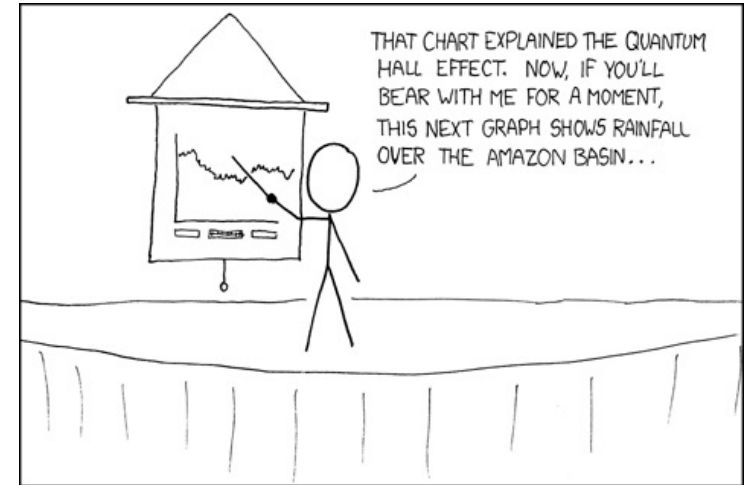


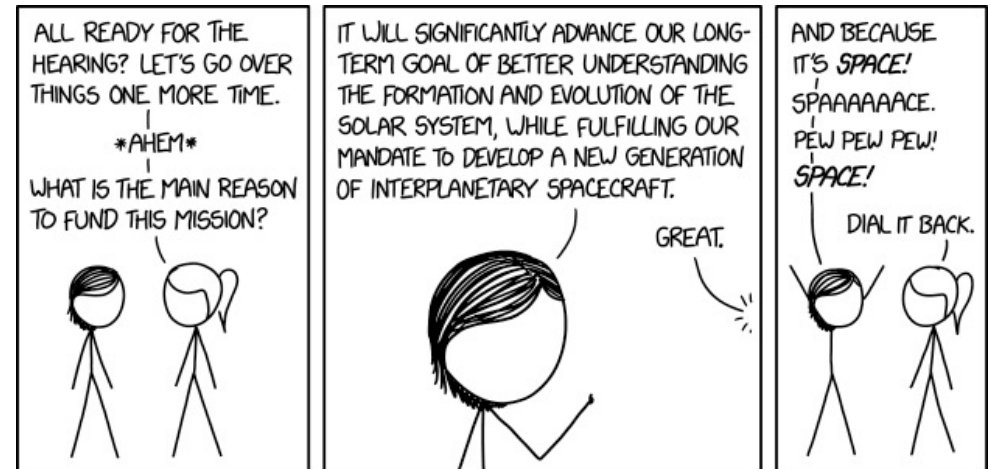
M3D4: Design new IPC variant

- Prelab discussion
- Determine improved IPC variant and design mutation primers
- Quiz beginning at 3:15pm

** Will use the societal/ethical implications in proposal



IF YOU KEEP SAYING "BEAR WITH ME FOR A MOMENT", PEOPLE TAKE A WHILE TO FIGURE OUT THAT YOU'RE JUST SHOWING THEM RANDOM SLIDES. xkcd



Important Mod 3 Due Dates

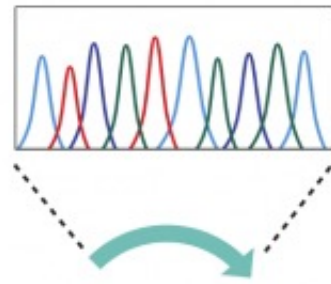
- **Research proposal presentation** (20%)
 - completed in teams and presented via Zoom
 - due Wednesday 5/19
- **Mini-report** (5%)
 - completed as a team and submitted via Stellar
 - due 5/14 at 10p
- Quiz (collectively 10%)
 - M3D4 (today!)
- Notebook (part of 10% Homework and Notebook)
 - due 5/13 at 10p
- Blog (part of 5% Participation)
 - due 5/20 at 10p via Slack (unless you have already completed 3 posts)

Mod 3 Overview

Research goal: Perform site-directed mutagenesis to alter the properties of a protein-based fluorescent sensor



Day 1:
examine sequence and
structural features of IPC



Day 2:
identify mutations in
variant IPC



Day 3:
examine effects of mutations
in variant IPC

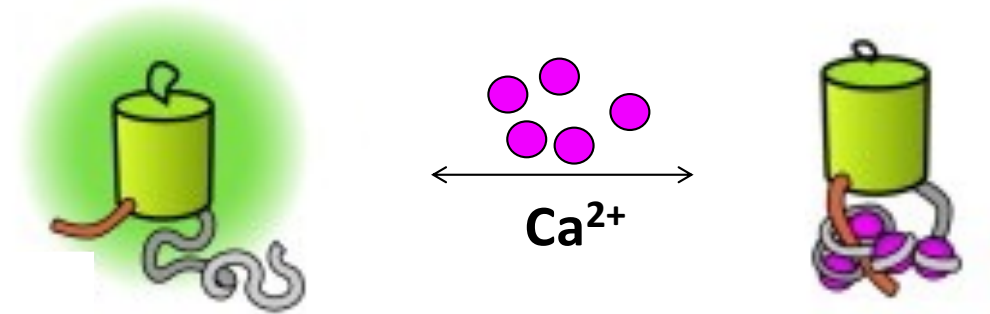


Day 4:
design new variant IPC

Create a new IPC variant

Bringing everything together!

