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Molecular probe

Molecular biology

- RNAs: mRNA
  tRNA
  rRNA
  RNAi
- DNAs: genome DNA
  cDNA
- Protein: mikro-makro
  mono-poly
  single-multi

Analysis:

- Identification human and animal disease
- Finger printing
- Sexing embryo
- Mutation pattern
- Biodiversity pattern
- Evolution
- Cloning DNA, cDNA, cRNA
- Transgenic
- Gene Function and Biomechanism

Human or Animal DNA/RNA

Isolate DNA

Isolate RNA

Amplify DNA target site

Reverse Transcription, cDNA

cRNA synthesis

DNA clone

cDNA clone

cRNA clone

Probe for blotting

DNA cloned with other gene target, reporter gene etc → transgenic

Probe for blotting, Immunohistochemistry, In situ hybridization, Screening culture-cells

RNA Analysis, Probe for Northern Blot, In situ hybridization

Probe or Marker

- Probe: a labeled, defined sequence used to search mixtures of nucleic acids for molecules containing a complementary sequence

Designing Probes

- If the sequence of a gene is partially known in an organism, a homologous probe can be made. If it is known in another organism, a heterologous probe can be made
- Probes are usually 20 to 30 bases long and can be labeled with radioactive tags or fluorescent tags.
- They can be synthesized by a company

Labeling of DNA or RNA probes

- Radioactive labeling: display and/or magnify the signals by radioactivity
- Non-radioactive labeling: display and/or magnify the signals by antigen labeling – antibody binding – enzyme binding - substrate application (signal release)
- End labeling: put the labels at the ends
- Uniform labeling: put the labels internally

End labeling

- **Single stranded DNA/RNA**
- **5’ -end labeling: polynucleotide kinase (PNK)**
- **3’-end labeling: terminal transferase**

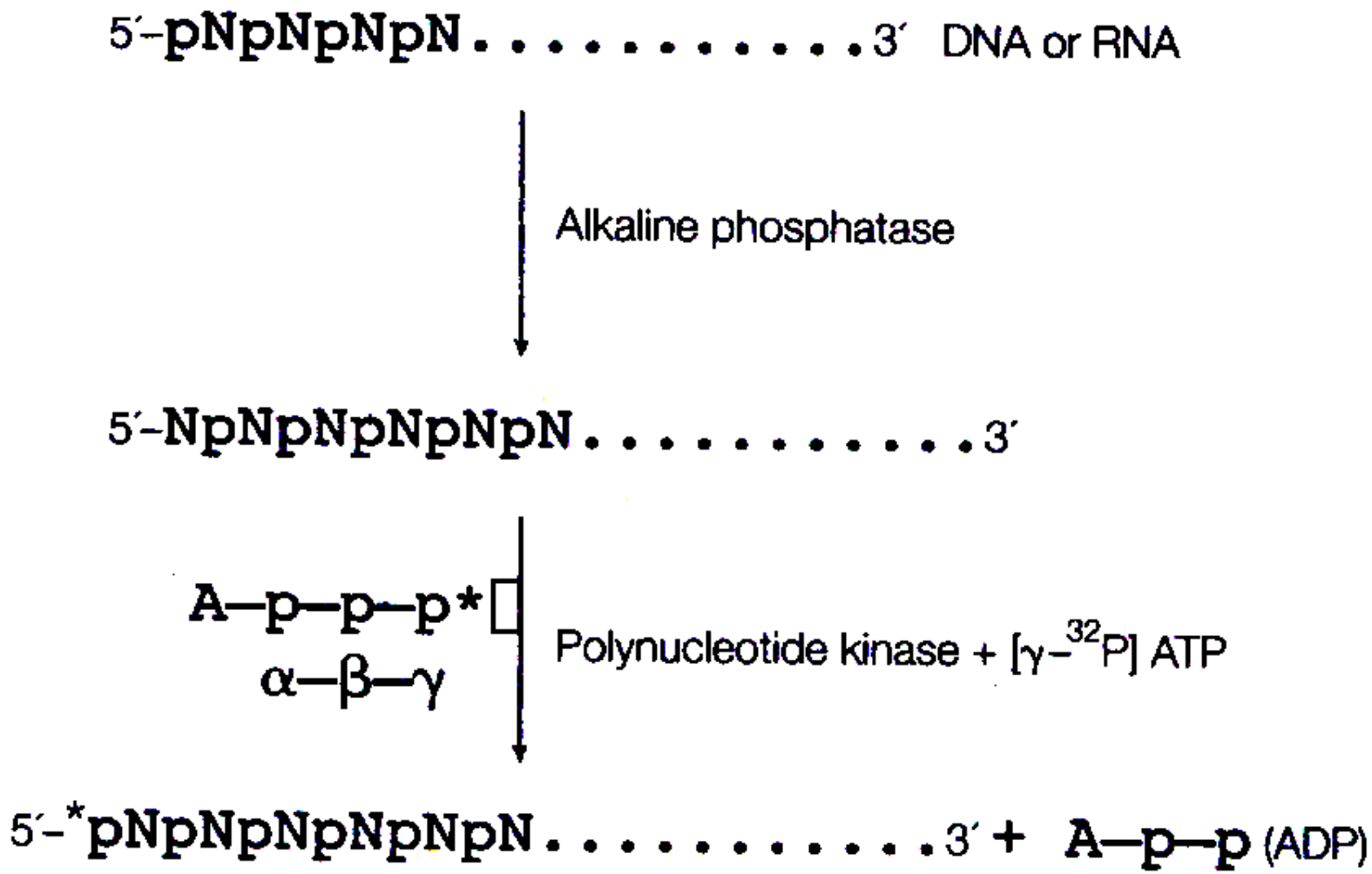
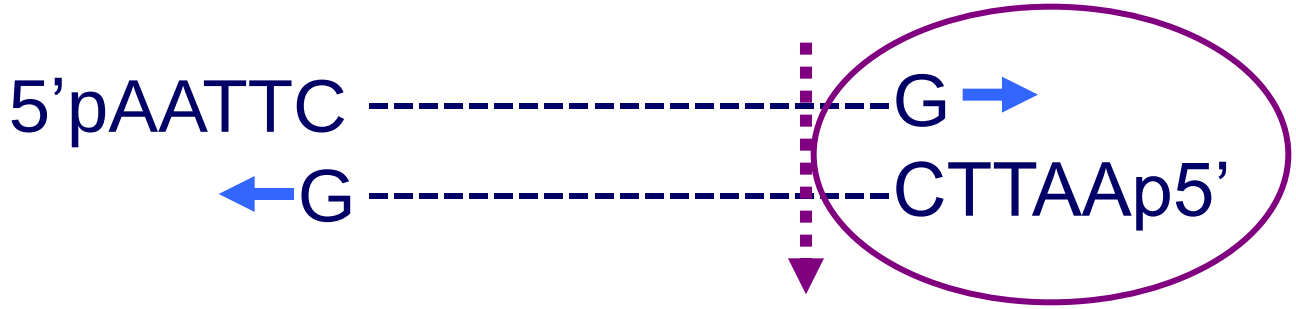


Fig. 2. 5′-End labeling of a nucleic acid molecule.

Labelling…

Labeling at both ends by kinase, then remove one end by restriction digestion



Labelling...

Uniform labeling of DNA/RNA

Nick translation:

DNase I to introduce random nicks →DNA polI to remove dNMPs from 3' to 5' and add new dNMP including labeled nucleotide at the 3' ends.

