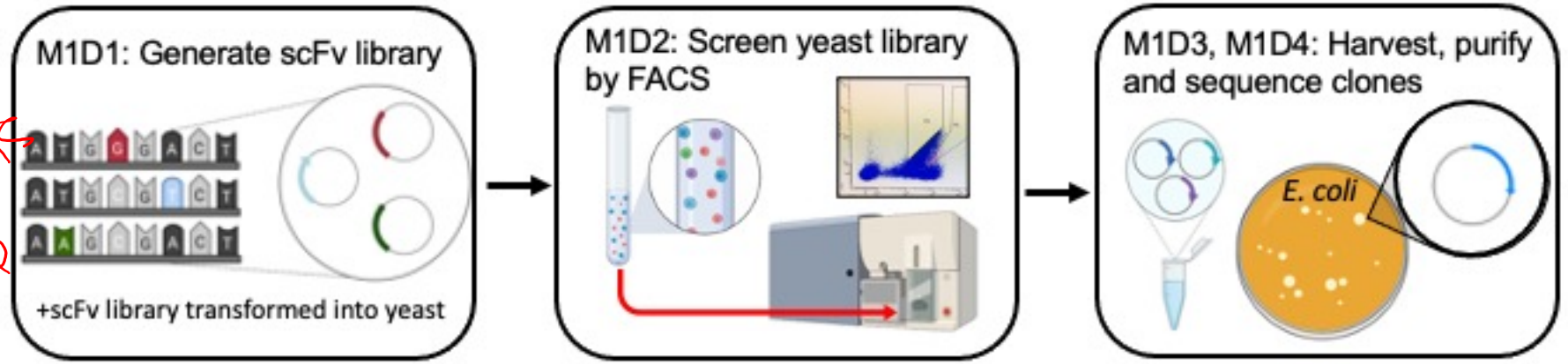
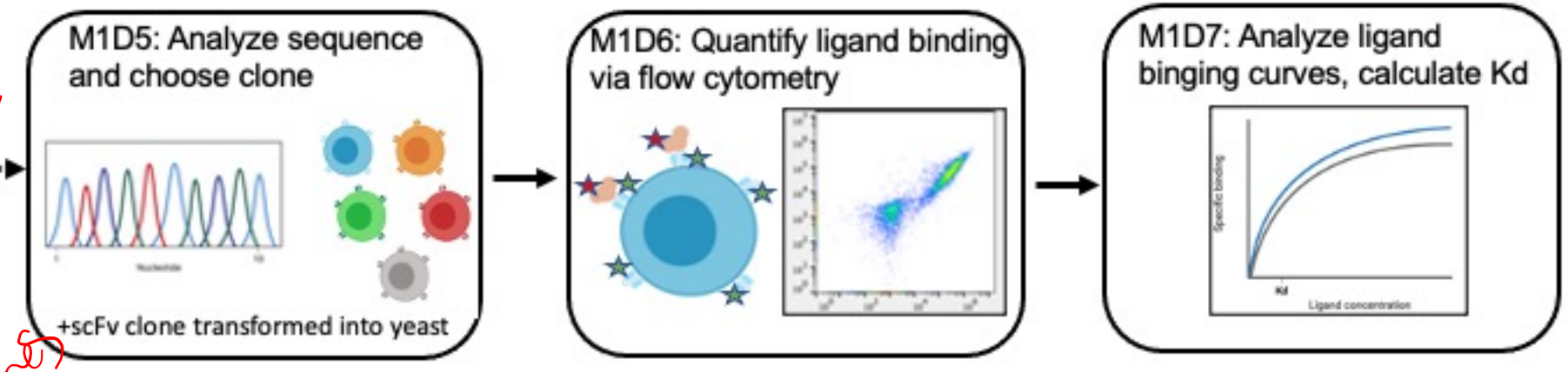
M1D5: Analyze clone sequences and choose clone to characterize

