Modern Tools of Molecular Biology

Restriction Enzymes, DNA Vectors, Molecular Cloning, High Throughput Sequencing and Protein/DNA Mapping

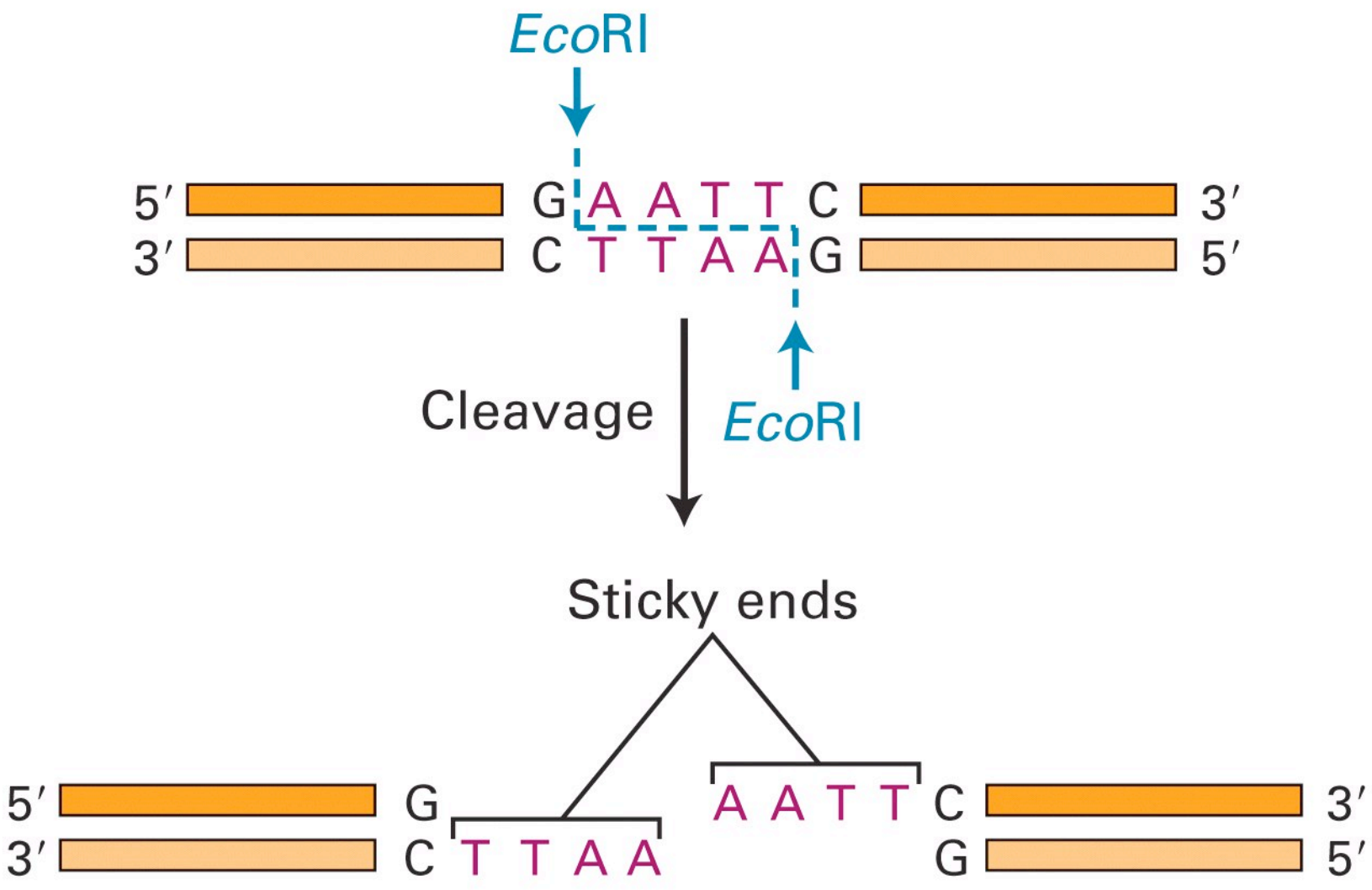
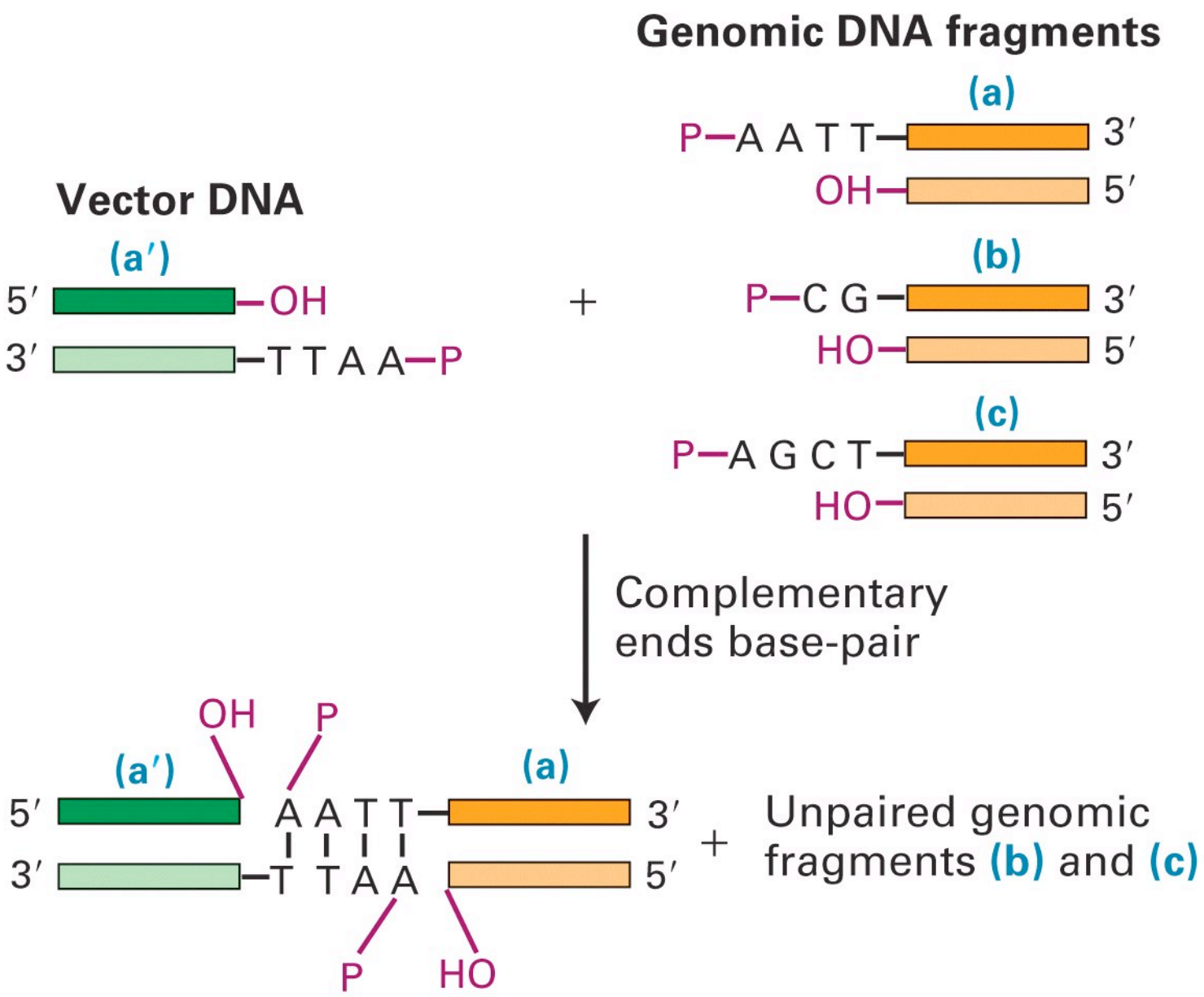
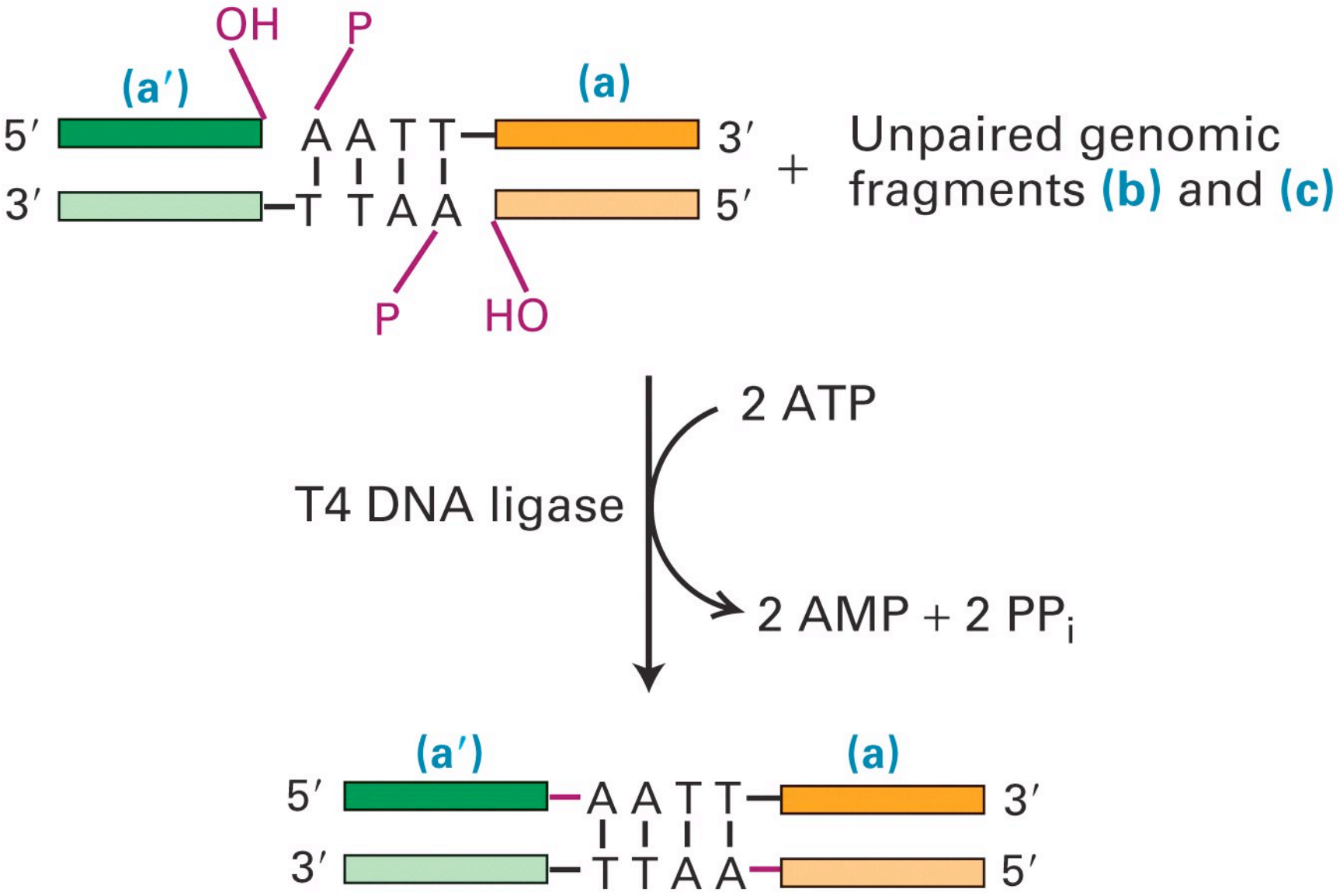

