

1/28/03

HC 70 A
Winter 2003
Professor Bob Goldberg

Learning Unit #3
Revised

HOW ARE GENES CLONED &
Engineered: The Nuts & Bolts

FACTOR VIII Story - A Case Study

Themes/Concepts

12.5 hr lecture
1/28
⊕
1 hr lecture
1/30

- ① Demonstration - Gel Electrophoresis
- ② Why Clone DNA - a Review
- ③ Tools to Clone DNA - a Review
- ④ Genome vs. cDNA Libraries
- ⑤ Using Probes to screen for specific clones
- ⑥ What is Factor VIII - Finding Genomic & cDNA clones
- ⑦ What are the properties & uses of Restriction Enzymes?
- ⑧ What are restriction maps used for?
- ⑨ ^{STOP} What are the properties of vectors used to make libraries?
- ⑩ What are the different vectors?
- ⑪ Creating a Genome library in a virus
- ⑫ Finding a large Factor VIII Gene - Walking the chromosome
- ⑬ Using PCR to amplify specific genes/mRNAs/DNA sequences

Why Clone Genes/mRNAs?

A Review

- ① Isolate specific genes / mRNAs from genome & population of mRNAs.
- ② Amplify specific gene / mRNA copies to obtain quantities for study.
- ③ Study Activity of gene / What it does & what function does it play in cell?
- ④ Study Structure of Gene / Sequence of Gene / mRNA - Introns? Exons? Switches?
- ⑤ Determine what protein encoded by gene / mRNA
- ⑥ Use gene / mRNA to make drugs in bacteria, animals, + / or plants.
- ⑦ Use gene / mRNA as probe to study genetic diseases / gene diversity / map genes
- ⑧ Use gene / mRNA as probe to identify & trace human diseases / pedigrees & DNA fingerprints
- ⑨ Use gene / mRNA probe for forensics & DNA identification
- ⑩ Use specific genes / mRNAs / switches to engineer organisms genetically

What are the tools required to Clone Genes & mRNAs?

A Review

- ① **DNA or mRNA** FROM ORGANISM / CELL TYPE (for mRNA) YOU WANT TO CLONE --- need to Isolate
- ② **Host Cells** For Vector Replication --- *E. coli* (prokaryote) or Yeast (eukaryote)
- ③ **Vectors** to Replicate DNA / Express Gene Coding Sequence --- PLASMID, VIRUS, COSMID, BAC, YAC
- ④ **Enzymes** to cut + join (engineer) DNA Sequences & Synthesize cDNA copies of mRNAs --- Restriction Enzymes, Ligase, DNA Polymerase, Terminal Transferase, Reverse Transcriptase →
Enzymes that naturally function in cells
- ⑤ **Probes** to identify Specific genes/sequence of DNA (e.g., switch) / mRNAs --- radioactive DNA/RNA probes, antibody probes

What are the Differences Between Genomic and cDNA Libraries?

A Library is a collection of individual clones

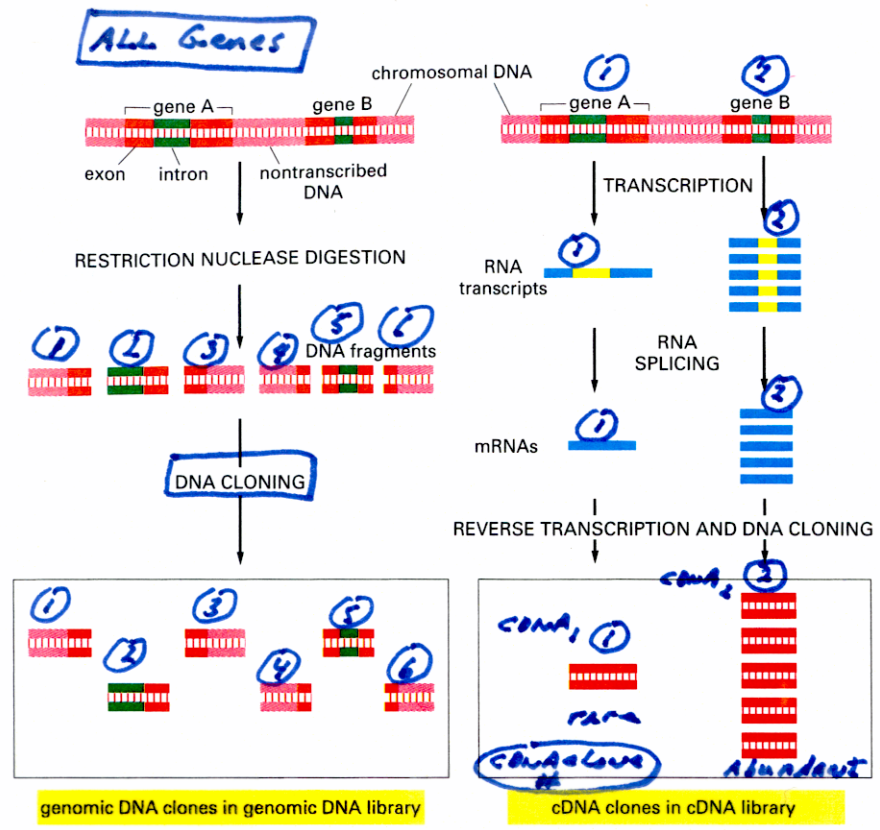


Figure 8-35 The differences between cDNA clones and genomic DNA clones derived from the same region of DNA. In this example gene A is infrequently transcribed, whereas gene B is frequently transcribed, and both genes contain introns (green). In the genomic DNA library, both the introns and the nontranscribed DNA (pink) are included in the clones, and most clones contain, at most, only part of the coding sequence of a gene (red). In the cDNA clones the intron sequences (yellow) have been removed by RNA splicing during the formation of the mRNA (blue), and a continuous coding sequence is therefore present in each clone. Because gene B is transcribed more abundantly than in gene A in the cells from which the cDNA library was made, it is represented much more frequently than A in the cDNA library. In contrast, A and B are in principle represented equally in the genomic DNA library.

- ① ALL Genes in Library
- ② EACH Gene/DNA represented equally in Genome Library

1 DNA_x → 1 DNA_x in library
 1 DNA_y → 1 DNA_y in library

Genomic Clones

- ① ALL Sequences in Genome → genes/switches
- ② Complete Gene - Exons + Introns to understand Gene Structure + Evolution + Mutation/Diseases
- ③ Needed For Genome Sequencing Projects

- ① ONLY mRNAs present in specific cells/organs in Library
- ② Subset of genes in genome
- ③ cDNA clones not present equally - present in proportion to amount of mRNA sequence in cell

1 mRNA_x → 1 cDNA_x
 100 mRNA_y → 100 cDNA_y

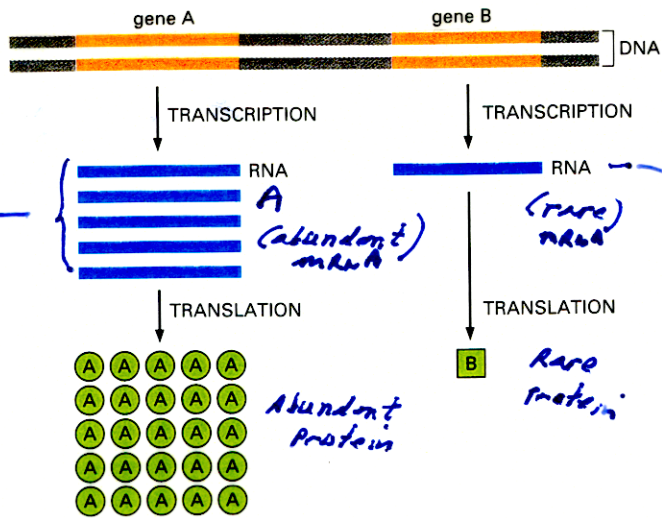
Easier to find Abundant cDNA in Library!

cDNA Clones

- ① ONLY Coding sequences: useful to identify proteins
- ② Subset of gene sequences - what genes active in specific cell - time of development - tumor? Profile of Active Genes
- ③ useful to identify specific "genes"
- ④ For DRUGS/BACTERIA expression

cDNA Clones Represent Gene Coding Sequences & Are Found in cDNA Libraries in Proportion to level Gene is Active in cell

Gene Active in Organ X



Gene active at different levels!!

5 cDNA clones for mRNA A

1 cDNA clone for mRNA B

∴ Find cDNA clones for Abundant mRNAs more frequently than Rare mRNA

What's consequence for screening specific cDNA clones from cDNA library?

How Identify specific Clones in Genomic and/or cDNA Libraries?

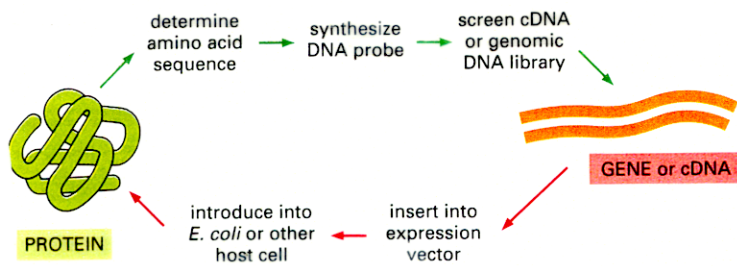


Figure 10-28 Knowledge of the molecular biology of cells makes it possible to experimentally move from gene to protein and from protein to gene. A small quantity of a purified protein is used to obtain a partial amino acid sequence. This provides sequence information that enables the corresponding gene to be cloned from a DNA library (see Figure 10-18). Once the gene has been cloned, its protein-coding sequence can be used to design a DNA that can then be used to produce large quantities of the protein from genetically engineered cells (see Figure 10-27).

FROM PROTEIN TO GENE

Isolate protein on the basis of its molecular function (e.g., enzymatic or hormonal activity)

Determine partial amino acid sequence of the protein

Synthesize oligonucleotides that correspond to portions of the amino acid sequence

Use oligonucleotides as probes to select cDNA or genomic clone encoding the protein from library

Sequence isolated gene

FROM GENE TO PROTEIN

Isolate genomic clone corresponding to an altered trait in mutants (e.g., nutritional auxotrophy, inherited disease, developmental defect)

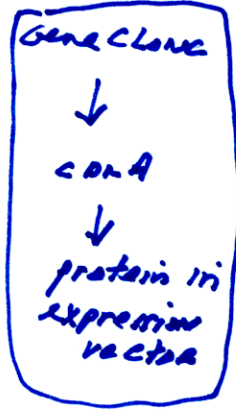
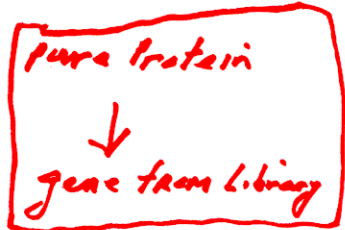
Use genomic DNA to isolate a cDNA for the mRNA encoded by the gene

Sequence the cDNA to deduce amino acid sequence of the encoded protein

Compare deduced amino acid sequence with that of known proteins to gain insight into function of the protein

Use expression vector to produce the encoded protein

→ STUDY



Screening Library

Genome

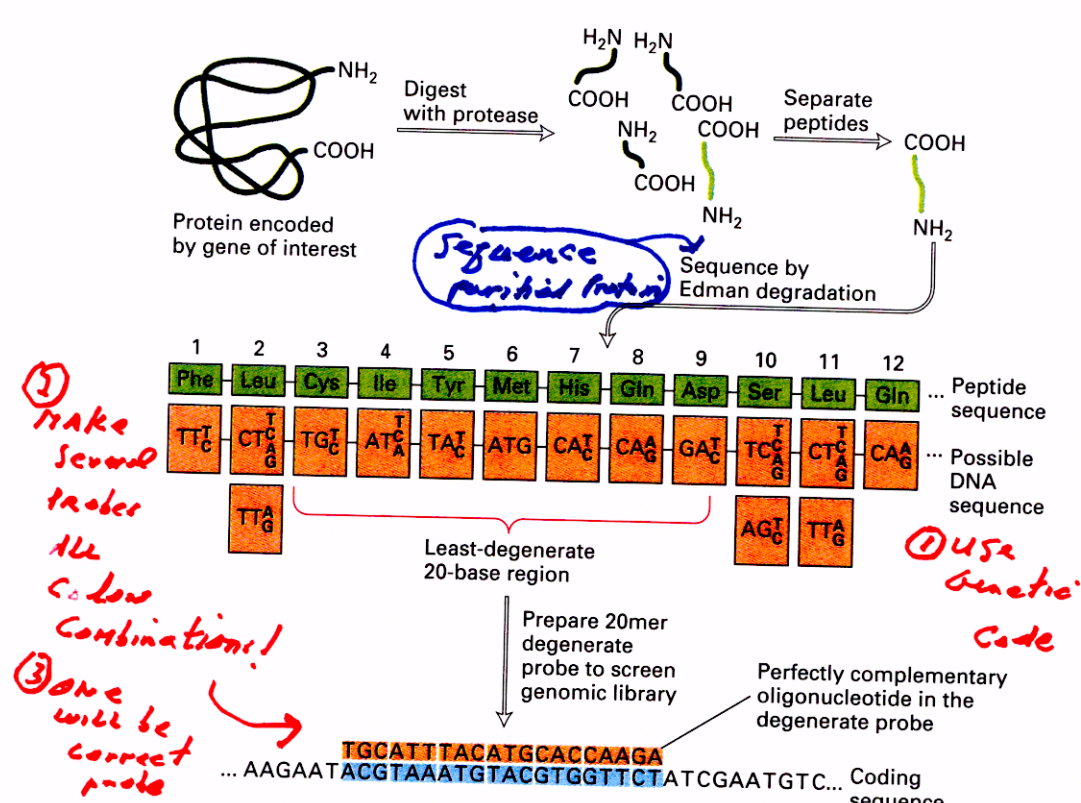
- ① Sequence → Database
- ② Probe from cDNA/Switch
- ③ Probe from pure mRNA
- ④ Synthetic Probe from Translated DNA Sequence & Genetic Code

cDNA

- ① Sequence → Database
- ② pure mRNA probe
- ③ Synthetic probe from translated protein sequence/genetic code
- ④ exon probe
- ⑤ Antibody probe using expression vector

A GENE PROBE CAN BE SYNTHESIZED FROM KNOWLEDGE OF PROTEIN SEQUENCE

Synthetic Genes!

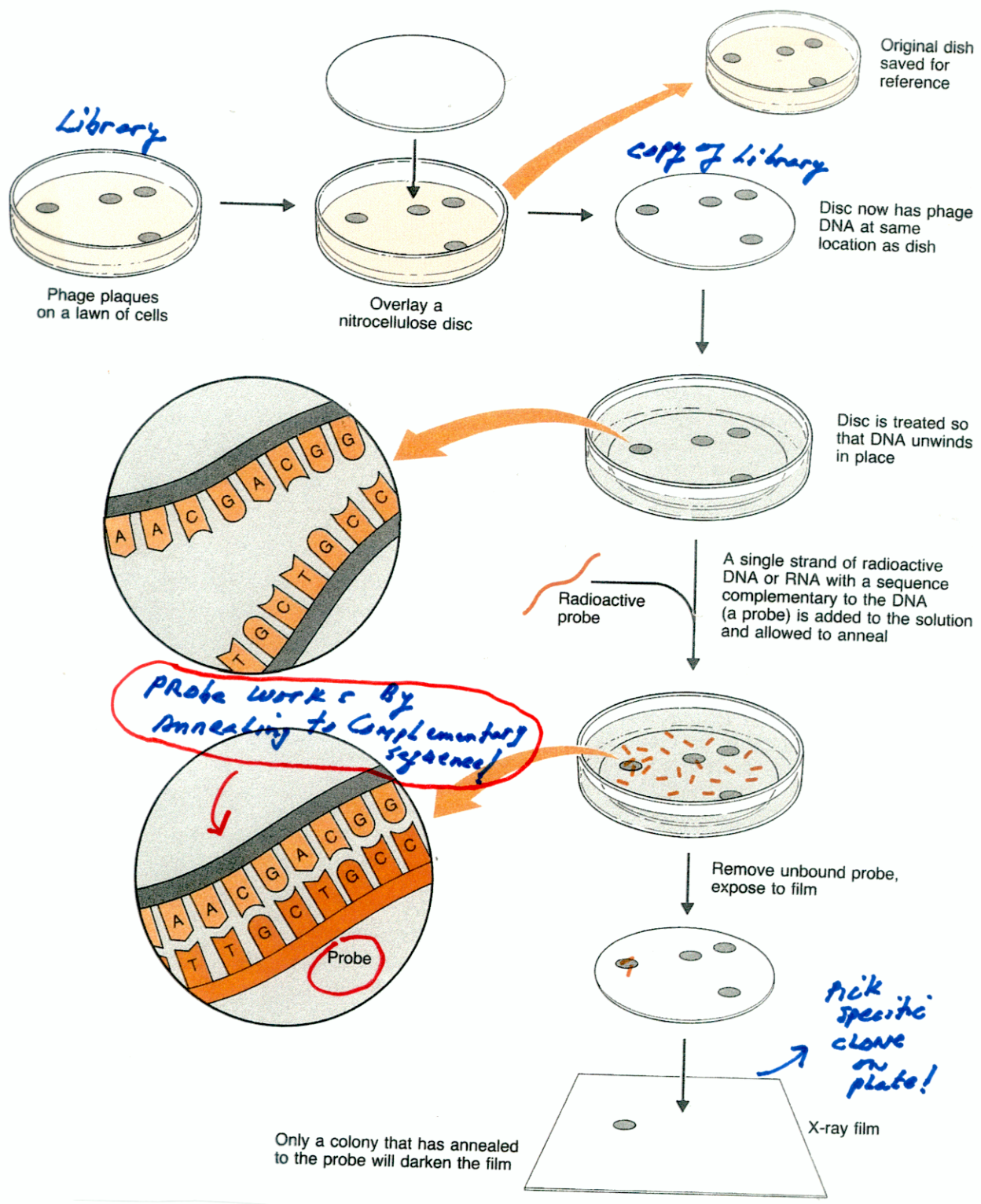


▲ FIGURE 7-19 Designing oligonucleotide probes based on protein sequence. An isolated protein is digested with a selective protease such as trypsin, which specifically cleaves peptide bonds on the carboxy-terminal side of lysine and arginine residues. The resulting peptides are separated, and several are partially sequenced from their N-terminus by sequential Edman degradation. The determined sequences then are analyzed to identify the 6- or 7-aa region that can be encoded by the smallest number of possible DNA sequences. Because of the degeneracy of the genetic code, the 12-aa sequence (light green) shown here theoretically could be encoded by any of the DNA triplets below it, with the possible alternative bases at the same

position indicated. For example, Phe-1 is encoded by TTT or TTC; Leu-2 is encoded by one of six possible triplets (CTT, CTC, CTA, CTG, TTA, or TTG). The region with the least degeneracy for a sequence of 20 bases (20-mer) is indicated by the red bracket. There are 48 possible DNA sequences in this 20-base region that could encode the peptide sequence 3-9. Since the actual sequence of the gene is unknown, a degenerate 20-mer probe consisting of a mixture of all the possible 20-base oligonucleotides is prepared. If a cDNA or genomic library is screened with this degenerate probe, the one oligonucleotide that is perfectly complementary to the actual coding sequence (blue) will hybridize to it.

