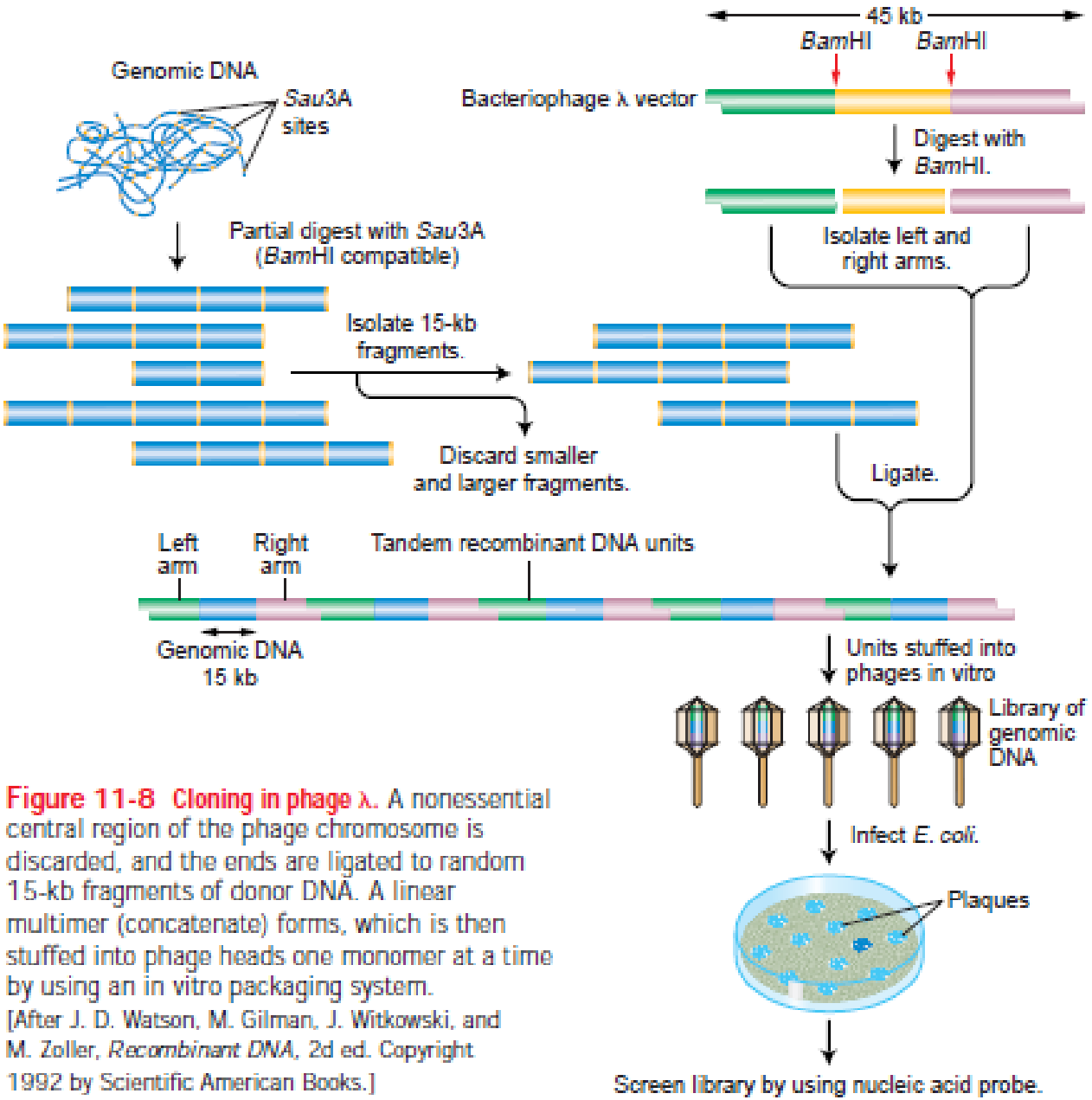
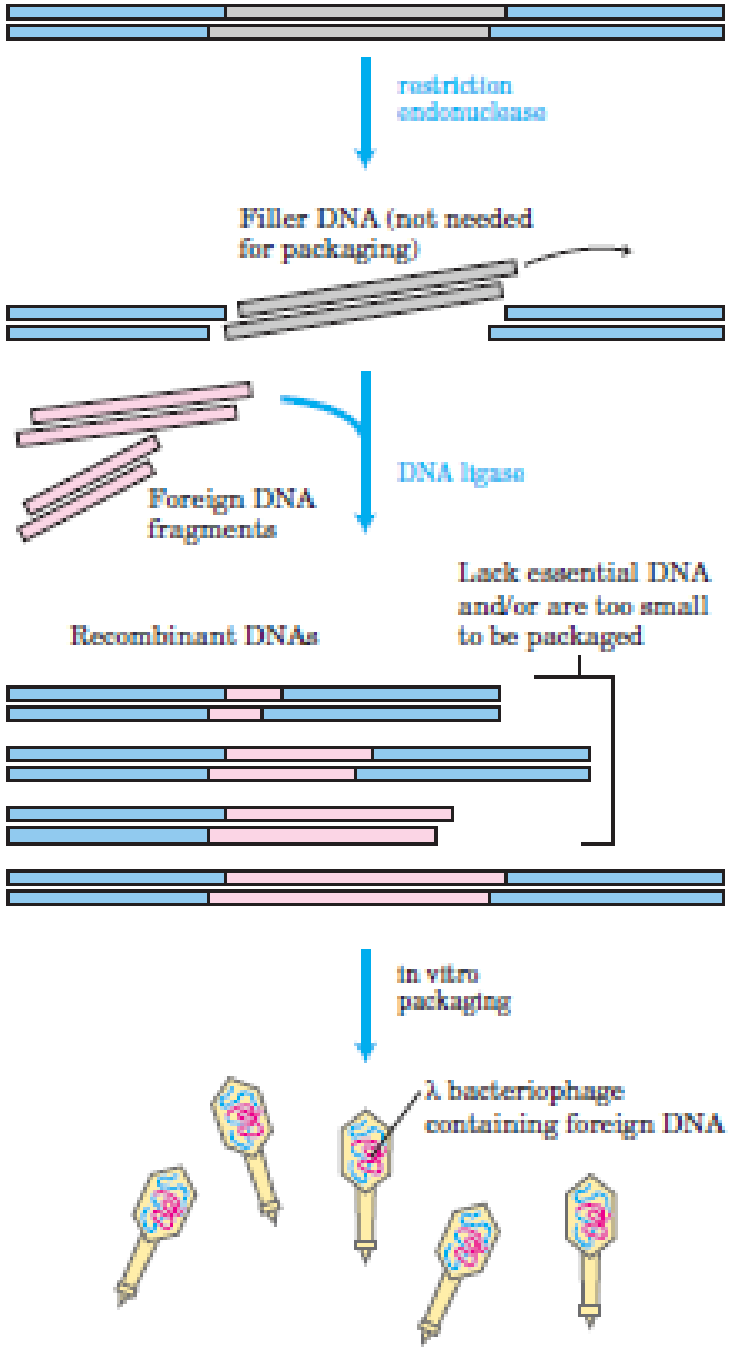


