

RESTRICTION ENZYMES

.



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ENZYMES

 Enzymes are proteins

 biological catalysts  help drive biochemical reactions.

 Enzyme names end with an ase (eg.,

endonuclease)

 Bacteria have evolved a class of enzymes that

destroy foreign DNA (eg. Virus DNA).

 protect bacteria from bacteriophages (Viruses).

 Bacteriophages cannot multiply if their DNA is

destroyed by the host.



RESTRICTION END/EXO NUCLEASES

 Restriction endonucleases RESTRICT viruses

 Viral genome is destroyed upon entry.

 Restriction endonuclease = Restriction

enzymes

 Endo (inside), nuclease (cuts nucleic acid)

 Exo(outside), nuclease (cuts nucleic acid)

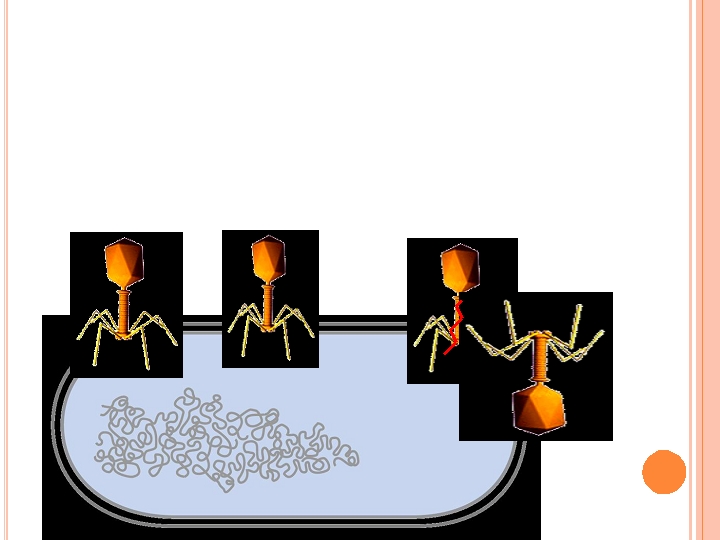
 Restriction endonuclease recognizes a short

and specific DNA sequence and cuts it from

inside.

 The specific DNA sequence is called

recognition sequence.

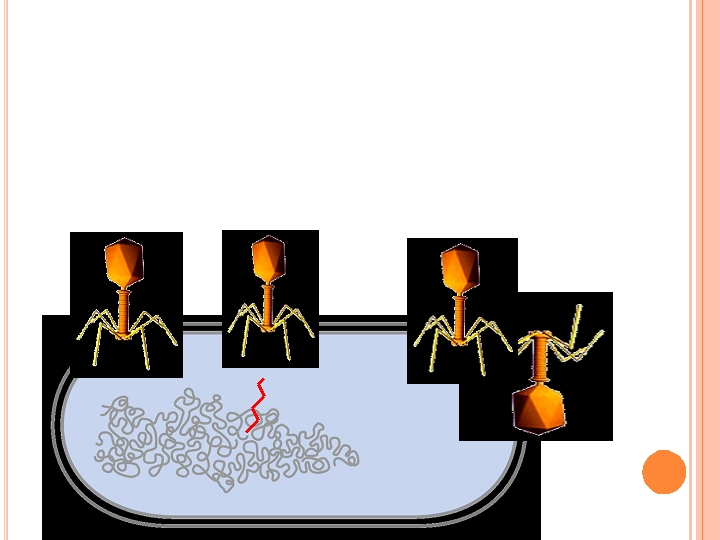


ORIGINS OF RESTRICTION ENZYMES

1)