Using a "Probe" to Identify Specific cDNA or Genomic Clones

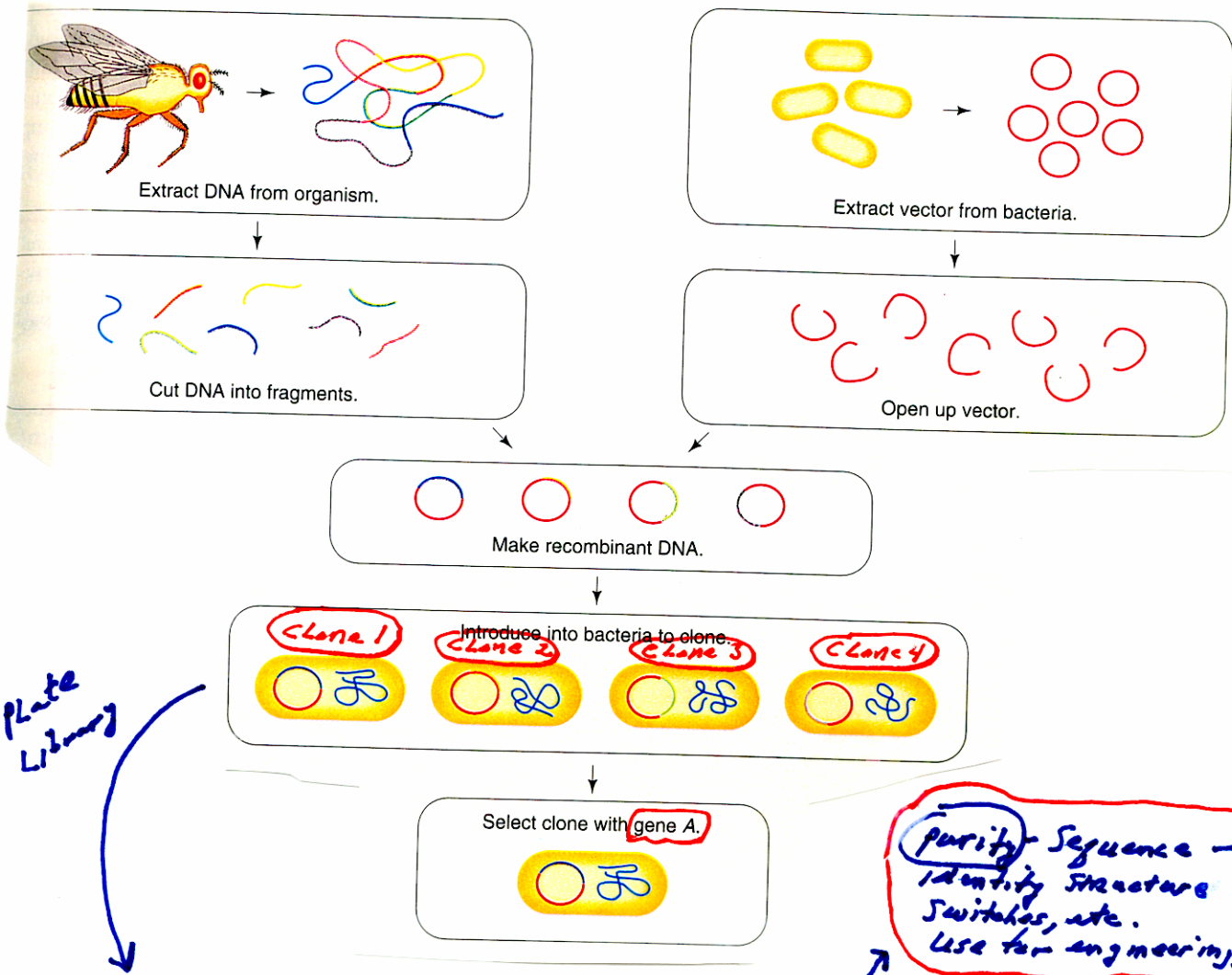
Probe is a specific sequence of DNA or mRNA - Generally Radioactive & used by annealing to detect specific clones!!



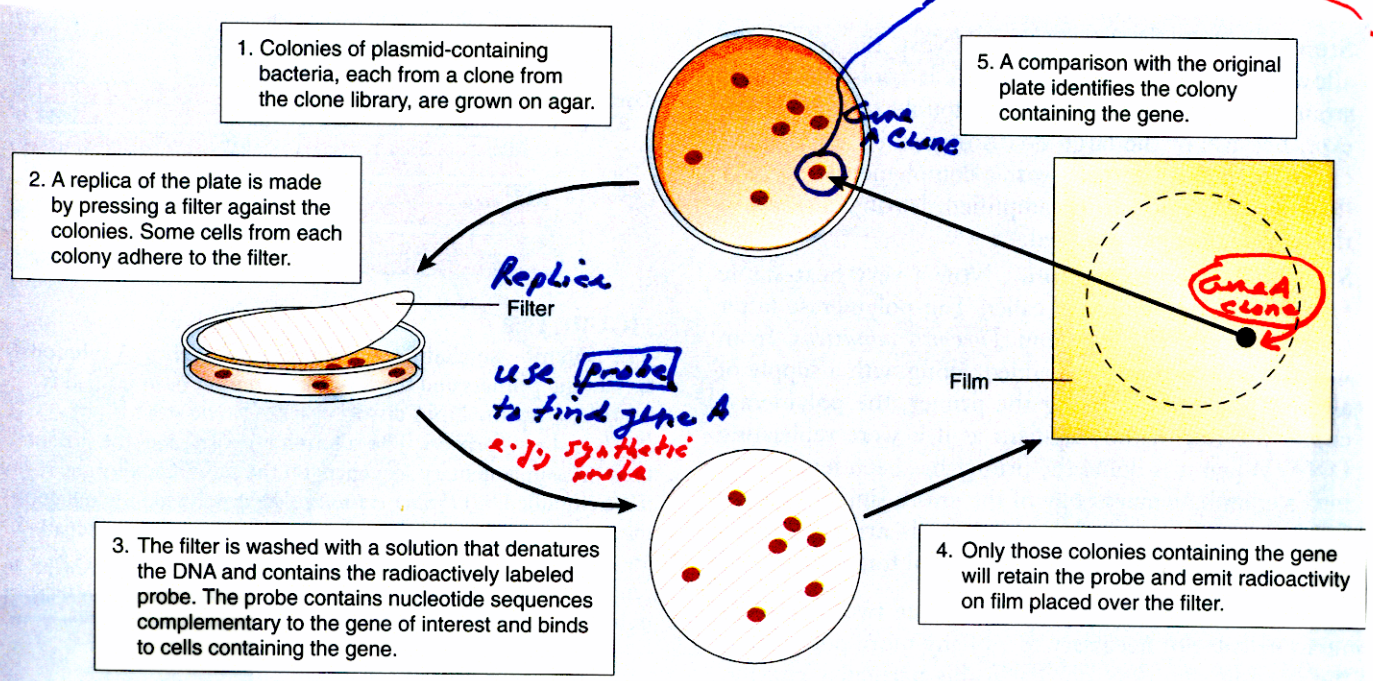
Probe works by annealing to complementary sequence!

Probes are generally specific cloned sequences & used for fingerprinting as well!

SELECTING A SPECIFIC CLONE FROM A GENOME LIBRARY



Purify Sequence - Identify Structure Switches, etc. Use for engineering, DNA fingerprints, to get acid, etc.



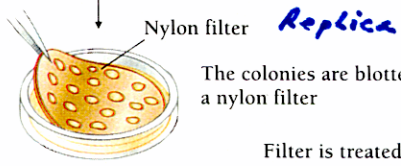
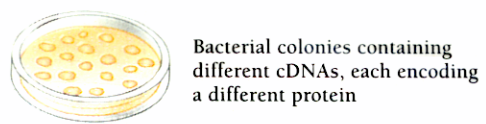
SELECTING A SPECIFIC cDNA CLONE FROM A cDNA LIBRARY

Using Nucleic Acid &/or Antibody Probes

Nucleic Acid Probes

- ① purified mRNA probe
 - ② protein sequence → probe
 - ③ gene probe
- any vector

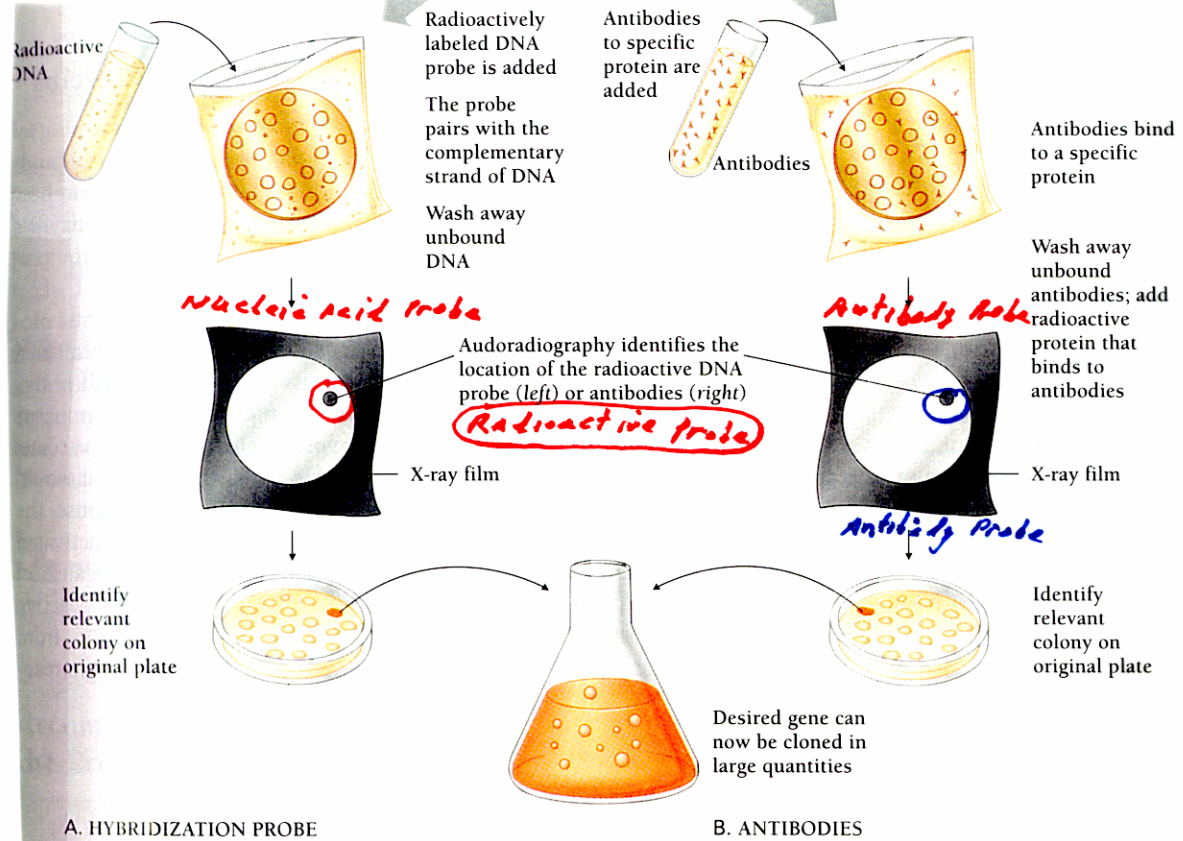
Plate cDNA Library



- Need Purified Protein
- ① Antibody Probe
- ② Need Expression Vector

Filter is treated to remove proteins, leaving DNA attached to filter

Filter is treated to keep proteins attached to filter

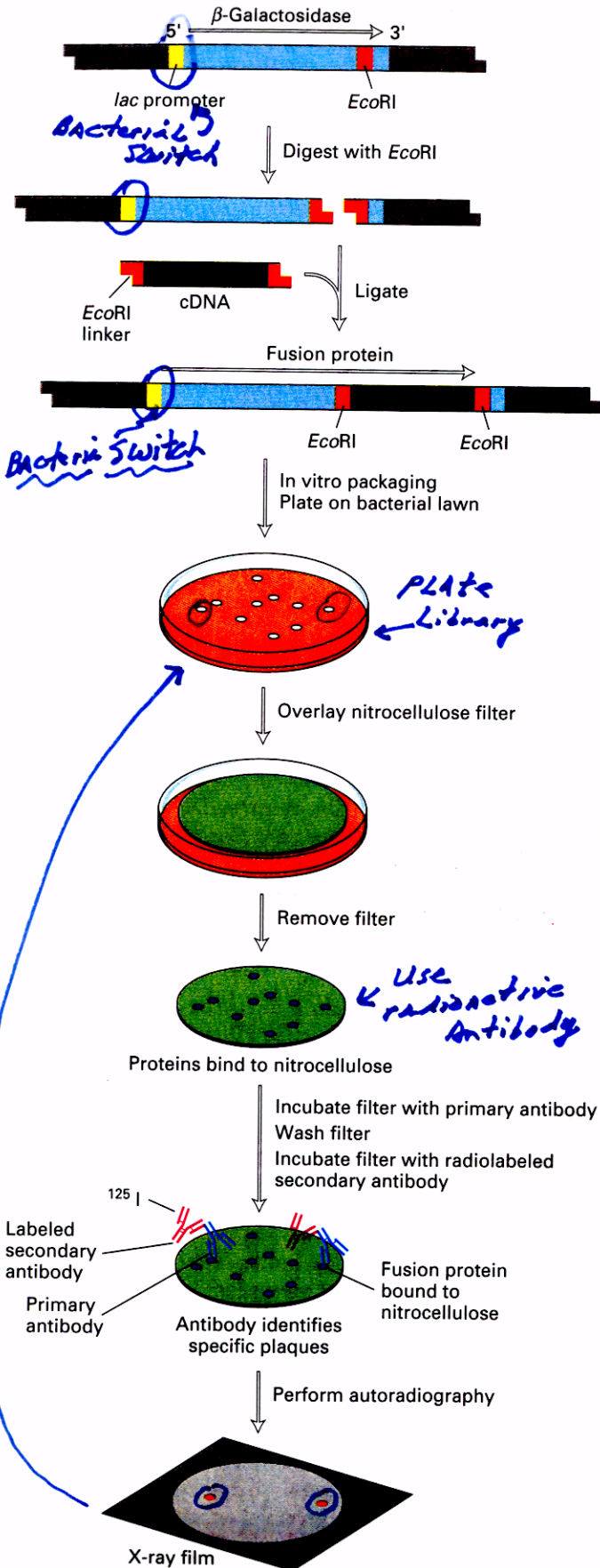


A. HYBRIDIZATION PROBE

B. ANTIBODIES

Figure 13-8 Two techniques for locating a gene. A. A hybridization probe locates a specific DNA sequence. B. Antibodies locate the protein product of the same sequence.

USING ANTIBODIES TO SELECT A SPECIFIC cDNA CLONE REQUIRES AN EXPRESSION VECTOR



Need to Purify Protein \rightarrow Induce Antibodies to Protein in Rabbit

◀ **FIGURE 7-21 Use of λ expression cloning to identify a cloned DNA based on binding of the encoded protein to a specific antibody.** The *lgt11* vector was engineered to express the *E. coli* protein β -galactosidase at high levels. The only *EcoRI* recognition site (red) in this vector lies near the 3' end of the β -galactosidase gene. If a cDNA (green), or protein-coding fragment of genomic DNA, is inserted into this *EcoRI* site in the correct orientation and proper reading frame, it will be expressed as a fusion protein in which most of the β -galactosidase sequence is at the N-terminal end and the protein sequence encoded by the inserted DNA is at the C-terminal end. Plaques resulting from infection with recombinant *lgt11* contain high concentrations of such fusion proteins. These proteins can be transferred and bound to a replica filter, which then is incubated with a monoclonal primary antibody (blue) that recognizes the protein of interest. Rinsing the filter washes away antibody molecules that are not bound to the specific fusion protein attached to the filter. Bound antibody usually is detected by incubating the filter with a second radiolabeled antibody (dark red) that binds to the primary antibody. Any signals that appear on the autoradiogram are used to locate plaques on the master plate containing the gene of interest. [Adapted from J. D. Watson et al., 1992, *Recombinant DNA*, 2d ed., Scientific American Books.]

Isolating the Human Factor VIII Gene & cDNA

A Case Study of Gene/mana Cloning

Table 13.2 Some Important Genetic Disorders

Disorder	Symptom	Defect	Dominant/Recessive	Frequency among Human Births
Cystic fibrosis	Mucus clogs lungs, liver, and pancreas	Failure of chloride ion transport mechanism	Recessive	1/2500 (Caucasians)
Sickle cell anemia	Poor blood circulation	Abnormal hemoglobin molecules	Recessive	1/625 (African Americans)
Tay-Sachs disease	Deterioration of central nervous system in infancy	Defective enzyme (hexosaminidase A)	Recessive	1/3500 (Ashkenazi Jews)
Phenylketonuria	Brain fails to develop in infancy	Defective enzyme (phenylalanine hydroxylase)	Recessive	1/12,000
Hemophilia	Blood fails to clot	Defective blood clotting factor VIII	Sex-linked recessive	1/10,000 (Caucasian males)
Huntington's disease	Brain tissue gradually deteriorates in middle age	Production of an inhibitor of brain cell metabolism	Dominant	1/24,000
Muscular dystrophy (Duchenne)	Muscles waste away	Degradation of myelin coating of nerves stimulating muscles	Sex-linked recessive	1/3700 (males)
Hypercholesterolemia	Excessive cholesterol levels in blood, leading to heart disease	Abnormal form of cholesterol cell surface receptor	Dominant	1/500

Royal Hemophilia Gene

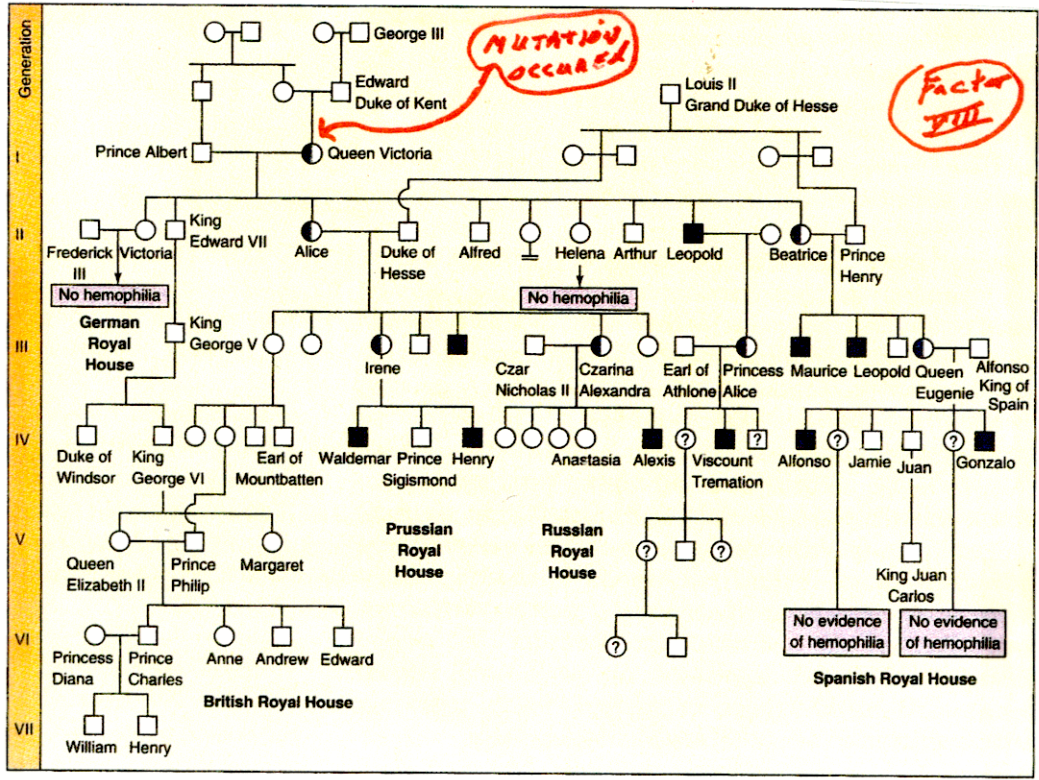


FIGURE 13.26 The Royal hemophilia pedigree. Queen Victoria's daughter Alice introduced hemophilia into the Russian and Austrian royal houses, and Victoria's daughter Beatrice introduced it into the Spanish royal house. Victoria's son Leopold, himself a victim, also transmitted the disorder in a third line of descent. Half-shaded symbols represent carriers with one normal allele and one defective allele; fully shaded symbols represent affected individuals.