Figure 11-8 Cloning in phage λ. A nonessential central region of the phage chromosome is discarded, and the ends are ligated to random 15-kb fragments of donor DNA. A linear multimer (concatenate) forms, which is then stuffed into phage heads one monomer at a time by using an in vitro packaging system.
[After J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright 1992 by Scientific American Books.]

Another advantage of using these modified viruses as vectors is that they enter bacteria much more easily than do plasmids.


restriction endonuclease
Filler DNA (not needed for packaging)
DNA ligase
Foreign DNA fragments
Lack essential DNA and/or are too small to be packaged
Recombinant DNAs
in vitro packaging
λ bacteriophage containing foreign DNA

A sample containing many copies of total genomic DNA is first mechanically sheared or partly digested by restriction enzymes into large fragments.

This process yields a nearly random population of overlapping DNA fragments.

These fragments are then separated by gel electrophoresis to isolate the set of all fragments that are about 15 kb long.

Synthetic linkers are attached to the ends of these fragments, cohesive ends are formed, and the fragments are then inserted into a vector, such as phage DNA, prepared with the same cohesive ends.

E. coli bacteria are then infected with these recombinant phages.

These phages replicate themselves and then lyse their bacterial hosts.

.

The resulting lysate contains fragments of human DNA housed in a sufficiently large number of virus particles to ensure that nearly the entire genome is represented.

These phages constitute a ***genomic library***. *Phages can be propagated indefinitely, and so the library* can be used repeatedly over long periods.

This genomic library is then screened to find the very small number of phages harboring the gene of interest.

For the human genome, a calculation shows that a 99% probability of success requires screening about 500,000 clones; hence, a very rapid and efficient screening process is essential. Rapid screening can be accomplished by DNA hybridization.

A dilute suspension of the recombinant phages is first plated on a lawn of bacteria.

Where each phage particle has landed and infected a bacterium**, a *plaque containing identical phages develops on the plate.***

A replica of this master plate is then made by applying a sheet of nitrocellulose.
Infected bacteria and phage DNA released from lysed cells adhere to the sheet
in a pattern of spots corresponding to the plaques.

Intact bacteria on this sheet are lysed with NaOH, which also serves to denature the DNA so that it becomes accessible for hybridization with a ^{32}P-labeled probe.

The presence of a specific DNA sequence in a single spot on the replica can be detected by using a radioactive complementary DNA or RNA molecule as a probe.

Autoradiography then reveals the positions of spots harboring recombinant DNA. The corresponding plaques are picked out of the intact master plate and grown. A single investigator can readily screen a million clones in a day.

