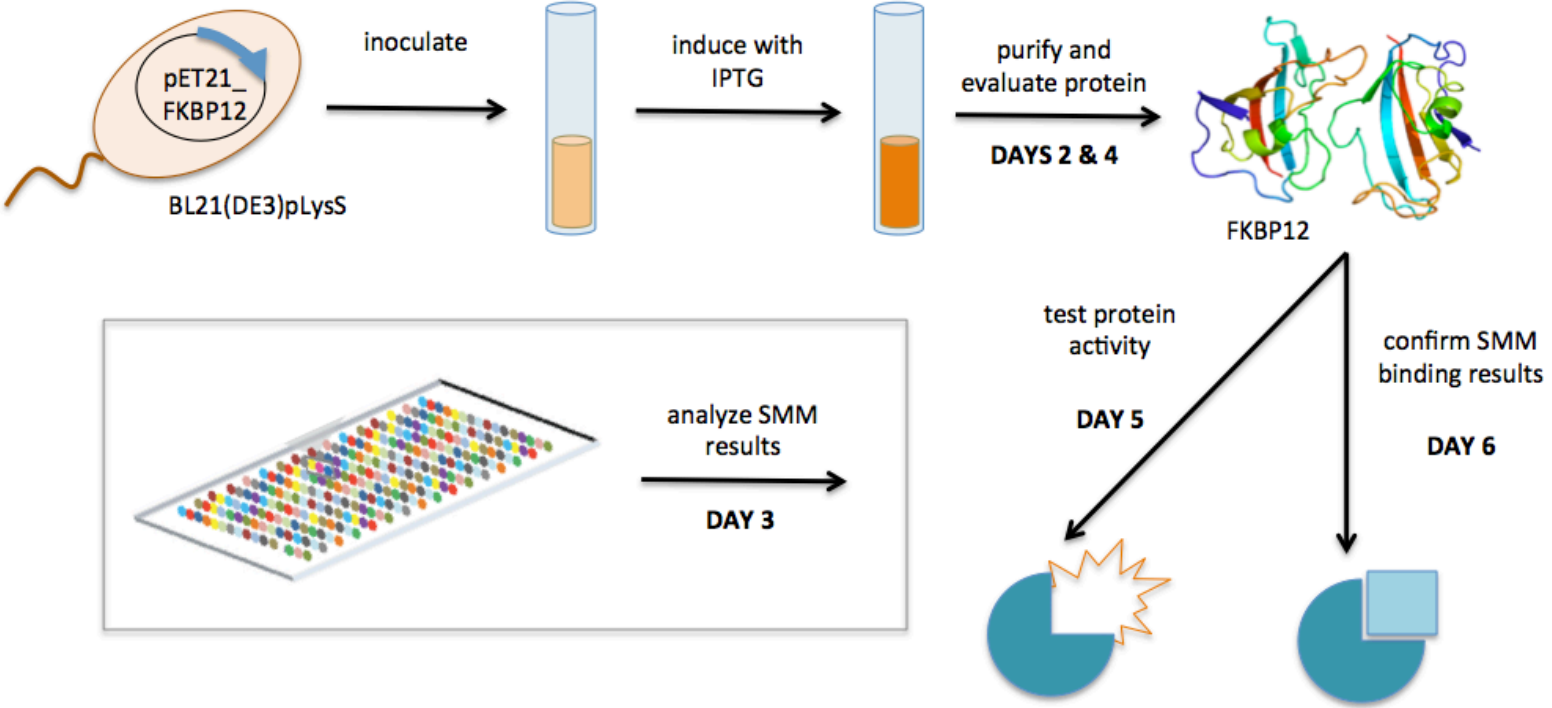
M1D4:
Evaluate protein purity and concentration

1. Laboratory quiz
2. Prelab discussion
3. Desalt & Concentrate protein solution
4. Visualize protein purity
5. Measure protein concentration

