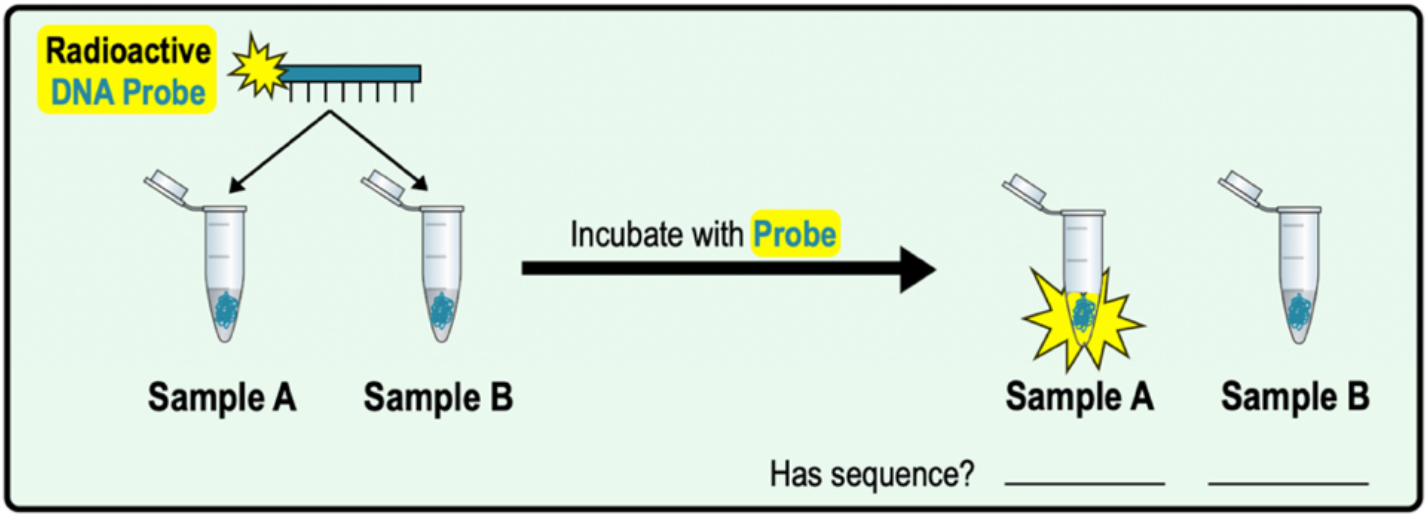
CONCEPT: SOUTHERN BLOTTING

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.

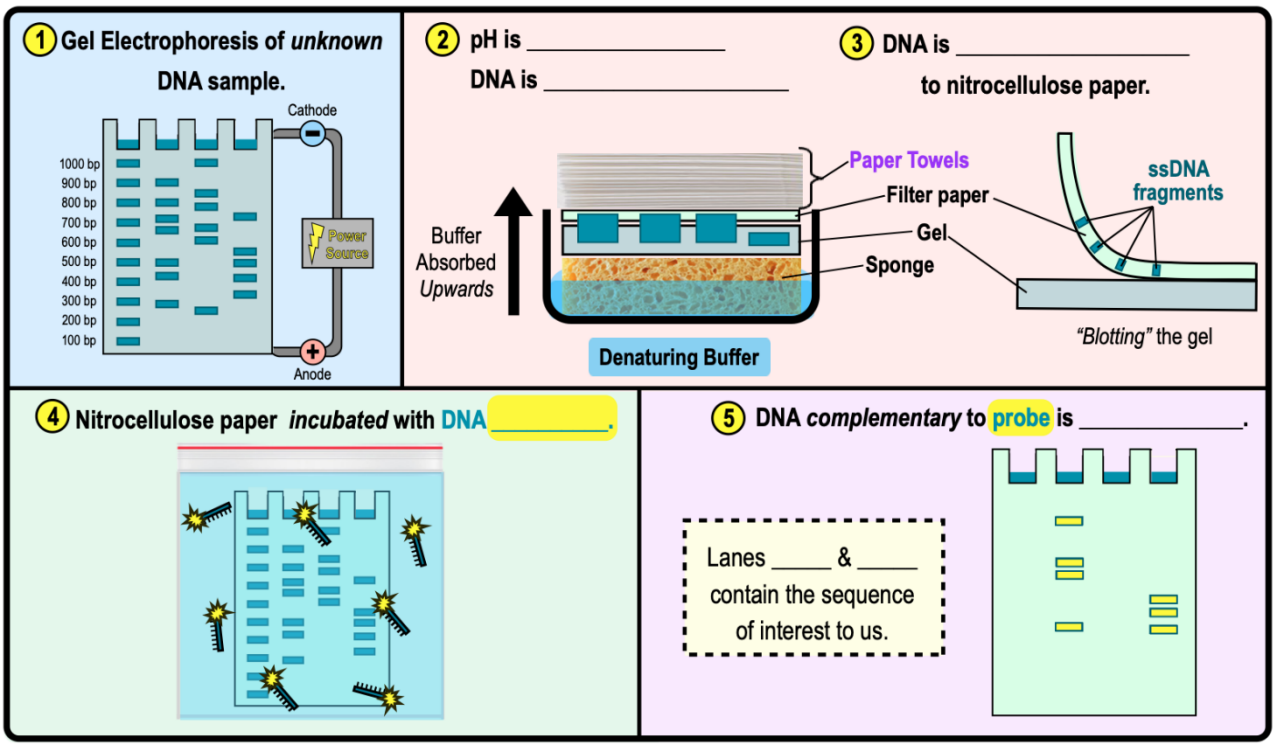
CONCEPT: SOUTHERN BLOTTING

- _____ **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.