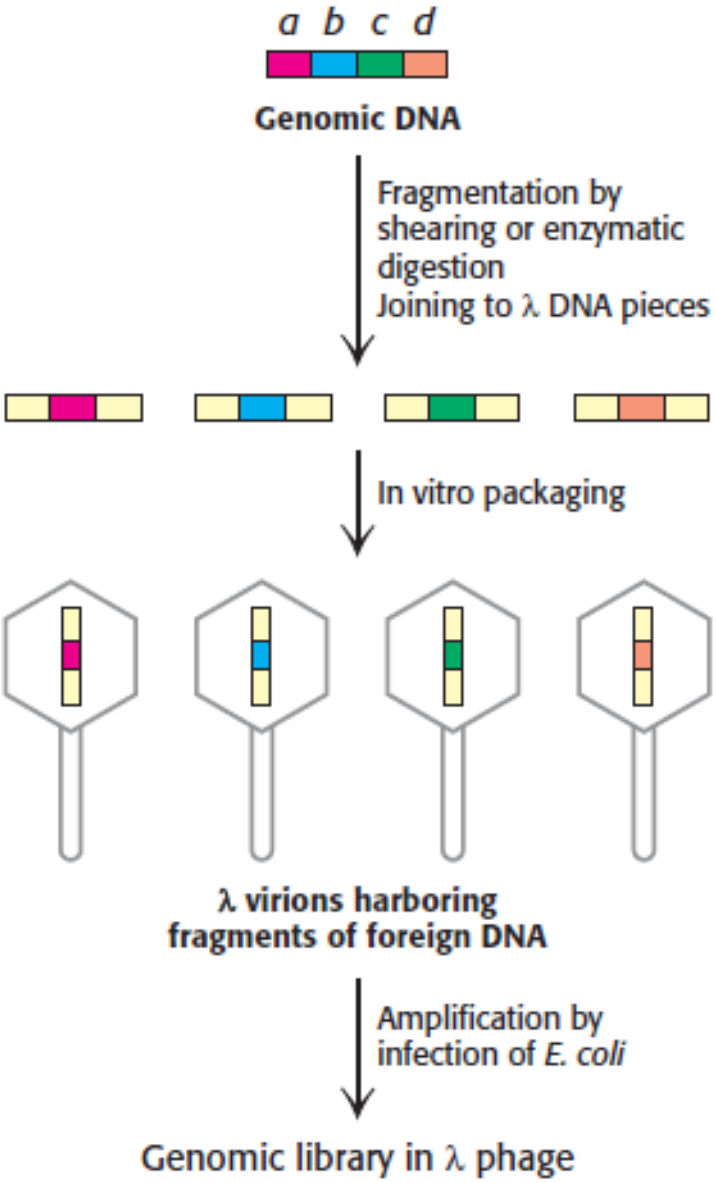


Figure 5.17 Creation of a genomic library. A genomic library can be created from a digest of a whole complex genome.

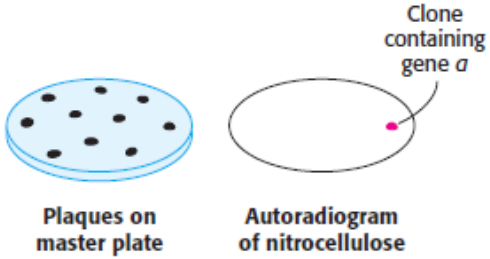


Figure 5.18 Screening a genomic library for a specific gene. Here, a plate is tested for plaques containing gene *a* of Figure 5.17.

Type of donor DNA

The choice of DNA to be used as the donor might seem to be obvious, but there are actually three possibilities.

• *Genomic DNA*. ***This DNA is obtained directly from*** **the chromosomes of the organism** under study. It is the most straightforward source of DNA. It needs to be cut up before cloning is possible.

• *cDNA.* ***Complementary DNA (cDNA) is a doublestranded*** **DNA version of an mRNA molecule.** In higher eukaryotes, an mRNA is a more useful predictor of a polypeptide sequence than is a genomic sequence, because the introns have been spliced out.

Chemically synthesized DNA. *Sometimes, a researcher* needs to include in a recombinant DNA molecule a specific sequence that for some reason cannot be isolated from available natural genomic DNA or cDNAs. If the DNA sequence is known (often from a complete genome sequence), then the gene can be synthesized chemically by using automated techniques.

- We take all the DNA from a genome, break it up into segments of the right size for our cloning vector, and insert each segment into a different copy of the vector, thereby creating a collection of recombinant DNA molecules that, taken together, represent the entire genome.

- We then transform or transduce these molecules into separate bacterial recipient cells, where they are amplified.

- The resulting collection of recombinant DNA-bearing bacteria or bacteriophages is called a **genomic library.**

- **Finding a specific clone of interest**
- **FINDING SPECIFIC CLONES BY USING PROBES A** library might contain as many as hundreds of thousands of cloned fragments. This huge collection of fragments must be screened to find the recombinant DNA molecule containing the gene of interest to a researcher.
- Such screening is accomplished by using a specific **probe that** will find and mark only the desired clone. There are two types of probes: those that recognize a specific nucleic acid sequence and those that recognize a specific protein.

Making genomic and cDNA libraries

Probes for finding DNA Probing for DNA makes use of the **power of base complementarity**.

Two single-stranded nucleic acids with full or partial complementary base sequence will "find" each other in solution by random collision.

Once united, the double-stranded hybrid so formed is stable.

This provides a powerful approach to finding specific sequences of interest. In the case of DNA, all molecules must be made single stranded by heating.

A single-stranded probe, labeled radioactively or chemically, is sent out to find its complementary target sequence in a population of DNAs such as a library.

