Lecture 6: Molecular Techniques

1. Restriction Mapping
2. Molecular cloning

    Inserts and vectors

    Restriction enzymes and ligase

    Fig. 9., 9.5b, 9.6; 9.7, 9.8, 9.9, 9.10, 9.11, 9.12

    Table 9.1, 9.2

    Read p279-293

Fig. 9..2

Fig.

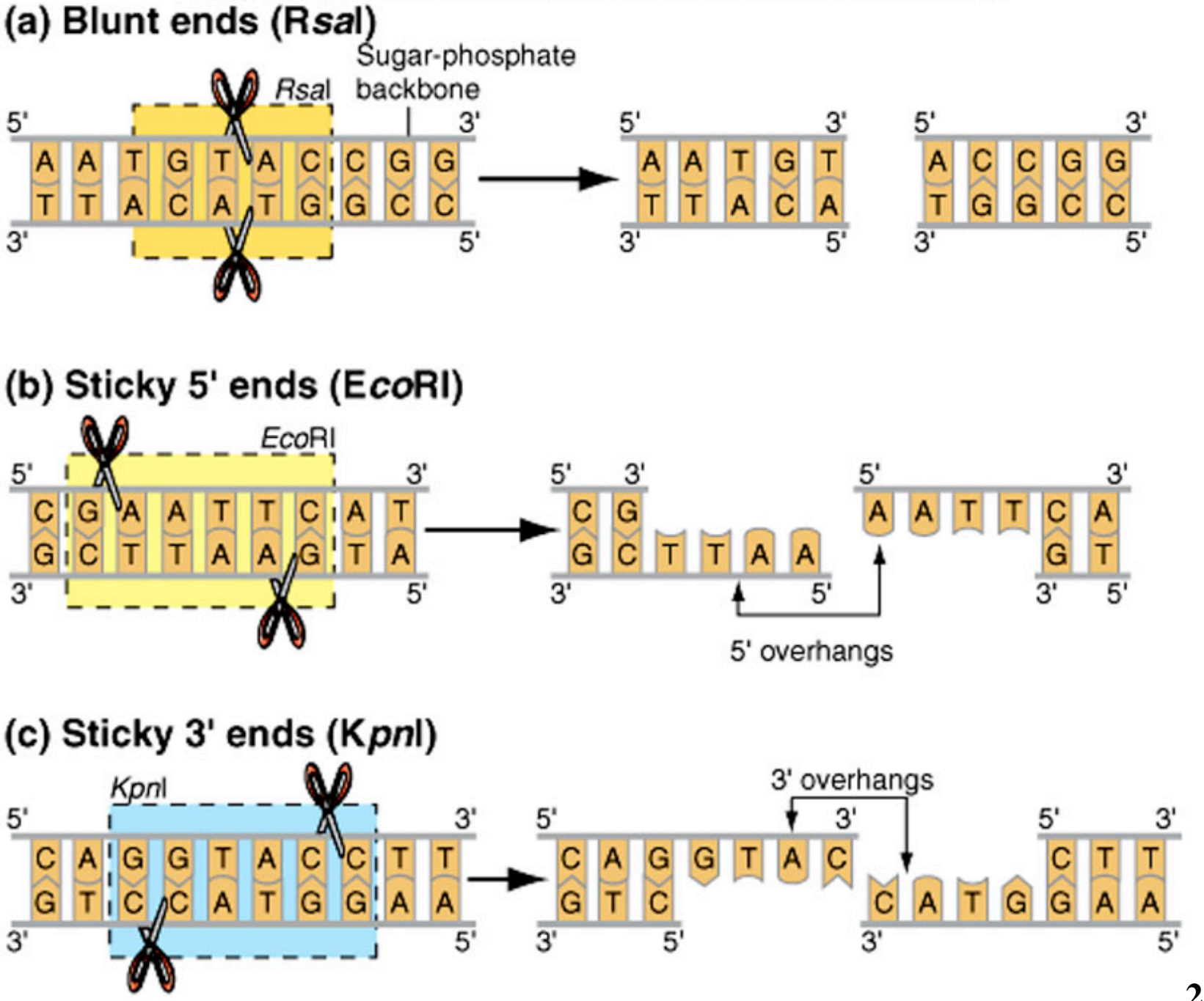


TABLE 9.1 Ten Commonly Used Restriction Enzymes

Enzyme	Sequence of Recognition Site	Microbial Origin
*Taq*I	5′ T C G A 3′ 3′ A G C T 5′	*Thermus aquaticus* YTI
*Rsa*I	5′ G T A C 3′ 3′ C A T G 5′	*Rhodopseudomonas sphaeroides*
*Sau*3AI	5′ G A T C 3′ 3′ C T A G 5′	*Staphylococcus aureus* 3A
*Eco*RI	5′ G A A T T C 3′ 3′ C T T A A G 5′	*Escherichia coli*
*Bam*HI	5′ G G A T C C 3′ 3′ C C T A G G 5′	*Bacillus amyloliquefaciens* H.
*Hind*III	5′ A A G C T T 3′ 3′ T T C G A A 5′	*Haemophilus influenzae*
*Kpn*I	5′ G G T A C C 3′ 3′ C C A T G G 5′	*Klebsiella pneumoniae* OK8
*Cla*I	5′ A T C G A T 3′ 3′ T A G C T A 5′	*Caryophanon latum*
*Bss*HII	5′ G C G C G C 3′ 3′ C G C G C G 5′	*Bacillus stearothermophilus*
*Not*I	5′ G C G G C C G C 3′ 3′ C G C C G G C G 5′	*Nocardia otitidiscaviarum*

