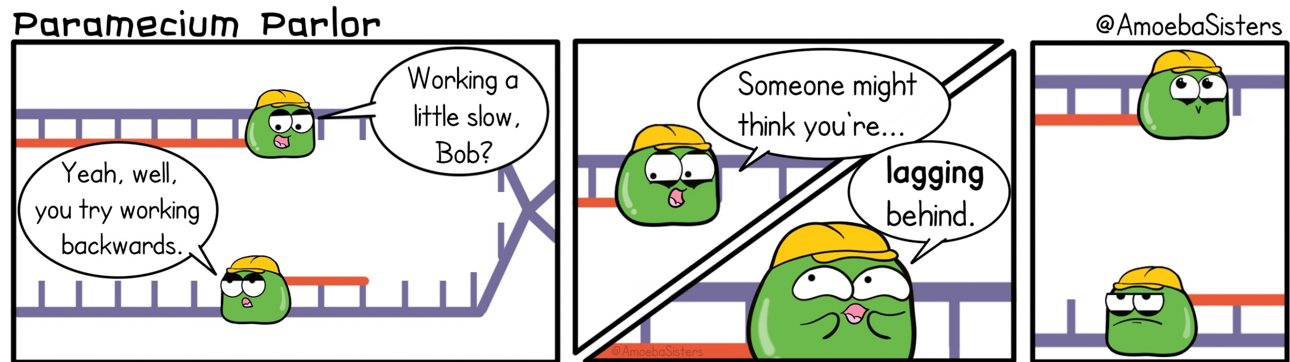
M2D2: Perform site-directed mutagenesis

1. Pre-lab discussion
2. Perform site-directed mutagenesis
3. Transform Fet4_mutant plasmid in bacteria cells



Mod2 Overview

Research goal: Increase the ability of the S.cerevisiae low-affinity iron permease, Fet4, to uptake cadmium.

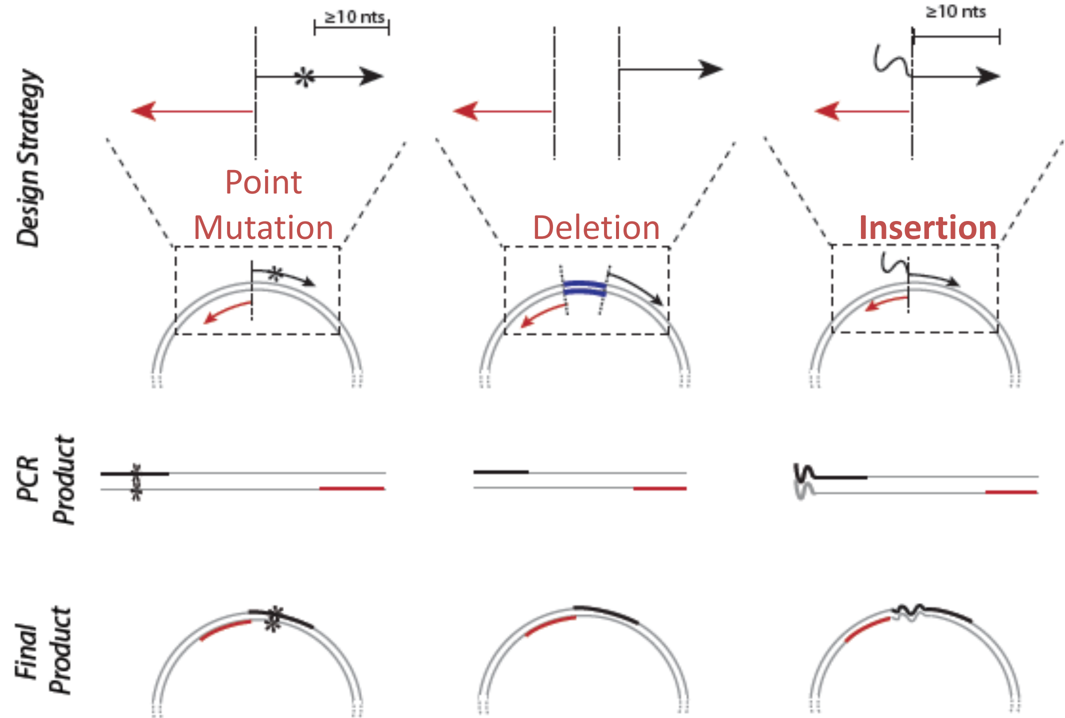
Last Lab:
Examined Fet4 and designed primers for site-directed mutagenesis