Probes as small as 15 to 20 base pairs will hybridize to specific complementary sequences within much larger cloned DNAs. Thus, probes can be thought of as "bait" for identifying much larger "prey."

The identification of a specific clone in a library is a two-step procedure.

First, colonies or plaques of the library on a petri dish are transferred to an absorbent membrane (often nitrocellulose) by simply laying the membrane on the surface of the medium.

The membrane is peeled off, **colonies or plaques clinging to the surface are lysed in situ, and the DNA is denatured.**

Second, the membrane is bathed with a solution of a single-stranded probe that is specific for the DNA being sought.

Generally, the probe is itself a cloned piece of DNA that has a sequence homologous to that of the desired gene.

The probe must be labeled with either a radioactive isotope or a fluorescent dye. Thus the position of a positive clone will become clear from the position of the concentrated radioactive or fluorescent label.

For radioactive labels, the membrane is placed on a piece of X-ray film, and the decay of the radioisotope produces subatomic particles that “expose” the film, producing a dark spot on the film adjacent to the location of the radioisotope concentration.

Such an exposed film is called an **autoradiogram.**

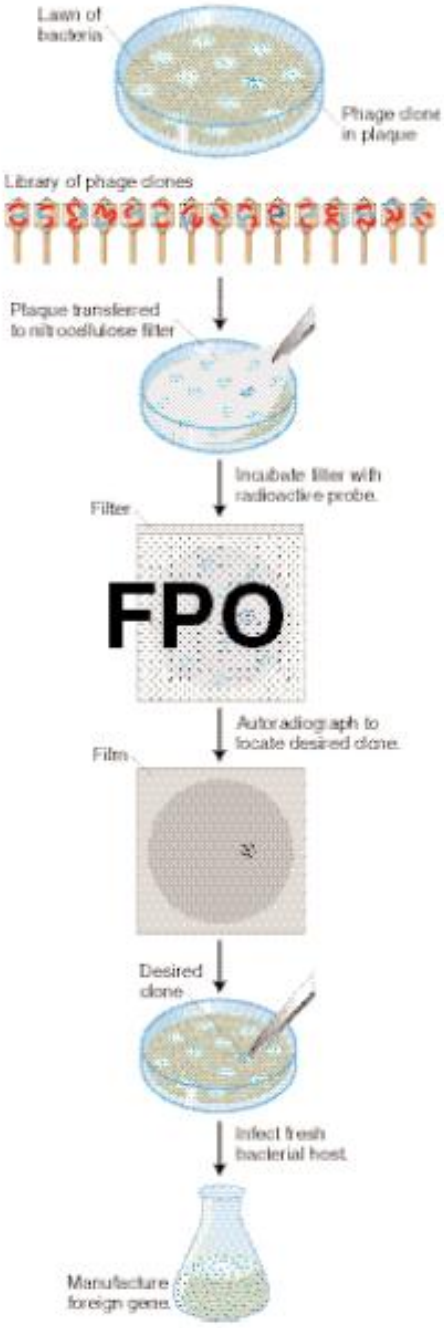


Figure 11-11 Using DNA or RNA probes to identify the clone carrying a gene of interest. The clone is identified by probing a genomic library, in this case made by cloning genes in λ bacteriophages, with DNA or RNA known to be related to the desired gene. A radioactive probe hybridizes with any recombinant DNA incorporating a matching DNA sequence, and the position of the clone having the DNA is revealed by autoradiography. Now the desired clone can be selected from the corresponding spot on the petri dish and transferred to a fresh bacterial host so that a pure gene can be manufactured. [After R. A. Weinberg, "A Molecular Basis of Cancer," and P. Leder, "The Genetics of Antibody Diversity." Copyright 1983, 1982 by Scientific American, Inc. All rights reserved.]

Southern blot

How can specific molecules be identified among the thousands of types in the cell? The most extensively used method for detecting specific macromolecules in a mixture is **probing.**

Electrophoresis fractionates a population of nucleic acid fragments on the basis of size.

The cut mixture is placed in a small well in a gelatinous slab (a gel), and the gel is placed in a powerful electrical field.

The electricity causes the molecules to move through the gel at speeds inversely proportional to their size.

After fractionation, the separated fragments are blotted onto a piece of porous membrane, where they maintain the same relative positions.

This procedure is called a **Southern blot.**

PROBING TO FIND A SPECIFIC NUCLEIC ACID IN A MIXTURE

After having been heated to separate the DNA strands and hold the DNA in position, the membrane is placed in a solution of the probe.

The single-stranded probe will find and bind to its complementary DNA sequence.

For example,

TAGGTATCG Probe
ACTAATCCATAGCTTA Genomic fragment
On the blot, this binding concentrates the label in one spot.