Fig. 9.6

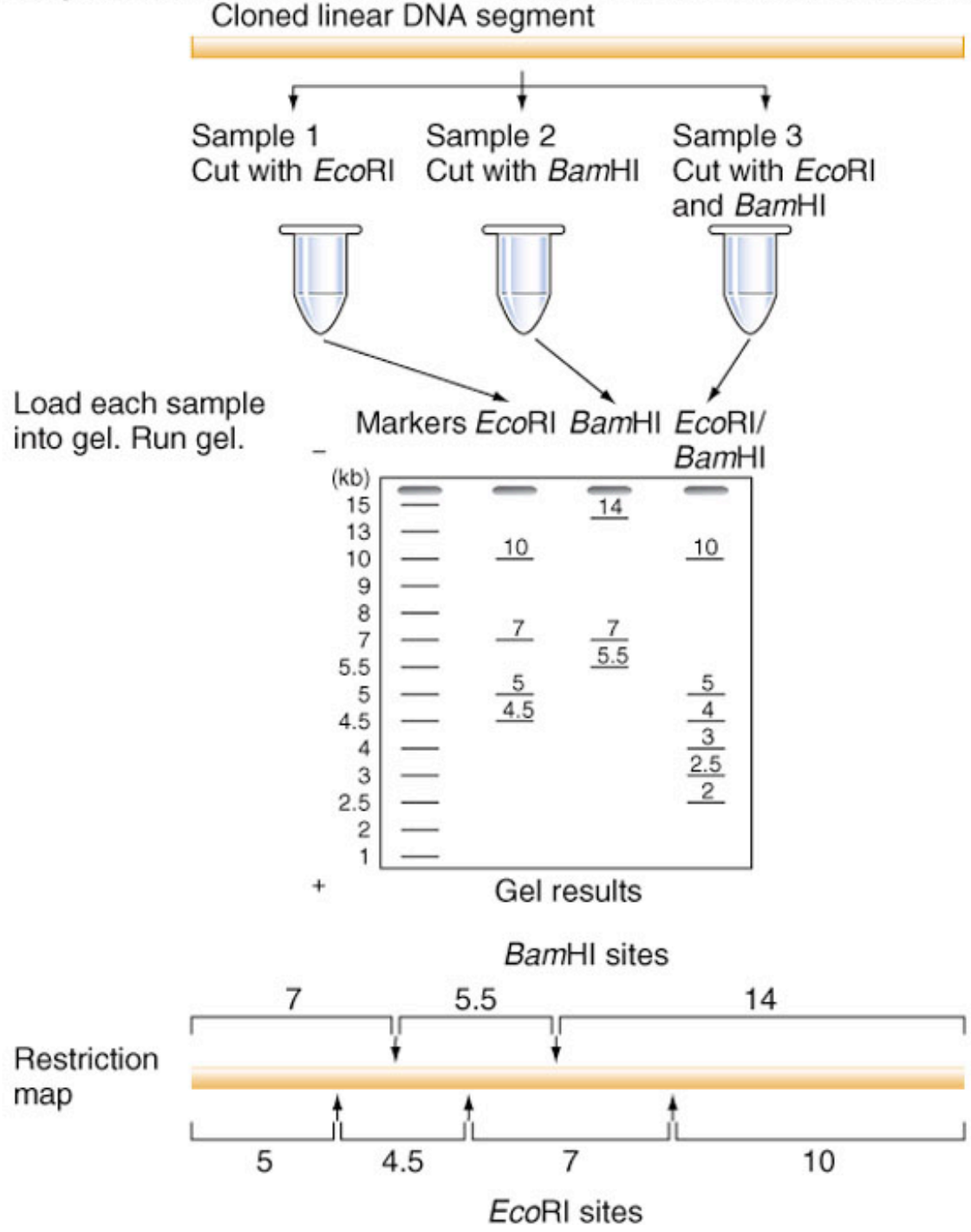
