

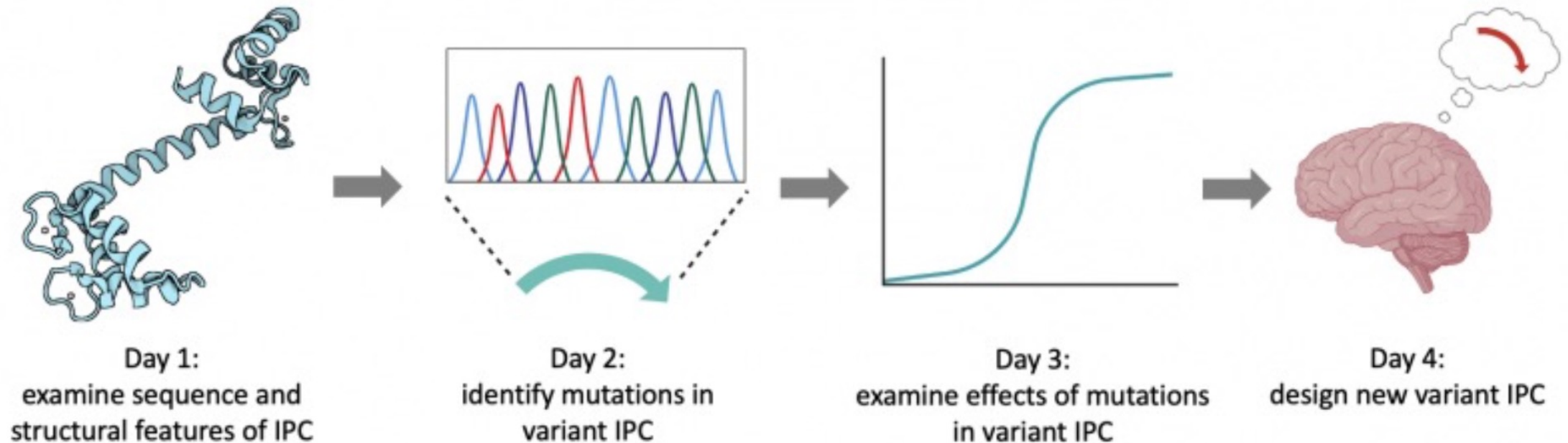
M3D2: Identify IPC mutations

- Prelab discussion
- Align sequences and identify IPC mutations
- Induce expression of IPC mutants
- Purify IPC protein



