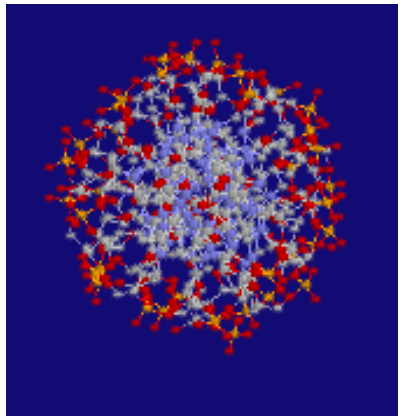
Gene Cloning 1



Week 9

Gene Cloning

- What is cloning?
- What is gene cloning? How does it differ from cloning a whole organism?
- Why do we clone genes?
- How do we clone genes?
- What is DNA library and cDNA library?

RESTRICTION ENZYMES

They are bacterial proteins (endonucleases) cutting DNA molecules from specific restriction sites.

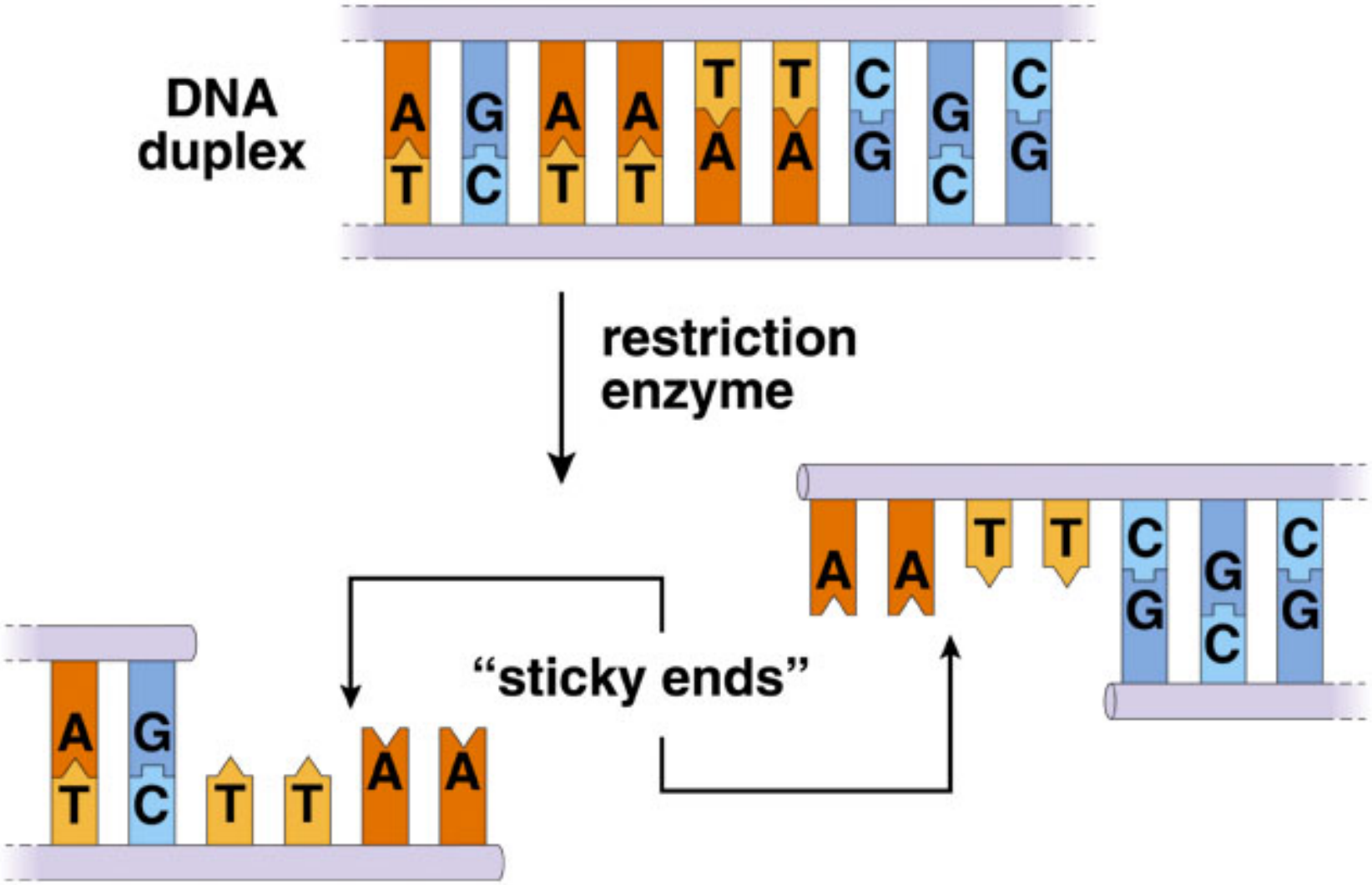
- restriction site: These are the 4-8 based specific DNA sequences those recognized by an restriction enzyme
- restriction fragment: Smaller DNA fragment separated from a larger DNA fragment following an digestion with one or more restriction enzymes
- hundreds of different RE are present, each has a specific restriction site