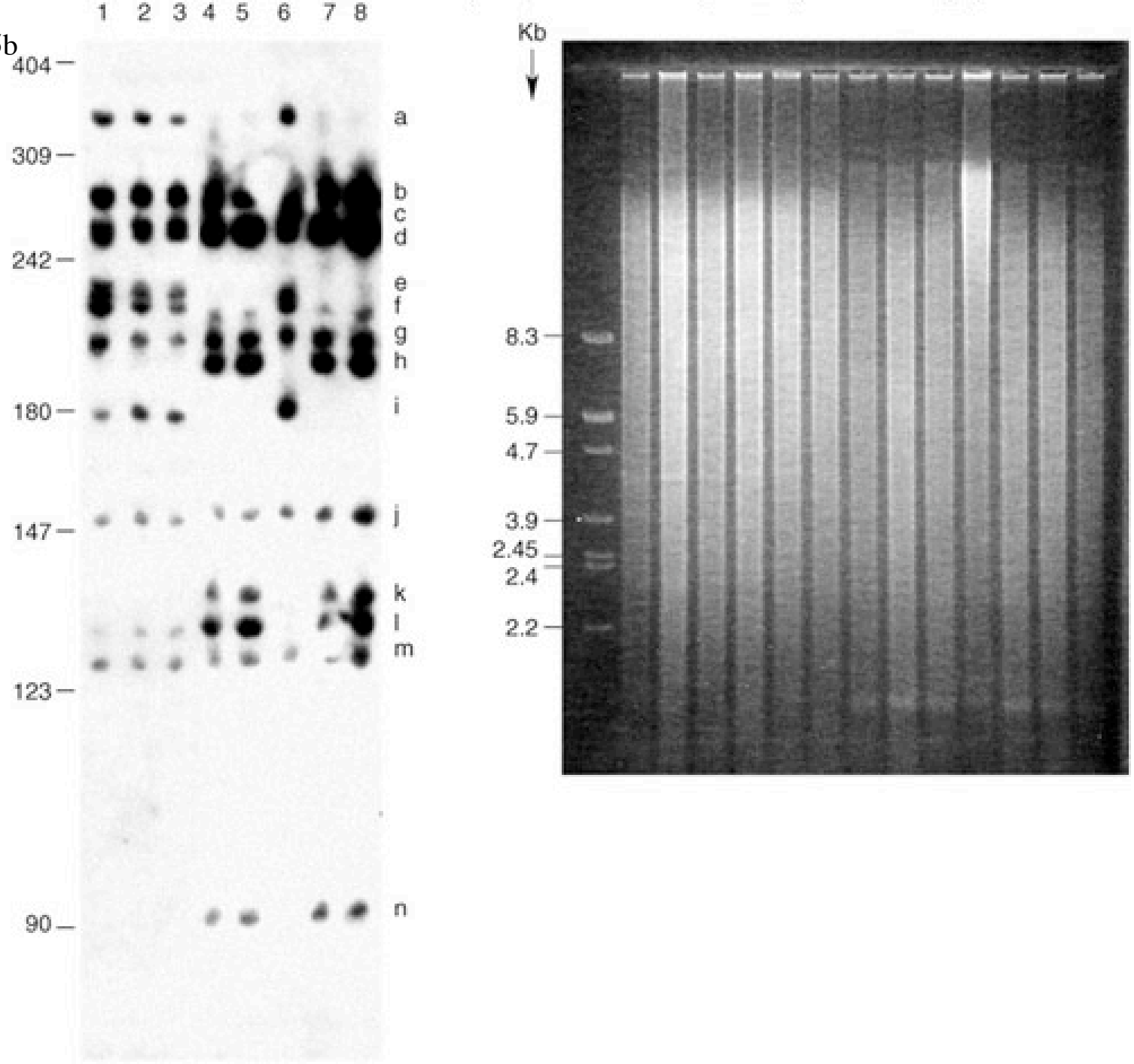


