

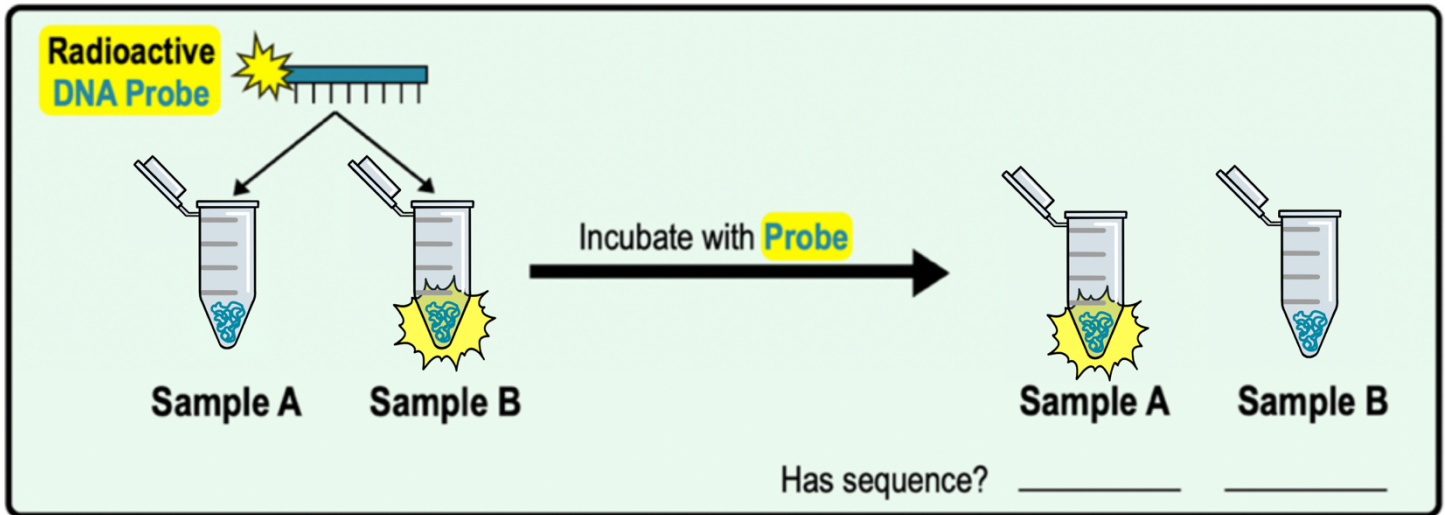
CONCEPT: SOUTHERN BLOTTING

Radioactive Probes

● After cloning a gene, the DNA can be used as a **probe** to detect the _____ sequence in an unknown DNA sample.

- _____: *radioactively* labeled molecules that are *visualized* using radioactive detection.
- **DNA probes** are _____-stranded DNA molecules complementary to a *specific* sequence of interest.

EXAMPLE: Radioactive probes are used to identify DNA that is complementary to it in different samples.



PRACTICE: A southern blotting technique is used to detect a specific _____ sequence from a blood or tissue sample.

- a) RNA
- b) DNA
- c) Protein
- d) Lipid

PRACTICE: A _____ is a single-stranded DNA molecule used in hybridization reactions to detect the presence of a particular gene in an assortment of DNA fragments.

(Hybridization Reactions: reactions that combine two complementary single-stranded DNA molecules)

- a) Plasmid.
- b) Vector.
- c) Probe.
- d) Blot.
- e) Polymerase.

