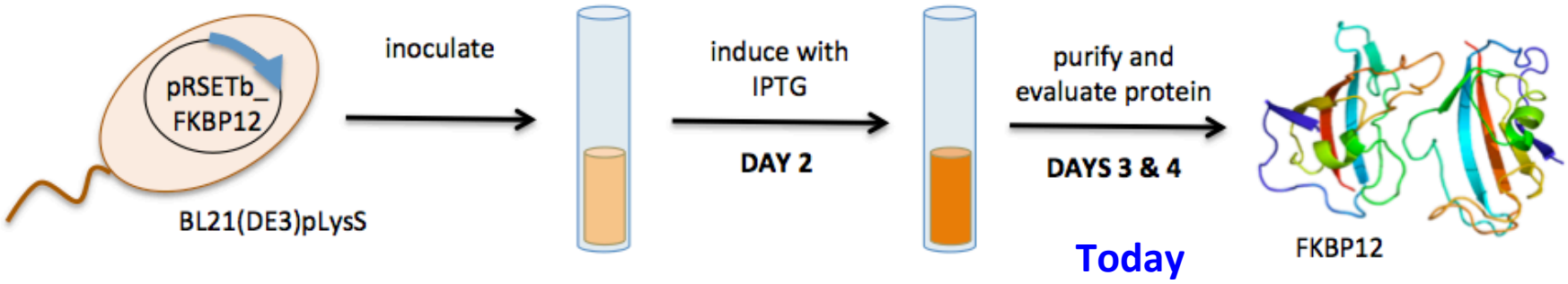
M1D4: Evaluate purity & concentration of FKBP12

1. Communication Workshop
2. Quiz
3. Prelab discussion
4. SDS-PAGE + Coomassie stain
5. BCA assay
6. Concentrate protein

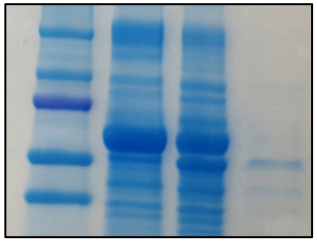
Announcements/Reminders:
3/6-7: M1 Quiz 2
3/12: Data Summary due
Welcome back, Leslie!

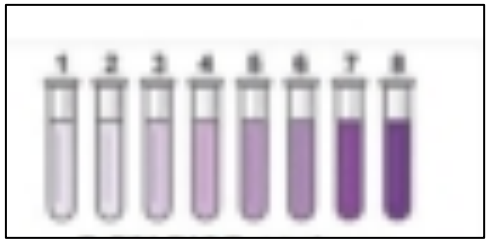
Office hours:
M 2-5pm (Noreen, 16-317)
T 4-5pm (Josephine, 56-341c)
W 4-5pm (Leslie, 56-341c)
R 10-11am (Josephine, 56-341c)
F 4-5pm (Leslie, 56-341c)
Email us for other times

Evaluate protein purity and concentration



Today





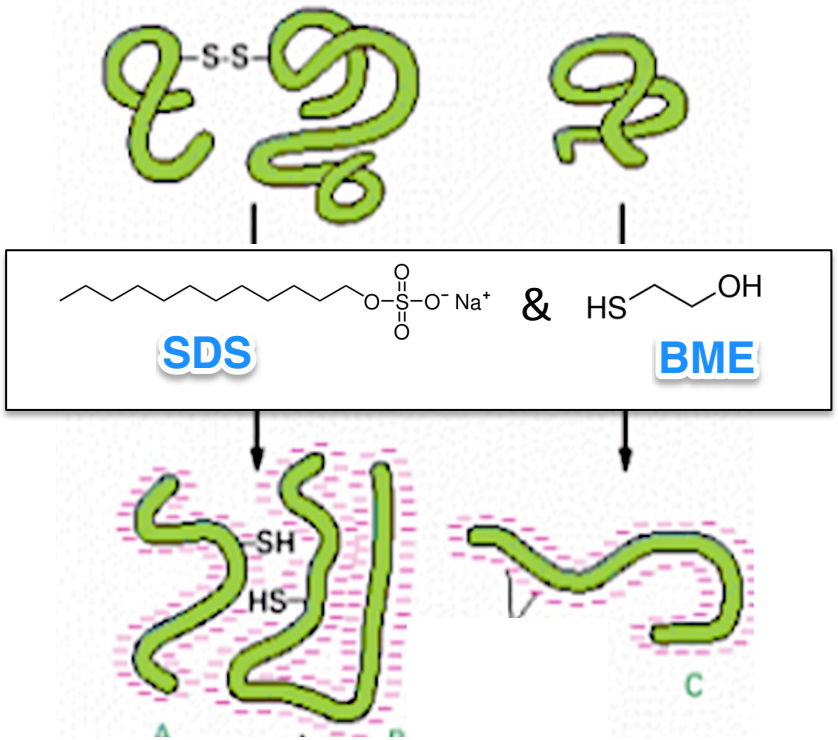
1. **SDS-PAGE**
   - Protein purity
   - Leaky expression of FKBP12 under T7 promoter?