- Comm Lab
- Prelab discussion
- Align scFv sequences to identify mutations

Overview of Mod1

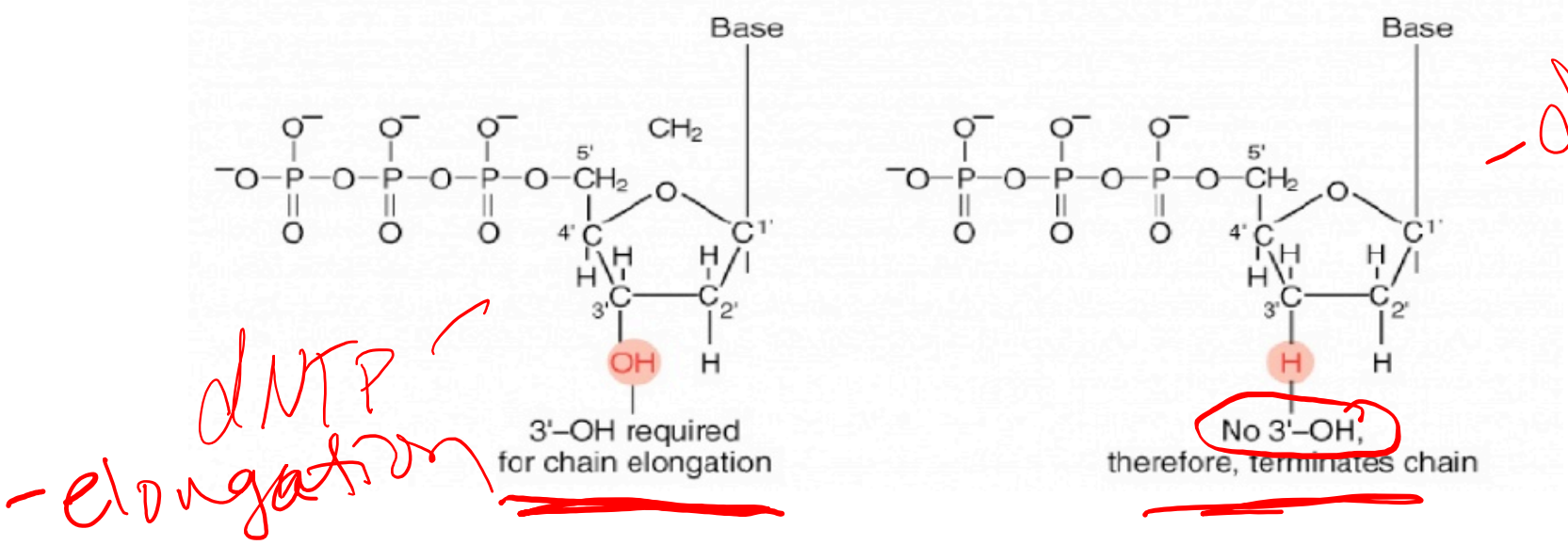
Research goal: Identify and characterize an antibody fragment (scFv) that shows improved binding to the antigen, lysozyme.



