RESTRICTION ENZYMES, PALINDROMES & C-DNA

Presentation by

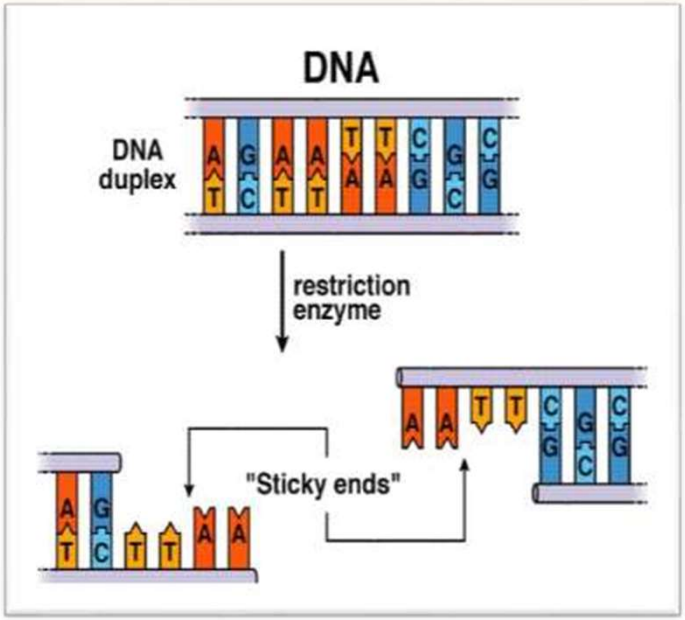
Dr. Girima Nagda

(Assistant Professor)

Department of Zoology, University College of Science,

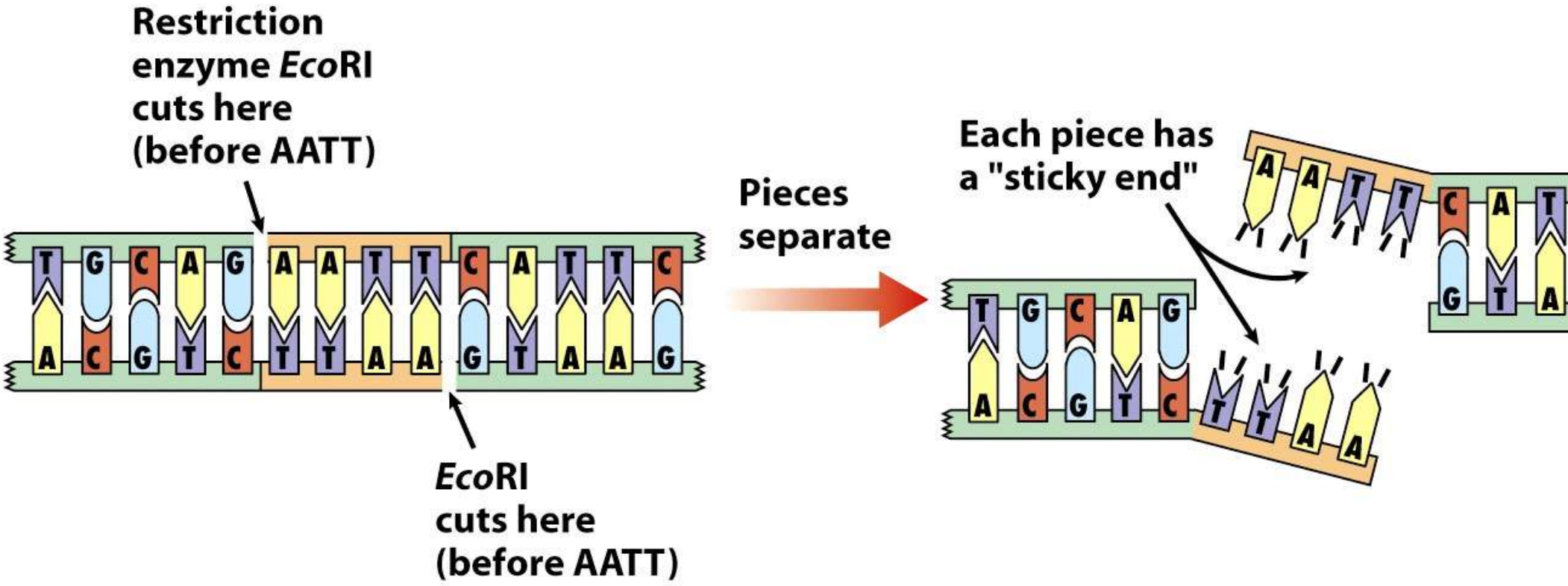
Mohanlal Sukhadia University, Udaipur (Raj.)