Bacteria produce restriction enzymes to protect

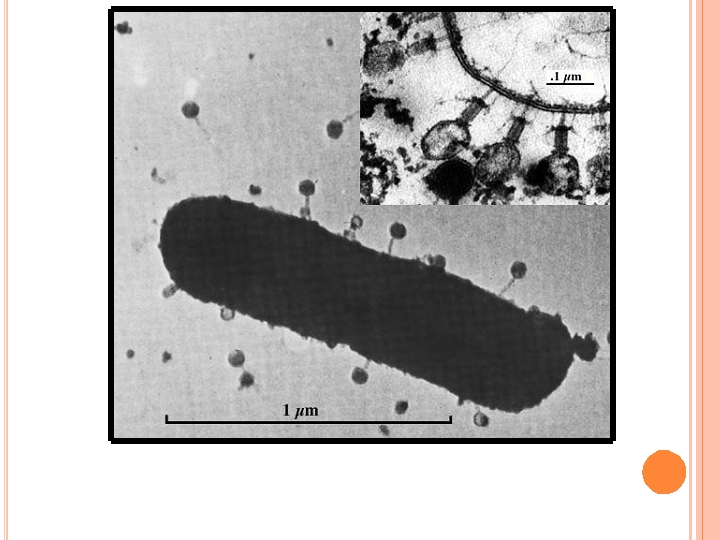
against invading viral DNA/RNA.



2)

The enzymes cut the invading DNA/RNA, rendering it

harmless.



ELECTRON MICROGRAPH OF

BACTERIOPHAGE ATTACK.



DISCOVERY

 In 1962, Werner Arber, a Swiss biochemist, provided

the first evidence for the existence of "molecular

scissors" that could cut DNA.

 He showed that *E. coli* bacteria have an enzymatic

"immune system" that recognizes and destroys foreign

DNA, and modifies native DNA to prevent self-

destruction.



 By the early 1970s these enzymes started to be

identified and purified.

 It was shown that each species of bacteria had its

own population of a SPECIFIC restriction

enzyme.

 Each enzyme recognized its own specific

sequence of DNA bases. It is at this sequence

that the DNA was cut.

 Smith,Nathans and Arber were awarded the

Nobel prise for Physiology and Medicine in 1978

for the discovery of endonucleases.



TYPES OF RESTRICTION ENDONULEASES

 There are the four distinct types of restriction

endonucleases: Type I,Type II, Type III And Type

IIs restriction endonucleases.

 Type I restriction endonucleases are complex

endonucleases and have recognition sequenses of

about 15 bp.They cleave the DNA about 1000 bp

away from the 5' end of the sequence "TCA"

located within the recognition site , EcoK, EcoB,

etc.



 Type II restriction endonucleases are remarkably

stable and induce cleavage either , in most cases

within or immediately outside their recognition

sequence, which are symmetrical. More then 350

different Type II endonucleases with over 100

different recognition sequences are known. They

require Mg+ ions for cleavage.The first Type II

enzyme to be isolated was Hind II in 1970.

 Only Type II are used for restriction mapping and

gene cloning in view of their cleavage only at specific

sites.



 Type III restriction endonucleases are

intermediate between the Type I and Type II

enzymes.They cleave DNA in the immidiate

vicinity of their recognition sites, e.g.,EcoPI,

EcoPI5 ,HinfIII, etc.

 Type I and Type III restriction enzymes are not

used in gene cloning.

 The Type IIs enzymes recognize asymmetric

target sites, and cleave the DNA duplex on one

side of the recognion sequence upto 20 bp away.



NOMENCLATURE

 Smith and Nathans (1973) proposed

enzyme naming scheme

 three-letter acronym for each enzyme derived

from the source organism

 First letter from genus

 Next two letters represent species

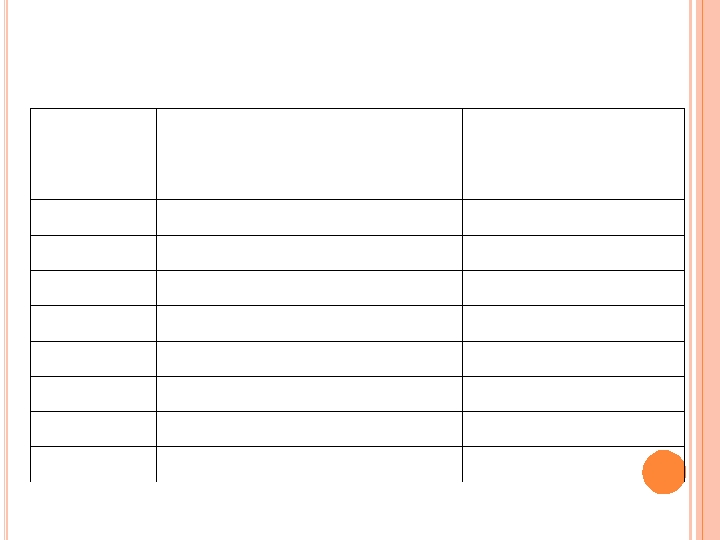
 Additional letter or number represent the

strain or serotypes

 For example. the enzyme *Hind*II was

isolated from *Haemophilus influenzae*

serotype d.



