Western/Northern/Southern Blot

Dr. Murat Erdem

Western Blot

- Western blotting is a well-established analytical technique for detecting, analyzing, and quantifying proteins.

- This method is widely used to detect specific protein molecules in complex samples such as tissue homogenates and cell lysates.

1

Preparation of protein samples

2

Resolve protein samples on SDS-PAGE gel.

3

Electrophoretically transfer fractionated proteins from gel onto membrane.

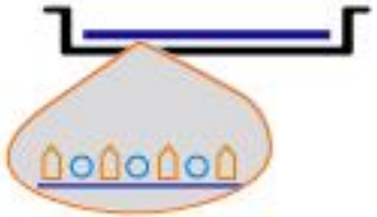
Transfer Sandwich

4

Block the membrane with neutral protein (BSA or milk casein).

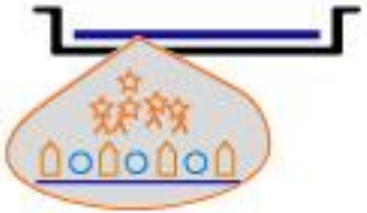
5

Incubate the membrane with primary antibody specific to desirable protein. 400



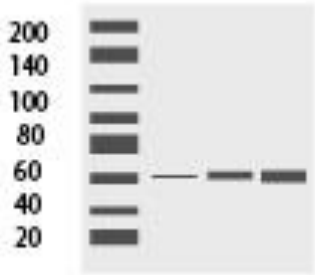
6

Incubate the membrane with HRP-labelled antibody specific to prey protein.



7

Incubate the blot with chemiluminescent HRP substrate and expose to film.



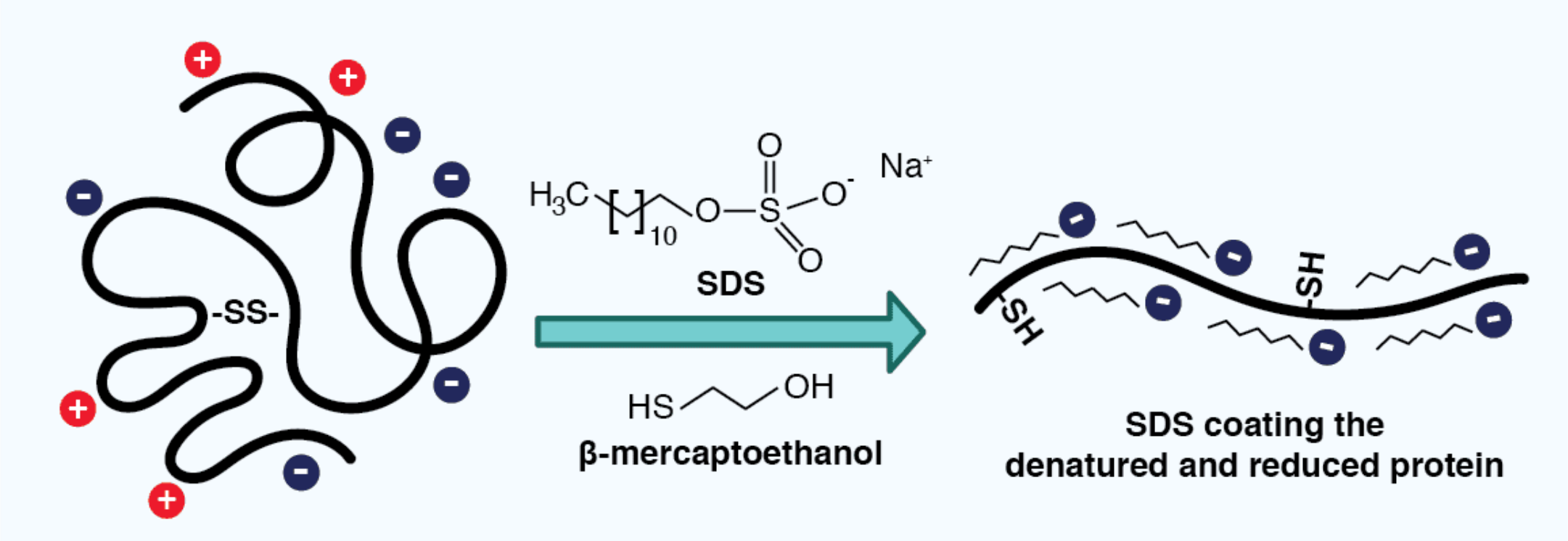
Sample preparation

- Proteins are extracted from different samples such as cells or tissues.
- Phosphatase and protease inhibitors are used to avoid the digestion of the sample at cold temperatures.
- Once the protein is extracted, the quantity of proteins needs to be determined (Bradford or BCA protein assay)

Sample preparation

- The protein samples are then appropriately diluted into a sample buffer containing glycerol, to increase the sample density, and bromophenol blue, to observe migration of the sample through the gel.
- Also, sodium dodecyl sulfate (SDS) and thiol reducing agents (DTT or β-mercaptoethanol) are added into sample buffer.
- A standard sample buffer is 2X, 4X or 6X Laemmli buffer.
- Protein samples are incubated at 950C for 5 minutes.

