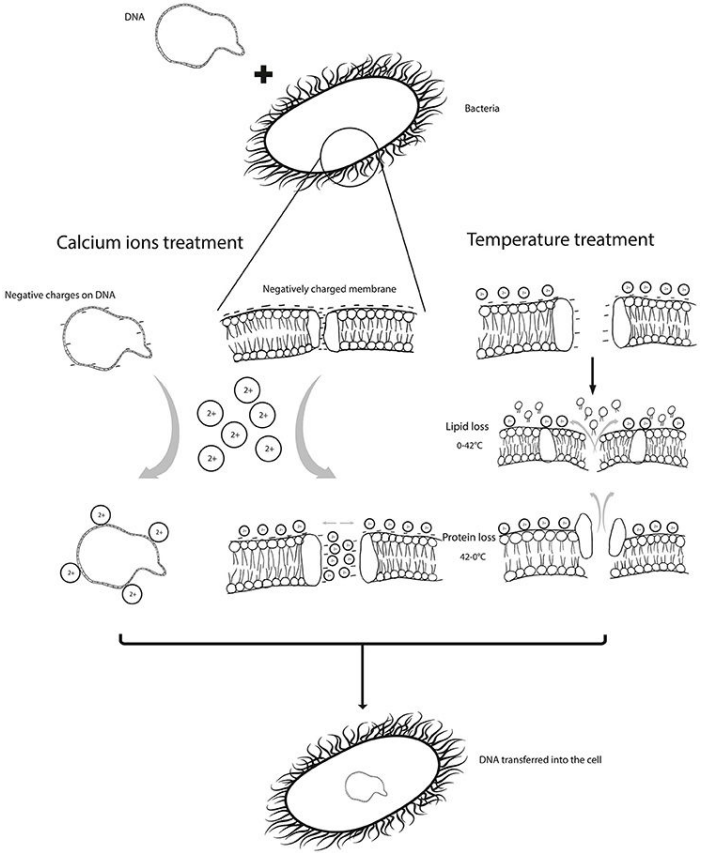






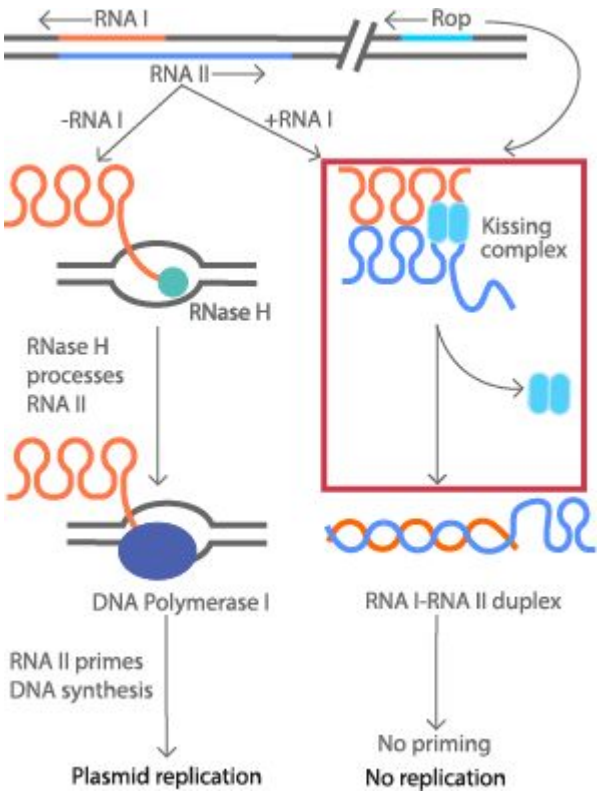
Plasmid Uptake by Calcium-Assisted Heat Shock

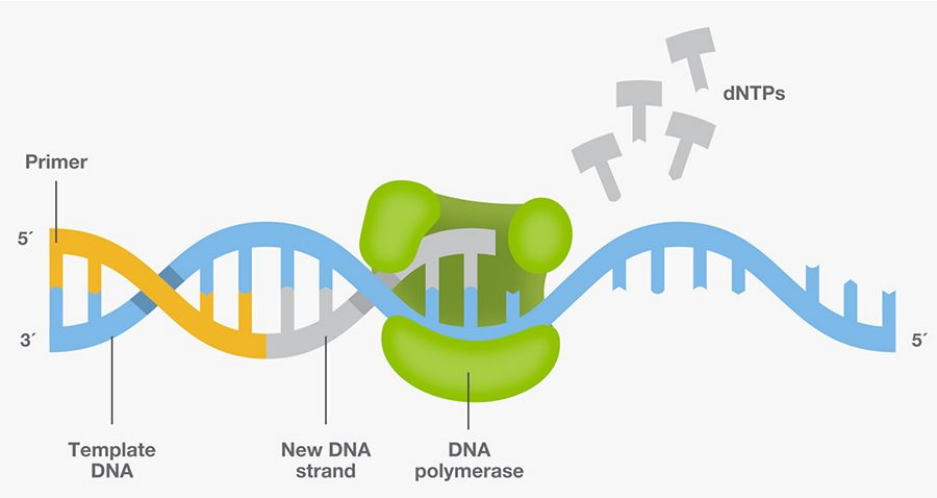




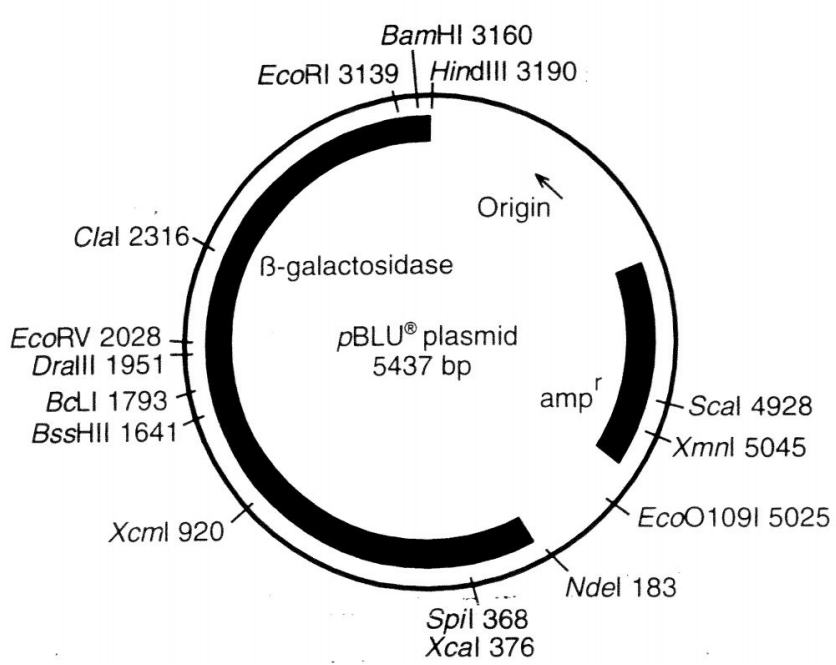
Asif et al, *Front Microbiol* 2017

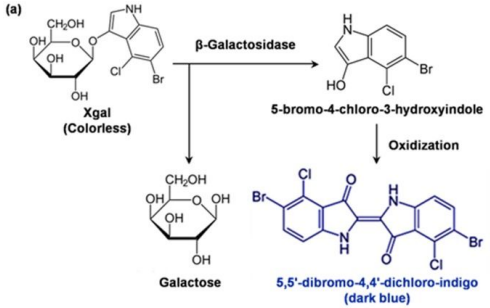
A Primer for This Week

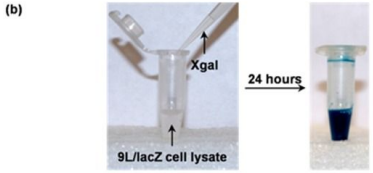


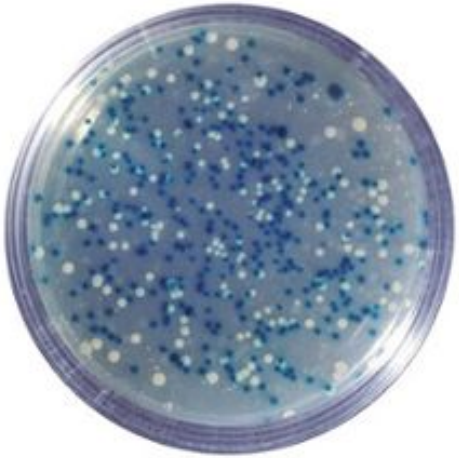


DNA-Programming w/ Plasmids



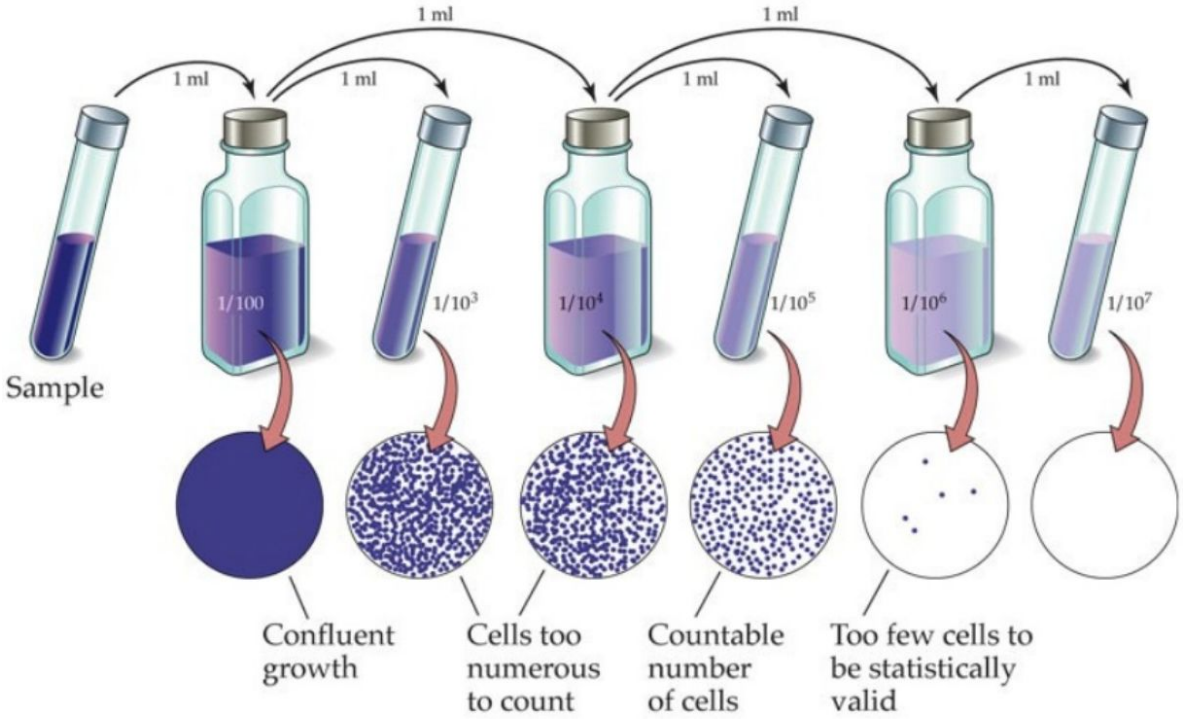






Viability Count (Colony Forming Units, CFU)

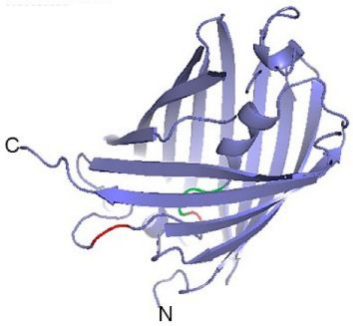
Serial Dilution



MICROBIAL LIFE, **Figure 6.4** © 2002 Sinauer Associates, Inc.

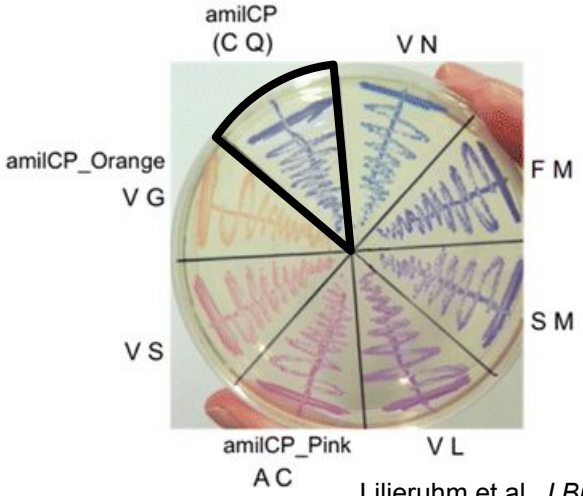
NextGenSynth Assignment:
DNA-Programmed Coral Color Changes

Think like a molecule.
-George Church



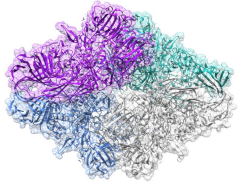
***A**cropora **mil**lepora **C**hromo**P**rotein*

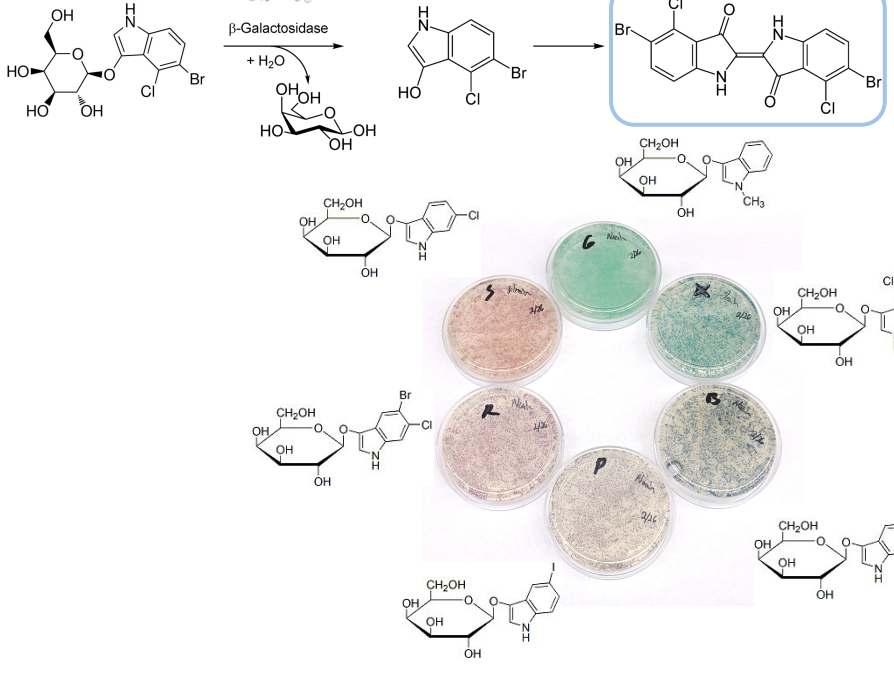
***A**cropora **mil**lepora*



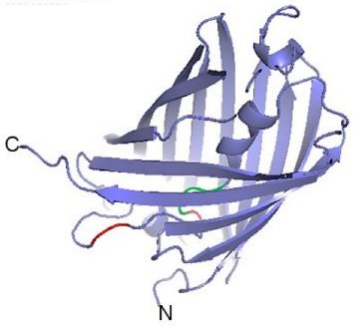
Liljeruhm et al, *J Biol Eng.* 2018

NextGenSynth Assignment:
DNA-Programmed Coral Color Changes

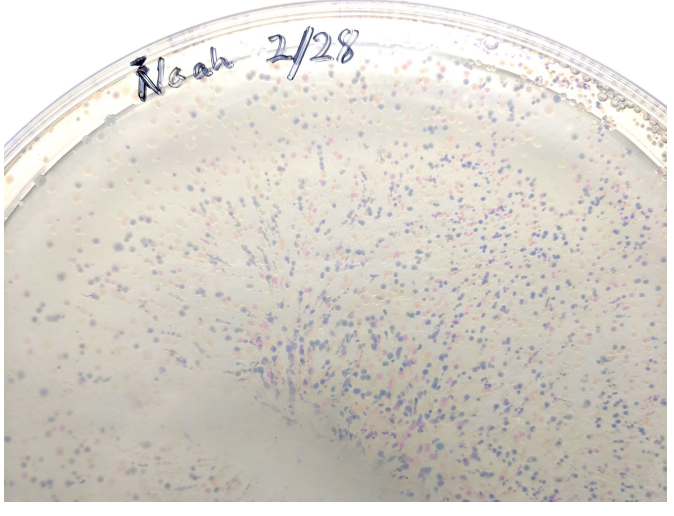




Think like a molecule.
-George Church



***A**cropora **mil**lepora **C**hromo**P**rotein*



Molecular Machinery:
A Tour of the Protein Data Bank

RCSB PDB PROTEIN DATA BANK
rcsb.org

Cells build many complex molecular machines that perform the biological jobs needed for life. Some of these machines are molecular scissors that cut food into digestible pieces. Others then use these pieces to build new molecules when cells grow or tissues need to be repaired. Some molecular machines form sturdy beams that support cells, and others are motors that use energy to crawl along these beams. Some recognize attackers and mobilize defenses against infection.