FEW RESTRICTION ENZYMES

**Enzyme Organism from which derived**

Bam HI

Eco RI

Hind III

Mbo I

Pst I

Sma I

Taq I

Xma I

*Bacillus amyloliquefaciens*

*Escherichia coli RY 13*

*Haemophilus inflenzae Rd*

*Moraxella bovis*

*Providencia stuartii*

*Serratia marcescens*

*Thermophilus aquaticus*

*Xanthamonas malvacearum*

**Target sequence**

**(cut at \*)**

**5' -->3'**

G\* G A T C C

G\* A A T T C

A\* A G C T T

\*G A T C

C T G C A \* G

C C C \* G G G

T \* C G A

C \* C C G G G



R-M SYSTEM





Restriction-modification (R-M) system.

 Endonuclease activity: cuts foreign DNA at the

recognition site

 Methyltransferase activity: protects host DNA from

cleavage by the restriction enzyme.

 Methyleate one of the bases in each strand

Restriction enzyme and its cognate modification

system constitute the R-M system



PROTECTION OF SELF DNA

 Bacteria protect their self DNA from

restriction digestion by methylation of its

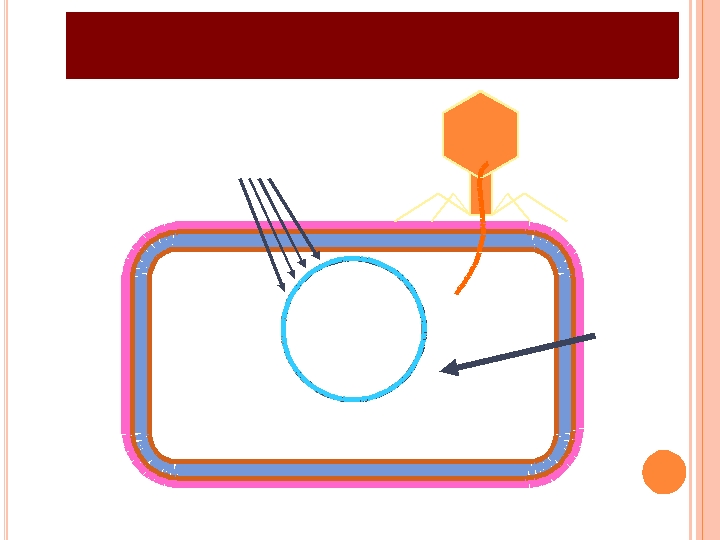
recognition site.

 Methylation is adding a methyl group (CH 3) to

DNA.

 Restriction enzymes are classified based on

recognition sequence and methylation pattern.