Hexanucleotide primered labeling:

Denature DNA → add random hexanucleotide primers and DNA pol → synthesis of new strand incorporating labeled nucleotide .

Labelling…

Strand-specific DNA probes:

e.g. M13 DNA as template
the missing strand can be re- synthesized
by incorporating radioactive nulceotides

Strand-specific RNA probes:

labeled by transcription

DNA hybridization can be used to identify specific DNA molecules

- Hybridization: the process of base-pairing between complementary ssDNA or RNA from two different sources

- Example:
- The Southern blot:
  - Electrophoretically separated DNA fragments are transferred to a nitrocellulose sheet.
  - A radioactively labeled probe for a DNA sequence of interest is bound to the nitrocellulose,
  - and bands are visualized with an autoradiogram.

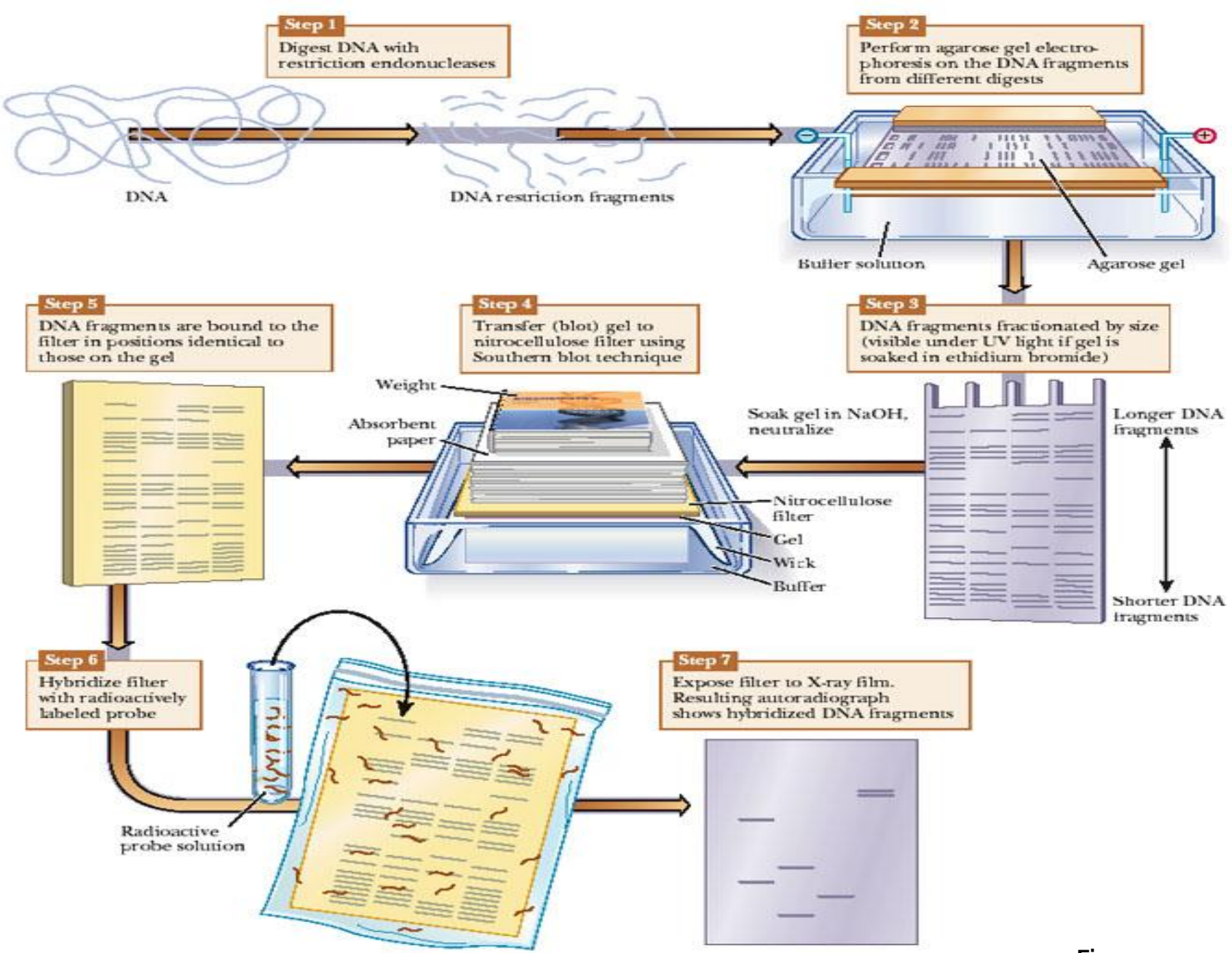


Fig. 13-23, p.357

Southern Blot: Detection of A Specific DNA Sequence Using a Probe

- can it be quickly screened for in a genomic library?
- What is replica plating, and how does it make library screens more efficient?
- What are some requirements for probing DNA with a complementary DNA fragment and how are these requirements met in screening a library with a probe?
- Describe how DNA probes can be labeled for detection of annealing to a complementary sequence. Which method(s) have the greatest sensitivity of detection?

Southern Blot: Detection of A Specific DNA Sequence Using a Probe

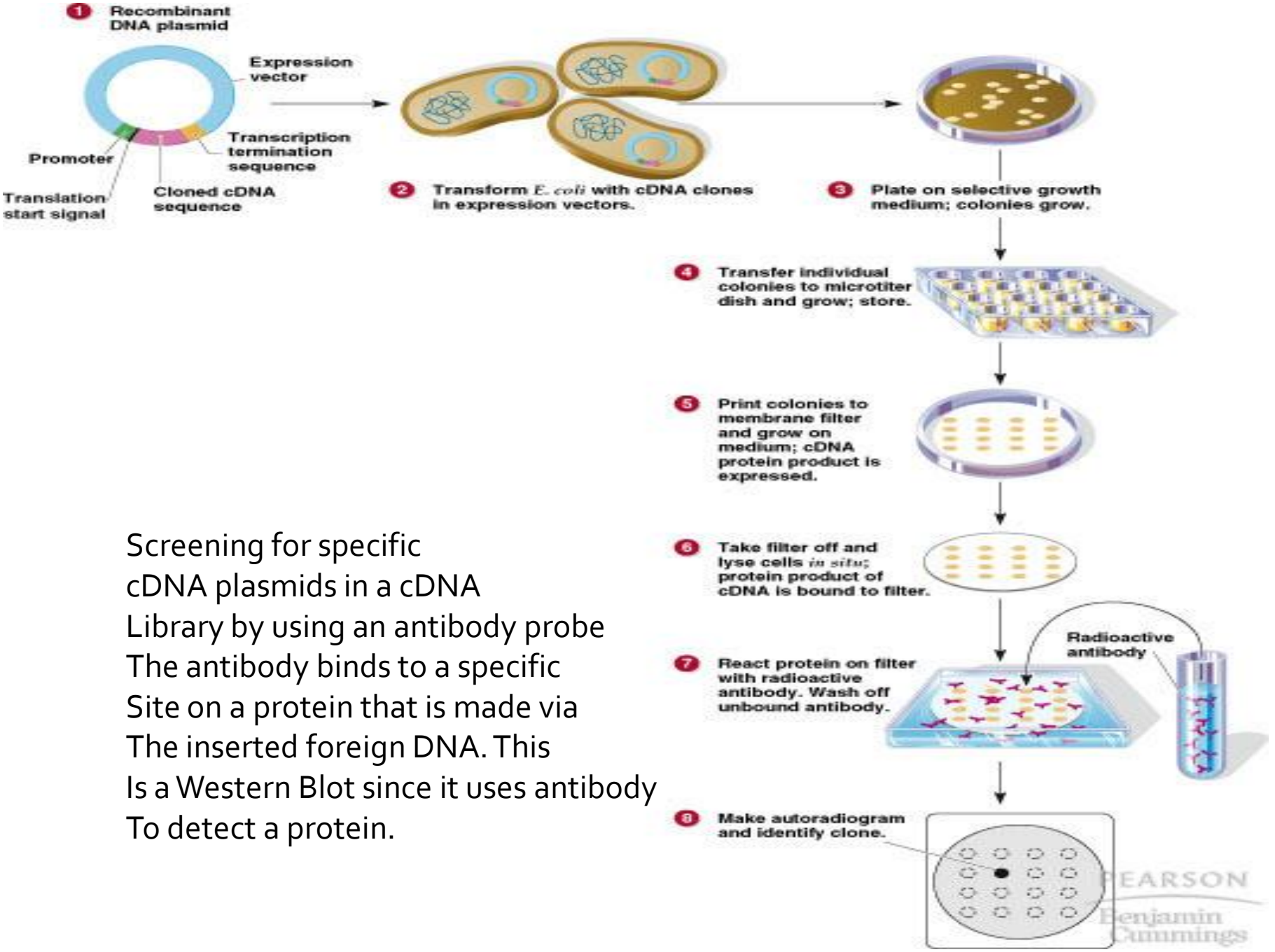
- Once some sequence information is available on a target gene, how About how long must a synthetic probe be in order to discriminate a target sequence in a genomic library?
- What is a heterologous probe, and what limitations are there to the use of these probes in screening genomic libraries?
- Describe how GenBank is used to design a heterologous probe that matches a consensus sequence.
- What can be done to design a probe specific to a target gene when there is no clear consensus sequence in the target gene?