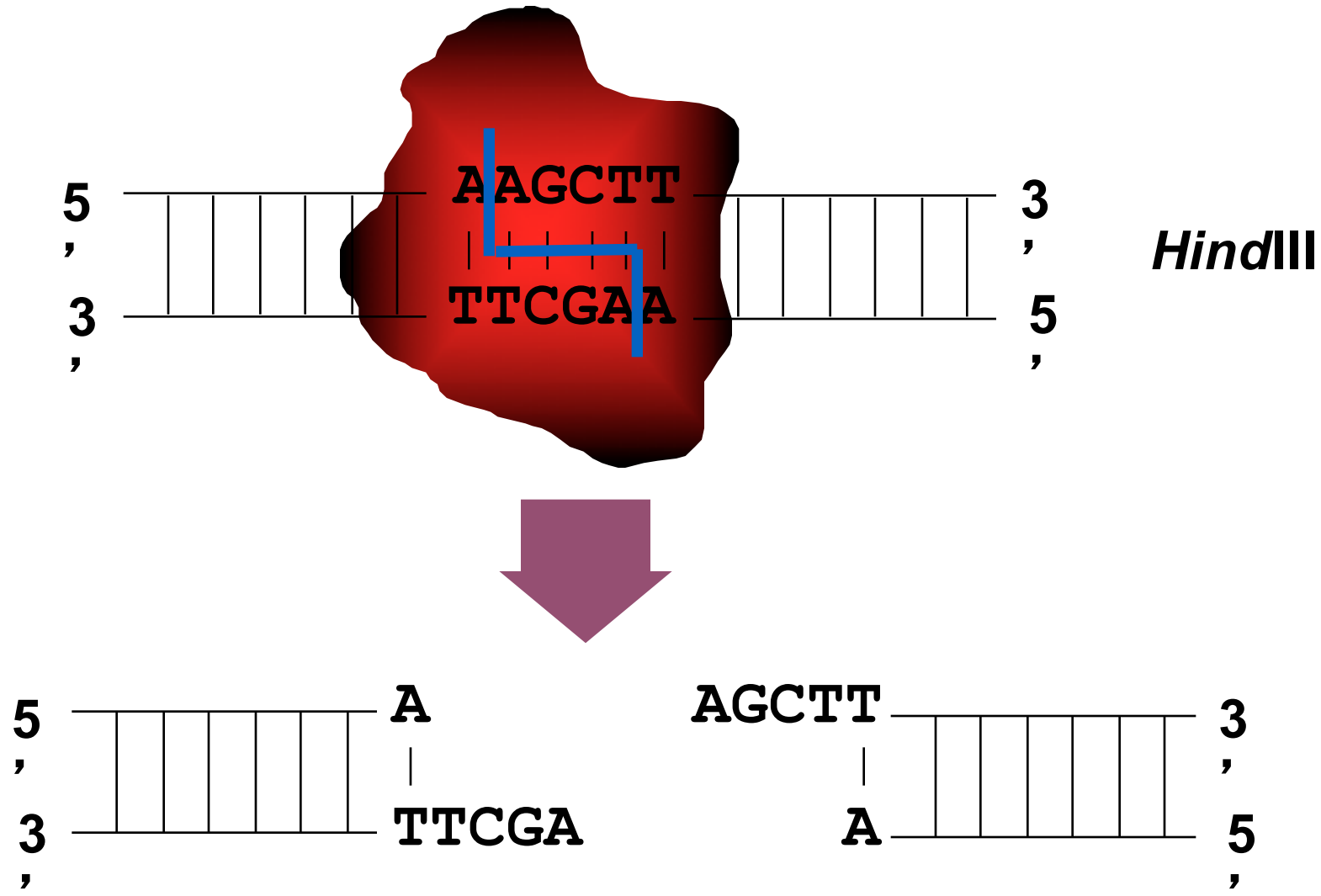
Nomenclature and Description of REs (EcoRI)		
Abbreviation	**Meaning**	**Definition**
E	*Escherichia*	genus
co	*coli*	species
R	RY13	strain
I	First identified	Identification priority