EcoRI
5′
3′
G A A T T C
C T T A A G
3′
5′
Cleavage
EcoRI
Sticky ends
5′
3′
G
C T T A A
A A T T C
G
3′
5′

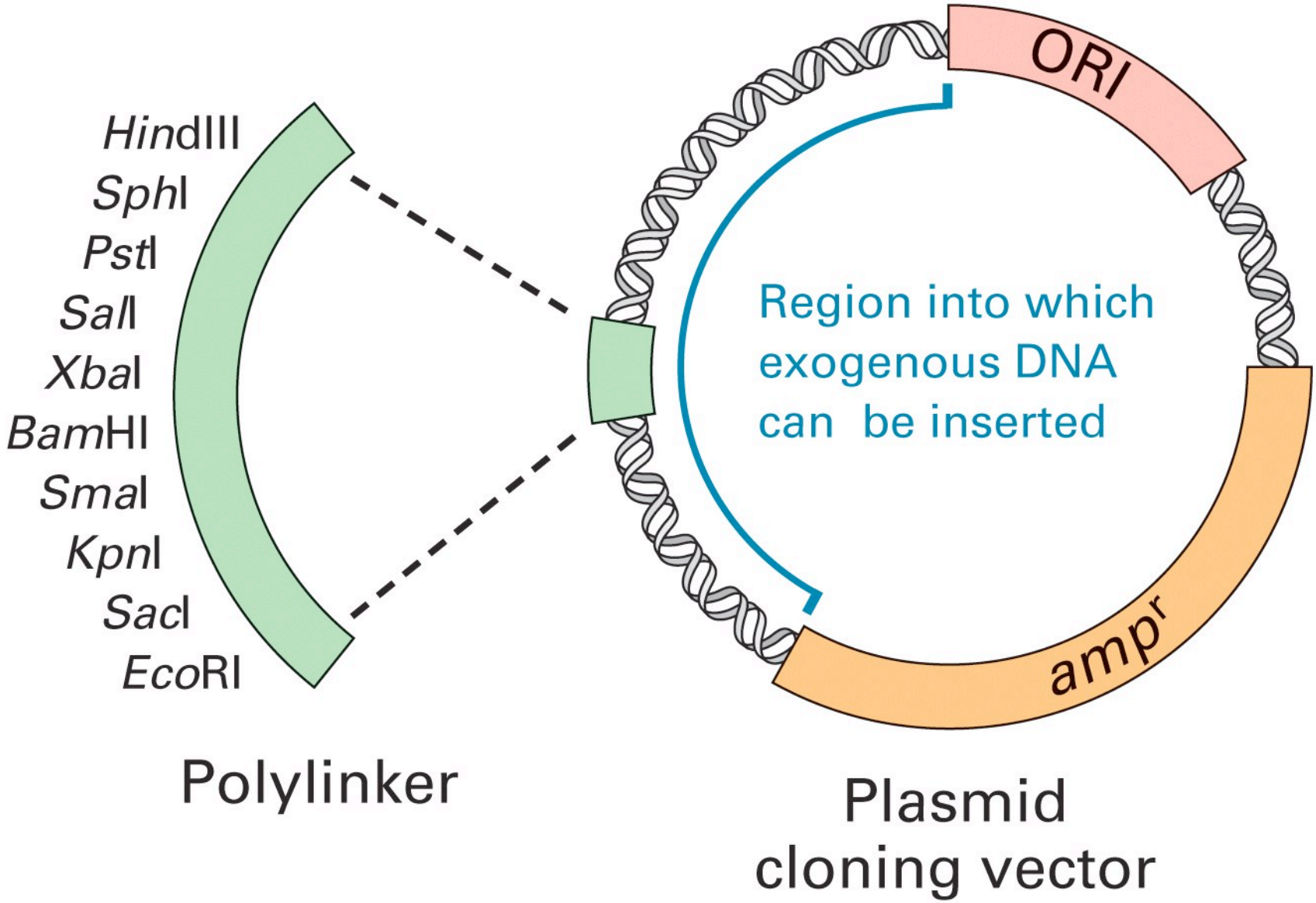
TABLE 9-1 Selected Restriction Enzymes and Their Recognition Sequences