Overview of Mod 1 experiments



First, must prepare FKBP12 for purity and concentration tests

Part 1: Desalt purified FKBP12

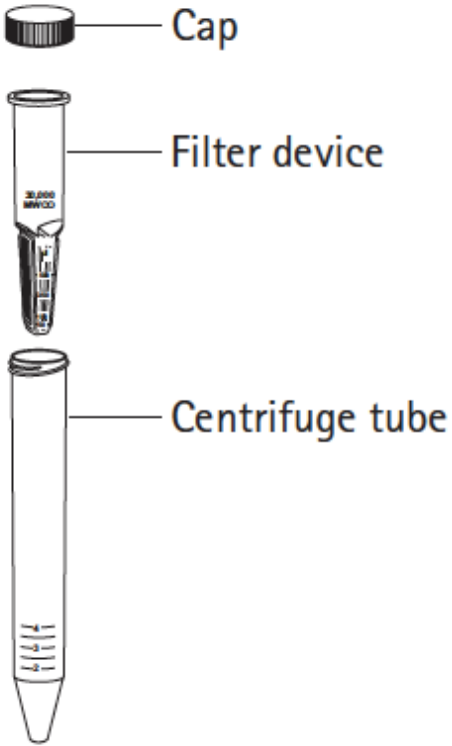
- Proprietary resin
- Retains ≥ 95% of salts and small molecules

keep flowthrough

Part 2: Concentrate purified FKBP12

- Centrifugal filter (3000 MWCO)
- Sample recovery ≥ 95%

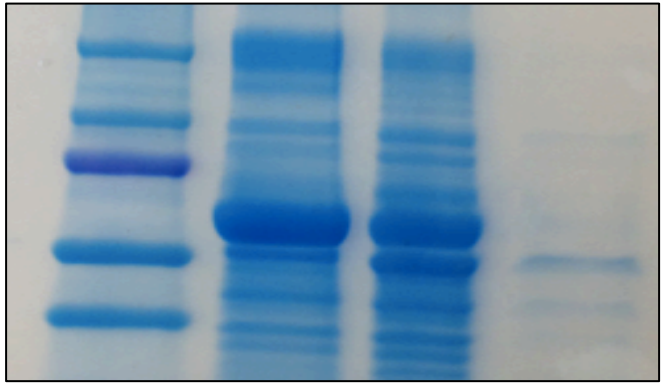
Important note on concentration procedure!

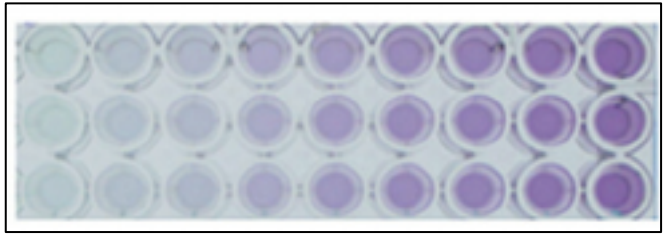


Protein is retained in the filter device, then removed with a pipet following centrifugation

concentration factor = $\frac{\text{starting volume}}{\text{final volume}}$

Evaluate FKPB12 purity and concentration





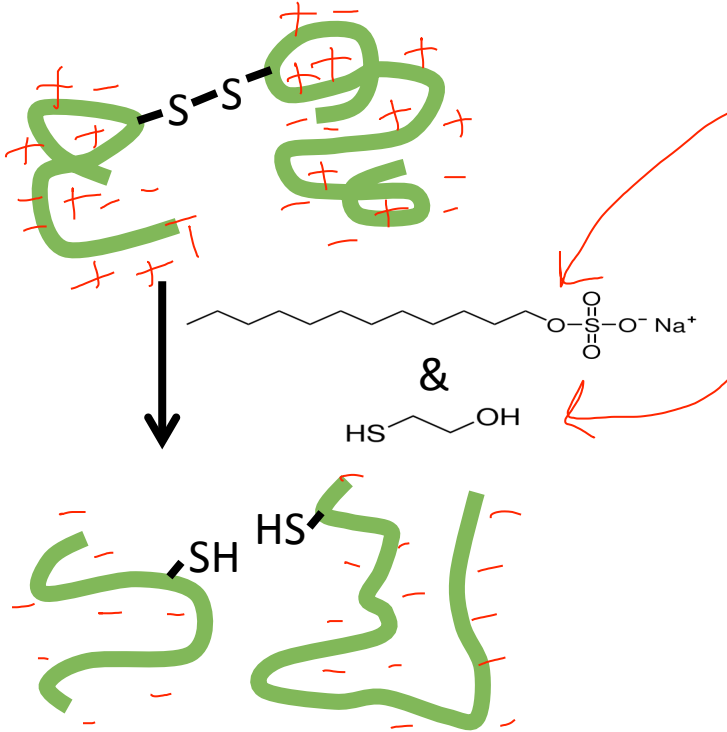
Part 3: Check purity using **SDS-PAGE**

- Visual examination of presence of other proteins in the sample (represented by bands in gel)
- Check for leaky expression of FKBP12 from T7 promoter

