Dr. Phillip Carpenter pcarpenter@med-pathway.com

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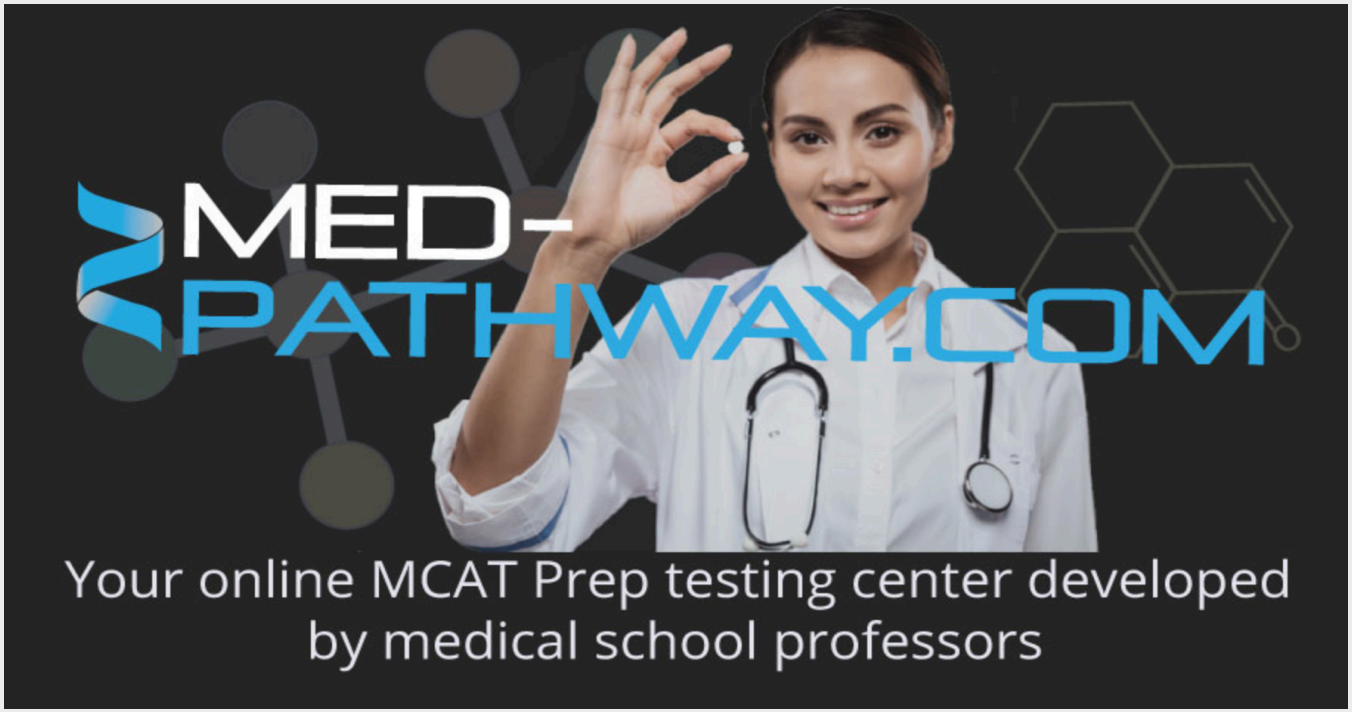
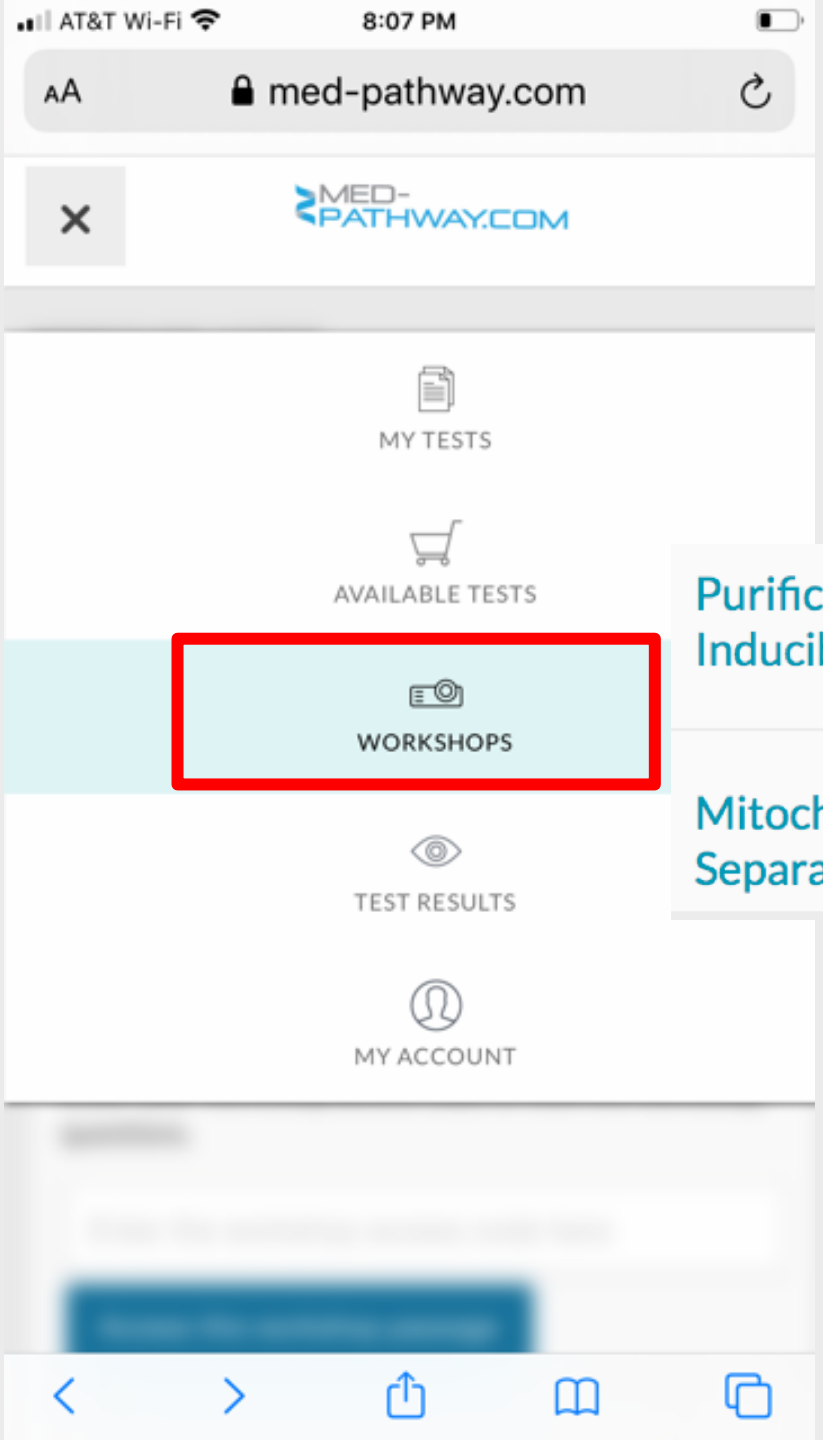
Lab Techniques Including Protein Purification & Separation Techniques

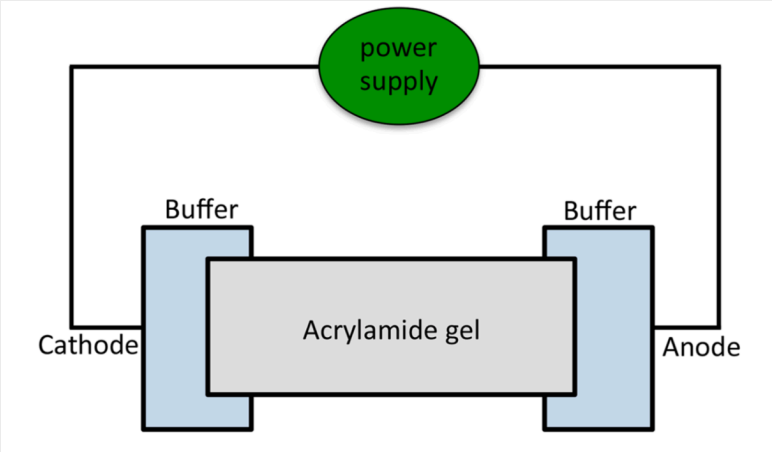




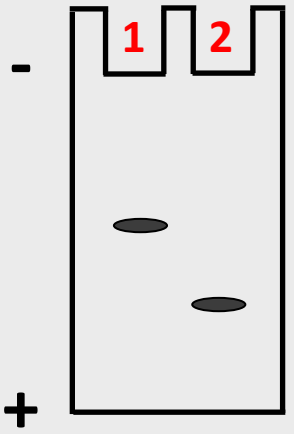
Purification of Hypoxia-Inducible Factor

