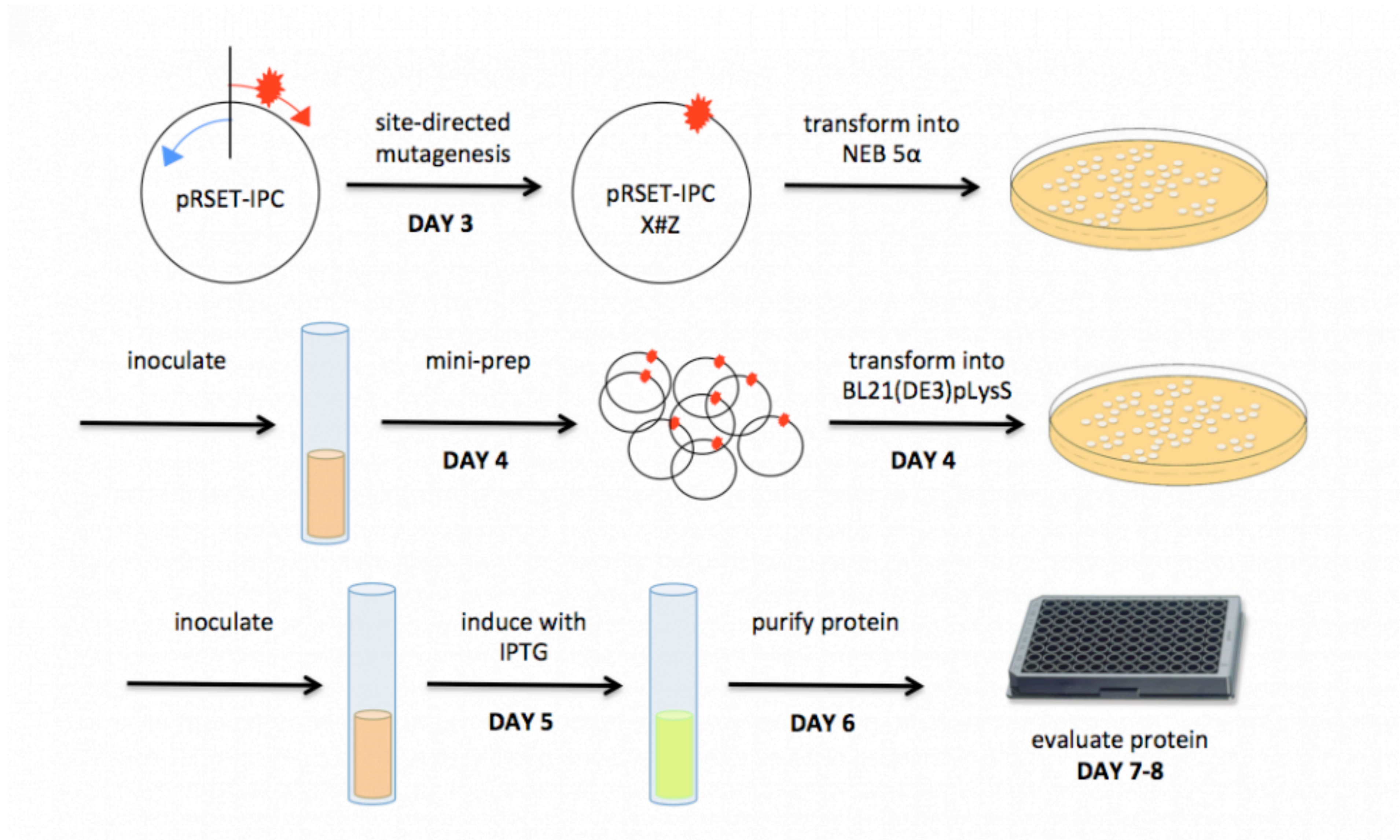
M1D3:Site-directed mutagenesis

02/11/16

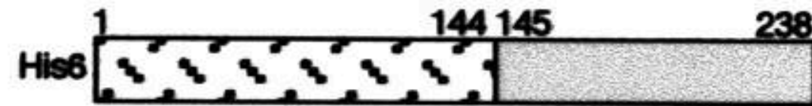
1. BE Communications lab workshop: figures and figure captions
2. Prelab Discussion
3. Set up site-directed mutagenesis reaction
4. Group paper discussion: Nagai et al.

