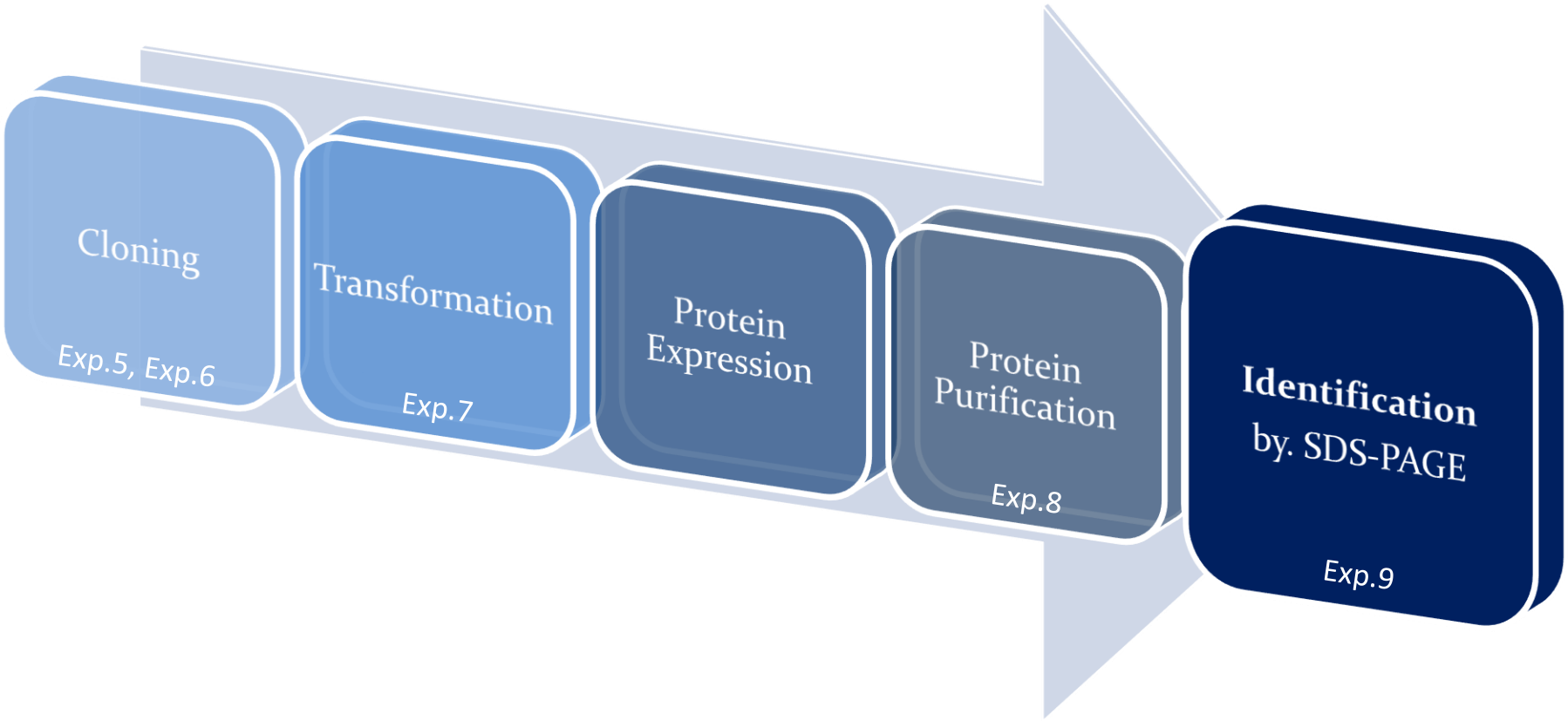
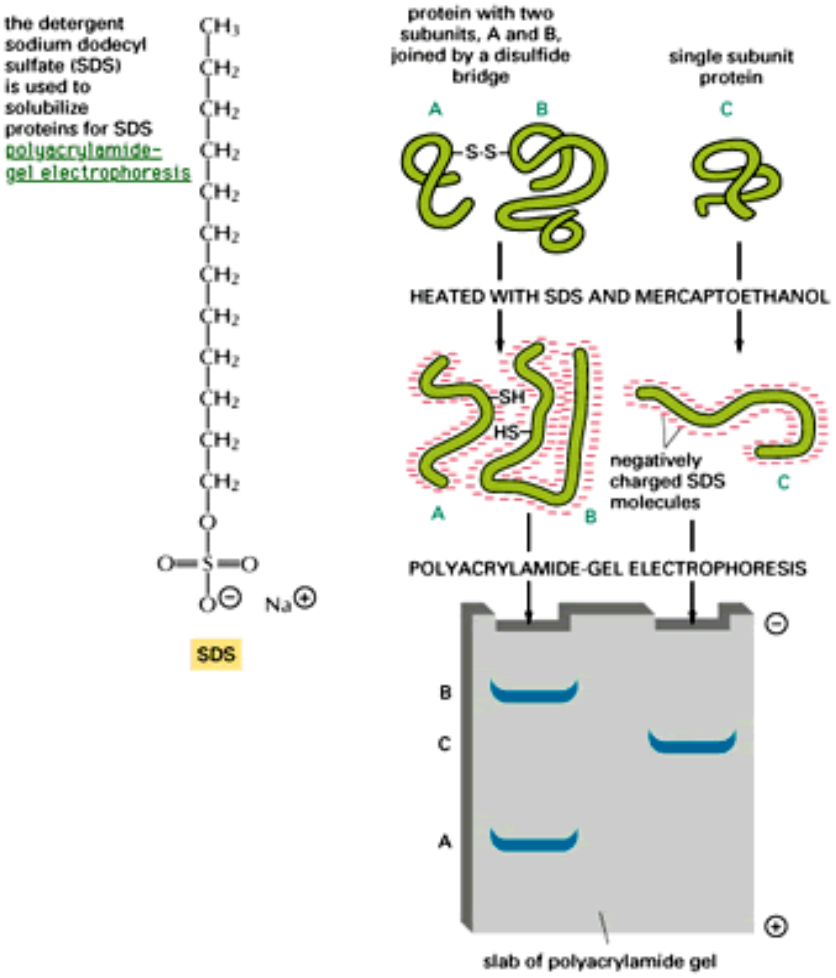
SDS-PAGE