Adenosine Triphosphate (ATP)
Glucose
Water

Researchers around the world are studying these molecules at the atomic level. These 3D structures are freely available at the Protein Data Bank (PDB), the central storehouse of biomolecular structures. A few examples from the ~100,000 structures held in the PDB are shown here at a magnification of about 3,500,000 times, with each atom represented as a small sphere. The enormous range of molecular sizes is illustrated here, from the water molecule (H2O) with only three atoms (shown at the left) to the ribosomal subunits with hundreds of thousands of atoms.

Digestive Enzymes: breaking food into small nutrient molecules
1. Amylase 1amd
2. Phospholipase 1poe
3. Deoxyribonuclease 2dnj
4. Lysozyme 1lz1
5. Pepsin 5pep
6. Trypsin 2ptc
7. Carboxypeptidase 3pta
8. Ribonuclease 5rsa

Blood Plasma Proteins: transporting nutrients and defending against injury
9. Factor X 1xka, 1ied
10. Thrombin 1ppb
11. Fibrin 1m1j, 2baf
12. Serum Albumin 1e7i

Viruses and Antibodies: engaging in constant battle in the bloodstream
13. Antibody 1igt
14. Rhinovirus 4rhv

Hormones: carrying molecular messages through blood
15. Glucagon 1gcn
16. Insulin 2hiu
17. Epidermal Growth Factor 1egf

Channels, Pumps and Receptors: getting back and forth across the membrane
18. Ras Protein 5p21
19. Beta2-Adrenergic Receptor/Gs Protein 3sn6
20. Acetylcholine Receptor 2bg9
21. Epidermal Growth Factor Receptor 1ivo, 2jwa, 2gs6
22. Rhodopsin 1f88
23. P-glycoprotein 4m2t
24. Potassium Channel 3lut
25. Calcium Pump 1su4
26. Cyclooxygenase 1prh

Photosynthesis: harvesting energy from the sun
27. Photosystem II 1s5l
28. Light-harvesting Complex 1rwt
29. Photosynthetic Reaction Center 1prc

Energy Production: powering the processes of the cell
30. Cytochrome c Oxidase (Complex IV) 1oco
31. Cytochrome c 3cyt
32. Cytochrome bc1 (Complex III) 1bgy
33. Succinate Dehydrogenase (Complex II) 1me6
34. NADH-Quinone Oxidoreductase (Complex I) 3m9s, 3rko
35. ATP Synthase 1e79, 1c17, 1f2p, 2a7u
36. Myoglobin 1mbd
37. Hemoglobin 4hhb

Storage: containing nutrients for future consumption
38. Ferritin 1fha

Enzymes: cutting and joining the molecules of life
39. Fatty Acid Synthase 2uvb, 2uvc
40. RuBisCo: Ribulose Bisphosphate Carboxylase/Oxygenase 1rcx
41. Green Fluorescent Protein 1gfl
42. Luciferase 2d1s
43. Glutamine Synthetase 2gls
44. Alcohol Dehydrogenase 2ohx
45. Dihydrofolate Reductase 1dhf
46. Nitrogenase 1n2c
47. Leucine Aminopeptidase 1lap
48. Beta-Lactamase 4bln
49. Catalase 1qqw
50. Thymidylate Synthase 2tsc
51. Tryptophan Synthase 1wsy
52. Aspartate Carbamoyltransferase 4at1
53. Hexokinase 1dgk
54. Phosphoglucose Isomerase 1hoa
55. Phosphofructokinase 4pfk
56. Aldolase 4ald
57. Triosephosphate Isomerase 2ypi
58. Glyceraldehyde-3-phosphate Dehydrogenase 3gpd
59. Phosphoglycerate Kinase 3pgk
60. Phosphoglycerate Mutase 3pgm
61. Enolase 5en1
62. Pyruvate Kinase 1a3w

Infrastructure: supporting and moving cells
63. Actin 1m8q
64. Myosin 1m8q
65. Microtubule 1tub
66. Collagen 1bkv (far left)

Protein Synthesis: building new molecular machines
67. Transfer RNA 4tna
68. Valyl-tRNA Synthetase 1gax
69. Threonyl-tRNA Synthetase 1qf6
70. Glutaminyl-tRNA Synthetase 1euq
71. Isoleucyl-tRNA Synthetase 1ffy
72. Phenylalanyl-tRNA Synthetase 1eiy
73. Aspartyl-tRNA Synthetase 1asy
74. Ribosome 1j5e, 1jj2
75. Elongation Factor Tu/tRNA 1ttt
76. Elongation Factor G 1dar
77. Elongation Factor Ts and Tu 1efu
78. Prefoldin 1fxk
79. Chaperonin GroEL/ES 1aon
80. Proline cis/trans Isomerase 2cpl
81. Heat Shock Protein Hsp90 2cg9
82. Proteasome 4b4t
83. Ubiquitin 1ubq

DNA: storing and reading genetic information
84. DNA 1bna
85. Restriction Endonuclease EcoRI 1eri
86. DNA Photolyase 1hez
87. Topoisomerase 1a36
88. RNA Polymerase 2e2i
89. lac Repressor 1lbh 1efa
90. Catabolite Gene Activator Protein 1cgp
91. TATA-binding Protein/ Transcription Factor IIB 1ais
92. DNA Helicase 4esv
93. DNA Polymerase 1tau
94. Nucleosome 1aoi
95. HU Protein 1pld
96. Single-stranded DNA-binding Protein 3a5u

Scale:
1nm 5nm 10nm
1nm (nanometer) =
10-9 millimeters

Extracellular Proteins | Membrane Proteins | Intracellular Proteins: Cytosol | Intracellular Proteins: Cytosol | Intracellular Proteins: Nucleus

Machines in the Microbial Factory

Escherichia coli

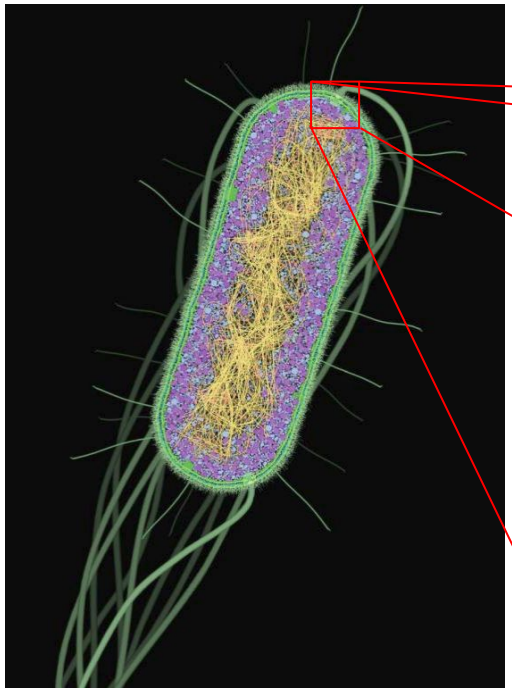
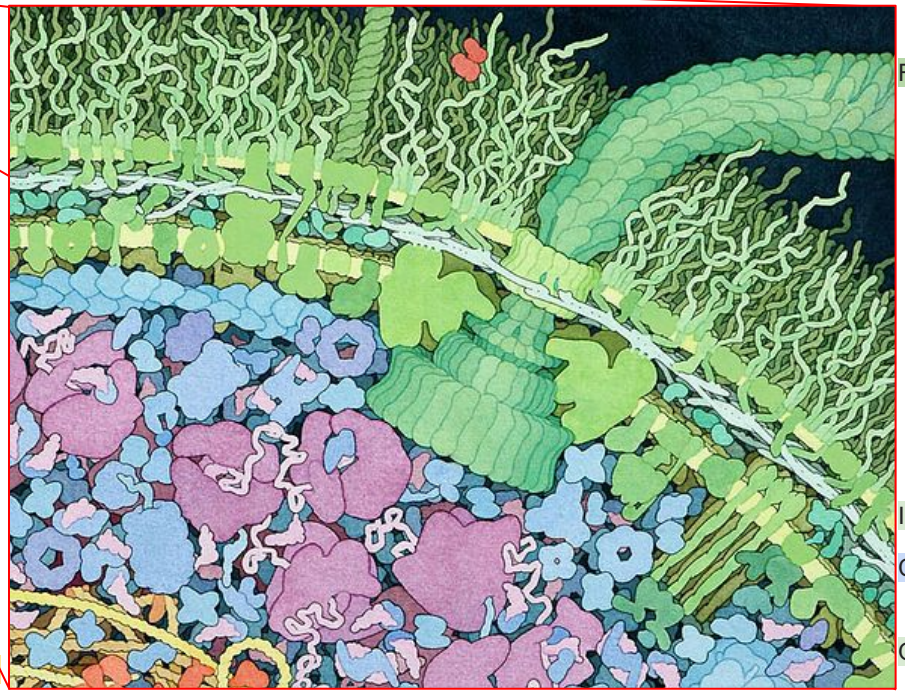
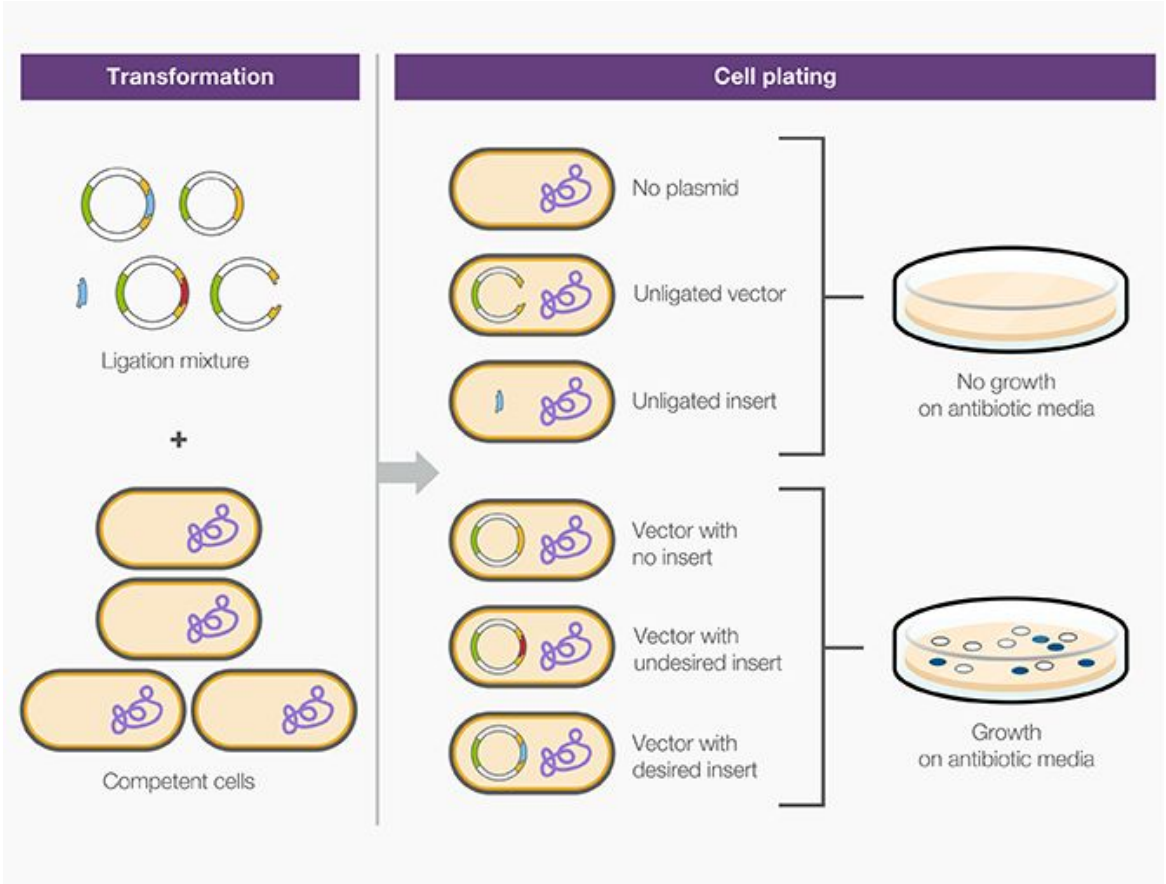
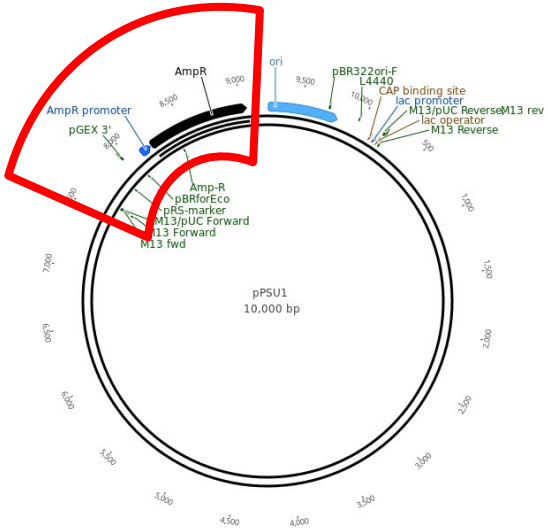


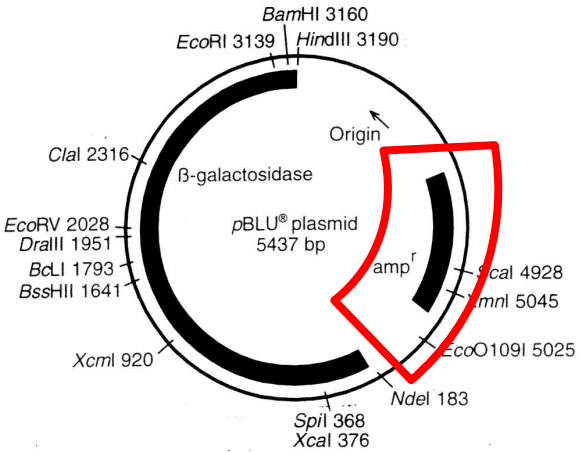
Image Credit: David Goodsell



Drug Selection of Transformed *E coli* Cells

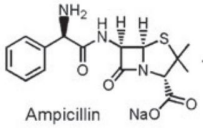


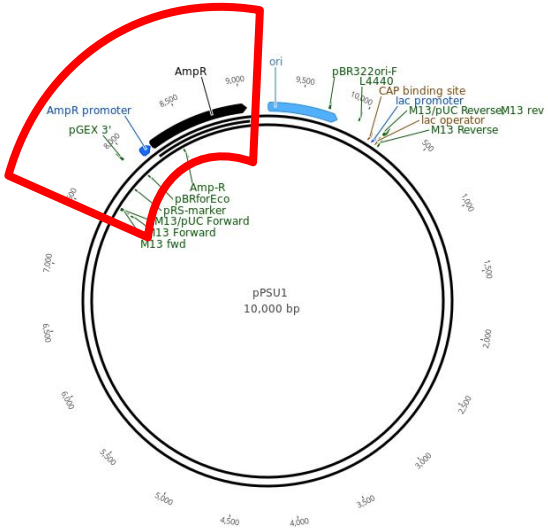


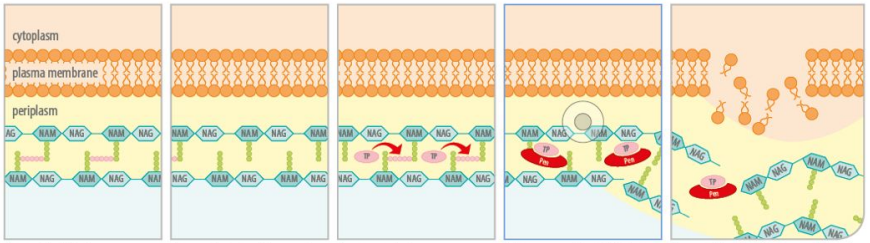


Drug Selection of Transformed *E coli* Cells

Penicillium mold



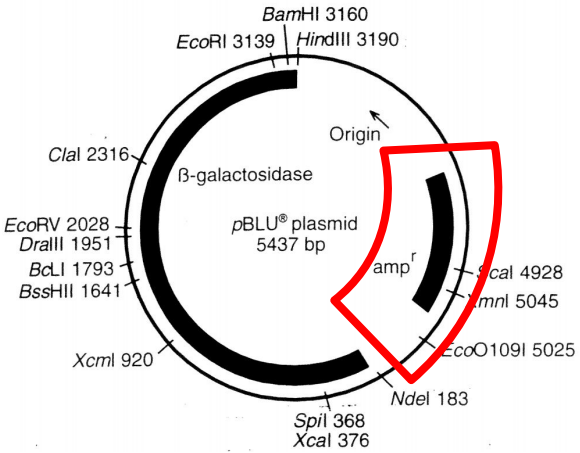




*For simplicity, only two layers of peptidoglycans (NAM-NAG) are shown. Normally the cell wall consists of many layers.