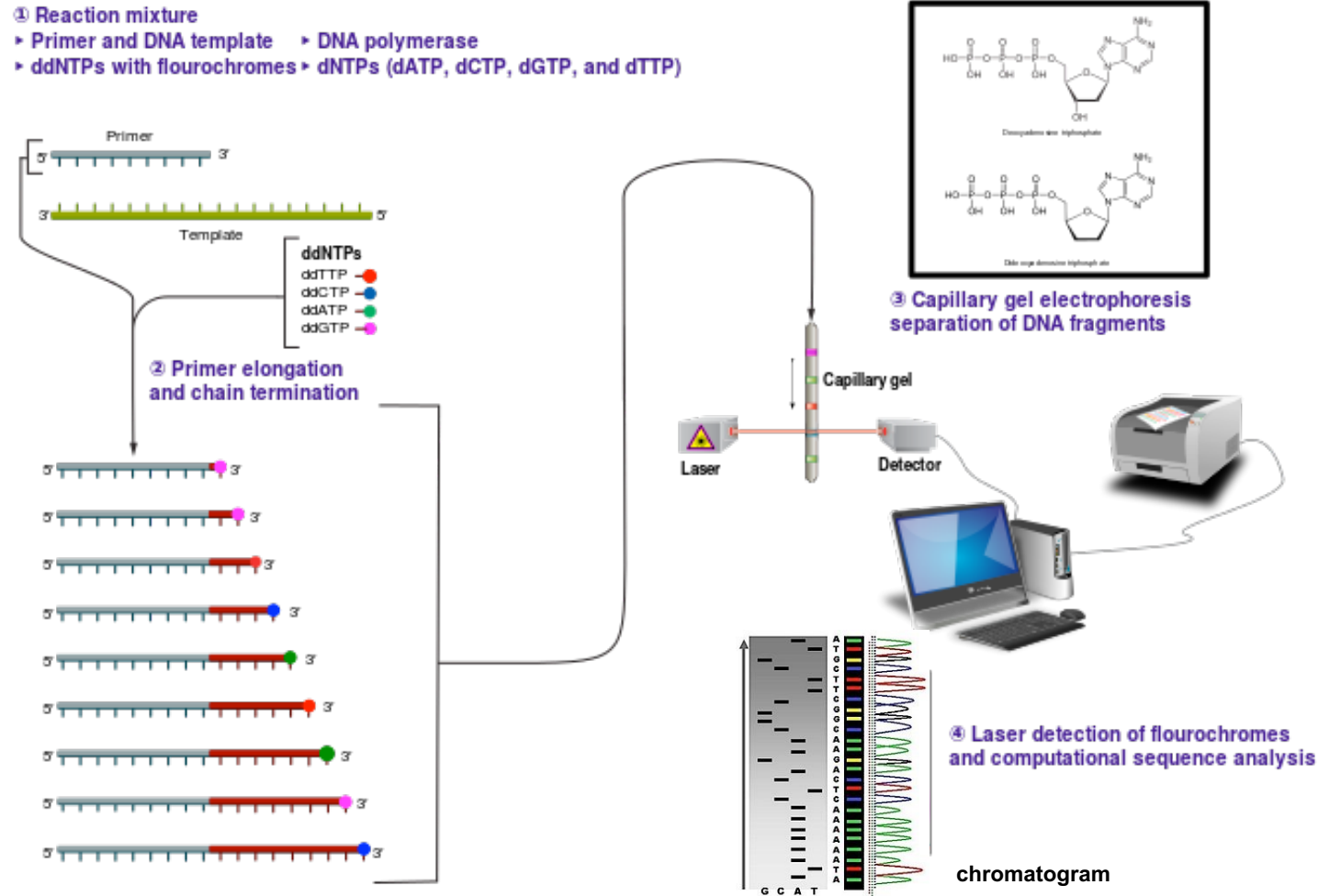
Mod3 experimental overview

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

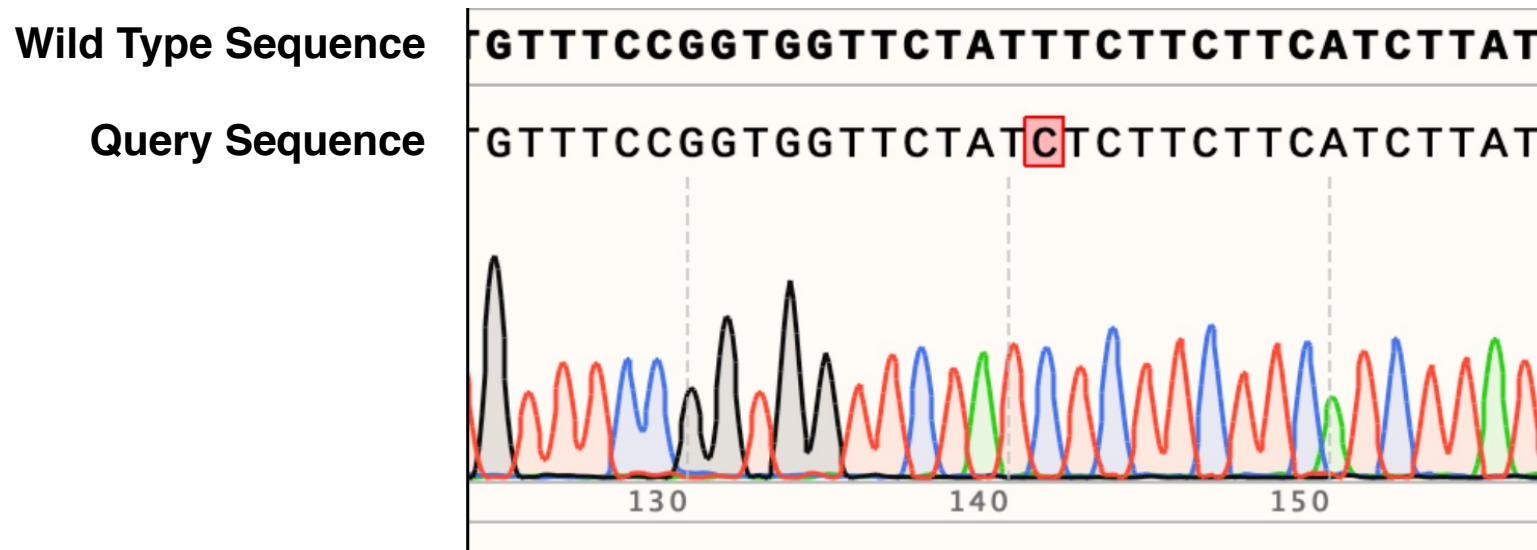


Mod1 Review: Sanger sequencing allows us to identify specific IPC mutations

- Di-deoxynucleotides (ddNTPs) terminate sequence elongation
- Each ddNTP attached to a fluorophore for detection
- ddNTP incorporated randomly and terminates elongating nucleotide chain