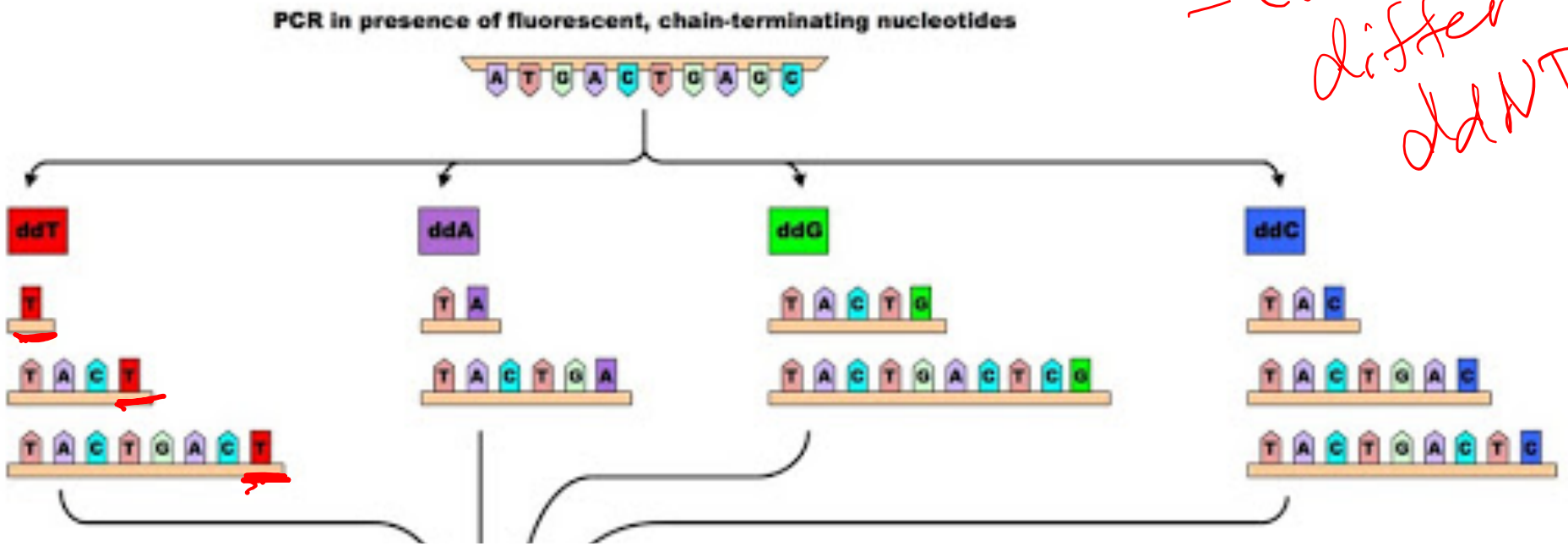


Sanger sequencing used to identify mutations in scFv clones

- Di-deoxynucleotides terminate sequence elongation
- 3’ hydroxy is lacking which prevents addition of subsequent base (required for nucleophilic attack at 5’ phosphate)