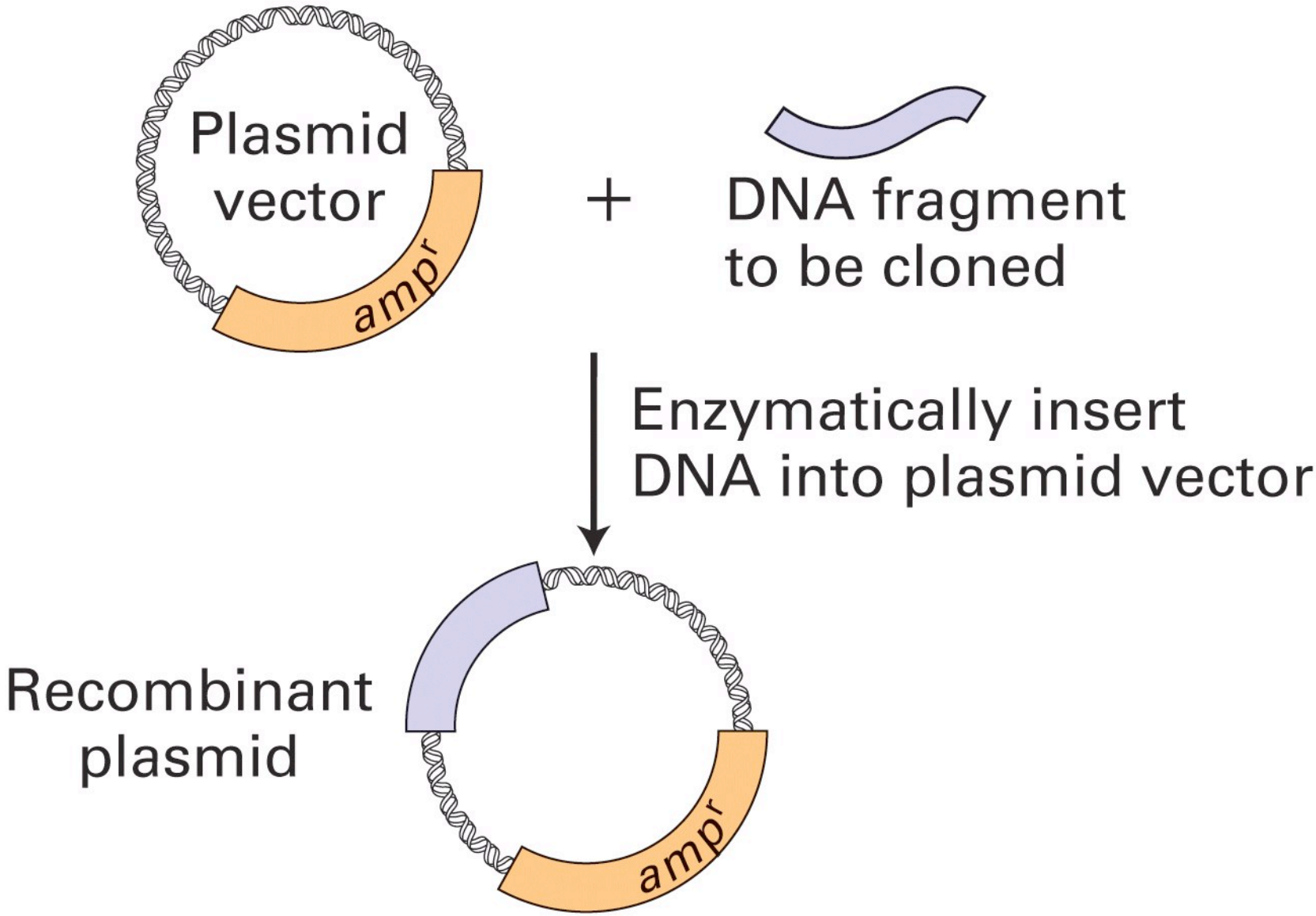
Enzyme	Source Microorganism	Recognition Site*	Ends Produced
*Pst*I	*Providencia stuartii*	↓ -C-T-G-C-A-G- -G-A-C-G-T-C- ↑	Sticky
*Sac*I	*Streptomyces achromogenes*	↓ -G-A-G-C-T-C- -C-T-C-G-A-G- ↑	Sticky
*Sal*I	*Streptomyces albue*	↓ -G-T-C-G-A-C- -C-A-G-C-T-G- ↑	Sticky
*Sma*I	*Serratia marcescens*	↓ -C-C-C-G-G-G- -G-G-G-C-C-C- ↑	Blunt
*Sph*I	*Streptomyces phaeochromogenes*	↓ -G-C-A-T-G-C- -C-G-T-A-C-G- ↑	Sticky
*Xba*I	*Xanthomonas badrii*	↓ -T-C-T-A-G-A- -A-G-A-T-C-T- ↑	Sticky

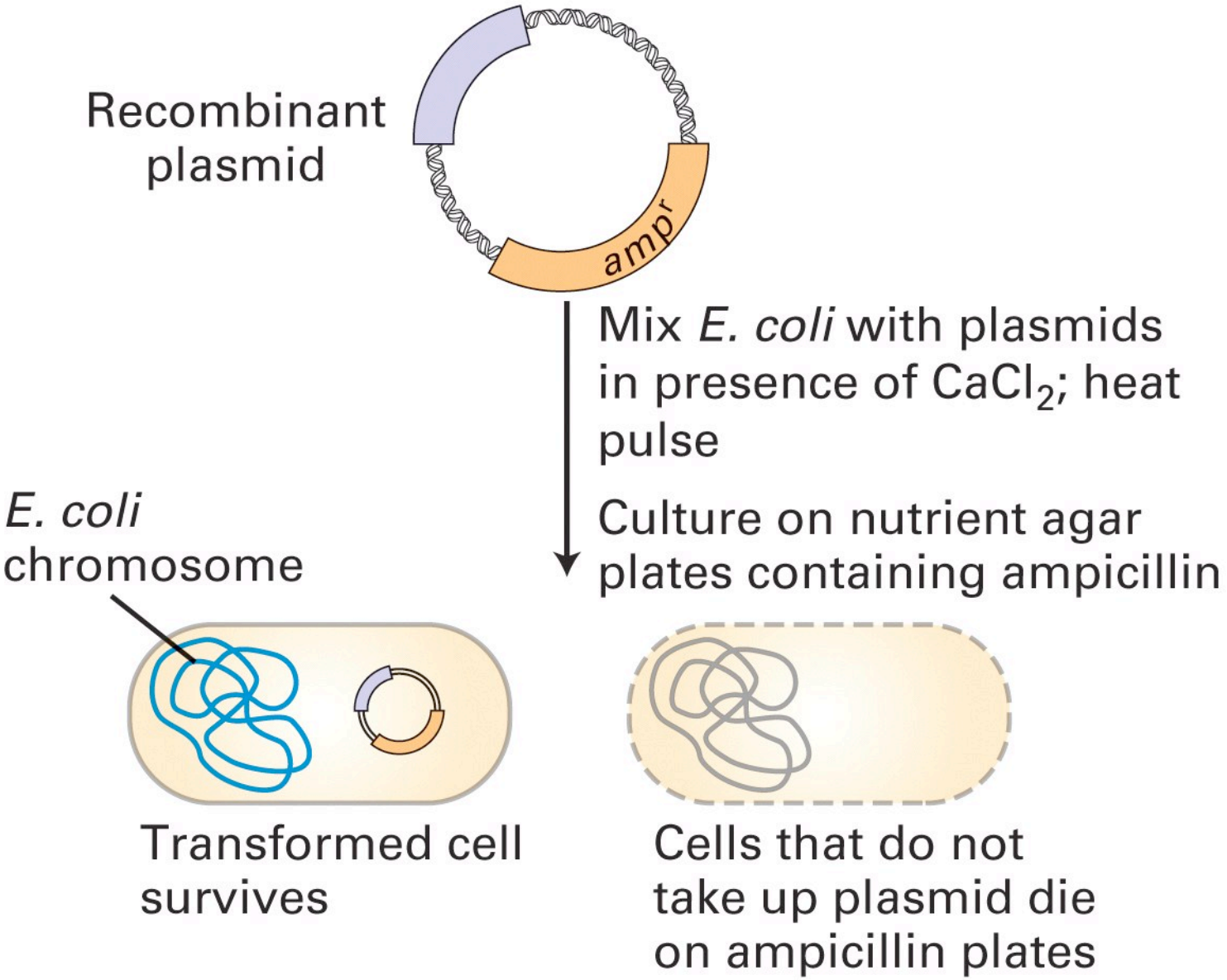
*These recognition sequences are included in a common polylinker sequence (see Figure 9-12).