Inner Membrane

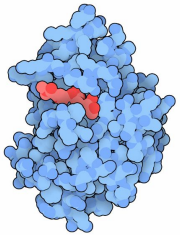
Cell Wall

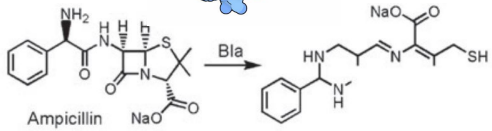


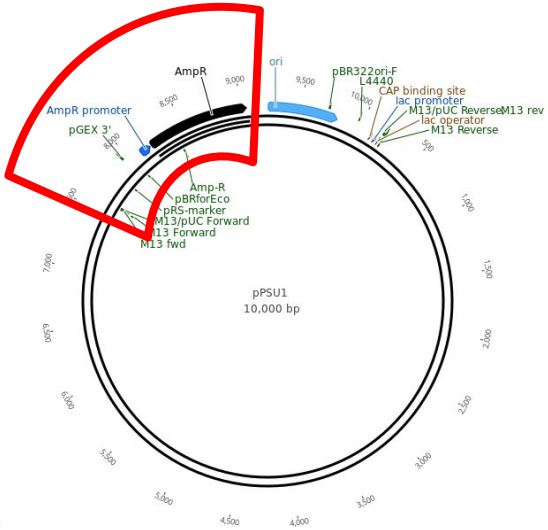
Drug Selection of Transformed *E coli* Cells

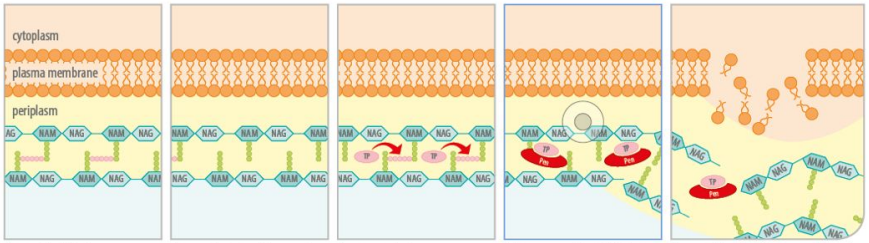
Penicillium mold

Beta-lactamase (Bla/amp^r)

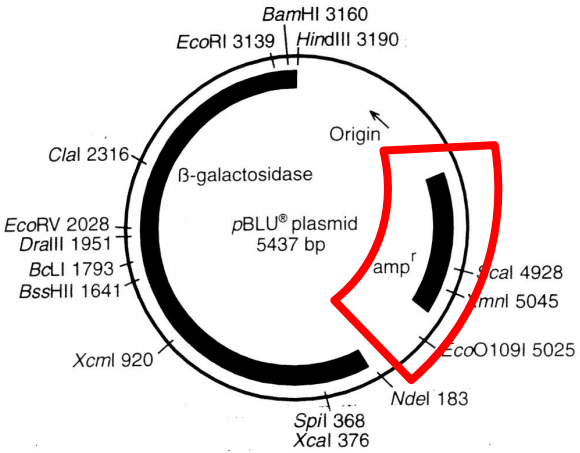






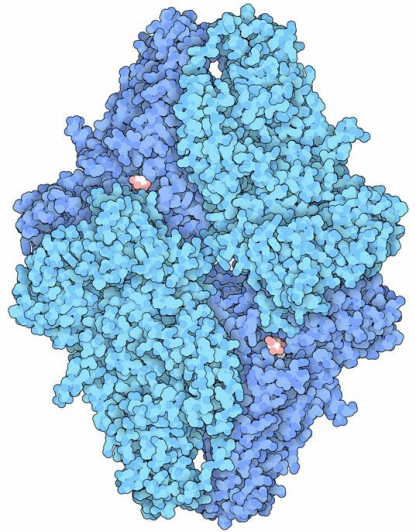


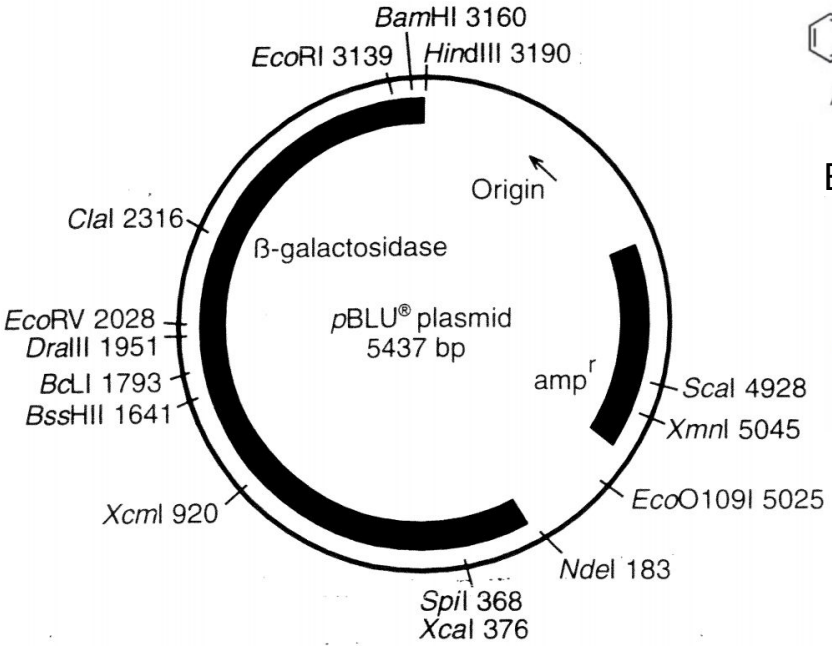
*For simplicity, only two layers of peptidoglycans (NAM-NAG) are shown. Normally the cell wall consists of many layers.

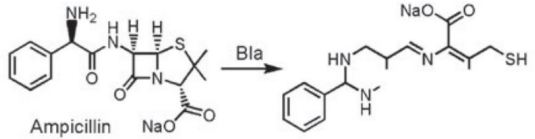


DNA-Programming w/ Plasmids

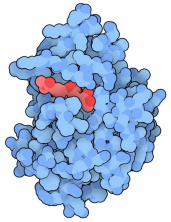
Beta-galactosidase (lacZ)

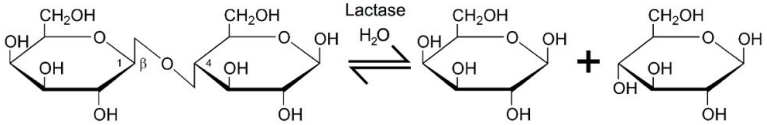






Beta-lactamase (Bla/amp^r)

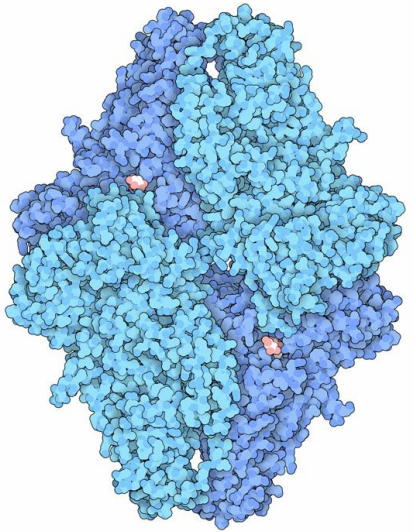


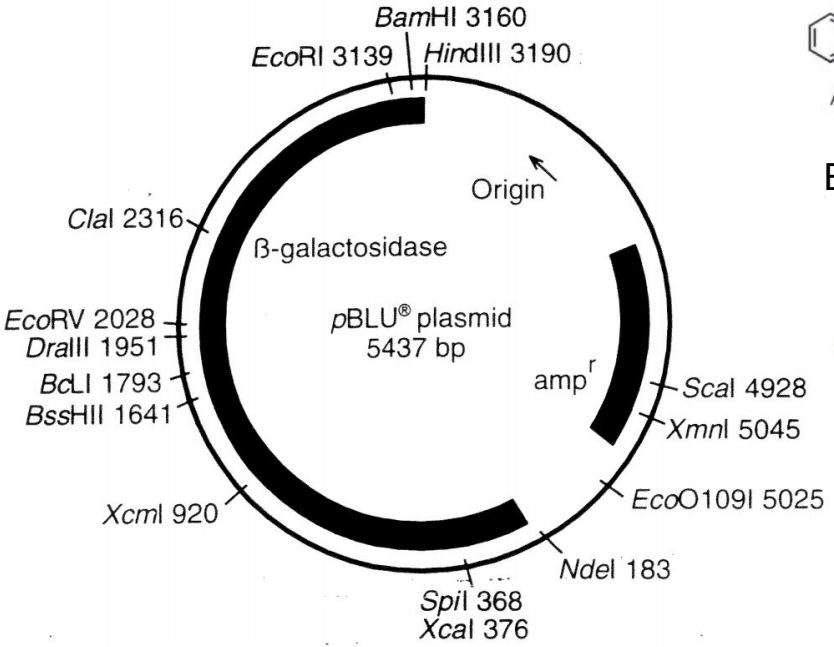


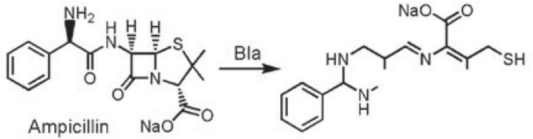
Lactose D-galactose D-glucose

DNA-Programming w/ Plasmids

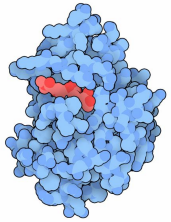
Beta-galactosidase (lacZ)

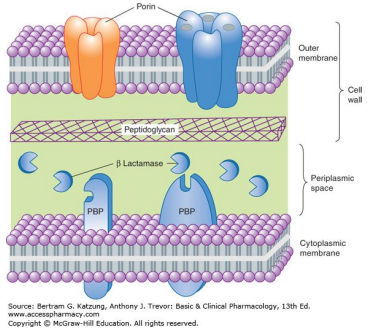


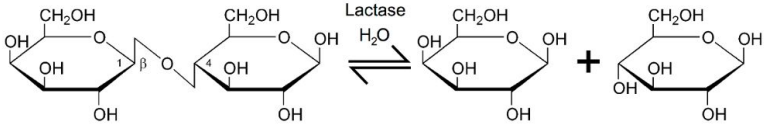


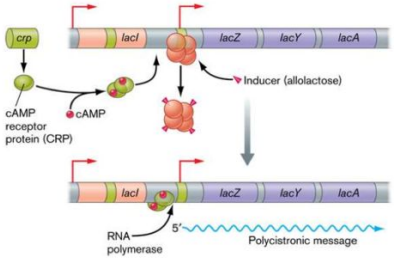


Beta-lactamase (Bla/amp^r)



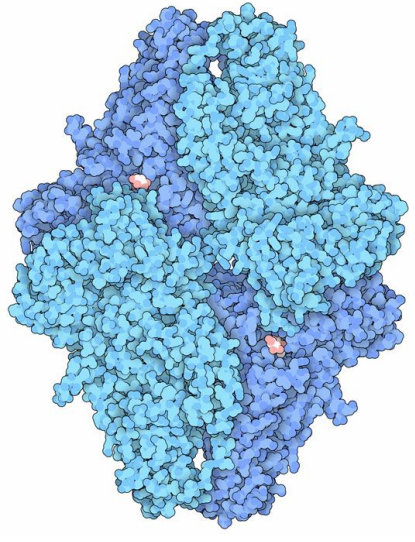


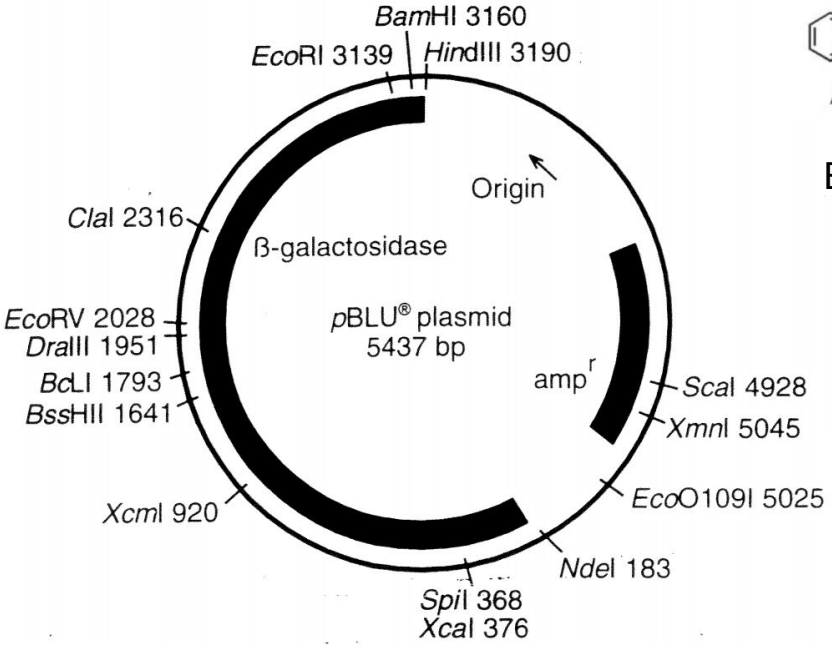


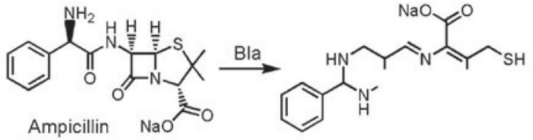


DNA-Programming w/ Plasmids

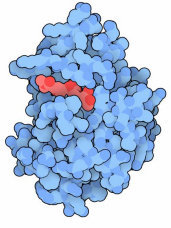
Beta-galactosidase (lacZ)