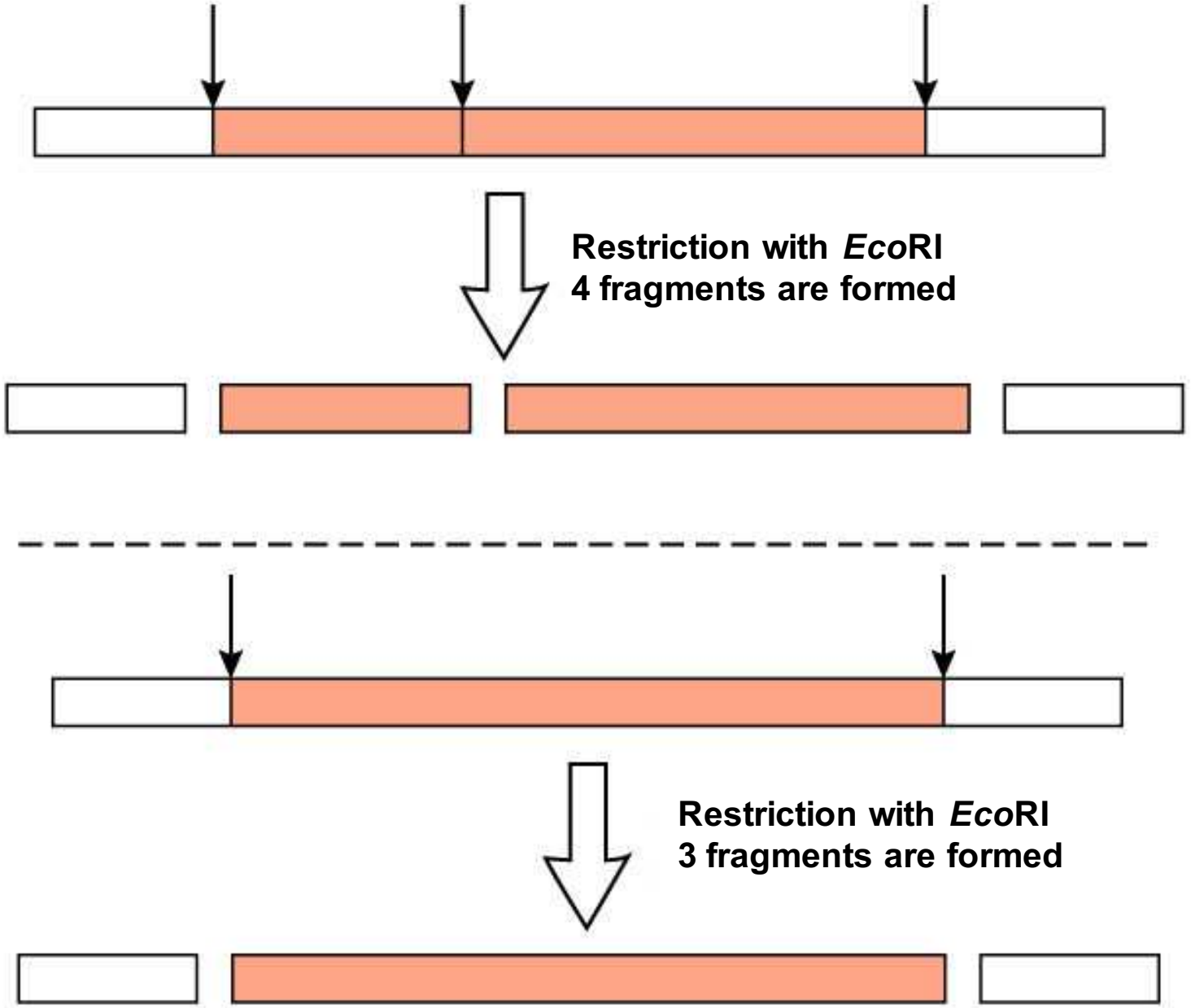
Table. Specific restriction sites of some of the REs