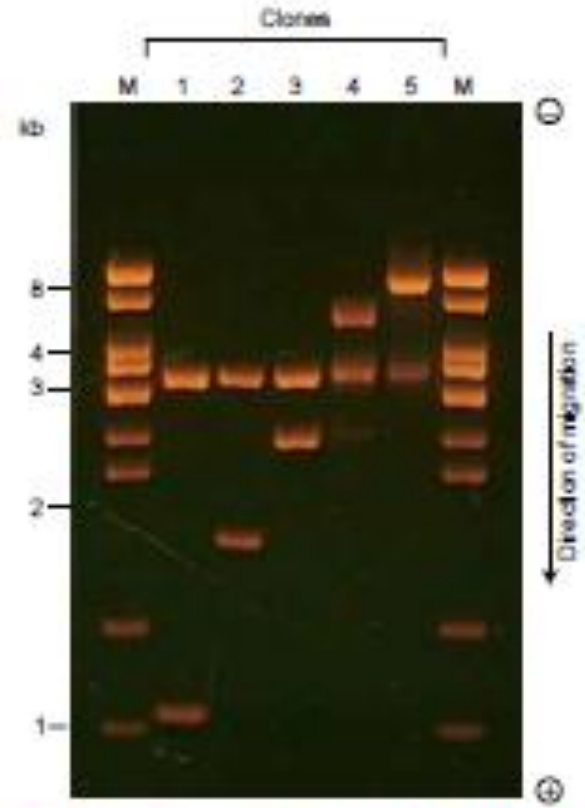


Figure 11-13 **Mixtures of different-sized DNA fragments separated electrophoretically on an agarose gel.** The samples are five recombinant vectors treated with *Eco*RI. The mixtures are applied to wells at the top of the gel, and fragments move under the influence of an electric field to different positions dependent on size (and, therefore, number of charges). The DNA bands have been visualized by staining with ethidium bromide and photographing under UV light. (*M* represents lanes containing standard fragments acting as markers for estimating DNA length.) [From

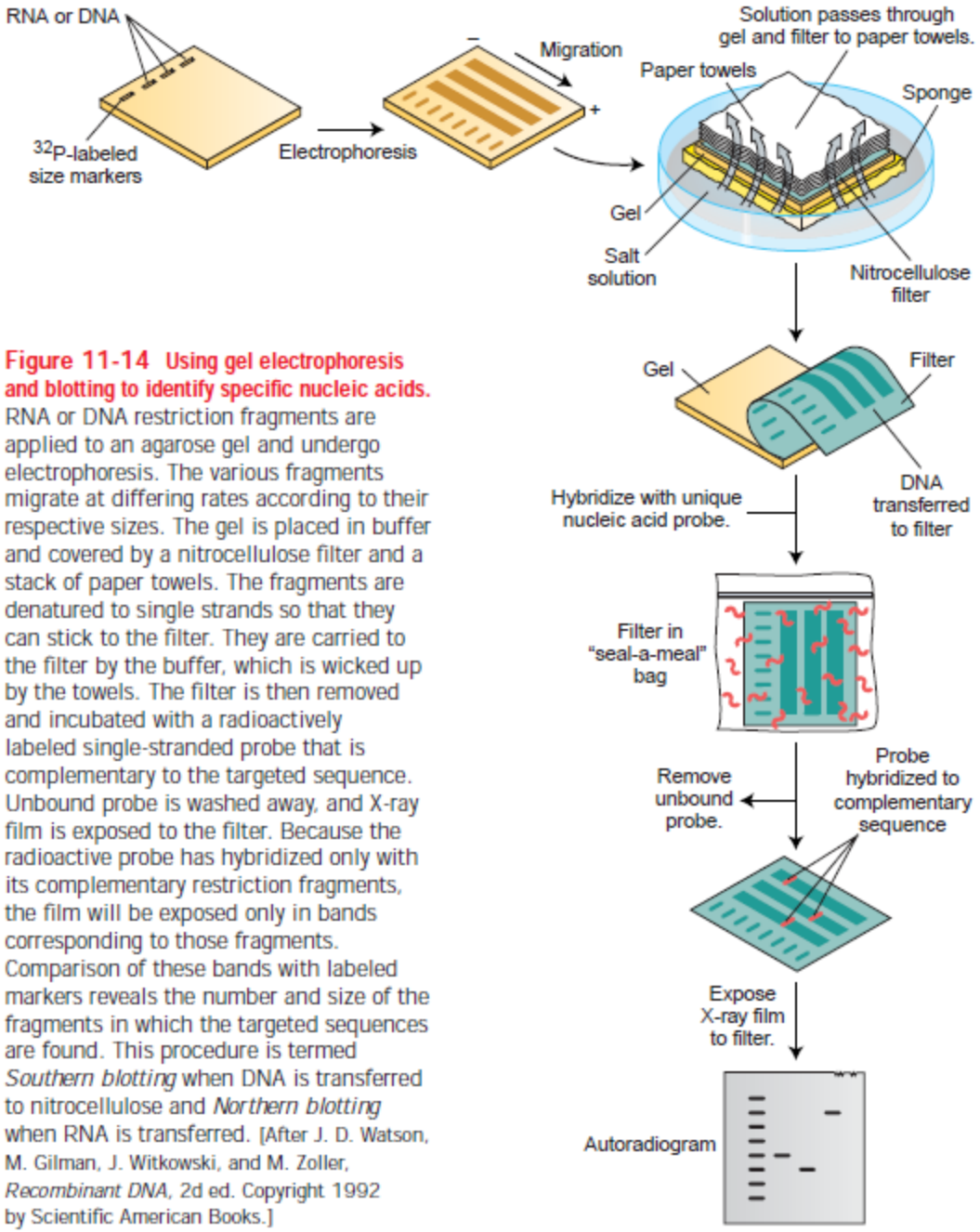
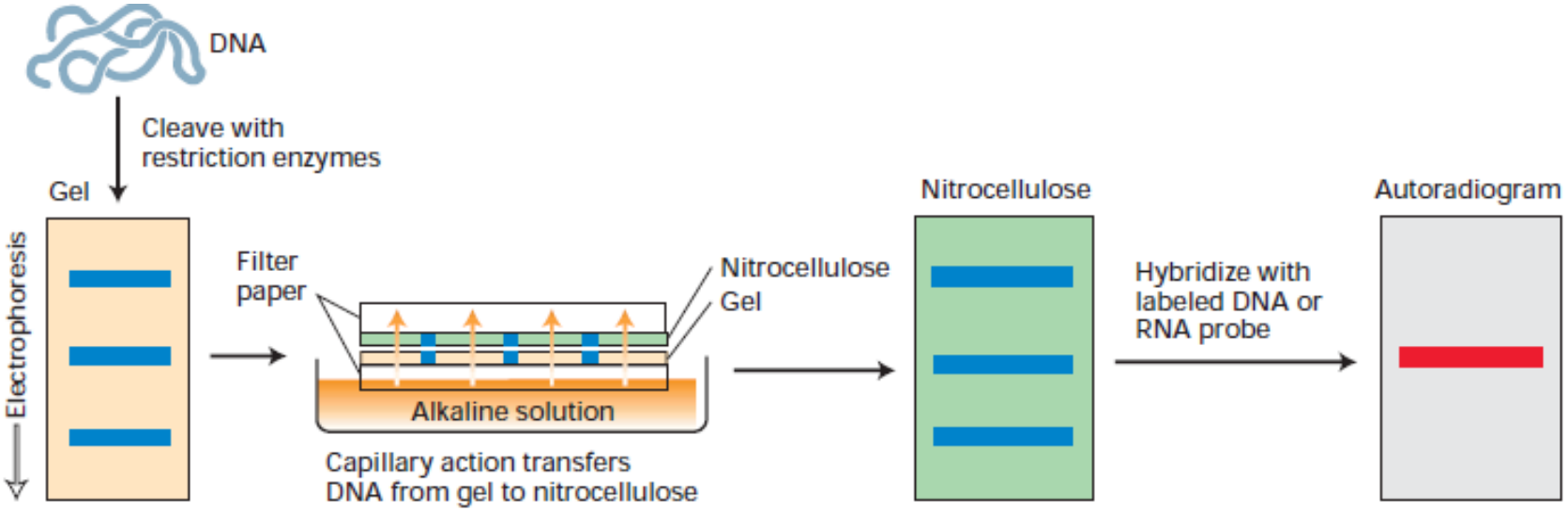