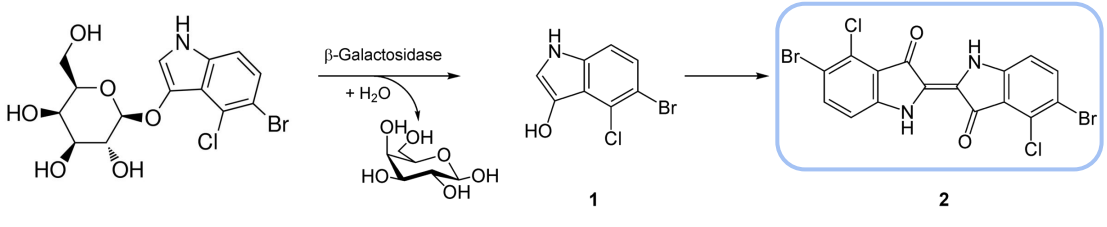


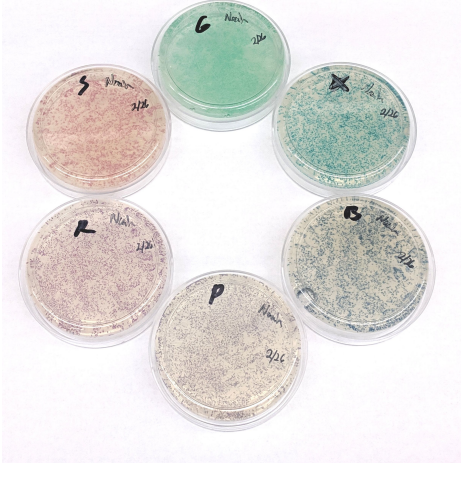




Beta-lactamase (Bla/amp^r)

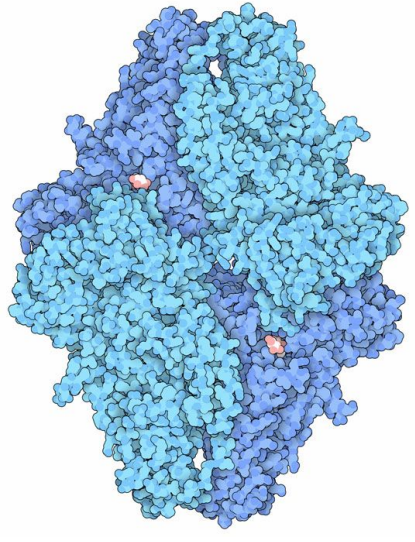


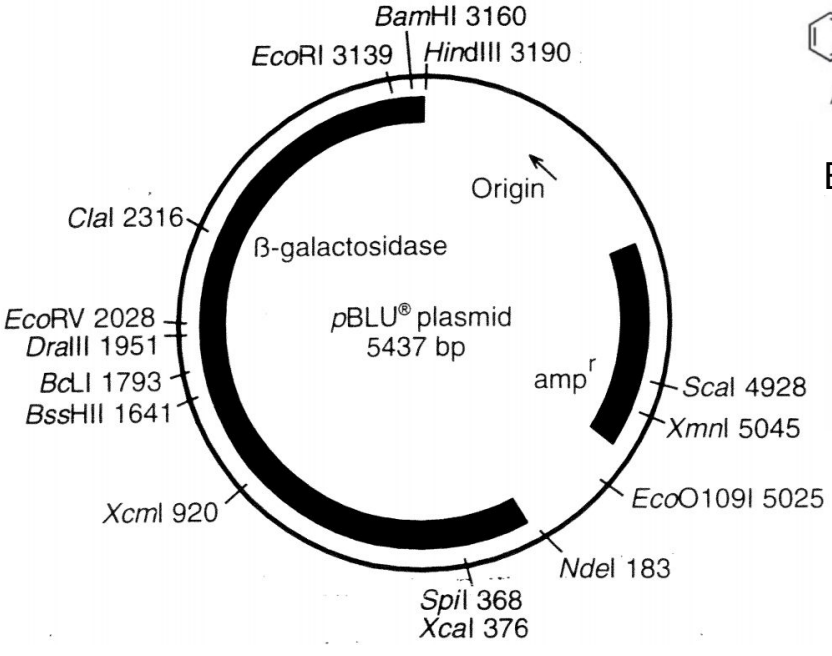


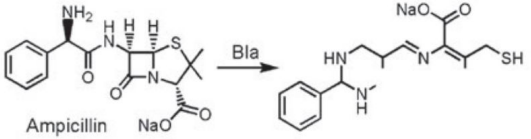


DNA-Programming w/ Plasmids

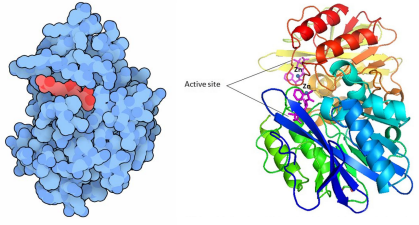
Beta-galactosidase (lacZ)

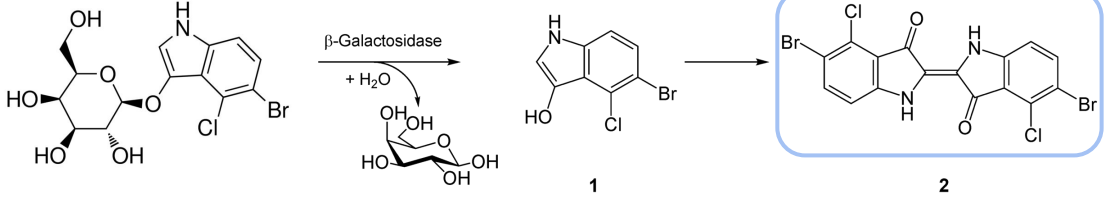


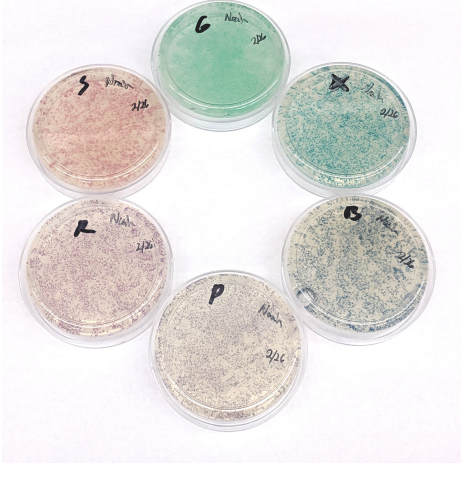




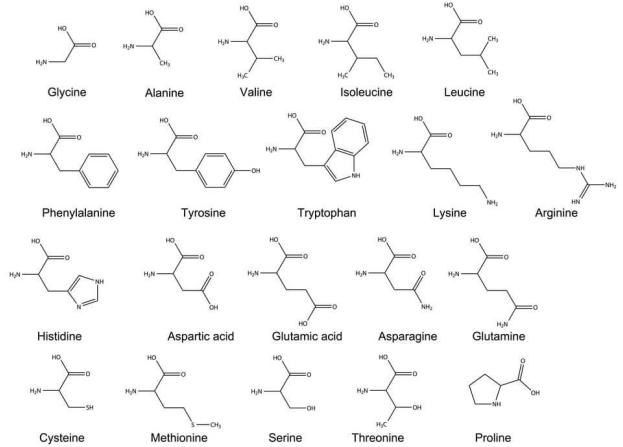
Beta-lactamase (Bla/amp^r)

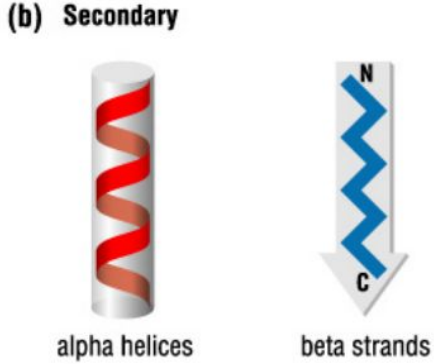


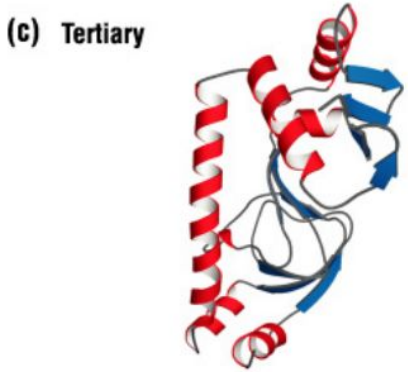




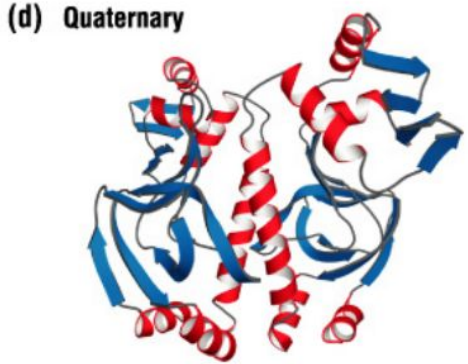
Hierarchy of Protein Structure





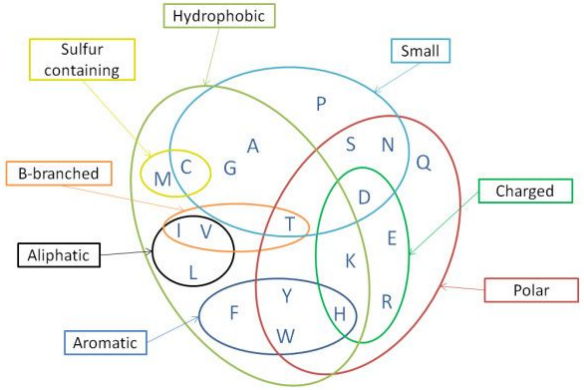


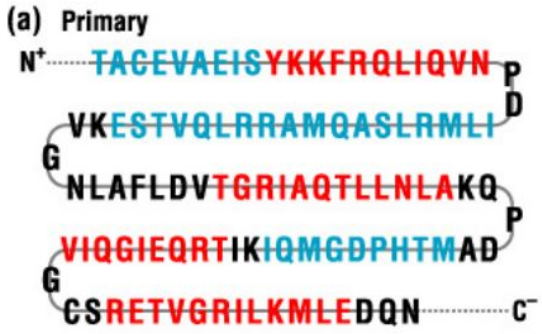
Cyclic-AMP Receptor Protein (CRP)

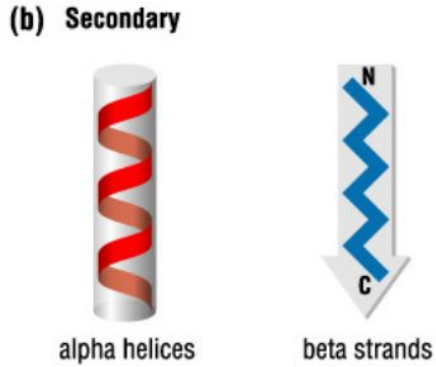


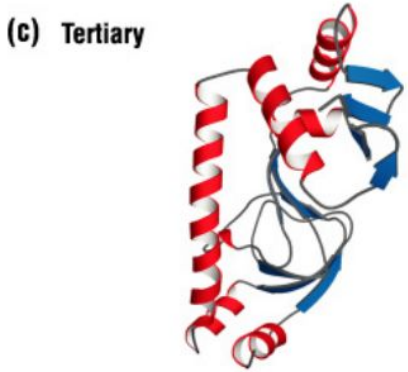
Homodimer (CRP-CRP)

Hierarchy of Protein Structure

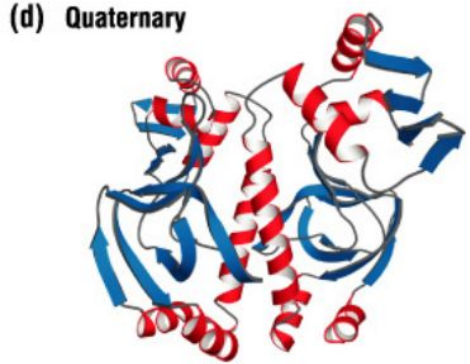






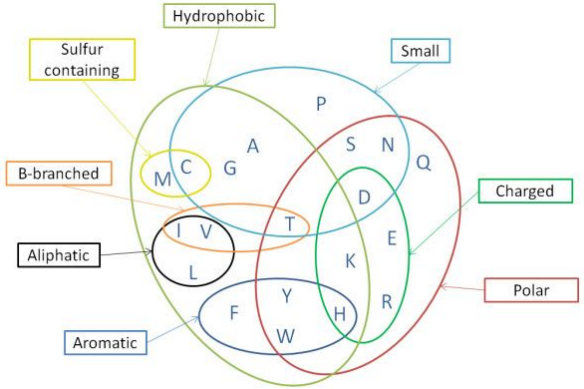


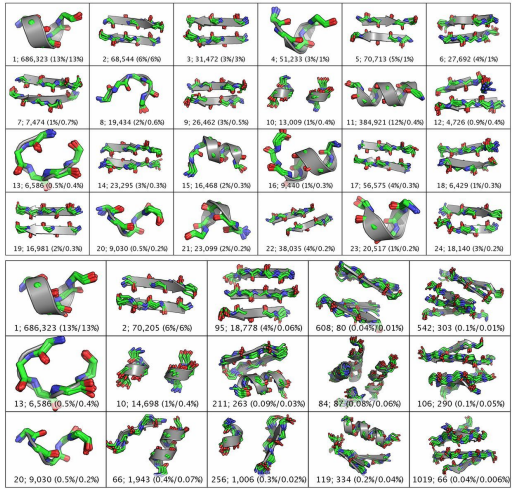
Cyclic-AMP Receptor Protein (CRP)

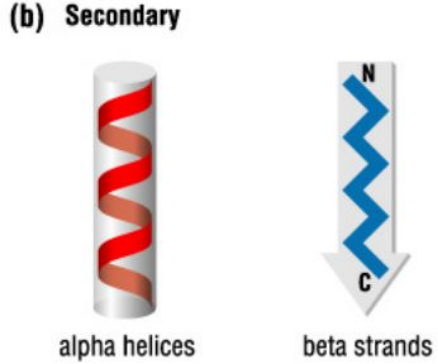


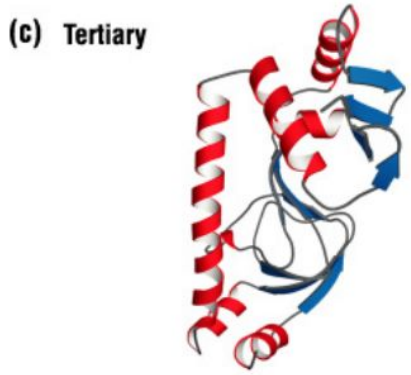
Homodimer (CRP-CRP)