FROM DISEASE TO GENE -
USING Purified Protein to
Identify Gene Involved in
Blood Clotting

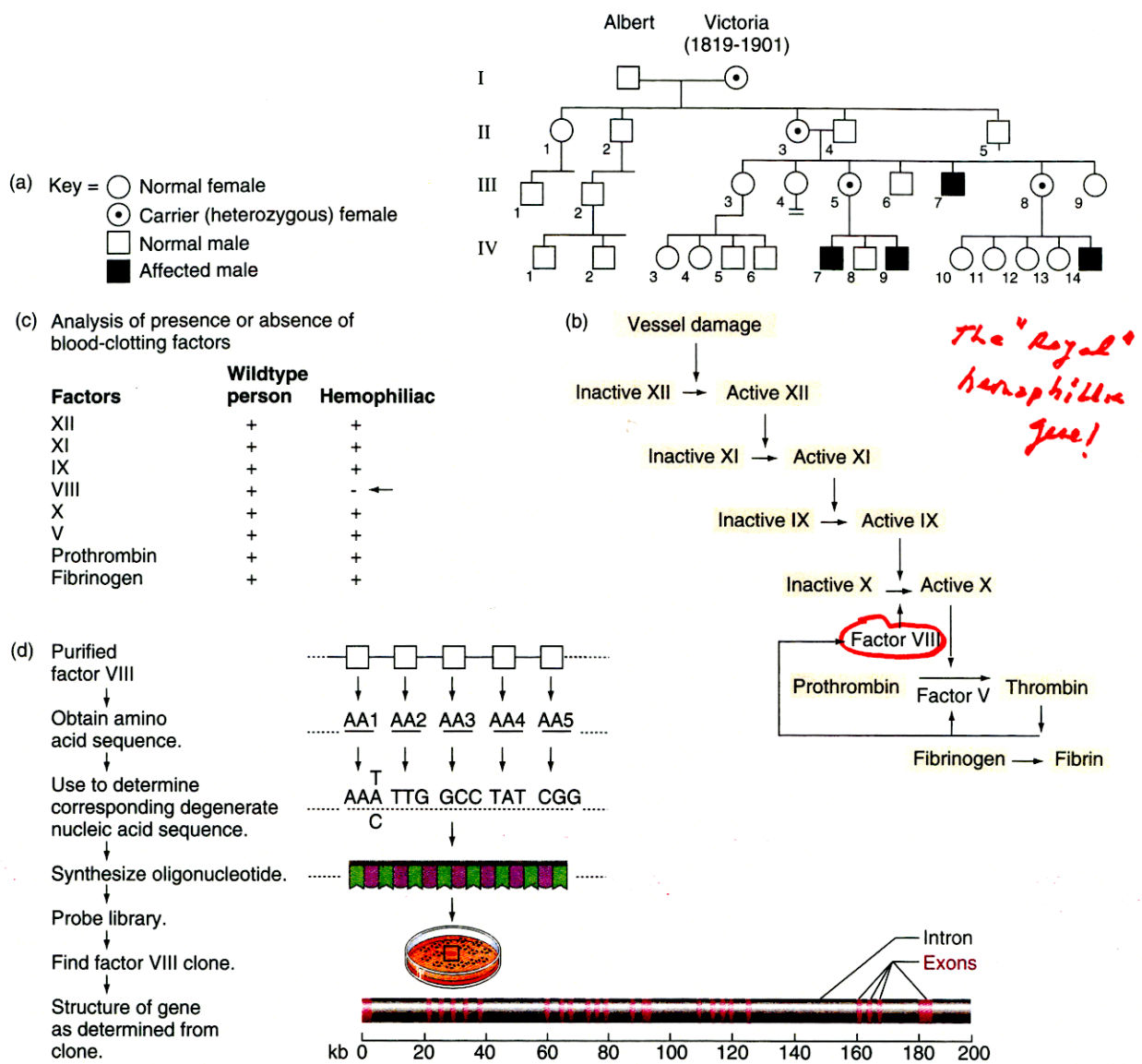
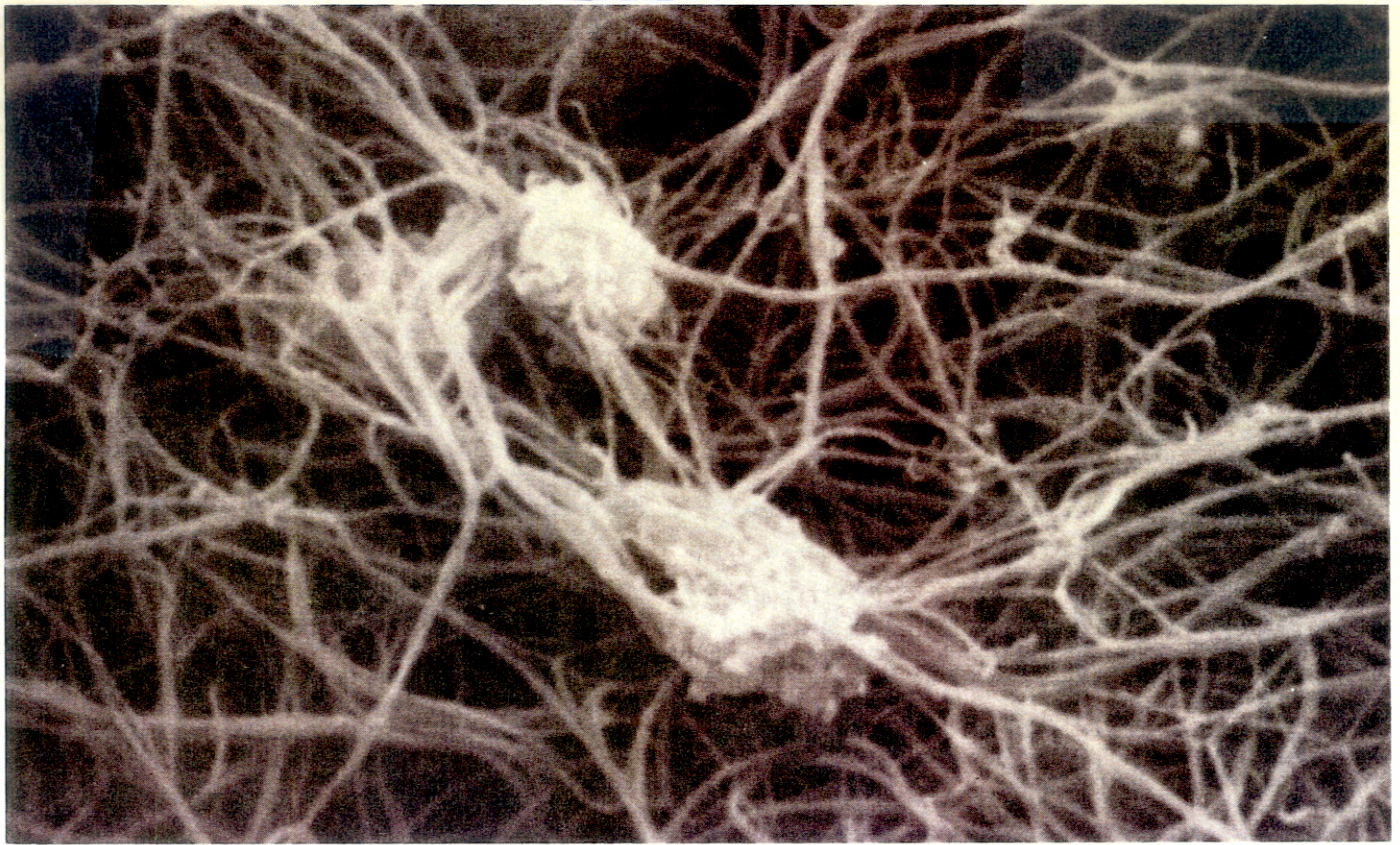


Figure 10.1 How geneticists identified the hemophilia A gene. (a) A pedigree of the royal family descended from Queen Victoria. This family tree uses the standard pedigree symbols. Black boxes represent males with hemophilia. (b) The blood clotting cascade. Vessel damage induces a cascade of enzymatic events that convert inactive factors to active factors. The cascade results in the transformation of fibrinogen to fibrin and the formation of a clot. (c) Many hemophiliac patients do not have an active form of Factor VIII. Blood tests can determine the presence or absence of the active form of each factor involved in the clotting cascade. The results of such analyses show that hemophiliacs, such as those found in Queen Victoria's pedigree, lack an active Factor VIII in their blood. (d) Starting with purified Factor VIII, scientists isolated DNA clones containing the *Factor VIII* gene. Researchers determined the amino-acid sequence of purified protein. Knowledge of this sequence enabled them to synthesize a degenerate oligonucleotide. They then used the oligonucleotide as a probe to screen a genomic library for clones containing all or parts of the gene. Finally, they sequenced the positive clones (that is, the clones with which the probe hybridizes) to determine the structure and coding sequence of the *Factor VIII* gene.

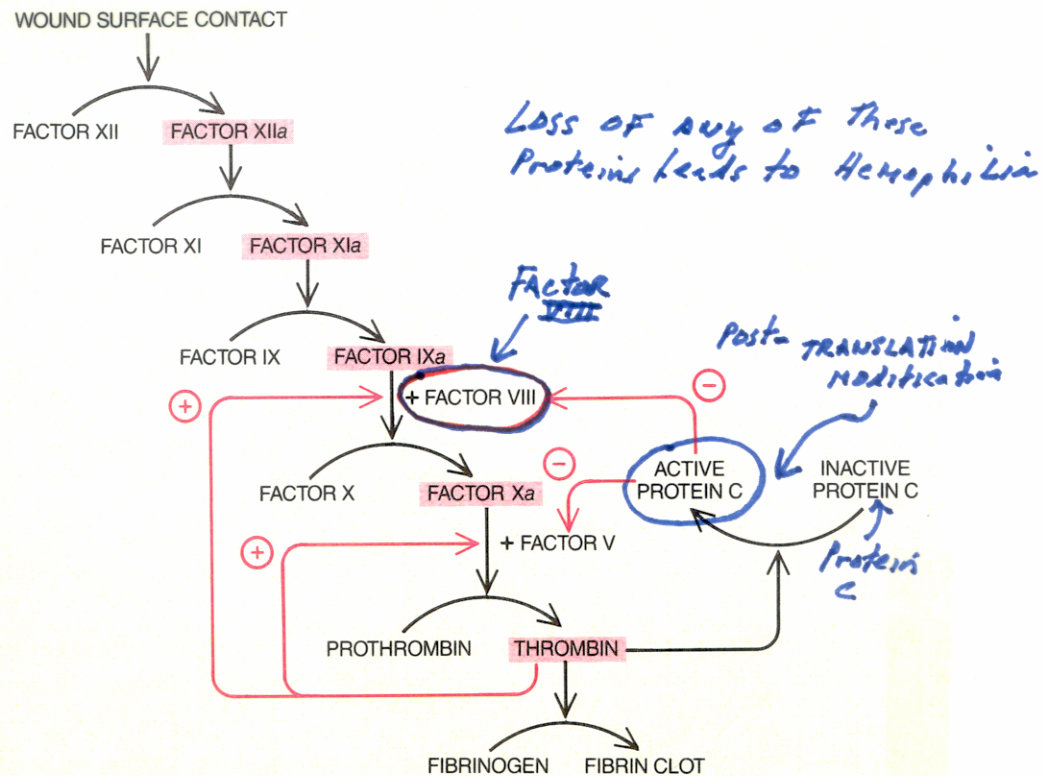
Protein → Gene

FORMATION OF A BLOOD CLOT



FIBRIN STRANDS stabilize a blood clot at the site of a wound by trapping the platelets that form the bulk of the clot. The electron micrograph, which was made by Jon C. Lewis of Wake Forest University, shows a clot formed in a suspension of platelets and fibrin.

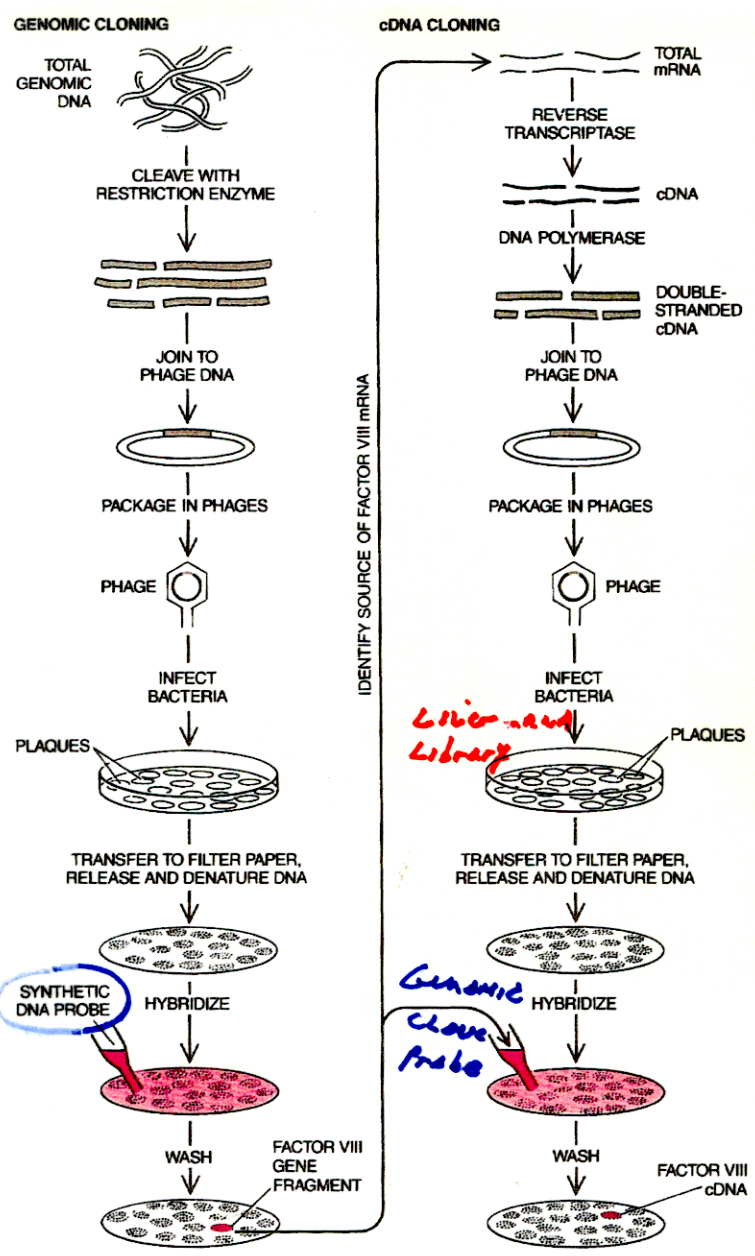
A clot in the bloodstream is the result of a complex cascade of enzymatic reactions culminating in the conversion of fibrinogen, a soluble protein, into insoluble fibrin strands. In hemophiliacs a crucial protein in the blood-clotting cascade is either missing or defective.



CLOTTING CASCADE begins when cell damage at a wound somehow activates the enzyme factor XII; it ends with the conversion of fibrinogen into fibrin by thrombin. At each step an inactive protein is converted into a protease, or protein-cutting enzyme (color), which activates the next protein. Some steps require cofactors such as factors VIII and V. The cascade includes positive- and negative-feedback loops (colored arrows). Thrombin activates factors VIII and V; it also deactivates them (by activating protein C), which helps to halt clotting. Some 85 percent of hemophiliacs lack factor VIII. The rest lack factor IX.

HOW CLONE FACTOR VIII GENE WHEN IT WAS NOT KNOWN WHERE GENE EXPRESSED?

- ① Make genome library because factor VIII gene is in genome!
 - ② Purify protein from blood - that's where it works (wasn't know where made)
 - ③ Reverse translate using the genetic code a portion of the protein sequence
 - ④ Synthesize a DNA probe complementary to factor VIII gene corresponding to protein sequence
 - ⑤ Screen genome library
- Entire gene or one clone?



- ① Use Gene probe to screen cDNA library for factor VIII cDNA clone
- ② How know what mRNA to use to make cDNA library?
- ③ Use gene probe to probe RNA blots containing mRNAs from all major organs (liver, kidney, blood, etc) -
- ④ Find factor VIII mRNA in liver - Made in liver - secreted into blood

GENE CLONING involves finding a specific gene among thousands in a human cell. The standard method, if one knows which cells make the desired protein, is to screen a copy DNA (cDNA) library derived by reverse transcription from the messenger RNA (mRNA) of those cells (right). In looking for the factor VIII gene, however, the authors did not know where the protein is produced. Hence they screened the entire human genome (left). Chromosomal DNA fragments were joined to the DNA of the bacterial virus phage lambda. Each phage contained one human DNA fragment; each phage multiplied and formed a plaque in a distinct region of a bacterial culture. To identify the plaque containing the factor VIII gene, the phages were blotted onto filter paper and broken open to release their DNA. The DNA was exposed to a radioactive probe: a small piece of synthetic DNA encoding part of factor VIII. The probe hybridized with part of the factor VIII gene, thereby labeling it. To produce factor VIII in cultured cells, it was still necessary to make factor VIII cDNA, which lacks the introns (noncoding sequences) that complicate the full gene. Now fragments of the cloned gene could serve as reliable probes, first for identifying cells that make factor VIII mRNA and then for finding factor VIII cDNA in the cDNA library.

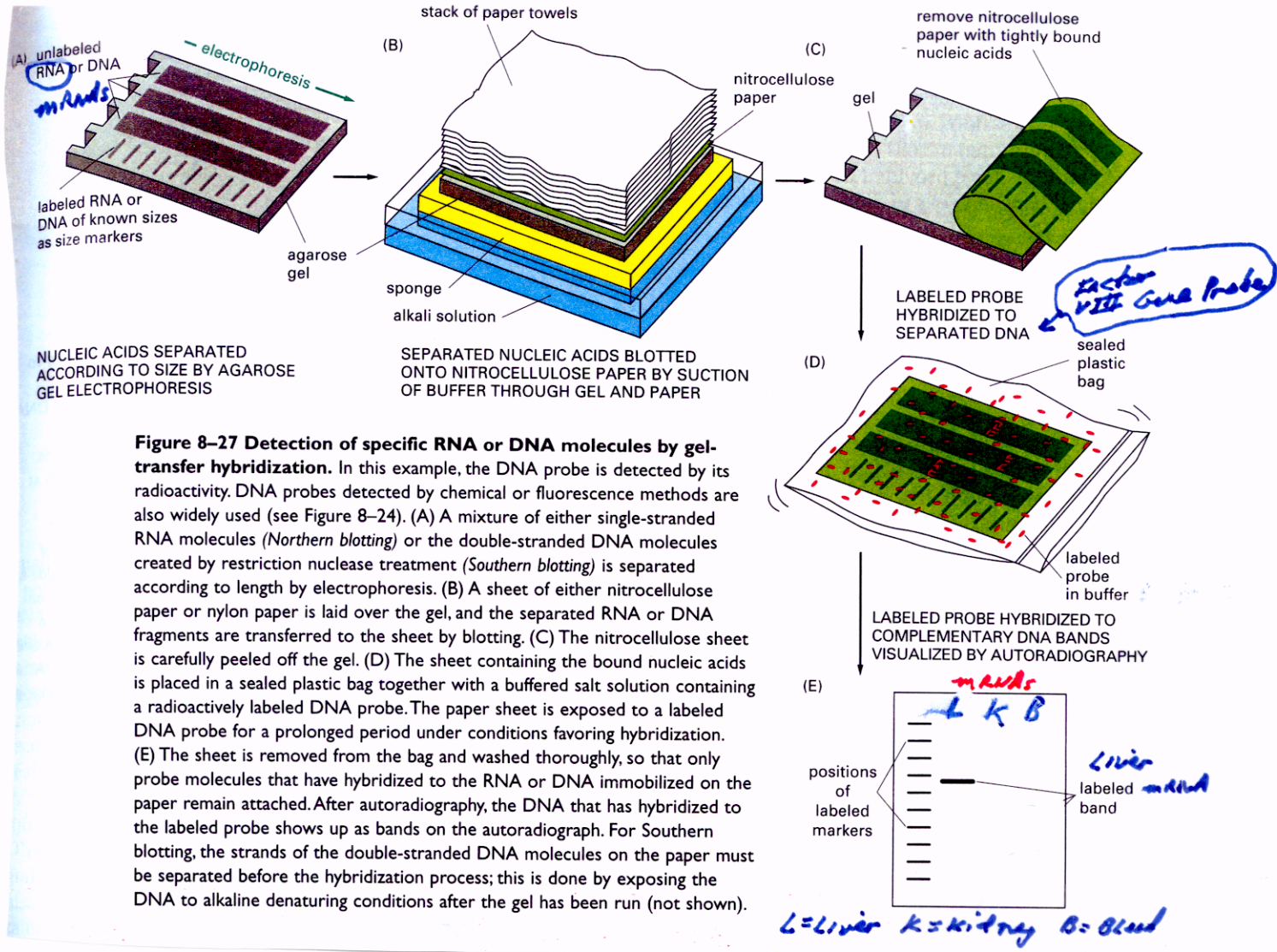
Why need cDNA?
Story continued!!

USING RNA Gel Blots and Factor VIII Gene Probe to Determine What Organ Factor VIII Gene is Expressed

RNA Gel Blots Follow Same Procedures AS DNA Gel Blots EXCEPT ① RNA used & ② RNAs are size-fractionated directly without using Restriction Enzymes that only digest DNA!

