

# M1D3: Site-directed mutagenesis

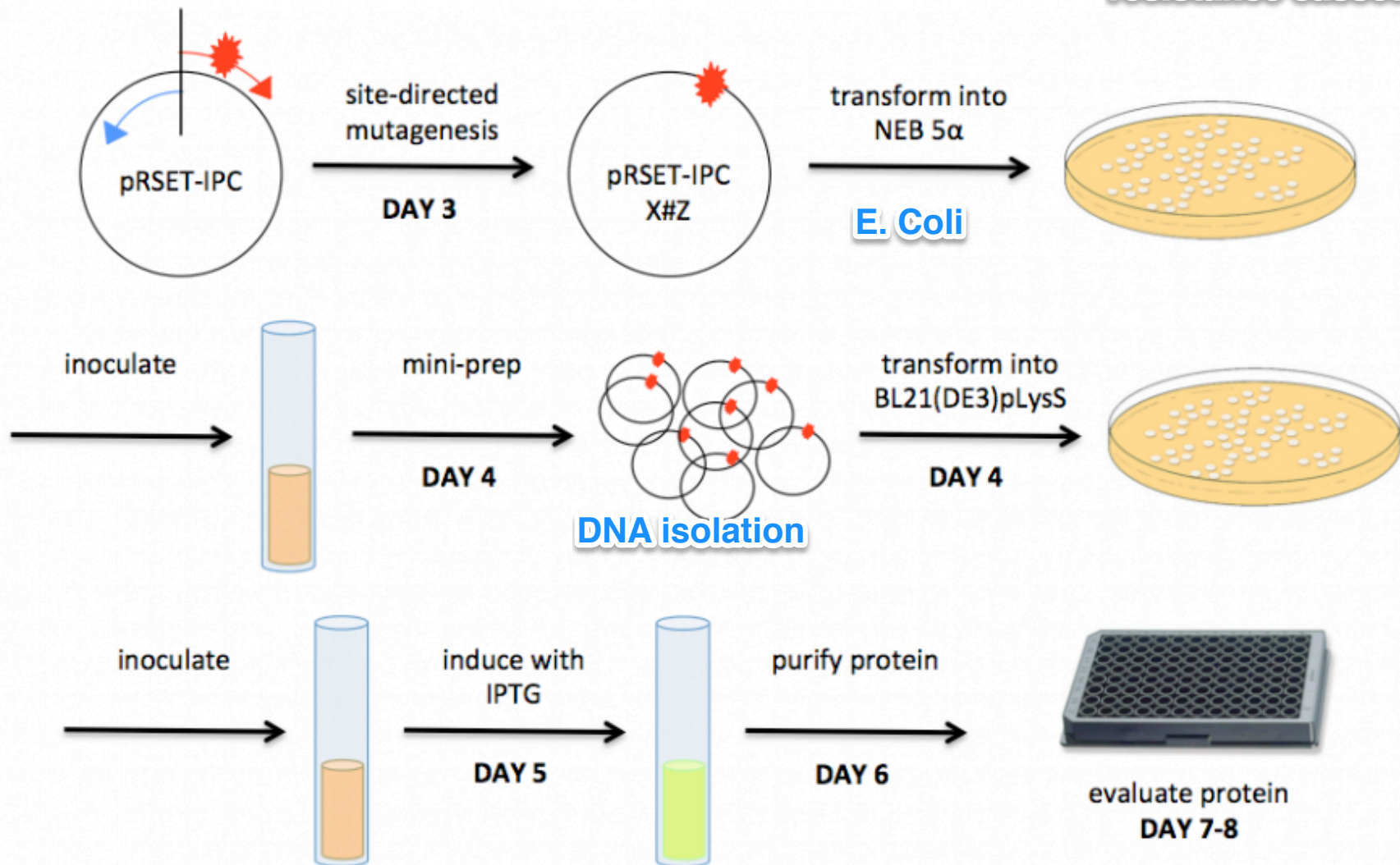
02/12/2016



# M1 experimental overview

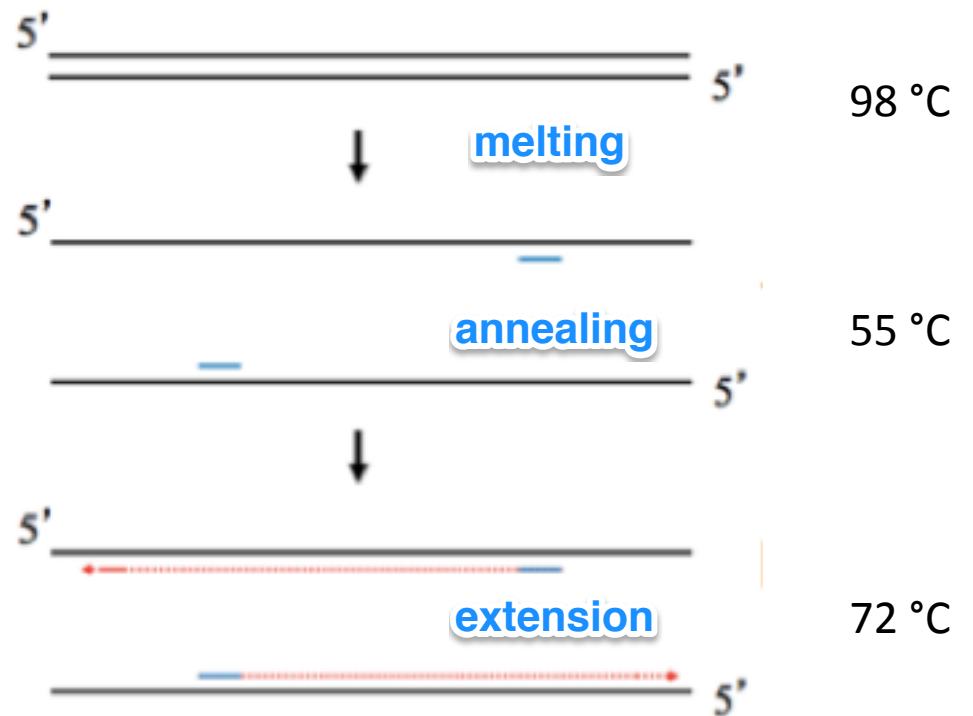
rational design or directed evolution?