Hierarchy of Protein Structure

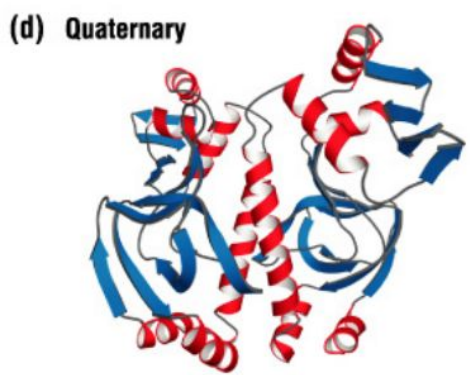






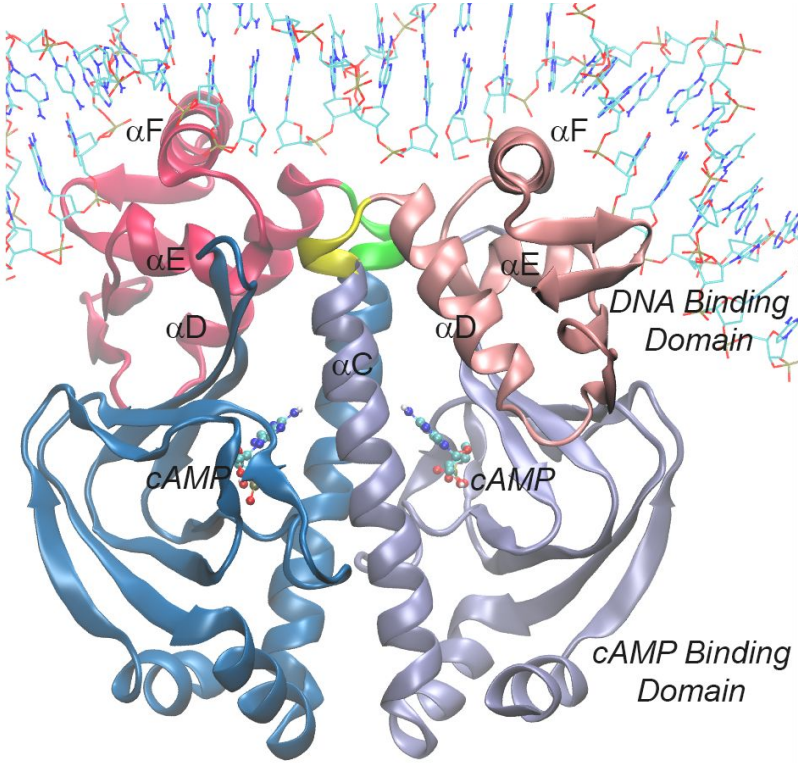


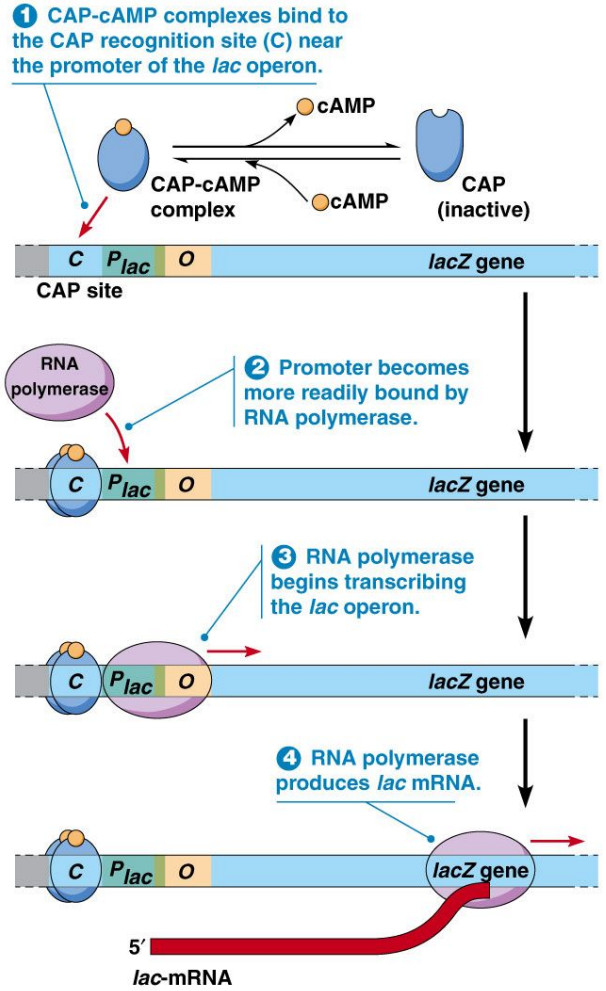
Cyclic-AMP Receptor Protein (CRP)



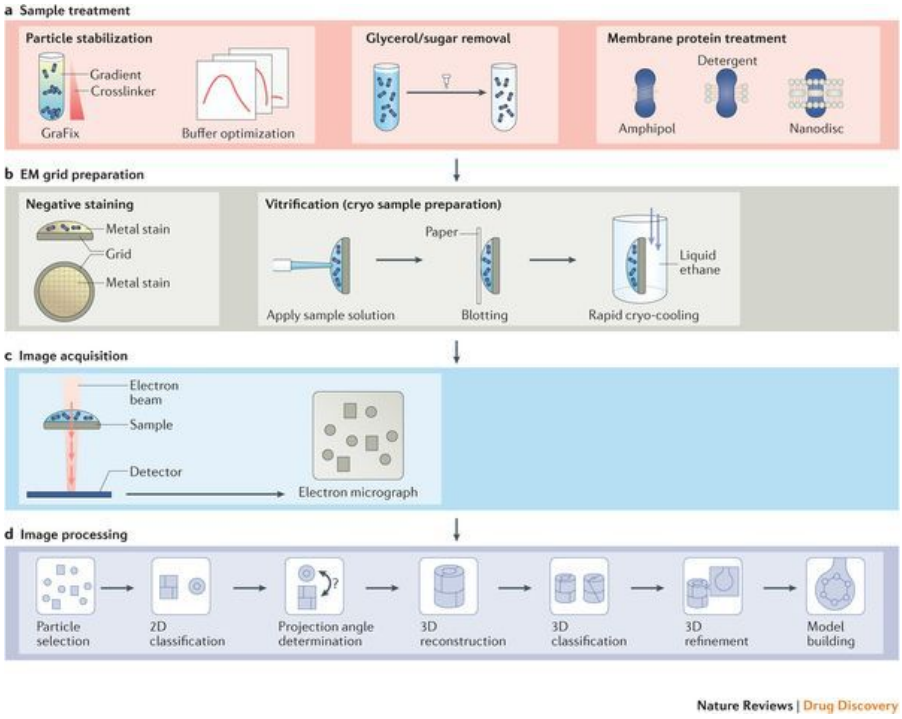
Homodimer (CRP-CRP)

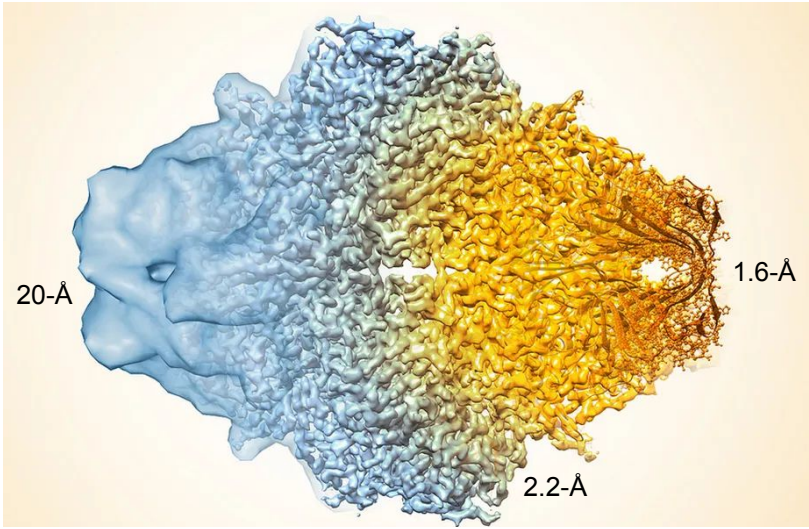
Modularity in Motifs and Domains

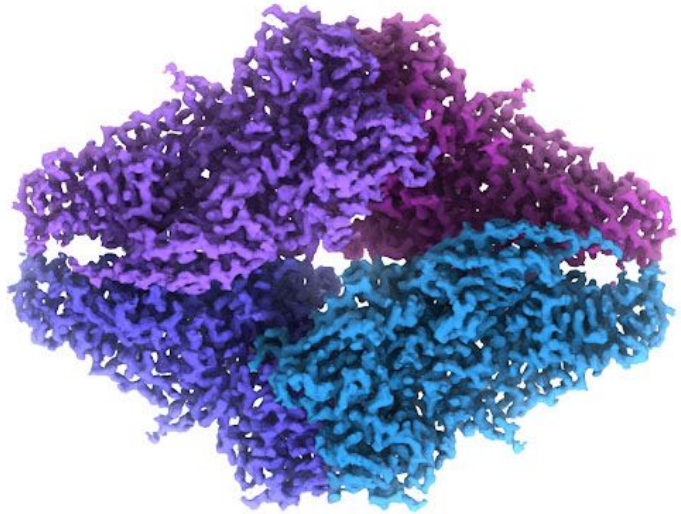




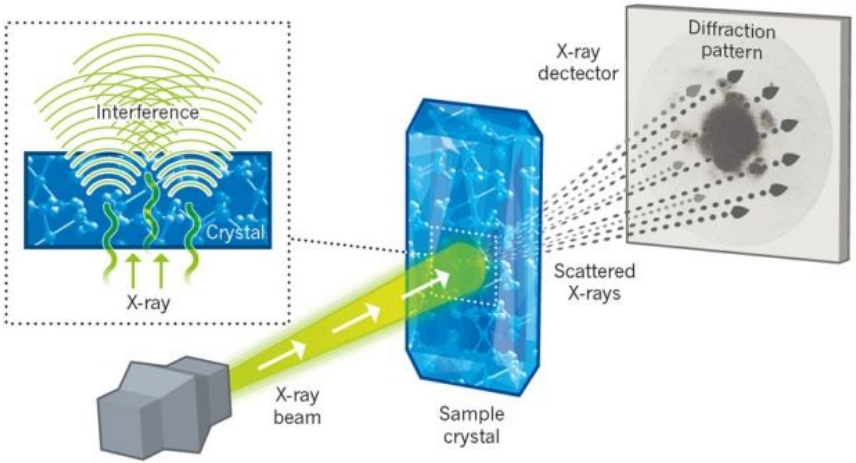
Solving for Structure: Cryo Electron Microscopy

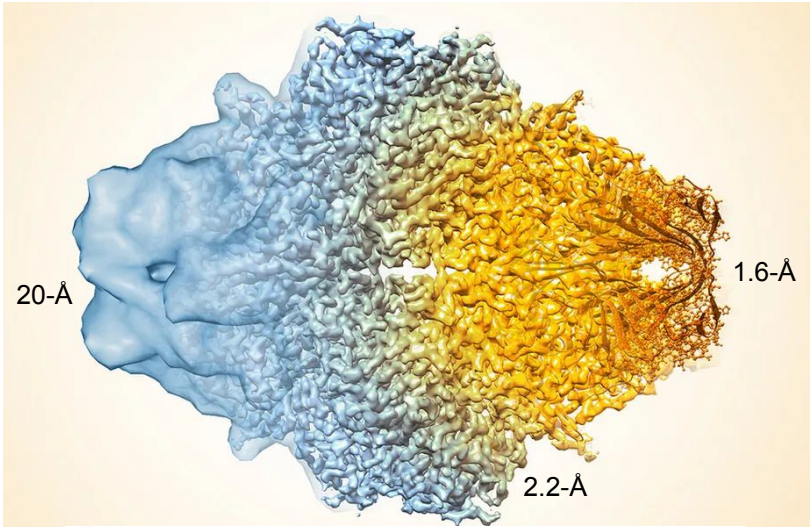


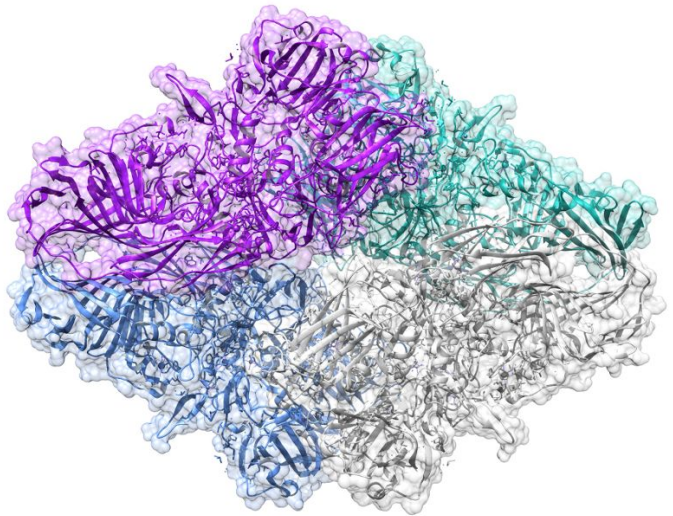




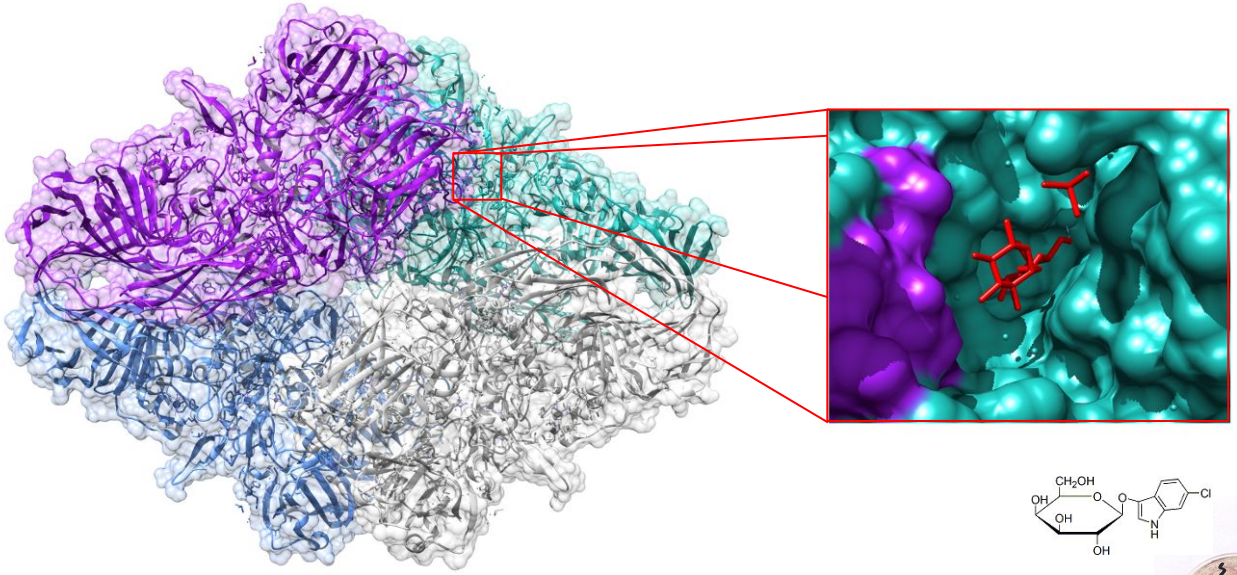
Solving for Structure: X-Ray Crystallography

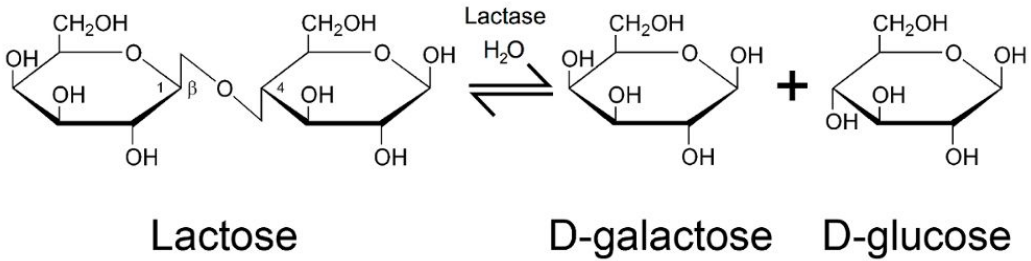


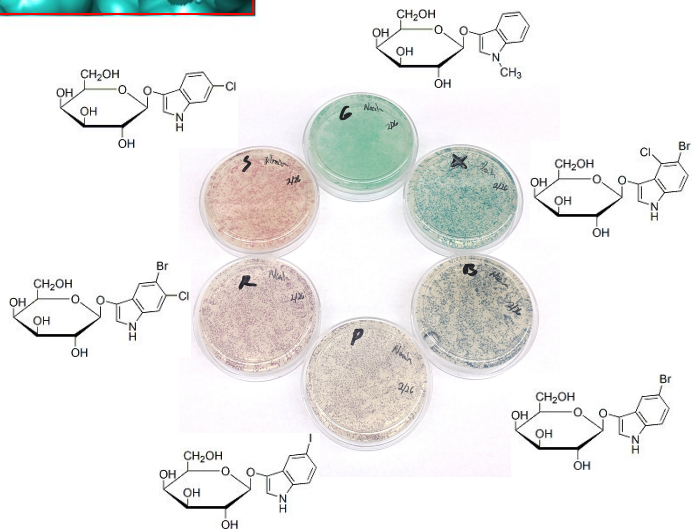




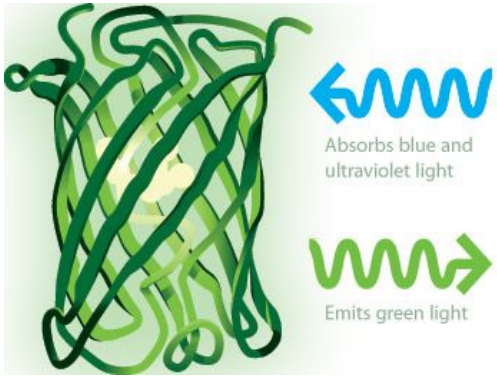
Color-Based Screens with Beta-galactosidase (lacZ)