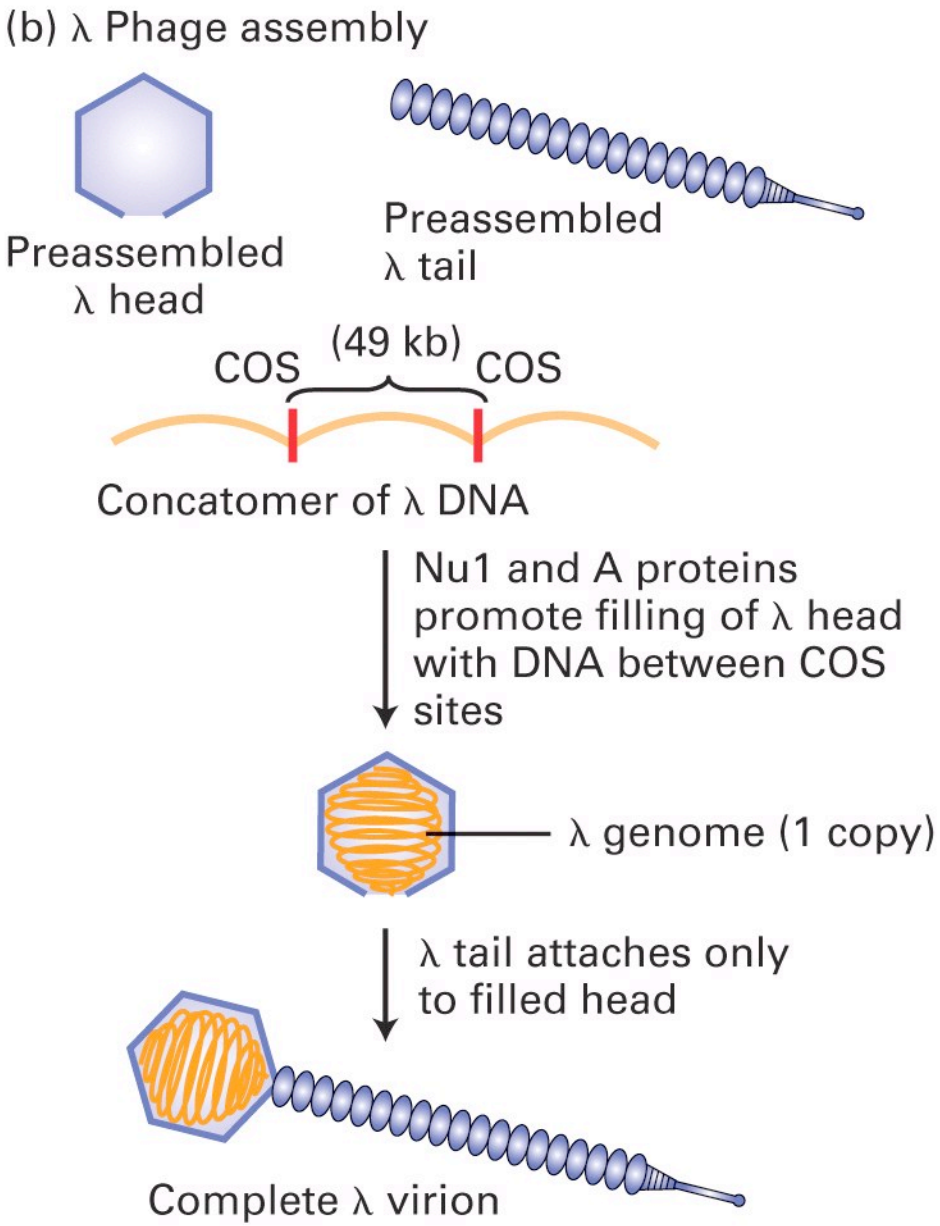

Genomic DNA fragments
(a)
P—A A T T
3′
OH
5′
Vector DNA
(a′)
5′
OH
3′
T T A A—P
+
(b)
P—C G
3′
HO
5′
(c)
P—A G C T
3′
HO
5′
Complementary ends base-pair
OH
P
(a′)
(a)
5′
A A T T
3′
3′
T T A A
5′
+
Unpaired genomic fragments (b) and (c)
P
HO

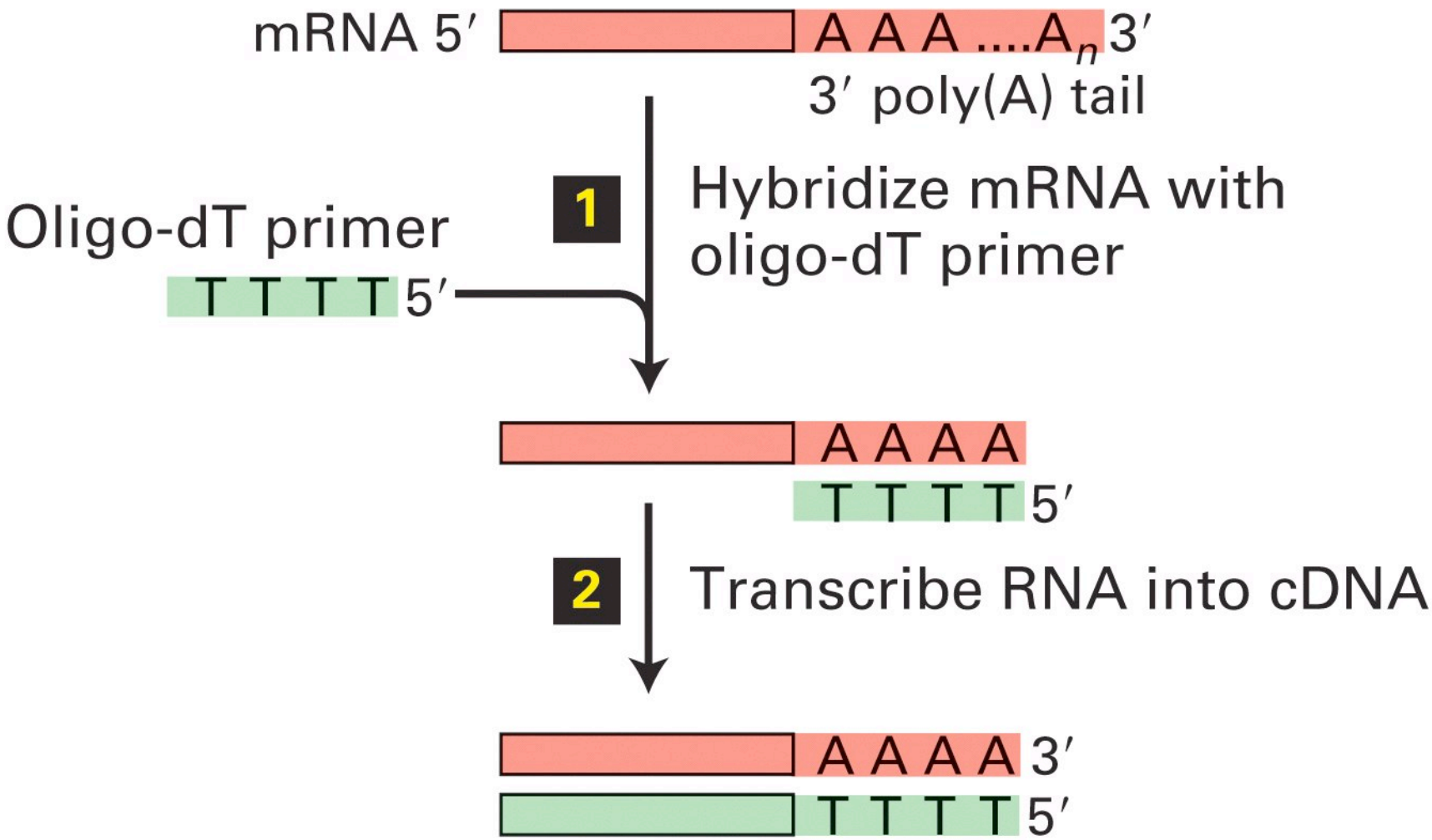

OH
P
(a′)
(a)
5′
A A T T
3′
3′
T T A A
5′
P
HO
+
Unpaired genomic fragments (b) and (c)
2 ATP
T4 DNA ligase
2 AMP + 2 PPi
(a′)
(a)
5′
A A T T
3′
3′
T T A A
5′