pRSET has an ampicillin resistance cassette



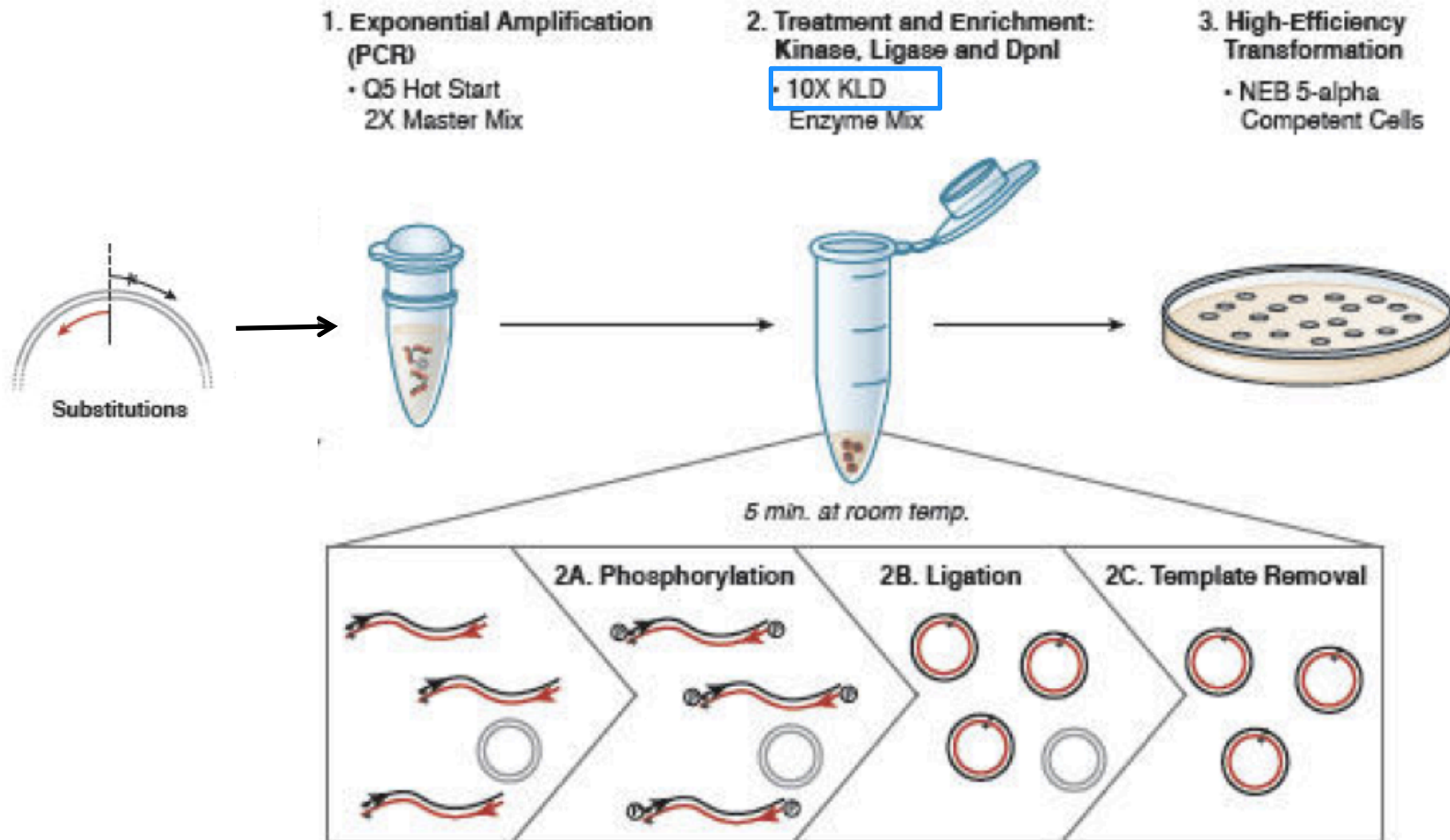
# SDM ingredients and cycling conditions

| SDM ingredients              |
|------------------------------|
| primers (0.5 $\mu\text{M}$ ) |
| template pRSET-IPC (25 ng)   |
| dNTPs                        |
| polymerase HF                |
| Mg <sup>2+</sup> cofactor    |
| buffer                       |
| water                        |



25 cycles

# SDM steps with NEB Q5 kit



template DNA was generated by bacteria, got methylated  
DpnI selectively digests methylated DNA

# Today in lab

- BE Communication Lab workshop
  - Figures & captions
- Reconstitute your primers
  - Add water for 100  $\mu$ M stocks
- Set up and start site-directed mutagenesis (SDM) reaction
  - KLD treatment & NEB 5 $\alpha$  *E.Coli* transformation tonight by faculty
- Group paper discussion



Happy Valentine's Day!  
Happy Presidents' Day!



Neither lecture nor lab next Tuesday / Wednesday!