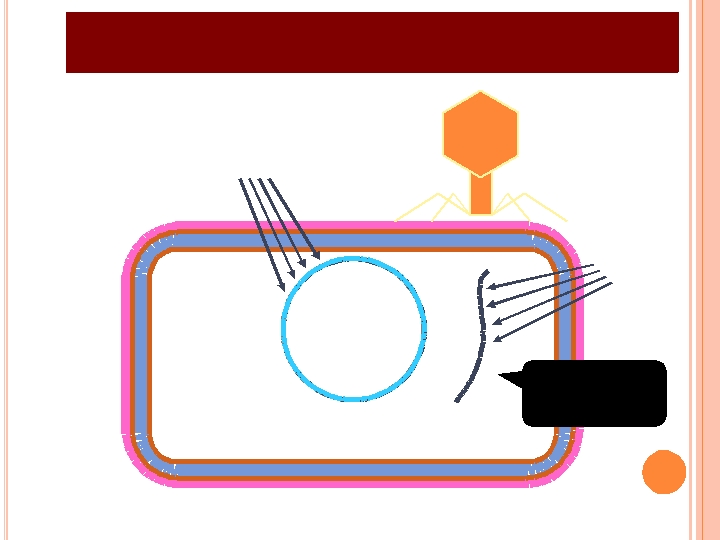


**REPELLING BACTERIOPHAGE ATTACK**

Methylation sites

Methylase

**M**



**REPELLING BACTERIOPHAGE ATTACK**

Methylation sites

Unmethylated

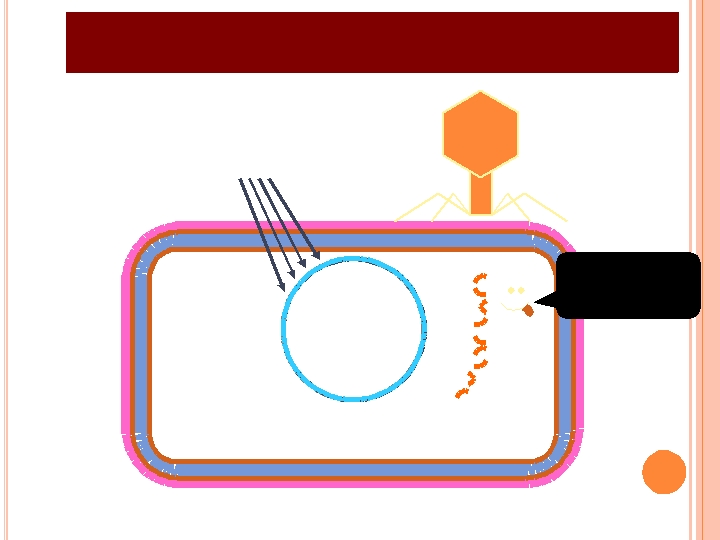
methylation

sites

**R**

Munch! Munch!

Munch . . .

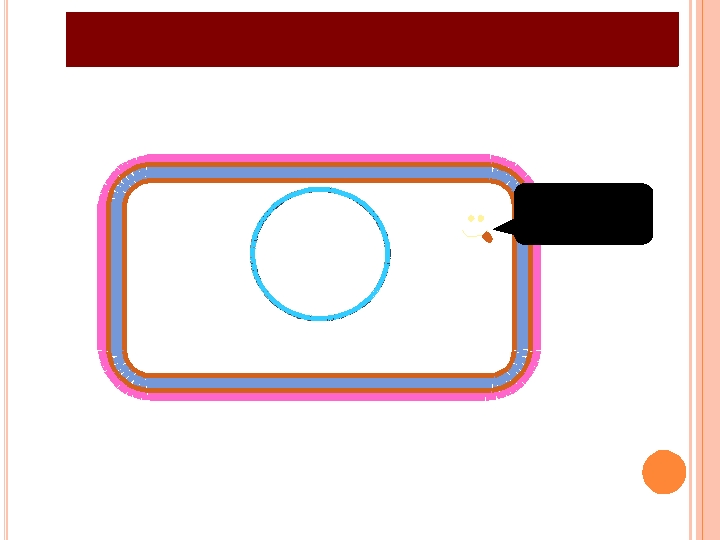


**REPELLING BACTERIOPHAGE ATTACK**

Methylation sites

Take that you

wicked virus!



**REPELLING BACTERIOPHAGE ATTACK**

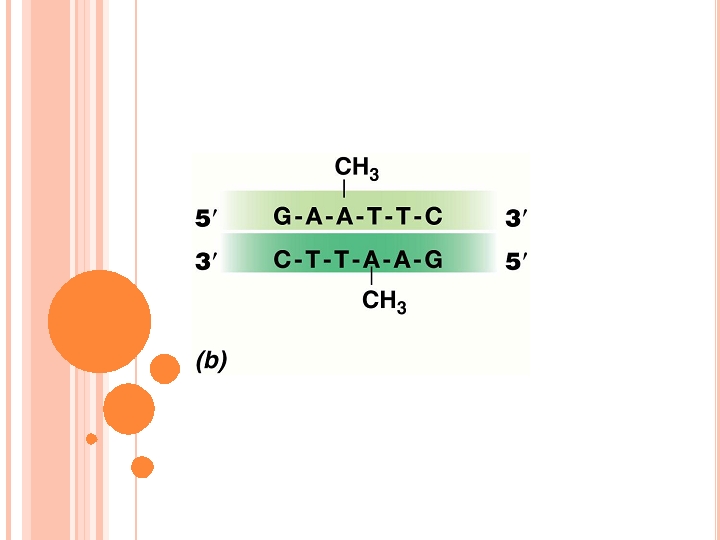
Take that you

wicked virus!