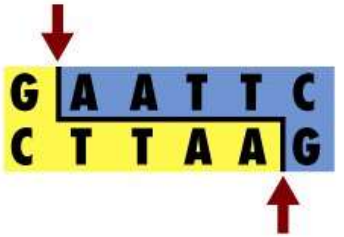
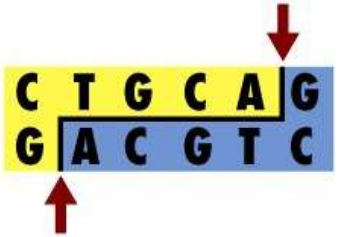
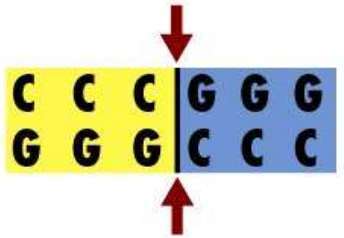
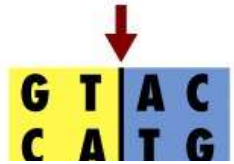
RESTRICTION ENZYME



- A special class of sequence-specific enzyme
- Found in bacteria
- Site-specific-cleave DNA molecules only at specific nucleotide sequence
- REases recognize DNA base sequence that are palindrome (MADAM)
- REase make staggered cuts with complementary base sequences for easy circulization

MOLECULAR SCISSORS/ RESTRICTION ENZYMES



Bacillus amyloliquefaciens H	***Bam* H1**	
Escherichia coli Ry13	***Eco* R1**	
Providencia stuartii 164	***Pst* 1**	
Serratia marcescens SB	***Sma* H1**	
Rhodopseudomonas sphaeroides	***Rsa* 1**	

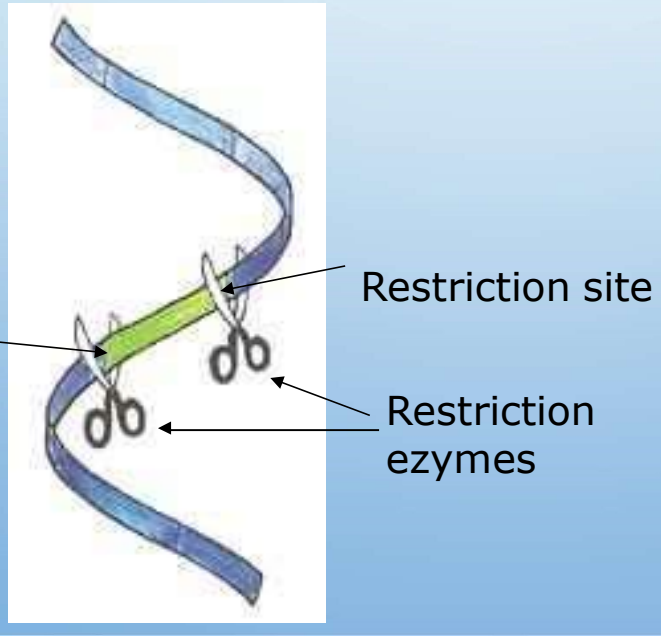
MOLECULAR TOOLS OF GENETIC ENGINEERING

- The genetic engineer's tool kit or molecular tool namely the enzymes are most commonly used in recombinant DNA experiments are

- Restriction endonucleases -DNA cutting Enzyme.
- DNA Ligases- DNA joining Enzyme.

RESTRICTION ENDONUCLEASES

Restriction enzymes act as molecular scissors and cut DNA at specific sites called restriction sites



RESTRICTION ENDONUCLEASES

- Named with particular reference to the bacteria from which they are isolated.