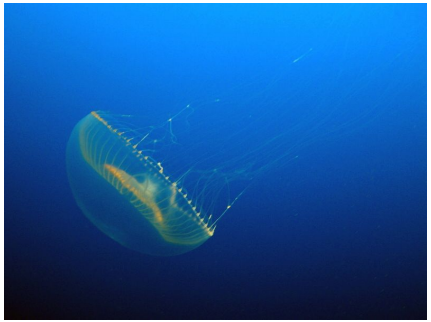




Genetically-Encoded Green Fluorescence



Aequorea victoria



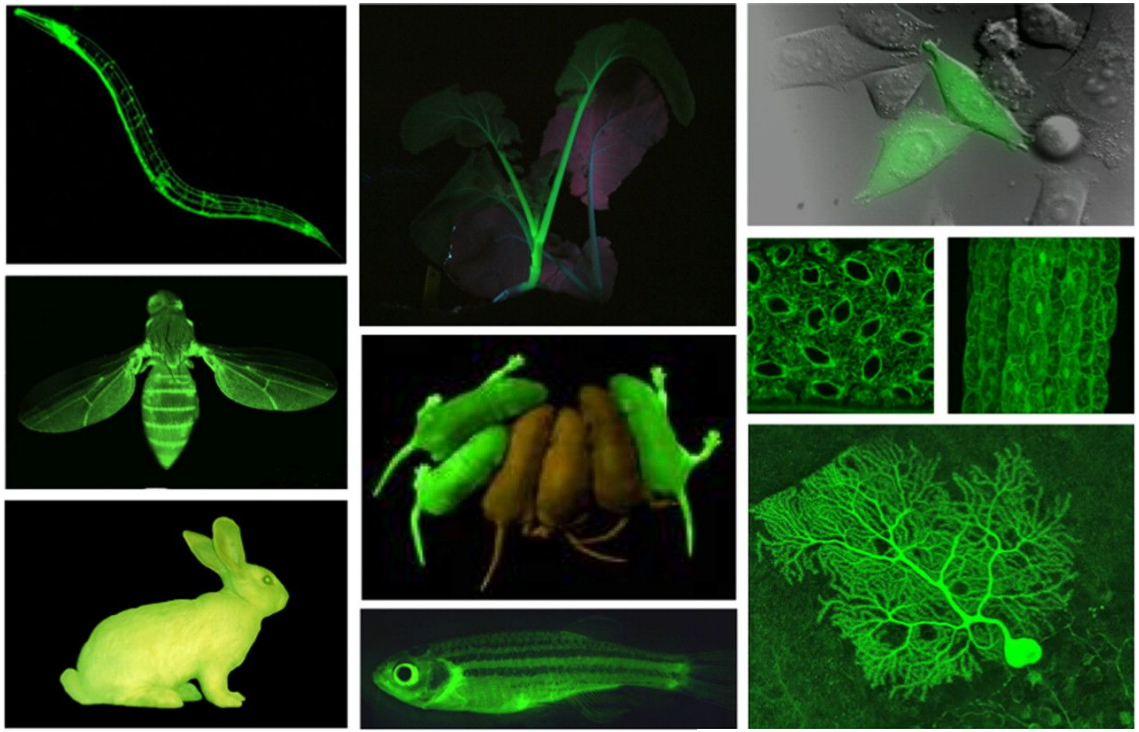
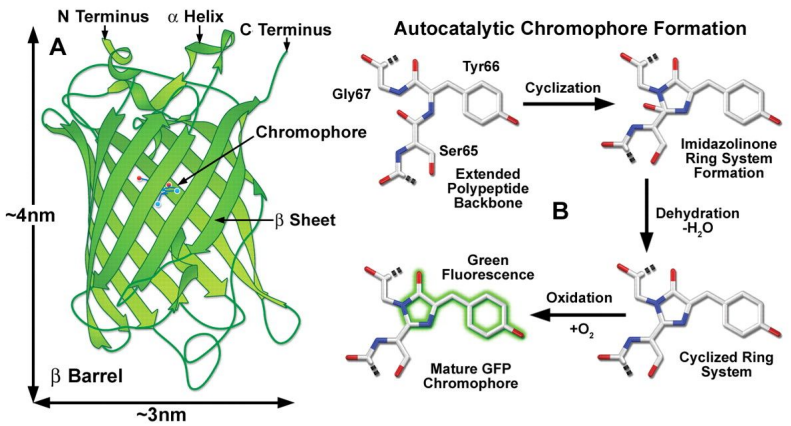
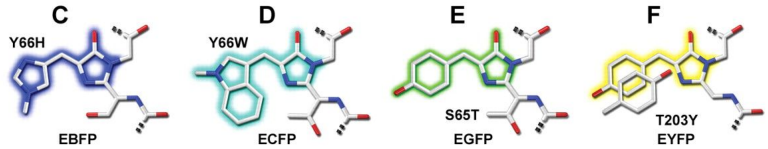


Image Credit: Roger Tsien Nobel Lecture

Chromophore Engineering





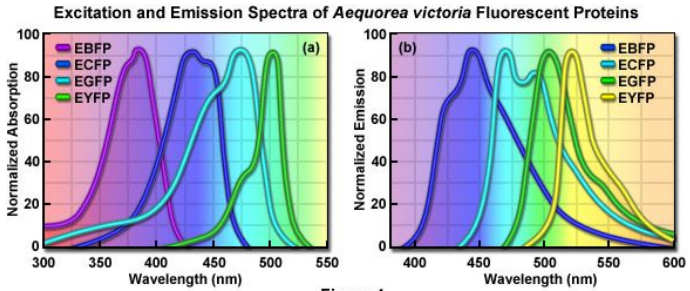
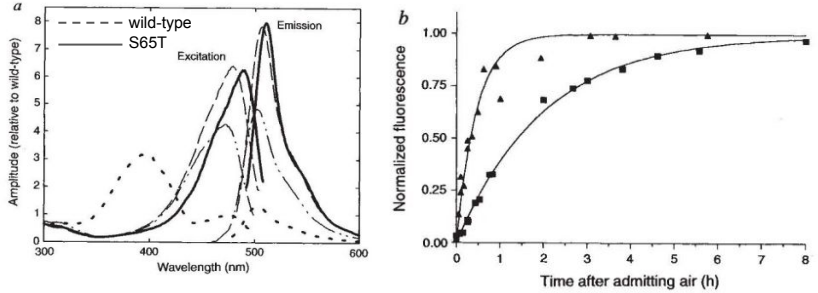


Figure 4



b, Rates of autoxidative fluorophore generation in wild-type (■) and S65T (▲) GFP, measured by development of fluorescence after admission of air to *E. coli* cultures anaerobically grown in GasPak pouches (Becton-Dickinson) for 3 days. Air was readmitted while transferring the cells to phosphate-buffered saline containing 8 mM NaN_3 as a metabolic inhibitor. The time course of subsequent fluorescence development measured the final oxidation step in the protein's self-modification to generate its internal fluorophore[8].

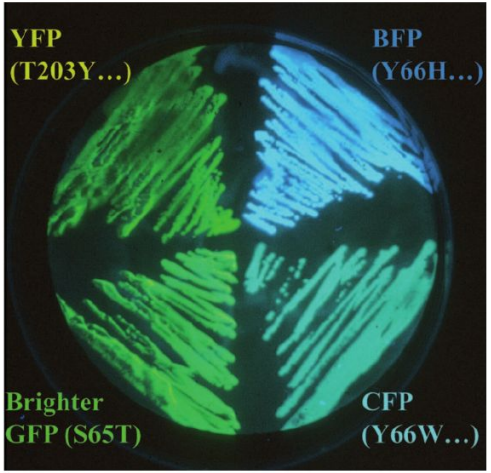
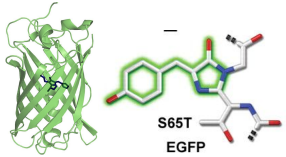
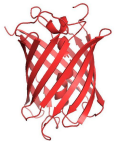
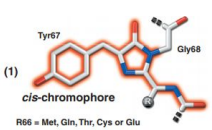


Image Credit: Roger Tsien Nobel Lecture

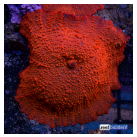
Fluorescent Spectrum & Structures







Discosoma



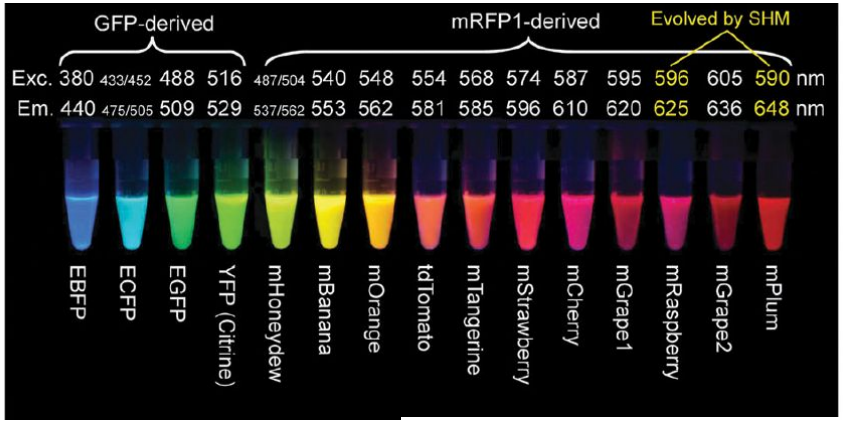
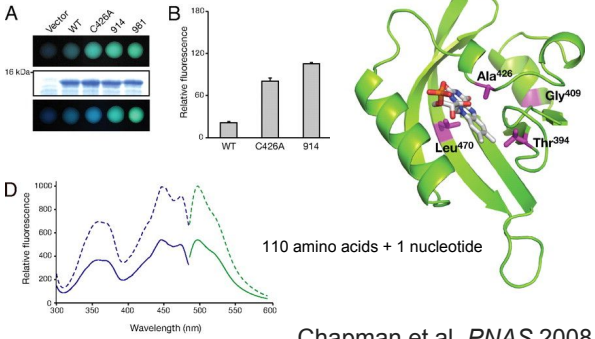
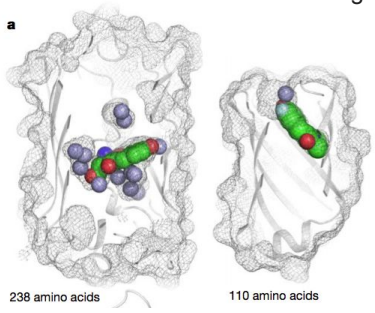


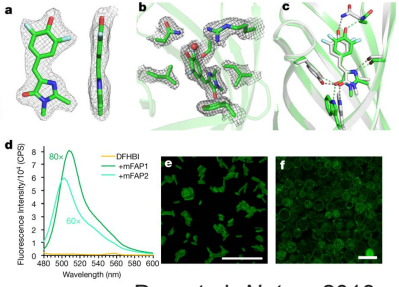
Image Credit: Roger Tsien Nobel Lecture

Arabidopsis thaliana

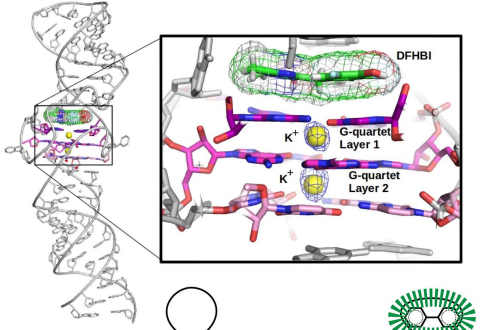


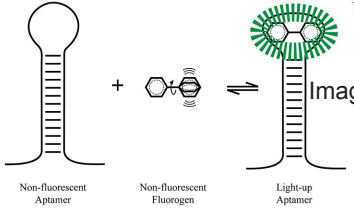
Chapman et al, *PNAS* 2008





Dou et al, *Nature* 2018





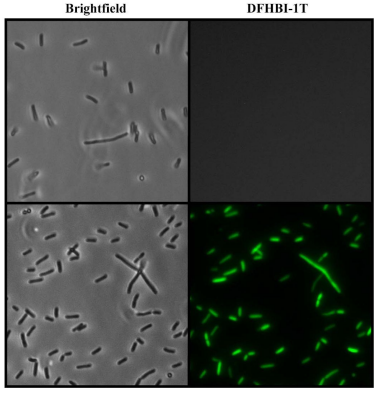
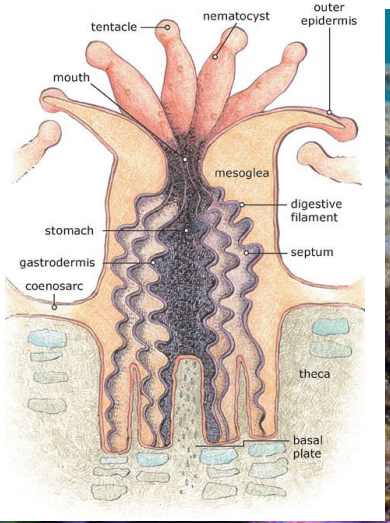


Image Credit: Roger Tsien Nobel Lecture

Protein Pigments

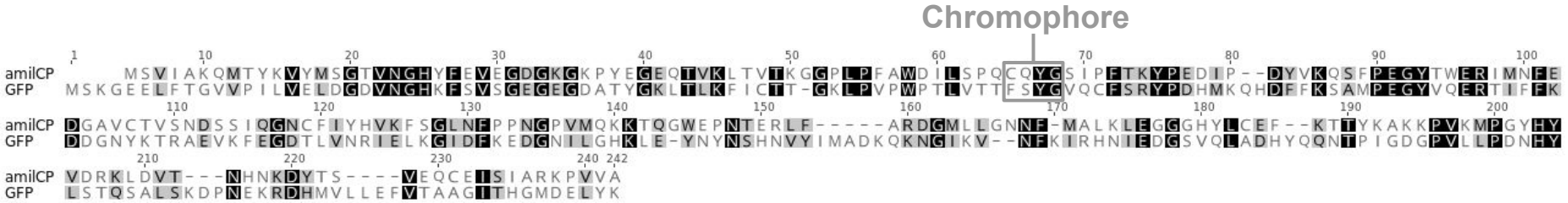


Acropora millepora

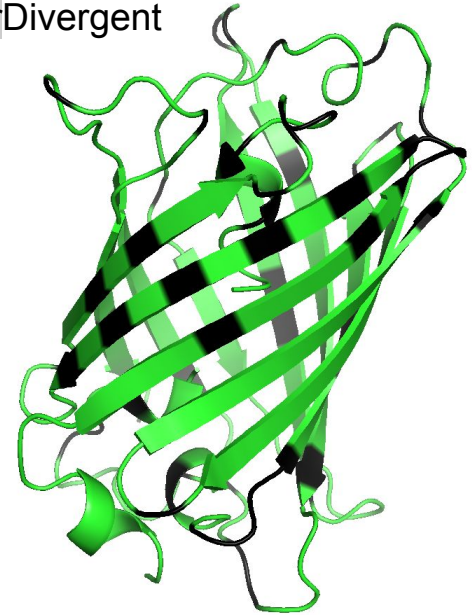
Protein Pigment Pellet Palette

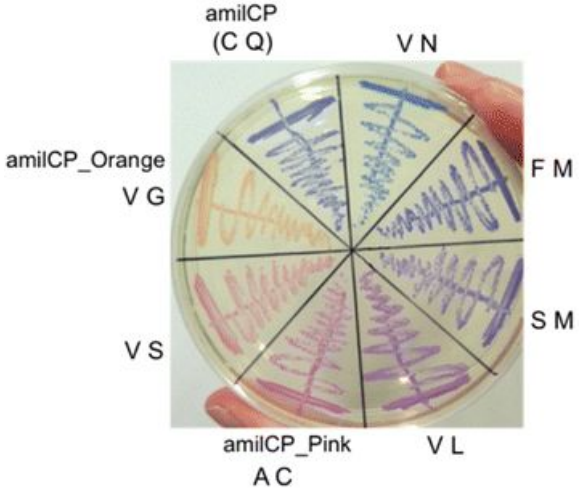
Pellet	BioBrick[16]	Name	Other names	Host eukaryote	Chromo-phore*	Excitation max. (nm)	Emission max. (nm)	Source ref.
S	K1033922	meffRed	meffRFP	*Montipora efflorescens*	FDYG	560	576	2
	K592012	eforRed	eforCP	*Echinopora forskaliana*	HMYG	589	609	2
	K1033927	asPink	asCP, asFP595	*Anemonia sulcata*	cMYG	568	595	19
	K1033925	spisPink	spisCP	*Stylophora pistillata*	LKYG	560	NF	2
	K1033913	scOrange	ScroogeOrange	Synthetic	cMYG			15
	K1033910	fwYellow	FezziwigYFP	Synthetic	LTYG	520	540	15
	K592010	amilGFP		*Acropora millepora*	FQYG	503	512	2
	K1033916	amajLime	amajCFP, amFP486	*Anemonia majano*	FKYG	458	486	18
S	K592011	cjBlue		*Cnidopus japonicus*	cQYG	610	NF	20
S	K1033902	meffBlue	Rtms5, NF pocilloporin	*Montipora efflorescens*	cQYG	592	NF	21
	K864401	aeBlue	aeCP597	*Actinia equina*	cMYG	597	NF	22
	K592009	amilCP		*Acropora millepora*	cQYG	588	NF	2
	K1033906	tsPurple	TinselPurple	Synthetic	cMYG			15
	K1033927	gfasPurple	gfasCP	*Galaxea fascicularis*	sQYG	577	NF	2

