Overview of Module 2

1. Name one protein function:

   Antibody, structural component, messenger, enzyme, regulatory

2. What is the purpose of a Western blot?

   Separate and identify target protein to better understand the protein's role and how protein mutations can result in disease state

3. What is the purpose of a BCA Assay? Which metal is present in BCA reagent?

   A BCA Assay allows us to calculate sample protein concentration using absorbance readings. The copper ion (Cu^{2+}) is reduced during the Biuret Reaction in a BCA Assay

4. What is the purpose of SDS-PAGE?

   Gel electrophoresis separates protein fragments by size.

5. Why do we need to transfer proteins from a gel to a membrane?

   The antibodies utilized in immunoblotting cannot bind to the protein when the protein is on the gel.

Practice Preparing a BCA Assay

1. **Calculate serial dilution volumes.** We will start with a 20 ug/mL BSA solution.
   a. Need 25 uL of standard for each well x 3 replicates = 75 uL per standard
   b. Always make a little extra: 80 uL per standard

BSA Concentration (ug/mL)	BSA Solution Added (uL)	Water Added (uL)	Total Volume (uL)	Final Volume (uL)
2000 ug/mL	160 uL of 2000 ug/mL	0	160	160 - 80 = 80
1000 ug/mL	80 uL of 2000 ug/mL	80	160	160 - 80 = 80
500 ug/mL	80 uL of 1000 ug/mL	80	160	160 - 80 = 80
250 ug/mL	80 uL of 500 ug/mL	80	160	160 - 80 = 80
50 ug/mL	32 uL of 250 ug/mL	128	160	160 - 16 = 144
5 ug/mL	16 uL of 50 ug/mL	144	160	160 - 0 = 160

0 ug/mL	0	160	160	160

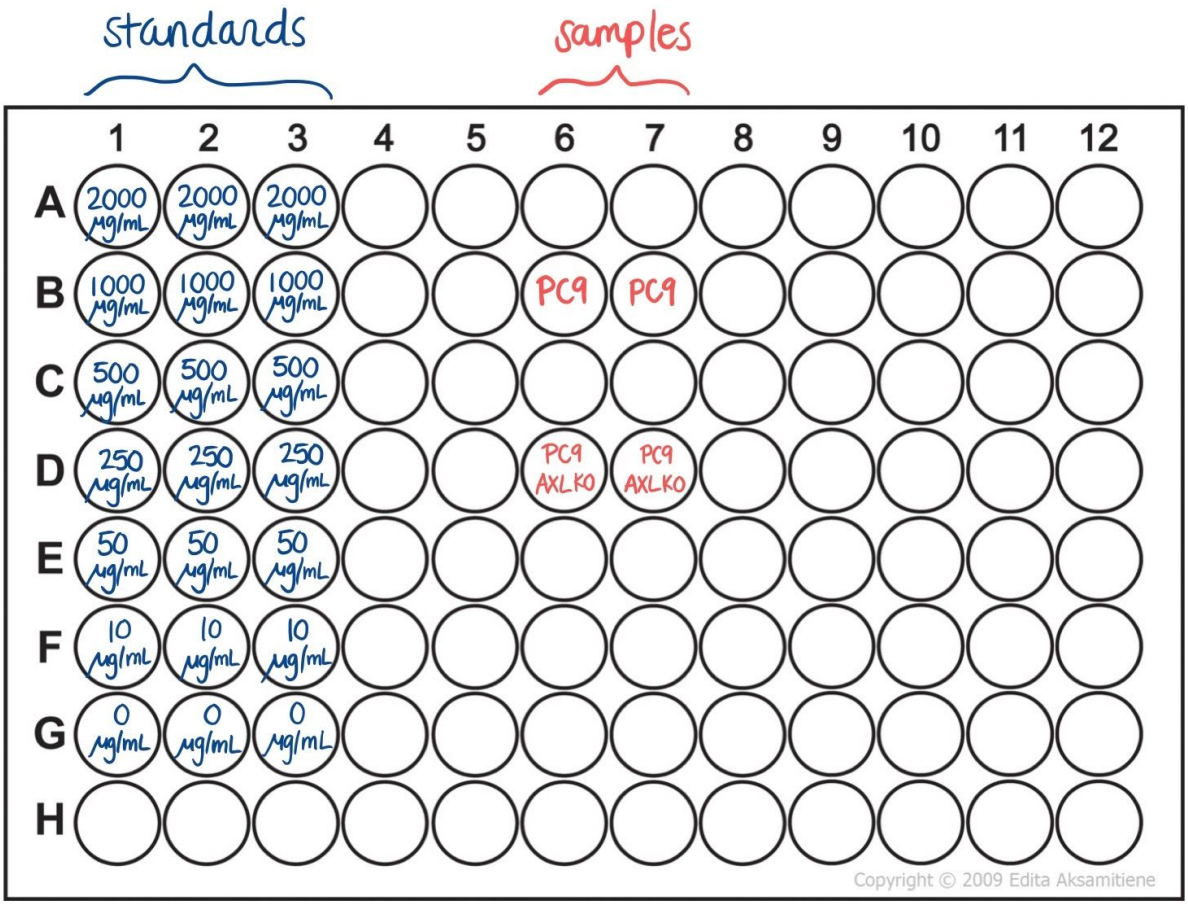
2. Design layout for 96 well plate:

a. Number of standards 7 x Number of repeats 3 = 21 wells

b. Number of samples 2 x Number of repeats 2 = 4 wells

c. Total number of wells = Standard wells 21 + Sample wells = 4 = 25 wells

d. Label the well plate below with standard and sample placement



3. Prepare reaction buffer:

a. Total number of wells 25 x 200 uL/well = 5000 uL reaction buffer

b. Always make a little extra: 5500 uL

c. Combine reagents A, B, and C in ratio 25:24:1 to make reaction buffer

i. Reagent A = (total volume of buffer 5500 uL)(25/50) = 2750 uL

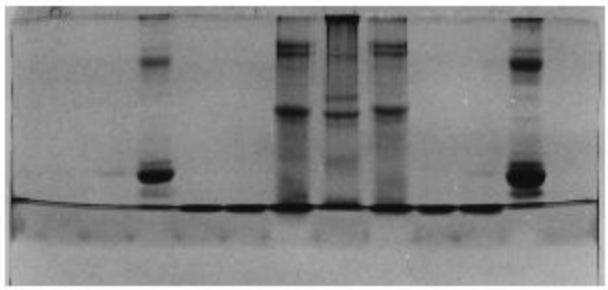
ii. Reagent B = (total volume of buffer 5500 uL)(24/50) = 2640 uL

iii. Reagent C =(total volume of buffer 5500 uL)(1/50) = 110 uL

Western Blot Hall of Shame (Challenge Question)

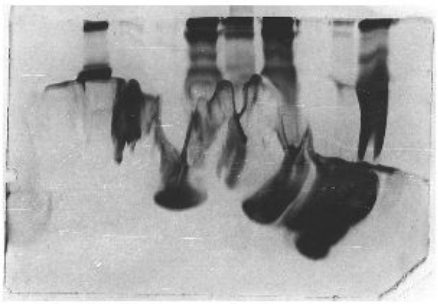
What do you think went wrong in the blots below?

A.



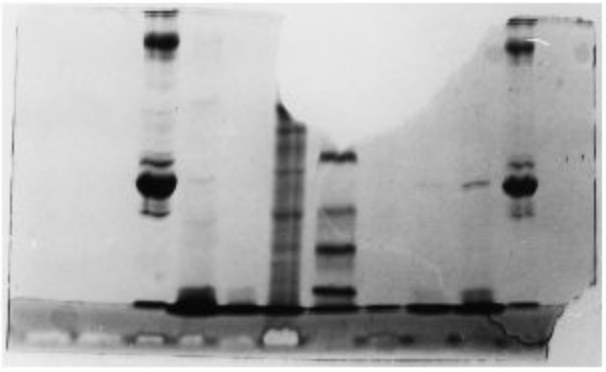
Stopped running the gel too soon → diffuse dye front

B.