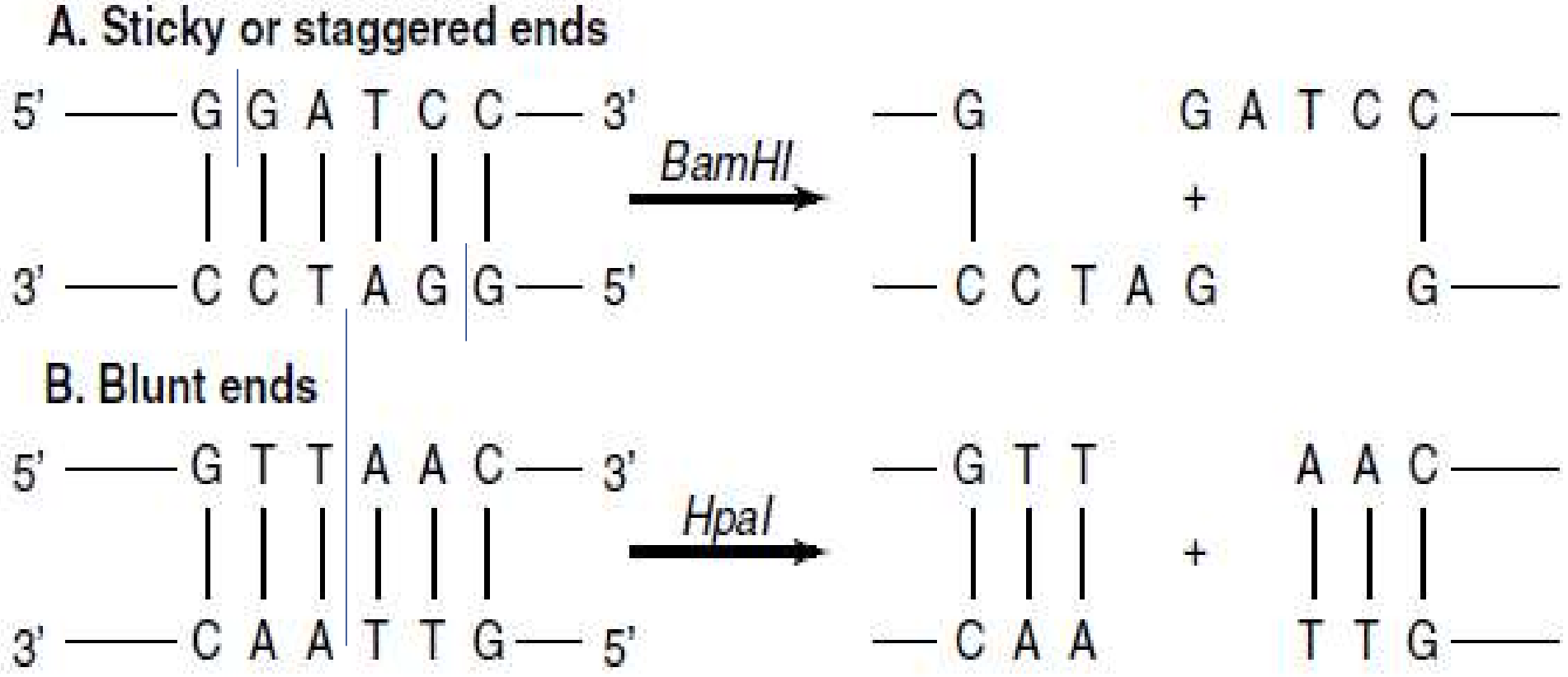
Eg. EcoRı

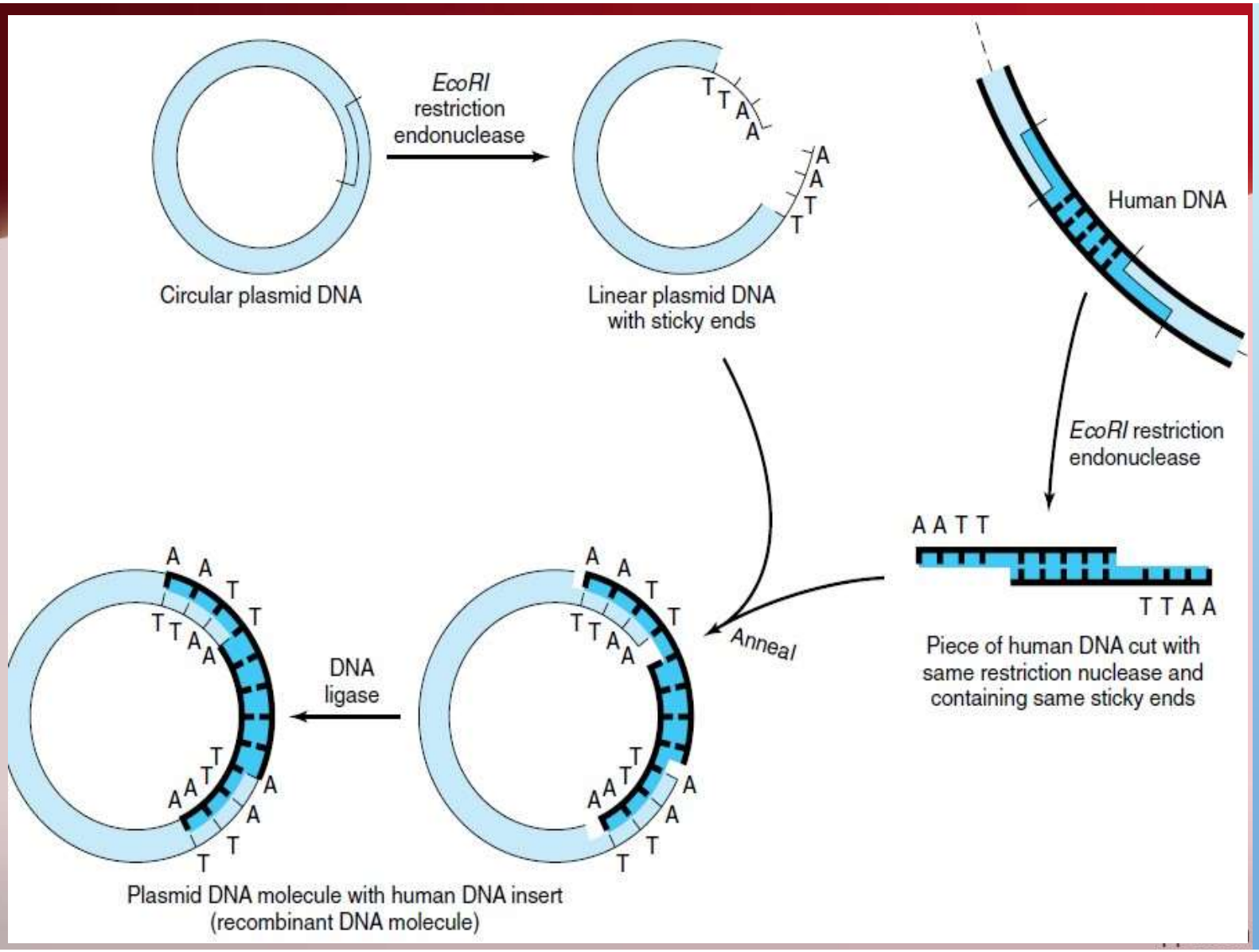
Eco RI, Eco RII, Eco RIII

:Types 3

PALINDROME

- MADAM
- EcoRI
- 5’GAATTC3’
- 3’CTTAAG5’