This Lab:
Perform site-directed mutagenesis and transform plasmids in bacteria

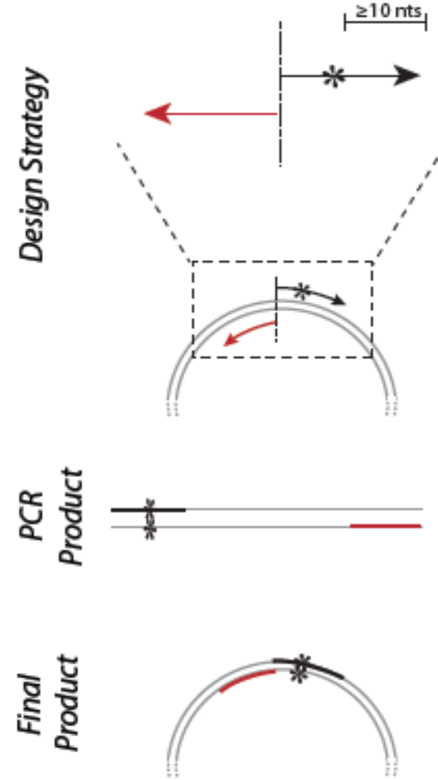
Next Lab:
Sequence clones and transform into yeast cells

Use site-directed mutagenesis (SDM) to engineer plasmid DNA

- NEB Q5 SDM kit
- Create specific, targeted changes in double-stranded plasmid DNA