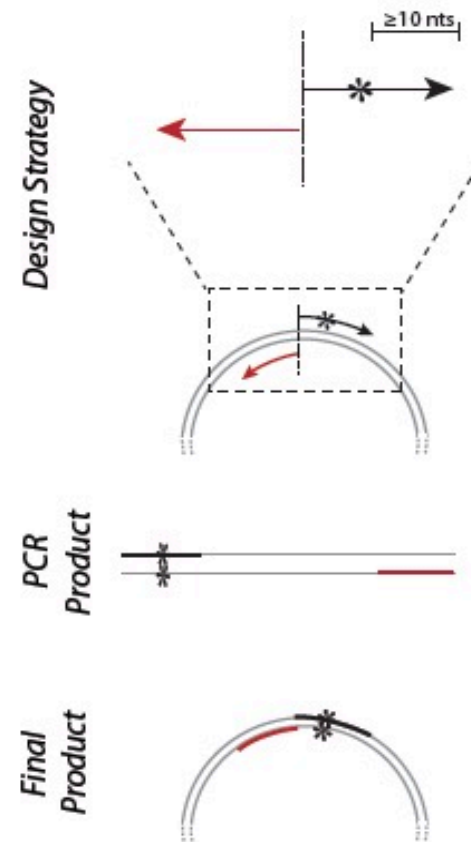
- Based on:
 - Structure
 - Sequence
 - Ca^{2+} binding curves of previous mutations



What amino acid on in the calmodulin sequence of IPC would you target to alter binding to calcium?

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

- NEB Q5 SDM kit:
Create specific, targeted changes in double-stranded plasmid DNA
- Forward primer:
- PCR product:
- Final product:



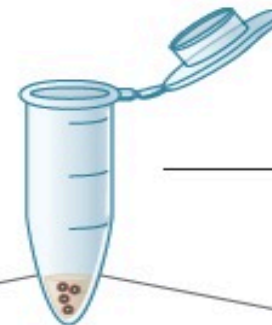
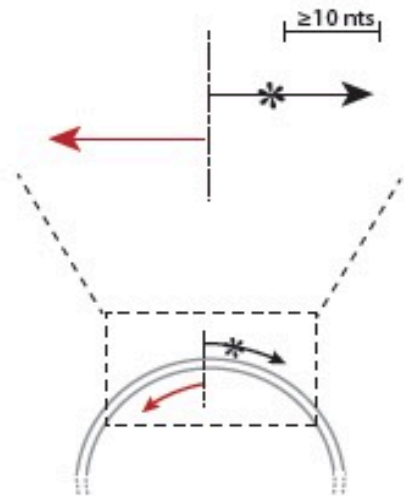
<https://www.neb.com/products/e0554-q5-site-directed-mutagenesis-kit#Product%20Information>

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

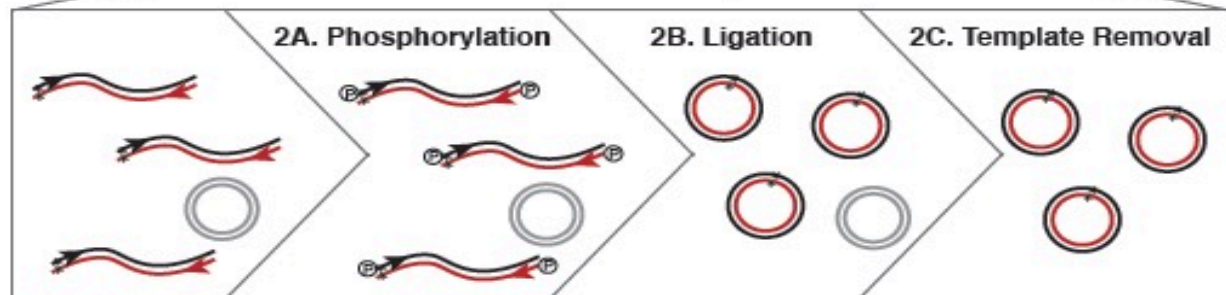
1. Exponential Amplification (PCR)
 - Q5 Hot Start 2x Master Mix

2. Treatment & Enrichment: Kinase, Ligase and Dpn1
 - 10x KLD enzyme mix

3. High-efficiency transformation
 - NEB 5-alpha competent cells



5 min. at room temp.



For Today:

- Design optimized primer to alter calmodulin binding to calcium
- Finish lab notebooks (specifically M3D3 due 5/13 by 10pm)
- Work on mini-report
- Quiz at 3:15pm



M3D3

Matlab trouble?
Talk to me...

Mini-Report: as always, read the wiki...

- 2-3 pages max!
 - Completed in teams
- Audience has high familiarity with your project
- What figures will you include?

For Thursday



- Thursday lab is entirely devoted to completing the mini-report
 - Should be able to complete it during lab, due at 10pm

This is it!

- It's been quite a semester...
 - Thank you for patience and engagement during a challenging time
- Feedback session on Thursday May 20 during lecture (11am)