Figure 11-14 Using gel electrophoresis and blotting to identify specific nucleic acids. RNA or DNA restriction fragments are applied to an agarose gel and undergo electrophoresis. The various fragments migrate at differing rates according to their respective sizes. The gel is placed in buffer and covered by a nitrocellulose filter and a stack of paper towels. The fragments are denatured to single strands so that they can stick to the filter. They are carried to the filter by the buffer, which is wicked up by the towels. The filter is then removed and incubated with a radioactively labeled single-stranded probe that is complementary to the targeted sequence. Unbound probe is washed away, and X-ray film is exposed to the filter. Because the radioactive probe has hybridized only with its complementary restriction fragments, the film will be exposed only in bands corresponding to those fragments. Comparison of these bands with labeled markers reveals the number and size of the fragments in which the targeted sequences are found. This procedure is termed *Southern blotting* when DNA is transferred to nitrocellulose and *Northern blotting* when RNA is transferred. [After J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright 1992 by Scientific American Books.]

https://www.youtube.com/watch?v=CSrUm-EgTK4


DNA
Cleave with
restriction enzymes
Gel
Electrophoresis
Filter
paper
Nitrocellulose
Gel
Alkaline solution
Capillary action transfers
DNA from gel to nitrocellulose
Nitrocellulose
Hybridize with
labeled DNA or
RNA probe
Autoradiogram

Probes for finding proteins

If the protein product of a gene is known and isolated in pure form, then this protein can be used to detect the clone of the corresponding gene in a library.

The process, requires two components. First, it requires an expression library, made by using expression vectors.

To make the library**, cDNA is inserted into the vector in the correct triplet reading frame** with a bacterial protein (in this case, -galactosidase), and cells containing the vector and its insert produce a “fusion” protein that is partly a translation of the cDNA insert and partly a part of the normal -galactosidase.