Part 4: Measure concentration using **BCA assay**

- Colorimetric assay
- Calculate concentration with standard curve

Part 3: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



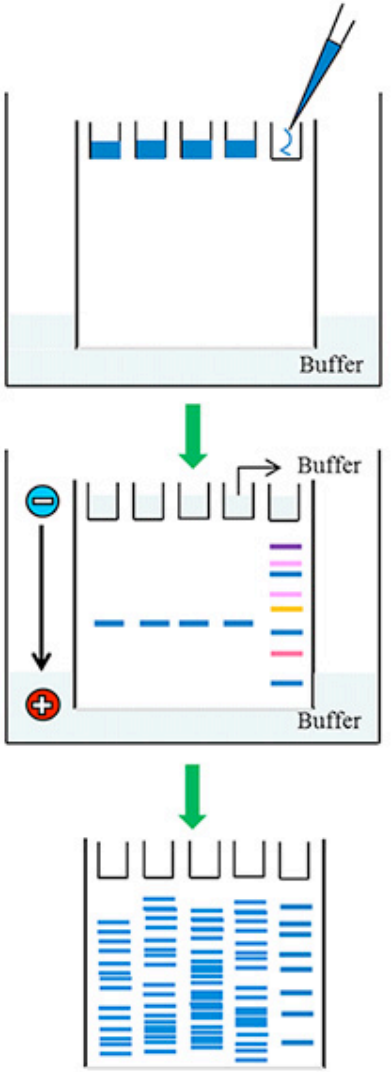
- Laemmli sample buffer / loading dye:
  - SDS 'coats' in negative charge
    denatures proteins
  - β-mercaptoethanol (BME)
    breaks S-S bonds
  - bromophenol blue
    tracker
  - glycerol
    weight
- Boiling:
  denatures higher structure

How are proteins separated with SDS-PAGE?

- Laemmli buffer and boiling results in linear and ⊖ charged proteins

- SDS-PAGE separates proteins by size

- Electrophoresis completed in TGS buffer
  - Tris-HCl buffer / pH
  - SDS maintains linear / ⊖ charge
  - Glycine ensures proteins enter gel



Loading your protein purification samples

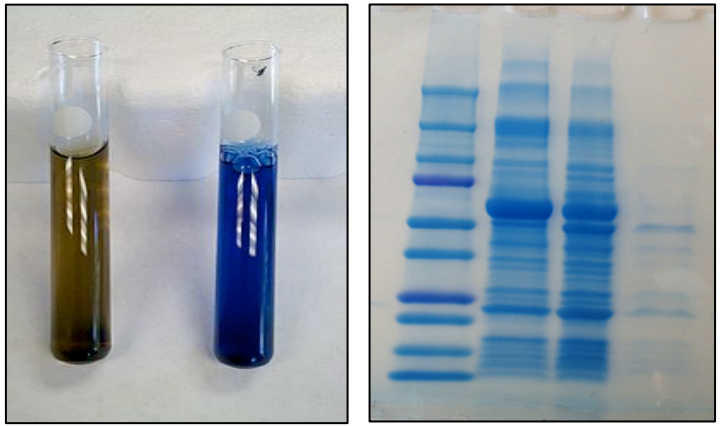
Consider the order of your samples:

- Figure will be in your Data Summary
- Samples:
  - Un-induced / induced cell lysates
  - Wash flow-through (2 samples)
  - Elution flow-through (2 samples)
  - Desalted and concentrated FKBP12
  - Stained and unstained ladders

How are proteins visualized with SDS-PAGE?

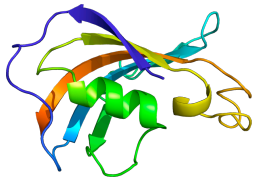
Coomassie brilliant blue G-250 dye applied to gel after electrophoresis

- Red if unbound (cationic form)
- Blue if bound to protein (anionic form)
- Hydrophobic and electrostatic interactions with basic residues
- Arg (also His, Lys, Phe, Trp)