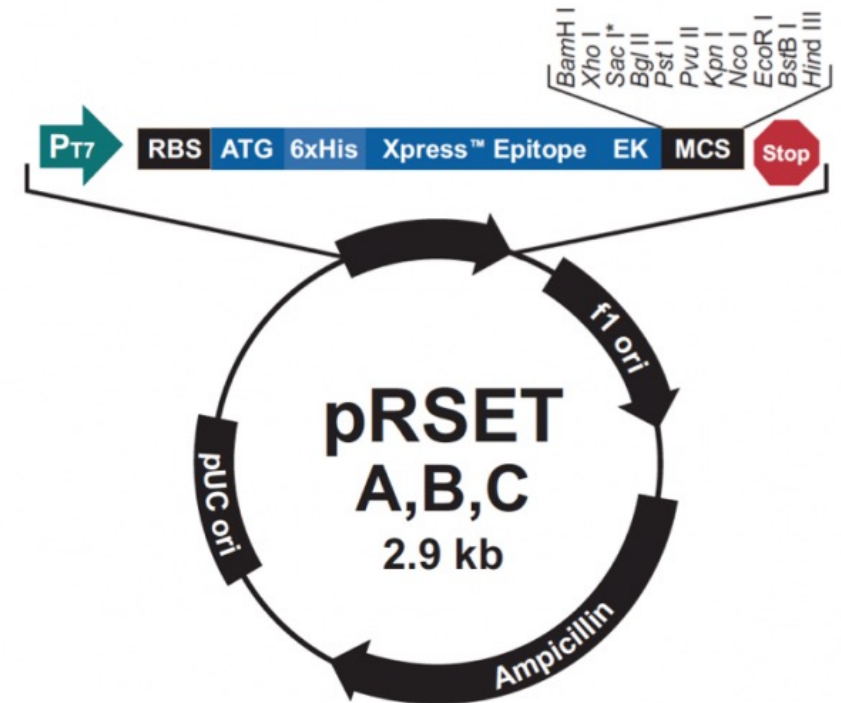
Sequence alignment: How do our mutants differ from wild type IPC?



- Think about the effect of sequence alignments into changes at the protein level
- Use your Snapgene file from M3D1 to identify regions of IPC where mutations are located

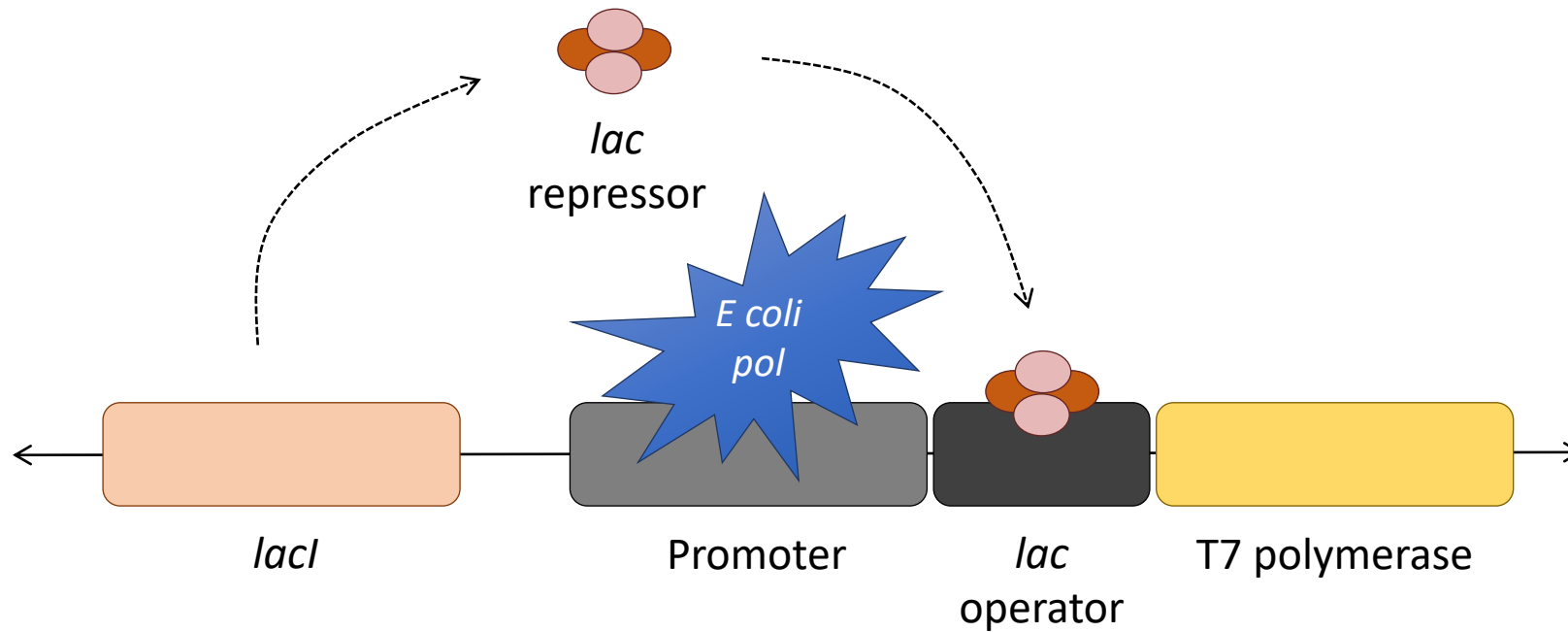
M2 Review (with a twist): Induce IPC protein expression