∴ Use Factor VIII Gene Probe to determine where gene is active & what located in body!

mRNAs - Liver, kidney, etc



only Liver mRNA hybridizes - Factor VIII Gene only in kidney + blood

USING RNA BLOTS TO DETERMINE Where Gene is Active

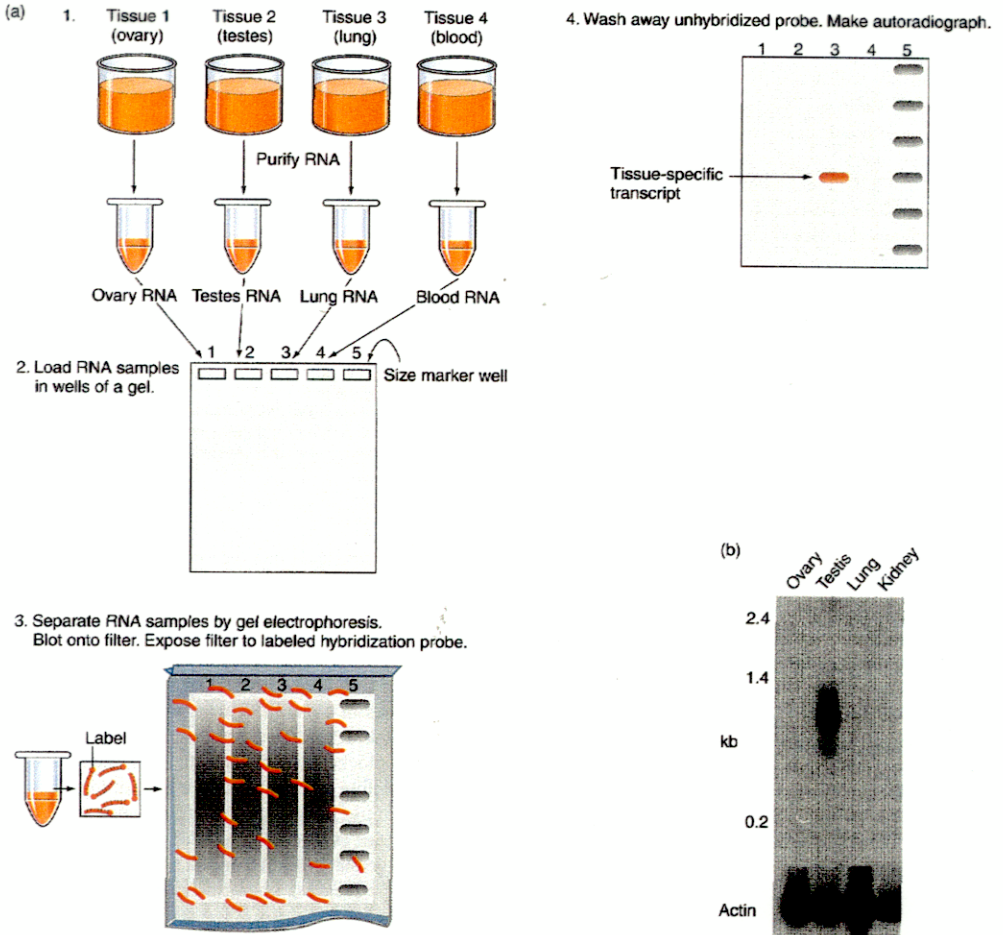


Figure 10.16 Northern blots: Snapshots of gene expression. (a) The protocol. (1) Purify RNA from each tissue to be examined for expression of the gene under investigation; here since you are looking at the *SRY* candidate for the testes-determining factor, the tissues to be examined are ovary, testes, lung, and blood. (2) Make an agarose gel and load each of the four RNA samples into a different well and load a fifth well with RNA size markers. Now subject the gel to an electric current that causes the RNA in each sample to migrate along a lane toward the bottom of the gel. The mobility of each RNA transcript in a sample depends on its size: smaller RNAs move faster, while larger RNAs migrate more slowly. When the smallest RNAs reach the bottom of the gel, turn off the current. Staining the RNAs in each lane would produce a smear reflecting the presence of so many RNAs of different sizes that they cannot be resolved from each other. (3) Blot the RNA within the gel and fix it to a filter so that each RNA molecule retains its position relative to all the other molecules. Expose the filter to labeled probe and allow the label to hybridize for several hours. (4) Wash away unhybridized probe. Place the filter on a film for autoradiography. Develop the film. You will see bands only in those lanes containing a tissue where the gene represented by the probe has been expressed. (b) Northern blot results obtained using the pY53.3 clone as a probe. This clone contains the *SRY* gene. The results show that *SRY* is expressed in the testes, but not the ovary, lung, or kidney. This result makes *SRY* a good candidate for the *TDF* locus. In a control experiment, researchers probed an identical blot with the same RNA samples using a clone containing the actin gene. As expected, a band of the same size appears in every lane. This control demonstrates the integrity of the RNA samples used in this study.

What Are the Properties of Restriction Enzymes Used in Genetic Engineering?

- ① present only in bacteria & have a defense function.
- ② Bind double-stranded DNA molecules only - Linear & Circular
- ③ Recognize a specific DNA sequence - 4, 6, or 8 bp
- ④ DNA Recognition Sequence a palindrome or sequence that is the same when "read" from either direction - i.e., strand of DNA.

$$\begin{array}{c}
 5' \downarrow \text{GAATTC} 3' \\
 3' \text{CTTAAG} 5' \\
 \uparrow \text{axis} \quad \text{where break bond}
 \end{array}$$

- ⑤ Some enzymes produce single-stranded complementary ("sticky") ends by digesting phosphodiester bonds within recognition sequence - bases that can anneal!

$$\begin{array}{ccc}
 5' \downarrow & & 3' \\
 -\text{GAATTC}- & \xrightarrow{\text{heat}} & \text{G} \quad \text{AATTC}- \\
 -\text{CTTAAG}- & & \text{CTTAA} \quad \text{G} \\
 3' \quad \uparrow 5' & & \text{stick ends} \quad \oplus
 \end{array}$$

Because recognition sites are present in all DNAs - DNA from different "sources" can be joined together 2 fragments

- ⑥ Restriction Enzymes recognize all double-stranded ends
- ⑦ # of restriction sites \propto to genome size

$$\text{Bacteria} \ll \text{HUMAN}$$

- ⑧ order of restriction sites reflects DNA sequence
 - ∴ unique DNA sequences have unique orders of restriction sites ∴ used for diagnostics - Markers!
 - ARE non-Random!!

There ARE MANY DIFFERENT RESTRICTION ENZYMES

TABLE 7-1 Selected Restriction Endonucleases and Their Restriction-Site Sequences

Source Microorganism	Enzyme*	Recognition Site (↓) [†]	Ends Produced
<i>Arthrobacter luteus</i>	AluI	AG↓CT	Blunt
<i>Bacillus amyloliquefaciens</i> H	BamHI	G↓GATCC	Sticky
<i>Escherichia coli</i>	EcoRI	G↓AATTC	Sticky
<i>Haemophilus gallinarum</i>	HgaI	GACGC+5↓	†
<i>Haemophilus influenzae</i>	HindIII	A↓AGCTT	Sticky
<i>Haemophilus parahaemolyticus</i>	HphI	GGTGA+8↓	†
<i>Nocardia otitiscaviarum</i>	NotI	GC↓GGCCGC	Sticky
<i>Staphylococcus aureus</i> 3A	Sau3AI	↓GATC	Sticky
<i>Serratia marcescens</i>	SmaI	CCC↓GGG	Blunt
<i>Thermus aquaticus</i>	TaqI	T↓CGA	Sticky

*Enzymes are named with abbreviations of the bacterial strains from which they are isolated; the roman numeral indicates the enzyme's priority of discovery in that strain (for example, AluI was the first restriction enzyme to be isolated from *Arthrobacter luteus*).
[†]Recognition sequences are written 5'→3' (only one strand is given), with the cleavage site indicated by an arrow. Enzymes producing blunt ends cut both strands at the indicated site; those producing sticky ends make staggered cuts, with cleavage occurring between the same nucleotides in each strand as shown in Figure 7-5a.
[‡]The cleavage sites for HphI and HgaI occur several nucleotides away from the recognition sequence. HgaI cuts five nucleotides 3' to the GACGC sequence on the top strand and ten nucleotides 5' to the complementary GTGCG sequence on the bottom strand. HphI cuts eight nucleotides 3' to the GGTGA sequence on the top strand and seven nucleotides 5' to the complementary CCACT sequence on the bottom strand.

SOURCE: R. J. Roberts, 1988, *Nucl. Acids Res.* 16(suppl):271.

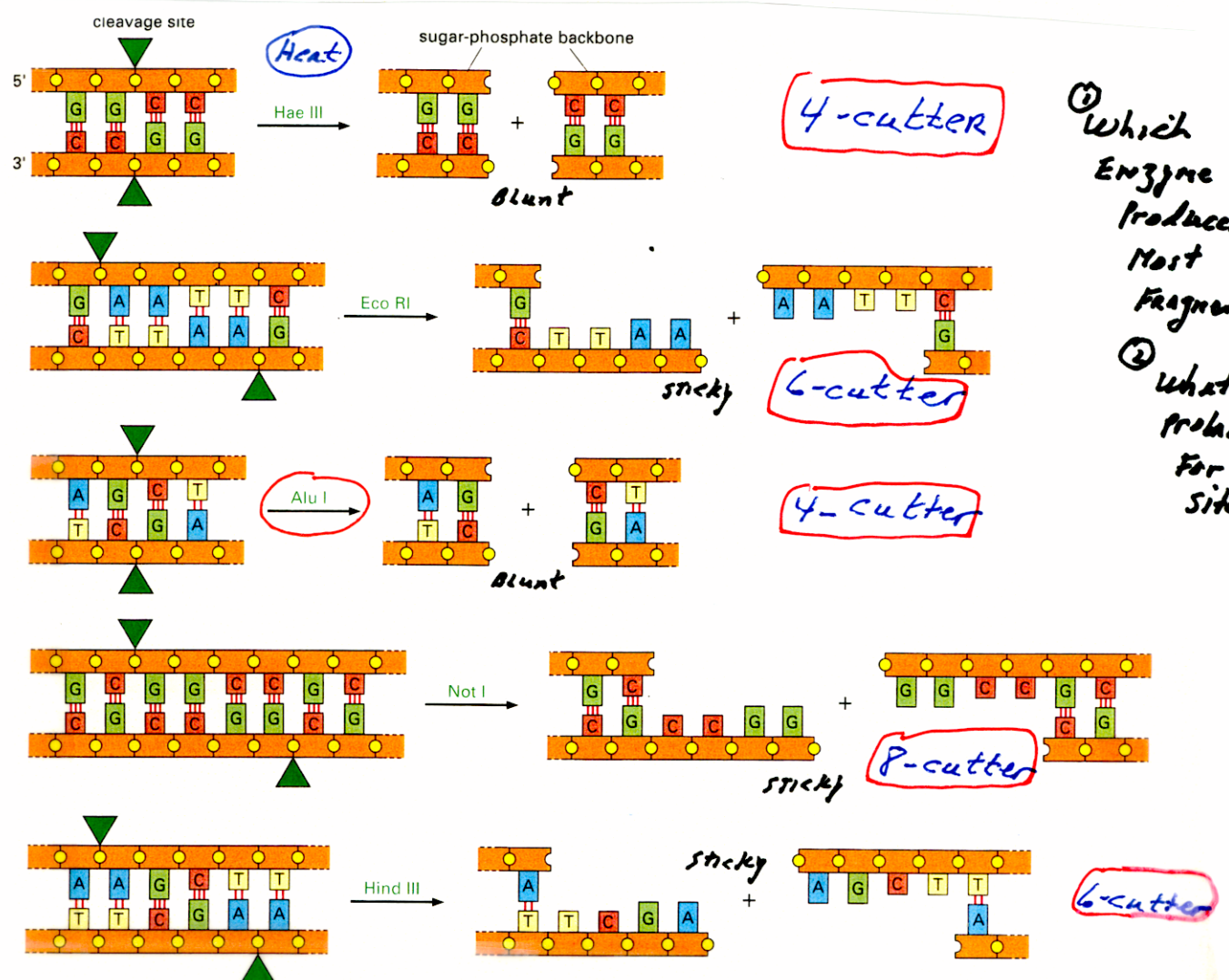


Figure 10-2 The nucleotide sequences recognized and cut by five widely used restriction nucleases. As shown, the target

HOW MANY DIFFERENT HUMAN DNA FRAGMENTS ARE GENERATED WHEN DIGESTED WITH DIFFERENT RESTRICTION ENZYMES?

Note: HUMAN Genome size is 3×10^9 b and it contains 40% G+C and 60% A+T bases.

Enzyme	Recognition Site	Number of Different DNA fragments
EcoRI	5' GAATTC 3' (6bp)	972,000 $\bar{x}_L = 3100$ bp (or) 1 site per 3100 bp
AruI	5' AGCT 3' (4bp)	10,800,000 $\bar{x}_L = 290$ bp (or) 1 site per 290 bp
NotI	5' GCCGCCGC 3' (8bp)	7,680 $\bar{x}_L = 390,000$ bp or 1 site per 390,000 bp

- How Make this calculation?
- How Many different Genomic clones would be generated with Each Enzyme?
- Which Enzyme(s) would you choose to make a Library? (19)

What ARE THE USES OF RESTRICTION ENZYMES?

- ① Cloning / Recombinant DNA - Creating recombinant DNA molecules
Moving parts of genes (switches, Introns, exons)
- ② Mapping Clones / Genes / Chromosomes

Maps provide guide posts - Mark positions in gene, plasmid, chromosome, genome, etc. Unique Sequence → Unique Map
Landmarks for DNA segments

③ DIAGNOSIS

- Specific Genes/Alleles e.g., Normal vs. Disease Gene (RFLP)
- Identity / Forensics e.g., Crime, Paternity, Lineage
- Presence of Pathogens e.g., Detect specific strain of bacteria

④ Ecology

- Species Identity
- Tracing Races to Geography
- Movement of Endangered Species

⑤ Anthropology

- Human Lineages
- Population Diversity
- Presence of Specific Pathogens

PROVIDES SPECIFIC FRAGMENT IDENTITY

CAN BE USED IN COMBINATION WITH PCR

RESTRICTION ENZYME SITES ARE
NON-RANDOM / SEQUENCE-BASED AND CAN
BE USED FOR MAPPING

① Map Genes, Chromosomes, Genome

② Maps of Genes CAN be used to:

- Study + Manipulate Gene Regions (e.g., Switch)
- cut out + clone specific Gene Regions
- Diagnosis / Identity Disease Genes / Specific Genes

③ Maps of Chromosomes CAN be used to:

- Mark-Map Gene Locations
- Identity Specific Chromosomes (e.g., Y chromosome)
- Identity Regions containing known Genes from other Studies - Markers for Genes

④ Maps of Genome CAN be used to:

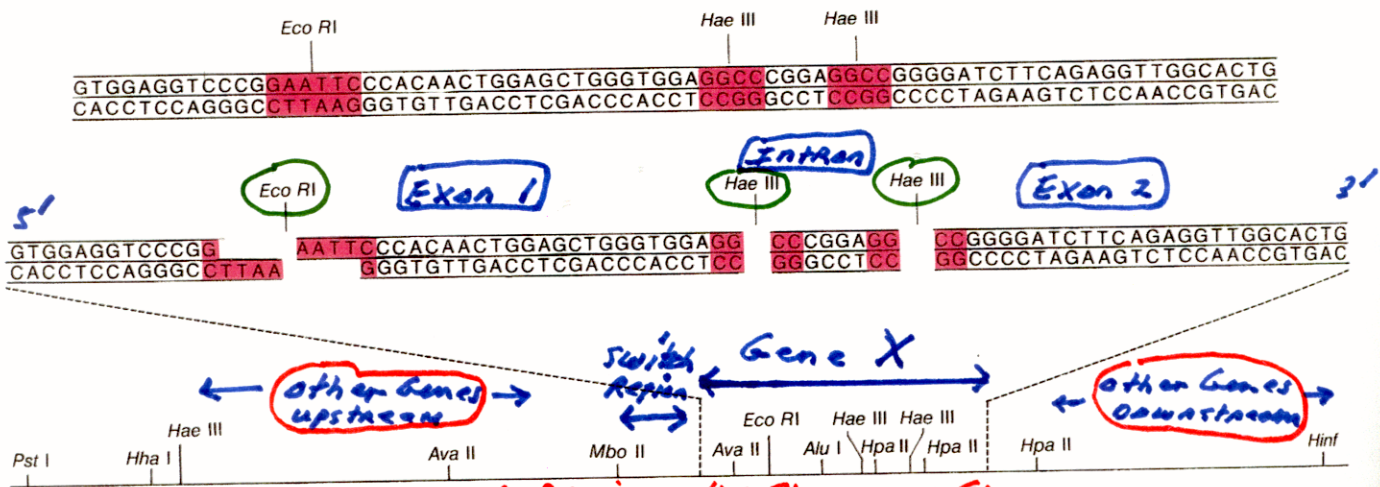
- Start Sequencing Entire Genome - know where Fragment being sequenced is!
- Create Recombinant Vectors using Vector Genome Map!

BASIS OF ALL Gene Manipulation
& Engineering - Need Maps to
know where you are!

Mapping Requires Cloned DNA Molecules - It is done
after recombinant DNA created or generated
from DNA sequence!

A RESTRICTION MAP OF A GENE PROVIDES Guideposts

MAP FOLLOWS SEQUENCE!



DNA CAN BE CUT into comparatively short lengths with the aid of restriction endonucleases, special enzymes that recognize specific base sequences at which they cause the molecule to come apart. For example, *Eco RI*, the first such enzyme discovered, recognizes a certain six-base sequence and cuts the molecule wherever this sequence appears, whereas *Hae III*, another restriction enzyme, operates at a certain four-base sequence. Since the probability of finding a partic-

ular four-base sequence is greater than that of finding a particular six-base sequence, one would expect *Hae III* to cut DNA more often than *Eco RI*. Accordingly one *Eco RI* site and two *Hae III* sites are represented in the DNA segment at the top, which corresponds to part of the gene coding for insulin in rat cells. The same DNA contains recognition sites for a number of other restriction enzymes, as is shown in the line diagram of a larger gene fragment at the bottom.

MAP ALLOWS Different Regions OF A Genomic CLONE +/- Gene to Be Identified & Manipulated

GEL ELECTROPHORESIS IS USED TO SEPARATE DNA FRAGMENTS BY SIZE

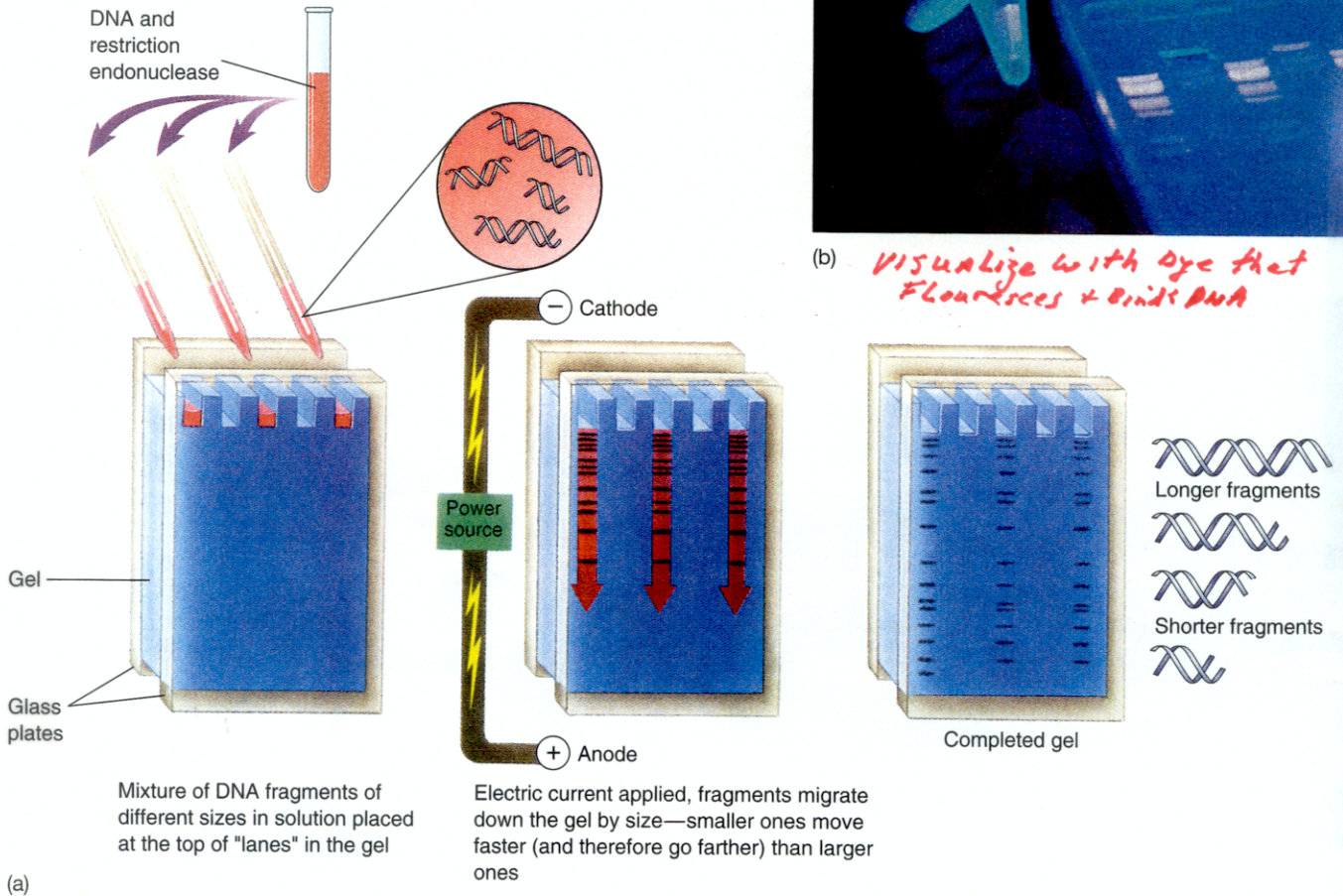


FIGURE 19.4

Gel electrophoresis. (a) After restriction endonucleases have cleaved the DNA, the fragments are loaded on a gel, and an electric current is applied. The DNA fragments migrate through the gel, with bigger ones moving more slowly. The fragments can be visualized easily, as the migrating bands fluoresce in UV light when stained with ethidium bromide. (b) In the photograph, one band of DNA has been excised from the gel for further analysis and can be seen glowing in the tube the technician holds.