2. **BCA assay**
   - Protein concentration

3. Concentrate protein

1) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

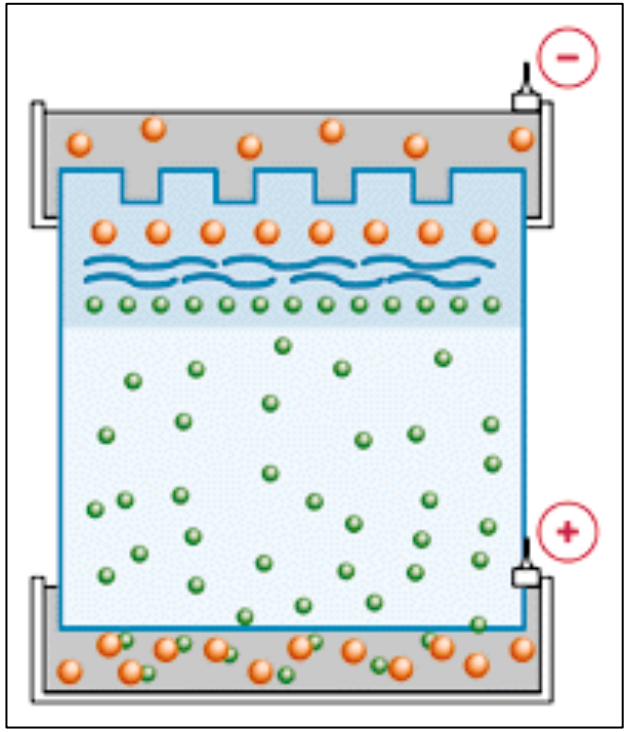
What gives proteins uniform charge and linear structure?



- Laemmli sample buffer / loading dye:
  - +SDS: detergent, denature proteins, coats protein with neg. charge
  - +β-mercaptoethanol breaks disulfide bonds
  - +bromophenol blue dye that runs at 3-5 kDa
  - +glycerol dense, viscous, helps protein sink in well
- Boiling denatures higher-order structures

1) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

How are the proteins separated?



Polyacrylamide gel

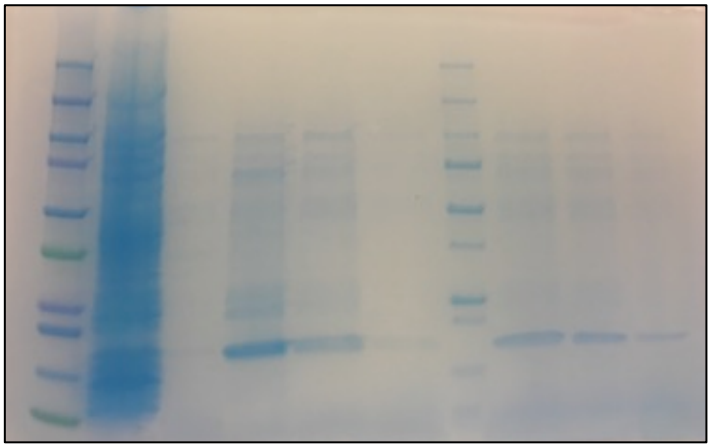
- TGS buffer
  - +Tris-HCl
  - +SDS, protein
  - +Glycine

- After Laemmli buffer and boiling, proteins are linear and negatively charged. Thus, SDS-PAGE separates proteins by __size____.