Roles of SDS and β-mercaptoethanol

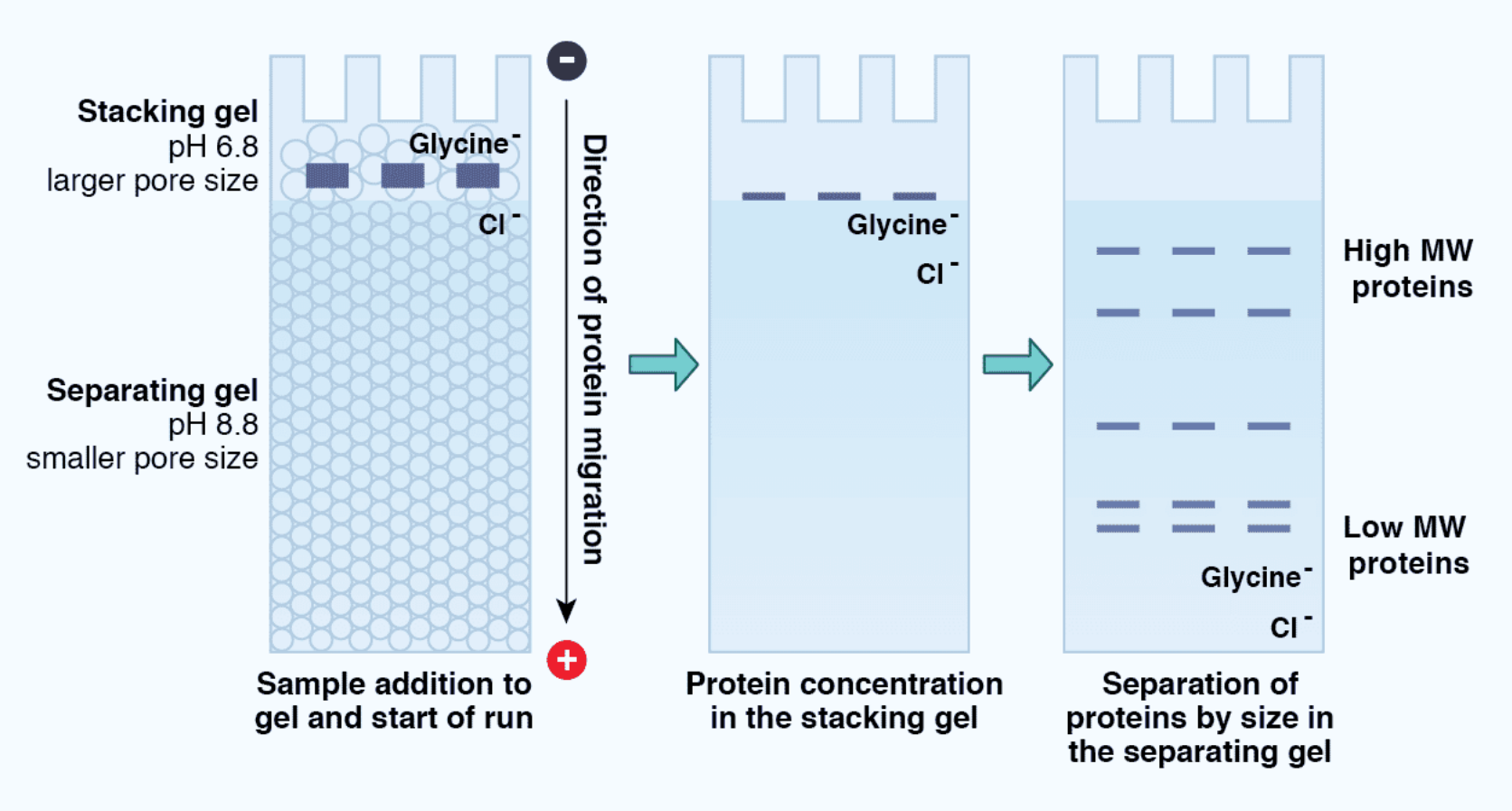


SDS-PAGE (Polyacrylamide Gel Electrophoresis)

- Electrophoresis is a laboratory technique used to separate DNA, RNA or protein molecules based on their size and electrical charge.

- Gel electrophoresis uses a gel as a sieving medium during electrophoresis.

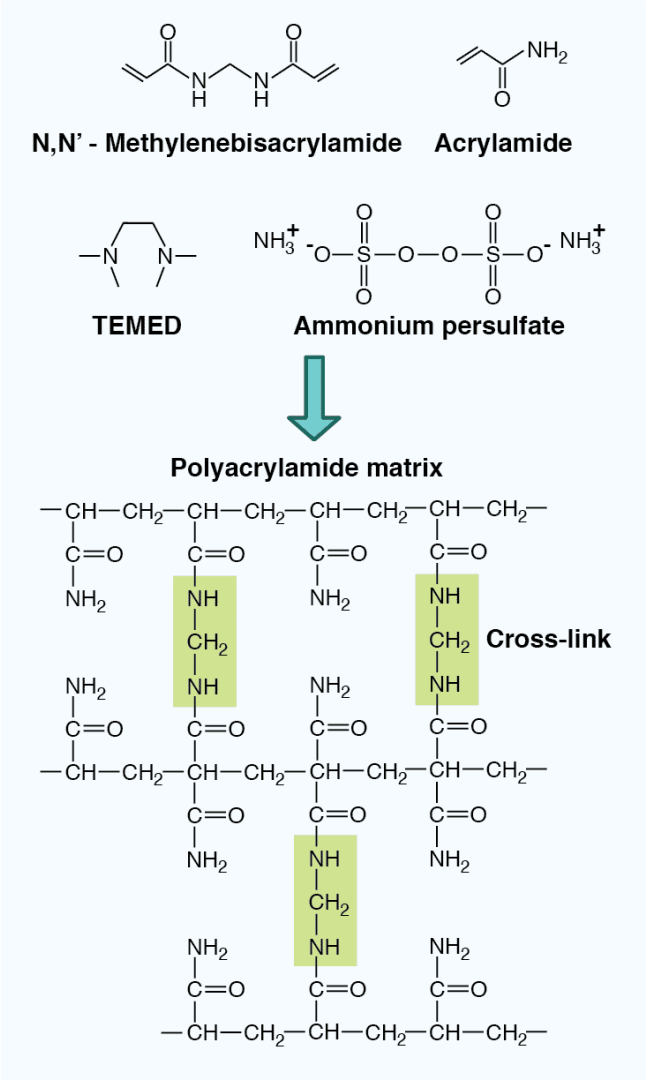
SDS-PAGE (Polyacrylamide Gel Electrophoresis)