Mitochondrial Permeability & Separation Chemistry

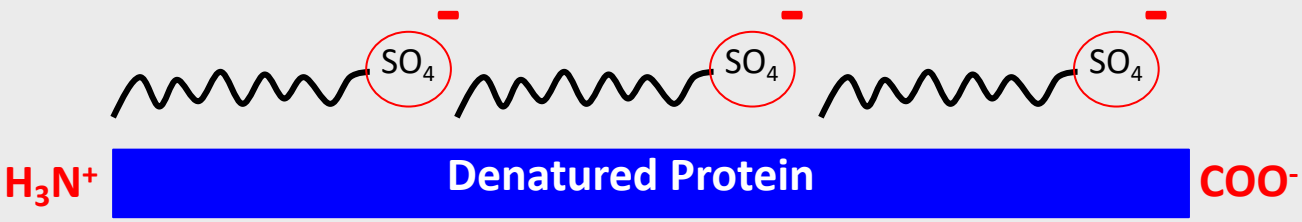
Gel Electrophoresis of Proteins



SDS PAGE



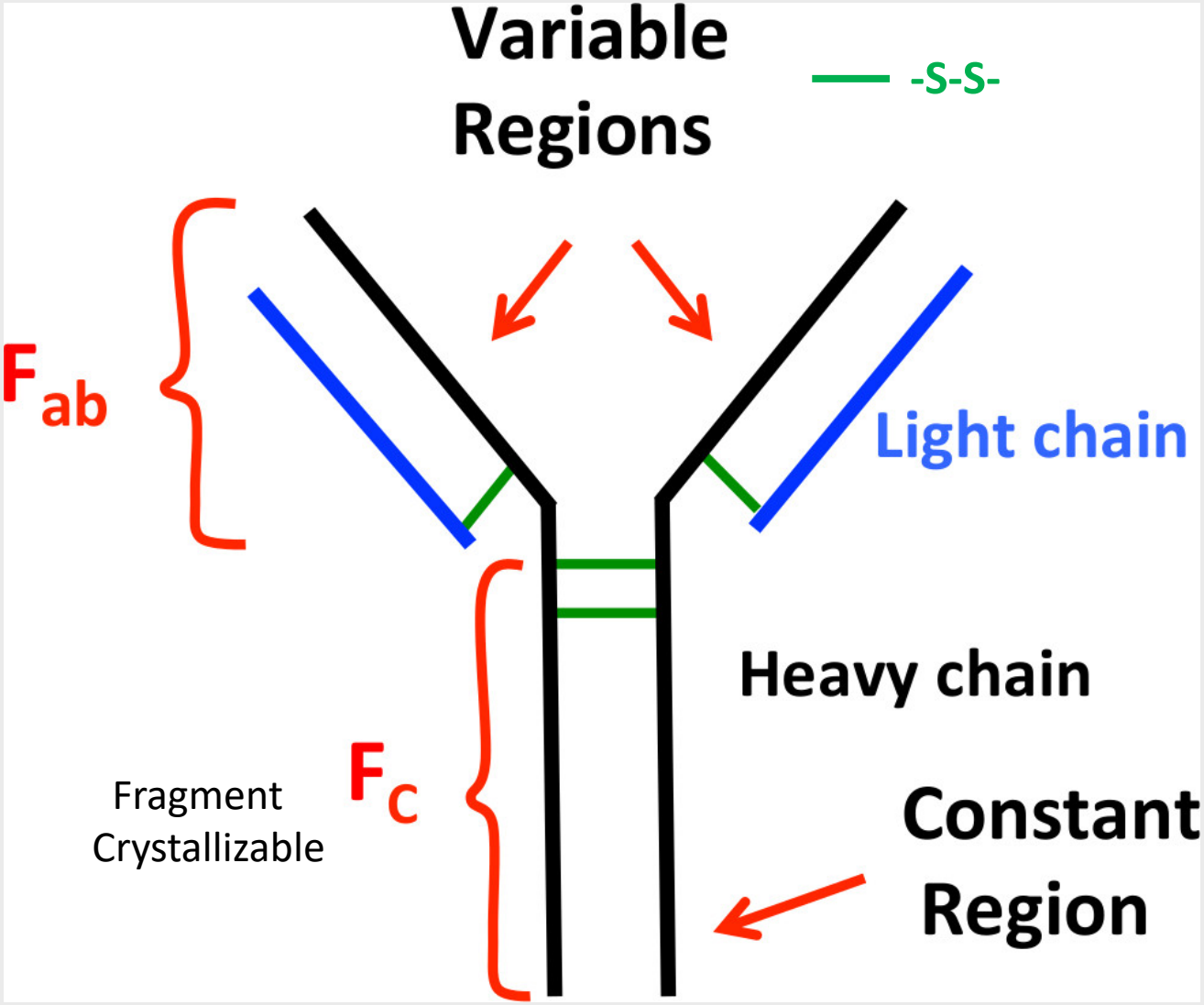
Sodium dodecyl sulfate (SDS) Denatures Proteins



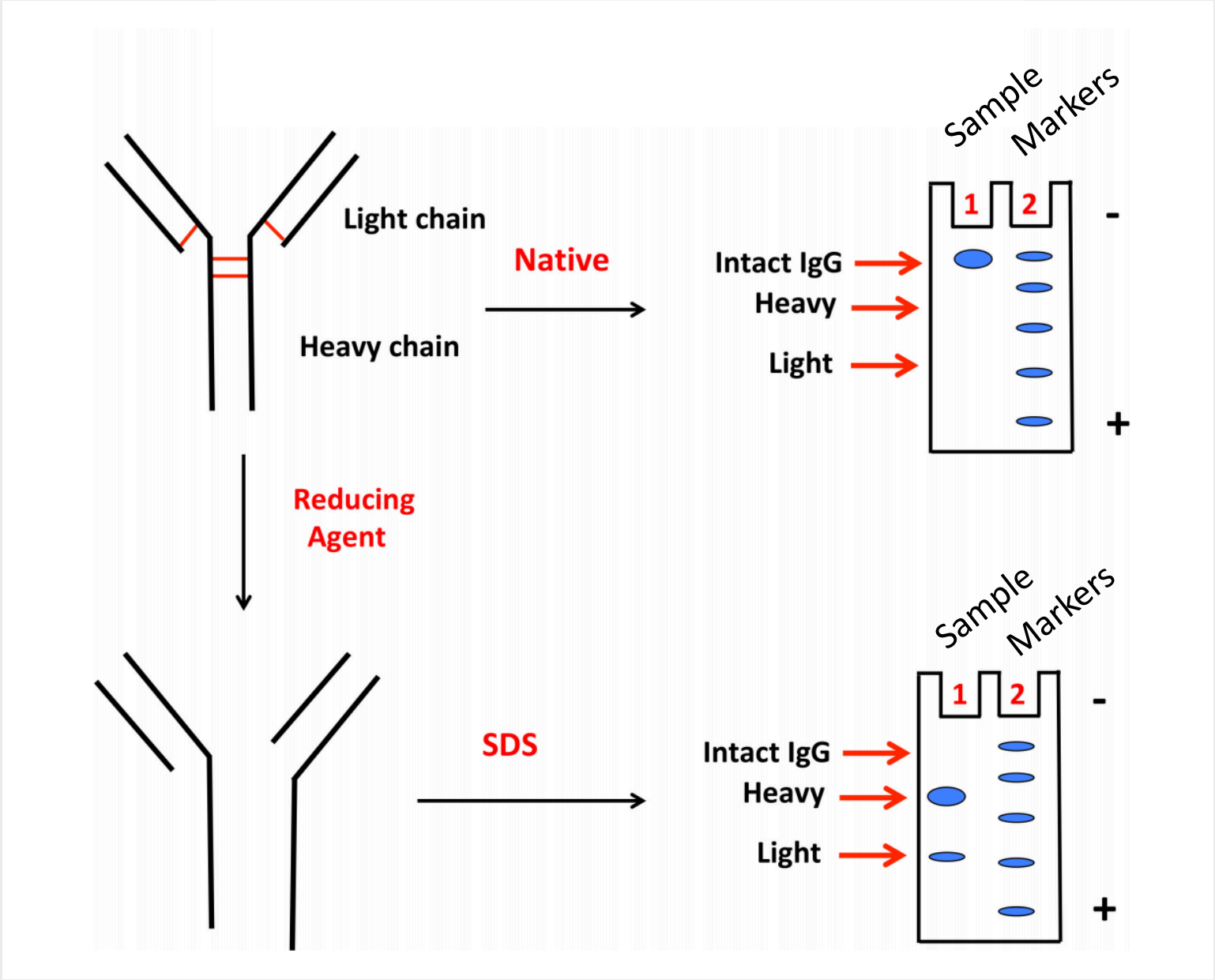
One SDS molecule per 2 Amino Acids
Normalizes the charge to mass ratio

Biological Techniques: Antibodies

Fragment Antigen Binding

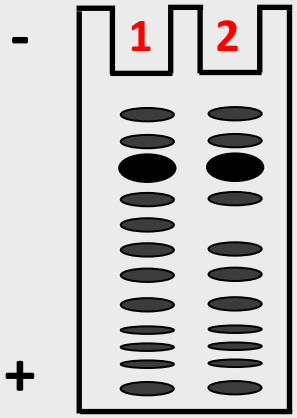


Native & Reducing Gels



Western Blotting

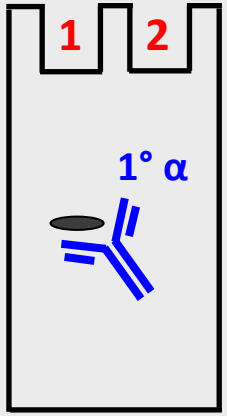
1. Run PAGE under Native or SDS reduced conditions.