Southern and Northern blotting

	DNA on blot	RNA on blot
1.	Genomic DNA preparation	RNA preparation
2.	Restriction digestion	-
3.	Denature with alkali	-
4.	Agarose gel electrophoresis	√
5.	DNA blotting/transfer and fixation	RNA
6.	Probe labeling	√
6.	Hybridization (temperature)	√
7.	Signal detection (X-ray film or antibody)	√

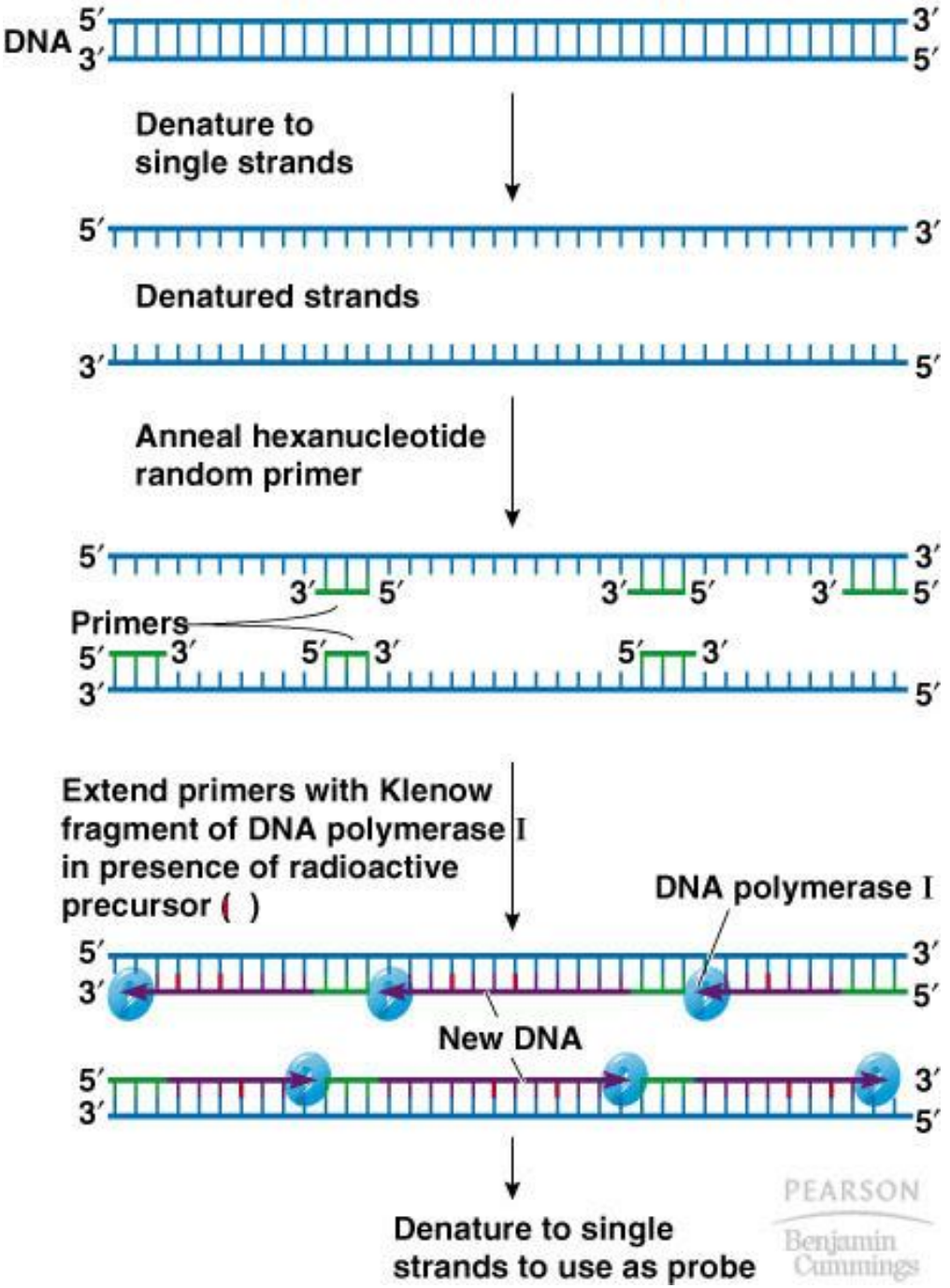
Screening

- What screening method can be used to identify a recombinant clone with a target gene in a cDNA library cloned into an expression vector? (Western Blot)
- What role does a plastic membrane play in this screening strategy? (protein product will stick to the membane)
- How can antibody binding be detected? (radioactivity is an inexpensive method!)
- Review the next slide—know how to do this!