- ① CAN "SEE" CLONED / PCR'd FRAGMENTS Directly
- ② NEED DNA gel Blot to "SEE" Specific fragments in Population of Genomic DNA

DNA Fragments Can Be Mapped Systematically on the Map Can Be Generated from a DNA Sequence

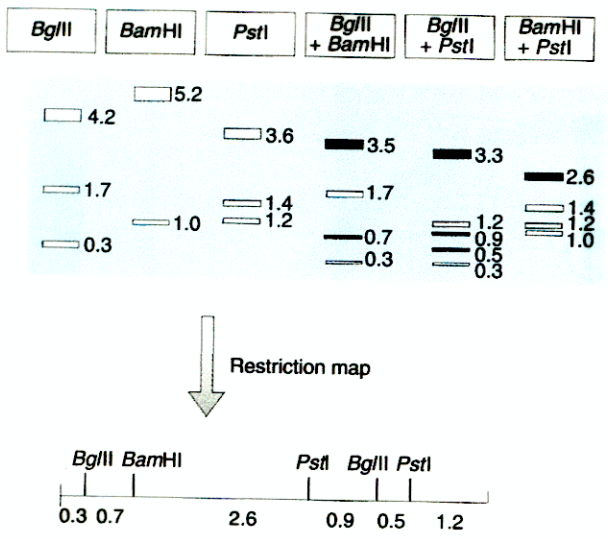


Figure 4.9: Generating a restriction map.

The size patterns from double digests provide information on the relative locations of restriction sites. The example shows size fractionation by agarose gel electrophoresis of restriction fragments following incubation of a 6.2 kb DNA fragment with the indicated enzymes. New bands in the double digests (i.e. not found in the original single digests) are indicated by black boxes. In the *Bgl*II + *Bam*HI double digest, the original 1.7 kb and 0.3 kb bands from the *Bgl*II digest alone are maintained, suggesting that these fragments do not have a *Bam*HI site, while the 4.2 kb *Bgl*II fragment is replaced by 3.5 kb and 0.7 kb fragments, suggesting that there is a *Bam*HI site within 0.7 kb from one end of the 4.2 kb *Bgl*II fragment. Similarly, in the *Bam*HI + *Pst*I double digest, the 1.4 kb and 1.2 kb fragments seen in the *Pst*I digest alone are maintained, suggesting that they lack a *Bam*HI site, while the 3.6 kb *Pst*I fragment is replaced by a 2.6 kb + 1.0 kb fragment, as a result of possession of an internal *Bam*HI site located 1.0 kb from one end. By comparing all three patterns of double digestion, the restriction map at the bottom can be deduced. *Note* that restriction mapping is often helped by the use of partial digests and also by end-labeling (Section 5.1.1).

MAPS OF TWO PLASMID VECTOR GENOMES

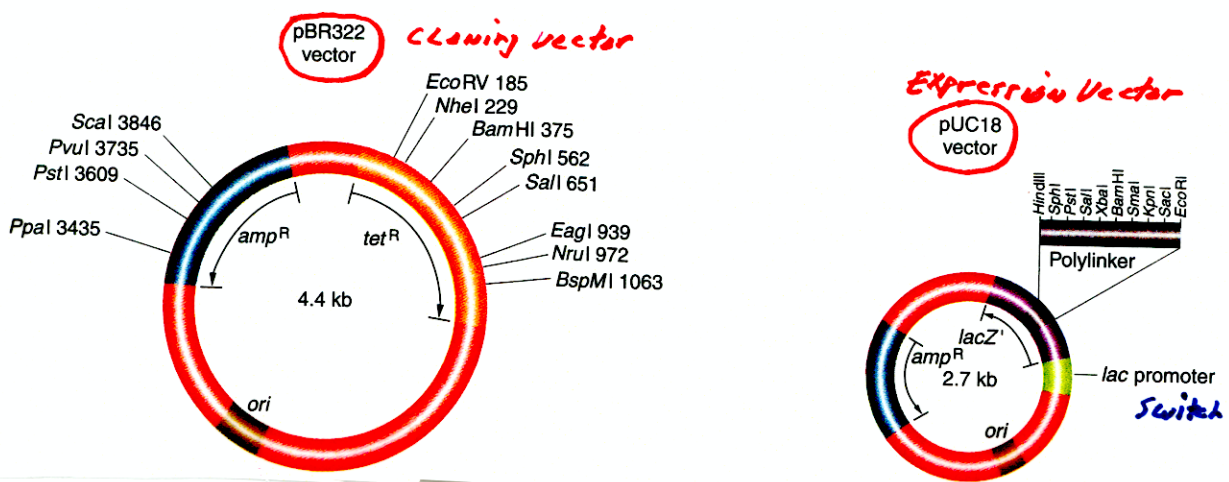
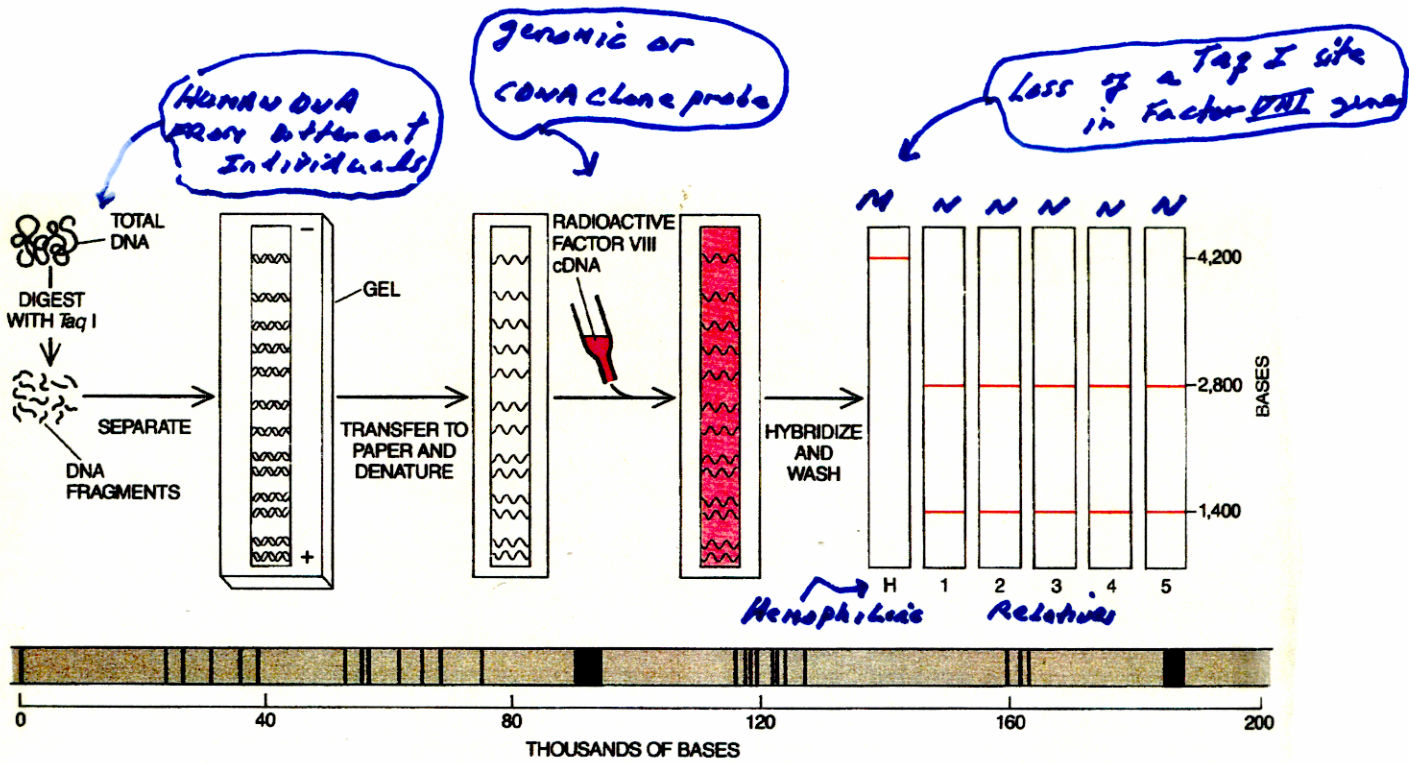


Figure 12-6 Two plasmids designed as vectors for DNA cloning, showing general structure and restriction sites. Insertion into pBR322 is detected by inactivation of one drug-resistance gene (*tet*^R), indicated by the Tet^S (sensitive) phenotype. Insertion into pUC18 is detected by inactivation of the β -galactosidase function of *Z'*, resulting in an inability to convert the artificial substrate X-Gal into a blue dye.

MAP OF THE FACTOR VIII GENE ALLOWS DISEASE ALLELES TO BE DETECTED



HEMOPHILIA-CAUSING MUTATIONS in the factor VIII gene can be detected by Southern blotting (*top*) if they happen to change the way the gene is fragmented by a restriction enzyme. DNA from blood cells is cut into millions of fragments, in this case with the enzyme *TaqI*. The fragments are separated according to size by electrophoresis, unraveled into single strands and blotted onto filter paper. The filter is bathed in a solution of radioactive factor VIII cDNA, which hybridizes only with fragments of the factor VIII

gene. The size of the hybridizing fragments is revealed by exposing X-ray film to the filter. In the example shown here a point mutation in the factor VIII gene of a hemophiliac (*H*) has eliminated a *TaqI* cleavage site. The 2,800- and 1,400-base fragments on the blot patterns of his relatives (*1-5*) are replaced by a single, uncut 4,200-base fragment. So far seven different mutations have been located on hemophiliac factor VIII genes (*bottom*). Four are point mutations, or changes of a single base (*dots*); three are extensive deletions (*bars*).

USE DNA Gel Blots & Factor VIII Probes to Investigate Presence of Mutant Alleles in FAMILIES (CARRIERS)

ONCE gene cDNA identified!

Restriction or Physical Maps of Chromosomes can be correlated with Gene Maps to provide Markers or Landmarks for Specific Genes

Chromosome 7

FIGURE 26-11
Probes in the CF region used for localizing and cloning the CF gene. Chromosome 7 is shown with its standard banding pattern. The CF gene had been mapped to the q31.2-q31.3 region and was known to lie between MET and D7S8, a distance of 1490 kb as determined by physical mapping. There are currently over 90 gene loci and over 400 polymorphic DNA fragments assigned to chromosome 7.

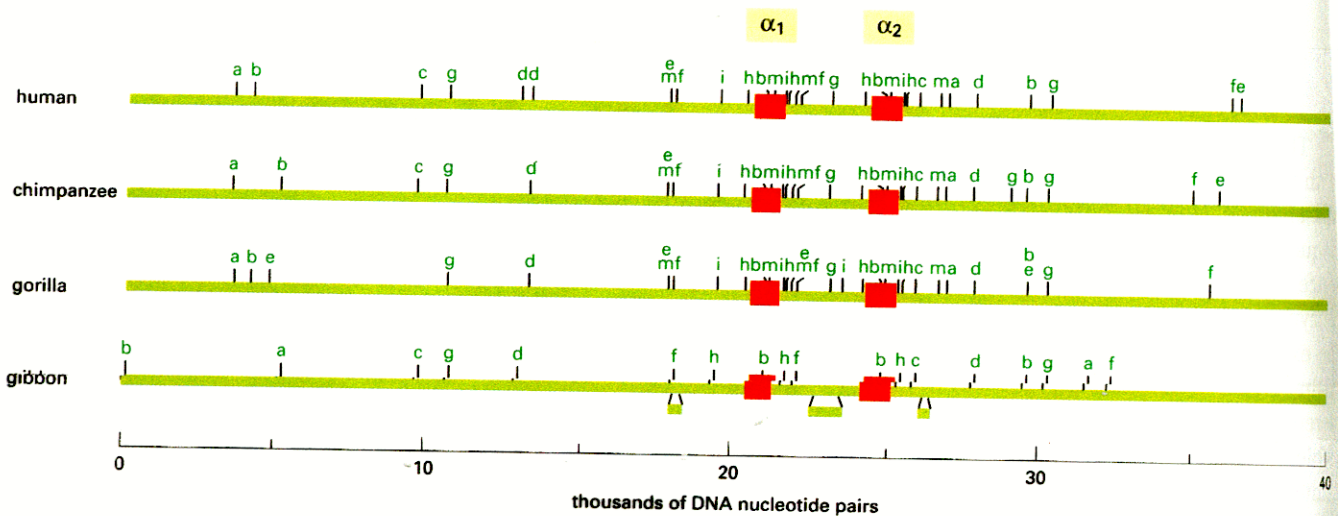
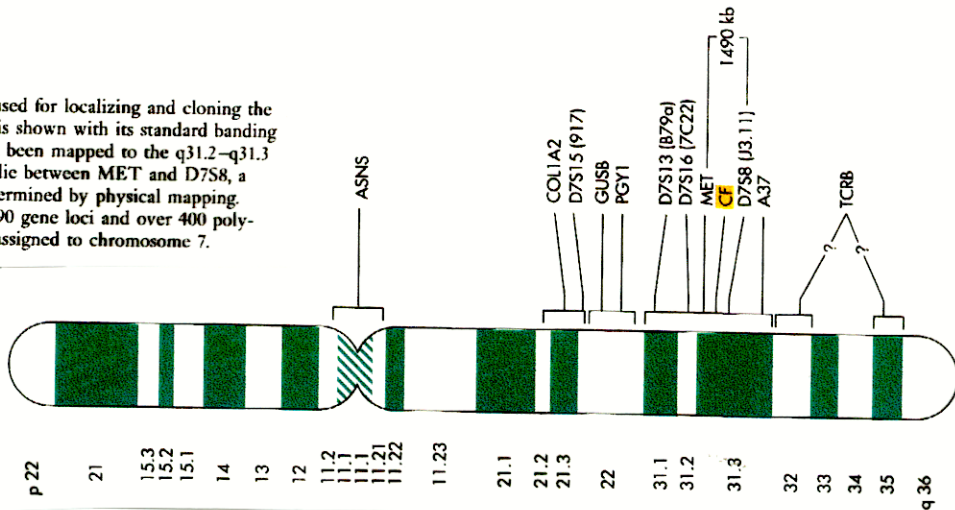


Figure 10-4 Comparison of the restriction maps of DNA containing the α -globin gene cluster from humans and other primates. The *thick green line* represents the chromosomal DNA containing the two α -globin genes (*red squares*) that are present in the primate genome (α globin is a subunit of hemoglobin, which contains two α -globin and two β -globin polypeptide chains). The *small letters* stand for sites cut by different restriction nucleases. The chimpanzee, the

primate most closely related to humans, has the most similar restriction map to ours, whereas the gibbon, which is the most distantly related, has the most dissimilar map. The *small green bars* under the main line in the gibbon map represent positions where additional DNA is present in the gibbon genome compared with the other species. (Courtesy of Elizabeth Zimmer and Allan Wilson.)

PROPERTIES OF VECTORS NEEDED TO CLONE AND MANIPULATE DNA FRAGMENTS

- ① Replication origin - Ability to replicate in Prokaryote &/or Eukaryotic cells
- ② Selectable / Distinguish from Non-recombinant Host Vectors - ① Antibiotic^r gene, ② Color marker gene, ③ ability to infect cells / package in virus
- ③ Unique / Single Restriction Sites for Cloning & Selection
Entire Sequence & Map available
- ④ Easily Re-introduced into host cells - ① Transformation, ② Infection
- ⑤ Easily Purified from host cell & manipulated
(e.g., ① plasmid, ② virus)
- ⑥ ALL are Genetically Engineered to meet cloning Experiment Needs - plasmid, expression plasmid, expression plasmid for animals, virus, plasmid/virus hybrid
E.G. - Plasmids Engineered to be small, have selectable markers, & unique cloning sites

THERE ARE A VARIETY OF VECTORS THAT HAVE BEEN ENGINEERED TO CLONE DNA FRAGMENTS AND CREATE GENOMIC + cDNA LIBRARIES

TABLE 8.2 Various Vectors and the Size of the Inserts They Carry

Vector	Form of Vector	Host	Typical Carrying Capacity (Size of Insert Accepted)	Major Uses
Plasmid	Double-stranded circular DNA	<i>E. coli</i>	Up to 15 kb	cDNA libraries; subcloning
Bacteriophage lambda	Virus (linear DNA)	<i>E. coli</i>	Up to 25 kb	Genomic and cDNA libraries
Cosmid	Double-stranded circular DNA	<i>E. coli</i>	30-45 kb	Genomic libraries
Phagemid	Virus convertible to plasmid	<i>E. coli</i>	Up to 12 kb	cDNA and genomic libraries
Bacteriophage P1	Virus (circular DNA)	<i>E. coli</i>	70-90 kb	Genomic libraries
BAC	Bacterial artificial chromosome	<i>E. coli</i>	100-500 kb	Genomic libraries
YAC	Yeast, artificial chromosome	Yeast	250-1000 kb (1 megabase)	Genomic libraries

① = USED IN LABS TODAY!

- ① PLASMIDS generally used for routine cloning + sequencing + cDNA libraries
- ② Genomic libraries usually made with VIRUS, BAC, or YAC vectors that can carry VERY LONG DNA segments (to keep # different clones in library small)
- ③ Vectors can be PLASMIDS, VIRUSES, or artificial chromosomes or combinations of these vectors!
With gene engineering - Can do anything!