Fig. 9.5b



Gel electrophoresis

2. Molecular Cloning

Inserts and vectors

TABLE 9.2 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	*E.coli*	Up to 15 kb	cDNA libraries; subcloning
Bacteriophage lambda	Virus (linear DNA)	*E.coli*	Up to 25 kb	Genomic and cDNA libraries
Cosmid	Double-stranded circular DNA	*E.coli*	30–45 kb	Genomic libraries
Bacteriophage P1	Virus (circular DNA)	*E.coli*	70–90 kb	Genomic libraries
BAC	Bacterial artificial chromosome	*E.coli*	100–500 kb	Genomic libraries
YAC	Yeast artificial chromosome	Yeast	250–2000 kb (2 megabases)	Genomic libraries

Restriction Digestion

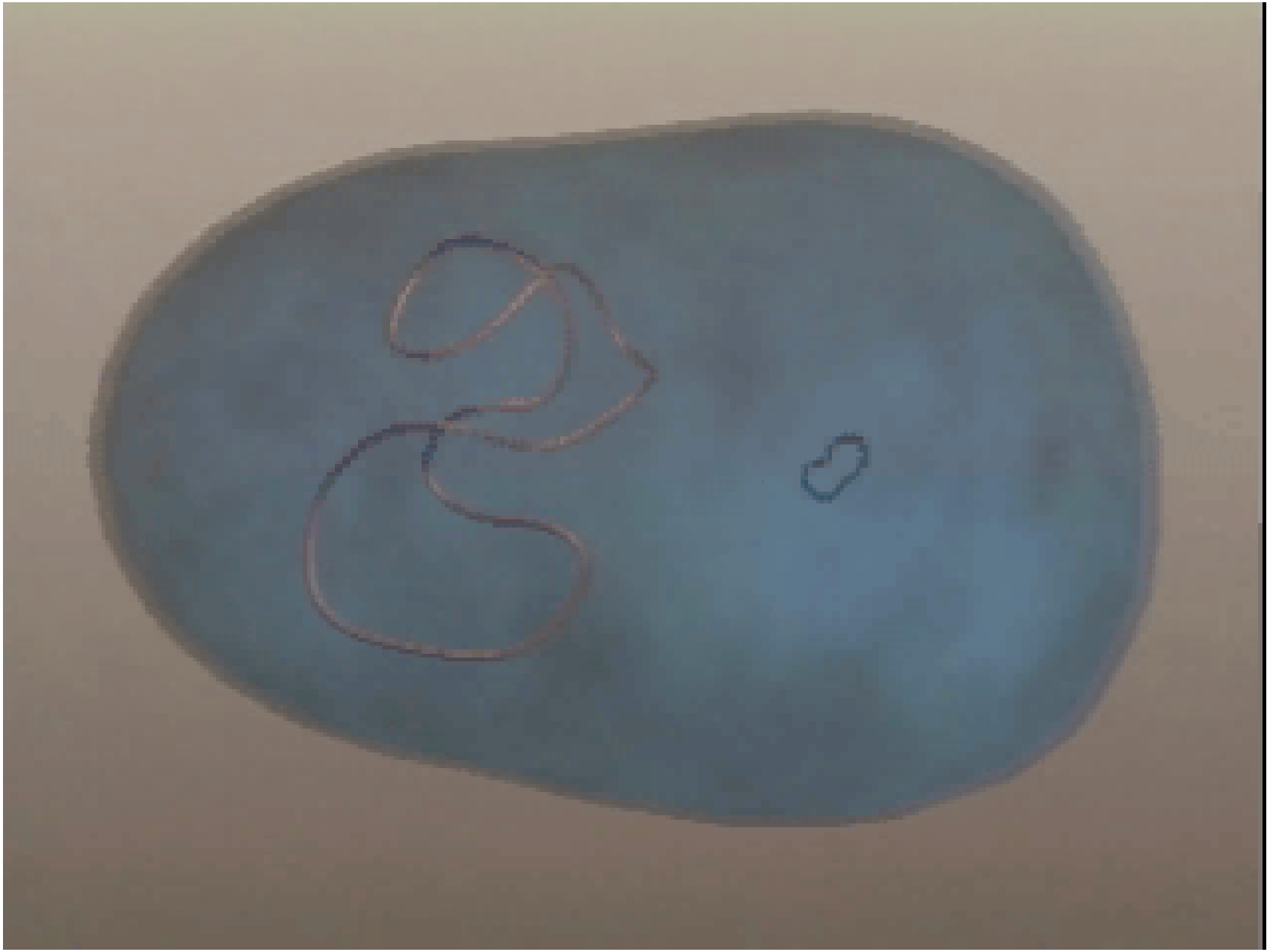


Fig. 9.7

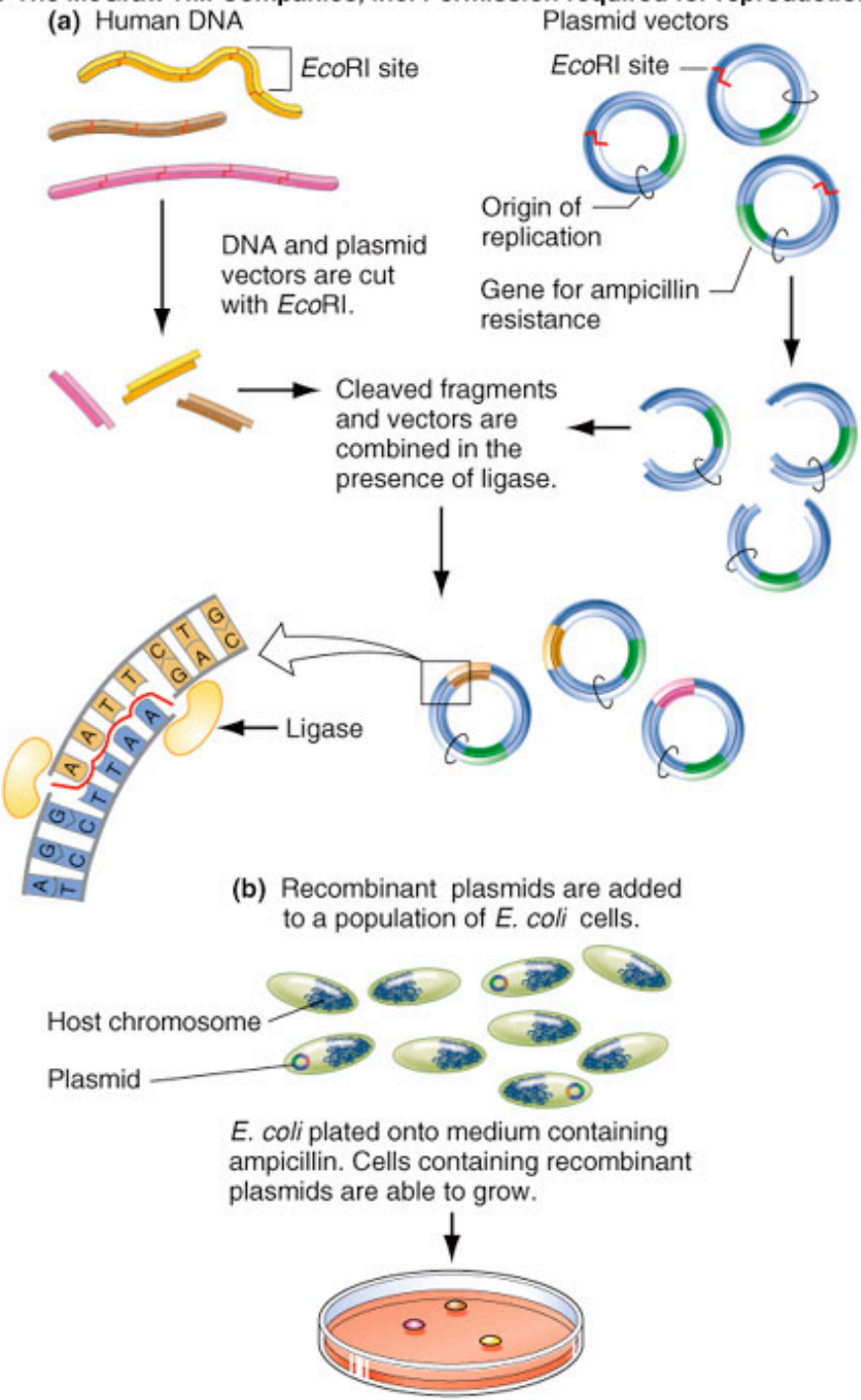


Fig. 9.8

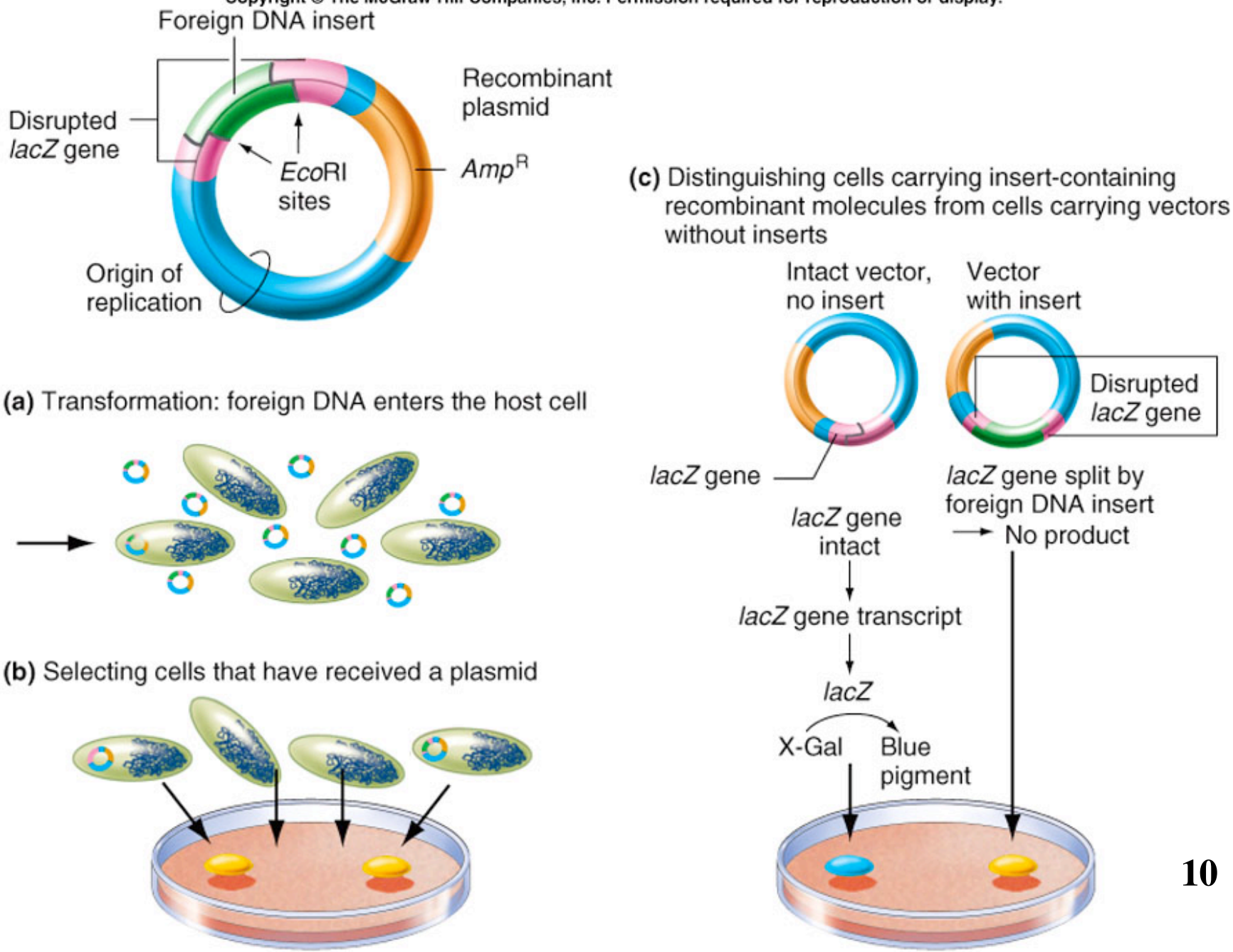
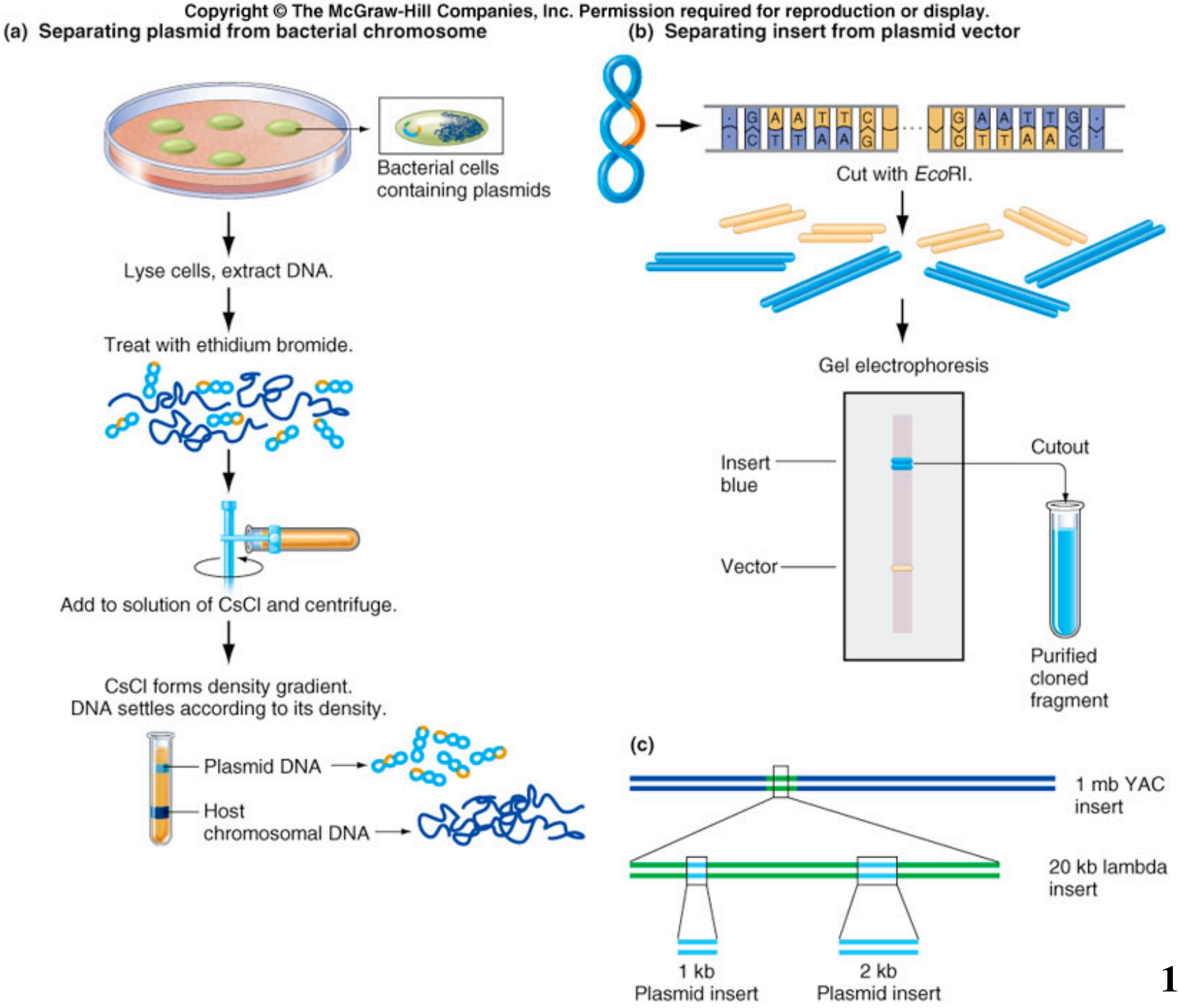


