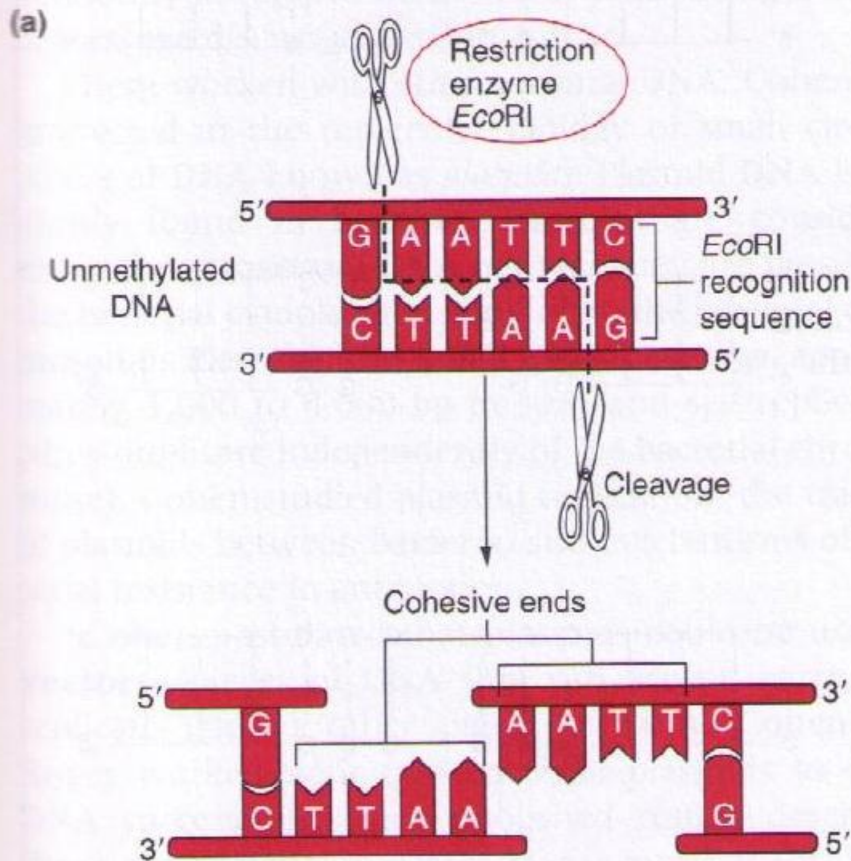


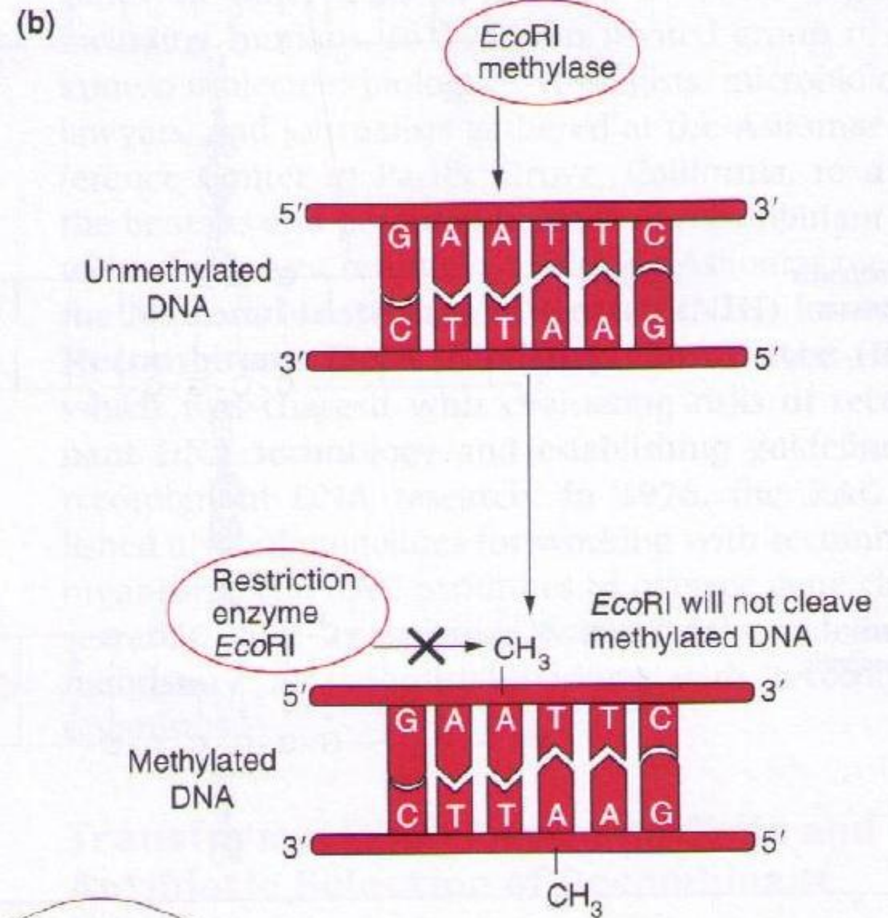


Figure 3.1 Restriction Enzyme Recognition Sequence and Enzyme Action (a) Digestion of DNA by *Eco*RI produces DNA fragments with cohesive ends. (b) Methylation of the recognition sequence by the enzyme *Eco*RI methylase blocks DNA cleavage by *Eco*RI.

Table 3.1 COMMON RESTRICTION ENZYMES

Source Microorganism	Enzyme	Recognition Sequence	Cut Products
Create Cohesive Ends			
Hemophilus influenzae	*Hin*dIII	5′ A-A-G-C-T-T 3′ 3′ T-T-C-G-A-A 5′	5′ A A-G-C-T-T 3′ 3′ T-T-C-G-A A 5′
Escherichia coli	*Eco*RI	5′ G-A-A-T-T-C 3′ 3′ C-T-T-A-A-G 5′	5′ G A-A-T-T-C 3′ 3′ C-T-T-A-A G 5′
Bacillus amyloliquefaciens	*Bam*HI	5′ G-G-A-T-C-C 3′ 3′ C-C-T-A-G-G 5′	5′ G G-A-T-C-C 3′ 3′ C-C-T-A-G G 5′
Thermus aquaticus	*Taq*I	5′ T-C-G-A 3′ 3′ A-G-C-T 5′	5′ T C-G-A 3′ 3′ A-G-C T 5′
Create Blunt Ends			
Arthrobacter luteus	*Alu*I	5′ A-G-C-T 3′ 3′ T-C-G-A 5′	5′ A-G C-T 3′ 3′ T-C G-A 5′
Haemophilus aegypticus	*Hae*III	5′ G-G-C-C 3′ 3′ C-C-G-G 5′	5′ G-G C-C 3′ 3′ C-C G-G 5′
Serratia marcescens	*Sma*I	5′ C-C-C-G-G-G 3′ 3′ G-G-G-C-C-C 5′	5′ C-C-C G-G-G 3′ 3′ G-G-G C-C-C 5′