Methylase and restriction endonucleases must

recognize the same sequences if they are to function

as an effective system



**FIGURE 11.1B**

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 Multi-subunit proteins .

 Function as a single protein complex .

 Contain

 two R (restriction) subunits.

 two M (methylation) subunits and .

 one S (specificity) subunit.

 Cleave DNA at random length from

recognition site.



RECOGNITION SEQUENCES

 Each restriction enzyme always cuts at the same

recognition sequence.

 Produce the same gel banding pattern (fingerprint).

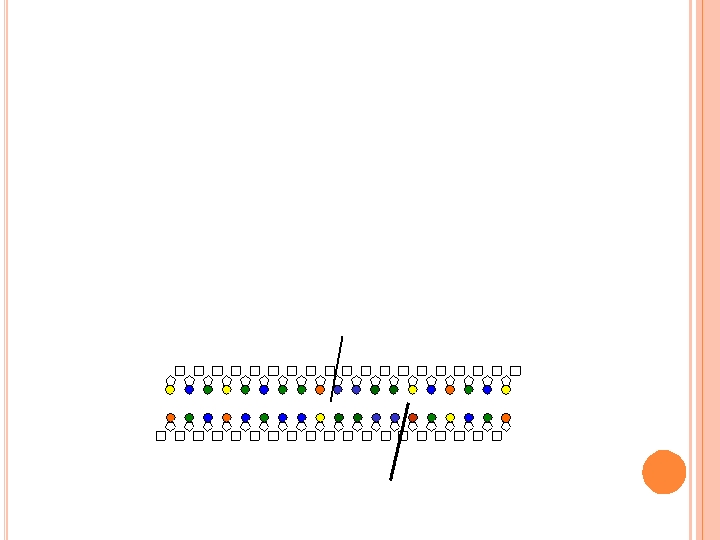
 Many restriction sequences are palindromic. For

example.

5' GAATTC 3'

3' CTTAAG 5'

(Read the same in the opposite direction (eg. madam, race car…)



RESTRICTION ENZYME ECORI

 Eco RI recognizes the sequence 5'….GAATTC…..

 A cut is made between the G and the A on each strand.

 This restriction enzyme cleaves the nucleotides 5'AATT

overhanging.

 These are known as "sticky ends" because hydrogen bonds

are available to "stick" to a complimentary 3'TTAA.

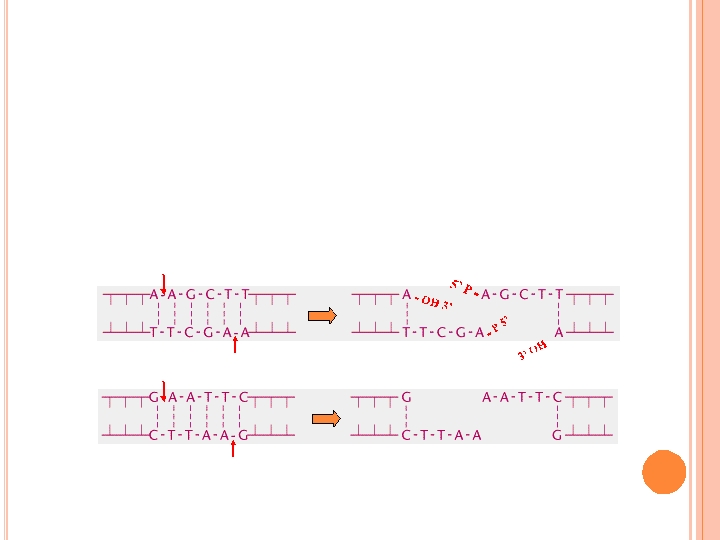
 Note: Restriction enzymes don't stop with one cut! They

continue to cut at every recognition sequence on a DNA

strand.

Restriction Enzyme

Cut from EcoRI



STICKY END CUTTERS

 Most restriction enzymes make staggered cuts.

