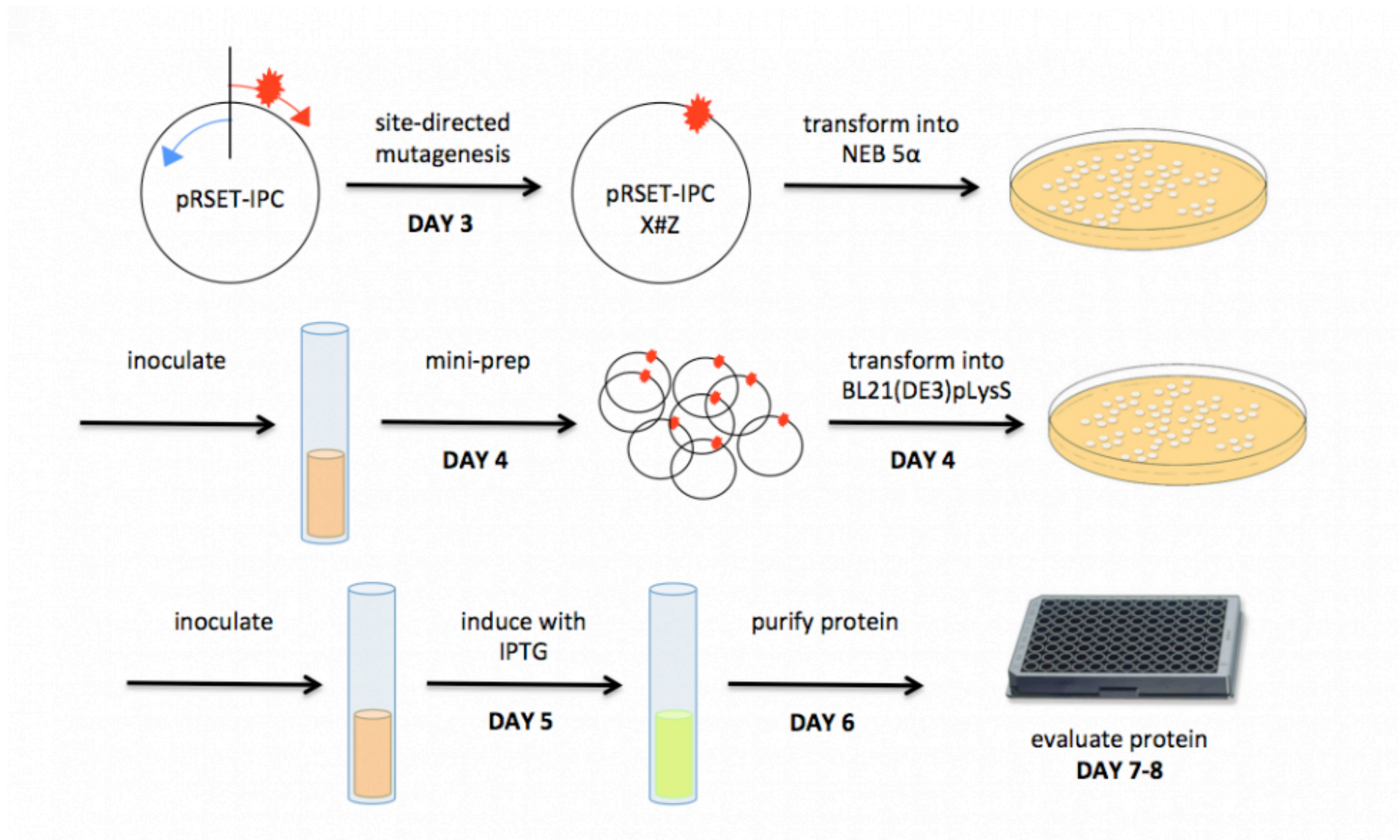


M1D3:Site-directed mutagenesis

02/11/16

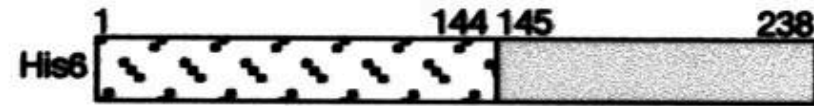
1. BE Communications lab workshop: figures and figure captions
2. Prelab Discussion
3. Set up site-directed mutagenesis reaction
4. Group paper discussion: Nagai et al.