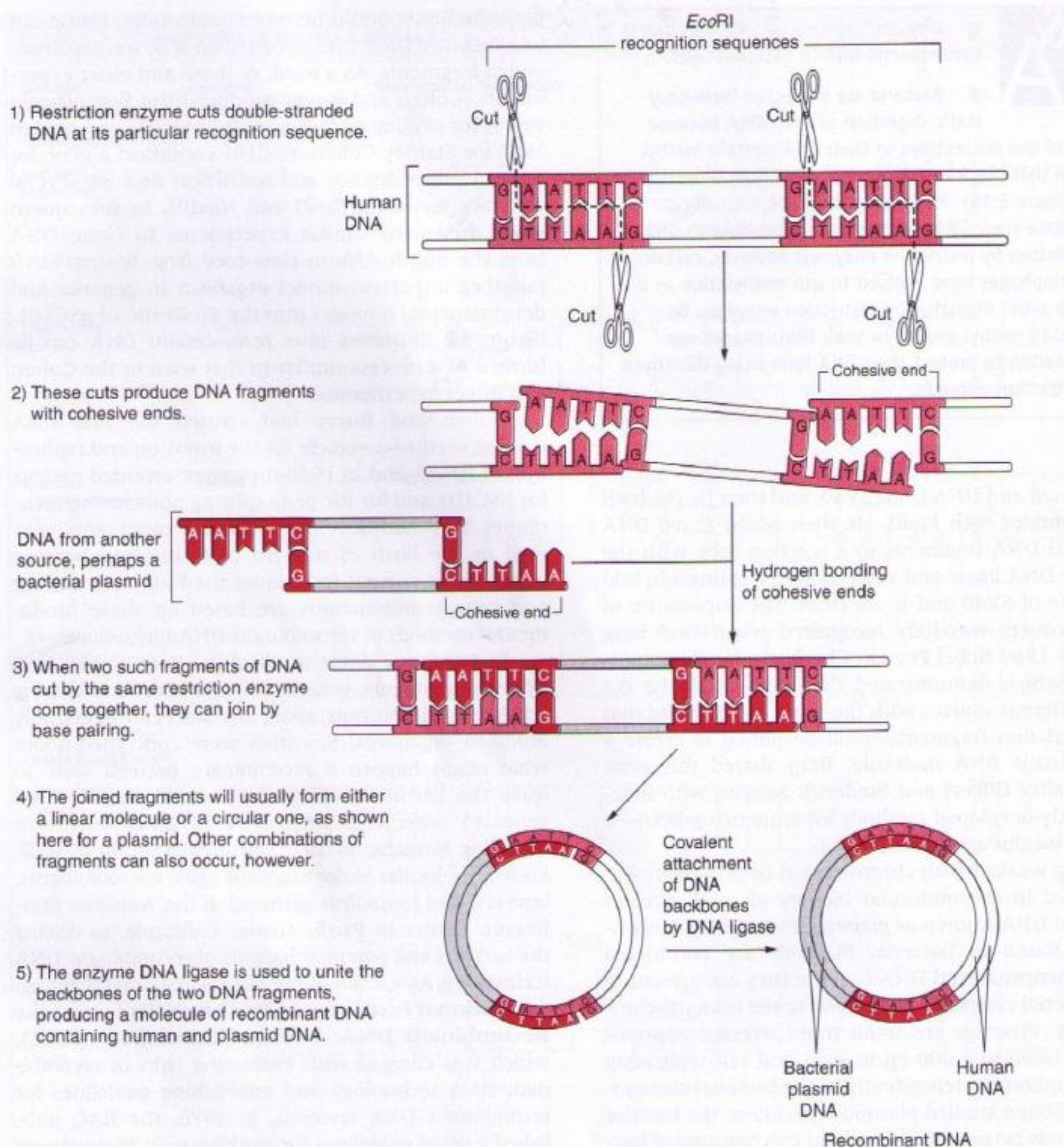


Figure 3.2 Creating Recombinant DNA The restriction enzyme *Eco*RI binds to a specific sequence (5'-GAATTC-3') and then cleaves the DNA backbone, producing DNA fragments. The single-stranded ends of the DNA fragments can hydrogen bond with each other because they have complementary base pairs. The enzyme DNA ligase can then catalyze the formation of covalent bonds in the DNA backbones of the fragments to create a piece of recombinant DNA.