The stacking gel has a lower concentration of acrylamide (4%), a lower pH 6.8

The separating gel has a higher acrylamide comcentration with pH 8.8

The chemical structure of a polyacrylamide matrix



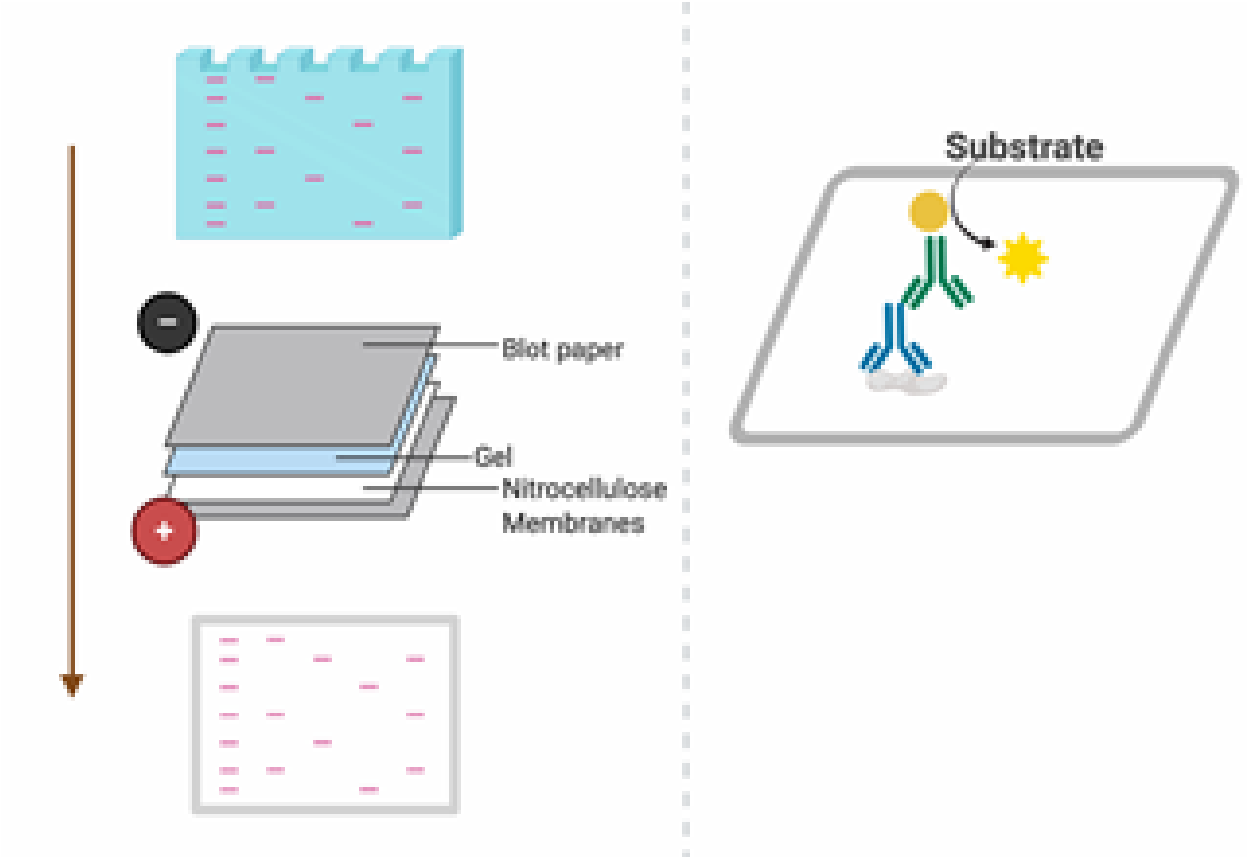
+SDS

Recommended gel percentages for different protein size ranges

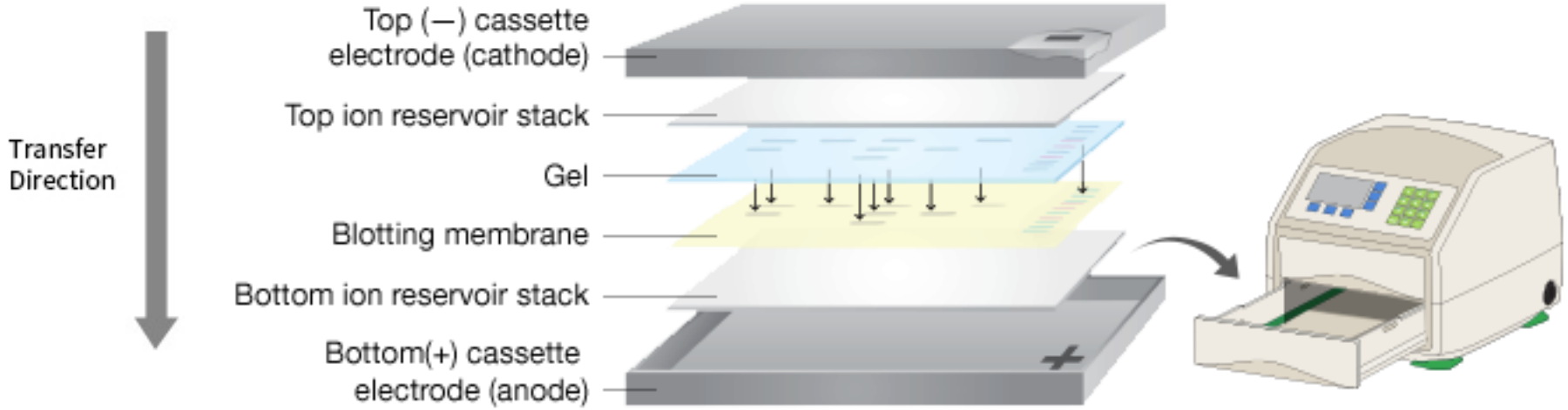
Protein Size Range	Recommended Gel Percentage
4 – 40 kDa	20%
12 – 45 kDa	15%
10 – 70 kDa	12.5%
15 – 100 kDa	10%
25 – 100 kDa	7.5%

Protein Transfer

- Seperated proteins according to molecular weight are tranfered from gel to membrane.
- The solid phase membranes are used in immunoblotting have a high affinity for proteins
- Nitrocellulose and polyvinylidene difluoride (PVDF).

