

Blotting Techniques:

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Page 1 of 32



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Page 8 of 32

1
DNA

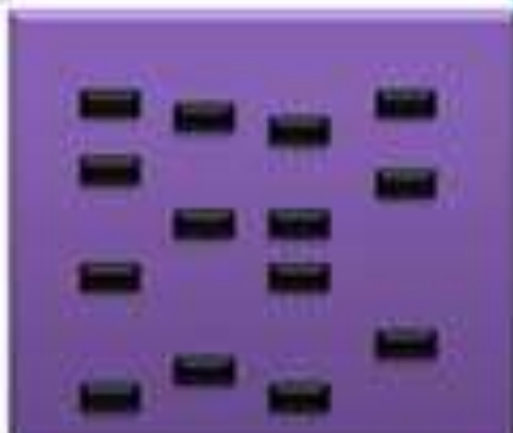
2
Separate DNA on an
Agarose Gel

3
Transfer or BLOT DNA
fragments from GEL to
Membrane

4
Membrane with
DNA bands
transferred to it

5
Radiolabeled probe
Incubated with
Membrane

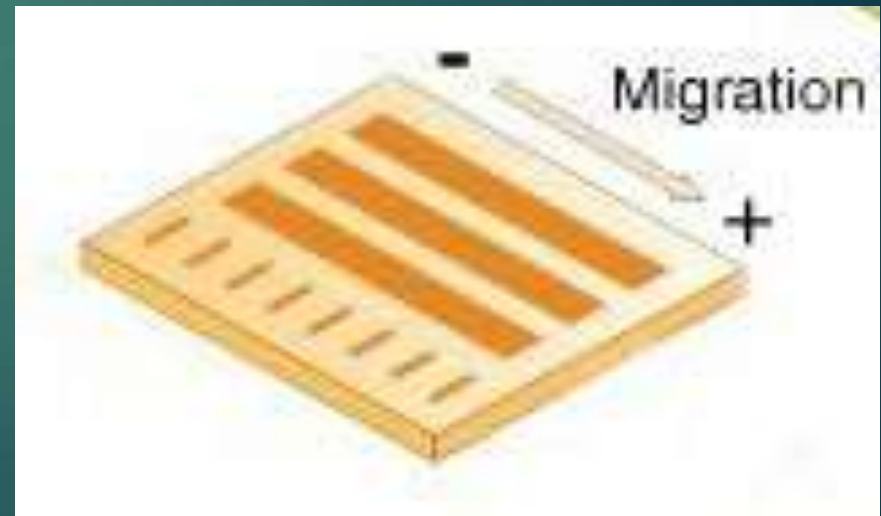
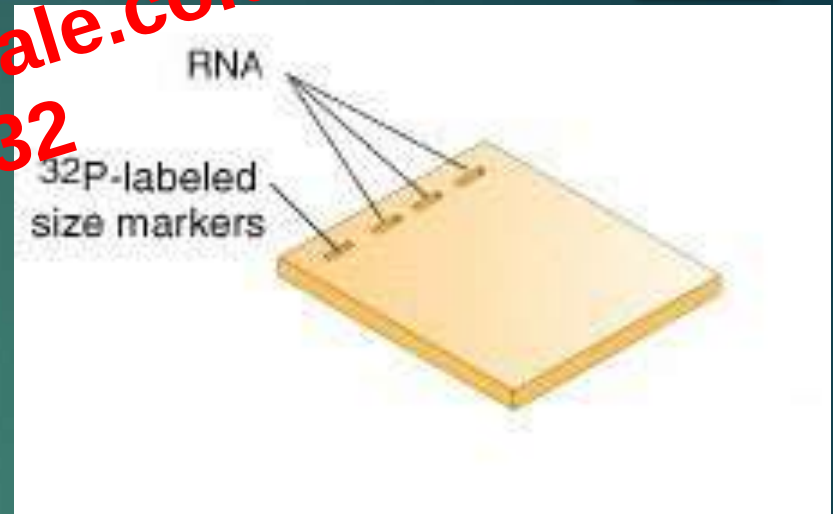
6
Bound DNA Bands
are Exposed on Film



Cont.....

2. Samples are loaded on gel and the RNA samples are separated according to their size on an agarose gel.

- ▶ The resulting gel following after the electrophoresis run.



Cont.....

4. The membrane is placed in a dish containing hybridization buffer with a labeled probe.

❖ Thus, it will hybridize to the RNA on the blot that corresponds to the sequence of interest.

5. The membrane is washed to remove unbound probe.

Cont...

3. The blot is incubated with a generic protein (such as milk proteins or BSA) which binds to any remaining sticky places on the nitrocellulose.
4. An antibody that is specific for the protein of interest (the primary antibody - Ab1) is added to the nitrocellulose sheet and reacts with the antigen. Only the band containing the protein of interest binds the antibody, forming a layer of antibody molecules .