Creating the Chromophore Colorwheel



Conserved Similar Divergent

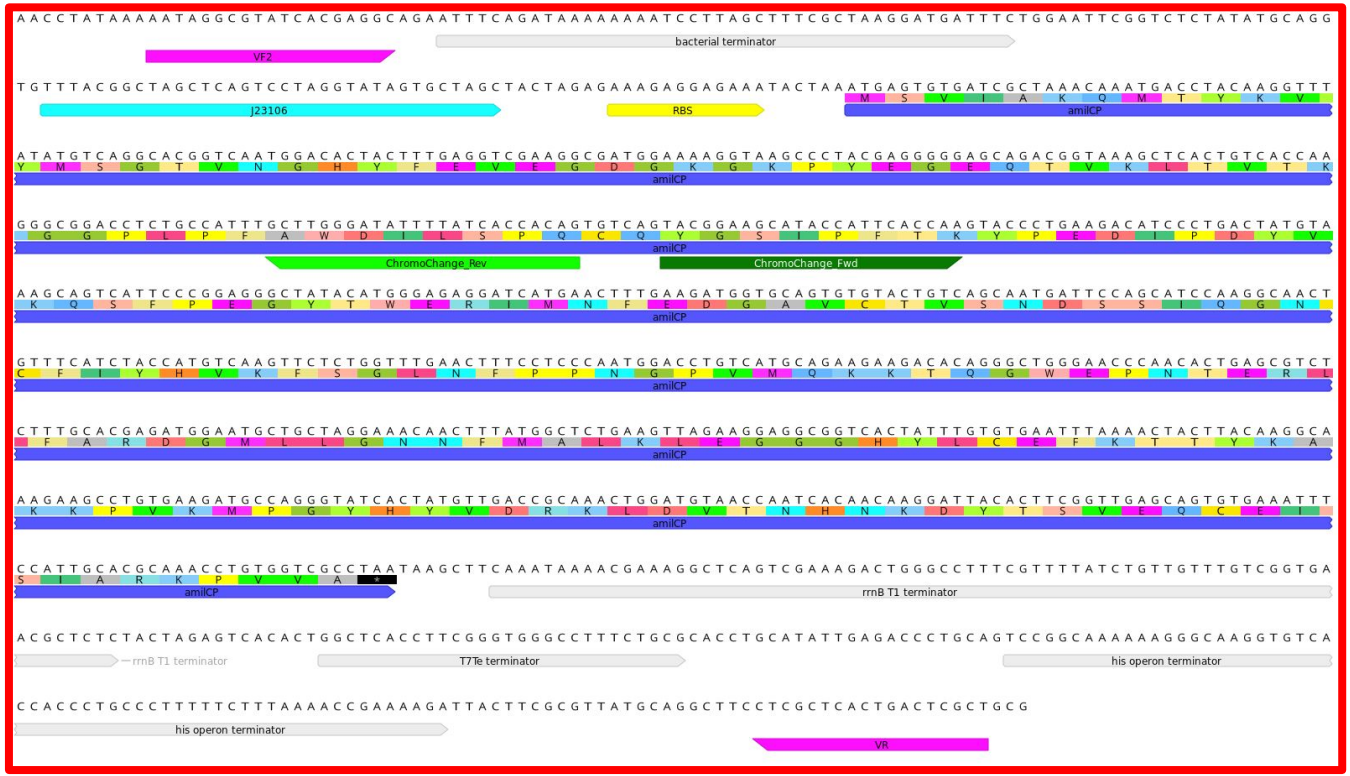




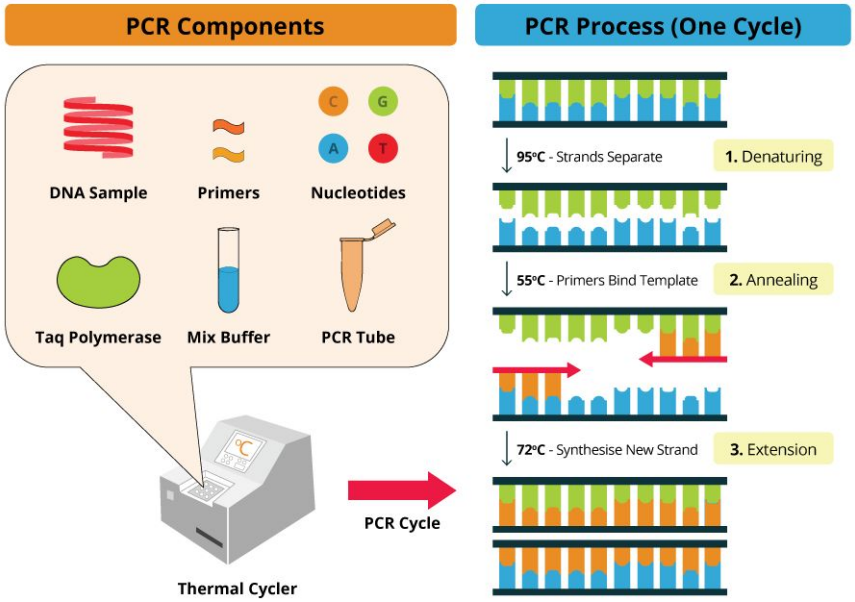
Part Map and Sequence for Assignment

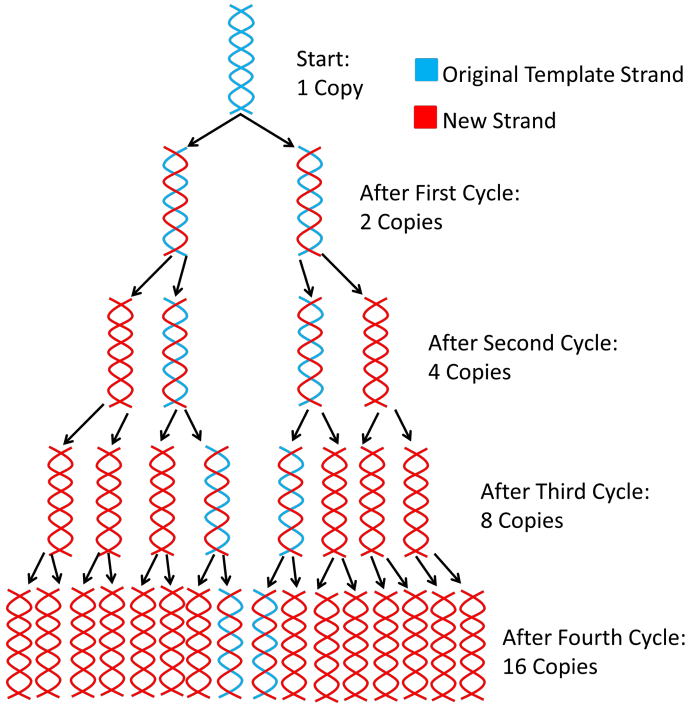
J23106: Promoter (Start DNA -> RNA)
RBS: Ribosome Binding Site (RNA -> Protein)
amilCP: Chromoprotein Coding Sequence (CDS)
Terminators: Transcription Stop (Stop DNA -> RNA)

VF2: Fwd Primer Site (PCR)
VR: Rev Primer Site (PCR)

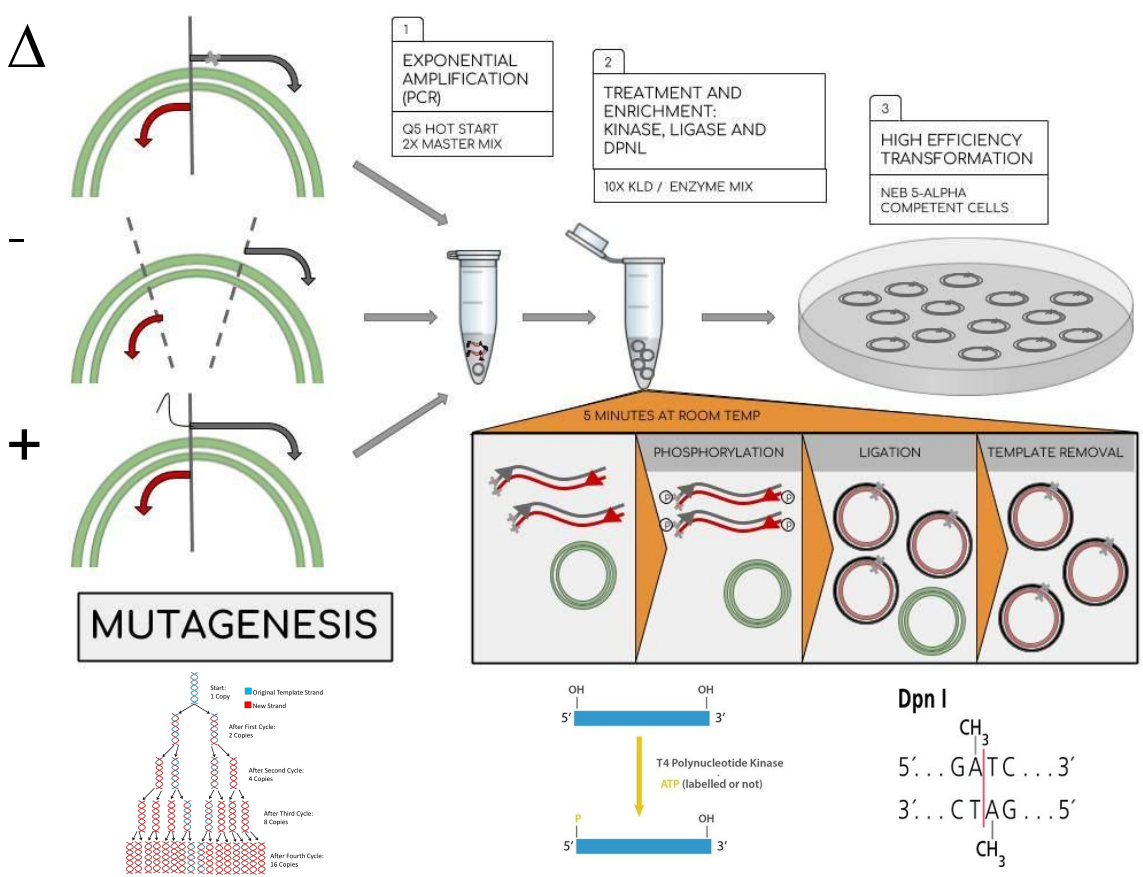


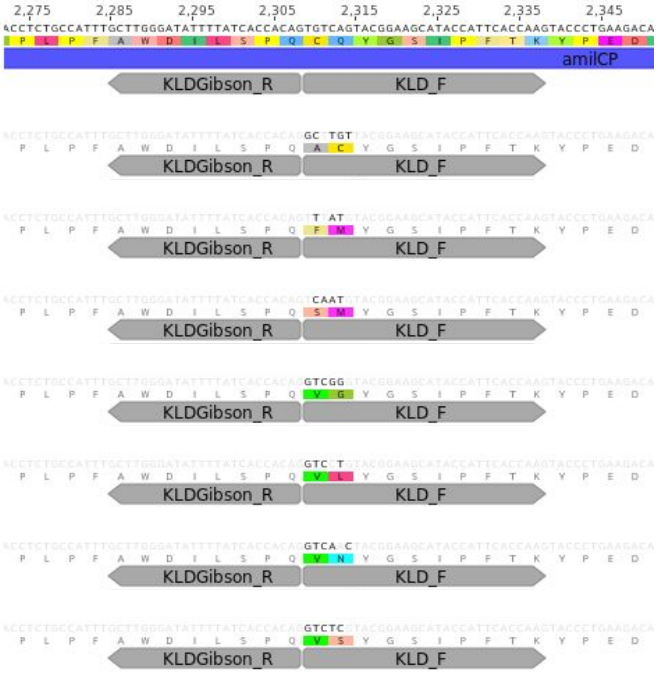
Copying and Changing DNA with Polymerase Chain Reaction



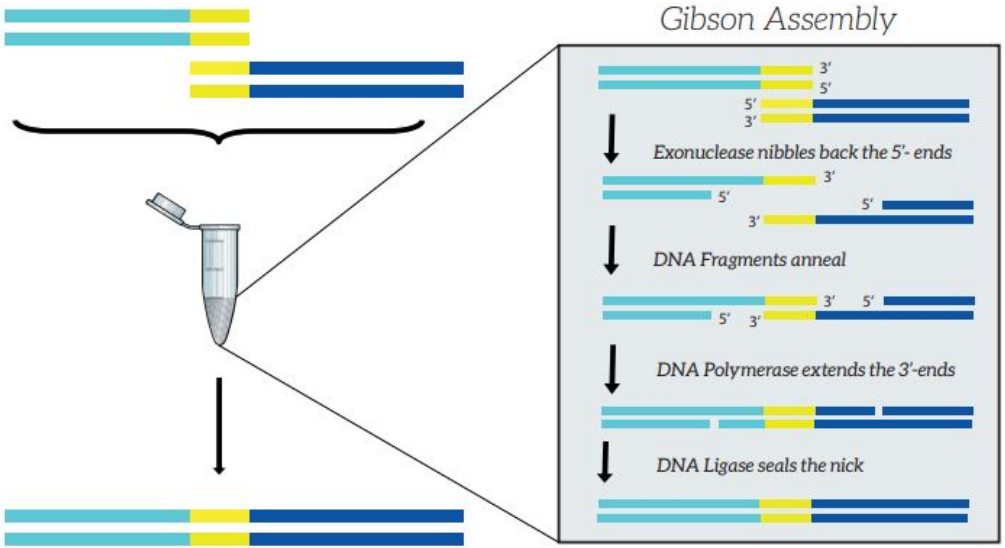


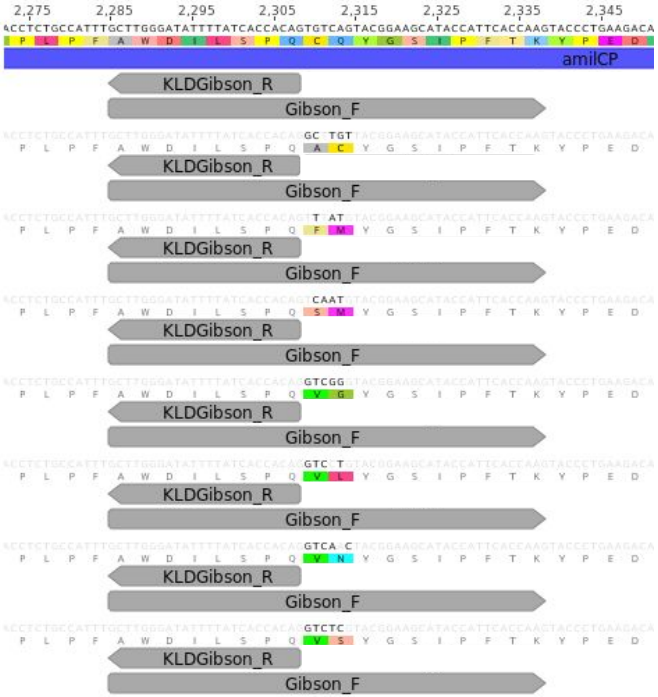
Point Mutagenesis with KLD (Kinase, Ligase,DpnI) Mix