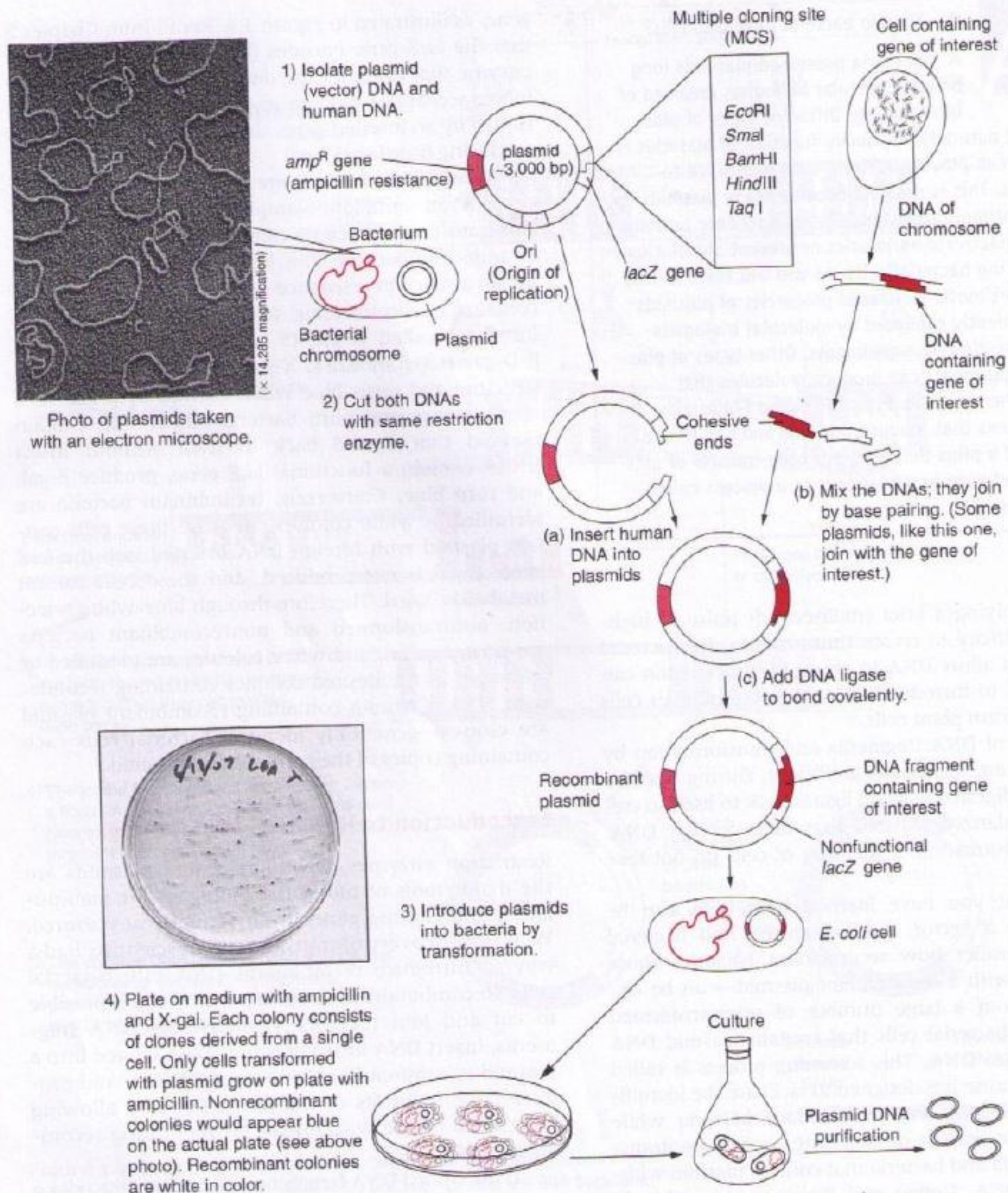


Figure 3.3 Cloning a Gene in a Plasmid and Blue-White Selection

Table 3.2 A COMPARISON OF DNA VECTORS AND THEIR APPLICATIONS

Vector Type	Maximum Insert Size (kb)	Applications	Limitations
Bacterial plasmid vectors (circular)	~6–12	DNA cloning, protein expression, subcloning, direct sequencing of insert	Restricted insert size; limited expression of proteins; copy number problems; replication restricted to bacteria
	DNA	DNA	
Bacteriophage vectors (linear)	~25	cDNA, genomic and expression libraries	Packaging limits DNA insert size; host replication problems
Cosmid (circular)	~35	cDNA and genomic libraries, cloning large DNA fragments	Phage packaging restrictions; not ideal for protein expression; cannot be replicated in mammalian cells
Bacterial artificial chromosome (BAC, circular)	~300	Genomic libraries, cloning large DNA fragments	Replication restricted to bacteria; cannot be used for protein expression
Yeast artificial chromosome (YAC, circular)	200–2,000	Genomic libraries, cloning large DNA fragments	Must be grown in yeast; cannot be used in bacteria
Ti vector (circular)	Varies depending on type of Ti vector used	Gene transfer in plants	Limited to use in plant cells only; number of restriction sites randomly distributed; large size of vector not easily manipulated