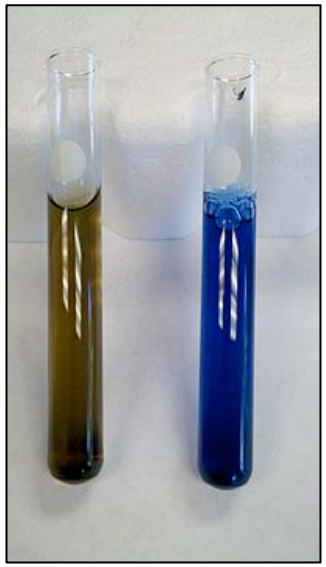
https://www.nationaldiagnostics.com/electrophoresis/article/multiphasic-buffer-systems

Load 6 samples + 2 ladders on SDS-PAGE gel

- Loading order considerations:
  - Think about figure(s) in your Results
  - Cell lysate – IPTG / + IPTG
  - Supernatant from 3 washes (+ IPTG)
  - Dialyzed FKBP12
  - Stained and unstained ladders

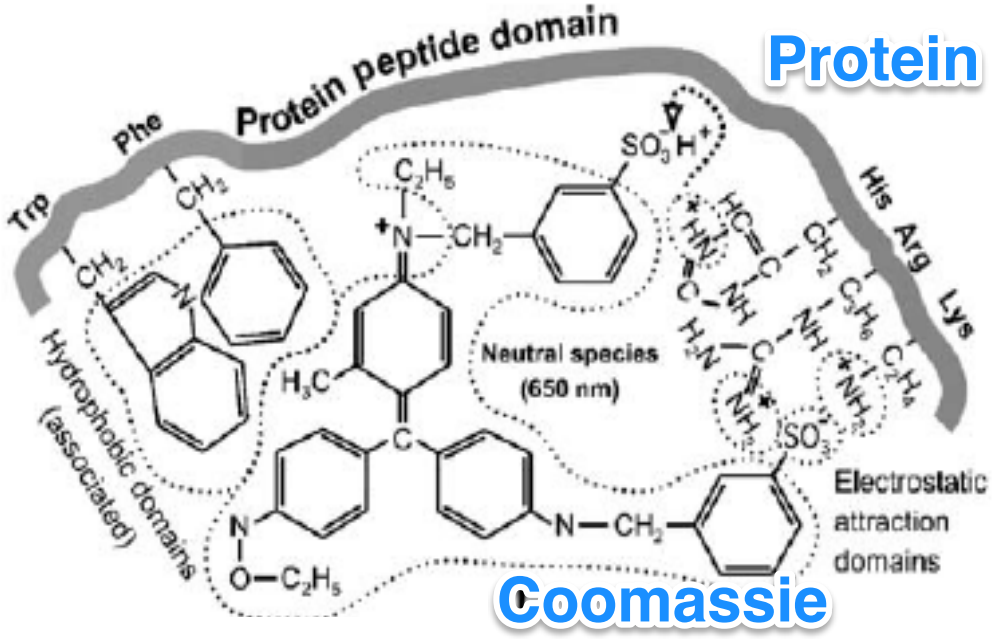


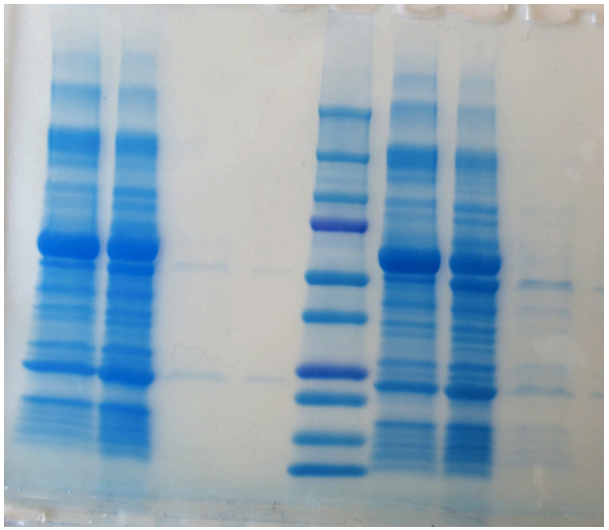
- 4-20 % acrylamide gel:
  - for 10-250 kDa proteins
  - FKBP12 ~ 12 kDa
    - 331 bps = 110 aa* ~110 Daltons/aa ~12 kDa
  - His-tag ~ 3 kDa
  - 6His-FKBP12~ 15 kDa



Visualize proteins using Coomassie colorimetric assay

- Coomassie brilliant blue G-250 dye
  - Red if unbound (cationic form)
  - Blue if bound to protein (anionic)
  - Hydrophobic & electrostatic interactions
  - Arg residues (also His, Lys, Phe, Trp)