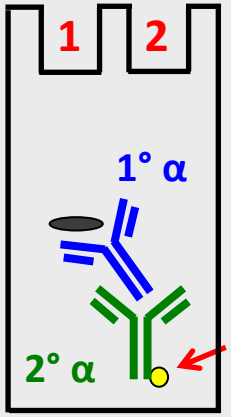
2. Transfer to membrane

3. Block membrane with Non-specific protein

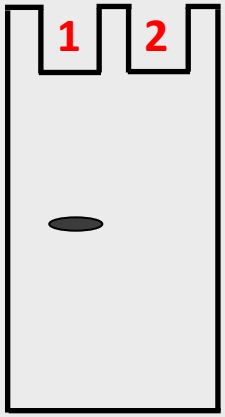
4. Add 1° antibody



5. Wash; Add 2°α



6. Wash, Detect



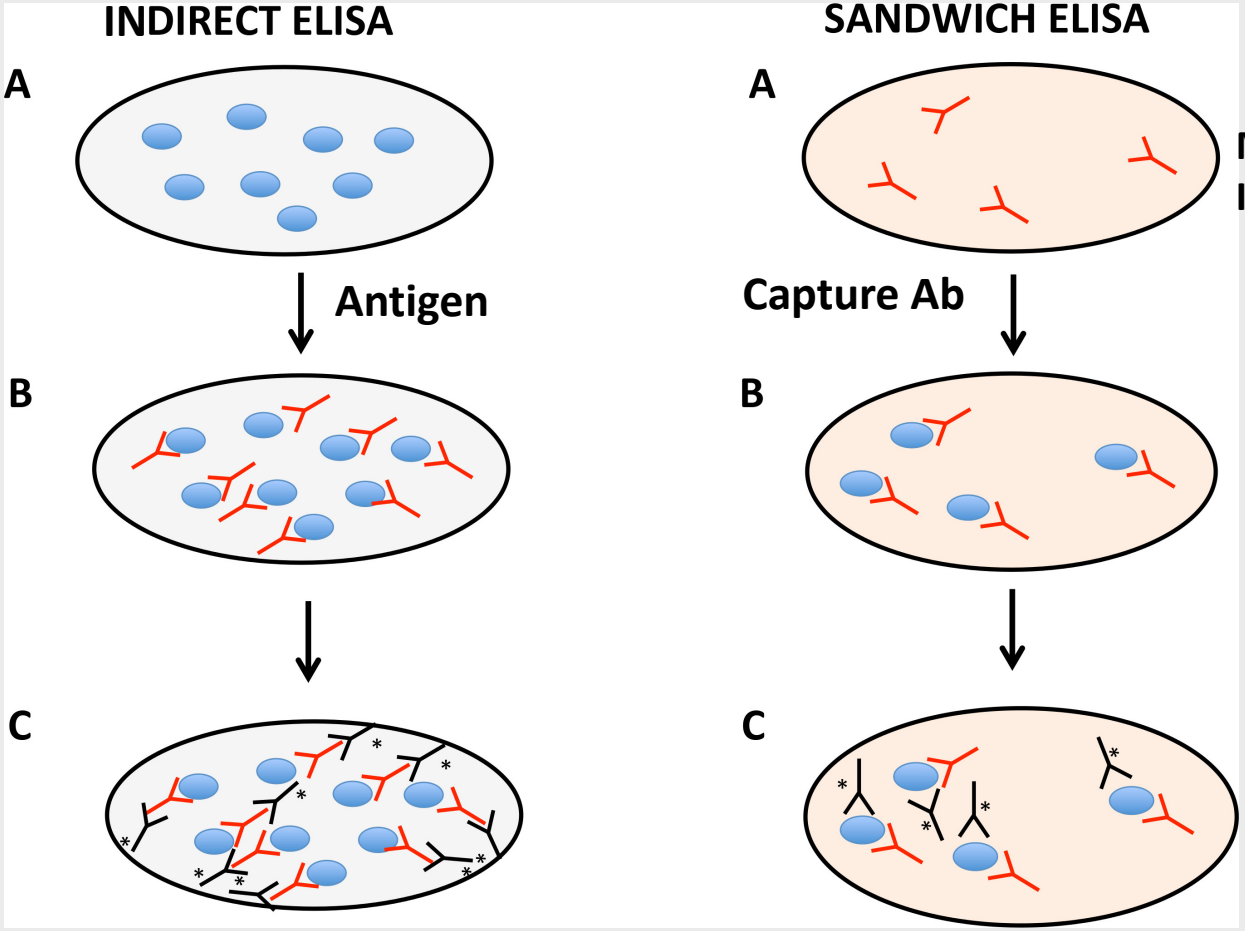
Controls?
Positive
Negative

ELISA Assays

2 Types

Determines [Ab]
In solution

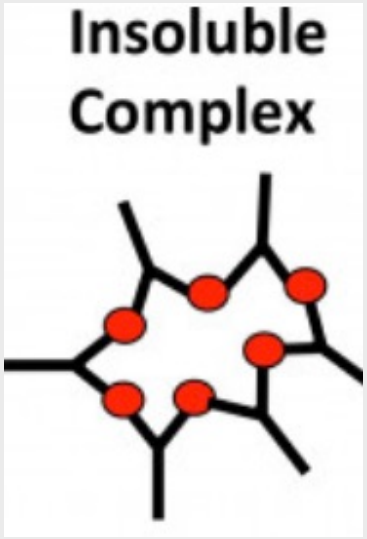
Measures [antigen]
In unknown sample



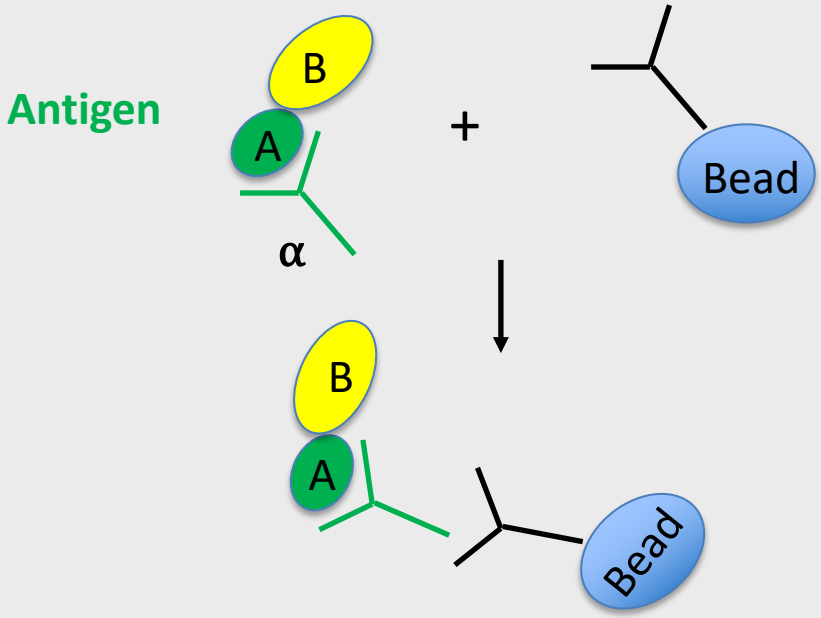
Controls:
No Antigen
No Primary Ab

Immunoprecipitation (IP)