Purpose of all vectors - Clone / Manipulate DNA / cDNA

TWO PLASMID VECTORS FOR EVERYDAY WORK

Cloning, cDNA cloning, sequencing, expression, etc.

up to 15 kb inserts

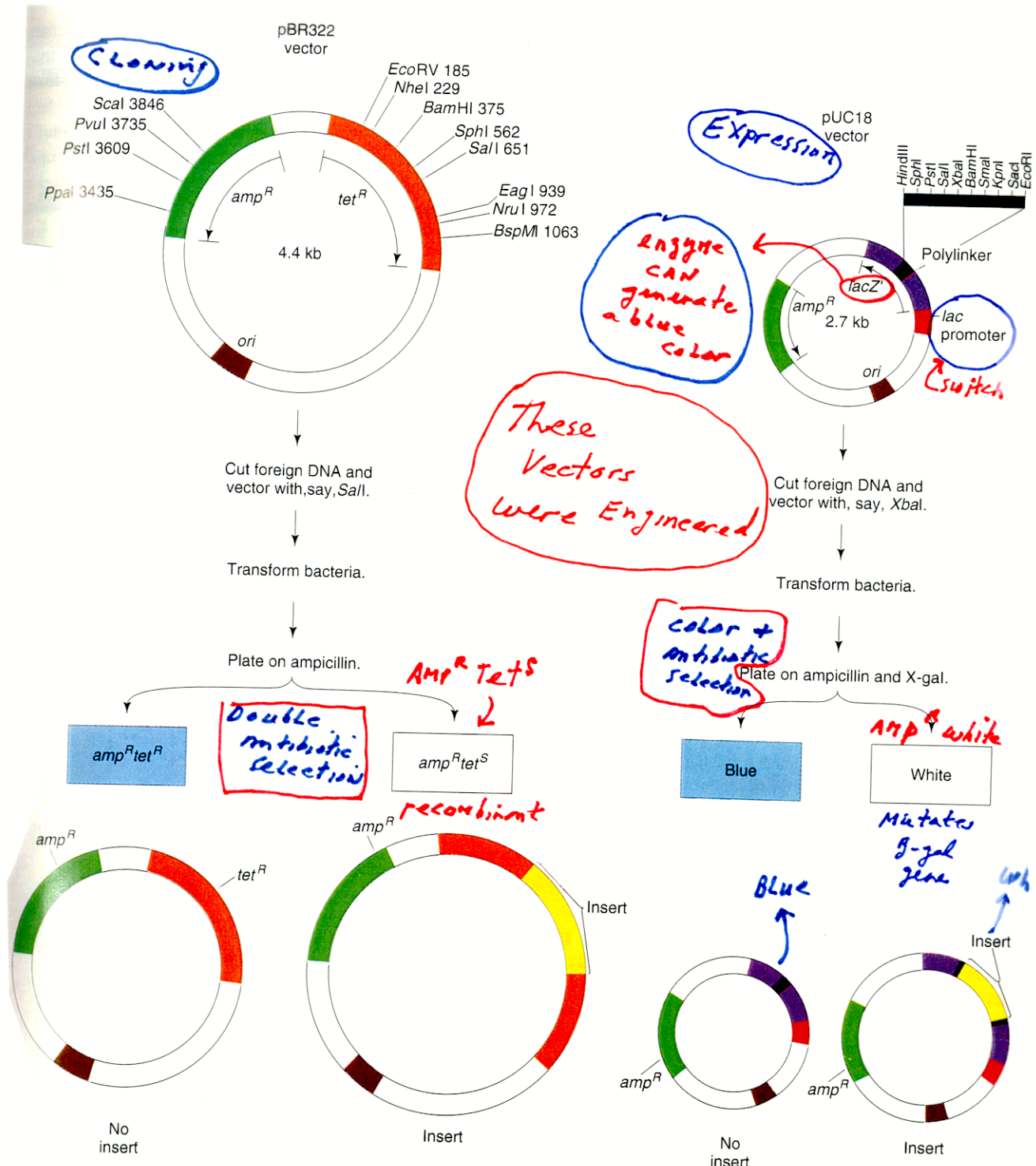


Figure 14-7 Two plasmids designed as vectors for DNA cloning, showing general structure and restriction sites. Insertion into pBR322 is detected by inactivation of one drug-resistance gene (*tet^R*), indicated by the *tet^S* (sensitive) phenotype. Insertion into pUC18 is detected by inactivation of the β-galactosidase function of Z', resulting in an inability to convert the artificial substrate X-gal into a blue dye.

CAN use *E. coli* β -galactosidase Gene to select for recombinant plasmids

pluc 18 vector

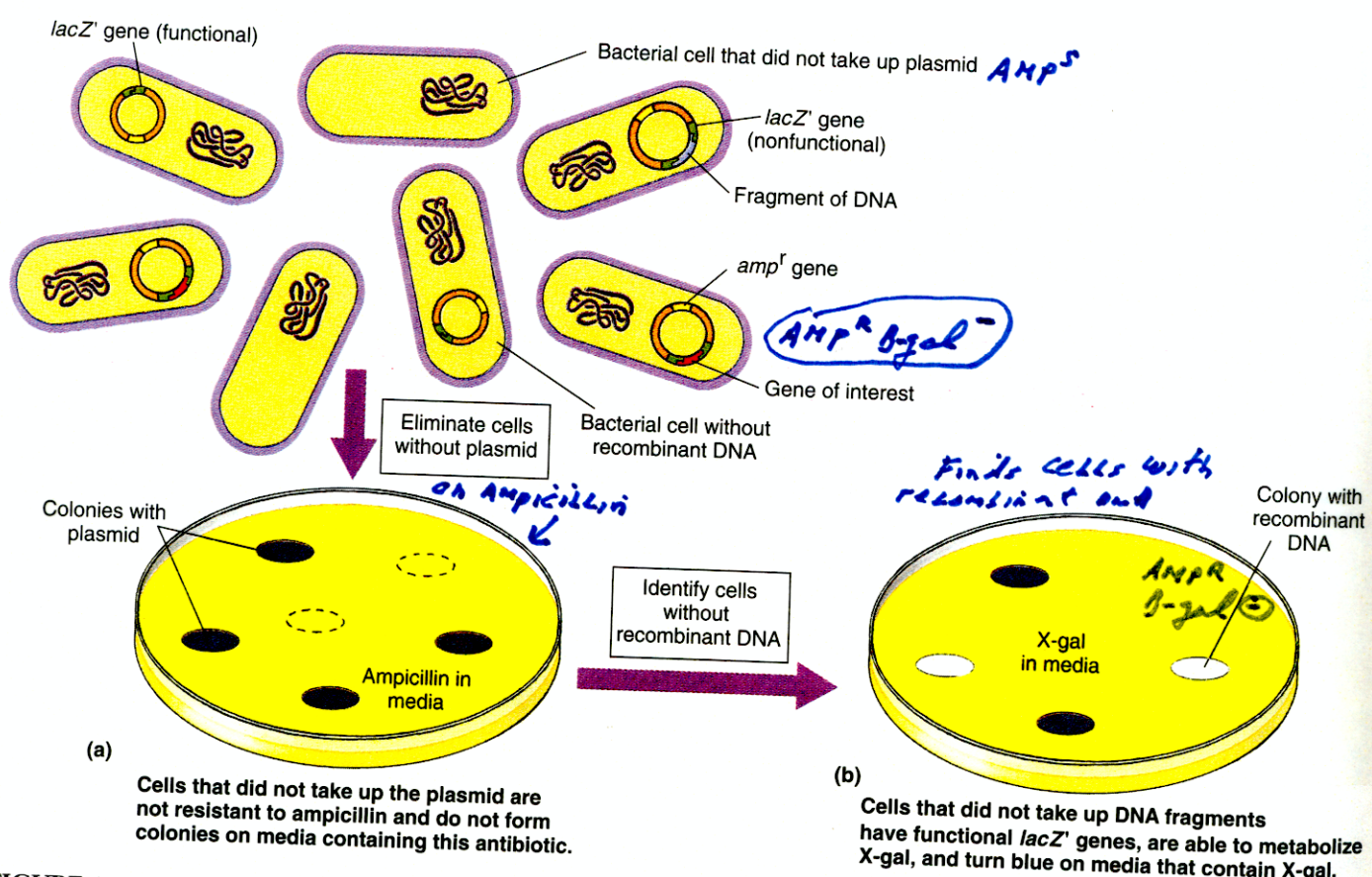


FIGURE 19.6

Stage 4-I: Using antibiotic resistance and X-gal as preliminary screens of restriction fragment clones. Bacteria are transformed with recombinant plasmids that contain a gene (*amp^r*) that confers resistance to the antibiotic ampicillin and a gene (*lacZ'*) that is required to produce β -galactosidase, the enzyme which enables the cells to metabolize the sugar X-gal. (a) Only those bacteria that have incorporated a plasmid will be resistant to ampicillin and will grow on a medium that contains the antibiotic. (b) Ampicillin-resistant bacteria will be able to metabolize X-gal if their plasmid does *not* contain a DNA fragment inserted in the *lacZ'* gene; such bacteria will turn blue when grown on a medium containing X-gal. Bacteria with a plasmid that has a DNA fragment inserted within the *lacZ'* gene will not be able to metabolize X-gal and, therefore, will remain colorless in the presence of X-gal.

X-gal \rightarrow Blue color

β -galactosidase enzyme

NORMALLY \downarrow

Lactose \rightarrow galactose + glucose

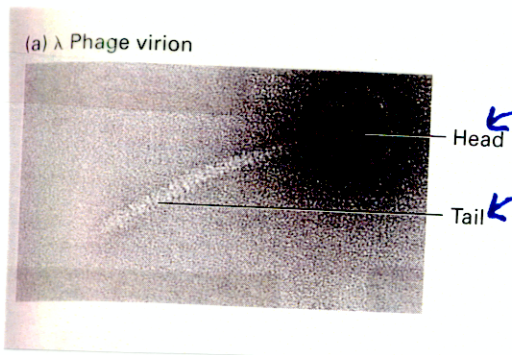
MILK sugar

USING THE LAMBDA (λ) BACTERIA VIRUS AS A VECTOR (E. coli virus)

MAX Delbruck
Father of phage!

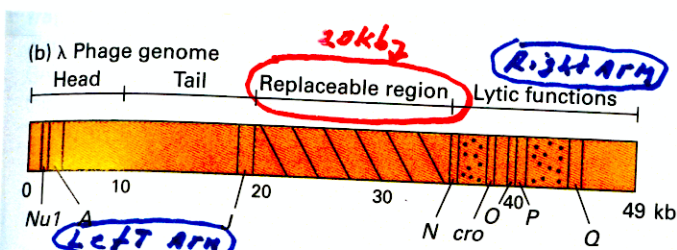
Advantages over Plasmids

- ① Use Natural Infection Process - Much higher efficiency of getting DNA into bacteria cells \therefore more clones/ μ g DNA + Easier to use in Lab
- ② CAN CLONE Long DNA segments. Excellent for genome libraries.
- ③ CAN CLONE DNA in VIRUS genome + self-assemble virus (DNA + proteins) in test tube!!



VIRUS contains Protein + DNA

- ① Head contains DNA
- ② Tail binds bacteria cell + injects DNA genome inside



λ Genome

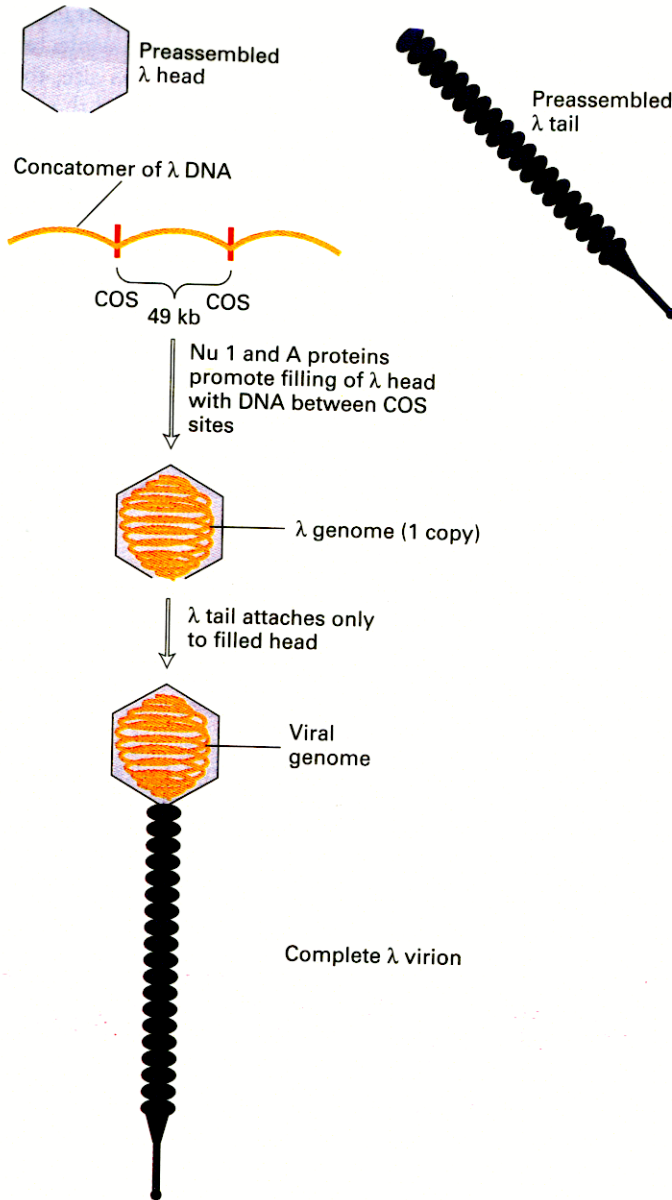
- ① 50,000 bp
- ② Genes needed for replication on right side or "ARM"
- ③ Genes needed for proteins - head + tail on left "ARM"
- ④ Genes in middle not needed & can be replaced with foreign DNA! 20kb

one of first genomes to be sequenced!

NOTE:
Restriction Enzymes "Fight" viral infection!

▲ FIGURE 7-10 The bacteriophage genome. (a) Electron micrograph of bacteriophage λ virion. The genome is contained within the head. (b) Simplified map of the λ phage genome. Genes encoding proteins required for assembly of the head and tail map at the left end; those encoding additional proteins required for the lytic cycle map at the right end. Some regions of the genome can be replaced by exogenous DNA (diagonal lines) or deleted (dotted area) without affecting the ability of λ phage to infect host cells and assemble new virions, permitting insertion of up to ≈ 25 kb of exogenous DNA between the J and N genes. There are about 60 genes on the λ genome. Only a few individual genes are shown in this diagram. Small numbers indicate positions in kilobases (kb). [Photograph courtesy of R. Duda and R. Hendrix.]

λ VIRUS CAN BE SELF-ASSEMBLED IN A TEST TUBE



Also packaged naturally in *E. coli* cells

▲ **FIGURE 7-11 Assembly of bacteriophage λ virions.** Empty heads and tails are assembled from multiple copies of several different λ proteins. During the late stage of λ infection, long DNA molecules called *concatomers* are formed; these multimeric molecules consist of copies of the λ genome linked end to end and separated by COS sites (red), a protein-binding nucleotide sequence that occurs once in each copy of the λ genome. Binding of the λ proteins Nu1 and A to COS sites promotes insertion of the DNA between two adjacent COS sites into an empty head. After the heads are filled with DNA, preassembled λ tails are attached, producing complete λ virions capable of infecting *E. coli* cells.

The LAMBDA Phage Infects *E. coli*, Replicates, & Destroys (Lyses) the Bacterial Cells

Phage Life Cycle

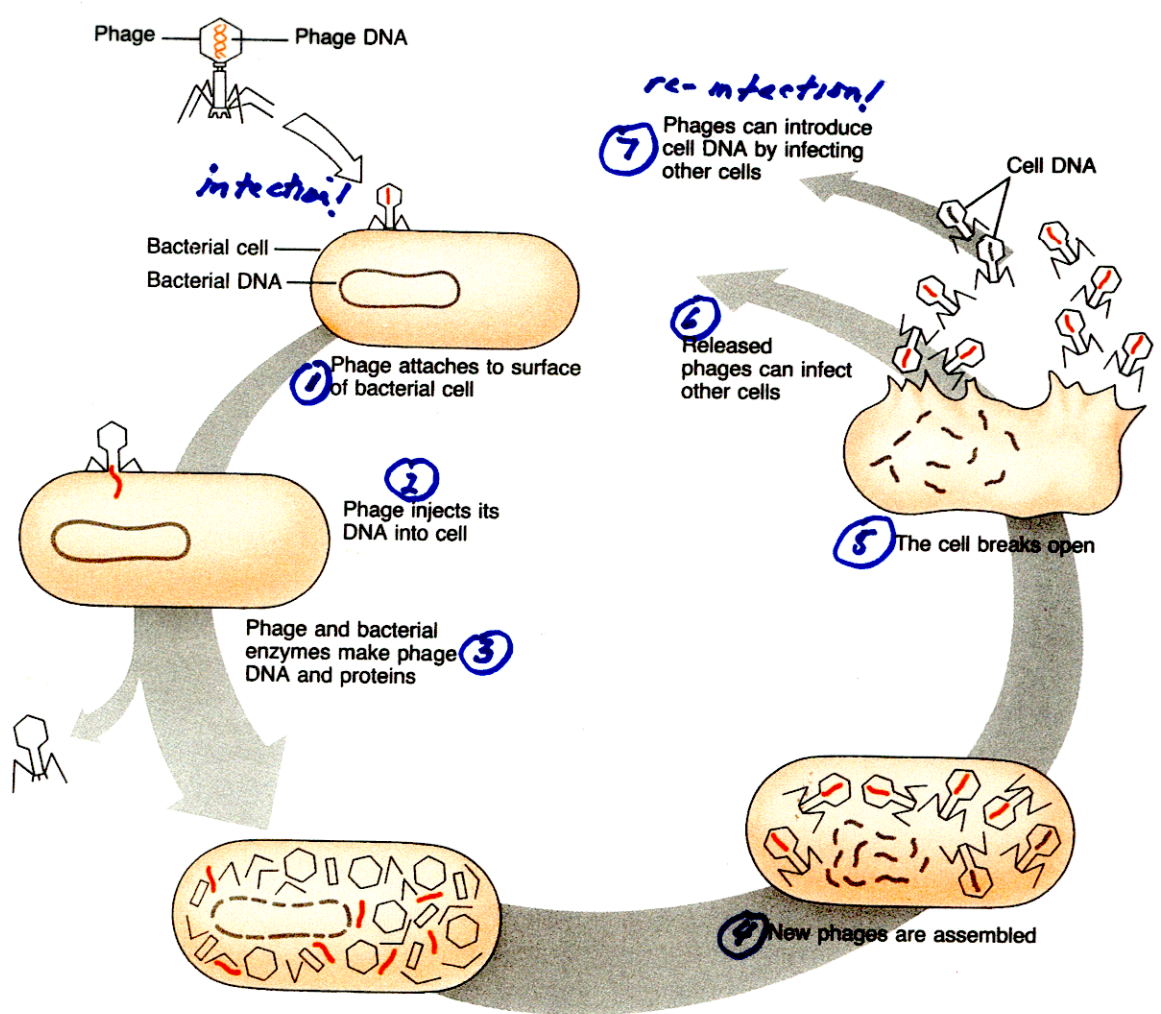
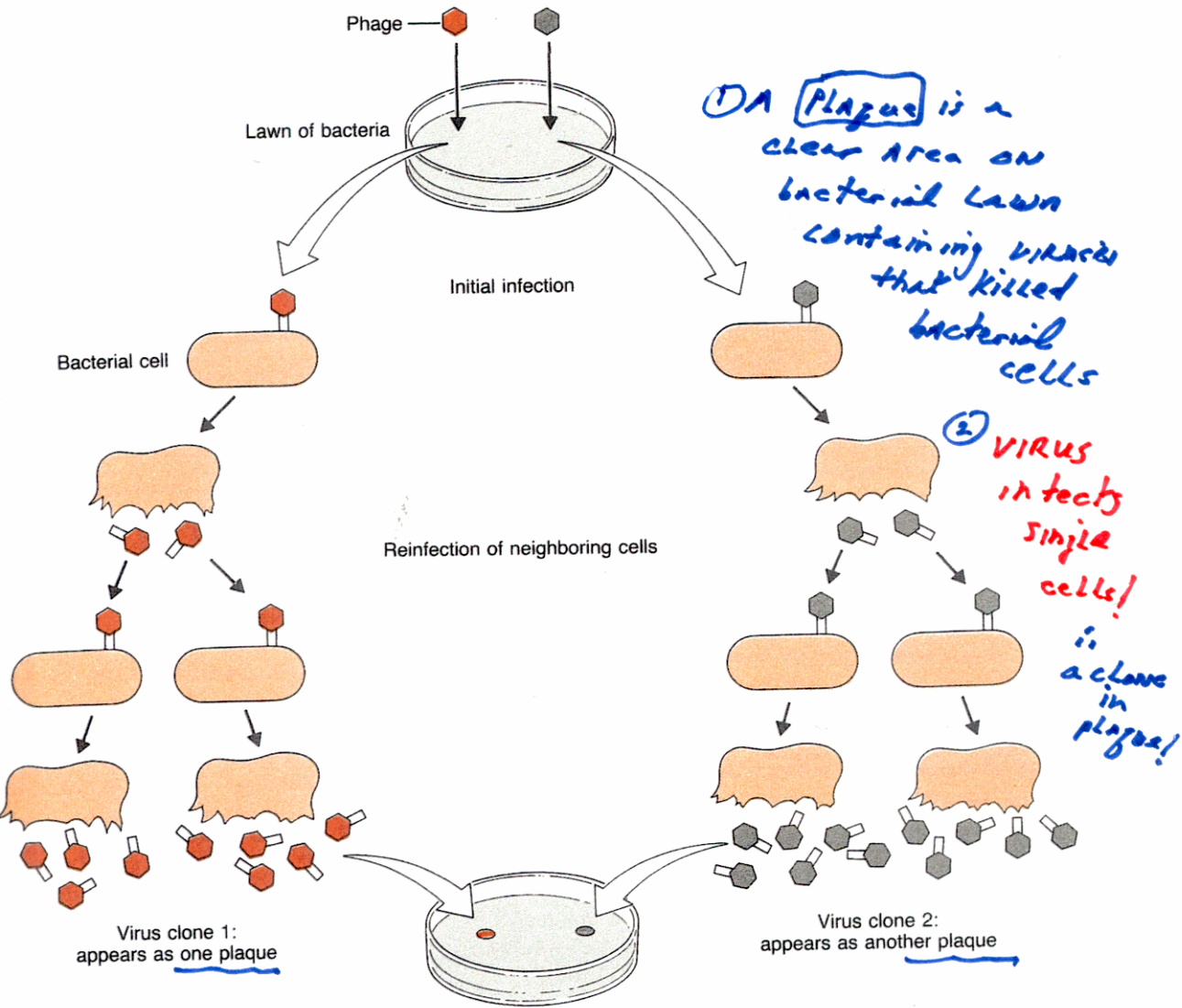


Figure 4.4 Events that occur when a phage infects a bacterial cell.