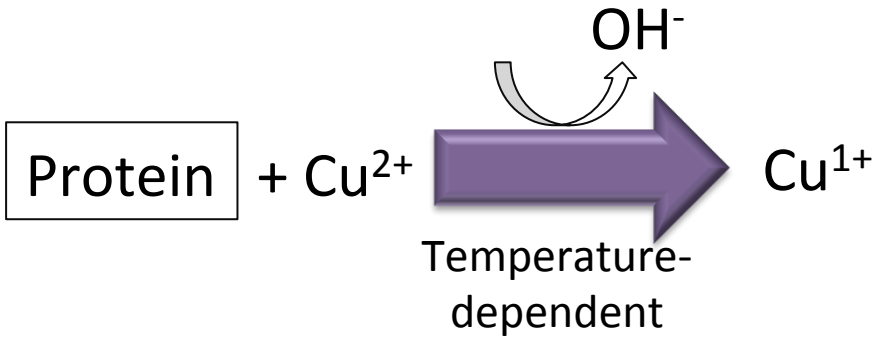


Georgiou *et al.* (2008) *Anal Bioanal Chem* **391**: 391-403

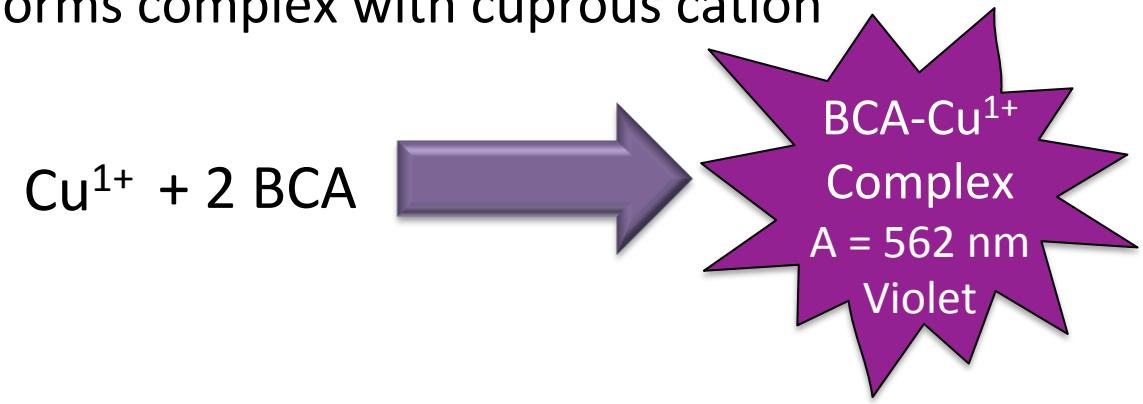
2) BCA protein assay—measure concentration

BCA: bicinchoninic acid (chromogenic reagent to detect Cu^{1+})

Step 1 Biuret reaction: chelation of copper with protein, reduce copper



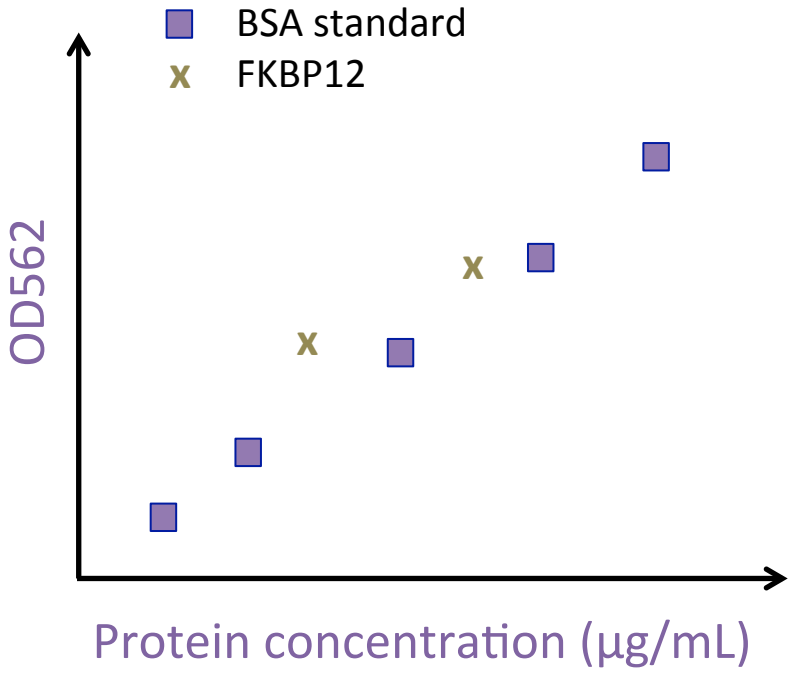
Step 2 BCA forms complex with cuprous cation