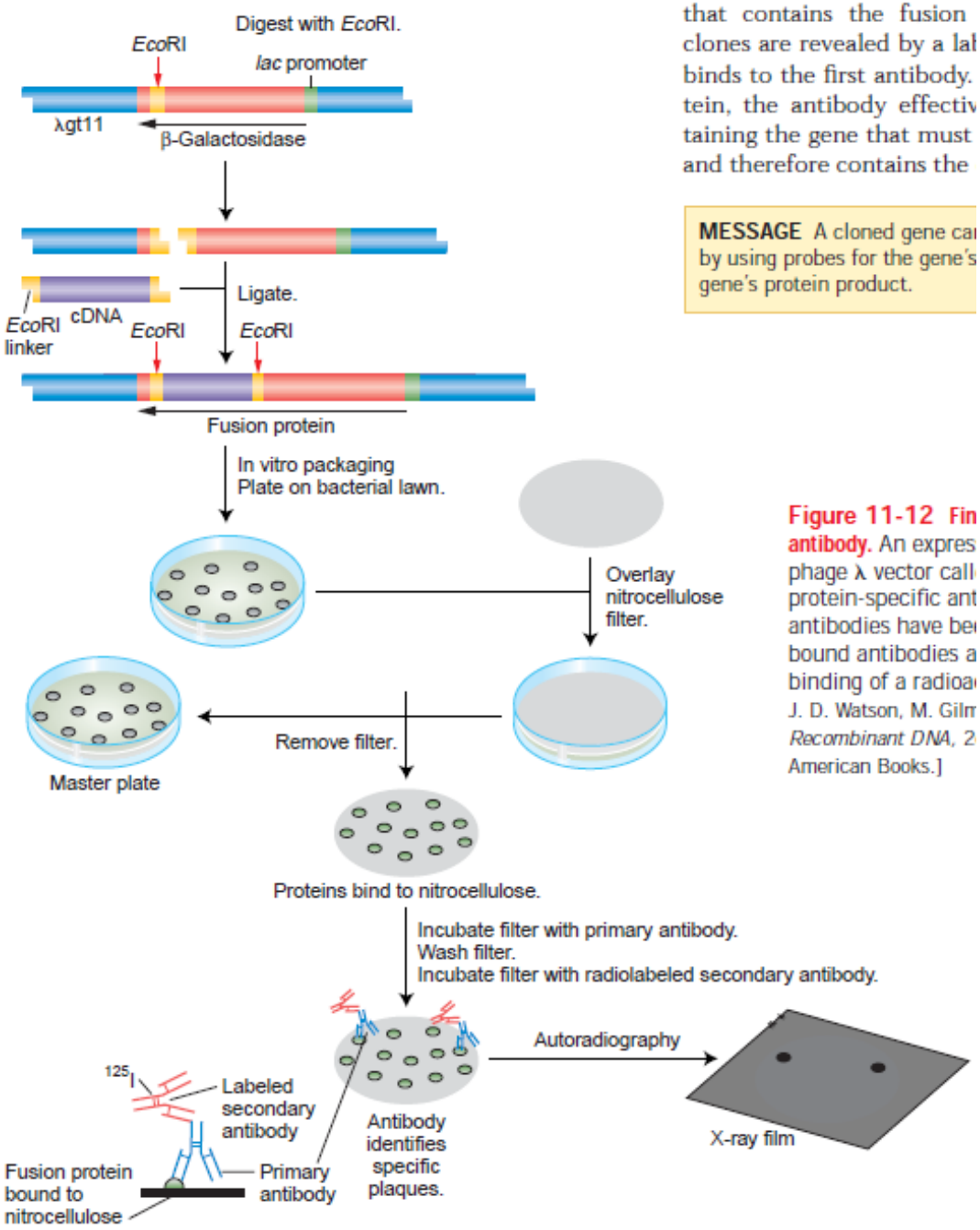
Second, the process requires an **antibody to the specific protein product of the gene of** interest. (An antibody is a protein made by an animal’s immune system that binds with high affinity to a given molecule.)

The antibody is used to screen the expression library for that protein.

A membrane is laid over the surface of the medium and removed so that some of the cells of each colony are now attached to the membrane at locations that correspond to their positions on the original petri dish.

The imprinted membrane is then dried and bathed in a solution of the antibody, which will bind to the imprint of any colony that contains the fusion protein of interest.

Positive clones are revealed by a labeled secondary antibody that binds to the first antibody.



that contains the fusion
clones are revealed by a lab
binds to the first antibody.
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gene's protein product.

**Figure 11-12 Fin
antibody.** An expres
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J. D. Watson, M. Gilm
Recombinant DNA, 2
American Books.]