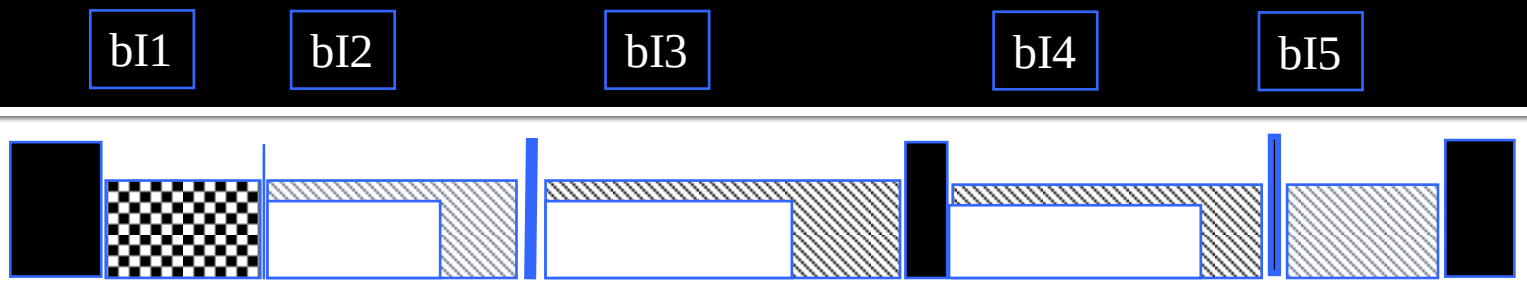


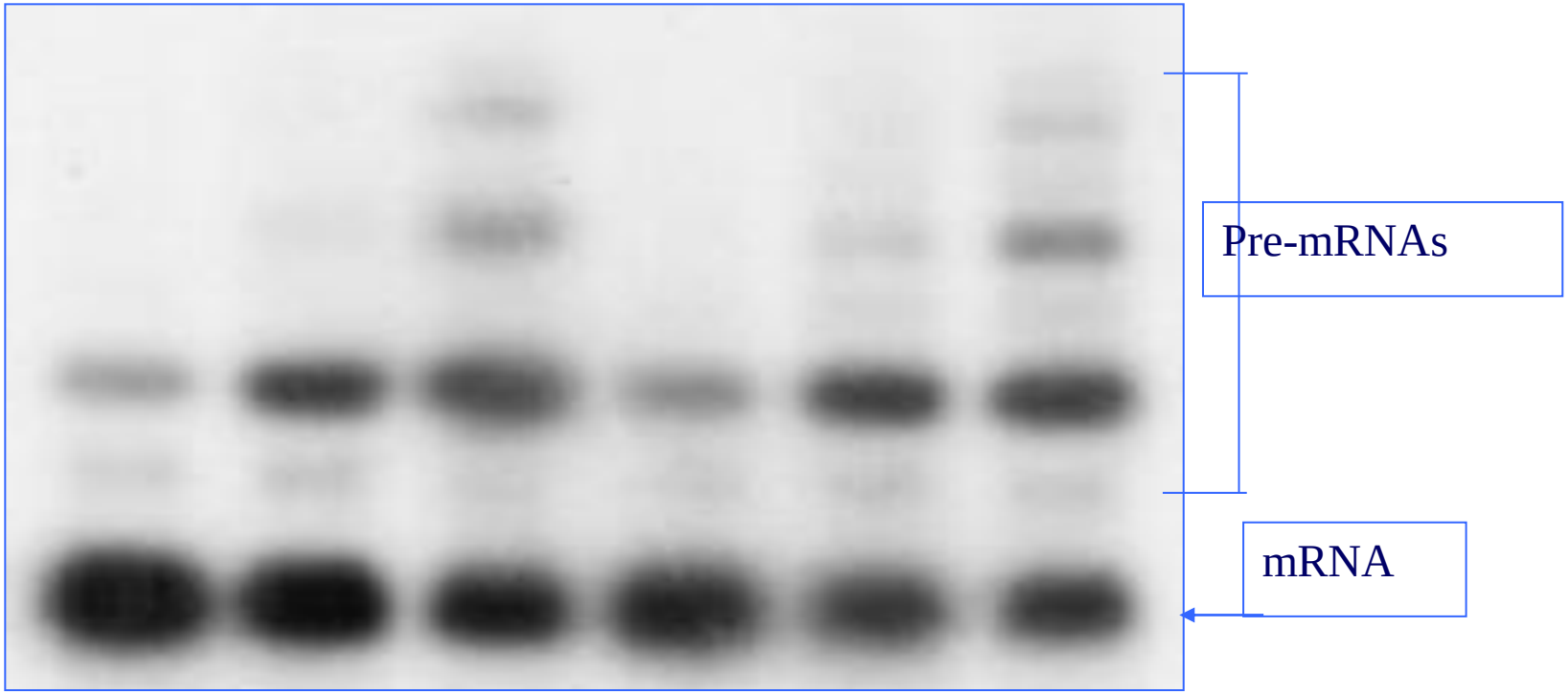
Screening for specific
cDNA plasmids in a cDNA
Library by using an antibody probe
The antibody binds to a specific
Site on a protein that is made via
The inserted foreign DNA. This
Is a Western Blot since it uses antibody
To detect a protein.

Radioactive
Labeling of DNA



Northern analysis COB RNAs in S. cerevisiae





Result of Northern Blot

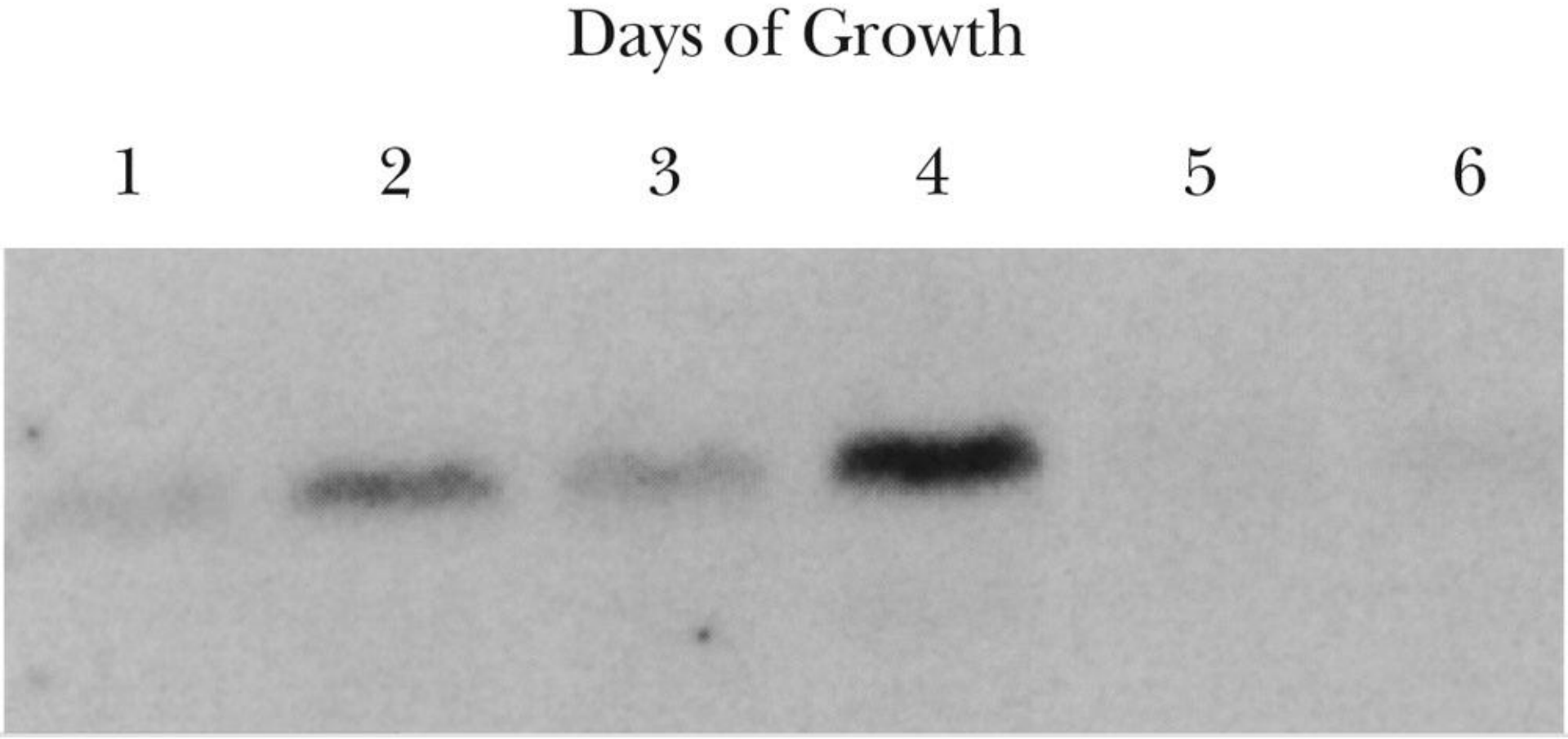
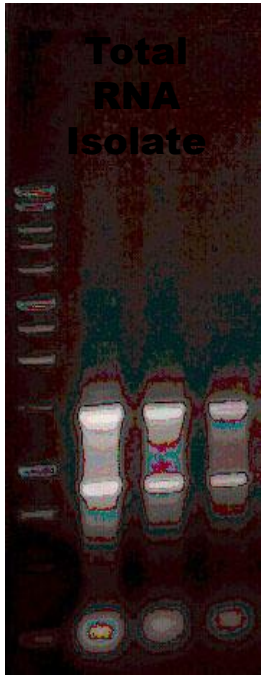