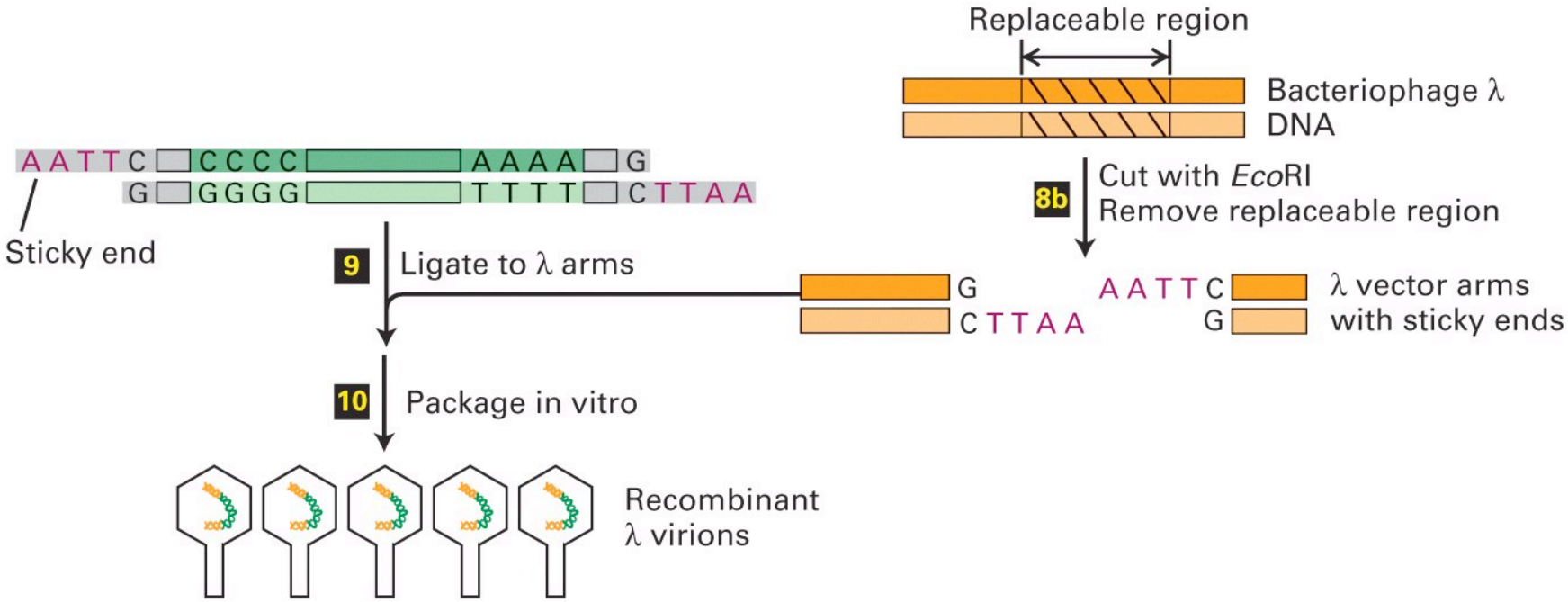

HindIII
SphI
PstI
SalI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI
Polylinker
ORI
Region into which
exogenous DNA
can be inserted
ampr
Plasmid
cloning vector

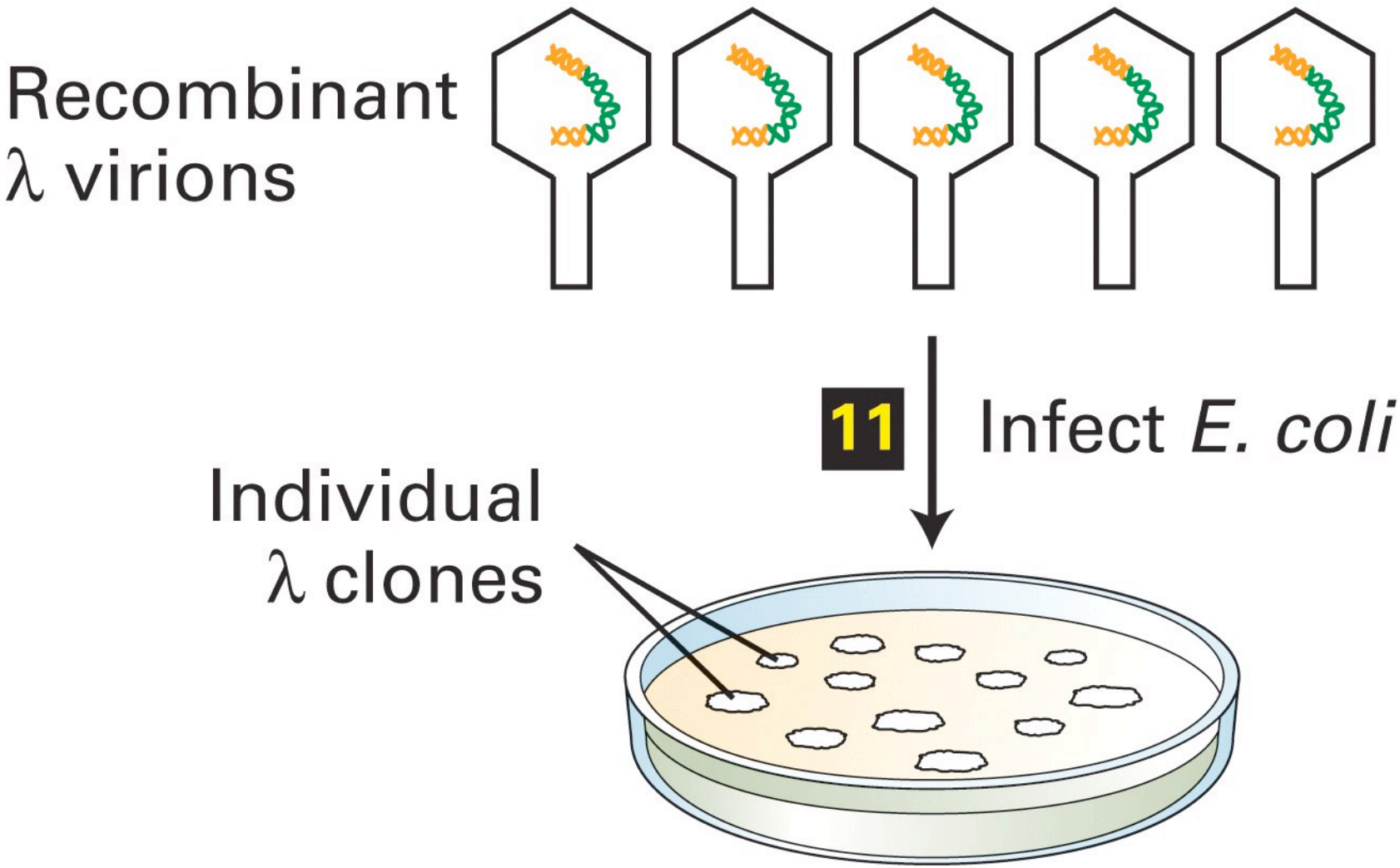

Plasmid vector
ampr
+
DNA fragment to be cloned
Enzymatically insert DNA into plasmid vector
Recombinant plasmid
ampr