M1 experimental overview

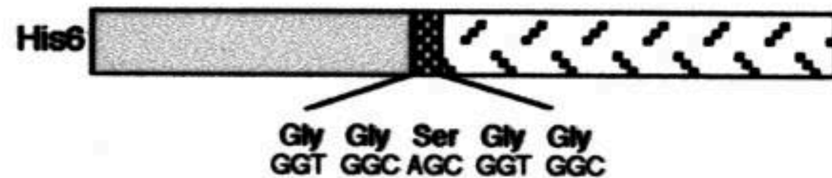


Inverse pericam (IPC) is dimmer with Ca^{2+}

EYFP (V68L/Q69K)

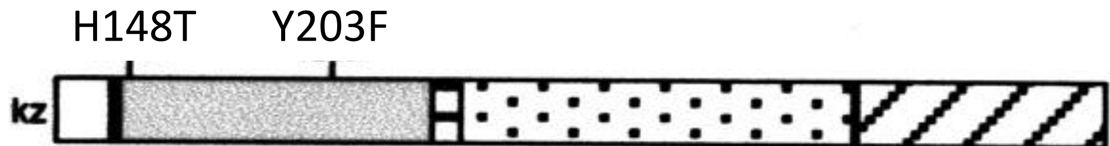


cpEYFP(V68L/Q69K)