- IPC gene is inducible
- T7 promoter requires the presence of **T7 polymerase** for expression
 - What is special about T7 polymerase?
 - How does this differ from a constitutive promoter (e.g. Amp)?

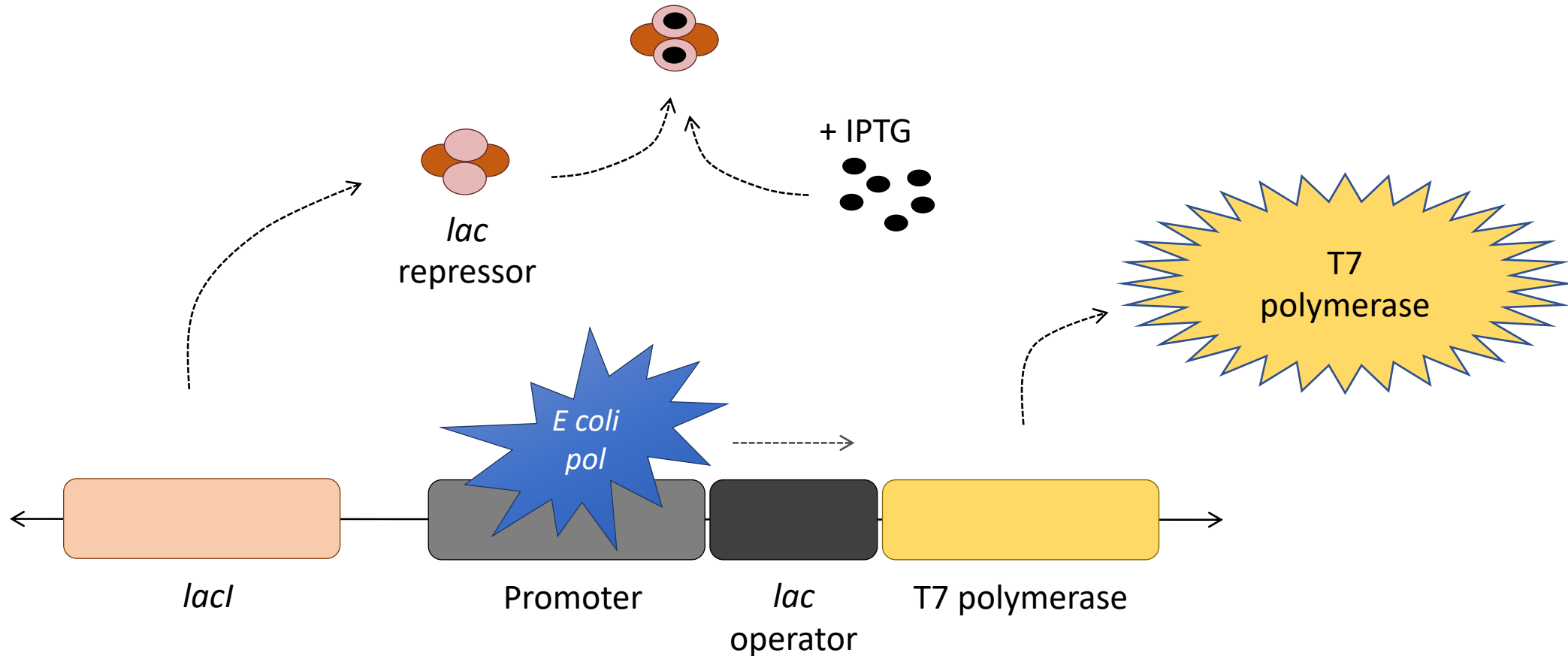


*Version C does not contain Sac I

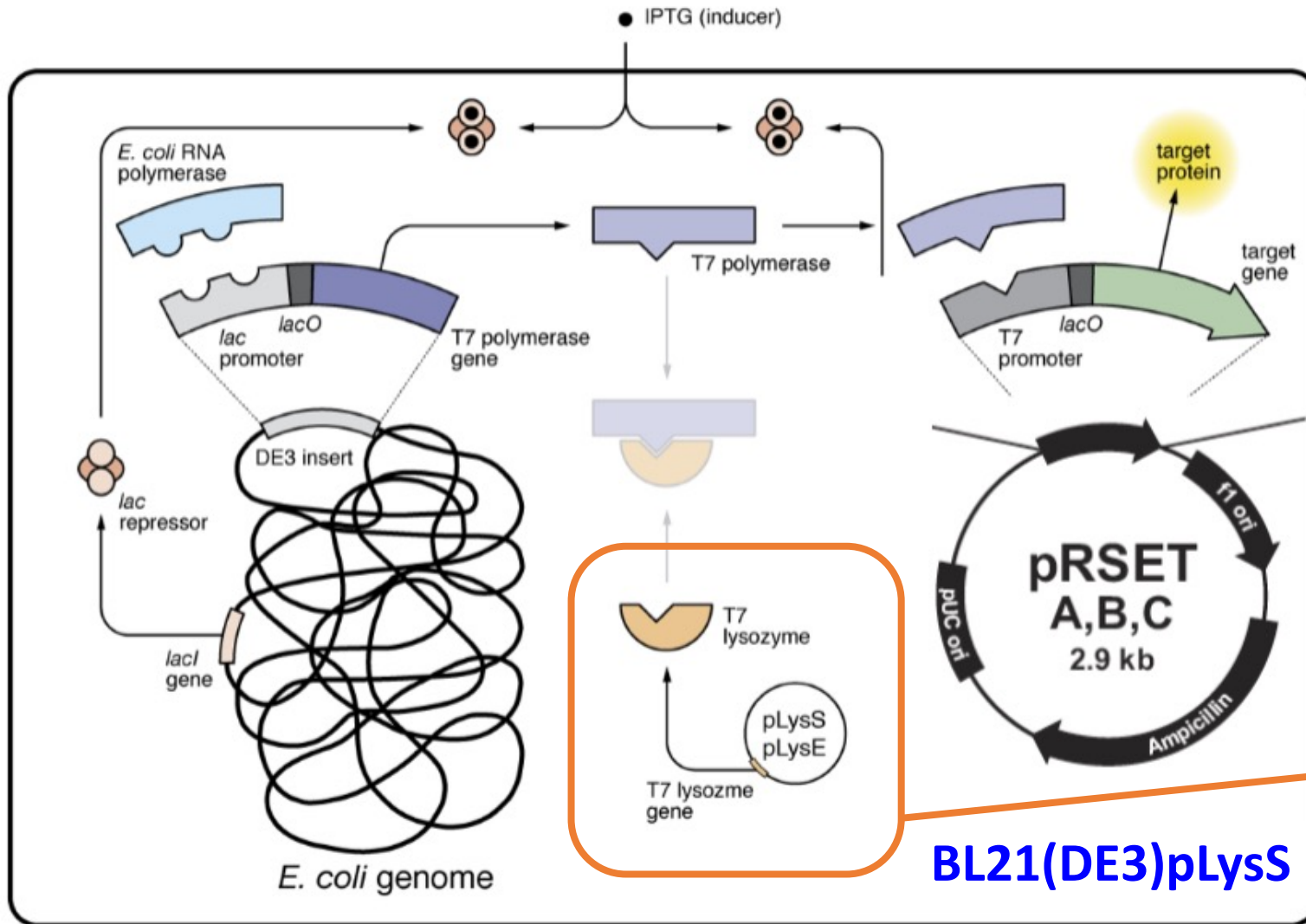
LacI repressor blocks expression of T7 polymerase in the absence of lactose