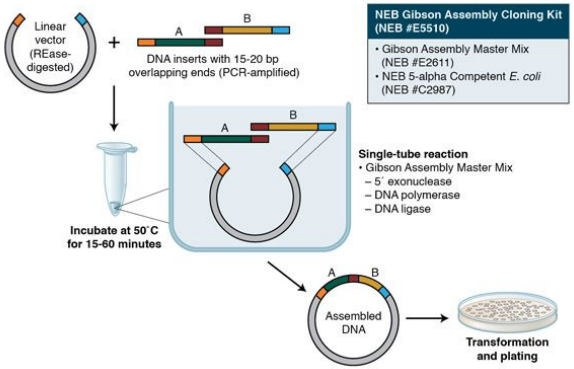


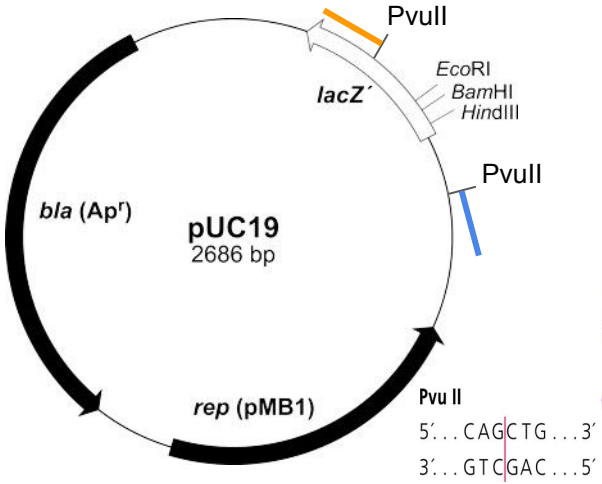
Gibson Assembly Mechanism

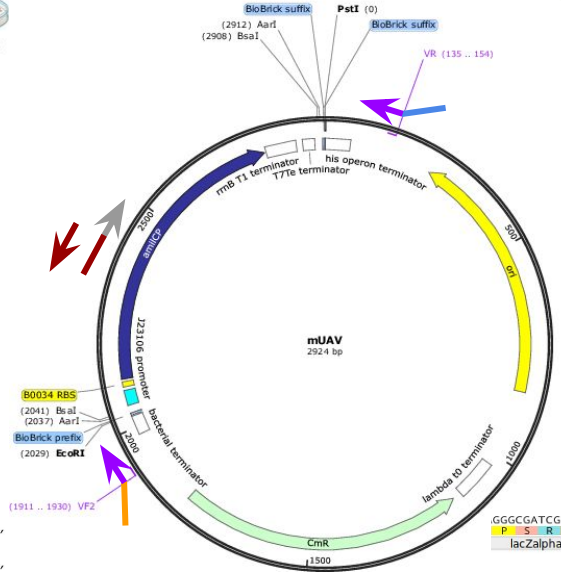


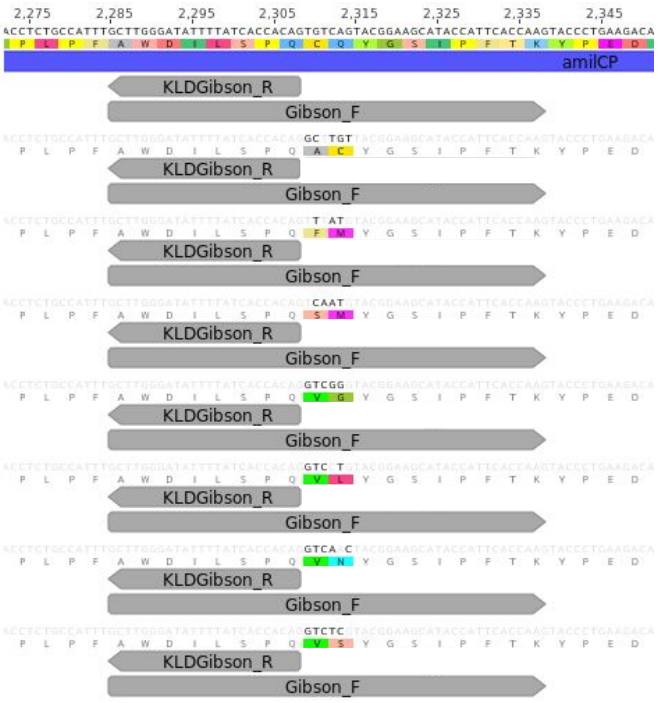


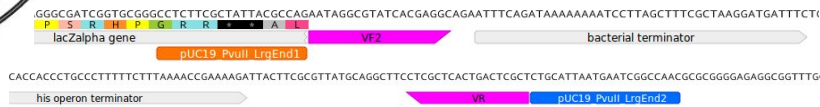
Gibson Assembly for Mutagenesis and Drug Selection Swap



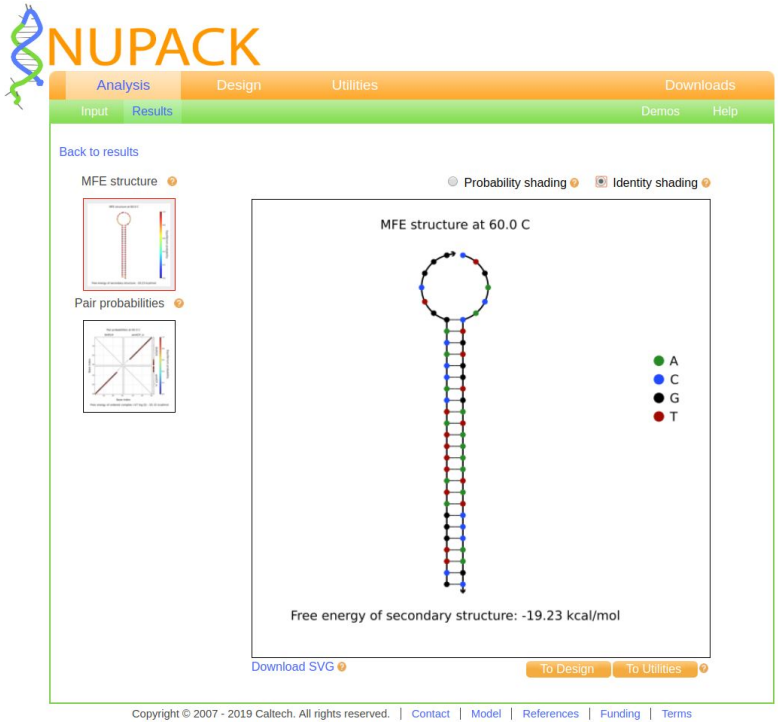


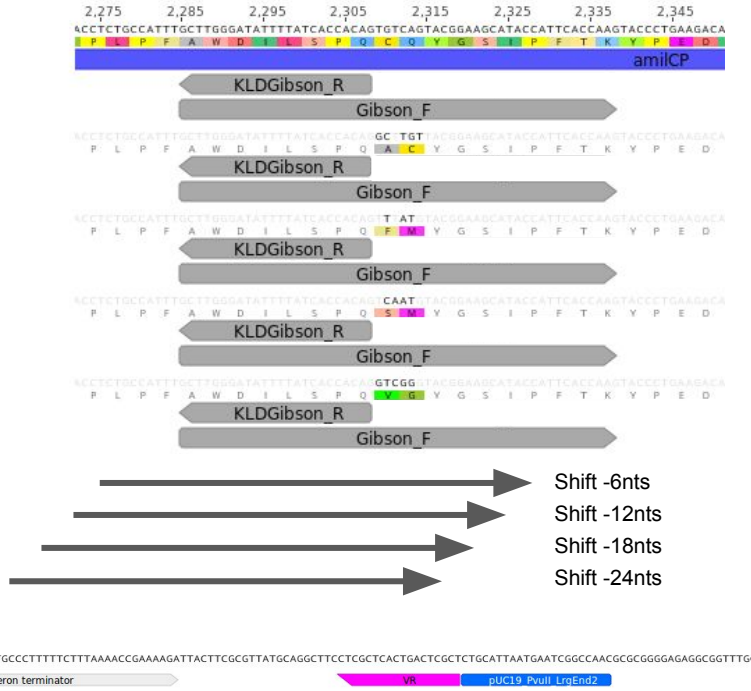




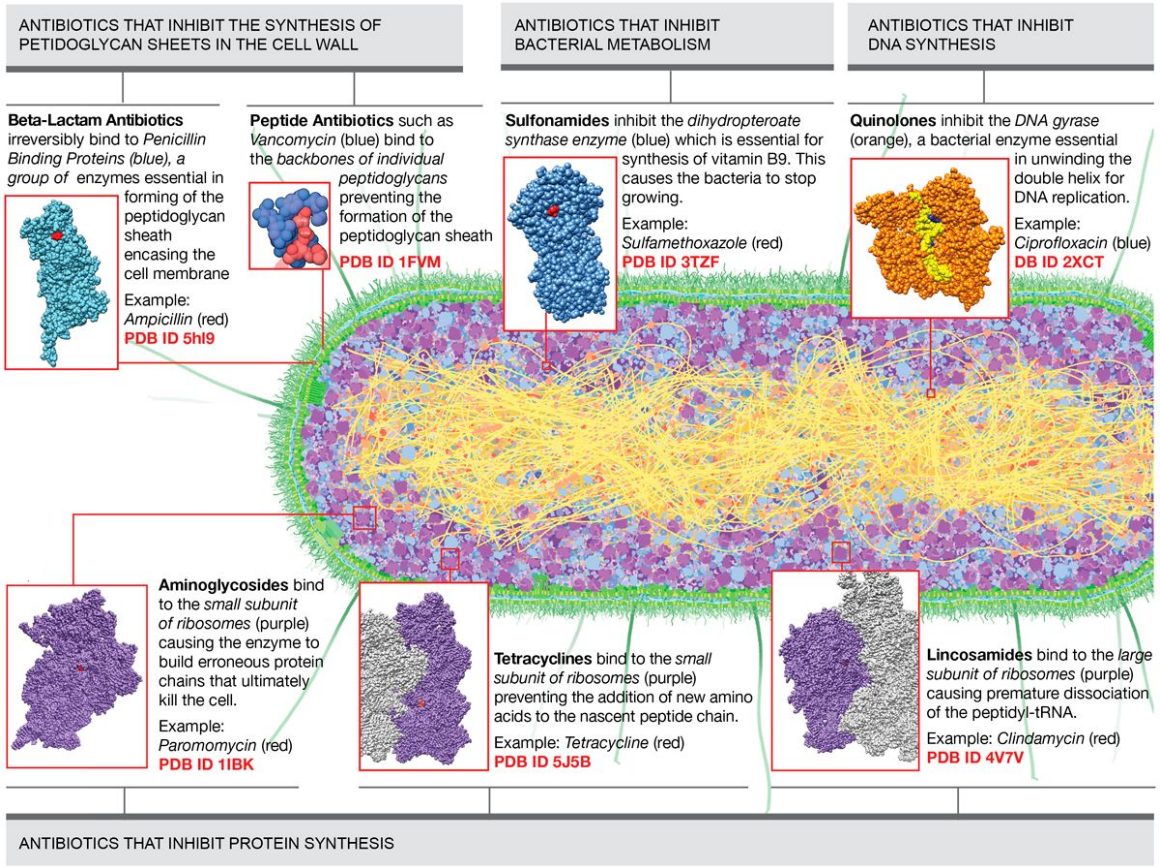


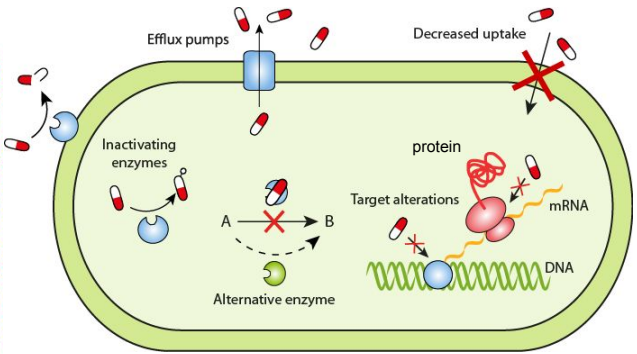
(Bonus) Primer Fitness Prediction



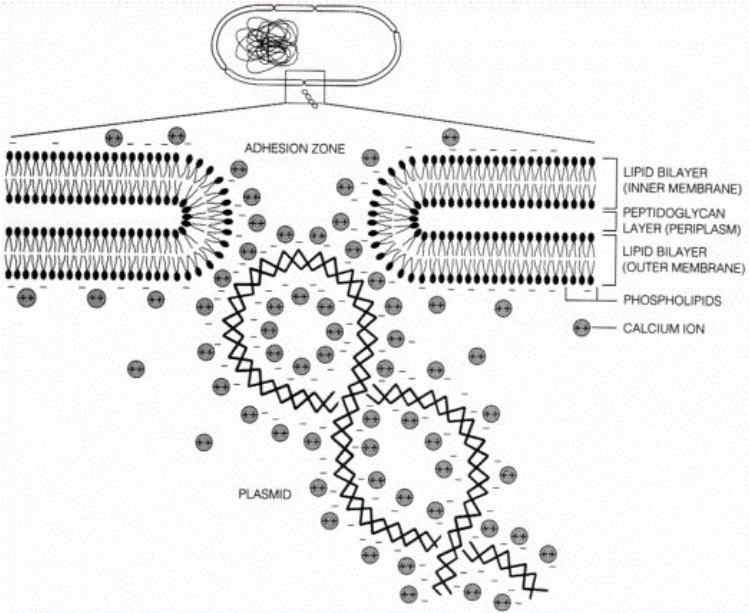


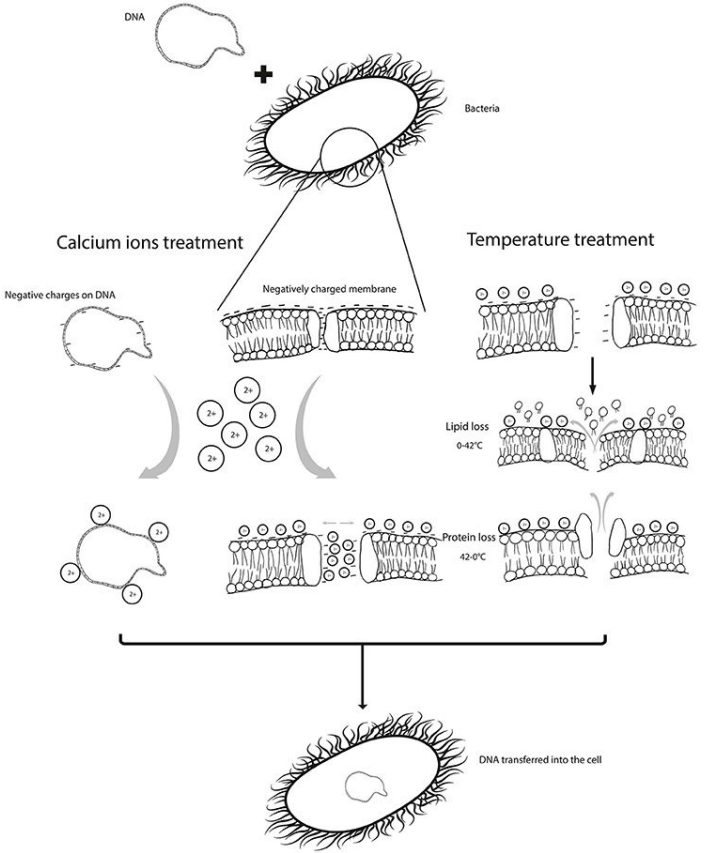
Alternative Antibiotic Selection





Plasmid Uptake by Calcium-Assisted Heat Shock





Asif et al, *Front Microbiol* 2017