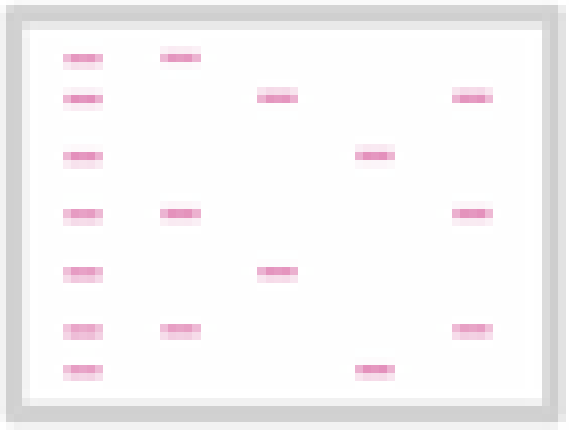


Protein Transfer

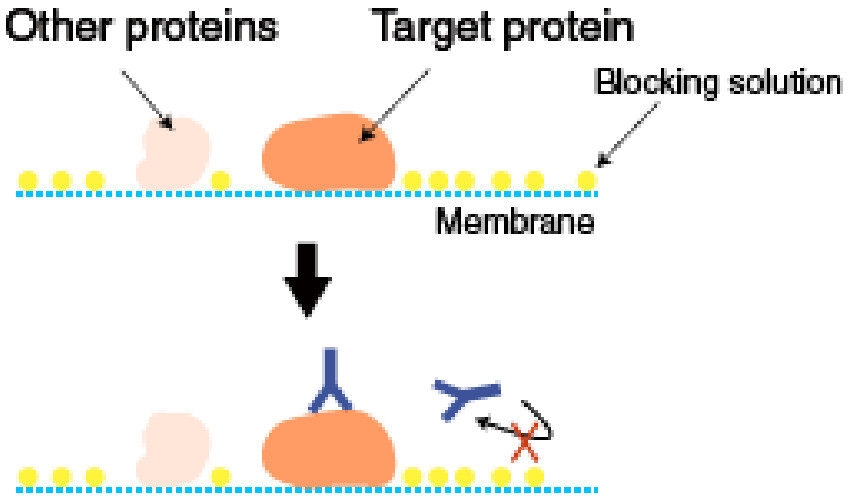


Blocking of Membrane with Neutral Protein

Blocking: Nonfat milk or BSA Fraction V is dissolved in Tris-buffered saline (TBS) or phosphate-buffered saline (PBS), and 0.1% Tween-20 can be added to help prevent non-specific interactions

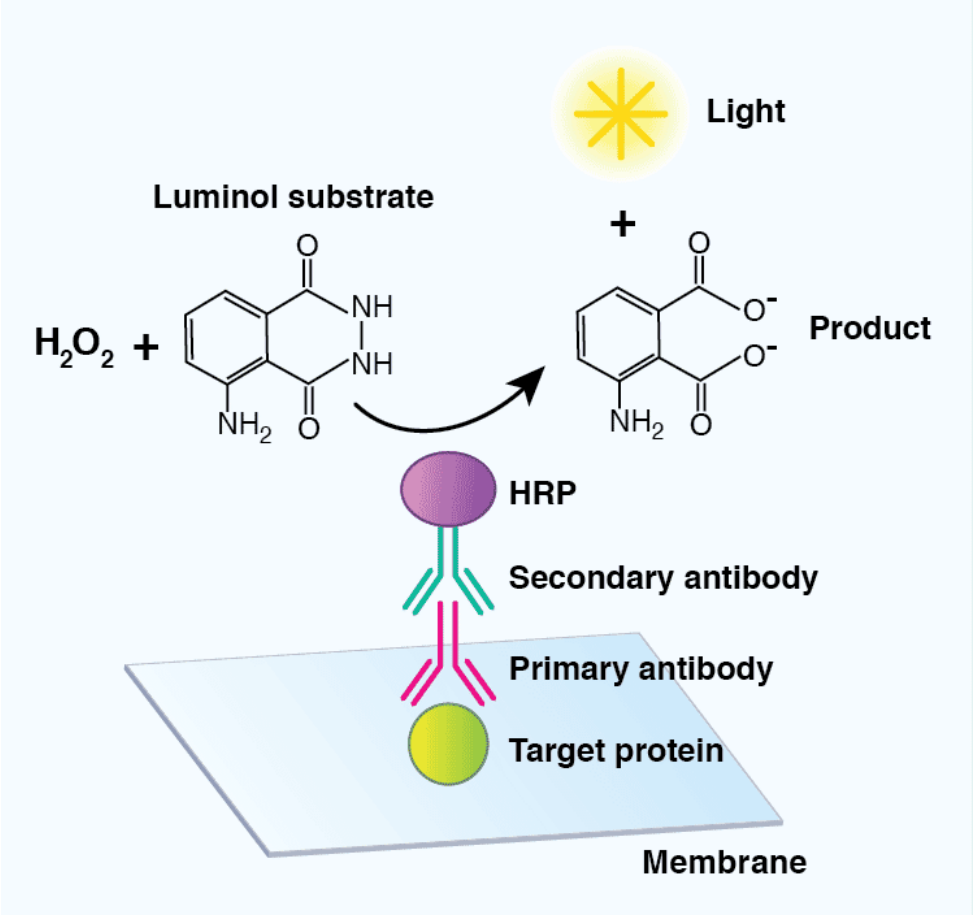


Membrane



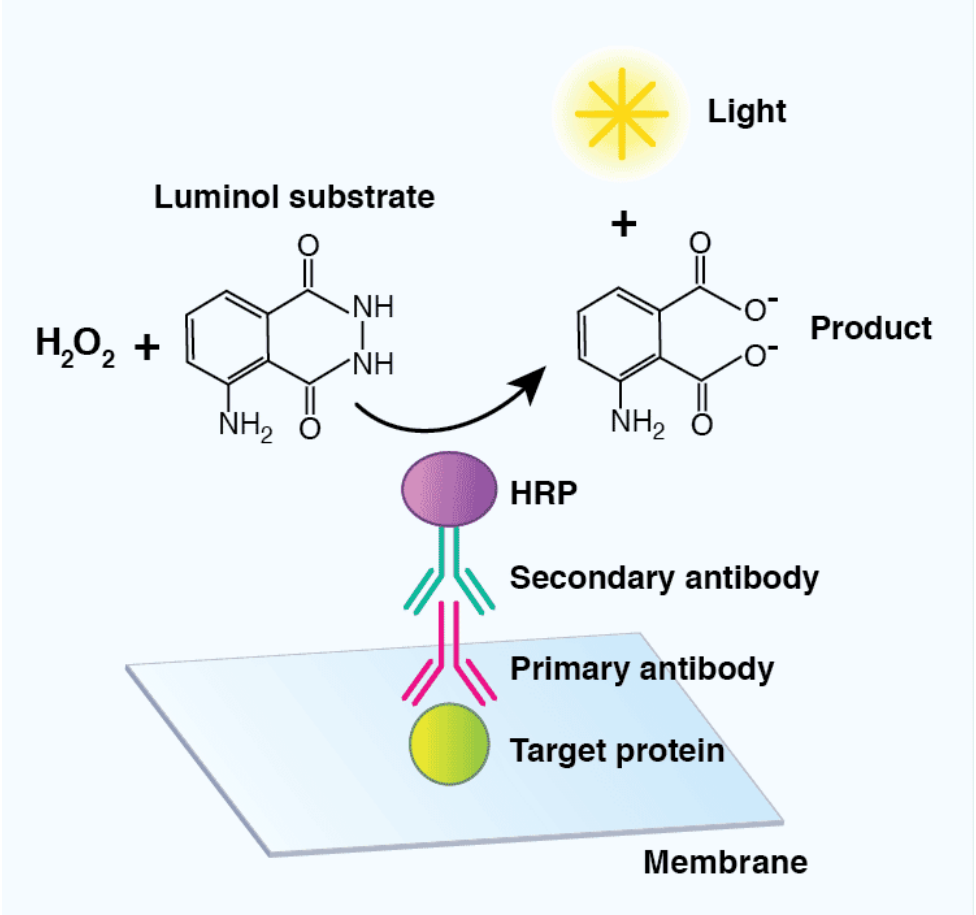
Antibody Incubations

- Primary antibody is diluted in TBST with 5% BSA and then added on the membrane. Membrane is incubated dor 1 hour.
- The membrane is washed with 5% BSA containing TBST 3 times.
- Secondary antibody is diluted in TBST with 5% BSA and then added on the membrane. Membrane is incubated dor 1 hour.
- The membrane is washed 3 times.