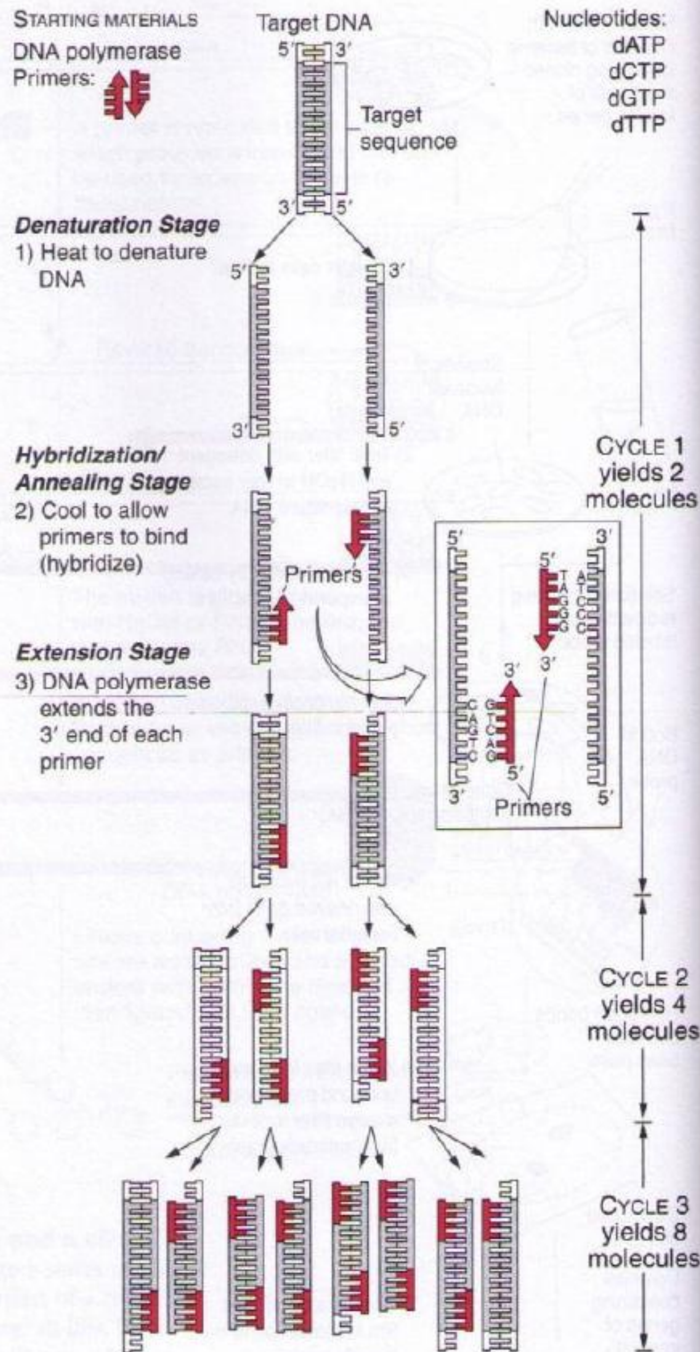


Figure 3.7 The Polymerase Chain Reaction

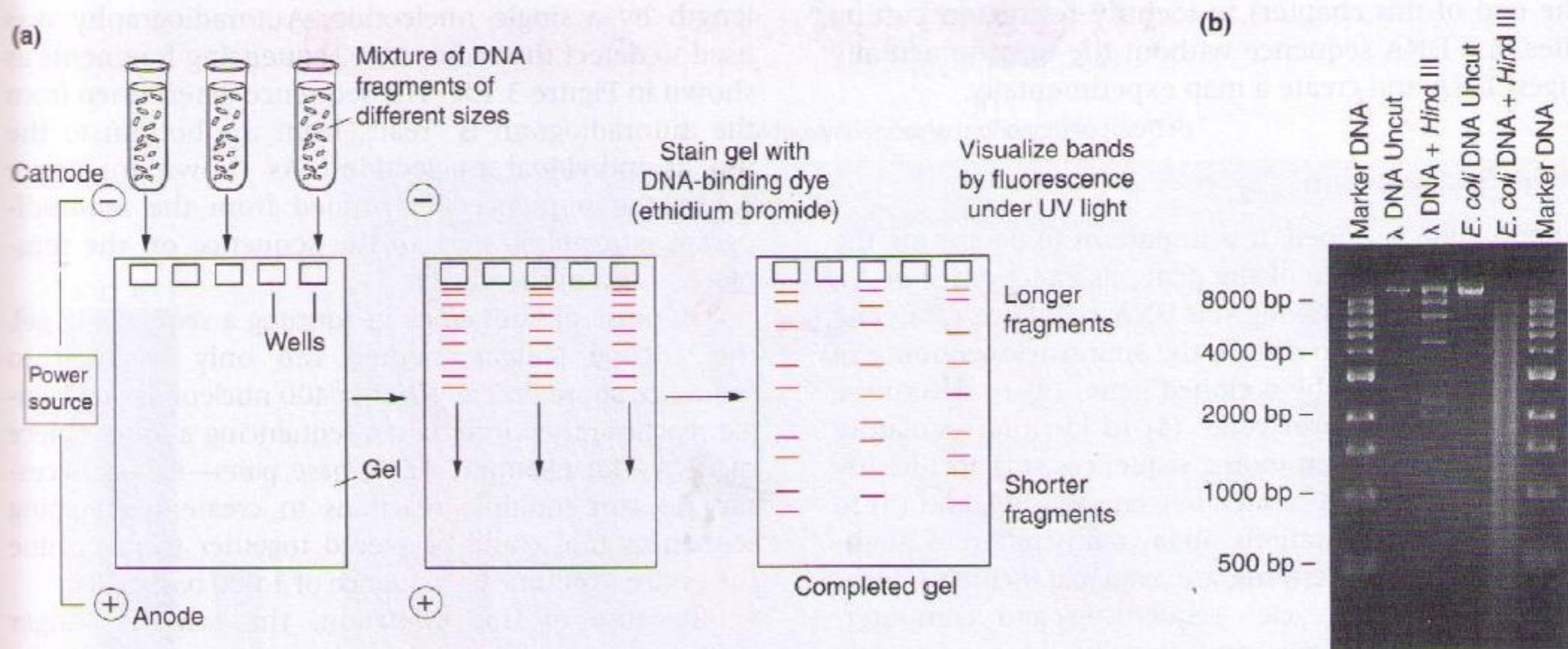