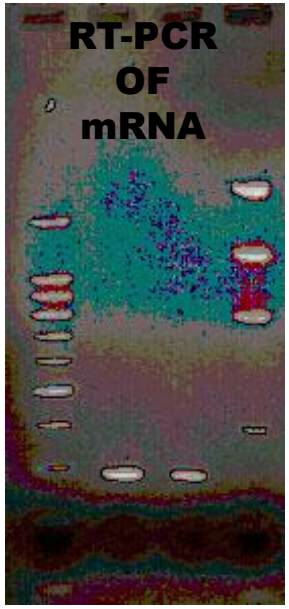
Fig. 13-29, p.362

Characterization of clones

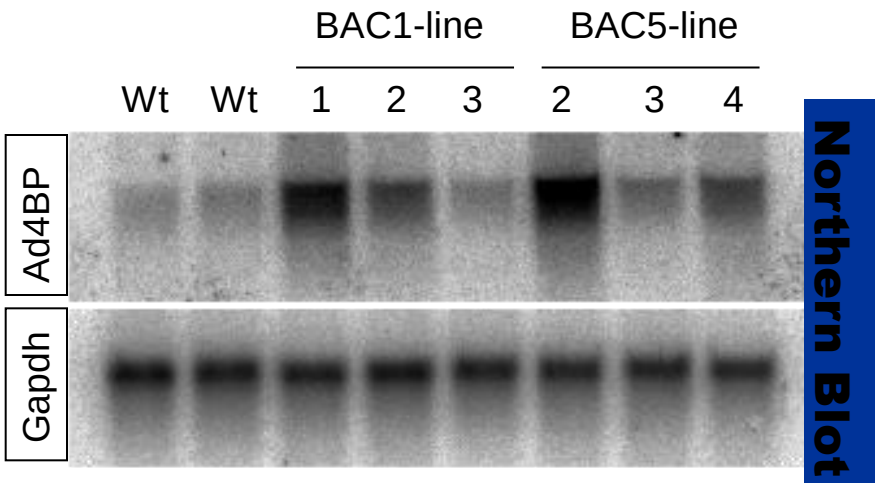
Blot type	Target	Probe	Applications
Southern	DNA	DNA or RNA	mapping genomic clones estimating gene numbers
Northern	RNA	DNA or RNA	RNA sizes, abundance, and expression
Western	Protein	Antibodies	protein size, abundance

mRNA Identification





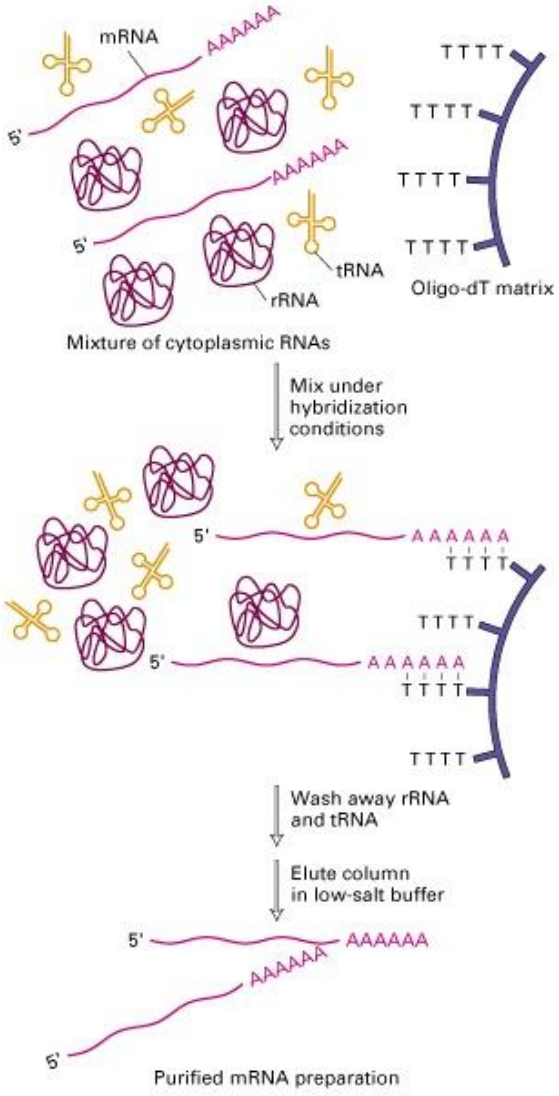
RT-PCR detect the mRNA with a pair specific primer



To detect level of mRNA of target gene of Transgenic-mice using Northern Blot Analysis with Specific Probe, and compare with control gene