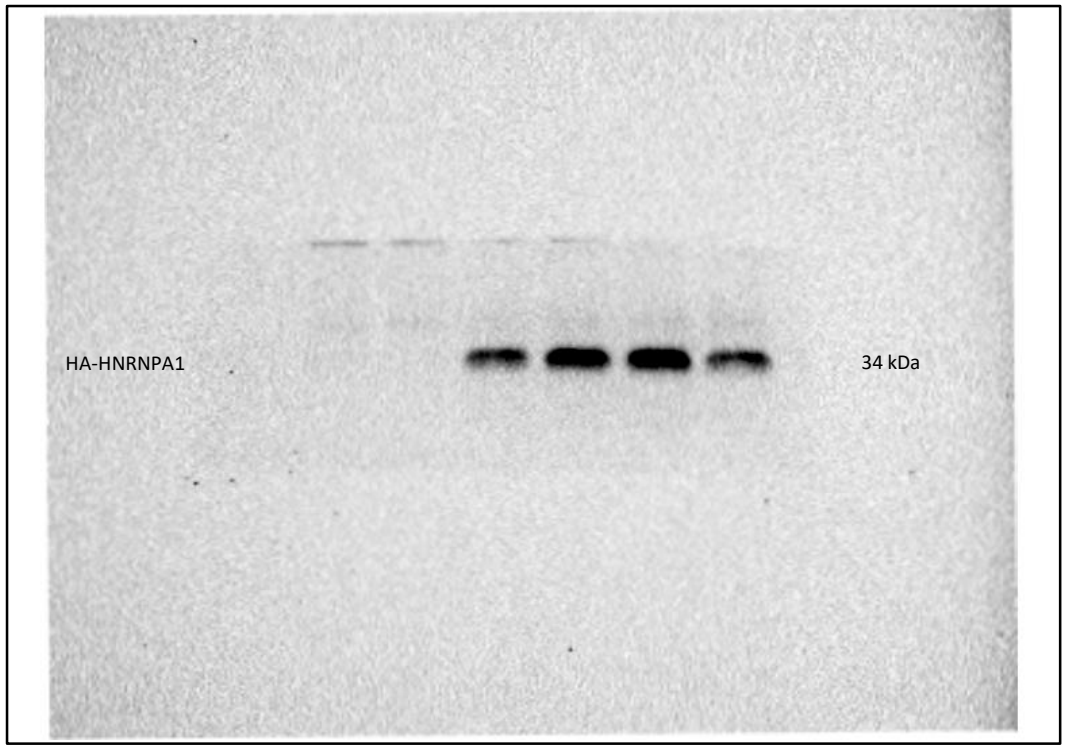


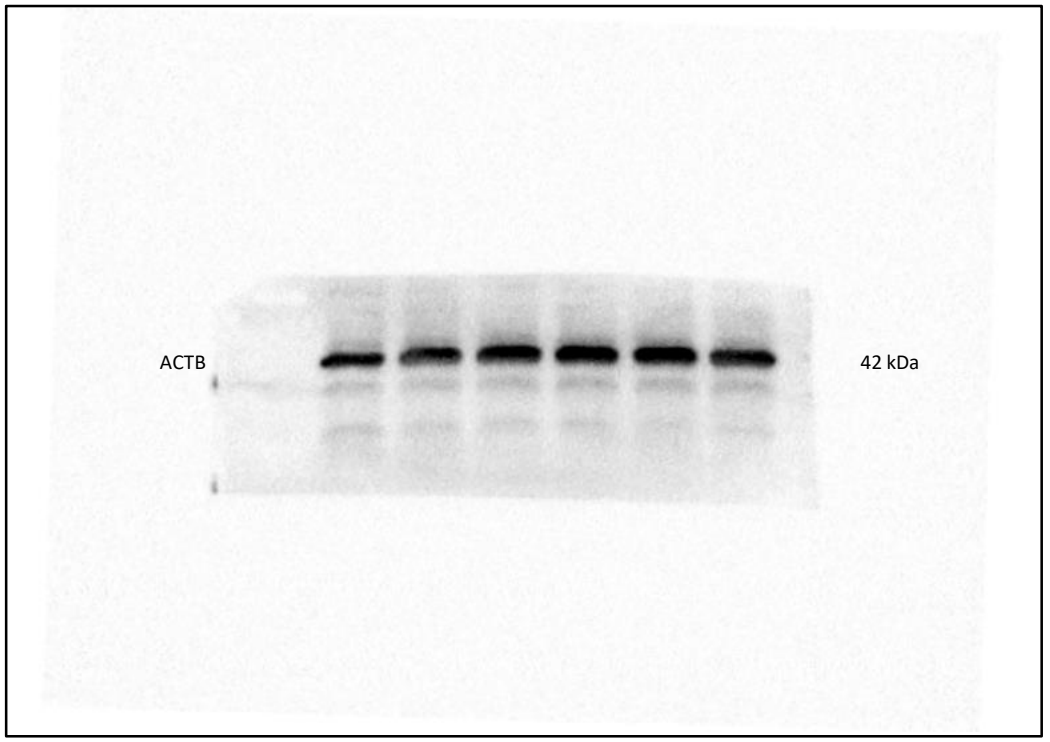
Imaging of Protein Bands

- A charge-coupled device camera-based imaging system, enclosed in a dark cabinet, is used for image processing.