Fig. 9.9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(a) Separating plasmid from bacterial chromosome
(b) Separating insert from plasmid vector
Bacterial cells containing plasmids
Lyse cells, extract DNA.
Treat with ethidium bromide.
Add to solution of CsCl and centrifuge.
CsCl forms density gradient.
DNA settles according to its density.
Plasmid DNA
Host chromosomal DNA
Cut with EcoRI.
Gel electrophoresis
Insert blue
Vector
Cutout
Purified cloned fragment
(c)
1 mb YAC insert
20 kb lambda insert
1 kb Plasmid insert
2 kb Plasmid insert

Genomic Library
Human: 3,000,000 kb/haploid genome
150 kb per insert--20,000 clones equals a genome
10kb per insert--300,000 clones (genome equivalent)

cDNA library

Expression Libraries

Fig. 9.10

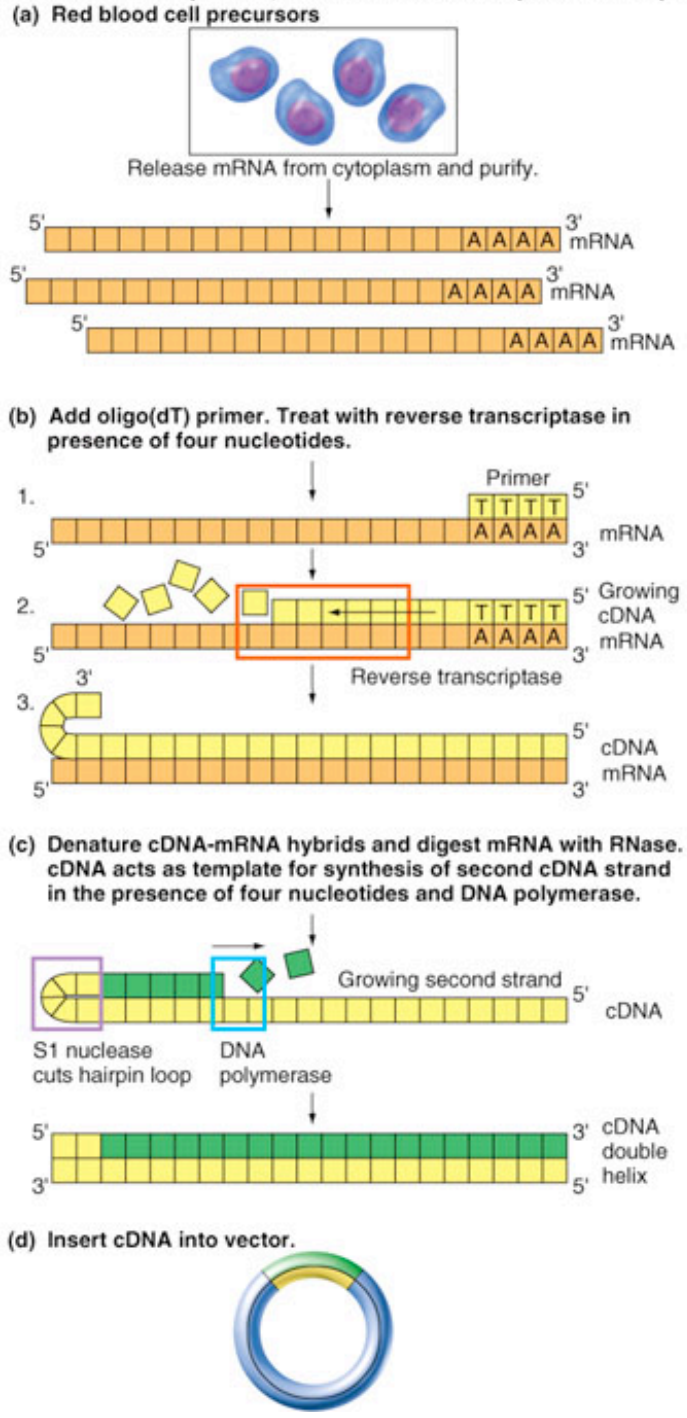

Fig. 9.11