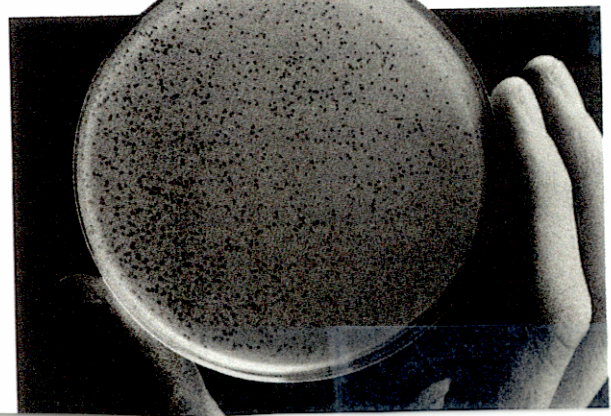
LAMBDA VIRUSES CAN BE GROWN ON A "LAWN" of *E. coli* in a Plate

PLAQUES CONTAIN VIRUS CLONES



Each plaque (clear spot) in the layer of bacteria growing on this Petri dish results from the destruction of bacteria by the descendants of a single phage.

SELECTION
= Killing Bacteria + Producing plaque



Using Phage & Natural Infection Process to Clone Genes

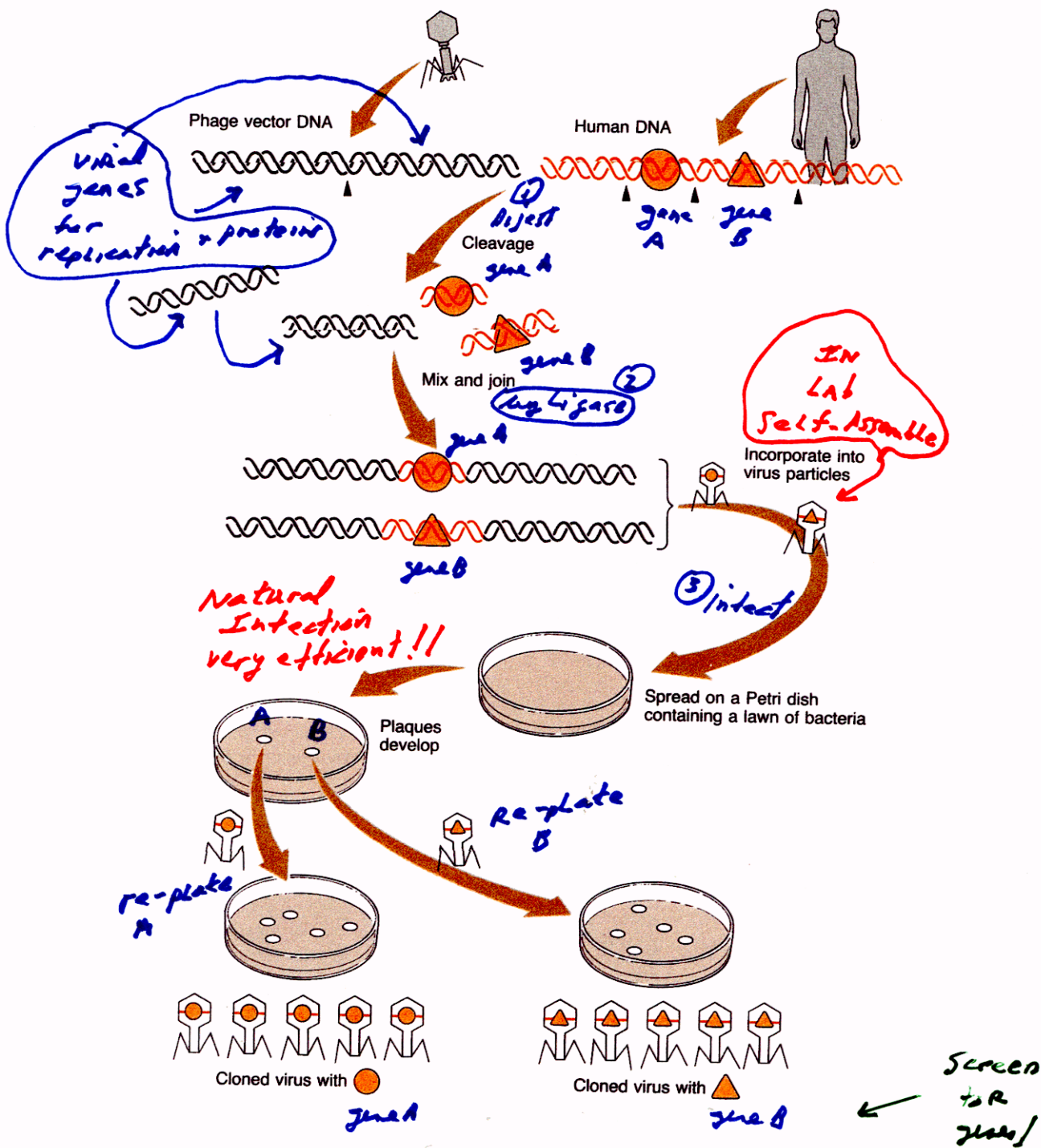
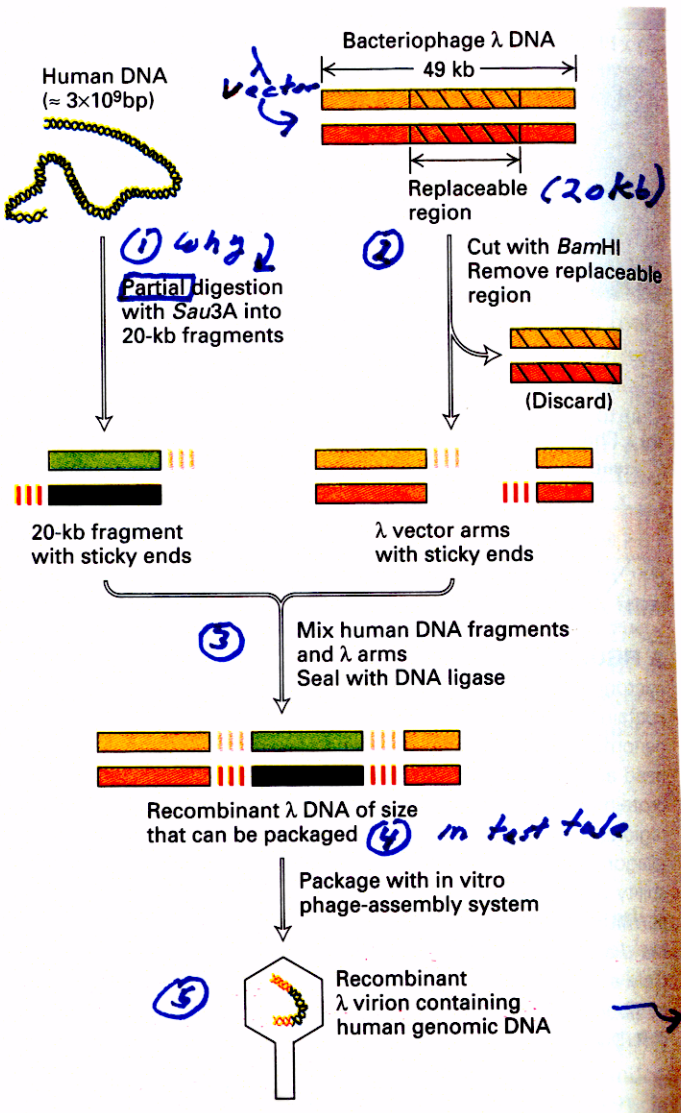


Figure 4.10 Molecular cloning of a mixture of foreign DNA inserts in a viral vector.

CLONING THE HUMAN GENOME AND SEARCHING FOR THE FACTOR UTII GENE

A COMPLETE HUMAN GENOME LIBRARY in λ phage vector

- ① Large DNA Fragments
- ② Keep Genes intact
- ③ Keep Clones in Library as few as possible to Find Gene - Easier to hunt thru 500,000 clones than 5,000,000!!



▲ FIGURE 7-12 Construction of a genomic library of human DNA in a bacteriophage λ vector. The nonessential regions in the right half of the λ genome (dotted areas in Figure 7-10b) usually are deleted to maximize the size of the exogenous DNA fragment that can be inserted. Then the λ DNA is treated to remove the central replaceable region. In this example, the replaceable region is cut out with *Bam*HI, and the total DNA from human cells is partially digested with *Sau*3A. These two restriction enzymes produce fragments with complementary sticky ends (red lines). The λ vector arms and ≈ 20 -kb genomic fragments are mixed, ligated, and packaged in vitro to produce recombinant λ phage virions, which are plated on a lawn of *E. coli* cells. In the diagrams of DNA regions, light and dark shades of the same color indicate complementary strands.

Why Partial or Incomplete Digestion of HUMAN DNA with Restriction Enzyme?

$Sau3A = 4bp = 5' GATC 3'$ \therefore 1 site every 280bp if digest to completion $\times 1 \times 10^7$ DNA fragments

$EcoRI = 6bp = 5' GAATC 3'$ \therefore 1 site every 3100bp if digest to completion (cleave every site) $\times 972,000$ DNA fragments!

- ① Complete Digestion produces fragments that are too small to clone in λ VIRUS (need 20kb)
- ② Complete Digestion would create huge genome libraries with large # clones to screen
- ③ Complete Digestion would break up genes on different DNA fragments - particularly if human genes big - \therefore would have one gene on many different clones - parts separated!
- ④ Complete Digestion provides no way to find neighbors of clones in genome - what's next to gene in chromosome!

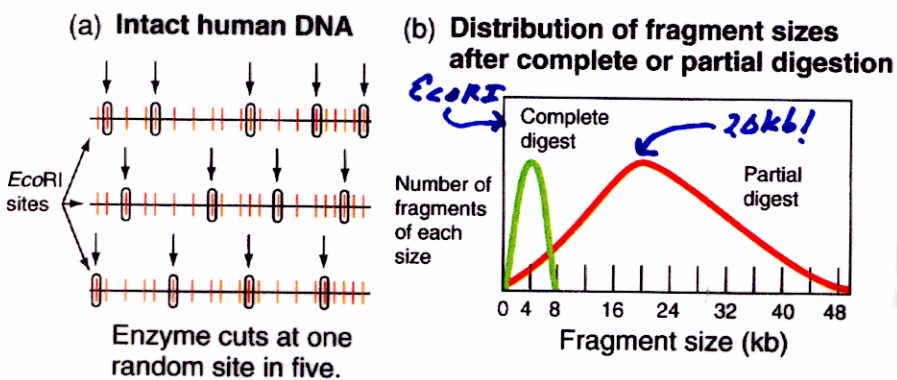
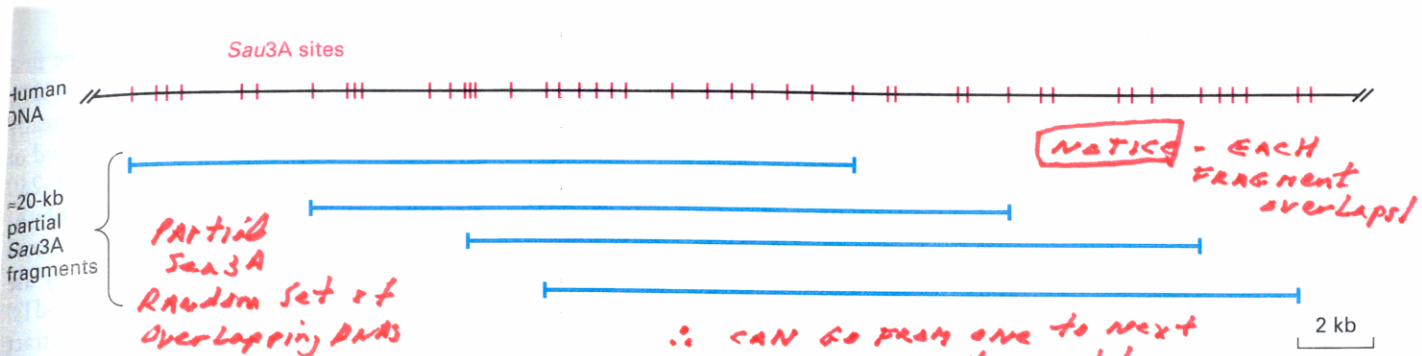


Figure 8.4 Comparison of results from partial and complete digests. (a) By reducing the time available for the reaction to occur, you can ensure that an enzyme actually cuts only a subset of the total recognition sites within a DNA sample. In this example, the chosen reaction time allowed only 1/5 of all *EcoRI* sites to be cut. The particular 20% of sites at which the cuts occur is totally random and different even on identical DNA molecules. (b) Most of the restriction fragments produced by partial digestion are larger than those produced by complete digestion with the same restriction enzyme.

PARTIAL DIGESTION PRODUCES A SERIES OF LARGE, OVERLAPPING DNA FRAGMENTS/CLONES!

CAN CONNECT ONE CLONE WITH ANOTHER!!
 BUILD UP CLONES OF EACH CHROMOSOME!!

CONSTRUCTING HUMAN GENOME LIBRARY BY PARTIAL DIGESTIONS CREATES A SET OF OVERLAPPING DNA FRAGMENTS/CLONES



▲ FIGURE 7-13 Production of overlapping restriction fragments by partial digestion of human genomic DNA with *Sau3A*. This restriction endonuclease recognizes the 4-bp sequence GATC and produces fragments with single-stranded sticky ends with this sequence on the 5' end of each strand. A hypothetical region

of human genomic DNA showing the *Sau3A* recognition sites (red) is shown at the top. Partial digestion of this region of DNA would yield a variety of overlapping fragments (blue) ≈20 kb long. Use of such overlapping fragments increases the probability that all sequences in the genomic DNA will be represented in a λ library.

∴ WOULD AN OVERLAPPING SET FOR EACH OF THE 24 CHROMOSOMES ALLOWING CLONES TO BE ORDERED FROM BEGINNING TO END BY RESTRICTION MAPPING BECAUSE EACH CHROMOSOME CONTAINS ONE DNA MOLECULE!

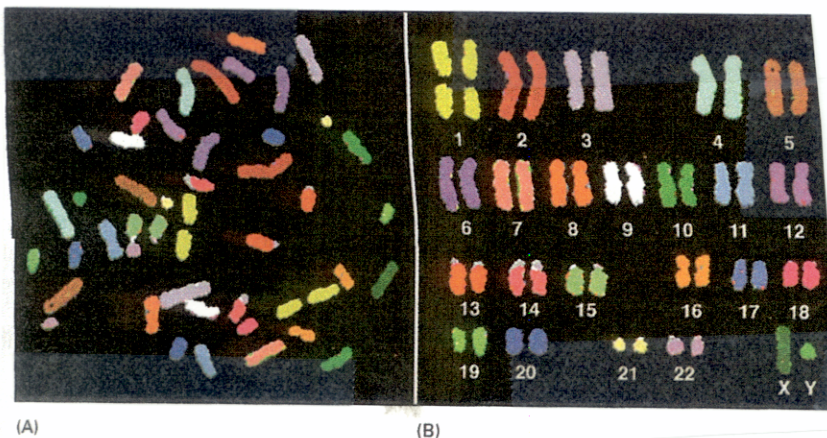


Figure 8-2 Human chromosomes.

∴ (A) The chromosomes as visualized as they originally spilled from the lysed cell. (B) The same chromosomes artificially lined up in order. This arrangement of the full chromosome set is called a karyotype. (From E. Schröck et al., *Science* 273:494-497, 1996.)

CONSTRUCTING THE HUMAN GENOME LIBRARY AND IDENTIFYING THE FACTOR VIII Gene

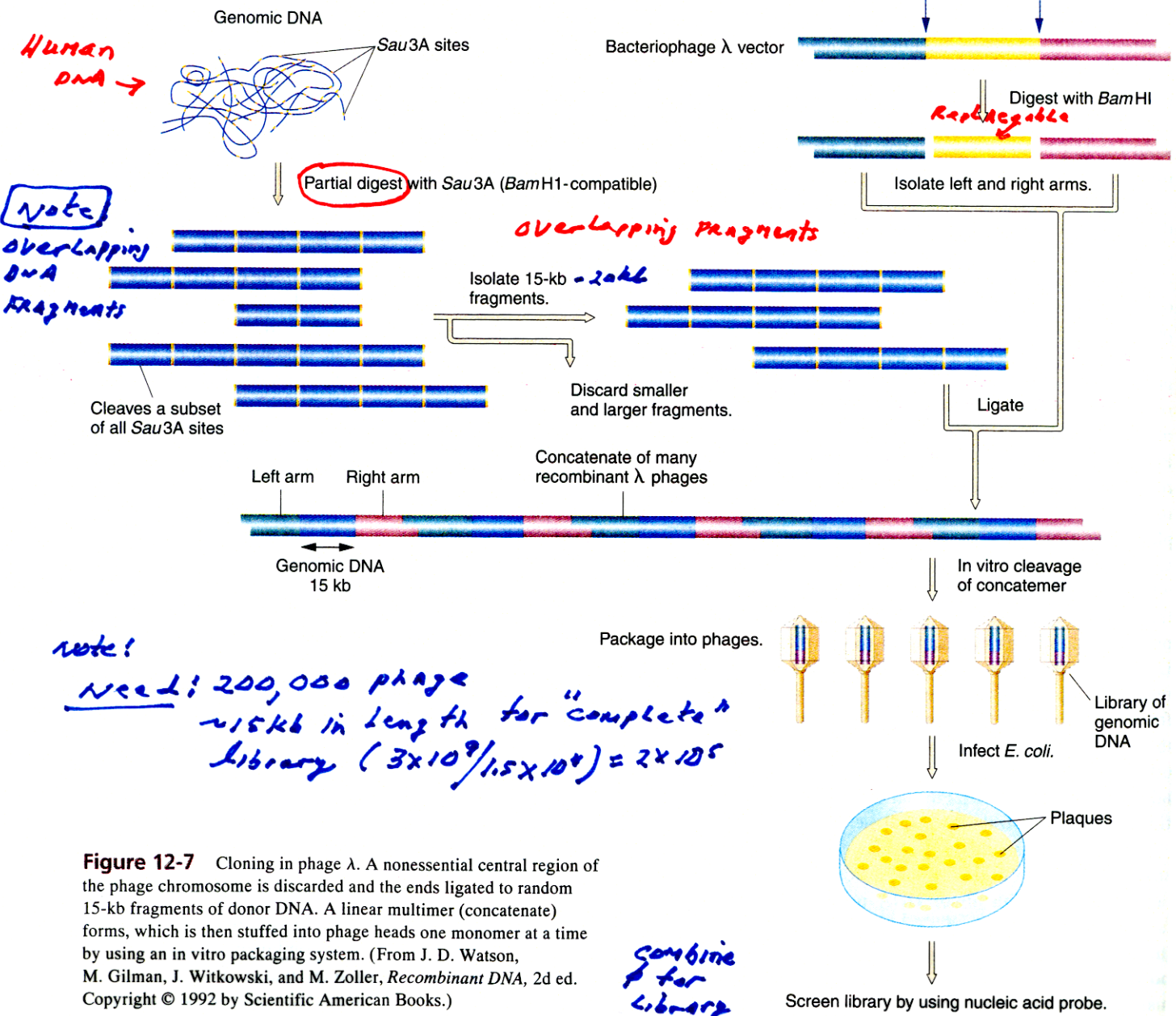
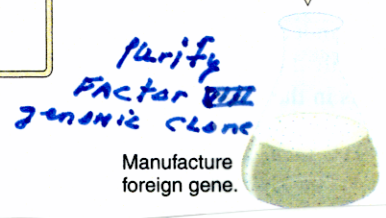
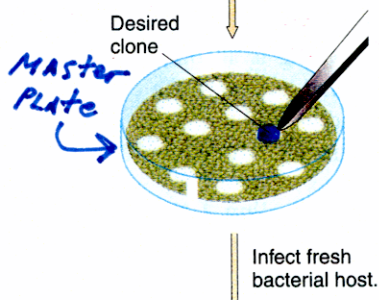
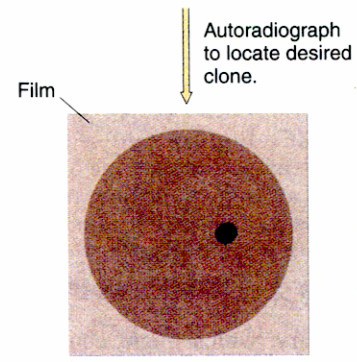
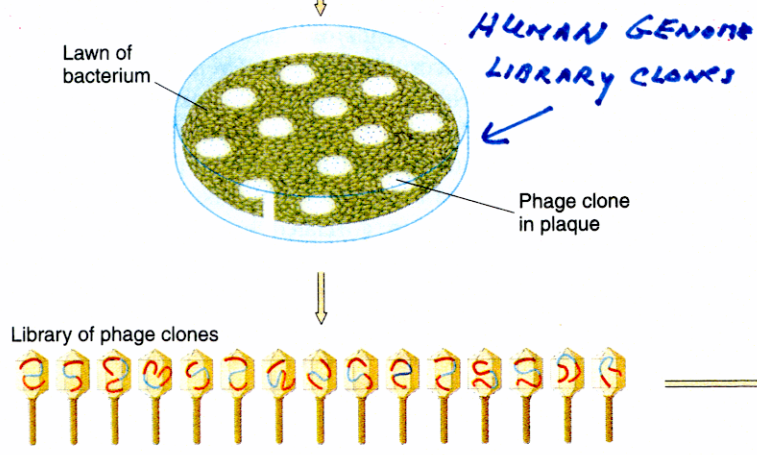
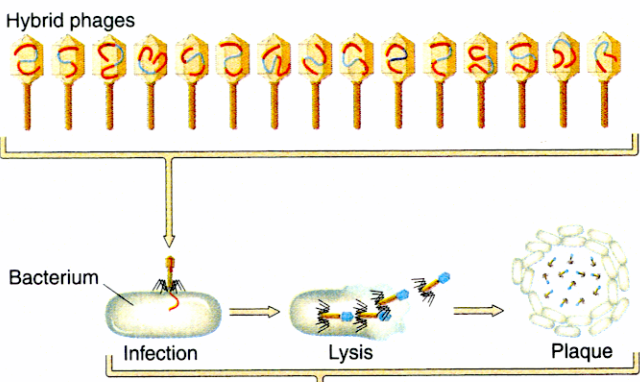
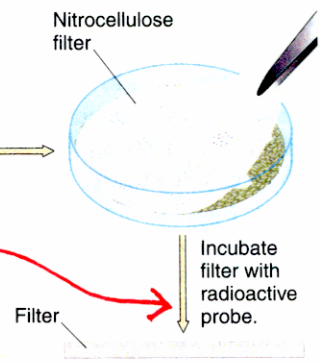
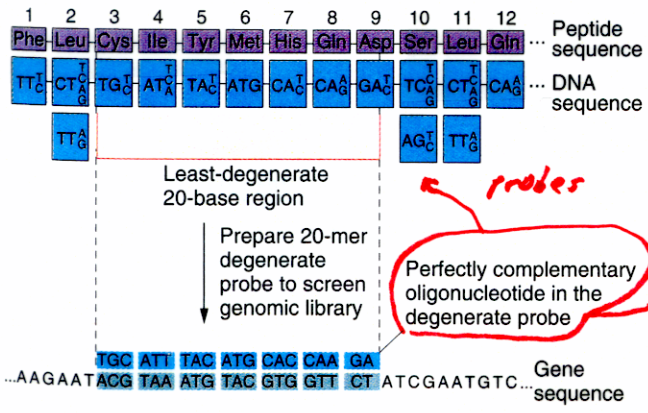