HA-Tag HNRNPA1 Expression in MDAMB231





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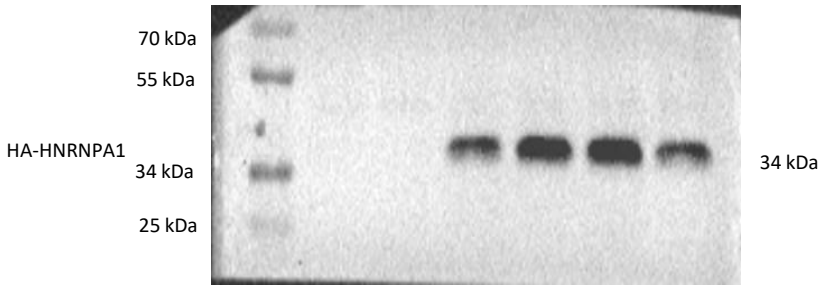

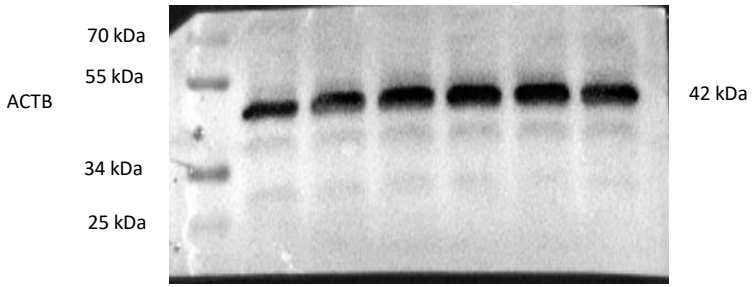

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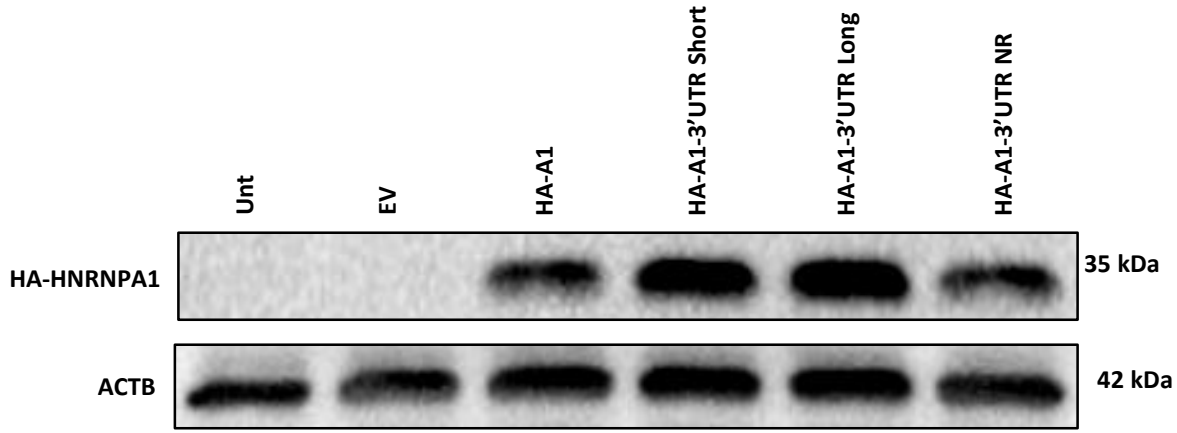


MDA-MB-231

48h Transfection
30ug Protein
8% SDS-PAGE gel used

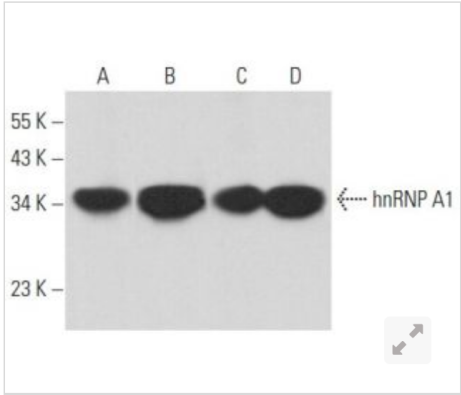
22-23.04.2020

Antibody Selection

scbt.com/p/hnrnp-a1-antibody-4b10

Player Cathrin Bri... | miRNAMap -- Sea... | GDS705 / 34987_... | microRNA target p... | GEO2R - GEO - N... | Knockdown of HN... | Reciprocal regulat...

Home / Antibodies / Primary Antibodies and ImmunoCruz® Conjugates / Transcription Regulators / hnRNP A1 Antibodies / **hnRNP A1 Antibody (4B10)**



Click on image or enlarge button to enlarge

hnRNP A1 Antibody (4B10): sc-32301. Western blot analysis of hnRNP A1 expression in PC-3 (A) and MDA-MB-231 (B) whole cell lysates and MCF7 (C) and SW480 (D) nuclear extracts.

hnRNP A1 Antibody (4B10): sc-32301

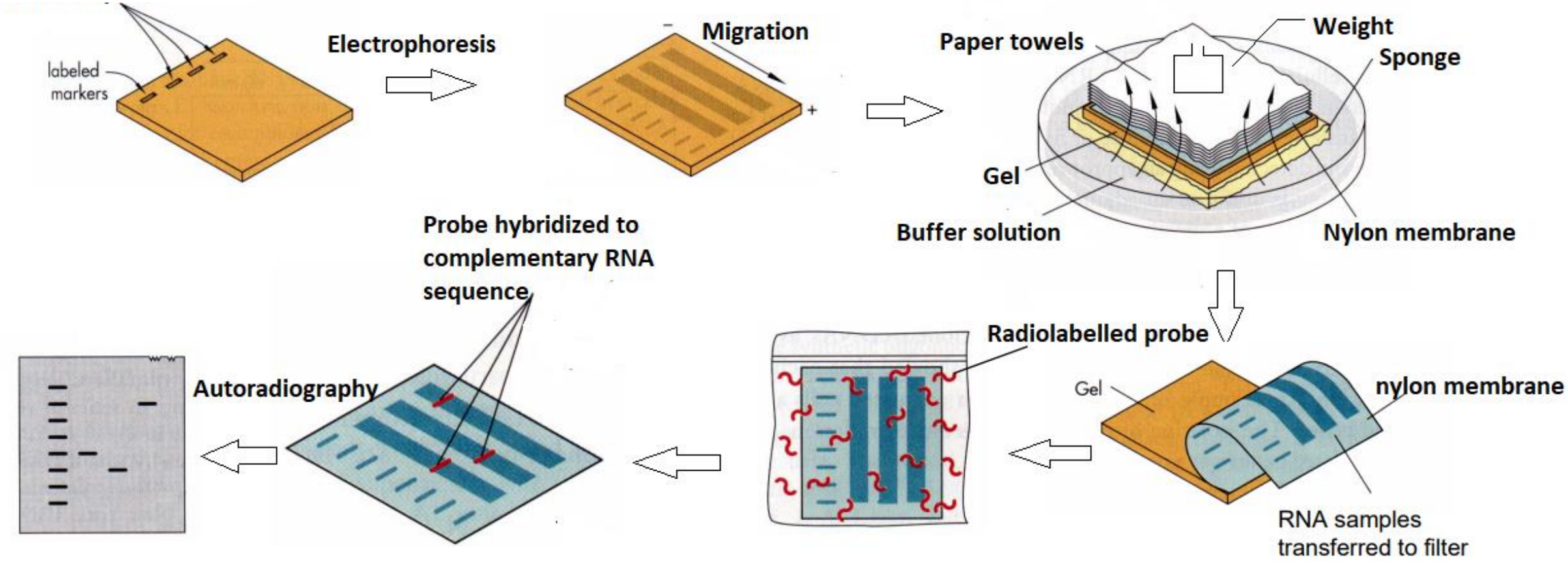
★★★★★ 4,8 (18) Write a review Ask a question