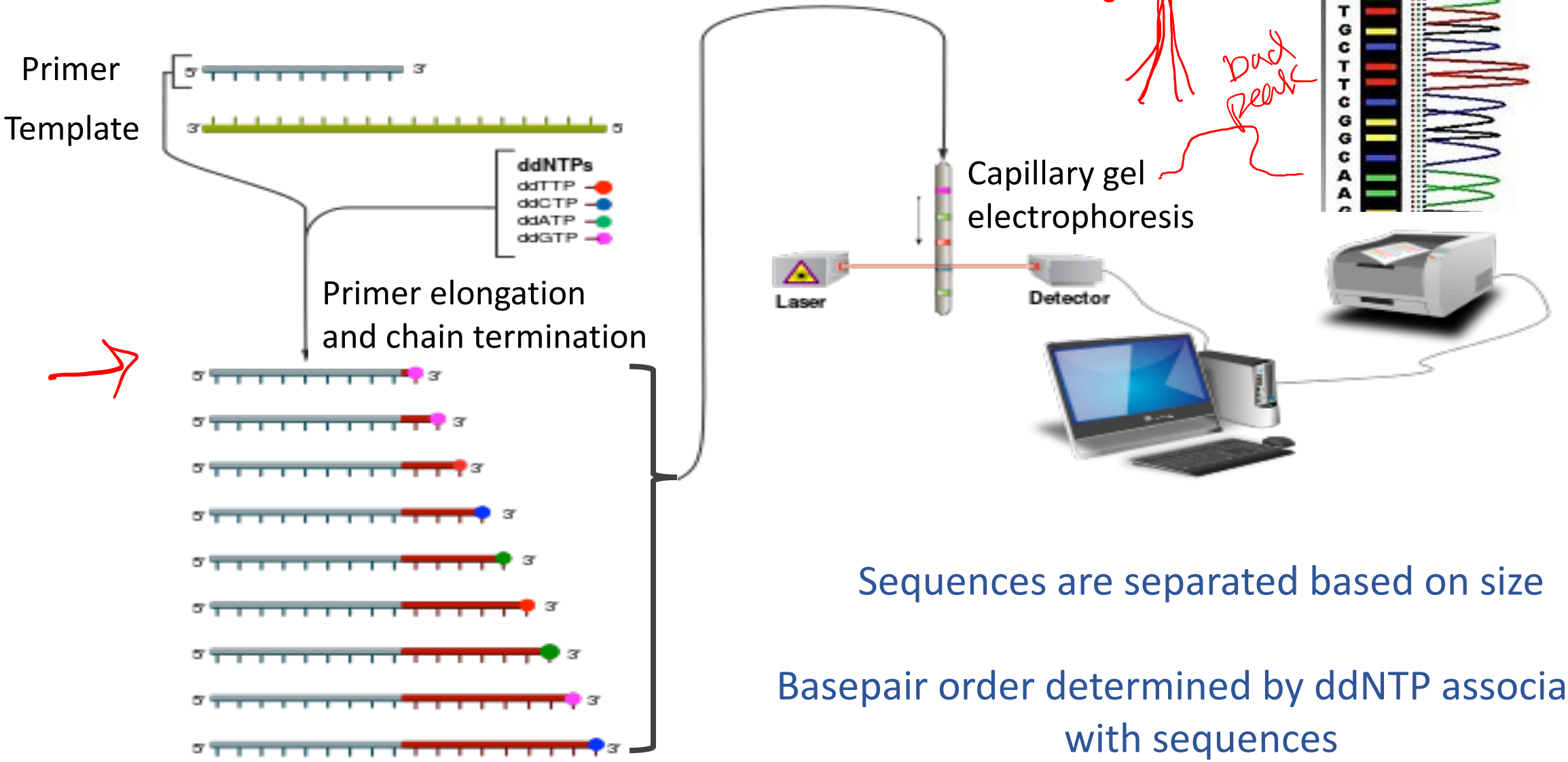


Sanger sequencing set-up



- [dNTPs] > [ddNTP]
- Each ddNTP attached to a fluorophore for detection
- ddNTP incorporated randomly and terminates elongating nucleotide chain

Sequence determined from chain termination products

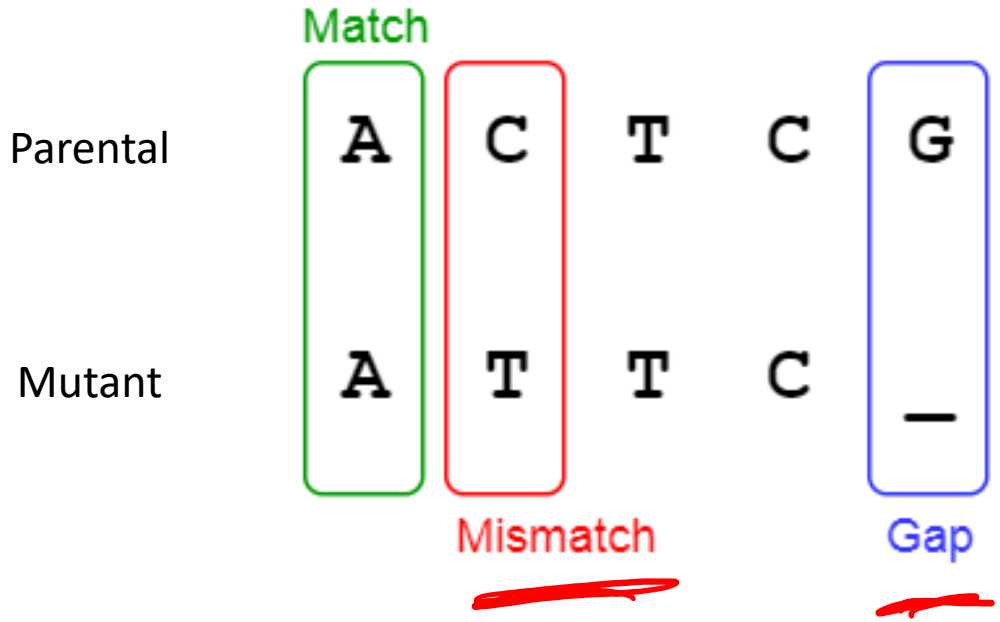


Sequences are separated based on size

Basepair order determined by ddNTP associated with sequences

Sequencing alignments will be used to identify mutations in scFv clones

- Use SnapGene or Benchling to compare clone sequence to parental sequence
- First, identify basepair changes in the sequence
- Then determine if basepair changes result in amino acid substitutions

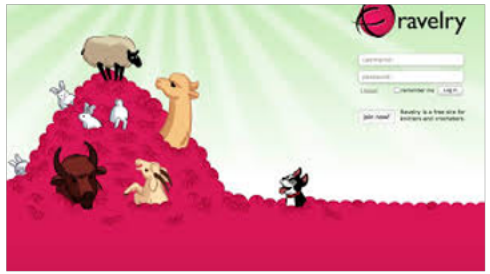


Notes on overview schematics…

How does Becky knit a scarf?