M1 experimental overview



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

EYFP (V68L/Q69K)



cpEYFP(V68L/Q69K)



Gly Gly Ser Gly Gly
GGT GGC AGC GGT GGC

pericam

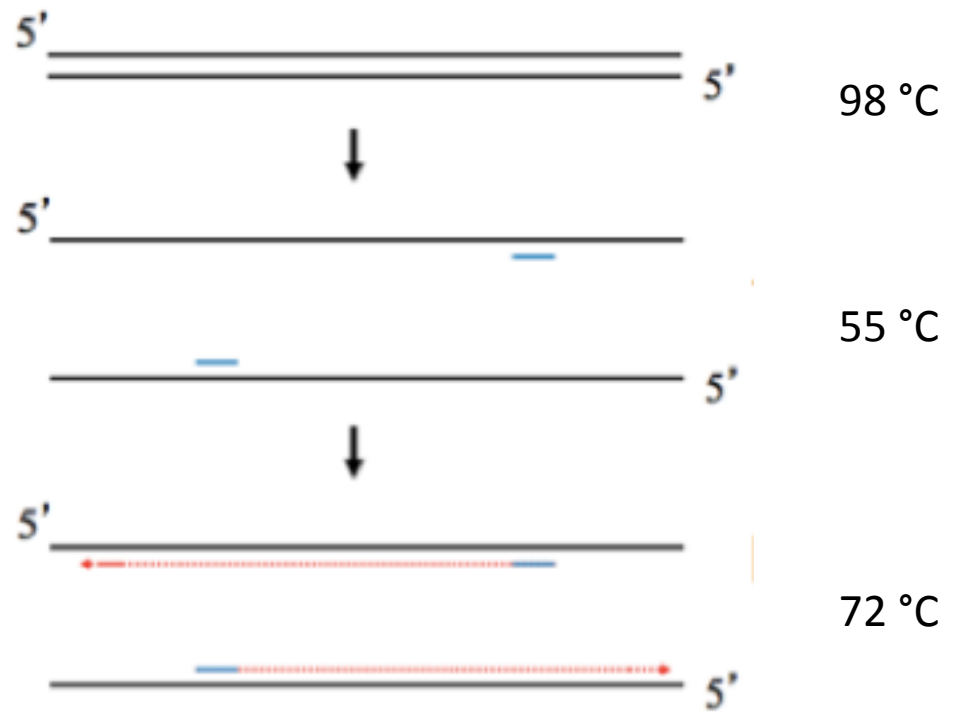
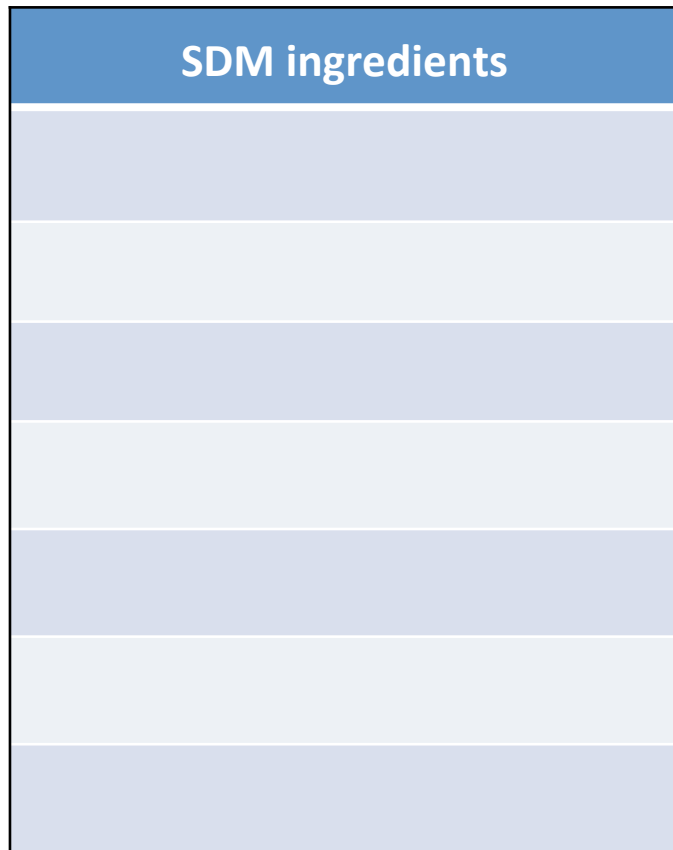