Datasheets

- hnRNP A1 Antibody (4B10) is a mouse **monoclonal** IgG_{2a} κ hnRNP A1 antibody, cited in **134 publications**, provided at **200 µg/ml**
- raised against full length partially purified hnRNP A1
- hnRNP A1 Antibody (4B10) is recommended for detection of hnRNP A1 of mouse, rat and human origin by WB, IP, IF, IHC(P) and ELISA; also reactive with additional species, including and bovine and canine
- Anti-hnRNP A1 Antibody (4B10) is available conjugated to **agarose** for IP; **HRP** for WB, IHC(P) and ELISA; and to either **phycoerythrin** or **FITC** for IF, IHC(P) and FCM
- also available conjugated to **Alexa Fluor® 488**, **Alexa Fluor® 546**, **Alexa Fluor® 594** or **Alexa Fluor® 647** for WB (RGB), IF, IHC(P) and FCM, and for use with RGB fluorescent imaging systems, such as iBright™ FL1000, FluorChem™, Typhoon, Azure and other comparable systems
- also available conjugated to **Alexa Fluor® 680** or **Alexa Fluor® 790** for WB (NIR), IF and FCM; for use with Near-Infrared (NIR) detection systems, such as LI-COR®Odyssey®, iBright™ FL1000, FluorChem™, Typhoon, Azure and other comparable systems
- Contact our Technical Service Department (or your local Distributor) for more information on how to receive a **FREE 10 µg sample** of **hnRNP A1 (4B10): sc-32301**.
- **m-IgG Fc BP-HRP** and **m-IgGκ BP-HRP** are the preferred secondary detection reagents for hnRNP A1 Antibody (4B10) for WB and IHC(P) applications. These reagents are now offered in bundles with hnRNP A1 Antibody (4B10) (**see ordering information below**).
- See **product citations (132)**

Nothern Blot and Southern Blot

RNA and DNA Samples



RNA or DNA isolation

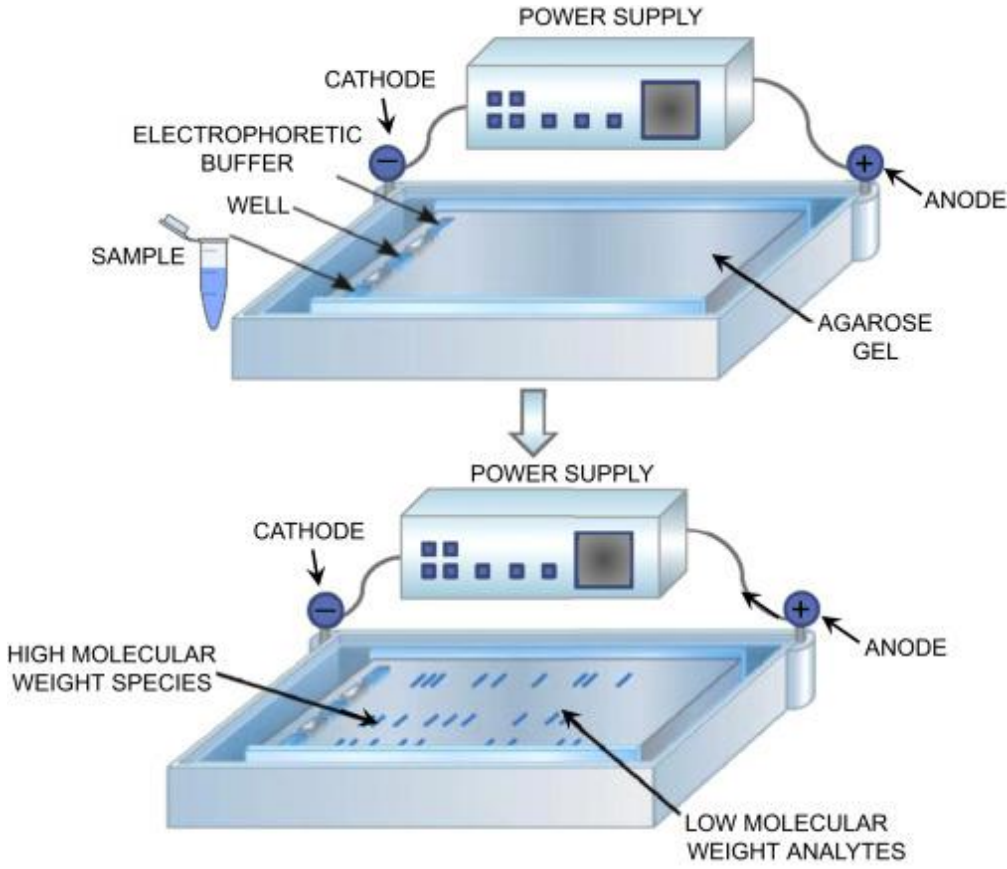
- RNA and DNA samples are extracted via Trizol reagent or any isolation kit.

- Extracted RNA is denatured by incubating at 60°C for 15 min in the presence of formamide, formaldehyde and MOPS buffer (pH 7.0).