 Staggered cuts produce single stranded

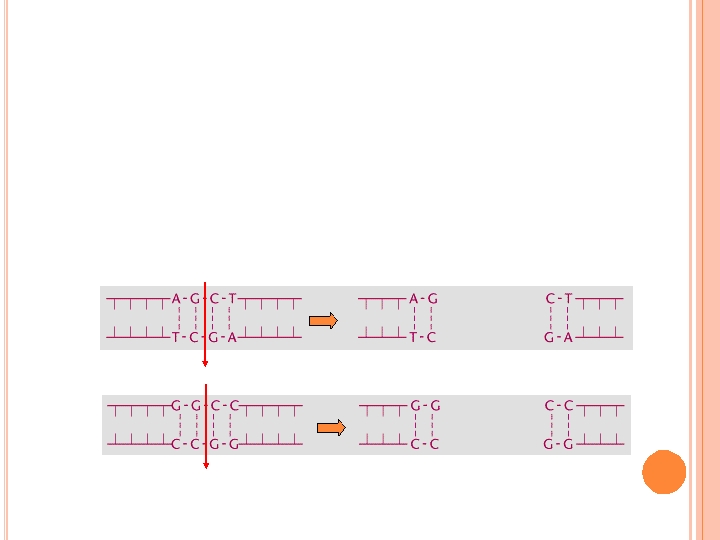
"sticky-ends".

 DNA from different sources can be spliced

easily because of sticky-end overhangs.

***Hin*dIII**

***Eco*RI**



BLUNT END CUTTERS

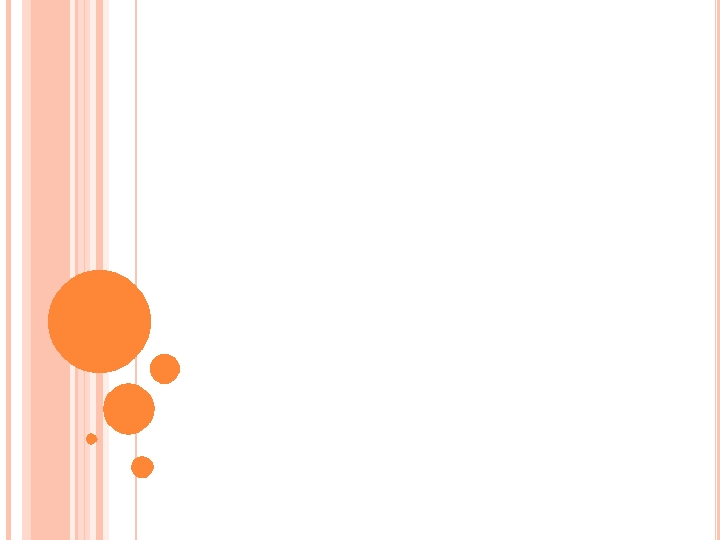
 Some restriction enzymes cut DNA at opposite base

 They leave blunt ended DNA fragments

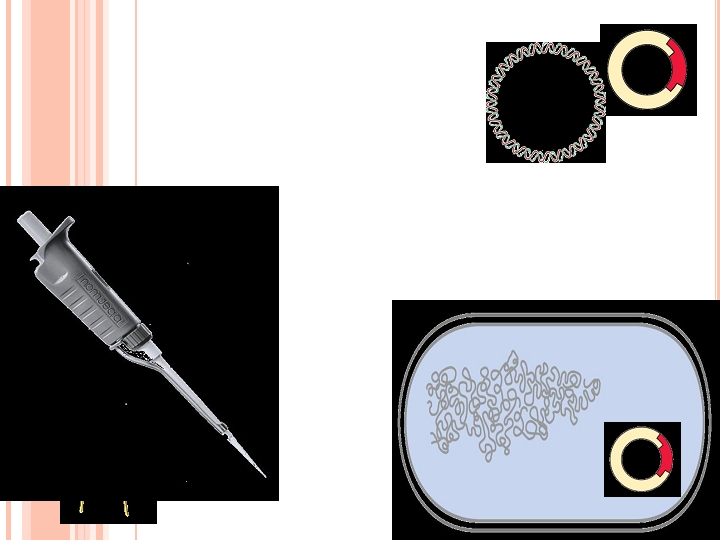
 These are called blunt end cutters

***Alu*I**

***Hae*III**



**APPLICATIONS**



**IN BIOTECHNOLOGY**

**Recombinant DNA and its Applications**



 Discovery of enzymes that cut and paste DNA

make genetic engineering possible.