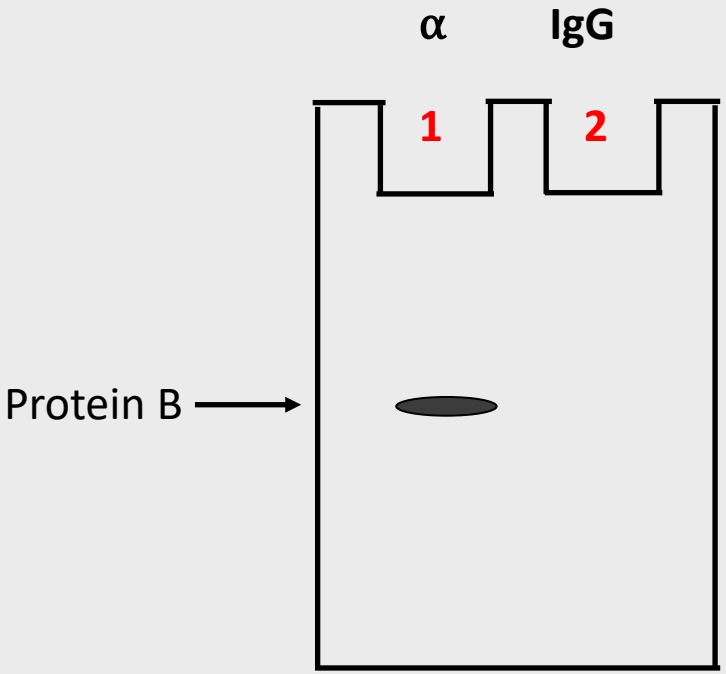
I



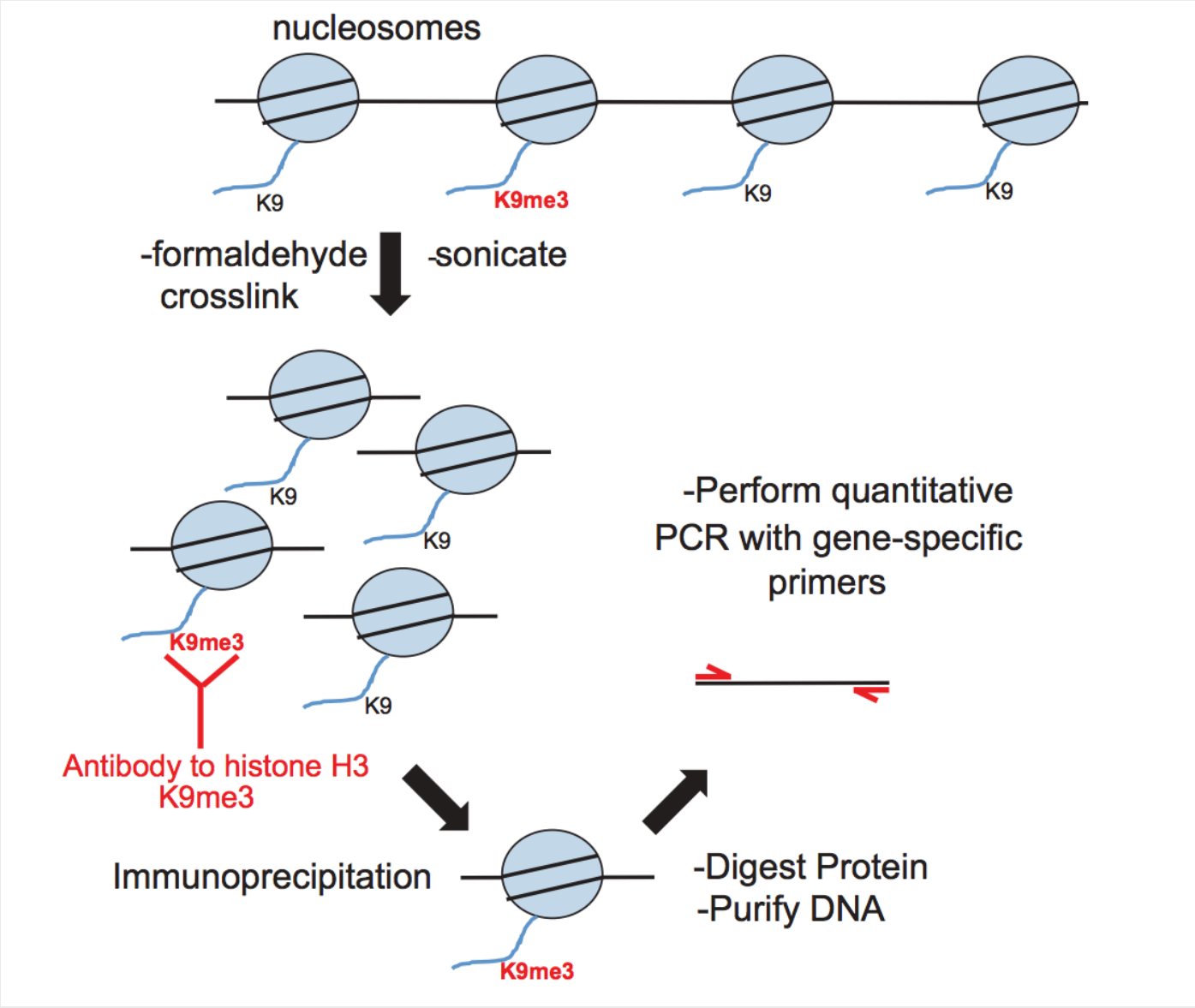
II



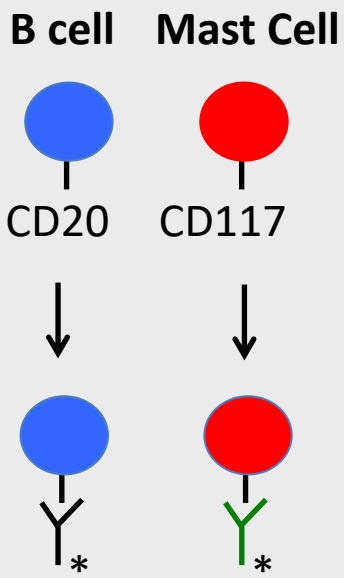
III IP/Western

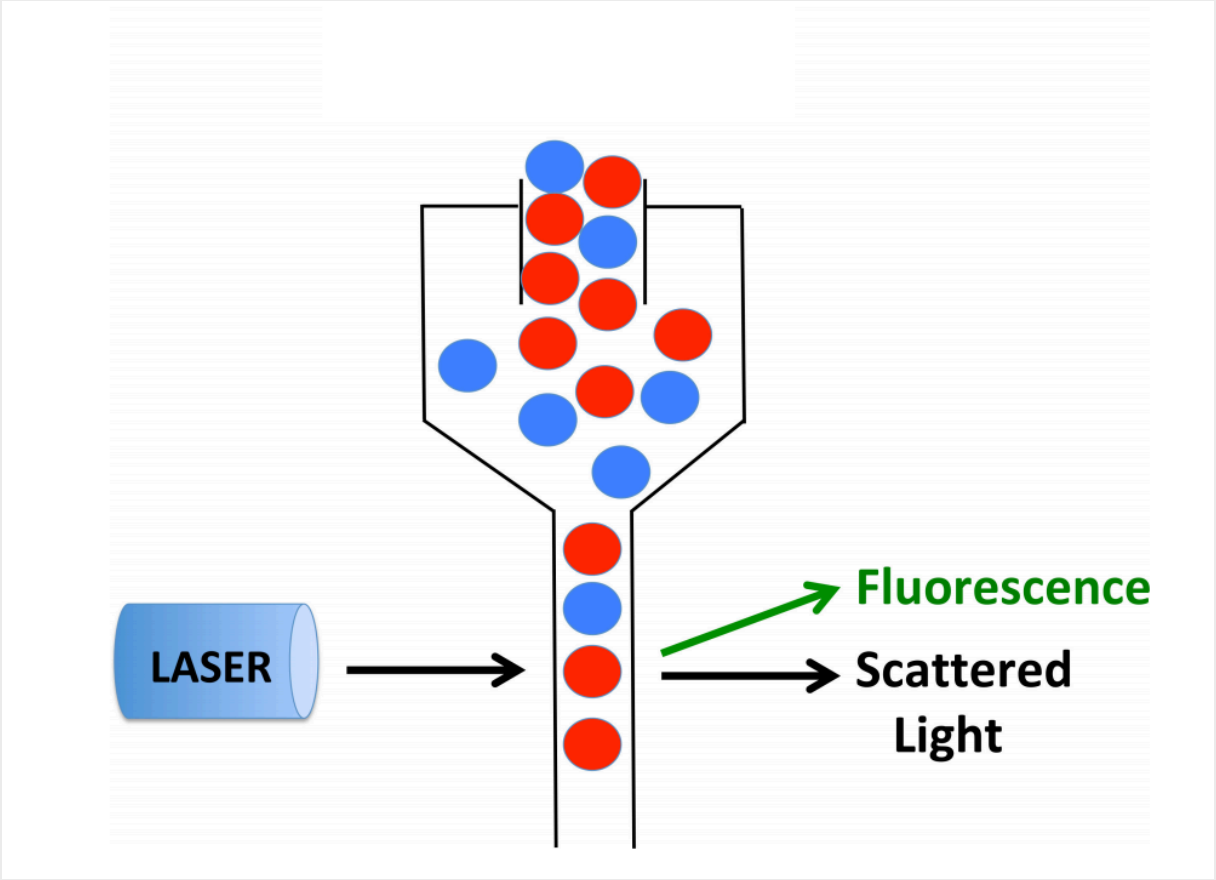


Chromatin Immunoprecipitation (ChIP)