Where are we now?



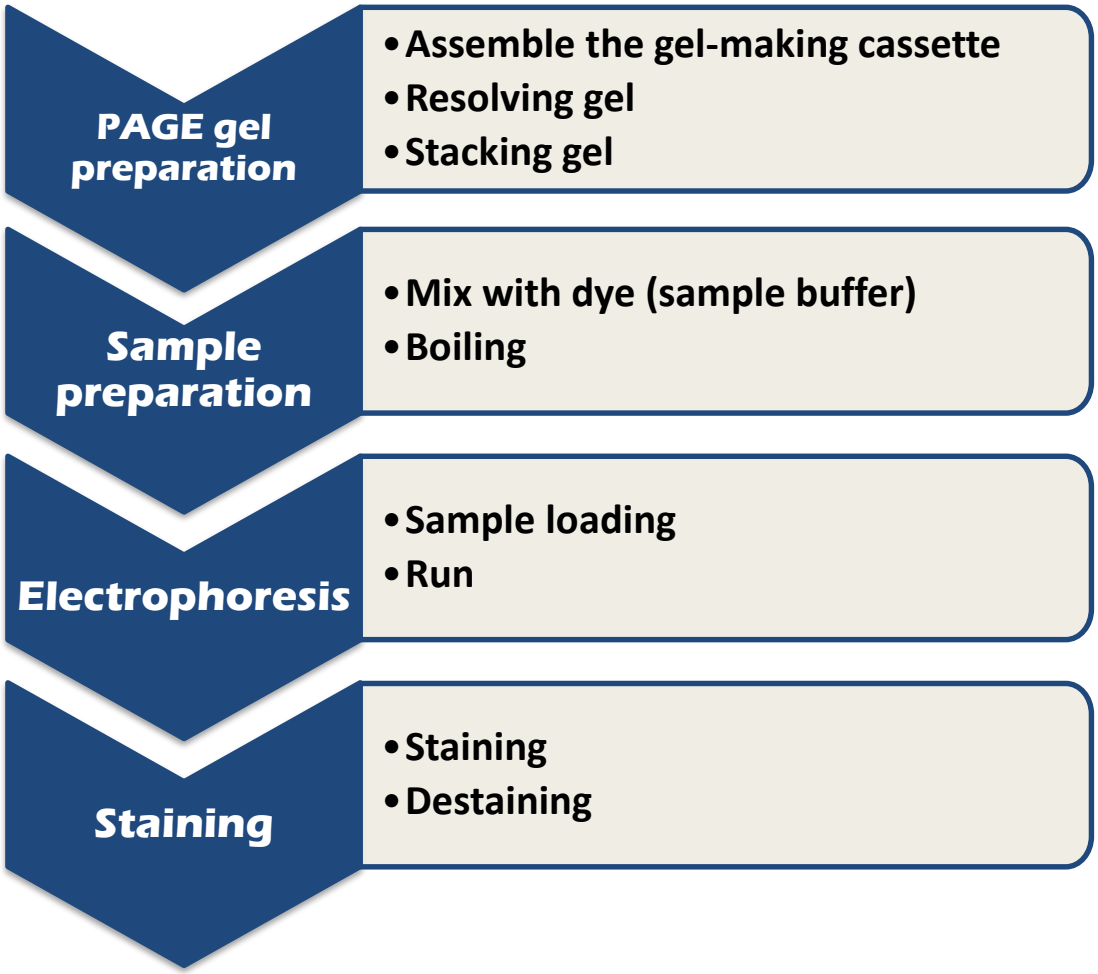
SDS-PAGE

Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis



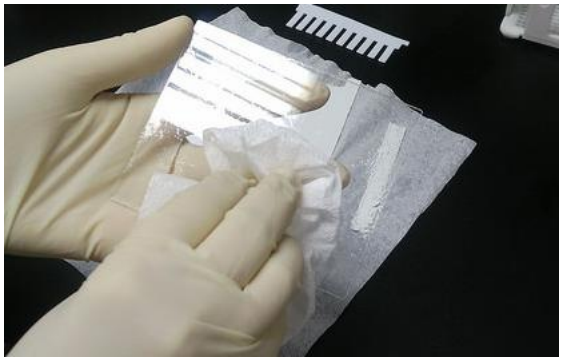
- Separate proteins according to their electrophoretic mobility
(a function of length or molecular weight as well as higher order protein folding, posttranslational modifications and other factor)

Today's Experimental Scheme



PAGE-gel Preparation

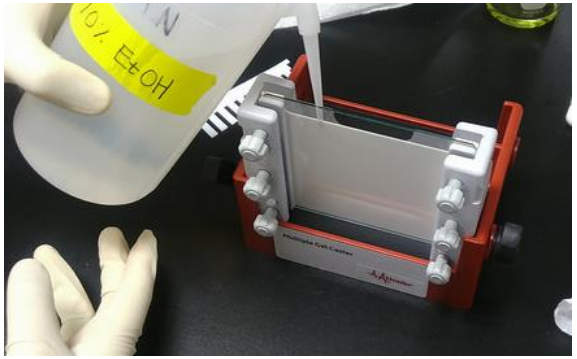
Step 1. Build up the gel-making cassette



1) Wipe the plates with 70% EtOH

2) Arrange the plates & spacers

3) Assemble the cassette



4) Check the leaking

PAGE-gel Preparation

Step 2. Mix the gel components

SDS-PAGE Gel			
Components	**Resolving gel**	Components	**Stacking gel**
	15 %		**5 %**
DW	1.15 ml	DW	1.34 ml
1.5 M Tris **(pH8.8)**	1.25 ml	1.0 M Tris **(pH6.8)**	250 ul
10 % SDS	50 ul	10 % SDS	20 ul
30 % Acrylamide mix	2.5 ml	30 % Acrylamide mix	330 ul
TEMED	3 ul	TEMED	3 ul
10 % APS (0.1 g/ml)	**50 ul**	**10 % APS (0.1 g/ml)**	**20 ul**
total	5 ml		2 ml

Add at last!

Just before you put the gel b/w the plates

Sample Preparation

Step 1. Mix your samples with 5X SDS-PAGE loading buffer

Sample 20ul + Buffer 5ul

※ Label each sample clearly not to be confused

Step 2. Boil the sample

※ The water or heat-block is hot! Be careful!