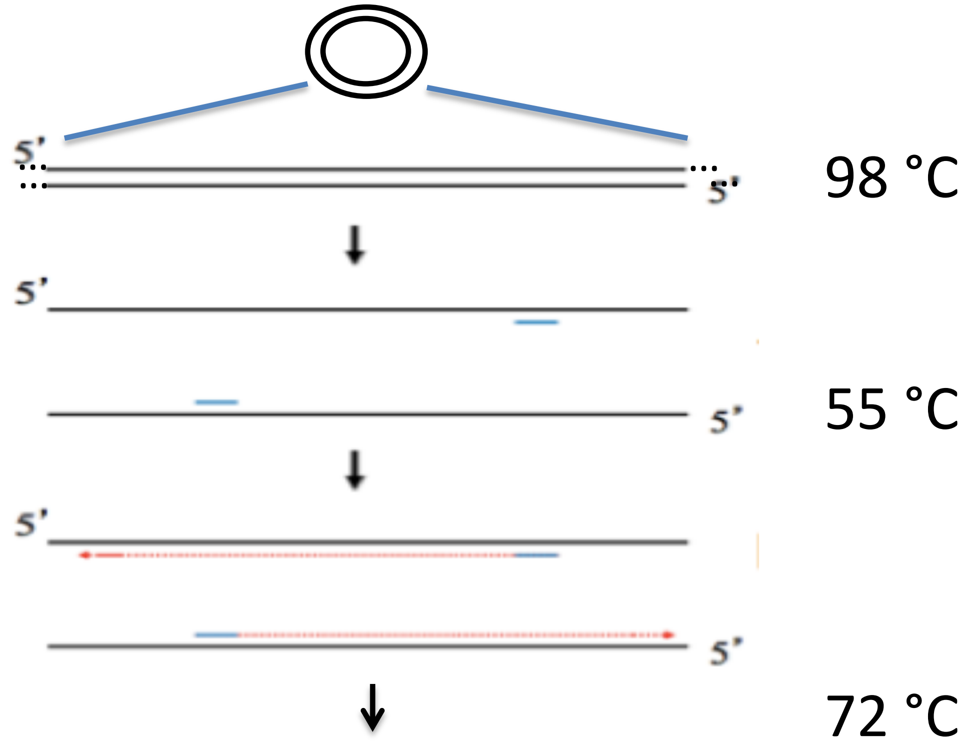


Point mutation of DNA via SDM



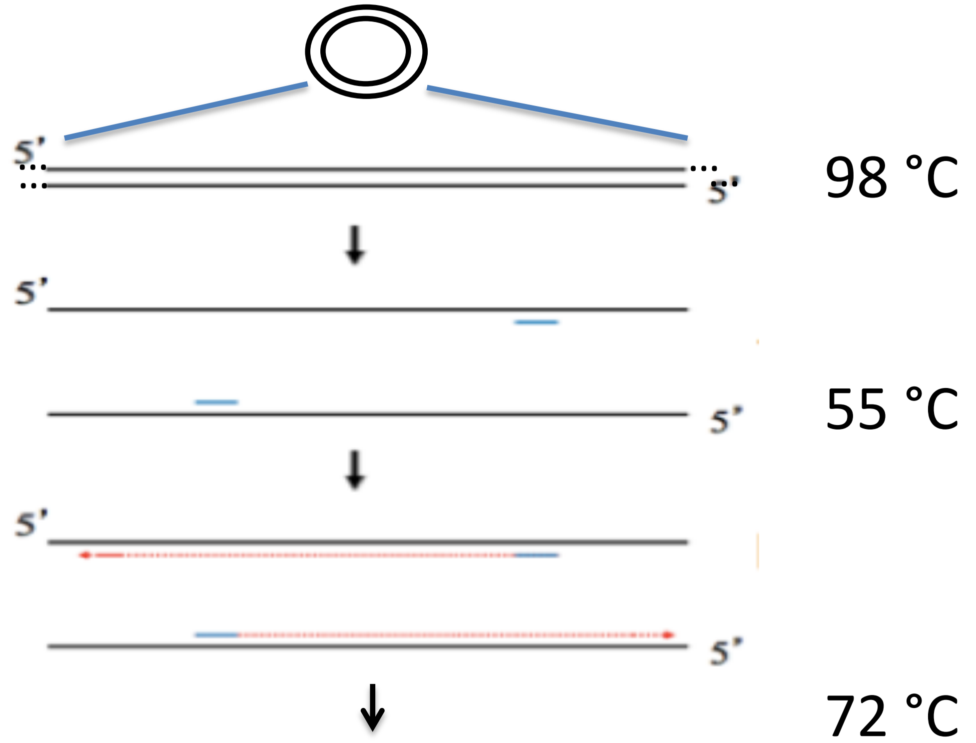
SDM Part 1: PCR amplification of DNA

Ingredients

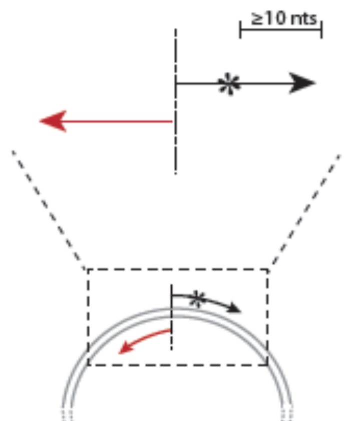


SDM Part 1: PCR amplification of DNA

Ingredients
Forward primer
Reverse primer
template
dNTPs
Polymerase
Buffer (pH, cofactors like Mg^{2+})
H_2O