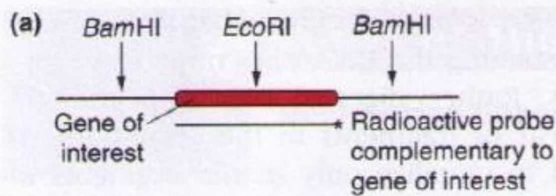
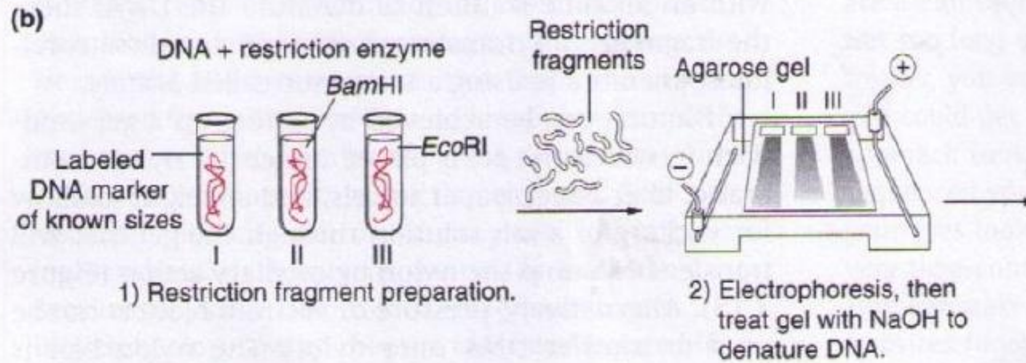
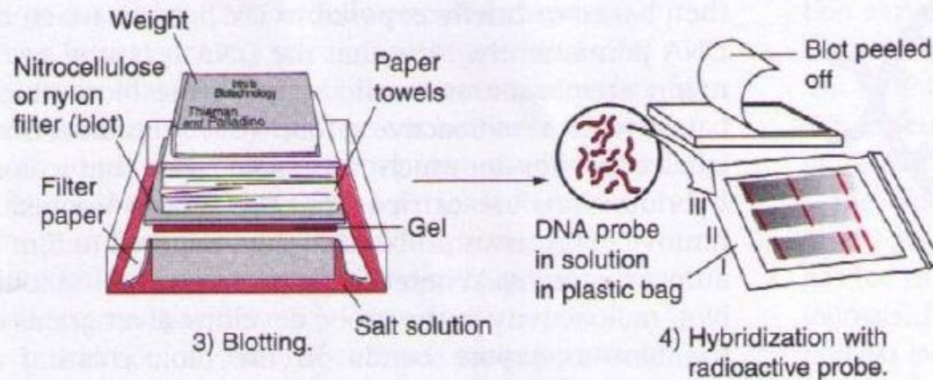