IPTG sequesters LacI repressor, allowing expression of T7 polymerase



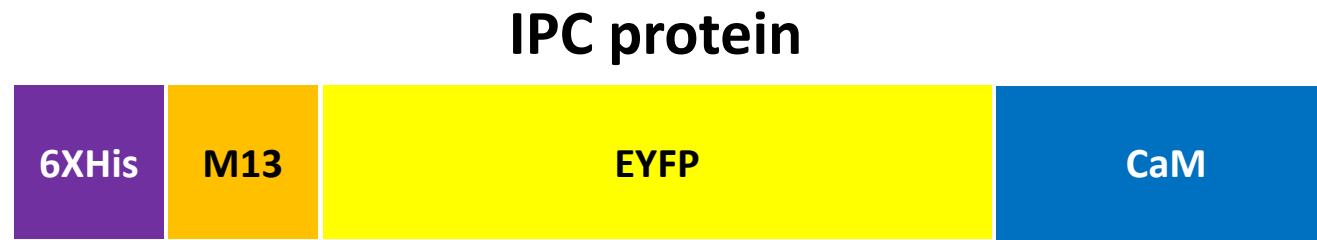
Inducing IPC expression: IPTG/Cm \rightarrow T7 pol \rightarrow IPC



T7 Lysozyme gene

- On a separate plasmid carried by the BL21 E. Coli
- Under basal conditions this plasmid expresses enough T7 lysozyme to degrade "leaky" T7RNAP
- Keep this plasmid in the E.Coli using chloramphenicol

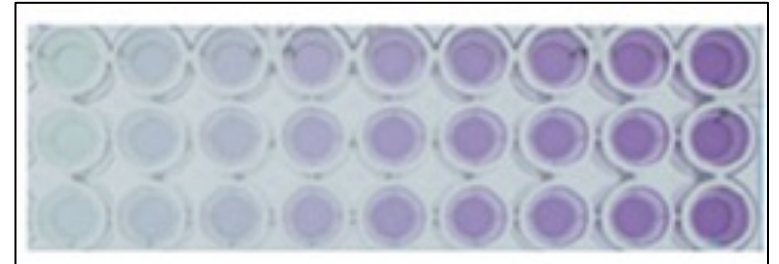
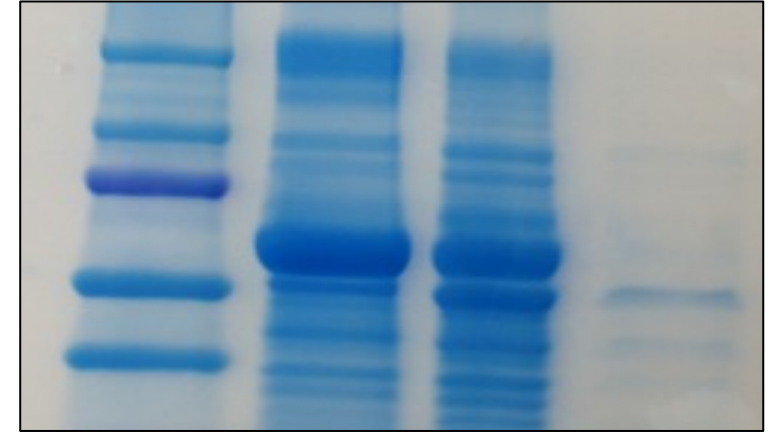
M2 Review: Protein purification of IPC via nickel-agarose resin



- Which protein domain is necessary for protein purification?
- What assays can we run to characterize the purity and efficiency of our purification?

M2 Review: Characterization of protein purification product

- Check purity using SDS-PAGE
 - Identifies presence of protein during purification procedure
 - Visual detection of other proteins in sample
- Measure concentration using microBCA assay
 - Colorimetric assay
 - Calculate concentration from standard curve
 - Used for dilute protein samples



For Today

- Work through wiki to determine the mutations in the IPC constructs
- Evaluate success of protein purification and determine concentration of IPC mutants

*** Divide and conquer this work!***

For M3D3...

- Work **with your lab partner** to write up a short description of your idea for the research proposal
 - Use the guidelines on the homework section of the wiki
- This does not have to be your final idea