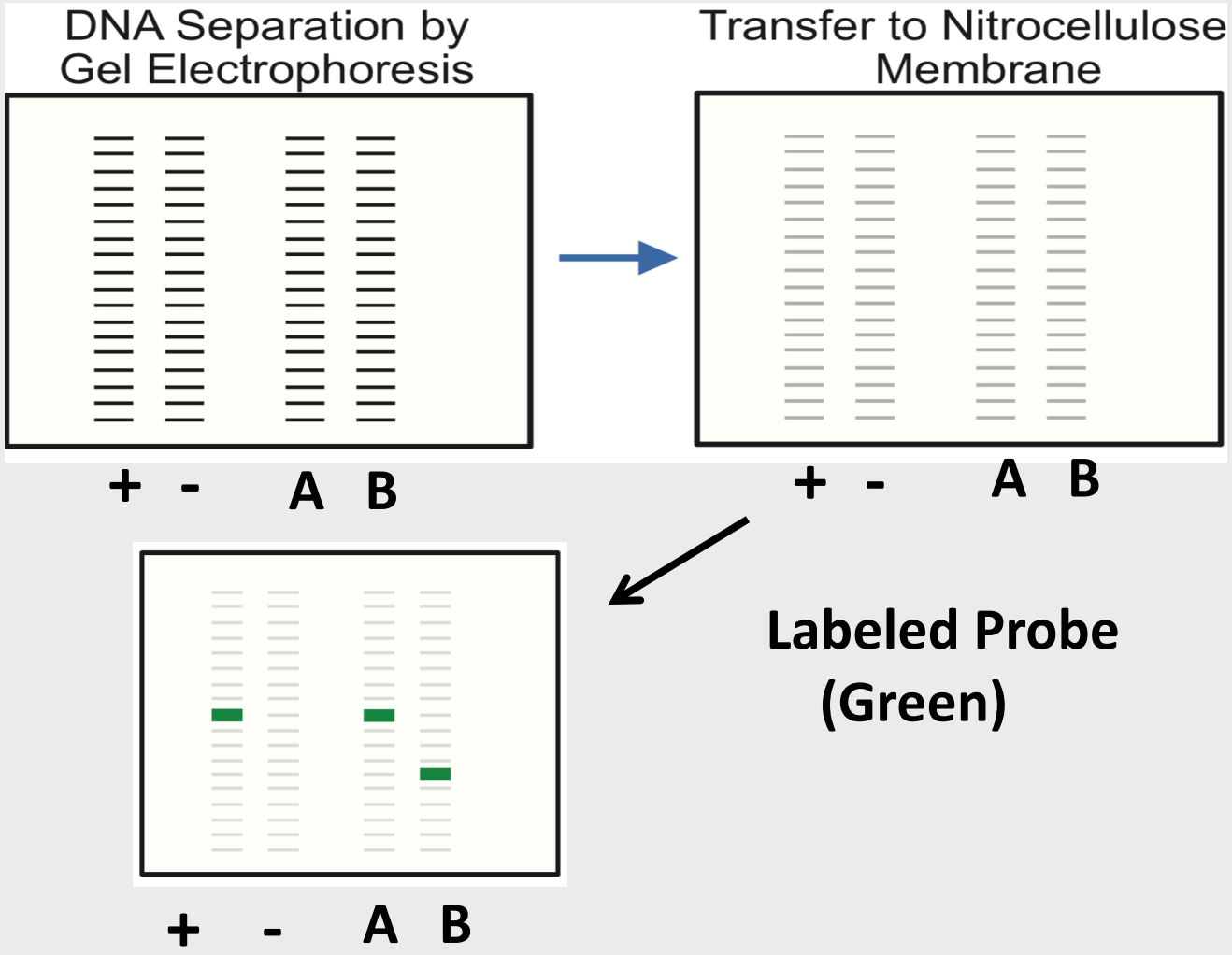


Flow Cytometry

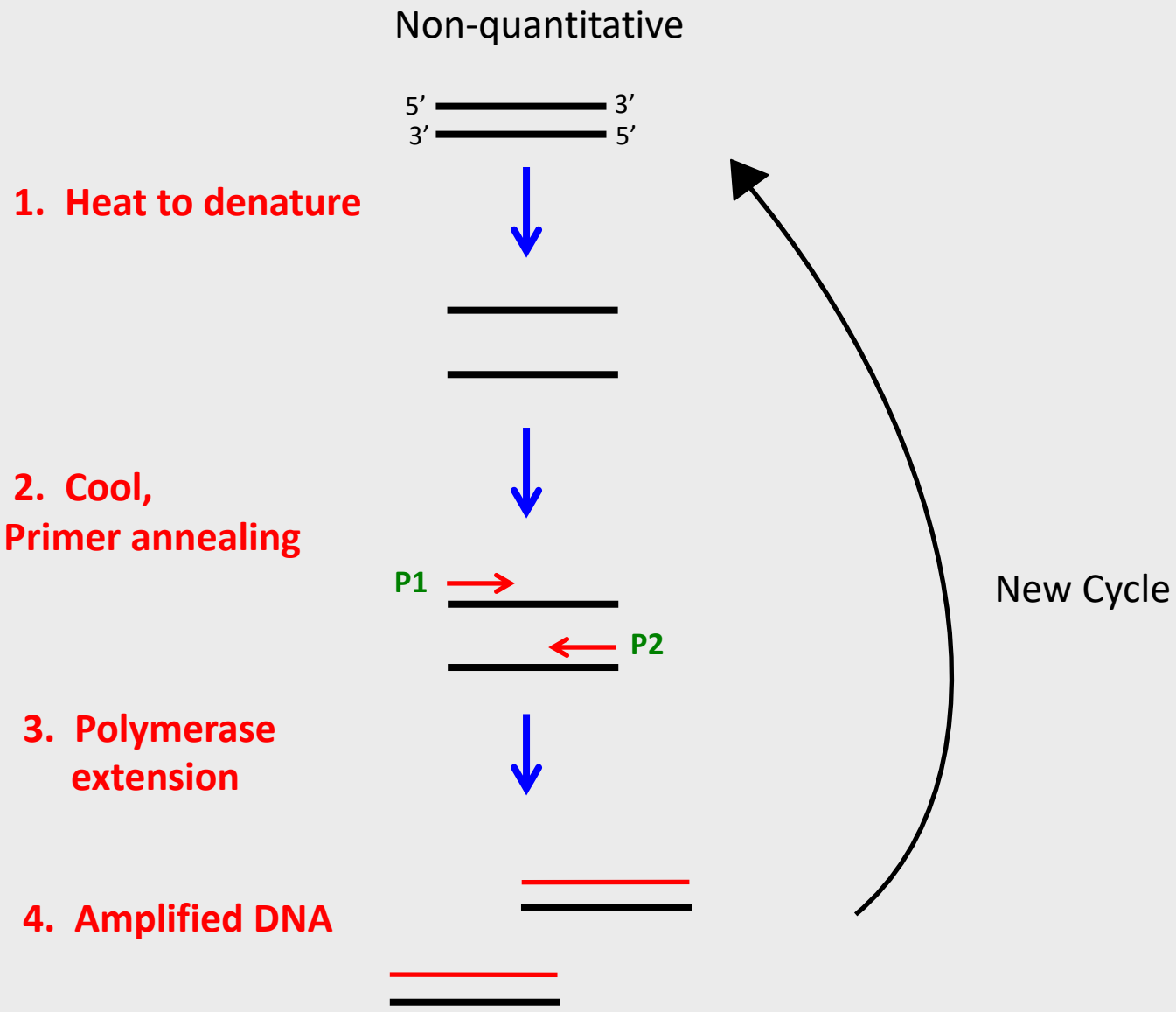




Nucleic Acid Techniques: Southern Blot

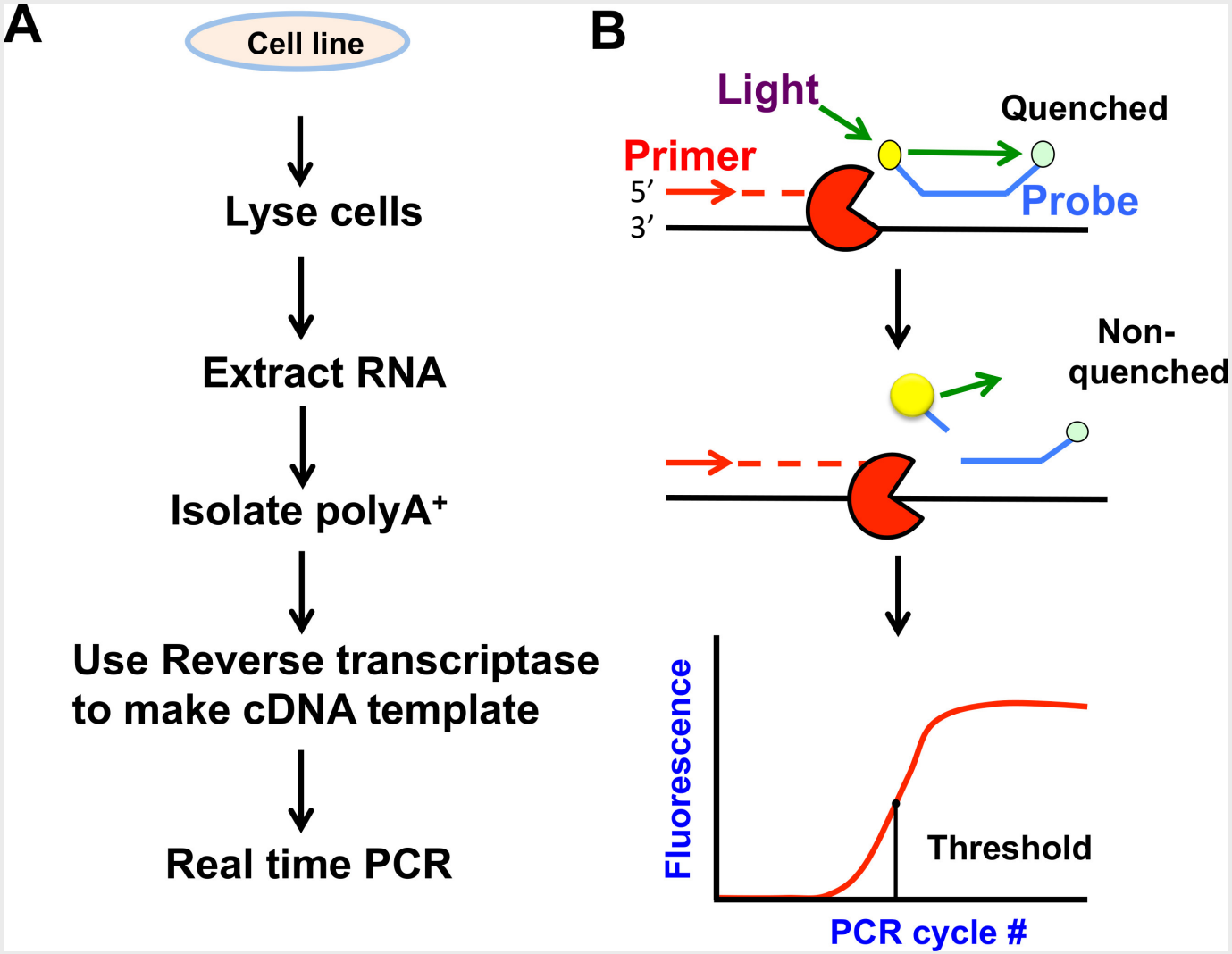


Polymerase Chain Reaction (PCR)