pericam

inverse-pericam



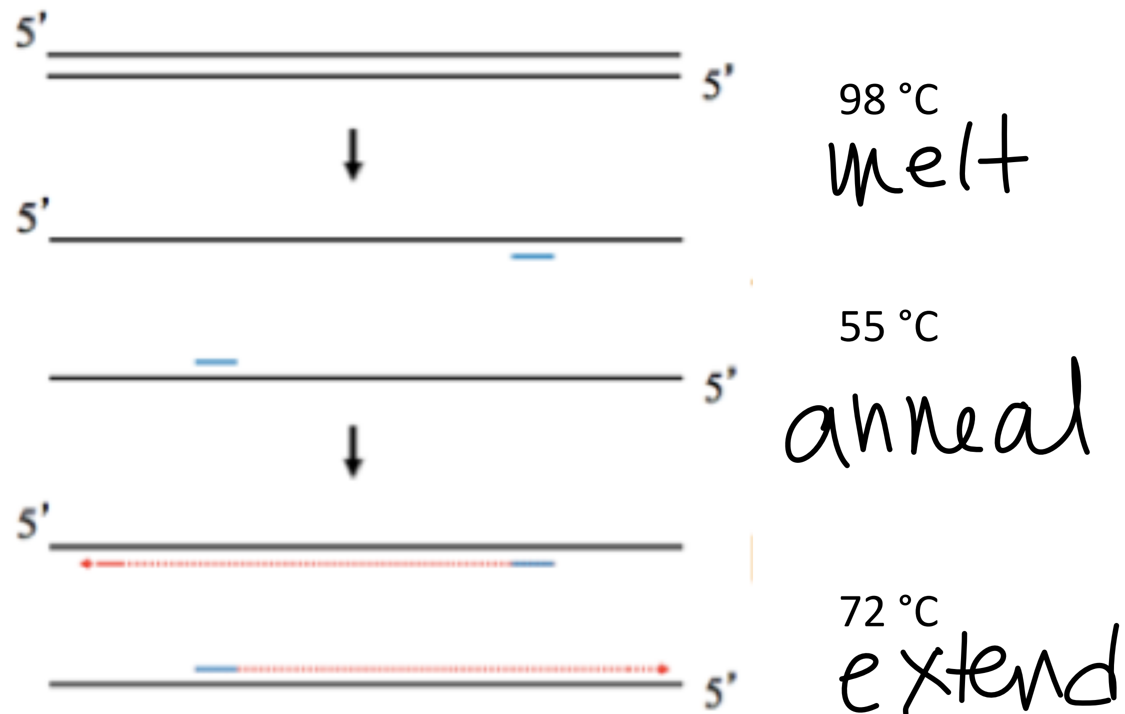
Directed Evolution or

★Rational Design★

SDM ingredients and cycling conditions

SDM ingredients
template
polymerase
primers
dNTPs
buffer
Mg++
H_2O

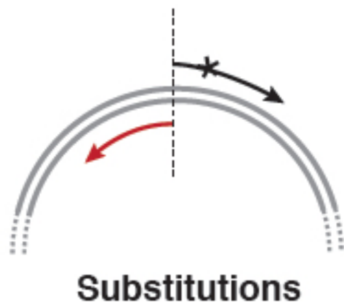
start circular



25 cycles

linear DNA

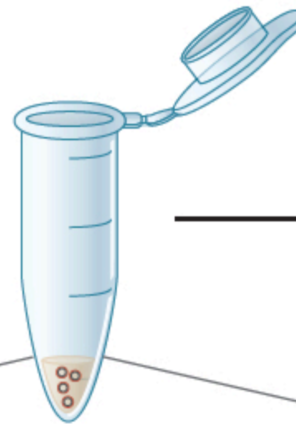
SDM steps with NEB Q5 kit

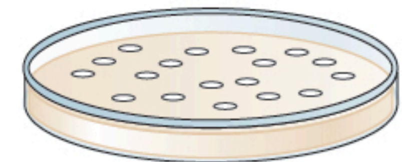


1. Exponential Amplification (PCR)
- Q5 Hot Start 2X Master Mix

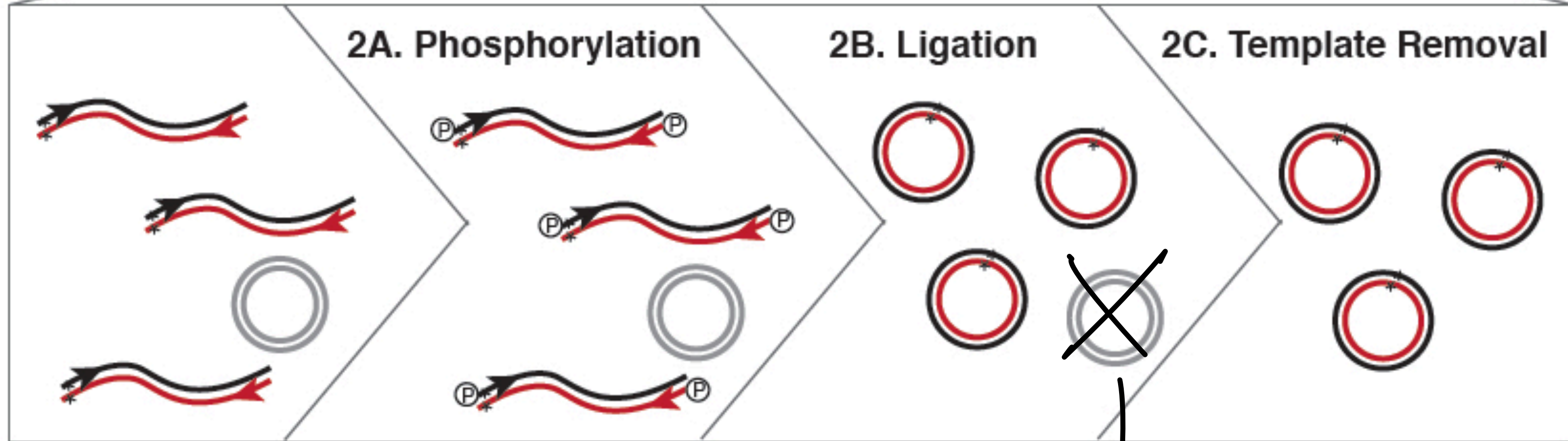
2. Treatment and Enrichment: Kinase, Ligase and DpnI
- 10X KLD Enzyme Mix