Figure 12-7 Cloning in phage λ. A nonessential central region of the phage chromosome is discarded and the ends ligated to random 15-kb fragments of donor DNA. A linear multimer (concatenate) forms, which is then stuffed into phage heads one monomer at a time by using an in vitro packaging system. (From J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright © 1992 by Scientific American Books.)

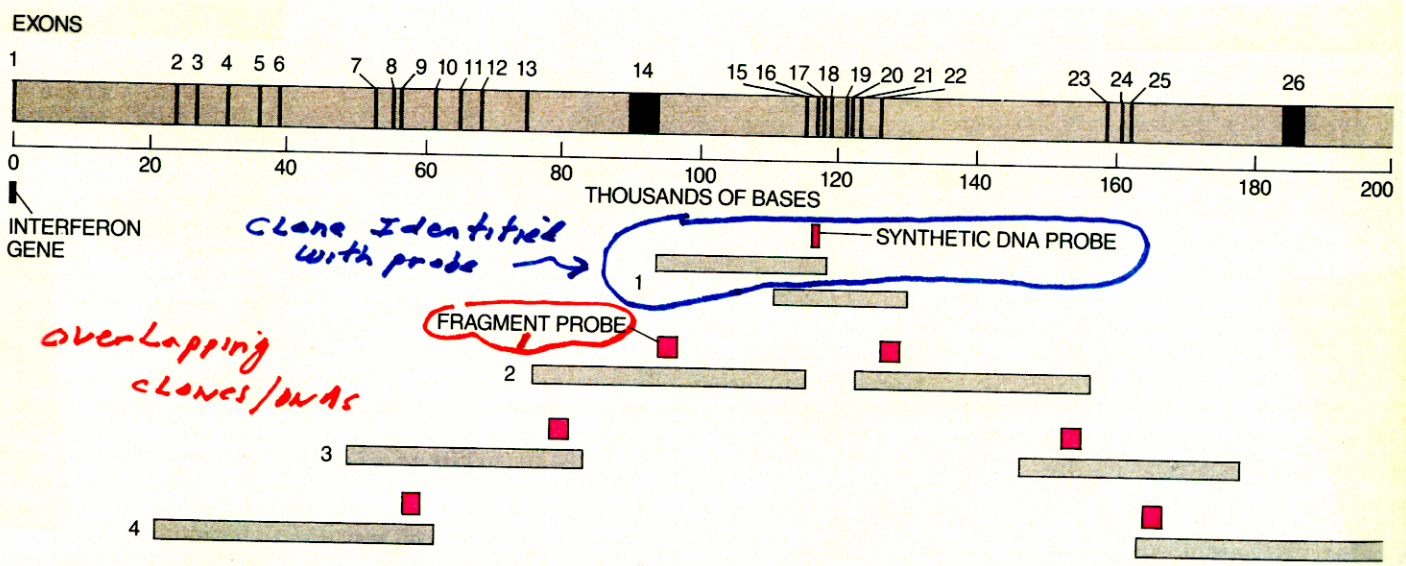
Finding the Factor VIII Gene

FACTOR VIII Protein Sequence
 ↳ Synthetic DNA Probe



SEQUENCE TO SEE IF IT MATCHES PROBE/PROBING

THE RESULT - THE FACTOR VIII GENE IS HUGE - 186,000 bp - The probe IDENTIFIED A CLONE CONTAINING ONLY ONE PART OF GENE!!!



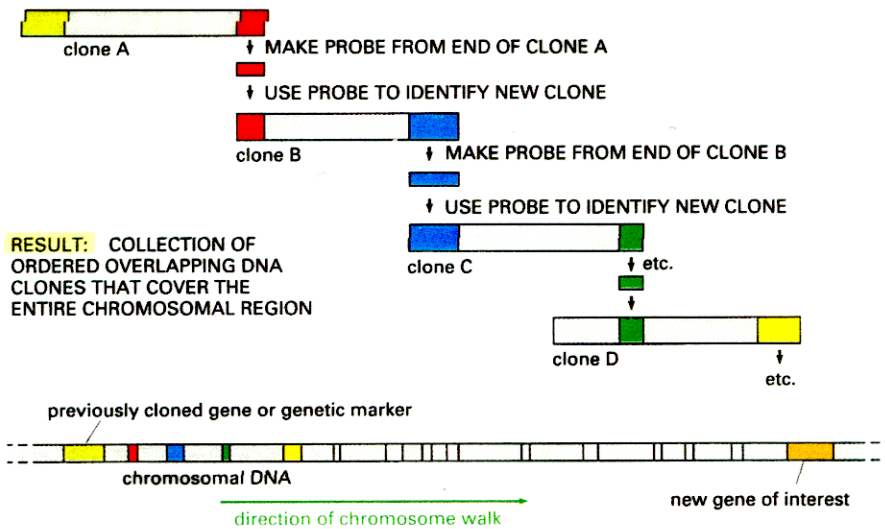
TREMENDOUS SIZE of the factor VIII gene, the largest gene cloned to date, forced workers to apply a cloning technique called chromosome walking. The factor VIII gene is 186,000 bases long. In contrast the interferon gene, which was cloned in 1980, incorporates only about 600 bases. Because the factor VIII gene is too large to fit into a single phage, segments of it were found in different plaques in the genomic library. When the library was screened

with a synthetic DNA probe, the probe hybridized with overlapping segments (1). Pieces of the segments then served as probes to re-screen the library and identify further segments (2). By repeating this procedure nearly all of the gene was identified (3, 4). (Its beginning was found once factor VIII cDNA was available as a probe.) Less than one-twentieth of the gene consists of exons, or coding sequences (black bands); the 26 exons are separated by 25 introns.

How FIND CLONES WITH Rest of Gene?

Remember - the library contains overlapping DNA clones ∴ CAN use one part of First clone to re-screen library & "WALK" to other gene regions - using restriction maps & sequencing as guides!

CHROMOSOME WALKING CAN BE USED TO FIND ALL PARTS OF FACTOR VIII Gene



(a) Chromosome walking

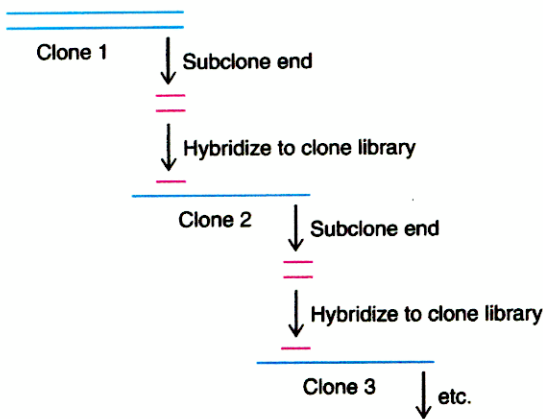
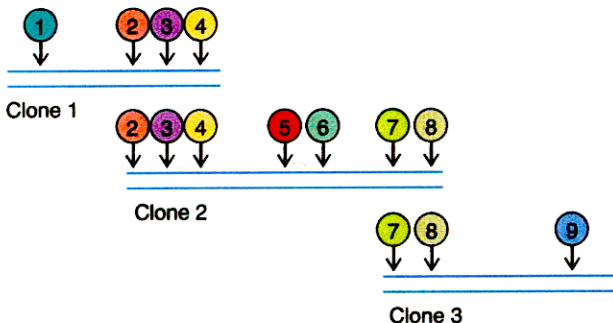


Figure 24.18 Mapping by chromosome walking. (a) Chromosome walking. To start the walk, choose a cloned piece of DNA (clone 1) and subclone one end of it. Then use this small end piece (red) as a probe to identify an overlapping clone (clone 2) in a library. Repeating the process, subclone the far end of clone 2 to generate a probe to identify yet another overlapping clone (clone 3). Repeat this cycle as many times as needed to build a set of overlapping clones spanning large stretches of DNA. (b) Physical mapping of restriction sites or STSs in each clone allows one to align the overlapping DNAs and build a map of the whole contig.

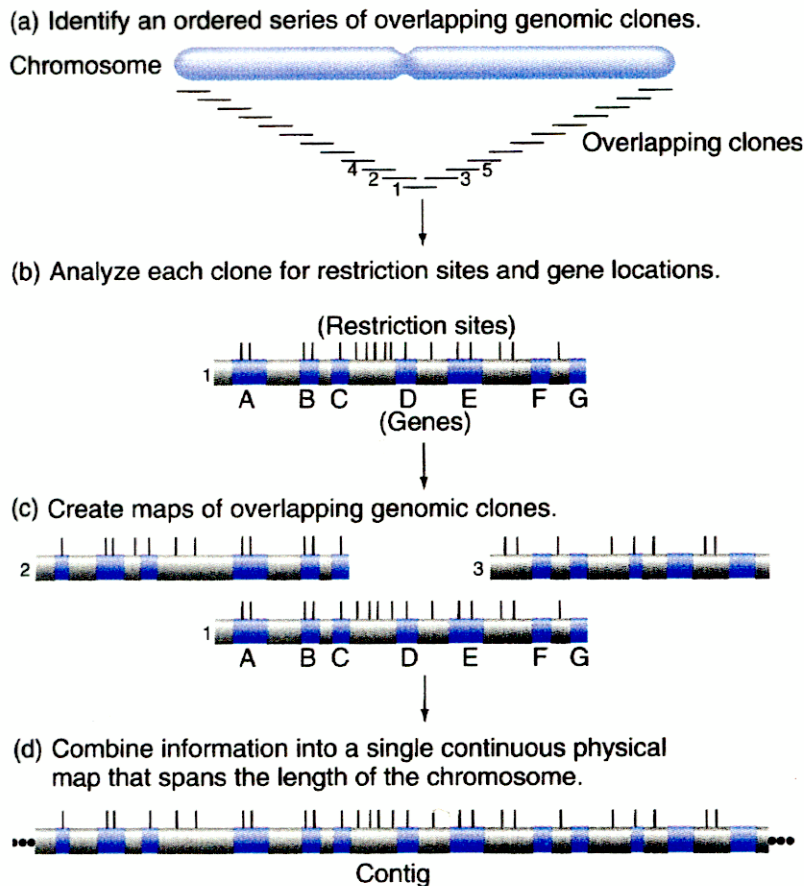
(b) Physical mapping (restriction sites and STSs)



Align using Restriction Maps/Sequence of Each Clone

CAN WALK DOWN AN ENTIRE CHROMOSOME & OBTAIN AN ENTIRE SET OF OVERLAPPING CLONES CONTAINING EVERY GENE IN CHROMOSOME

- ① Used to Sequence Human Genome
- ② Used to Map Genes to Chromosomes
- ③ Used for MARKERS (RFLPs) to identify & follow disease Genes



There are 24 sets of clones for HUMAN genome
 22 Autosomes + X chromosome + Y chromosome

Figure 10.5 Building a whole-chromosome physical map. (a) To produce a whole-chromosome physical map, you first order a set of overlapping genomic clones that extend from one end of the chromosome to the other. Subsequent figures describe various methods of obtaining this ordered set of clones. (b) You next map the restriction sites of each clone in the set through restriction analysis, and analyze individual restriction fragments in other ways, such as Northern blot analysis, to identify transcription units. (c) Computers overlay the different types of maps for each clone onto the overlapping clones to obtain a continuous map. (d) The result is a single continuous map extending the length of the chromosome.

USING PCR TO AMPLIFY SPECIFIC DNA SEQUENCES + mRNAs

Need DNA Sequence First!

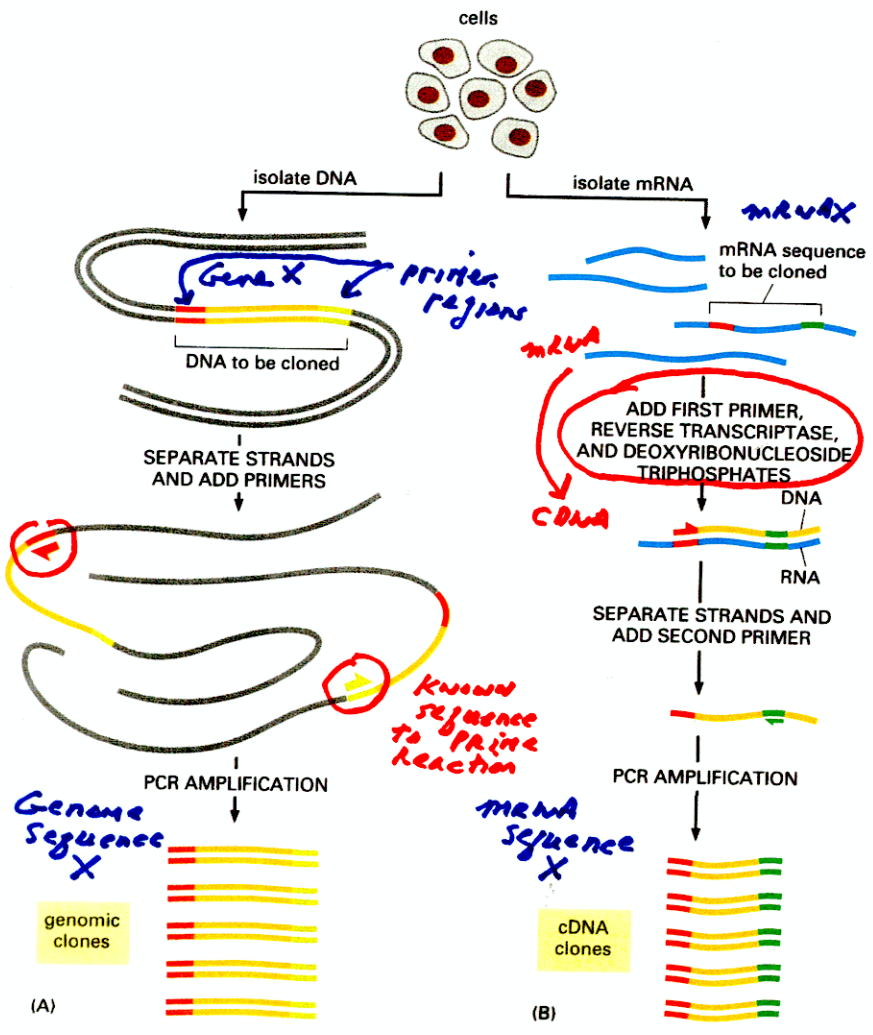


Figure 10-23 Use of PCR to obtain a genomic or cDNA clone. (A) To obtain a genomic clone using PCR, chromosomal DNA is first purified from cells. PCR primers that flank the stretch of DNA to be cloned are added, and many cycles of the PCR reaction are completed (see Figure 10-22). Since only the DNA between (and including) the primers is amplified, PCR provides a way to obtain selectively a short stretch of chromosomal DNA in an effectively pure form. (B) To use PCR to obtain a cDNA clone of a gene, mRNA is first purified from cells. The first primer is then added to the population of mRNAs, and reverse transcriptase is used to make a complementary DNA strand. The second primer is then added, and the single-stranded DNA molecule is amplified through many cycles of PCR, as shown in Figure 10-22.

↓
 Clone in vector
 or
 use directly

↓
 Clone in vector
 or
 use directly

PCR is A Powerful
DIAGNOSTIC TOOL!

HIV TEST in BLOOD - HIV is an RNA VIRUS

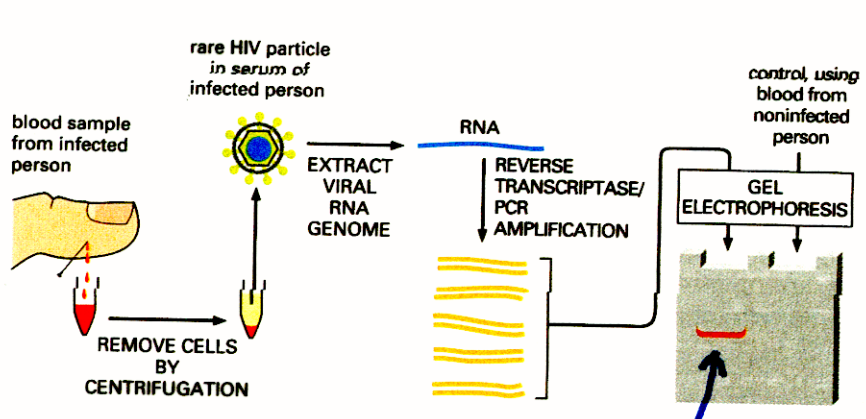


Figure 10-24 Use of PCR to detect the presence of a viral genome in a sample of blood. The genome of the human immunodeficiency virus (HIV), the cause of AIDS, is a single-stranded RNA molecule (see Figure 9-30). Because of its ability to enormously amplify the signal from every single molecule of nucleic acid, PCR is an extraordinarily sensitive method for detecting trace amounts of virus in a sample of blood or tissue without the need to purify the virus. In addition to HIV, many viruses that infect humans are now monitored in this way.

CAN USE TO OBTAIN DNA
Fingerprints / Disease Gene Markers /
Pathogen Identity, etc. By digesting
OR sequencing DNA bands produced by
PCR

ONLY Need DNA from one cell!
RNA