BCA/Copper complex absorbance at 562nm is linearly proportional with protein concentration

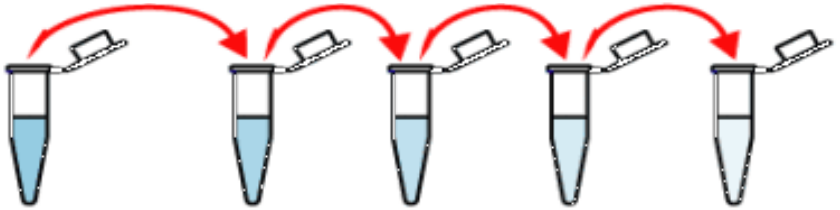
Standard curve for BCA/Copper complex absorbance (562nm) to determine protein concentration

Calibration with bovine serum albumin (B**S**A), 5 – 250 µg/mL

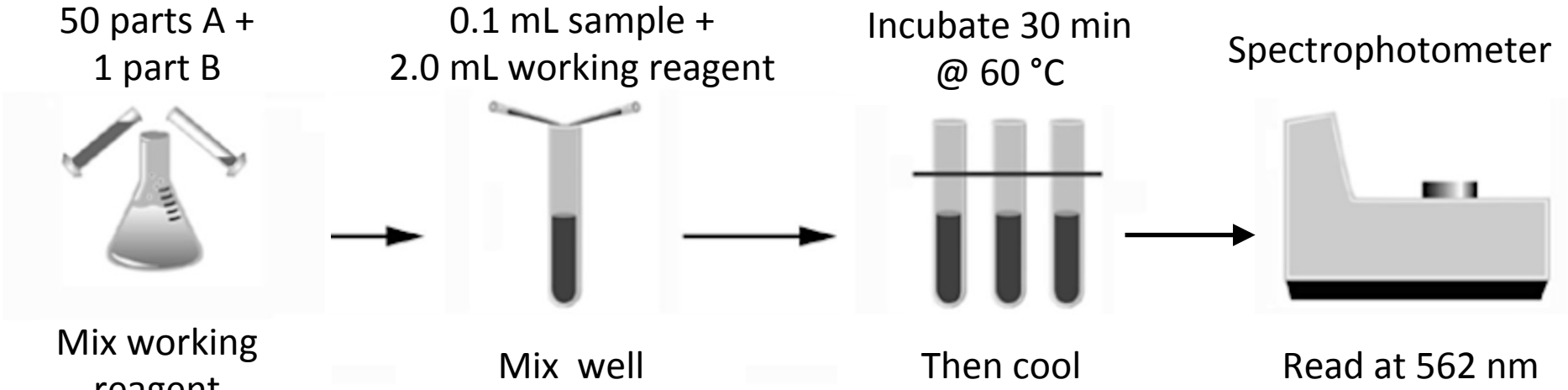


Be careful!

- Fresh tips from dilution to dilution
- Mix well between dilutions
- Pay careful attention to volumes



Schematic of BCA Assay protocol



There exist several protein concentration assays

assay	absorption	mechanism	detection limit	advantages	disadvantages
UV absorption	280 nm	tyrosine and tryptophan absorption	0.1-100 ug/ml	small sample volume, rapid, low cost	incompatible with detergents and denaturating agents, high variability
Bicinchoninic acid	562 nm	copper reduction (Cu^{2+} to Cu^{1+}), BCA reaction with Cu^{1+}	20-2000 ug/ml	compatible with detergents and denaturating agents, low variability	low or no compatibility with reducing agents
Bradford or Coomassie brilliant blue	470 nm	complex formation between Coomassie brilliant blue dye and proteins	20-2000 ug/ml	compatible with reducing agents, rapid	incompatible with detergents
Lowry	750 nm	copper reduction by proteins, Folin-Ciocalteu reduction by the copper-protein complex	10-1000 ug/ml	high sensitivity and precision	incompatible with detergents and reducing agents, long procedure

Table 1. Common total protein assays.