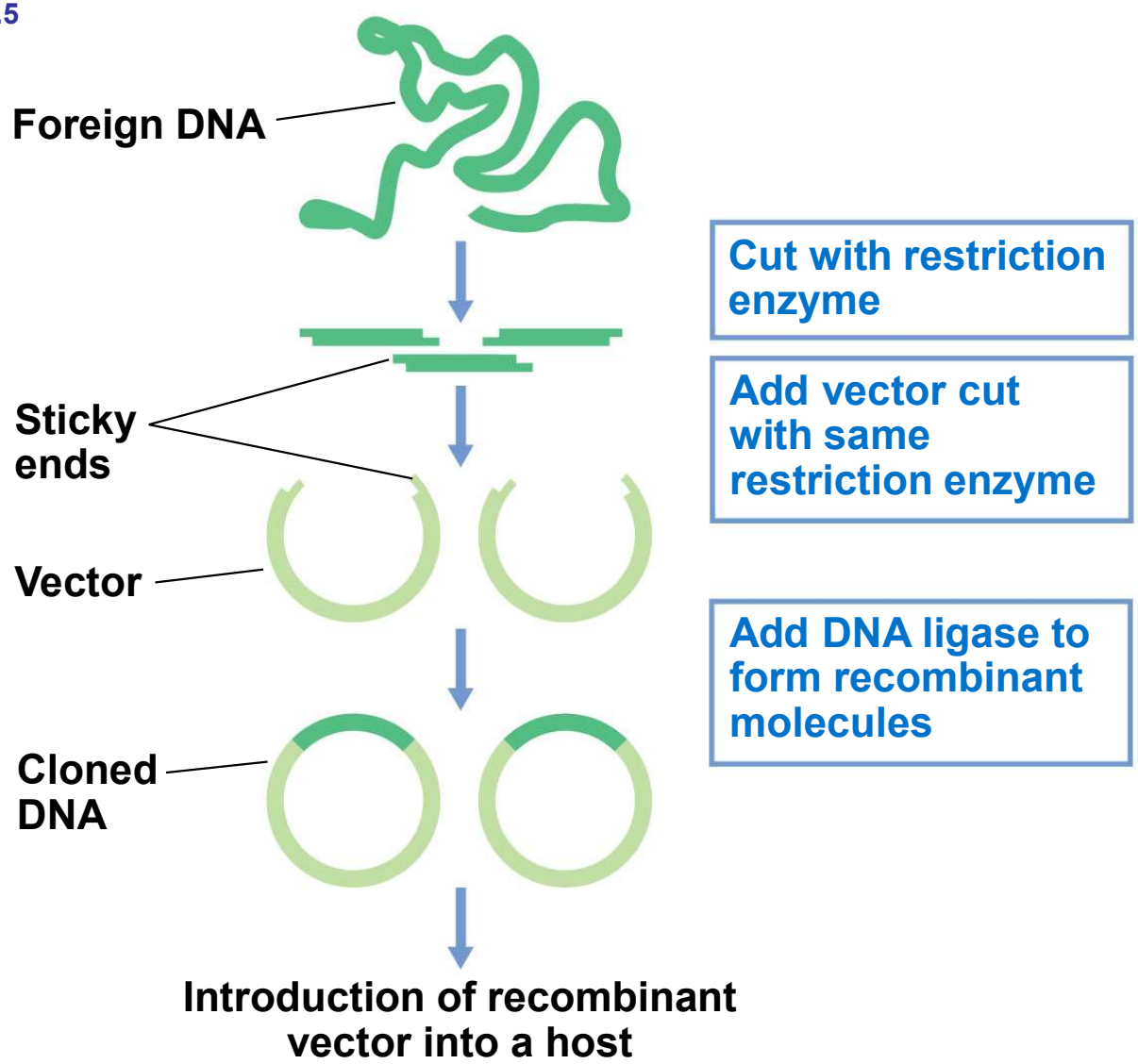

A. Sticky or staggered ends
5' — G G A T C C — 3'
3' — C C T A G G — 5'
BamHI
— G
G A T C C —
+
— C C T A G
G —
B. Blunt ends
5' — G T T A A C — 3'
3' — C A A T T G — 5'
HpaI
— G T T
A A C —
+
— C A A
T T G —


EcoRI
restriction
endonuclease
Circular plasmid DNA
Linear plasmid DNA
with sticky ends
Human DNA
EcoRI restriction
endonuclease
A A T T
T T A A
Piece of human DNA cut with
same restriction nuclease and
containing same sticky ends
Anneal
DNA
ligase
Plasmid DNA molecule with human DNA insert
(recombinant DNA molecule)

Endonuclease	Sequence Recognized Cleavage Sites Shown	Bacterial Source
BamHI	↓ GGATCC CCTAGG ↑	*Bacillus amylo-liquefaciens* H
BglII	↓ AGATCT TCTAGA ↑	*Bacillus glolbigii*
EcoRI	↓ GAATTC CTTAAG ↑	*Escherichia coli* RY13
EcoRII	↓ CCTGG GGACC ↑	*Escherichia coli* R245
HindIII	↓ AAGCTT TTCGAA ↑	*Haemophilus influenzae* R_d

HhaI	↓ GCGC CGCG ↑	*Haemophilus haemolyticus*
HpaI	↓ GTTAAC CAATTG ↑	*Haemophilus parainfluenzae*
MstII	↓ CCTNAGG GGANTCC ↑	Microcoleus strain
PstI	↓ CTGCAG GACGTC ↑	*Providencia stuartii* 164
TaqI	↓ TCGA AGCT ↑	*Thermus aquaticus* YTI