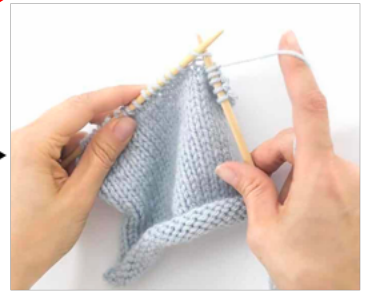
too much info

Buy beautiful yarn



Choose a pattern

Cast on 25 stitches



Knit

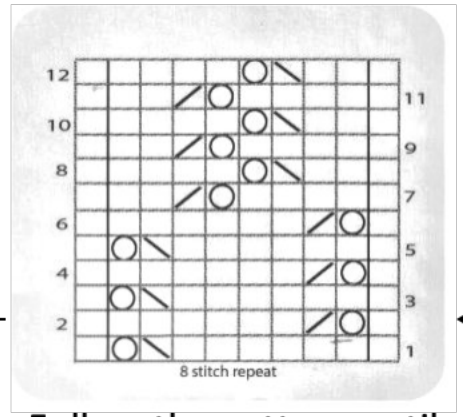
Purl

K & P & K & P

Measure size. It doesn't match the recommended gauge

Frog it!

Cast on 40 stitches



Follow the pattern until time to cast off

Block scarf to wear

What should be in the Title and Caption?

Title: State what is shown / represented in the schematic

Caption:

- Explain the flow of information using concise / clear language
- Expand on text shown in figure labels to eliminate excess wordiness / clutter from the figure
- Define all abbreviations / jargon / labels / symbols

Revised example:

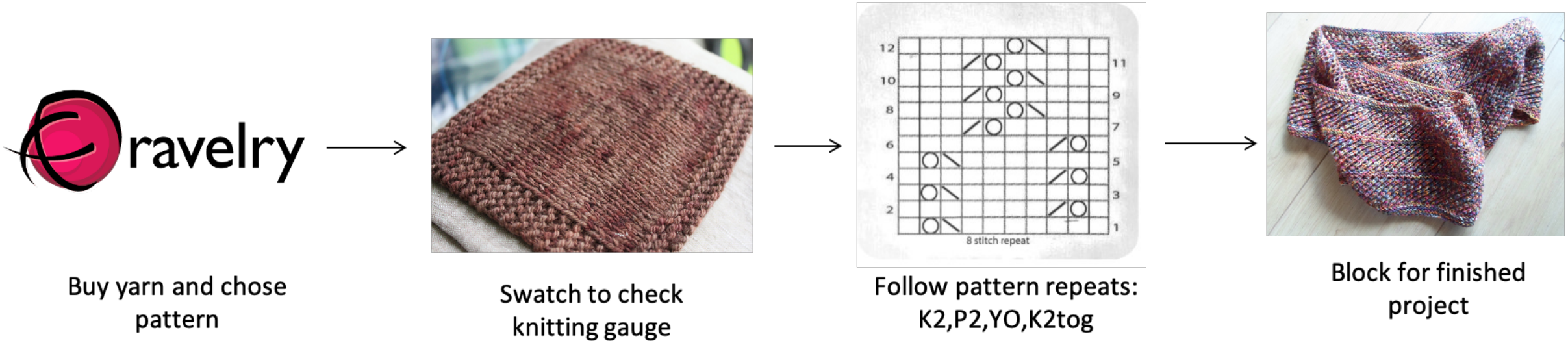


Figure 1: Becky's knitting process. Becky follows a specific protocol to knit a scarf. She choses her yarn and checks the pattern before following the written pattern and blocking to complete the project. K2= knit two, P2= purl 2, YO= yarn over, K2tog= knit two together

Mini-presentation outline

- Mini-presentation should be in bullet form
- Be quantitative when stating results (NOT "this was more/less than...")
  - For outline, ok to have placeholders
- Submit to Stellar

Category	Elements of a strong presentation	Weight
Introduction	• Introduce yourself and the research • Summarize the background information necessary to understand the research • Provide a clear and concise description of the central question / hypothesis	25%
Methods & Data	• Provide ONLY the method information necessary to understand the results • Give complete and concise explanations of the results • Relate the results to the central question	25%
Summary & Conclusions	• Highlight the key finding(s) relevant to the central question / hypothesis	25%
Organization	• Give a logical, easy-to-follow narrative • Include transition statements	15%
Delivery	• Show confidence / enthusiasm and speak clearly • Use appropriate language (technical or informal, as appropriate) • Be mindful of the time limit (3 minutes +/- 15 seconds!)	10%

The mini-presentation will be graded by Dr. Noreen Lyell with input from Dr. Leslie McClain, and Dr. Becky Meyer.

For today…

- Identify mutations in scFv clone sequences
- Work on M1D4 wiki (if not completed)

For M1D6 (Thurs. 3/11)…

- Create an overview schematic about the Mod1 research

- Write a bulleted outline of mini-presentation