3. High-Efficiency Transformation
- NEB 5-alpha Competent Cells

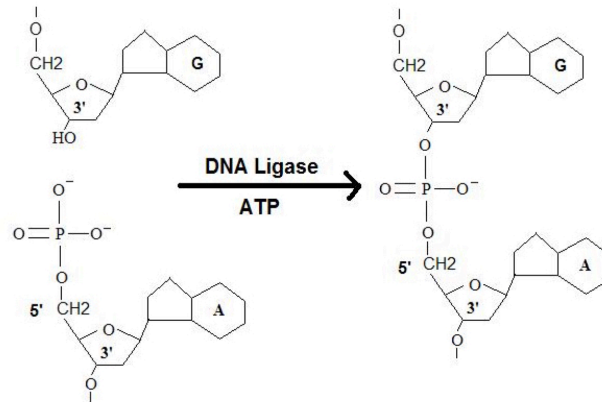




2. Kinase, Ligase, DpnI



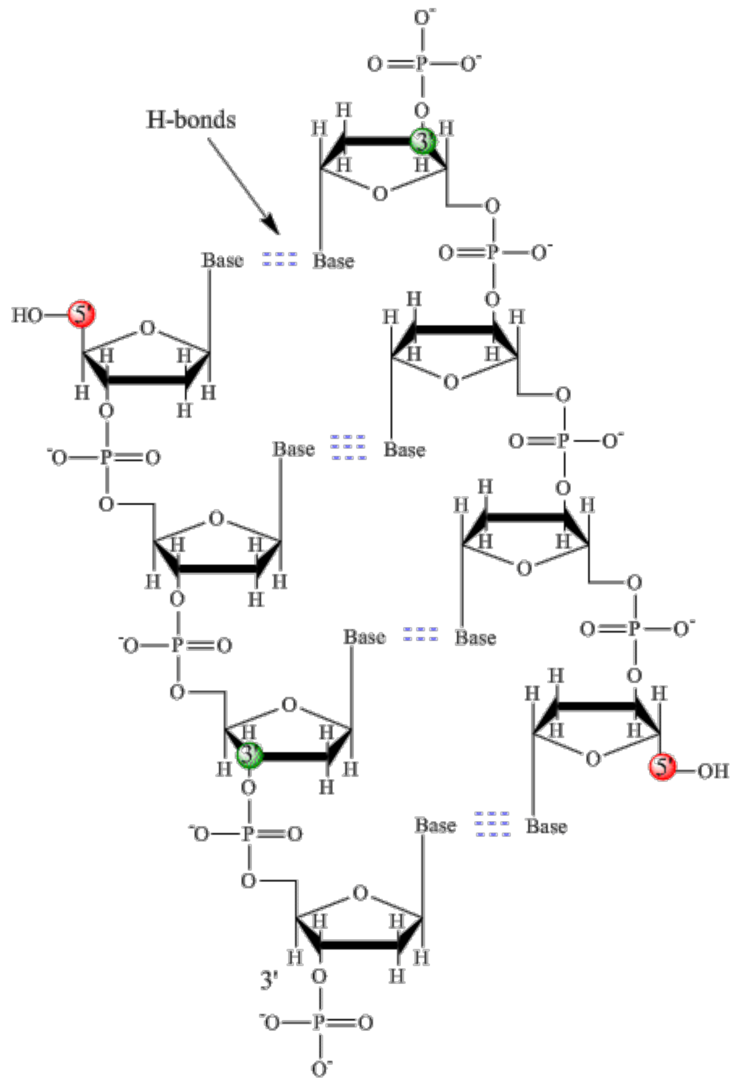
DpnI - cuts methylated DNA

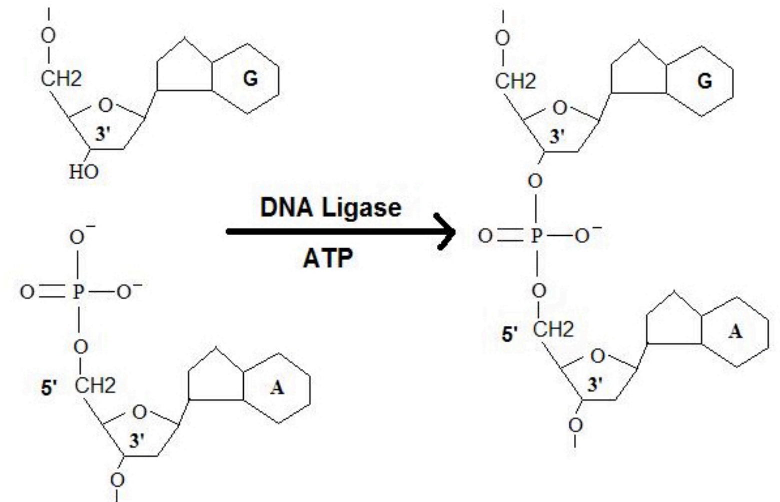


amp. in bacteria → methylated

Today in lab:

1. Calculate volume of water to reconstitute primers
2. Make primer stock and working dilution
3. Set up site-directed mutagenesis (SDM) reaction
4. Put SDM reaction in thermocycler
5. Nagai paper discussion


H-bonds
Base
Base
HO
5'
3'
OH
O
P
H
3'


CH2
HO
3'
5'
G
A
DNA Ligase
ATP