H148T Y203F

inverse-pericam

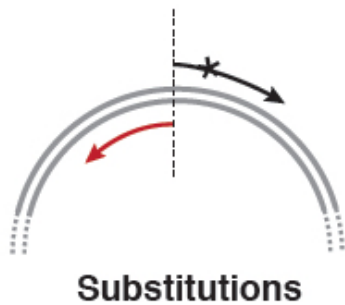


SDM ingredients and cycling conditions



25 cycles

SDM steps with NEB Q5 kit



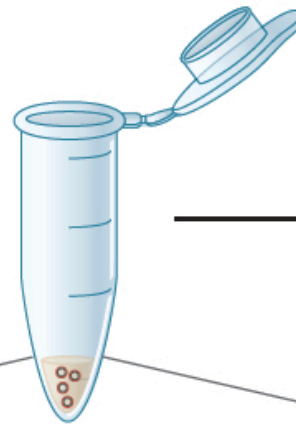
1. Exponential Amplification (PCR)

- Q5 Hot Start
- 2X Master Mix



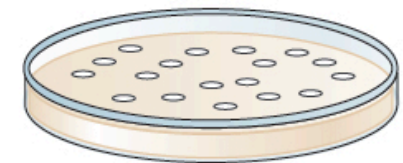
2. Treatment and Enrichment: Kinase, Ligase and DpnI

- 10X KLD
- Enzyme Mix



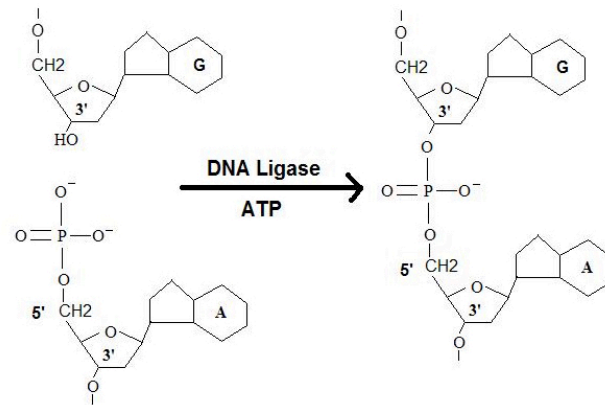
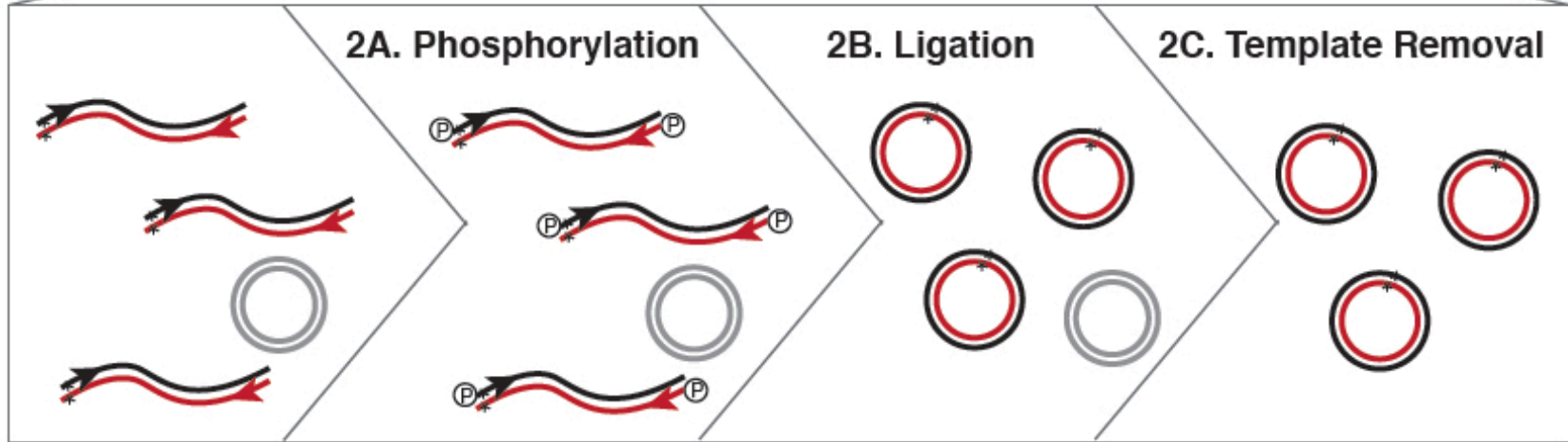
3. High-Efficiency Transformation

- NEB 5-alpha
- Competent Cells



2. Kinase, Ligase, Dpnl

5 min. at room temp.



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