Acrylamide mixture was poured evenly into mold or wells were overloaded with protein → smearing

C.



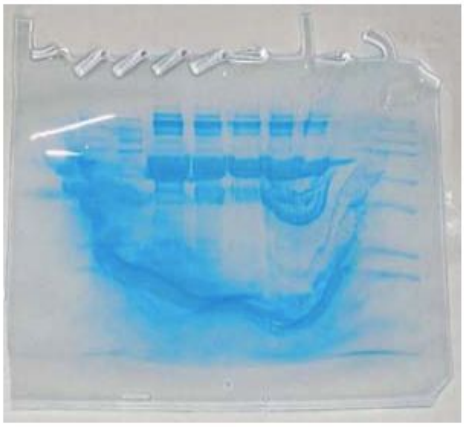
gel broke during handling process (can still use pieces and try to transfer to membrane)

D.

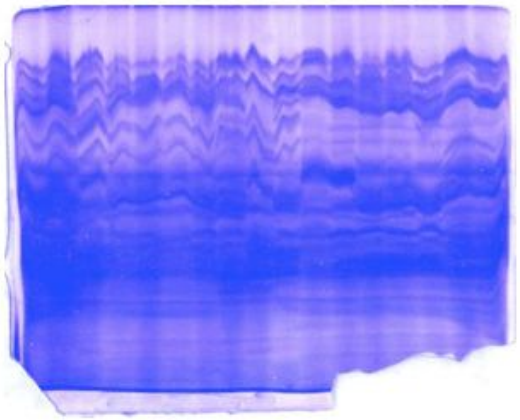
Acrylamide mixture was too dense (too crosslinked) to allow proteins to travel down

E.

F.

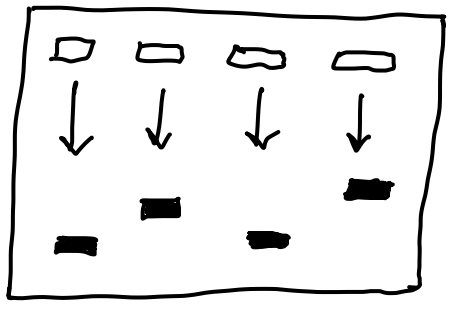


someone opened the gel box with the power on, disrupting the electric field



run with high current and overheated, resulting in warping

Mathematical Derivation of protein Displacement ($\vec{x}$) Using Force Equation.



Let's start with the force equation from Physics:

$$\vec{F} \equiv m\vec{a} \qquad (1)$$

By definition, acceleration is defined as the second time derivative of displacement ($\vec{x}$):

$$\vec{a} \equiv \frac{d^2\vec{x}}{dt^2} \qquad (2)$$

Combining eq. (1) and (2):

$$\vec{F} = m\frac{d^2\vec{x}}{dt^2} \qquad (3)$$

Now, we have a separable differential equation. To solve this, we move and integrate twice:

$$\frac{d^2\vec{x}}{dt^2} = \frac{\vec{F}}{m}$$

$$\int \frac{d^2\vec{x}}{dt} = \int \frac{\vec{F}}{m}\, dt$$

$$\frac{d\vec{x}}{dt} = \frac{\vec{F}}{m}t + \vec{A} \qquad (4)$$

$$\int \frac{d\vec{x}}{dt} = \int \left(\frac{\vec{F}}{m}t + \vec{A}\right)$$

$$\vec{x} = \frac{\vec{F}}{2m}t^2 + \vec{A}t + \vec{B} \qquad (5)$$

Let's assume $\vec{F} = \alpha$ is constant, and the same force is applied to all protein samples. Equation (5) becomes:

$$\vec{x} = \frac{\vec{\alpha}}{2m}t^2 + \vec{A}t + \vec{B} \qquad (6)$$

What happens if m increases?

$\vec{x}$ decreases. Thus, larger proteins won't travel as far.

On the other hand, what happens if m decreases?

$\vec{x}$ increases. So smaller proteins will travel further.