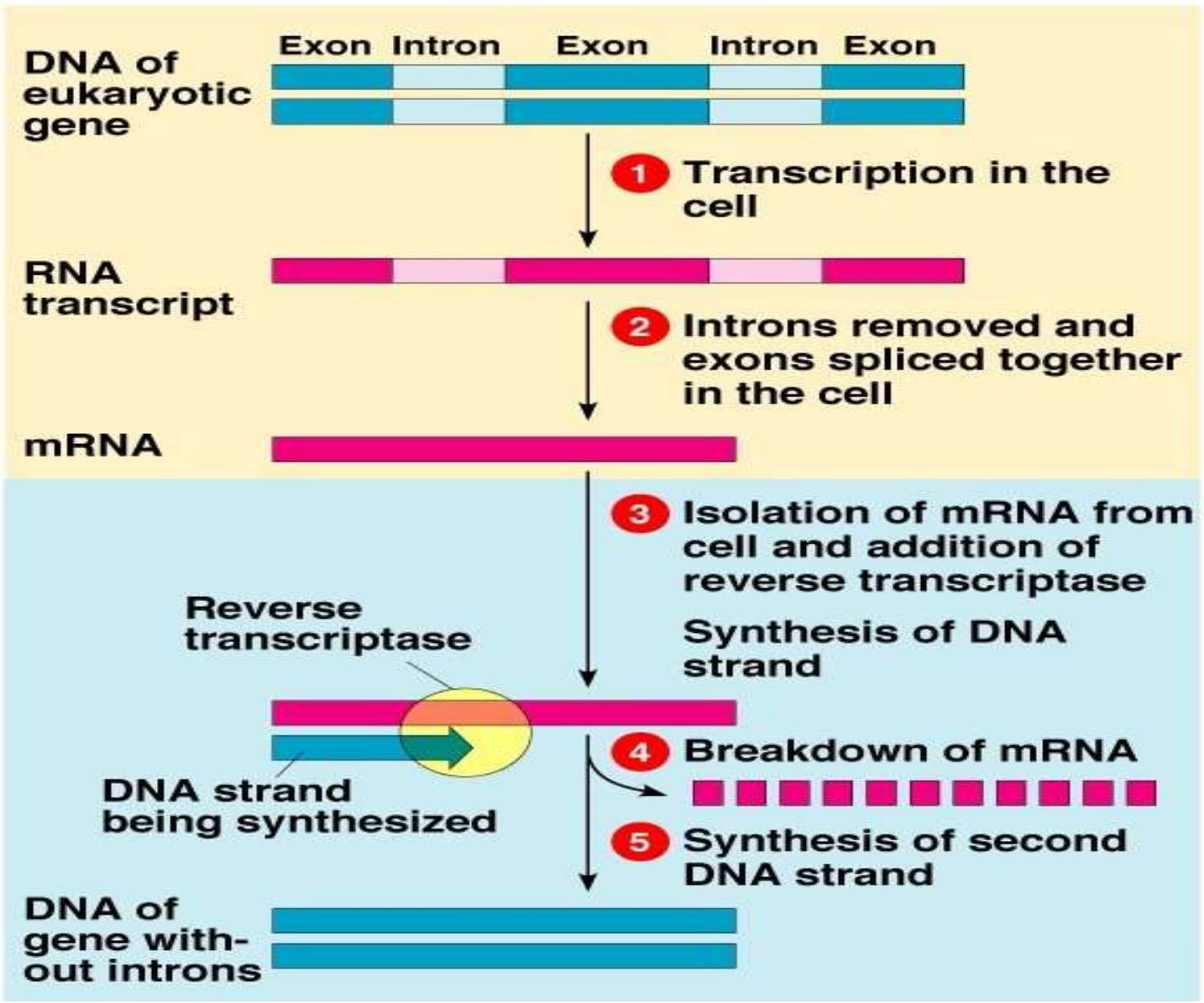
Figure 11.5



CDNA LIBRARY
COMPLEMENTARY DNA LIBRARY

- cDNA libraries contain those DNA sequences that appear as mRNA molecules, and these differ from one cell type to another.

- This mRNA can be used as a template to make a complementary dsDNA (cDNA) molecule using the enzyme reverse transcriptase.



Exons: Coding sequences; form part of protein

Introns: non coding sequences; removed from mRNA

Newly formed RNA= naïve RNA/ RNA transcript

THANK YOU