Ref. Fatchiyah et al. BBRC 2006, 341: 1036-1045

Complementary DNA (cDNA) libraries are prepared from isolated mRNAs



Preparation of a cDNA library

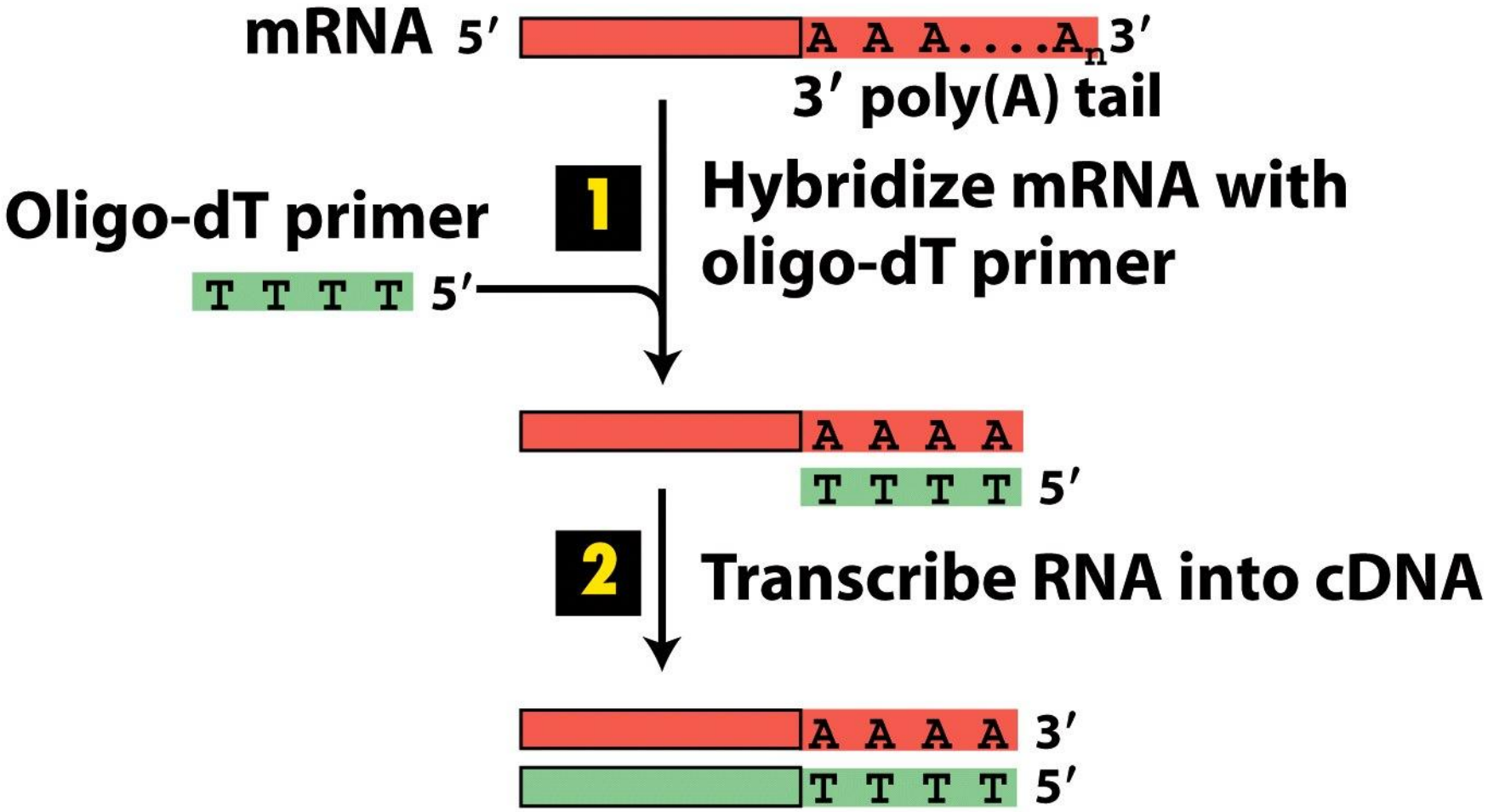


Figure 5-15 part 1
Molecular Cell Biology, Sixth Edition