3) Concentrate the FKBP12 protein

- Use centrifugal filter
- Record the total starting volume of protein solution
- Record the final volume after centrifugation

$$\text{Concentration factor} = \frac{\text{Starting volume}}{\text{Final volume}}$$

New FKBP12 concentration ≈

Old concentration (BCA) * conc. factor

*

M1D5 has a hefty homework assignment due—start on it soon!

- Data figure, represents evaluation of purified protein
  - 1 figure = 1 message; Either SDS-PAGE *or* BCA graph
  - Remember title & caption
- Results subsection related to figure
  - Bullet point format—see homework description on wiki
- Methods subsections (complete with your partners)
  - M1D3 protein purification
  - M1D4 evaluation of protien puritiy and concentration
  - Paragraph form, past tense
- Meet with BE fellow, submit a short summary about your meeting(1-2 paragraphs)

Tips for writing Methods

- Include enough information to replicate the experiment
  - List manufacturers name and location (City, ST)
  - Be **concise and clear** in your description
- Use subsections with descriptive titles
  - Put in logical order
  - Begin with topic sentence to introduce purpose
- Use clear and concise full sentenes
  - NO tables and lists
  - Passive voice expected
- 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.”

What can you improve in this example?

“Template DNA and primers were mixed with

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

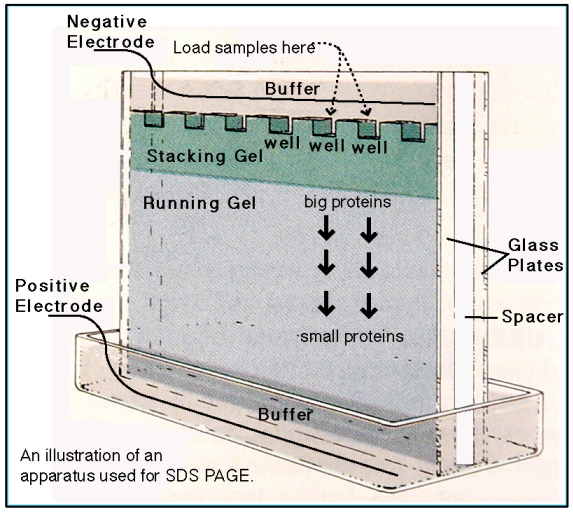
was added to 50 uL. A tube without template

was prepared and labeled control.”

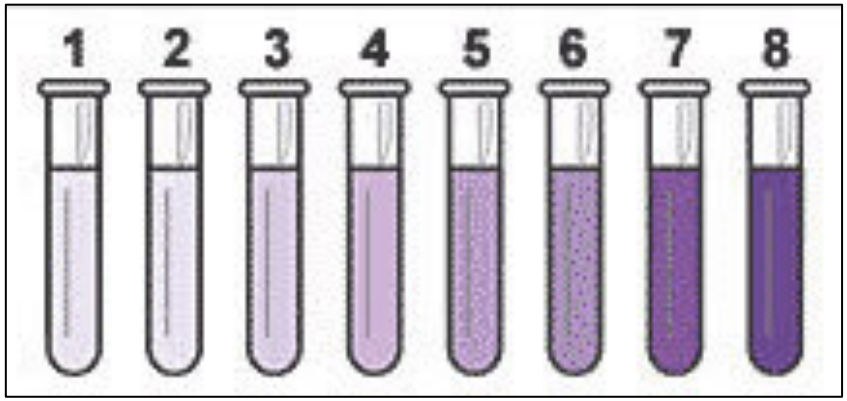
Revised example…

"*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."

Today in lab:

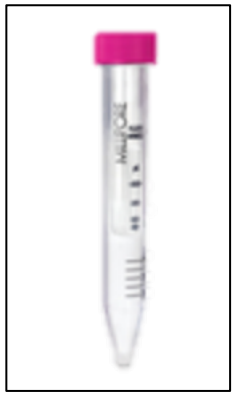


1. SDS-PAGE
   - boil samples
   - load samples in lanes
   - run at 200 V for 30 min
   - rinse with water
   - stain with Coomassie



2. BCA assay
   - prepare BSA standards
   - prepare working reagent
   - incubate at 60°C for 30min
   - measure OD_{562}

3. Concentrate protein



Today in lab:

Red, Pink, Purple, White, and Gray Teams:

- Start with Part 2 (SDS-PAGE)
- First round of electrophoresis

Orange, Yellow, Green, and Blue Teams:

- Start with Part 2 #1-4 (prepare samples for SDS-PAGE, but do not boil or load)
- Then move on to Part 3 (BCA)
- Second round of electrophoresis