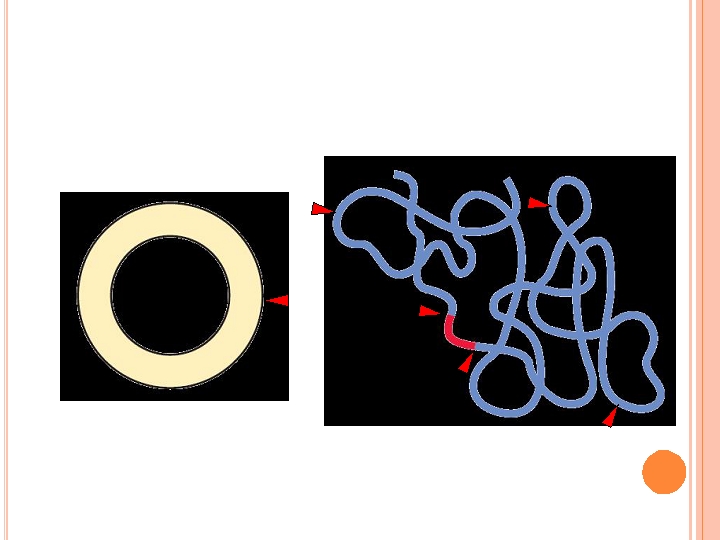
 Restriction enzyme cuts DNA and generates

fragments.

 Ligase joins different DNA fragments.

 DNA fragments from different species can be

ligated (joined) to create Recombinant DNA.

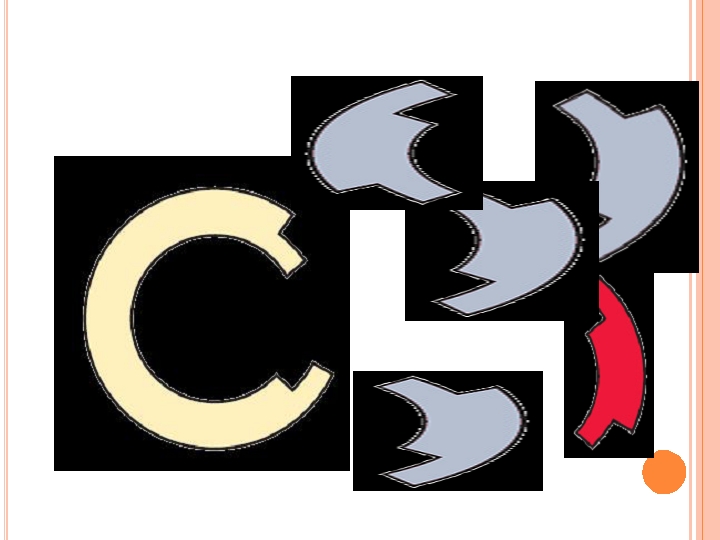


DNA FROM TWO SOURCES

(RESTRICTION SITES LABELED)