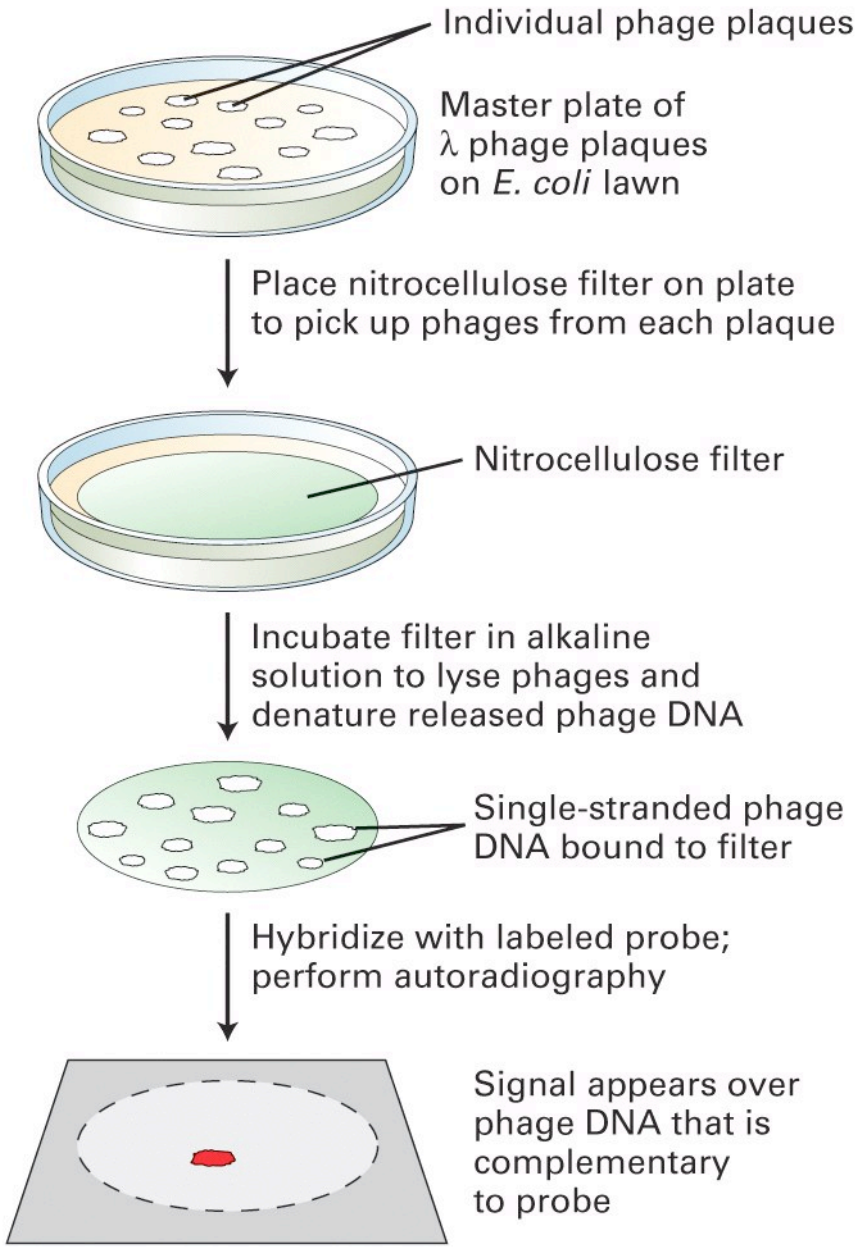

Recombinant plasmid
ampr
Mix E. coli with plasmids in presence of CaCl2; heat pulse
Culture on nutrient agar plates containing ampicillin
E. coli chromosome
Transformed cell survives
Cells that do not take up plasmid die on ampicillin plates


(b) λ Phage assembly
Preassembled λ head
Preassembled λ tail
COS
(49 kb)
COS
Concatomer of λ DNA
Nu1 and A proteins promote filling of λ head with DNA between COS sites
λ genome (1 copy)
λ tail attaches only to filled head
Complete λ virion

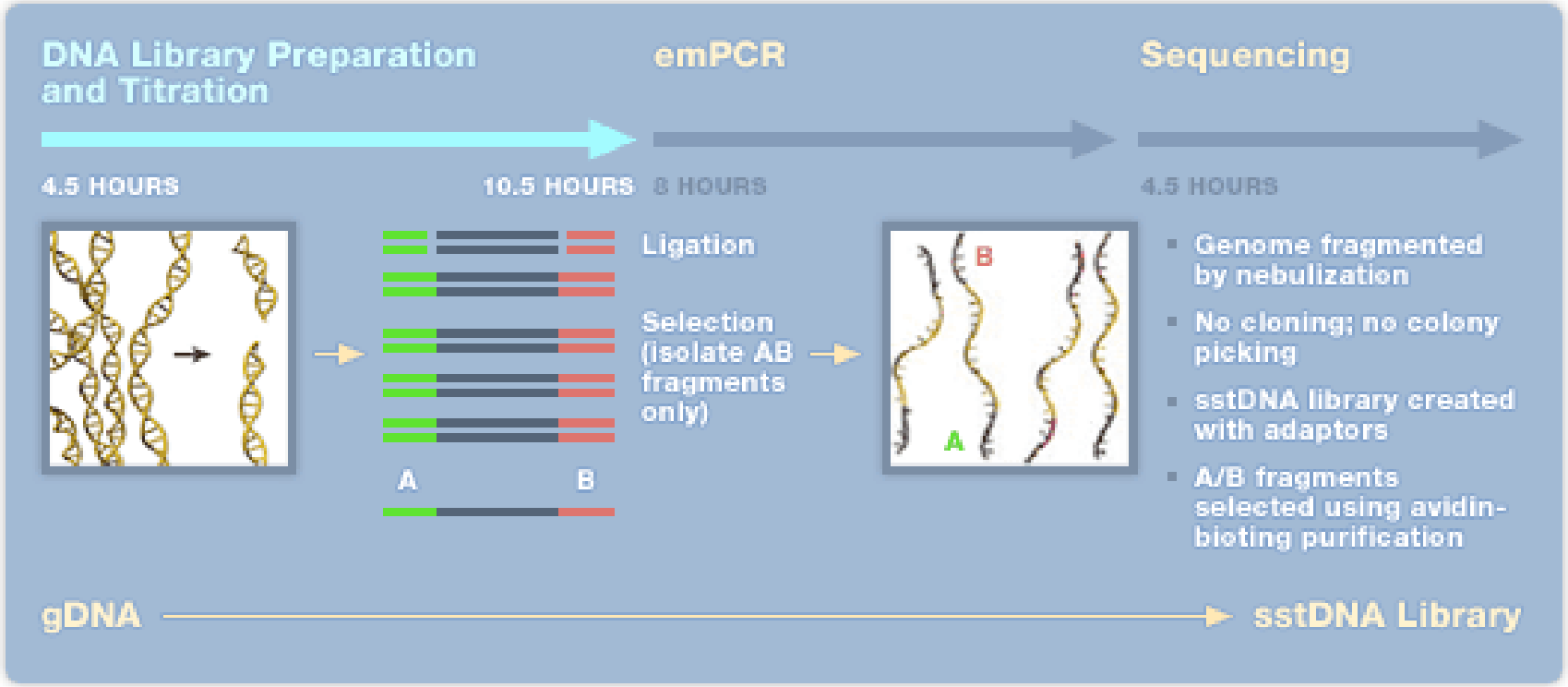

mRNA 5′
A A A ....A$_n$ 3′
3′ poly(A) tail
Oligo-dT primer
T T T T 5′
1
Hybridize mRNA with oligo-dT primer
A A A A
T T T T 5′
2
Transcribe RNA into cDNA
A A A A 3′
T T T T 5′


Replaceable region
Bacteriophage λ
DNA
8b
Cut with EcoRI
Remove replaceable region
A A T T C
C C C C
A A A A
G
G
G G G G
T T T T
C T T A A
Sticky end
9
Ligate to λ arms
G
C T T A A
A A T T C
G
λ vector arms
with sticky ends
10
Package in vitro
Recombinant
λ virions