- Extracted DNA is digested with restriction endonucleases for DNA fragmentation

Gel Electrophoresis

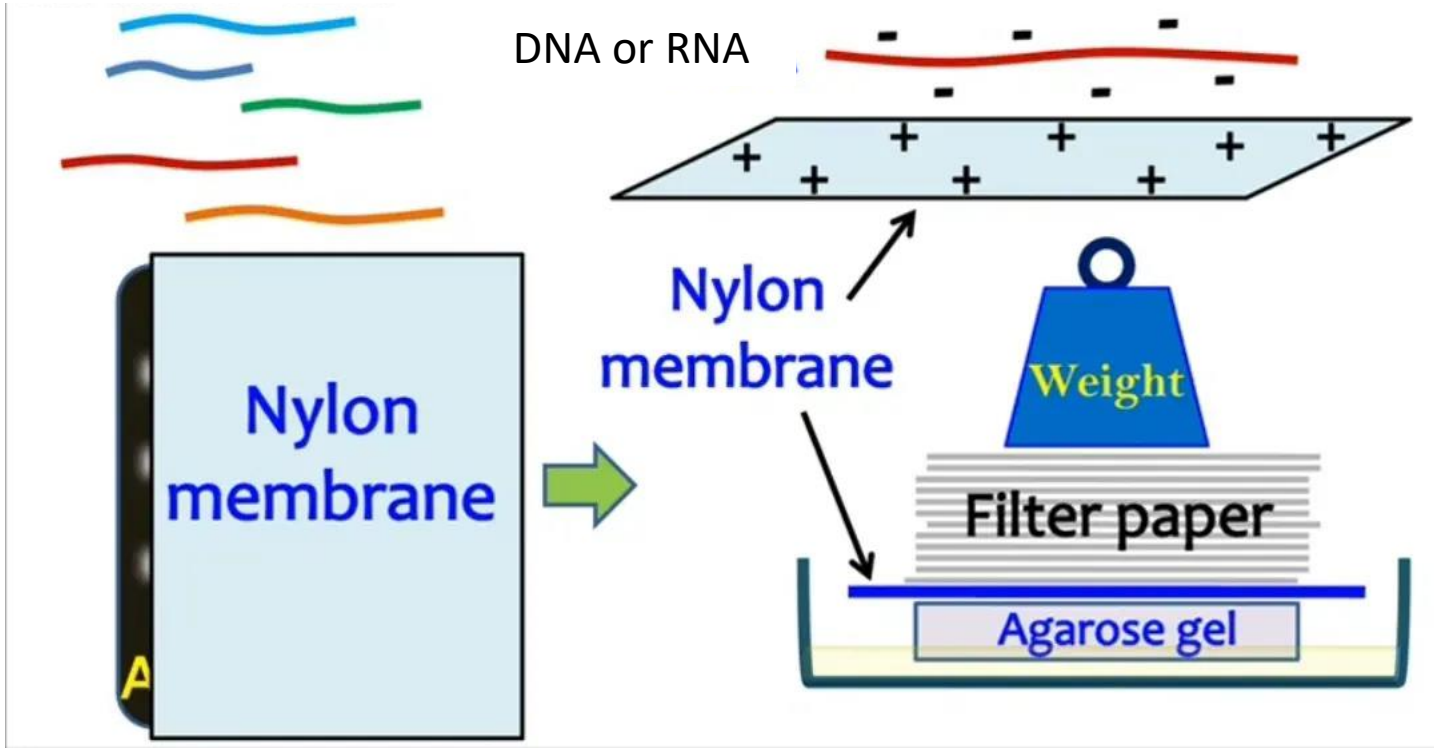
- The RNA or DNA samples are most commonly separated on agarose gels containing formaldehyde as a denaturing agent for the RNA to limit secondary structure
- The samples were electrophoresed on a 0.9% agarose gel containing 2.2 M formaldehyde
- DNA samples are most commonly separated on agarose gels
- Gel is soaked in NaOH for denaturation of dsDNA into ssDNA



DNA and RNA Transfer

Seperated RNA /DNA molecules according to molecular weight

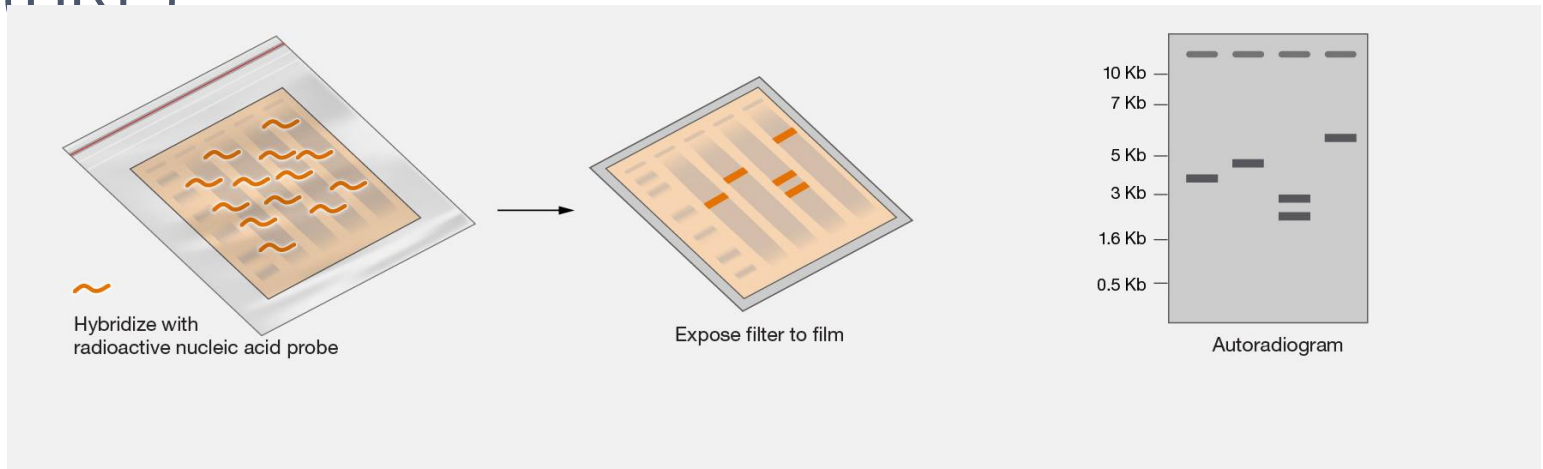
Nucleic acid Blot



UV Crosslinking

Probes

- Probes for northern blotting are composed of nucleic acids with a complementary sequence to the target DNA or RNA molecule
- Probes can be DNA, RNA, or oligonucleotides with a minimum of 25 complementary bases to the target sequence
- The probes must be labelled either with radioactive isotopes (32P) or with chemiluminescence in which alkaline phosphatase or horseradish peroxidase (HRP)



THANK YOU