Step 3. Prepare protein loading tips

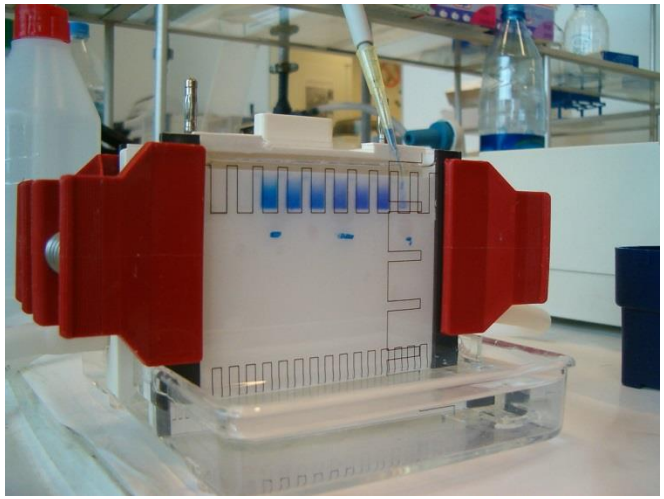
Electrophoresis

Step 1. Set up the equipment

1) Put up the casting cradle on the bottom chamber
2) Fix your gel to the casting cradle
3) Pour the 1X SDS-running buffer to fill the chamber

Step 2. Loading sample

1) Carefully load your sample to the well
   20 ul for each sample
2) Load the protein size-marker : 5 ul



Step 3. Running electrophoresis

150V, 60min

※ Check the electrode (black-black / red-red) before closing the upper chamber

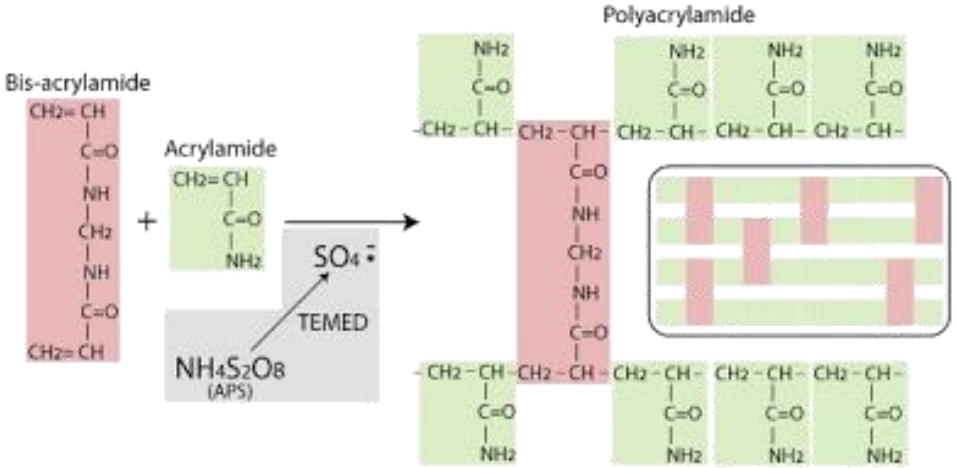
Staining

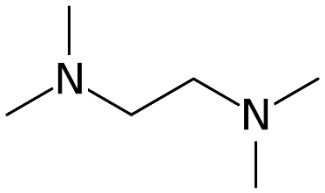
Step 1. Pour the staining solution
then **shake for 30min**

Step 2. Remove staining solution
and **pour de-staining solution**

※Staining solution is re-usable.
Do not discard to the sink and collect it to the assigned bottle.