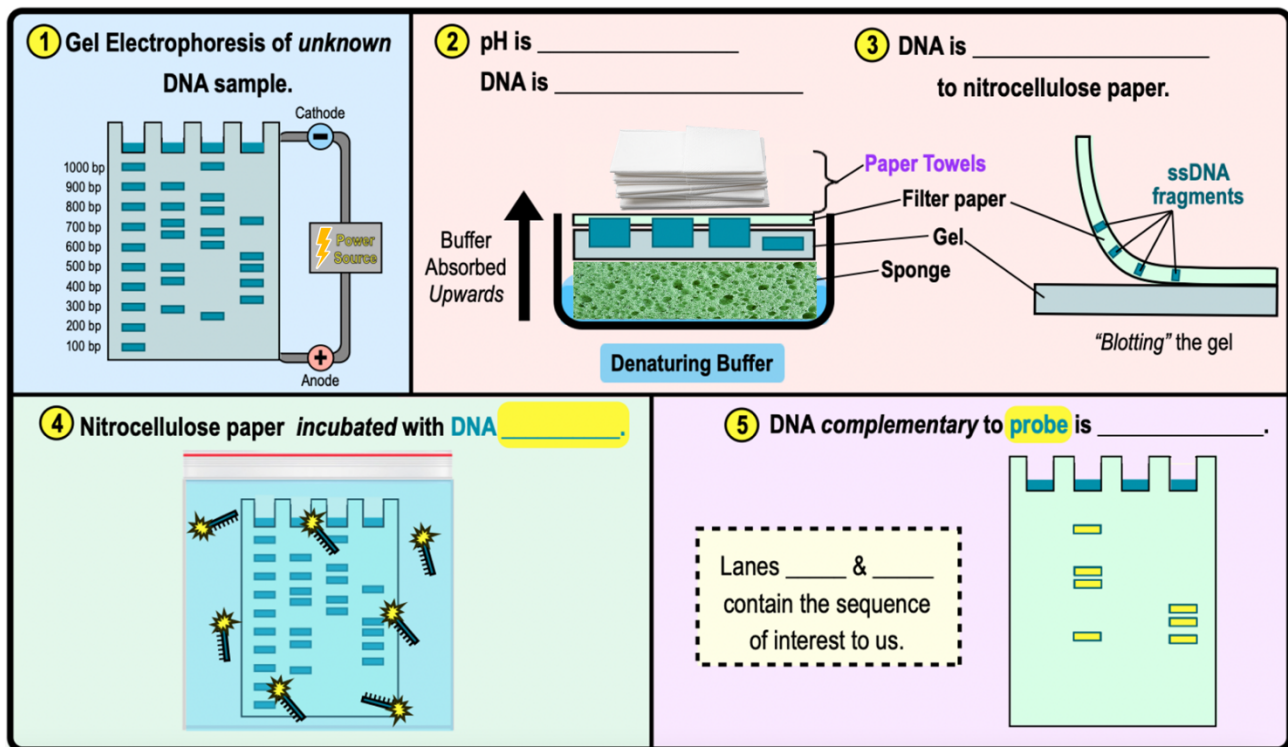
CONCEPT: SOUTHERN BLOTTING

- **Blotting:** technique used to *rapidly* detect a *specific* DNA sequence using **DNA probes**.
 - *Northern Blotting* detects _____ sequences & *Western Blotting* detects specific _____.

Steps of Southern Blotting

- 1 Unknown DNA sample is fragmented & separated by _____ using *gel electrophoresis*.
- 2 DNA on the gel is *denatured* to _____-stranded DNA (ssDNA) by incubating it with **denaturing buffer**.
 - **Filter paper** used to "**blot**" the gel & absorb the **denaturing buffer** as it migrates through to a **paper towel stack**.
- 3 ssDNA is _____ to the **nitrocellulose filter paper** when **denaturing buffer** is absorbed/migrated.
- 4 **Filter paper** is removed & incubated with the **radioactive probe** _____ to the sequence of interest.
- 5 **Radioactive filter paper** is analyzed & visible bands are ones that _____ to the **DNA probe** in step 4.

EXAMPLE: Analyze the Southern Blot below and decide which samples contain the DNA sequence of interest.



PRACTICE: A geneticist wants to see if her patient has Gene X. The geneticist takes a blood sample from her patient and prepares a southern blot. How will the geneticist know if her patient possesses Gene X?

- a) A radioactive probe, which is complementary to Gene X, will bind to the membrane.
- b) A radioactive probe, which has the same sequence as Gene X, will bind to the membrane.
- c) A radioactive probe will be absent from the membrane.
- d) A southern blot cannot confirm if the patient possesses Gene X.

CONCEPT: SOUTHERN BLOTTING

PRACTICE: Place the following steps of Southern Blotting in the correct order.

- a) ____: Filter paper is incubated with the labeled DNA probe which anneals to the ssDNA fragments.
- b) ____: Analyze gel to determine the presence of the DNA sequence of interest.
- c) ____: Separate DNA fragments by size using gel electrophoresis.
- d) ____: Denature DNA by soaking gel in a basic solution.
- e) ____: Fragment unknown DNA sample(s) using restriction enzymes.

PRACTICE: By analyzing the Southern Blot results below, which of the samples contains the gene of interest?

- a) A only.
- b) A & C.
- c) B & D.
- d) A, C, & E.
- e) E only.
- f) None.