Real Time(RT) - PCR Assays

Quantification of mRNA; largely replaces Northern Blots

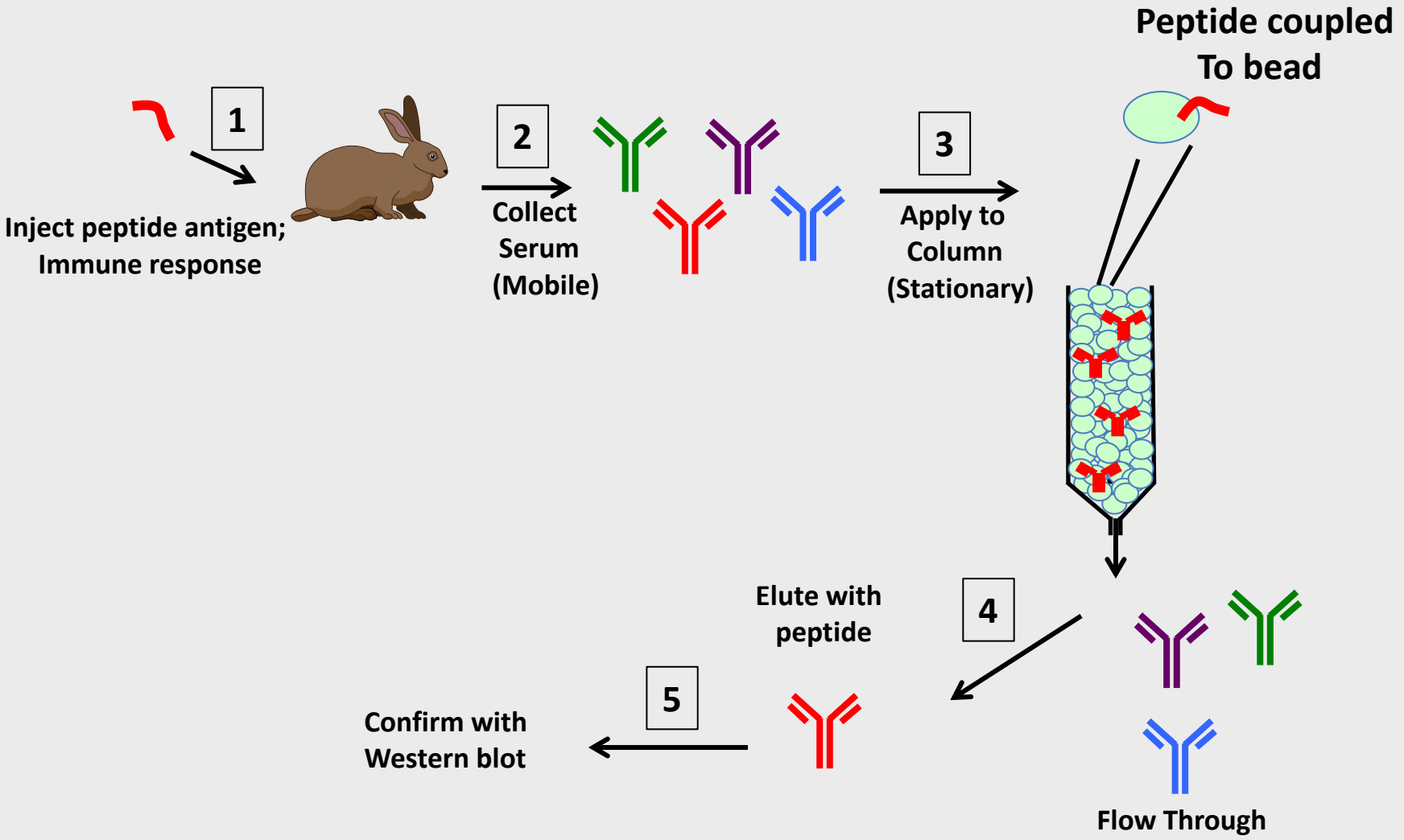


Chromatography

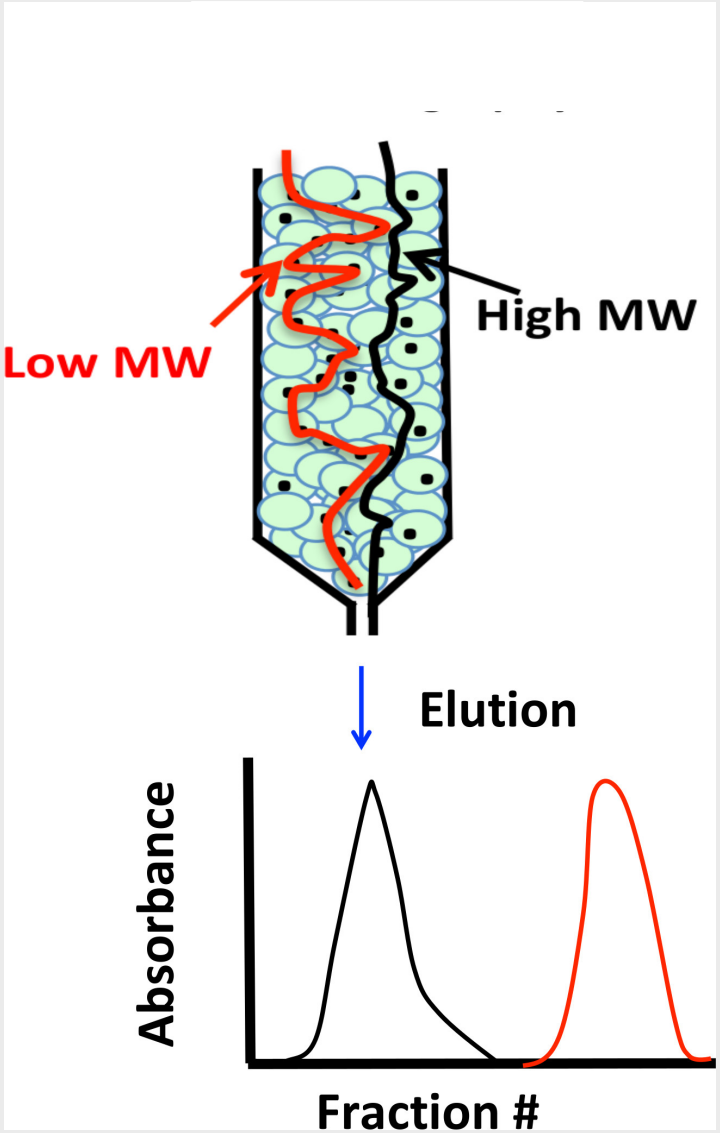
Separation of substances based upon their physical behavior in both mobile and stationary phases