Polyacrylamide Gel





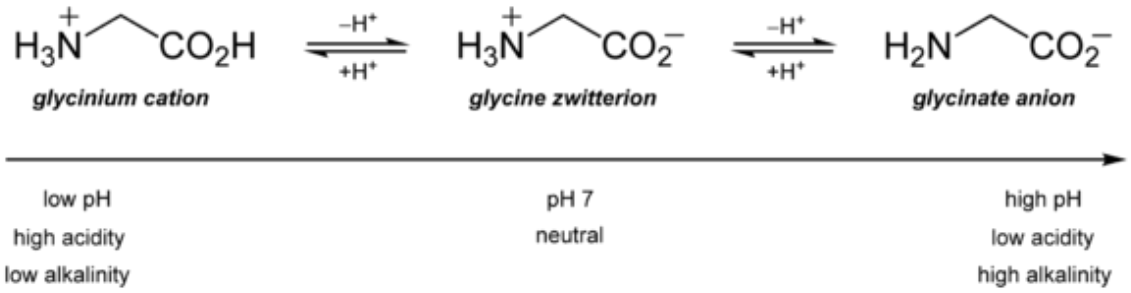
TEMED

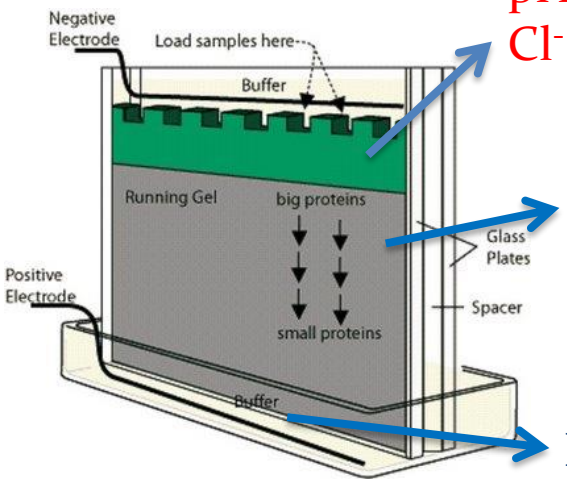
- Acrylamide cross-linked by N,N'-methylene bis-acrylamide.

- The polymerization is initiated by APS(ammonium persulfate) free radical, which is generated by TEMED.

※ Acrylamide and bis-acrylamide is known to have strong neurotoxicity. Be careful!

Resolving & Stacking Gel





Stacking gel
pH 6.8
Cl^- > pro > Gly

Resolving gel
(Running gel)
pH 8.8
Cl^- > Gly > pro

Buffer
(Tris-Glycine buffer)

- In large-pored stacking gel, proteins are caught between Cl^- and Glycine. It concentrates all proteins in one band, and makes the gel resolution better.
- Proteins are separated in resolving gel by molecular weight.

Loading Buffer Components

250mM Tris-HCl pH6.8

5% 2-mercapto ethanol

10% SDS

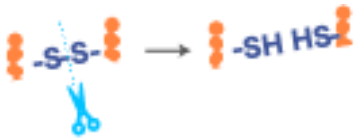
0.2% bromophenol blue

50% glycerol

- 2-mercapto ethanol

used as a reducing agent

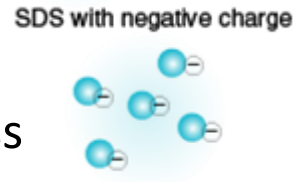
disconnect disulfide bond



- SDS (Sodium Dodecyl Sulfate)