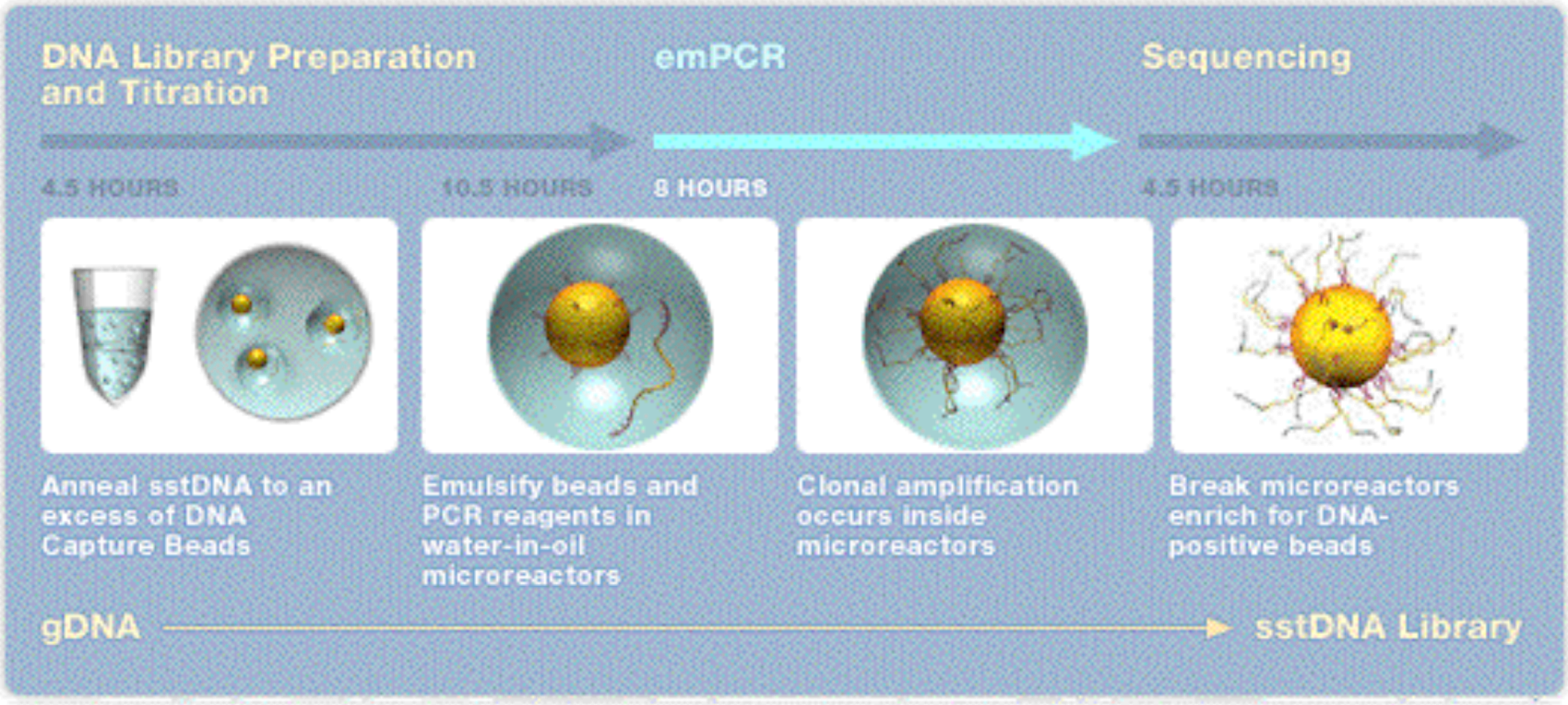

Recombinant
λ virions
11
Infect *E. coli*
Individual
λ clones


Individual phage plaques
Master plate of
λ phage plaques
on E. coli lawn
Place nitrocellulose filter on plate
to pick up phages from each plaque
Nitrocellulose filter
Incubate filter in alkaline
solution to lyse phages and
denature released phage DNA
Single-stranded phage
DNA bound to filter
Hybridize with labeled probe;
perform autoradiography
Signal appears over
phage DNA that is
complementary
to probe

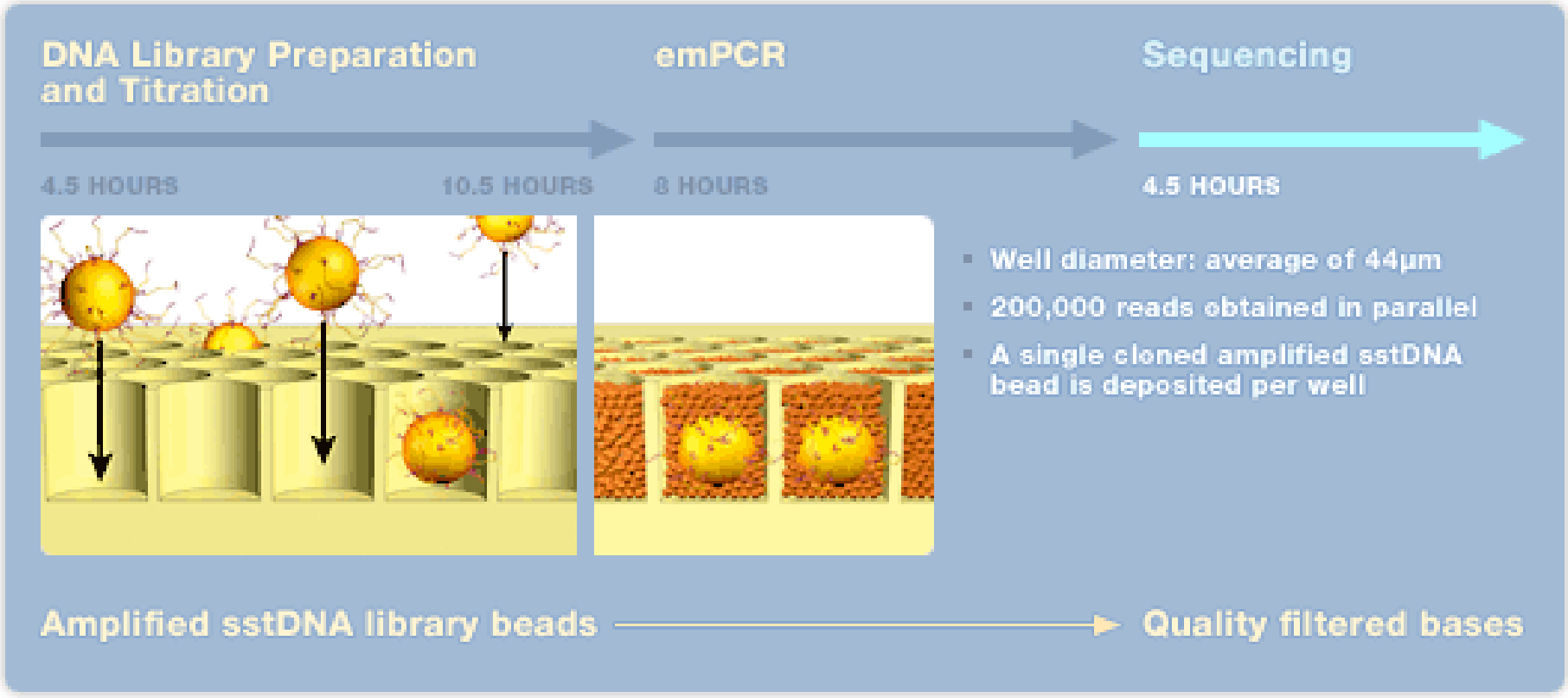
454 : High Through-put DNA Sequencing



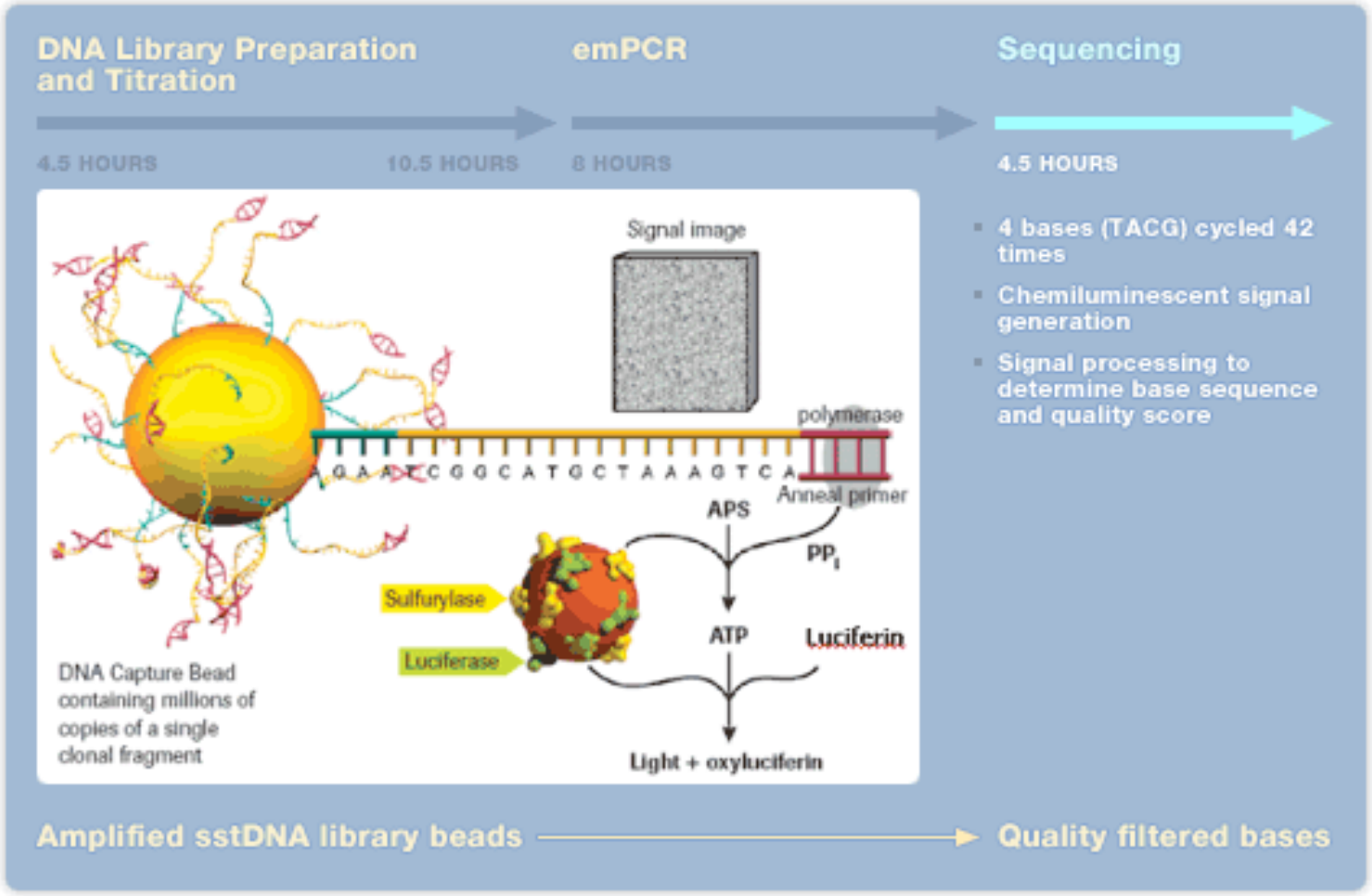
Bead based technology for rapid parallel sequencing