Antibody Purification: Affinity Columns

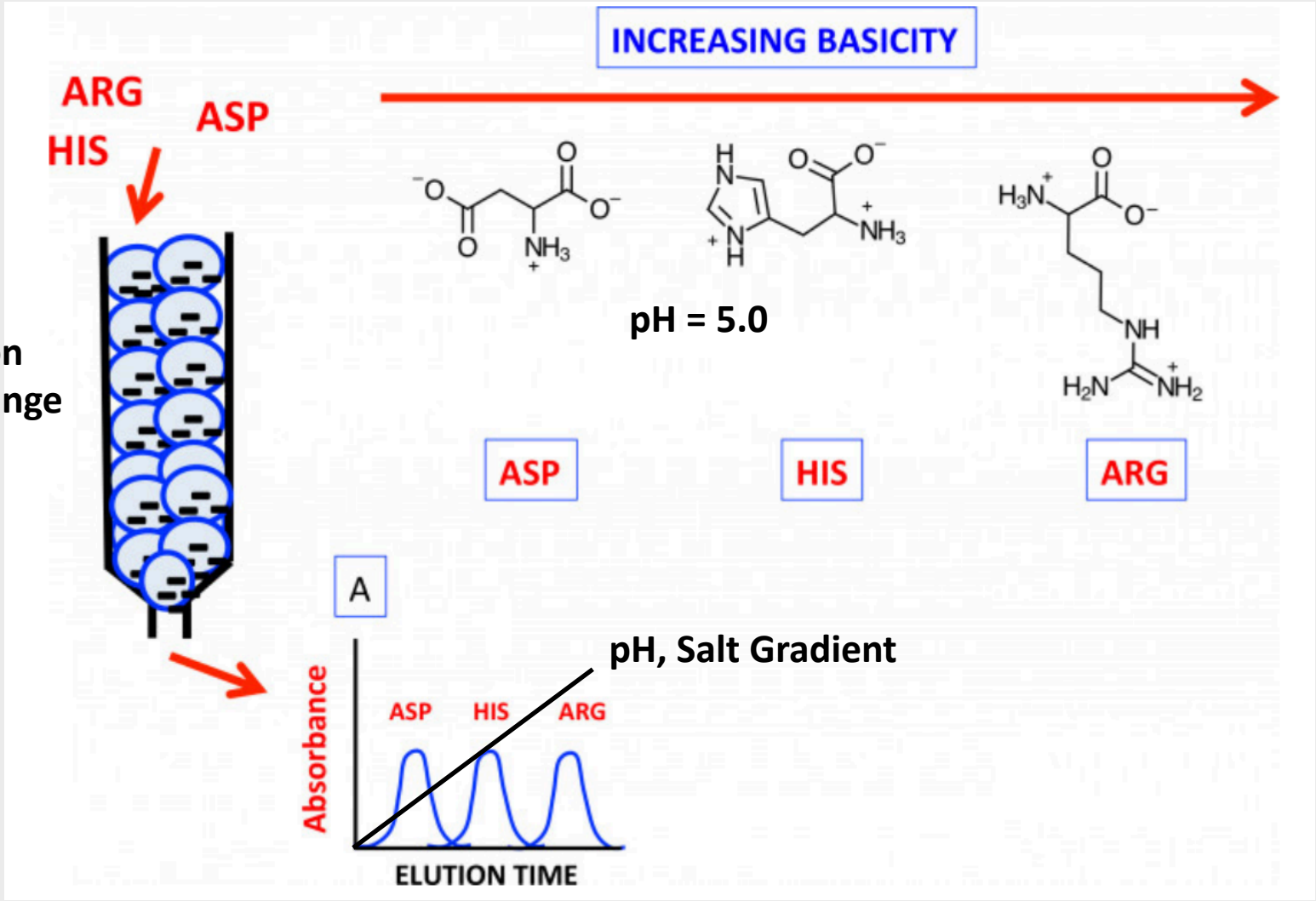
Objective: Generate & Purify Antibodies



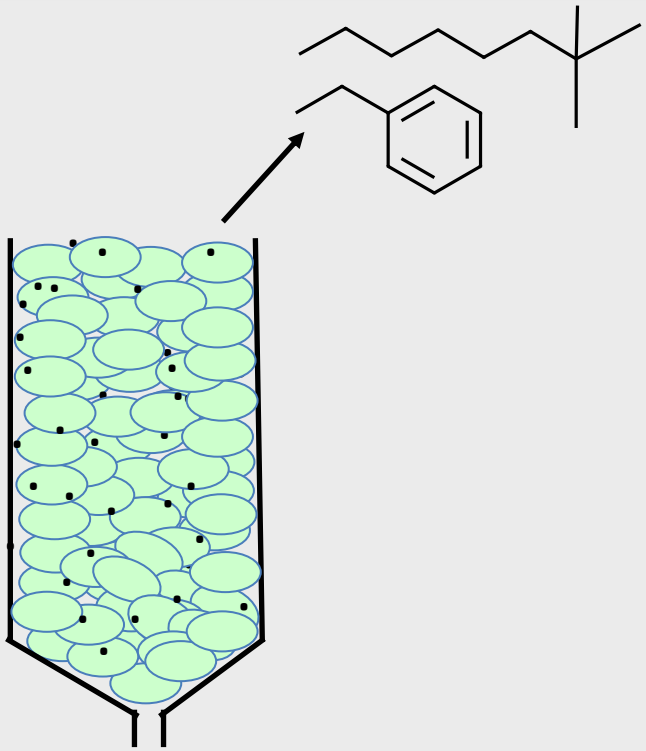
Gel Filtration Chromatography

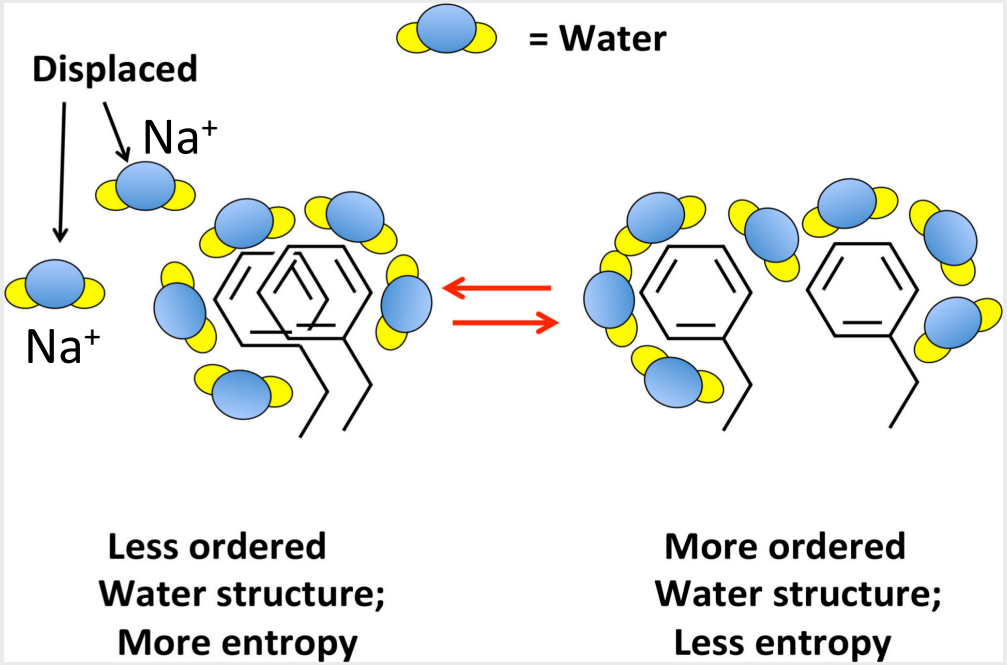


Ion Exchange Chromatography



Hydrophobic Chromatography

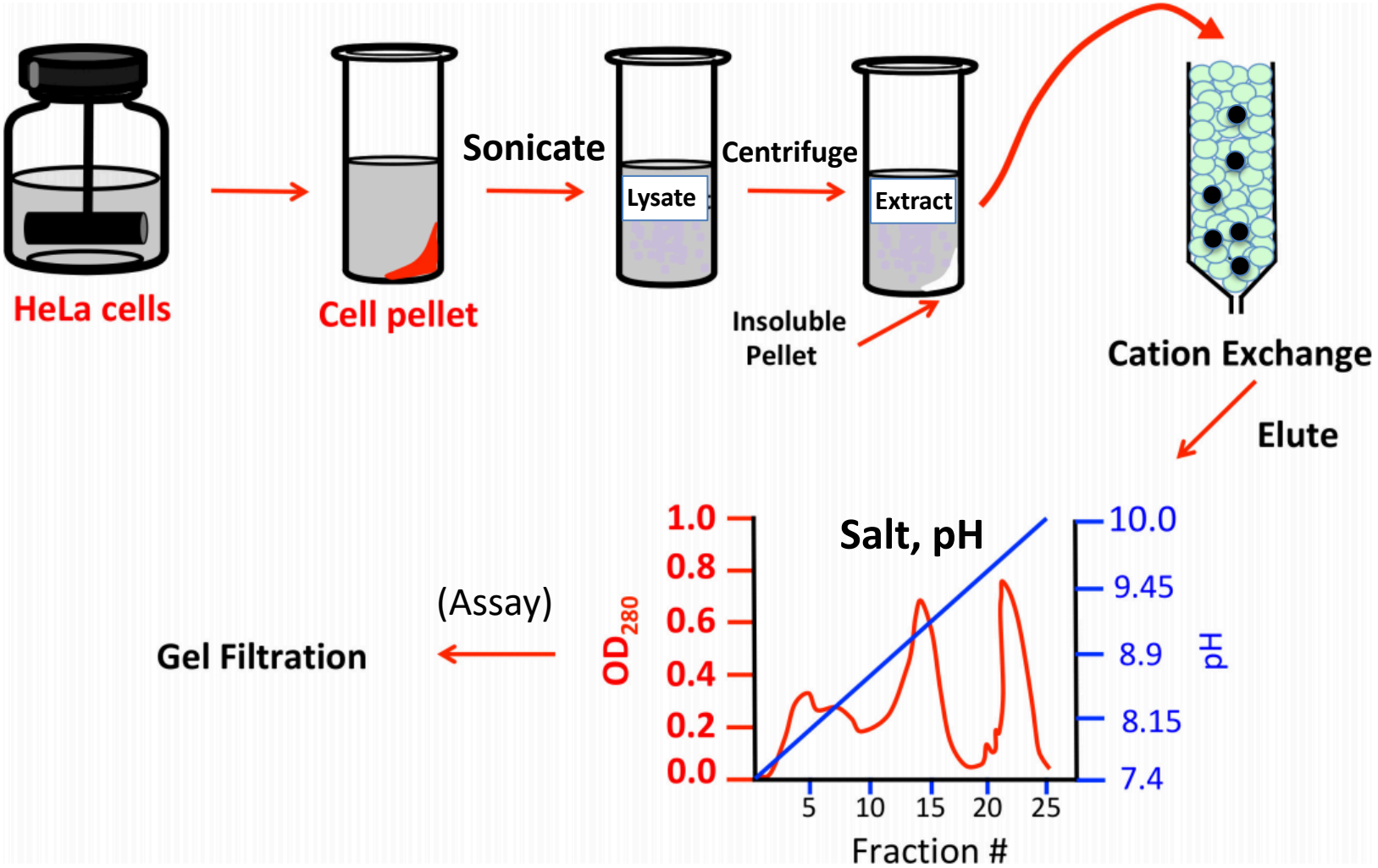




The Hydrophobic effect: **Δ**G = H - T **Δ**S

Binding occurs in High Salt as this
Displaces solvation & exposes hydrophobic sequences

Protein Purification Protocols



Purification Tables

Purification Step	Protein (mg)	Enzyme (Units)	Specific Activity
1. Crude lysate	740	4800	6.48
2. Crude extract	620	4200	6.77
3. 45-55% Ammo Sulfate cut	130	2541	19.5
4. DEAE pooled	65	2400	36.9
5. Gel filtration	4.2	1702	404

Specific Activity = Enzyme Units/mg

Purification Tables

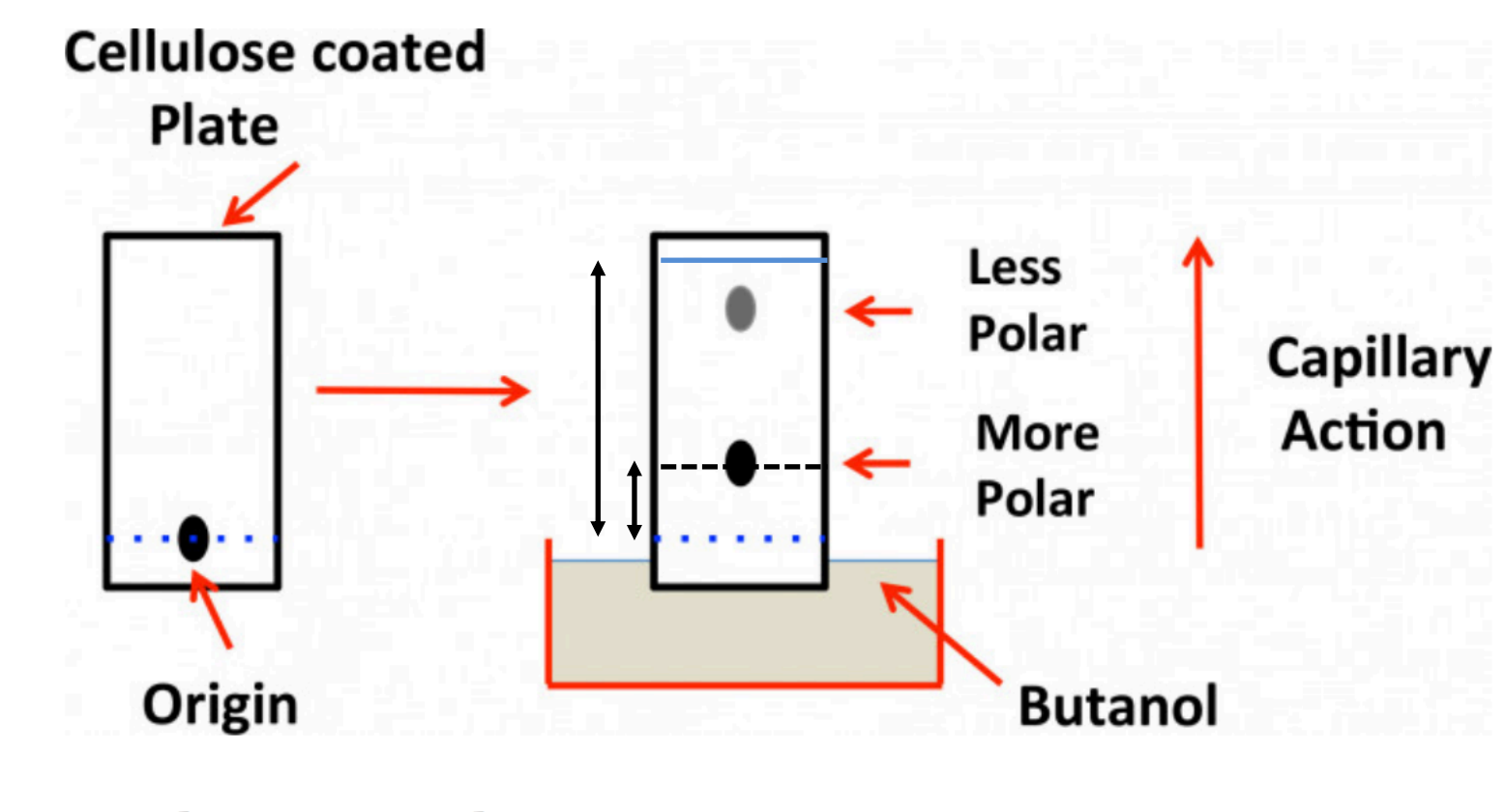
1. Specific Activity = Enzyme Units/mg

2. % Yield = Enzyme Units for a given step/Enzyme Units for First step

3. Fold Purification = Specific activity of Step/Specific activity of Step 1

Purification Step	Protein (mg)	Enzyme (Units)	Specific Activity	% Yield	Fold Purified
1. Crude lysate	740	4800	6.48	100	1
2. Crude extract	620	4200	6.77	87.5	1.04
3. 45-55% Ammo Sulfate cut	130	2541	19.5	52.9	3.0
4. DEAE pooled	65	2400	36.9	50.0	5.69
5. Gel filtration	4.2	1702	404	35.4	62.03