Figure 3.11 Agarose Gel Electrophoresis (a) DNA fragments can be separated and visualized by agarose gel electrophoresis. (b) Photograph of an agarose gel stained with ethidium bromide. Lanes labeled as "Marker DNA" were loaded with commercially prepared DNA size standards. These serve as a ladder of fragments of known size that are used to determine the size of experimental samples of DNA being analyzed. The lane labeled "λ DNA uncut" shows high molecular weight uncut chromosomal DNA from phage λ; "λ DNA + *Hind*III" shows a series of discrete fragments created when λ DNA is digested with the restriction enzyme *Hind*III. The lane labeled "*E. coli* DNA uncut" contains undigested chromosomal DNA and the adjacent lane shows *E. coli* chromosomal DNA digested with *Hind*III (*E. coli* DNA + *Hind*III). Notice how the *Hind*III-digested *E. coli* DNA produces a smear of bands unlike the set of discrete fragments visualized with *Hind*III-digested λ DNA. This smearing is due to the large size of the *E. coli* chromosome and the large number of cutting sites for *Hind*III; so many fragments are created that it is not possible to visualize discrete bands.







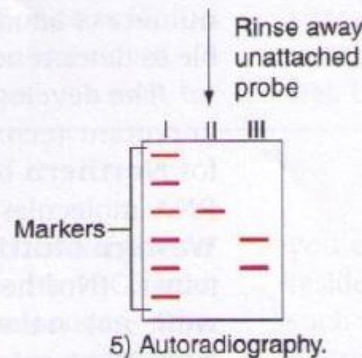


Figure 3.15 Southern Blot Analysis of DNA Fragments (a) Region of DNA for a gene of interest to be studied by Southern blot analysis (b). Steps involved in Southern blotting: (1) The DNA samples to be analyzed are digested with restriction enzymes. (2) Then the mixtures of the restriction fragments from each sample are separated by electrophoresis. (3) When the samples are blotted, capillary action pulls a salt solution upward from a filter paper wick through the gel, transferring the DNA to a nylon filter or blot. The single strands of DNA stick to the blot, positioned in bands exactly as on the gel. (4) The blot is exposed to a solution containing a radioactively labeled probe (a single-stranded DNA complementary to the DNA sequence of interest), which attaches by base pairing to restriction fragments of complementary sequence. (5) Film is laid over the blot. The radioactivity in the bound probe exposes the film to form an image corresponding to specific DNA bands on the blot that base pair with the probe.