used as an ionic detergent

even distribution of charge/mass



- Boiling for denaturation

Electrophoretic Mobility & Size

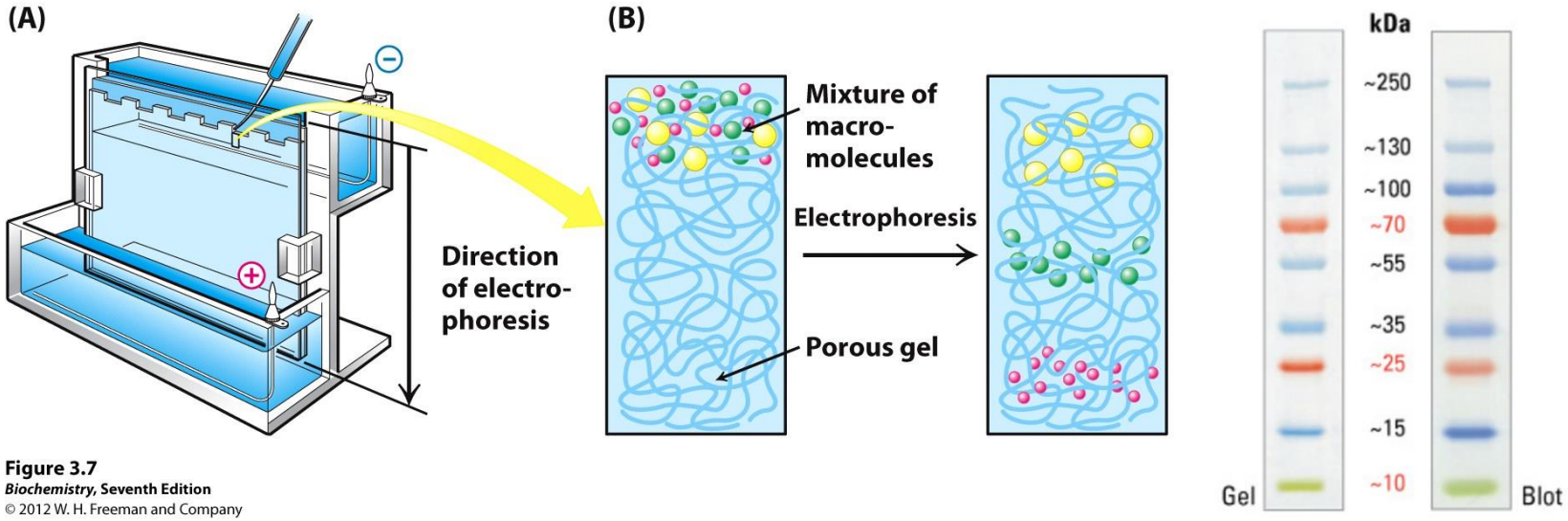


Figure 3.7
***Biochemistry*, Seventh Edition**

- Speed of migration is proportional to the size of the proteins ; **smaller polypeptides running faster than larger polypeptides**

- Compare the size of your protein to the size of GFP (26.9 kDa).

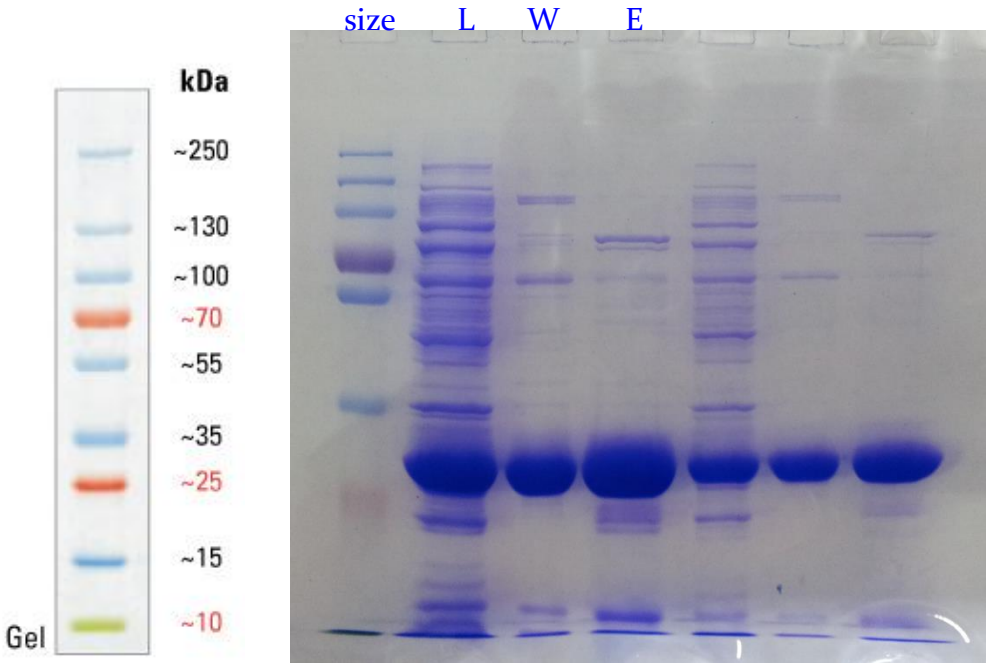
Linear Range of Protein Size for Effective Separation

Acrylamide concentration (%)	Linear range of separation (kD)
15	**12 - 43**
10	16 – 68
7.5	36 – 94
5.0	57 – 212

15% gel is appropriate for GFP

Result Analysis

Example.



Target Protein = 28.5 kDa