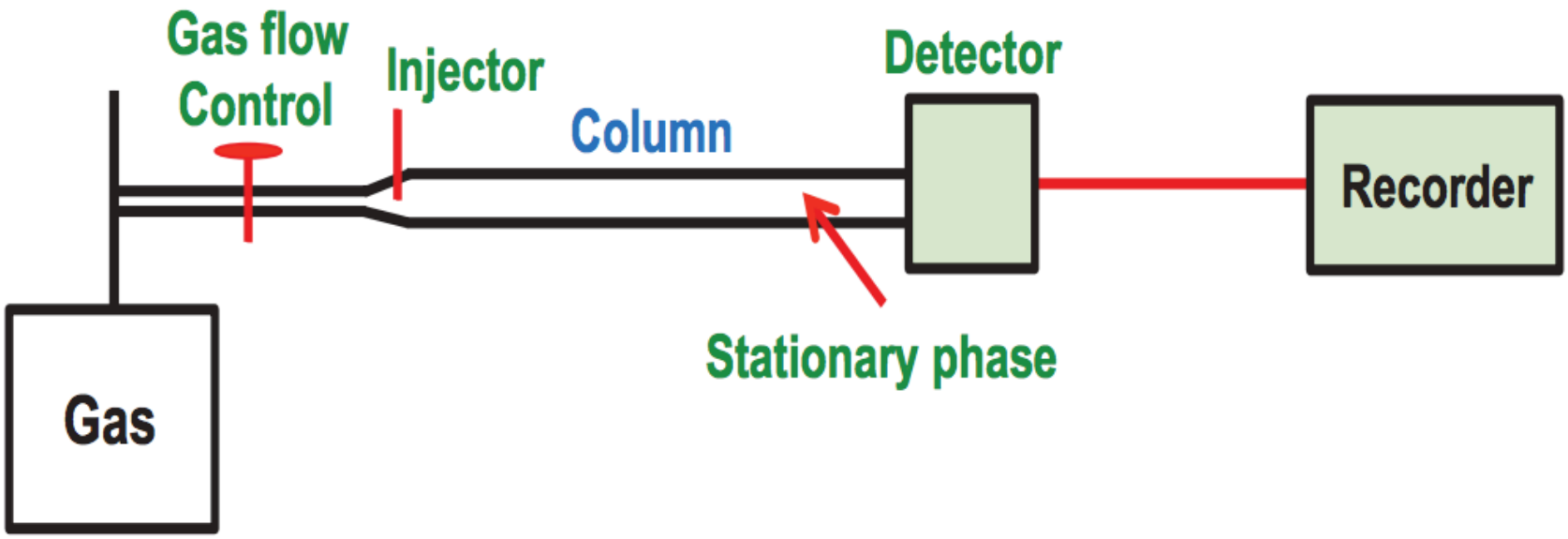
Thin Layer Chromatography (TLC)



Polar is Lower & Slower

R_f = Retardation factor =
Distance migrated/distance of solvent front

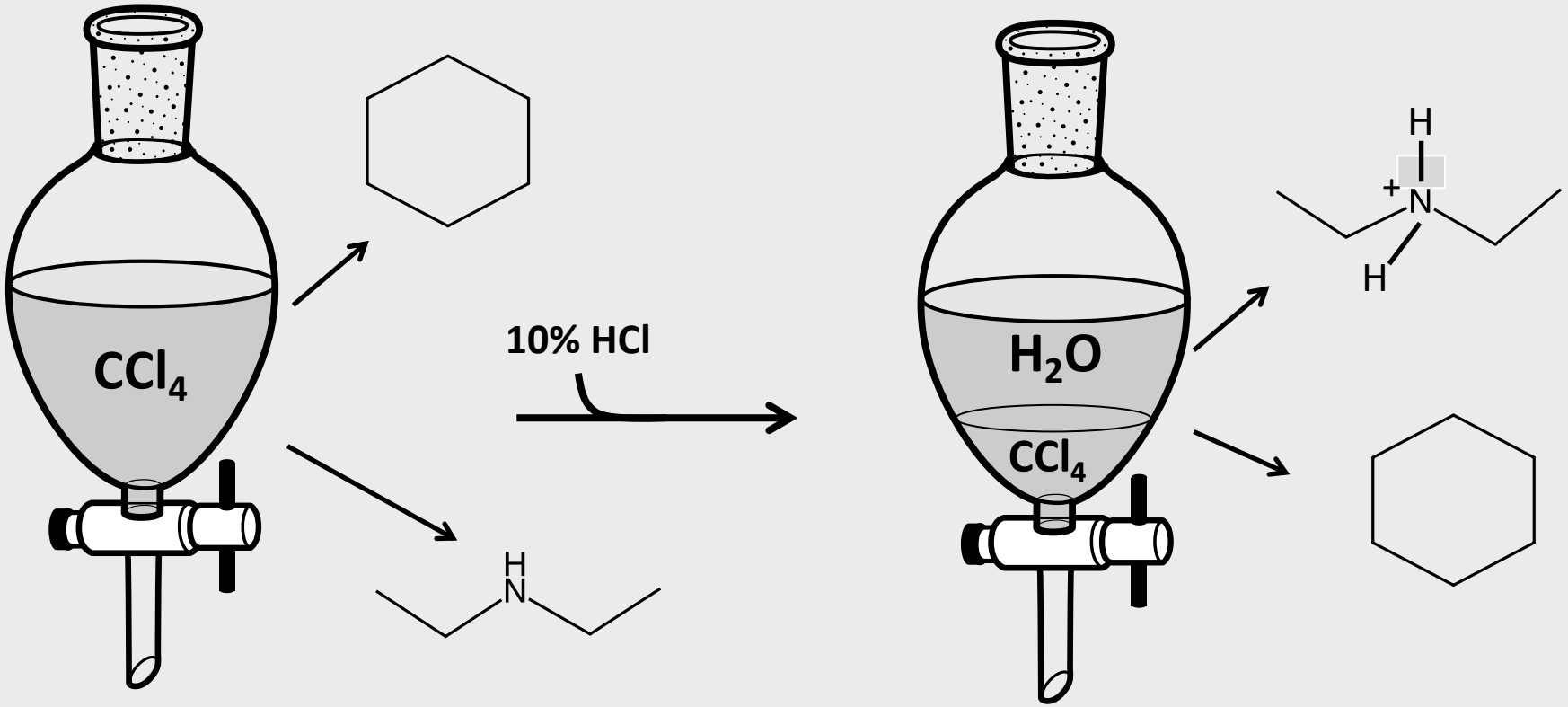
Gas Chromatography



1. Vaporize sample
2. Add gas
3. More volatile components

Move through faster (lower retention time)

Extractions

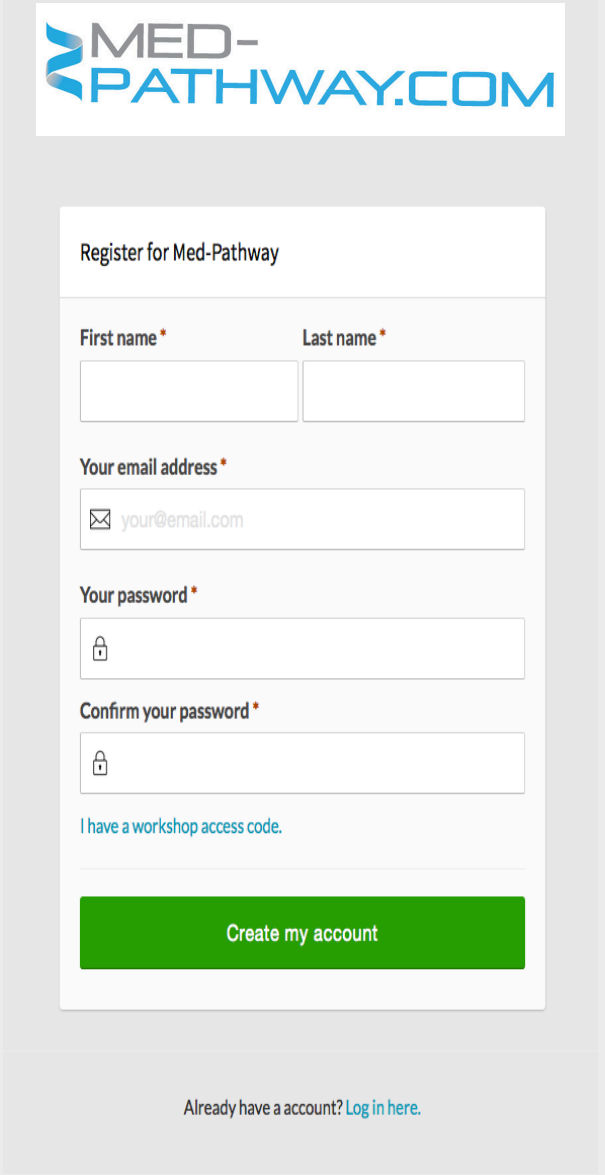


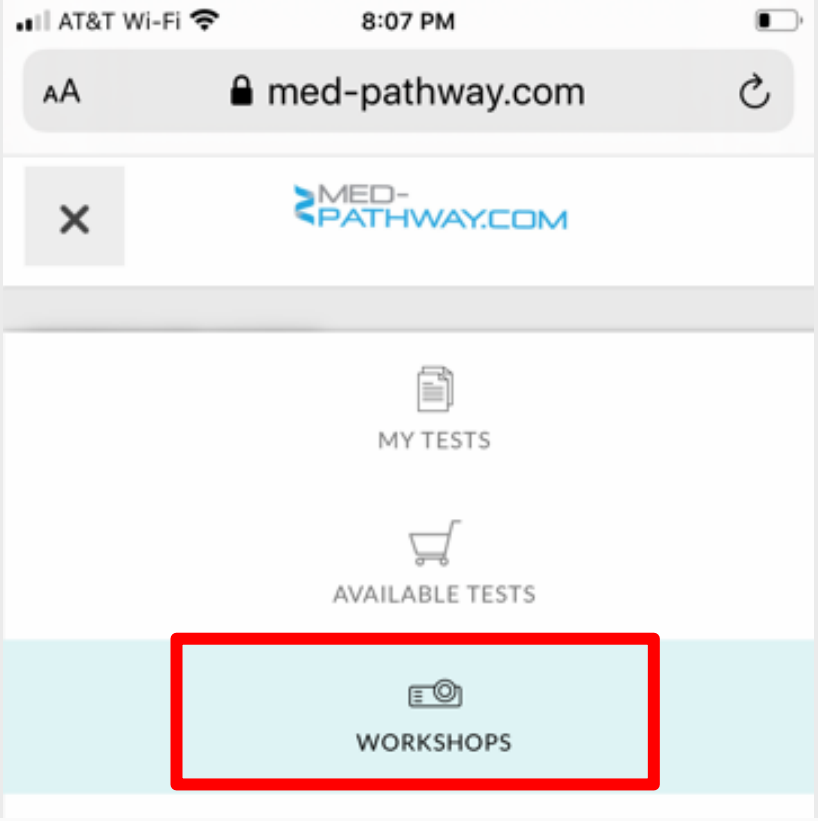
Partition Coefficient

$$K_i = [\ ]_{Organic} / [\ ]_{Water}$$

Workshop Passages

www.med-pathway.com/register





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Passcodes

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