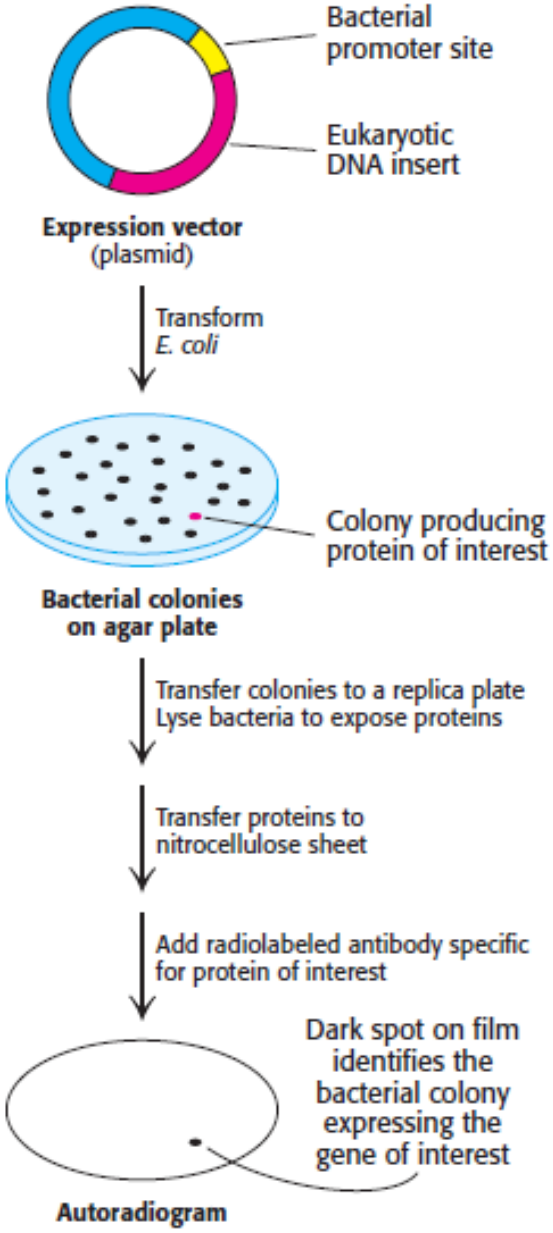

Figure 5.30 Screening of cDNA clones. A method of screening for cDNA clones is to identify expressed products by staining with specific antibody.

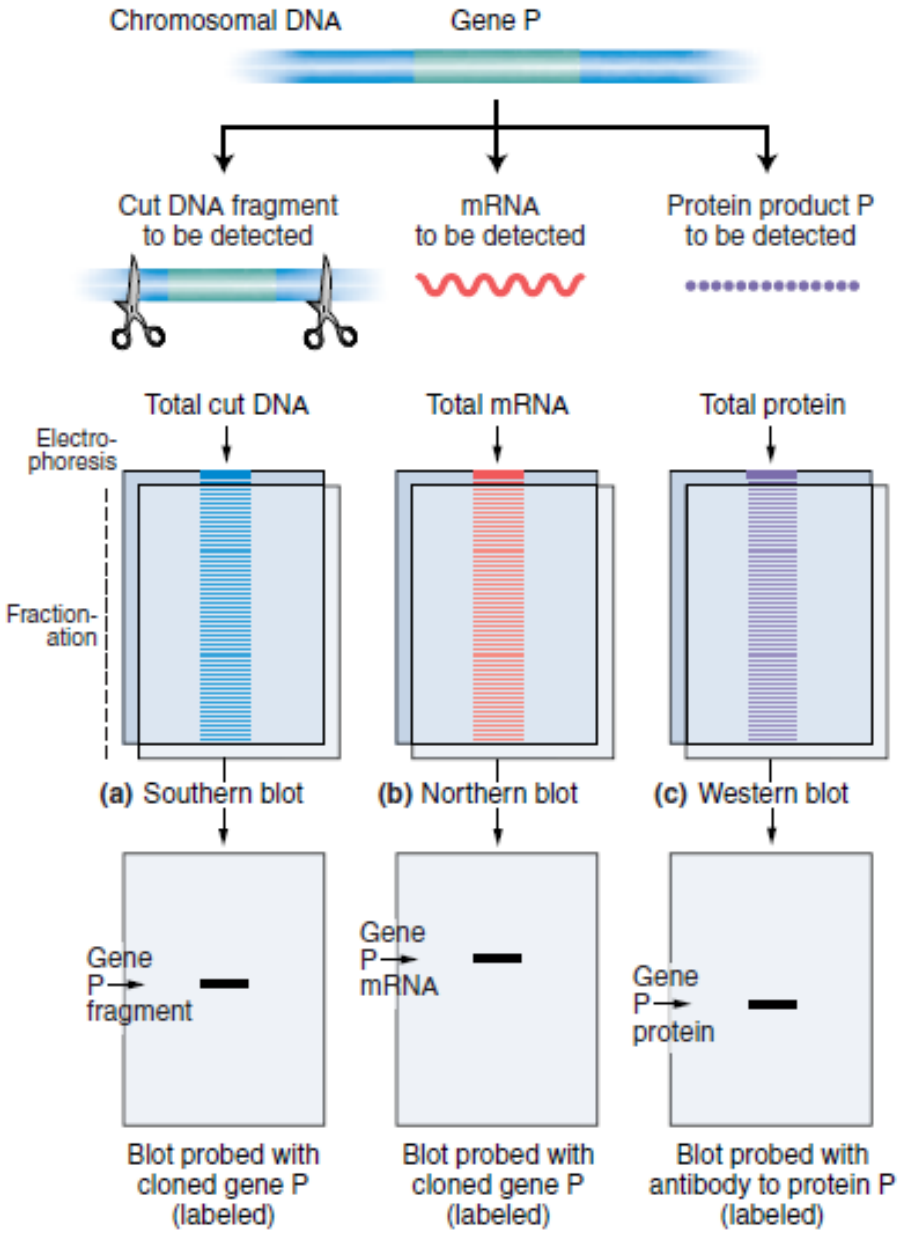

Figure 1-14 Probing DNA, RNA, and protein mixtures.