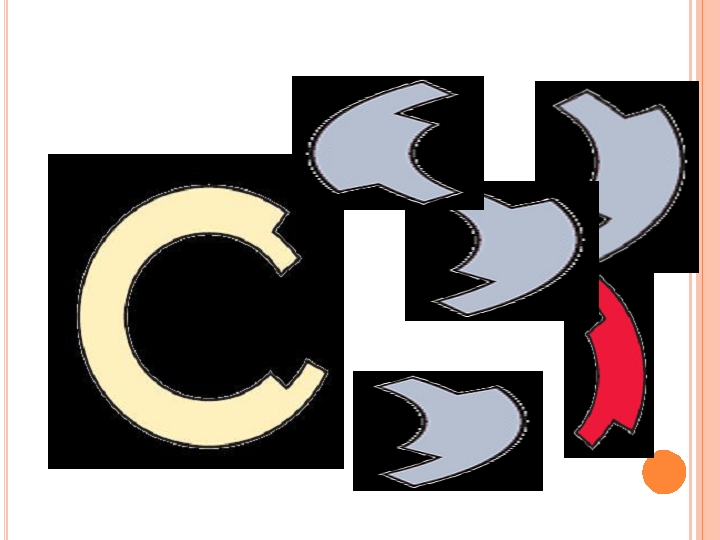
Circular DNA

Linear DNA

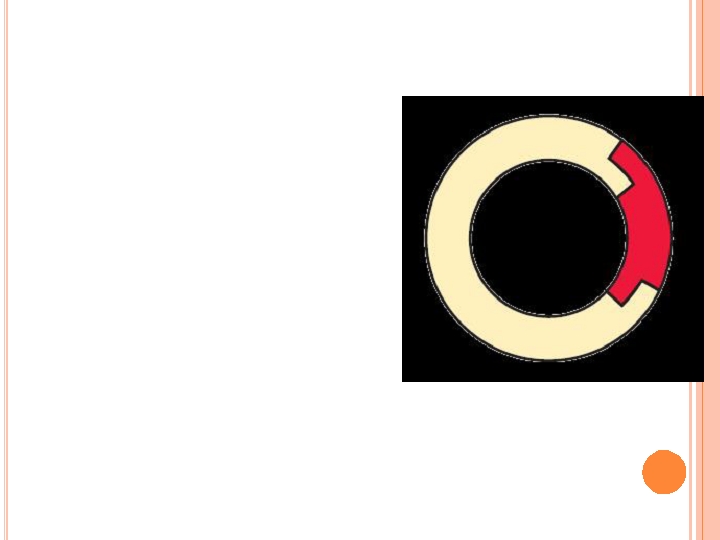


APPLICATION OF RESTRICTION

ENZYMES



ADDING DNA LIGASE



RECOMBINANT DNA PLASMID

 Many possible

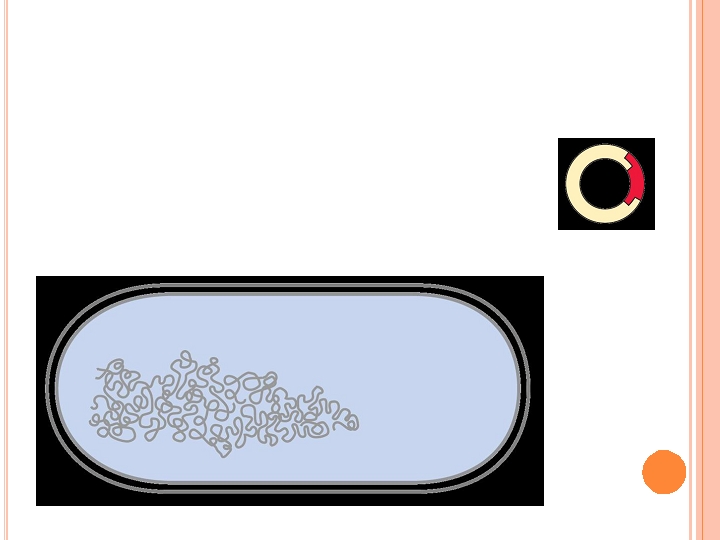
recombinant DNA

plasmids can be

produced, but this was

the desired plasmid

for the experiment.

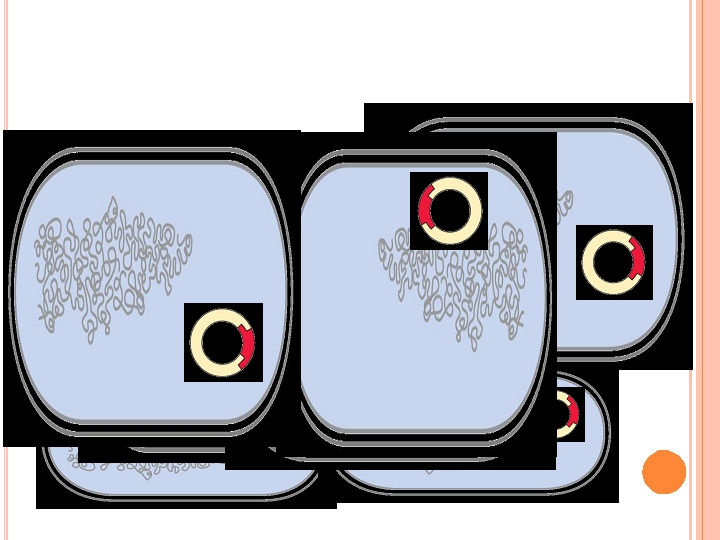


PLASMID DNA INSERTION

DNA plasmids can be inserted into

bacteria using a variety of laboratory

processes.



TRANSGENIC COLONY ALLOWED TO

GROW



SOME APPLICATIONS OF RECOMBINANT

DNA TECHNOLOGY

Bacteria, Yeasts, and Plants can

all be modified to produce

important pharmaceuticals,

enriched foods, and industrial

products.



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Thank You