Organism	**RE Name**	**Restriction site**
Escherichia coli	*Eco*RI	G⁻ AATTC
Escherichia coli	*Eco*RII	⁻ CCAGG
Haemophilus influenzae	*Hind*II	GTPPy⁻ PuAC
Haemophilus hemolyticus	*Hha*I	GCG⁻ C
Bacillus subtilus	*Bsu*RI	G⁻ CC
Brevibacterium albidum	*Bal*I	TGG⁻ CCA
Thermus aquaticus	*Taq*I	T⁻ CGA

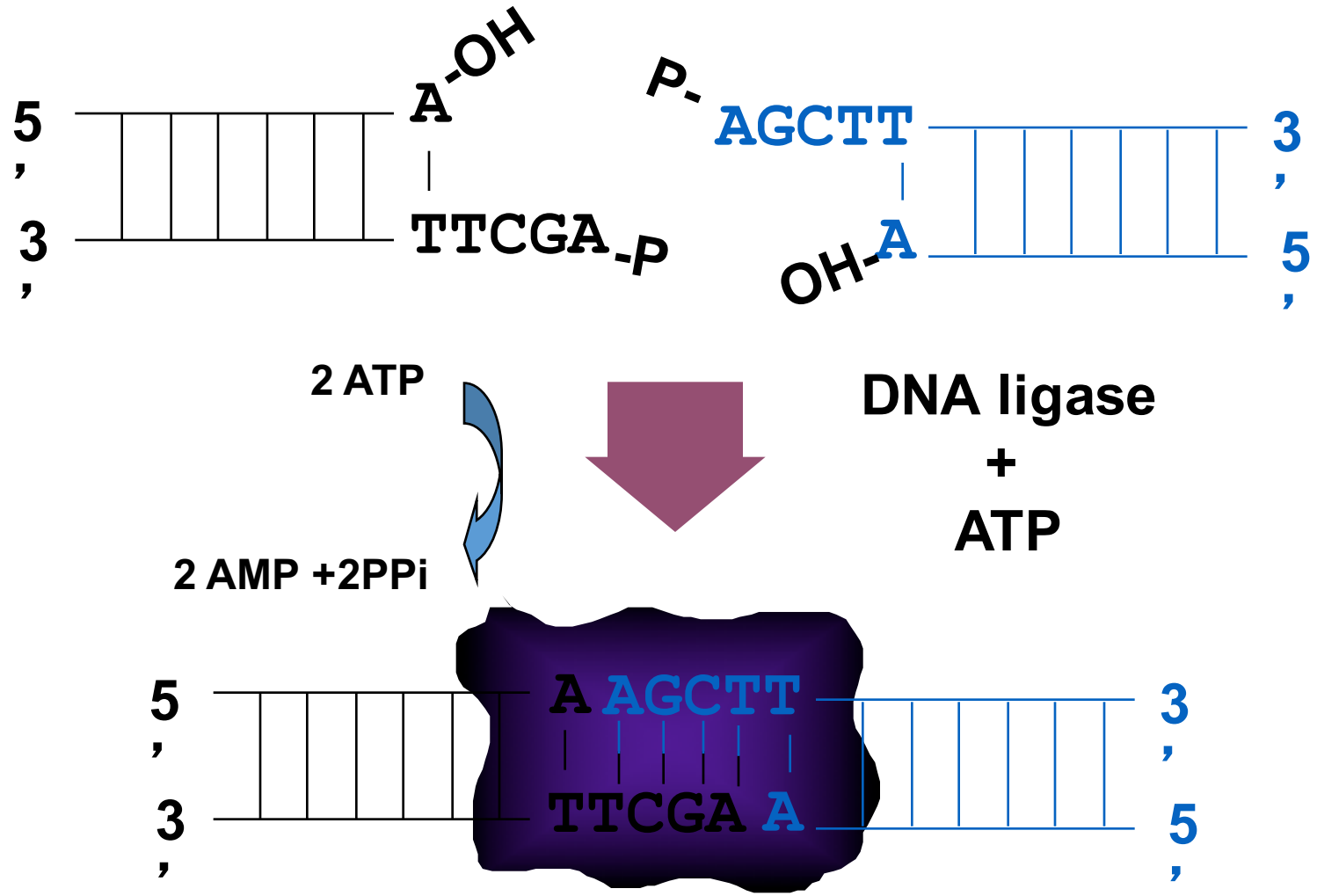
RE sites


DNA
duplex
A
T
G
C
A
T
A
T
T
A
T
A
C
G
G
C
C
G
restriction
enzyme
A
A
T
T
C
G
G
C
C
G
"sticky ends"
A
T
G
C
T
T
A
A


5'
3'
AAGCTT
TTCGAA
3'
5'
*Hind*III
5'
3'
A
TTCGA
AGCTT
A
3'
5'


Restriction with *Eco*RI
4 fragments are formed
Restriction with *Eco*RI
3 fragments are formed

DNA CLONING: DNA LIGATION



Cloning (Definition)

- Greek - clone, twins
- Isolationand mass production of a specific DNA fragment (clone) out of a complex DNA mixture,
- Asexual production of a content of an individual
- A group containing copy/copies of whole or part of a macromolecule
- Individual genetically similar to her/his parents formed from a single somatic cell of her/his parent (dictionary)

Stages of Gene Cloning

- Isolation and purification of a gene carrying DNA (or RNA) fragment from a complex DNA molecule,
- Determination of the location of the gene of interest