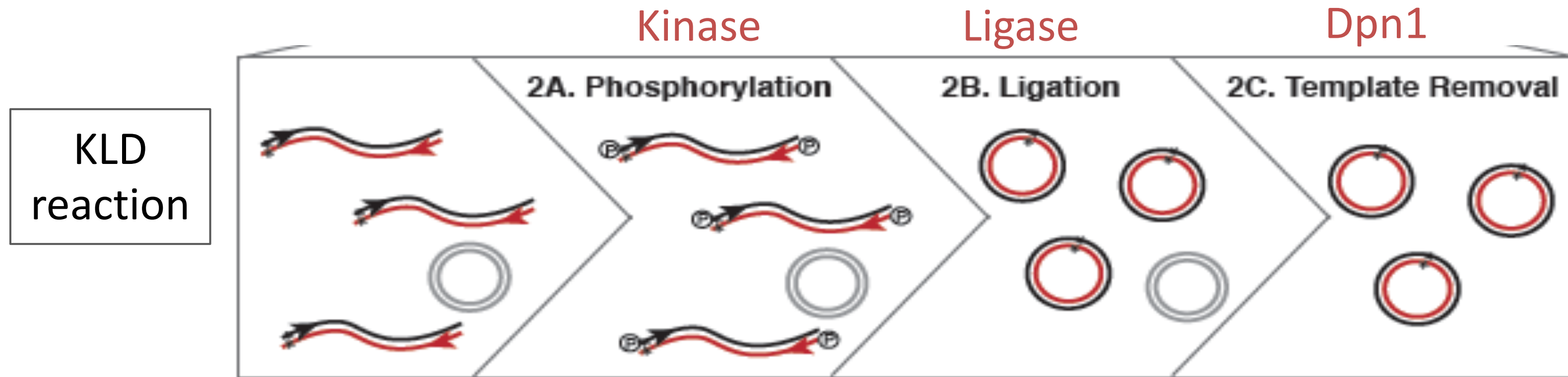


SDM Part 2: Recover circular plasmid product using Kinase-Ligase-Dpn1 (KLD) enzyme mix



1. Exponential Amplificaion (PCR)
   - Q5 Hot Start 2x Master Mix
2. Treatment & Enrichment: Kinase, Ligase and DpnI
   - 10x KLD enzyme mix
3. High-efficiency transformation
   - NEB 5-alpha competent cells

Phosphorylation video:
https://www.neb.com/tools-and-resources/video-library/the-mechanism-of-dna-phosphorylation



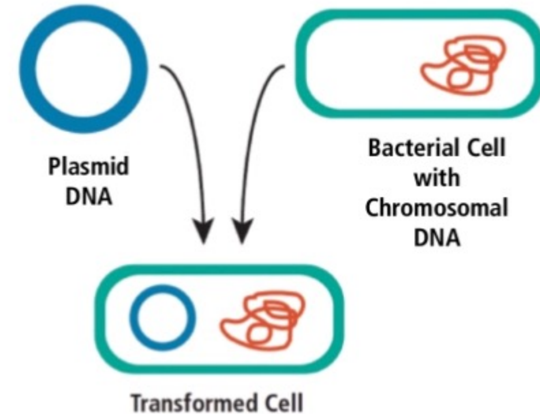
K: Kinase phosphorylates blunt ends, promotes ligation

L: Ligase repairs phosphate backbone, circularizing the plasmid

D: Dpn1 cleaves methylated DNA, which removes the template DNA

Review of bacterial transformation

1. Incubation
2. Heat shock
    1. DNA taken up by competent cells
3. Recovery
4. Selection



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For today

1. Use downtime to prepare upcoming assignments:
    1. Work on Data Summary Draft in downtime (Due Saturday 3/18)
    2. Work on blog post (Due Monday 3/20)

For M2D3…

1. Carefully read selected journal article and choose four figures key to showing main conclusion(s) of paper
2. Email Noreen with preferred presentation date