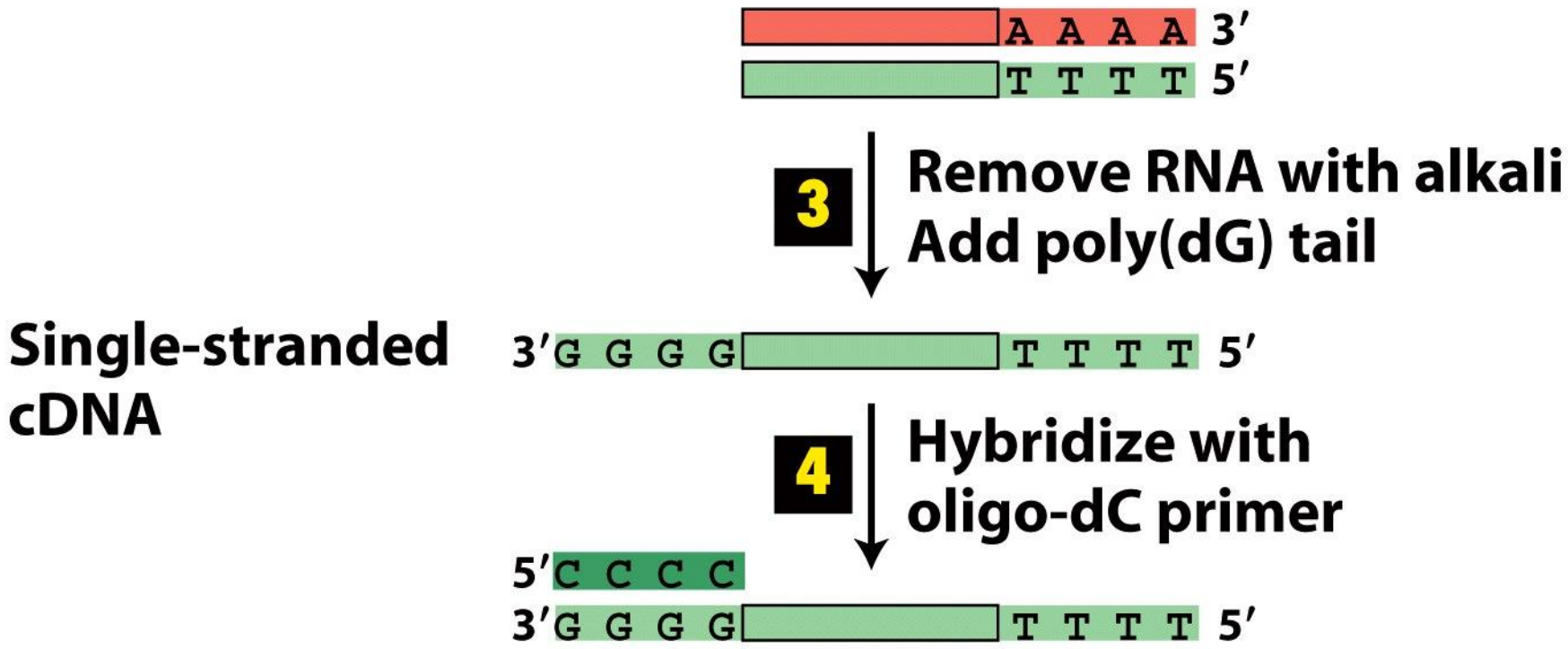


Figure 5-15 part 2
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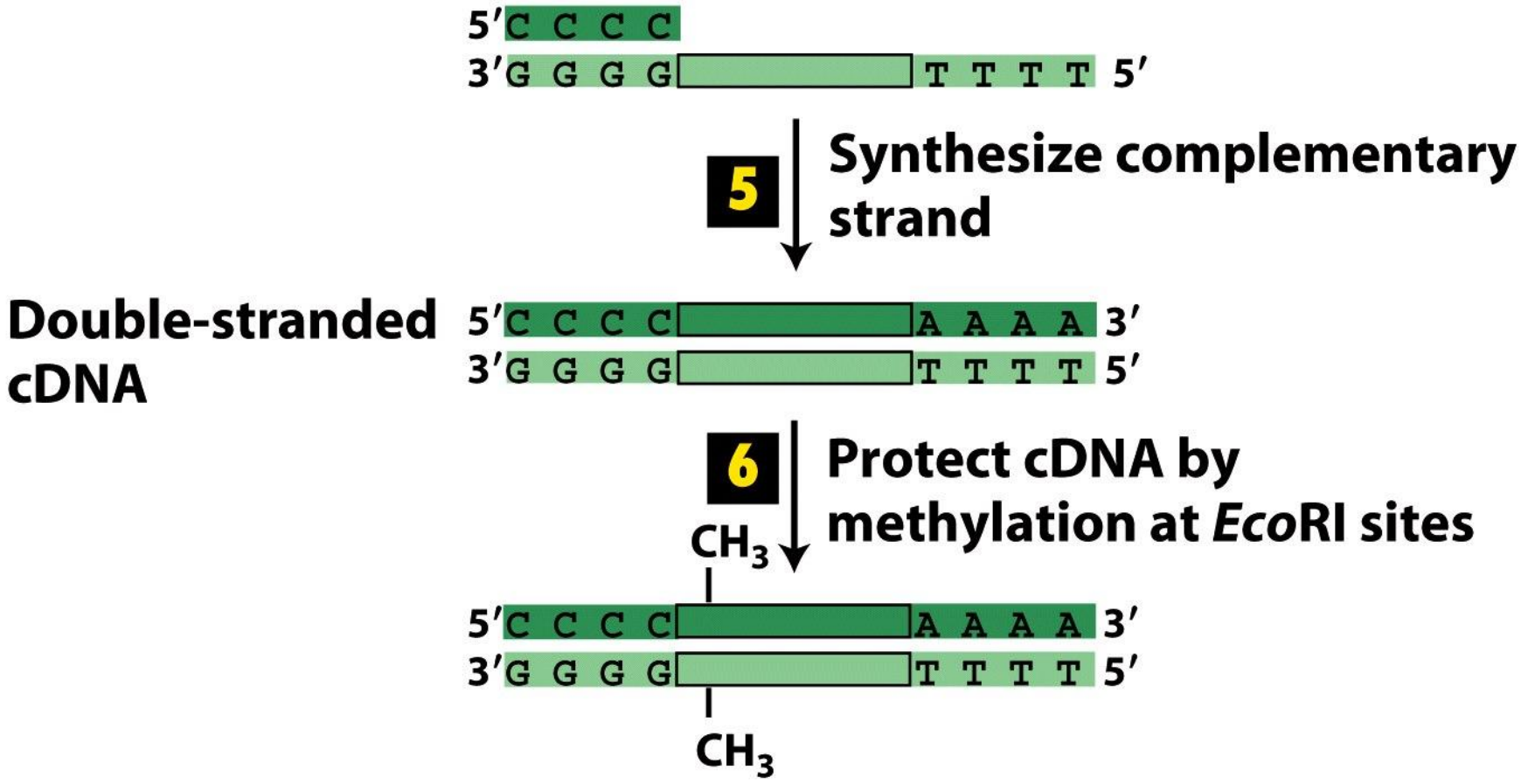

Figure 5-15 part 3
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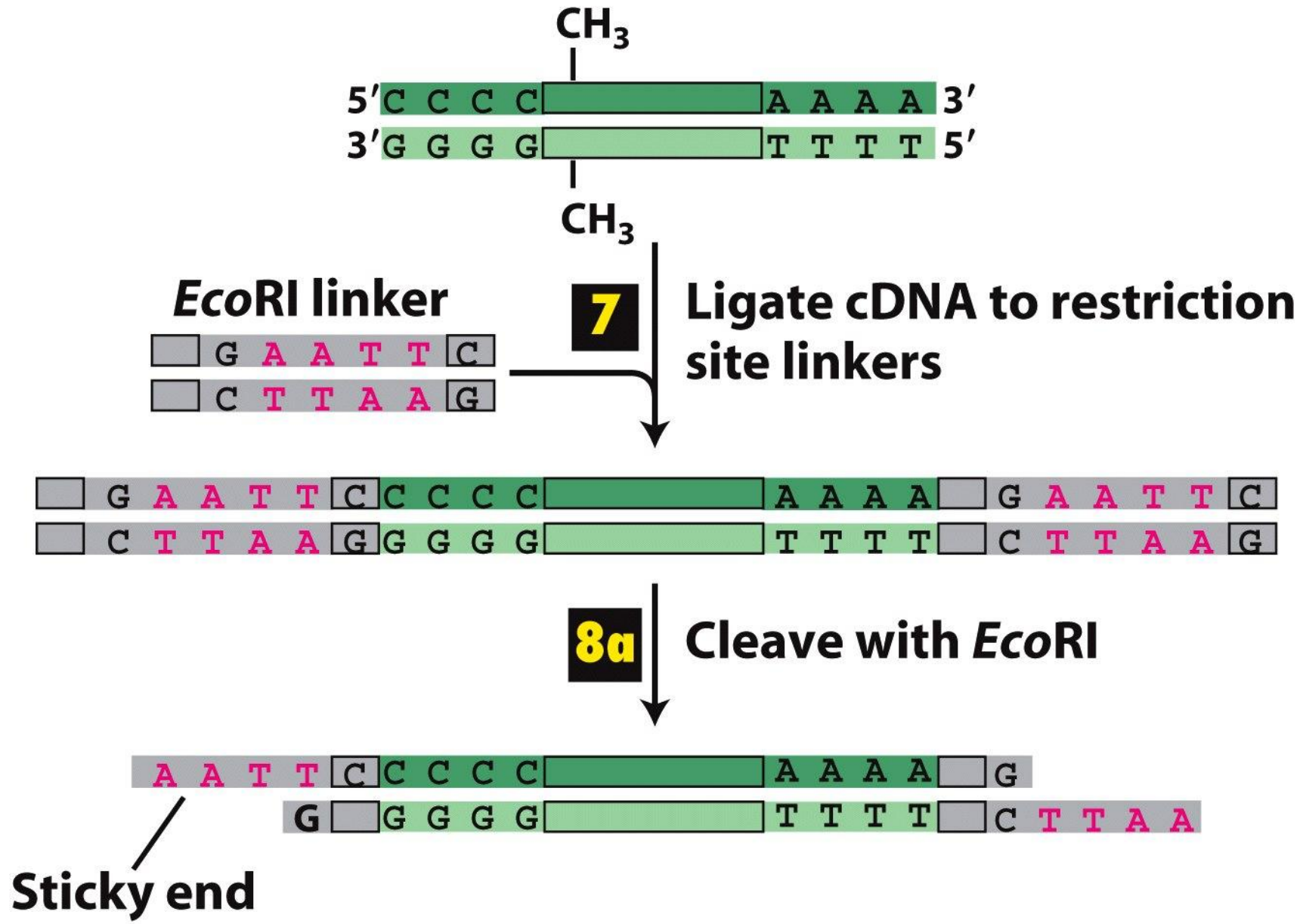