Part 4: Bicinchoninic acid (BCA) protein assay

Step 1: Biuret reaction; chelation of copper with protein, reduction of copper



+ Cu^{2+} $\xrightarrow[\text{Temperature-dependent}]{OH^-}$ Cu^{1+}

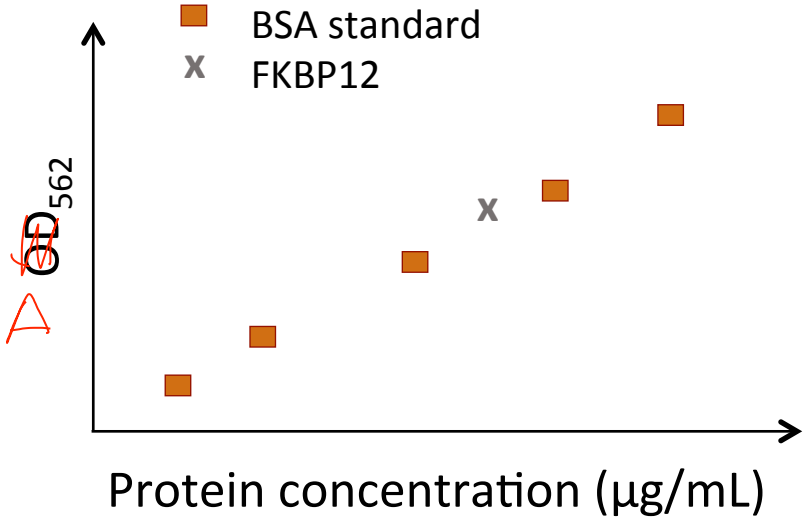
Step 2: BCA complexes with cuprous cation

Cu^{1+} + 2 BCA $\longrightarrow$ $BCA\text{-}Cu^{1+}$ complex (A = 562 nm, violet)

BCA/Cu^{1+} absorbance at 562 nm linearly proportional to protein concentration

Standard curve generated using serial dilutions of BSA

- Use fresh tips between tubes
- Mix well between dilutions
- Be mindful of volumes



Methods schematic of BCA assay protocol

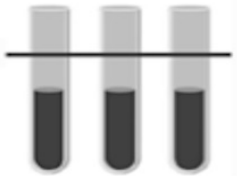
50 parts A +
1 part B

Mix working reagent

0.1 mL sample +
2.0 mL working reagent

Mix well

Incubate 30 min
@ 60 °C



Then cool

Spectrophotometer

Read at 562 nm

For today…

- R

For M1D5…

- Craft Data Summary slide for FKBP12 evaluation results
- Write Methods section for protein purification / evaluation
  - Include M1D2 protein purification and M1D4 evaluation tests
  - Completed in teams!!!

Notes on Data Summary and data slides…

Data Summary to be completed using PowerPoint

Each figure should relay one message

- Subpanels should be related to single conclusion
- Include SDS-PAGE image and / or BCA graph
- Remember the title and caption!!

Text should be related to results in the figure

- See guidelines in homework description
- Write in bullets!!

Pro tips for writing a methods section

Include enough information to replicate the experiment

- List manufacturer's name and location (Company, City, ST)
- Be **concise and clear** in your description

Use subsections with descriptive titles

- Put in logical order, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in full sentences and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write concentrations (when known) rather than volumes

Eliminate 20.109 specific details

- Example "labeled Row A, Row B…"
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say "completed by teaching faculty."

How can you improve this example?

"Template DNA and primers were mixed with

20 uL of 2.5X Master Mix in a PCR tube. Water

was added to 50 uL. A tube without template

was prepared and labeled control."

Be specific, what was your template? And from what will it be amplified? How much?

What is the sequence of the primers? And and what was the final concentration in the reaction?

"Template DNA and primers were mixed with

Give the final concentration, not the stock concentration.

This information can be assumed by your reader.

20 uL of 2.5X Master Mix in a PCR tube. Water

The volume is not important, just the concentration. Also, include manufacturer information for purchased reagents.

Your reader will know that reaction mixes are prepared in water.

was added to 50 uL. A tube without template

Because the final concentrations are reported, the final volume is not important.

was prepared and labeled control."

Though including a no template control is important, consider a more concise way to include this information by omitting unnecessary details.

Revised example…

"*FKBP100* was amplified from pcDNA3-FK (1 ng/uL) with primers pr1 (5' …AGA… 3') and pr2 (5' …CTC… 3'), each at 2 pmol/uL, using 1X Master Mix (5Prime, City, ST). A control with no template was included."

BE MINDFUL OF
SUBJECT AND VERB