Multi-well Reactions

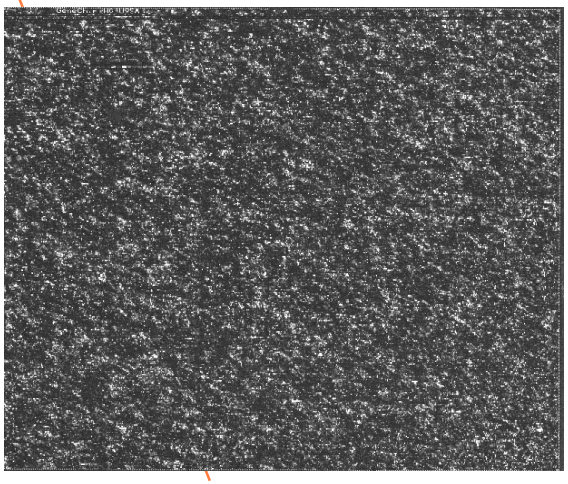


Chemi-luminescent sequencing



Micro Array Technology

Raw data image with >6,500,000 different complementary probes

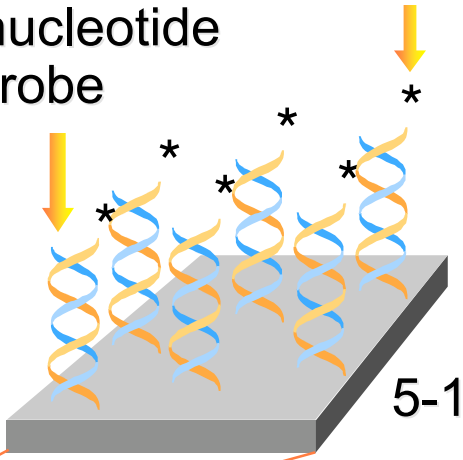


1.28 cm

Hybridized probe cell

RNA/DNA target

Oligonucleotide probe



5-11µm

Thousands of copies of a specific oligonucleotide probe in each feature

High Probe Densities Enable New Applications

Feature Size

	18um	11um	8um	5um	1um
Features	**500,000**	**1,300,000**	**2,600,000**	**6,500,000**	**163,000,000**

Commercial expression arrays

Commercial genotyping arrays

Exon, tiling and 500K Arrays

Future development

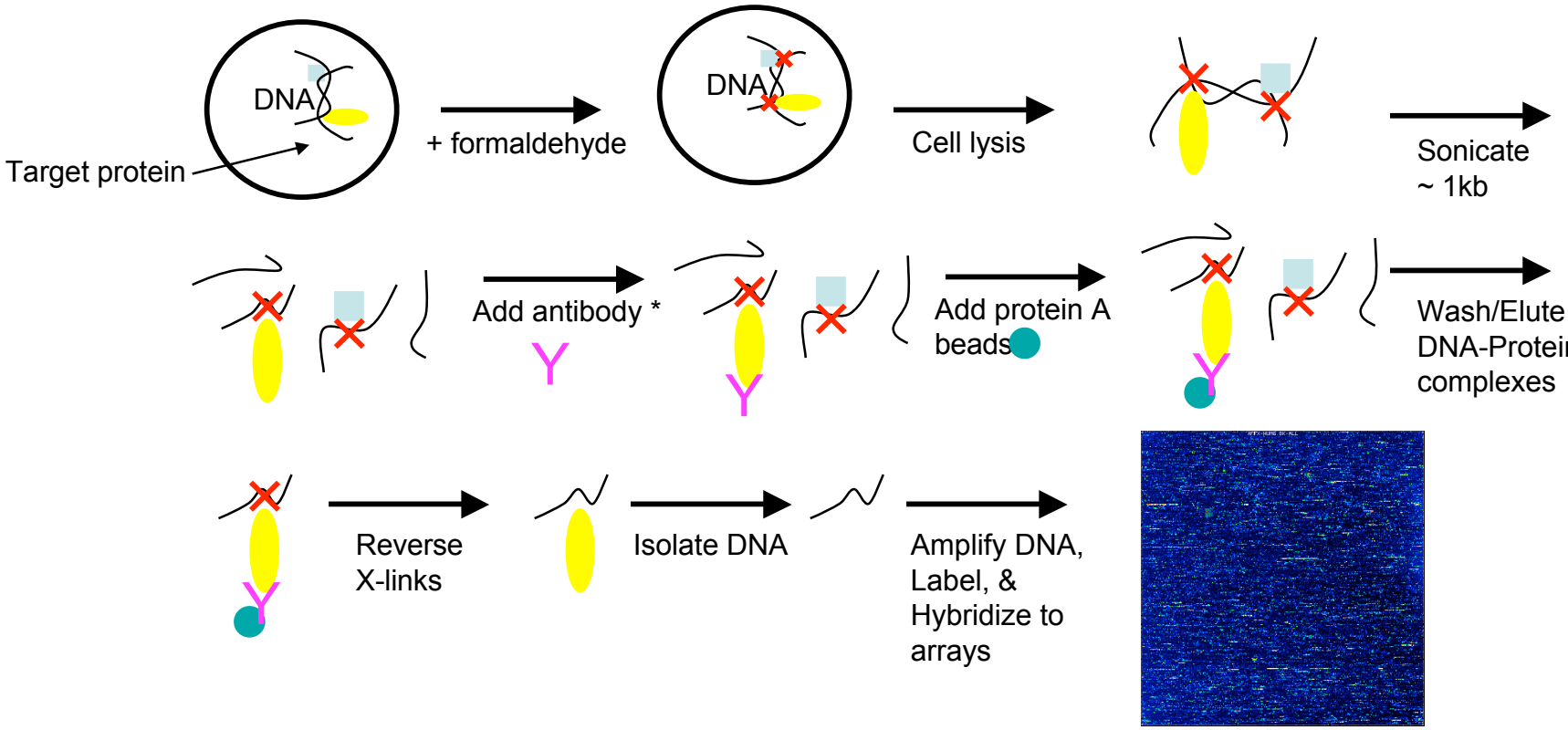
Advances in Genechip technology enable applications requiring high data content

Tiling Array Applications

- Mapping regions of transcription
- ChIP on chip experiments (Chromatin IP)
- Chromosomal origins of replication
- DNA methylation
- Copy number analysis
- SNP discovery

Genome reference arrays for multiple applications

Chromatin Immunoprecipitation



Chromatin IP Assay

- Any nucleotide fraction that can be immunoprecipitated can be interrogated on tiling arrays
- DNA binding proteins
  - Transcription factors
  - Modified histones
  - Structural proteins
- Assay is more complex than typical transcription mapping assay
  - Chromatin-protein immunoprecipitation
  - Purify, amplify and label DNA fragments
- Data analysis more comprehensive than typical transcription assay