Figure 5-15 part 4
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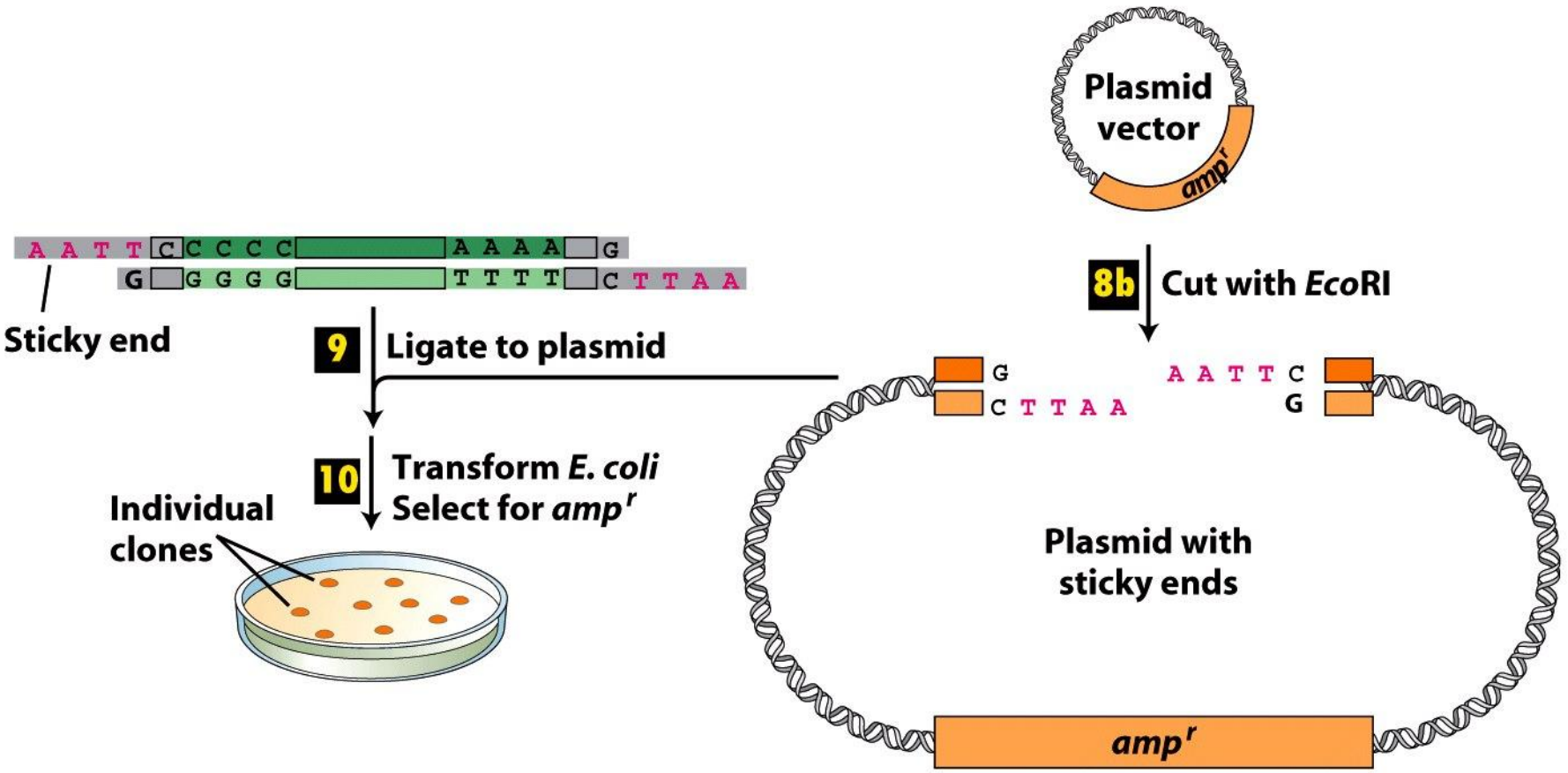

Figure 5-15 part 5
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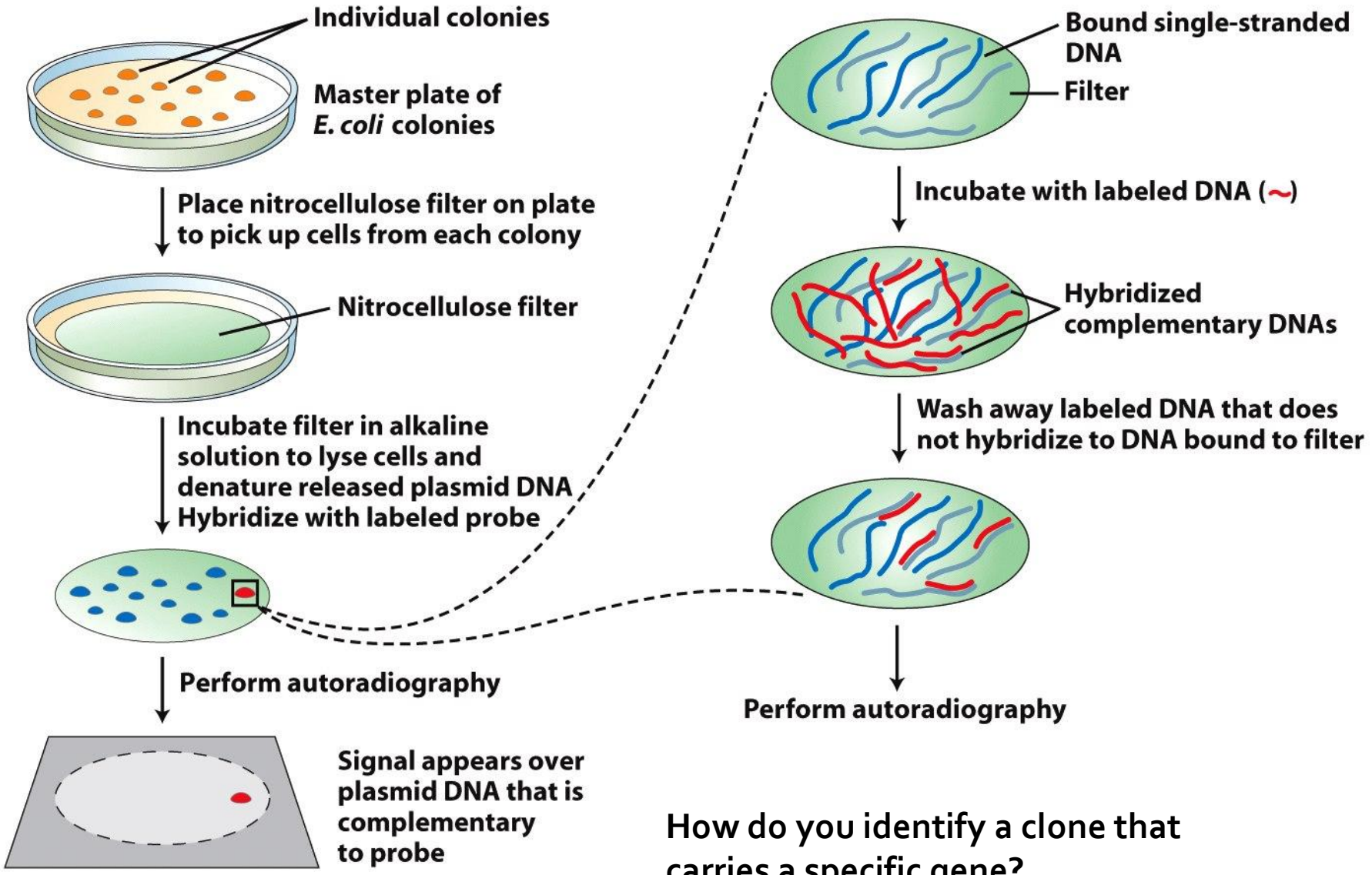

How do you identify a clone that carries a specific gene?

Figure 5-16
Molecular Cell Biology, Sixth Edition