- Incision of the gene
- Isolation of carrier vector DNA
- Insertion of gene DNA into the vector DNA (Recombination)
- Electroporation and/or transformation of established recombinant vector DNA to recipient cell (procaryotic/eucaryotic)
- Selection
- Control of gene products

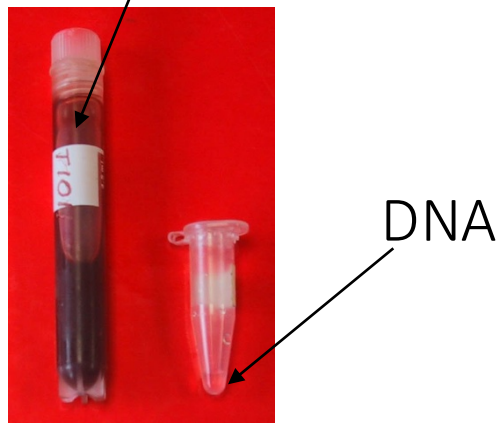
Do not forget! All organisms can be cloned but in a different way!!!

Why do we clone DNA?

- Determination of a nucleotide sequences of a specific gene after isolation --- Nowadays better ways for the purpose!!!
- Determiantion of control DNA sequences
- Investigation of protein/enzyme/RNA functions
- Detection of mutations i.e. Detection of gene defects related to specific diseases
- Production of substances in organisms for specific purposes i.e. İnsulin production İnsulin üretimi, establishment of resistant species

How do we clone DNA?

- DNA is extracted from organic material i.e. blood, tissue, from another bacteria
- REs, i.e. *EcoR*I, *Hind*III, cut DNA into smaller fragments
- Different DNA fragments cut by the same enzyme could be ligated or recombined



Restriction enzymes

Materials used in DNA Cloning

RESTRICTION ENZYMES

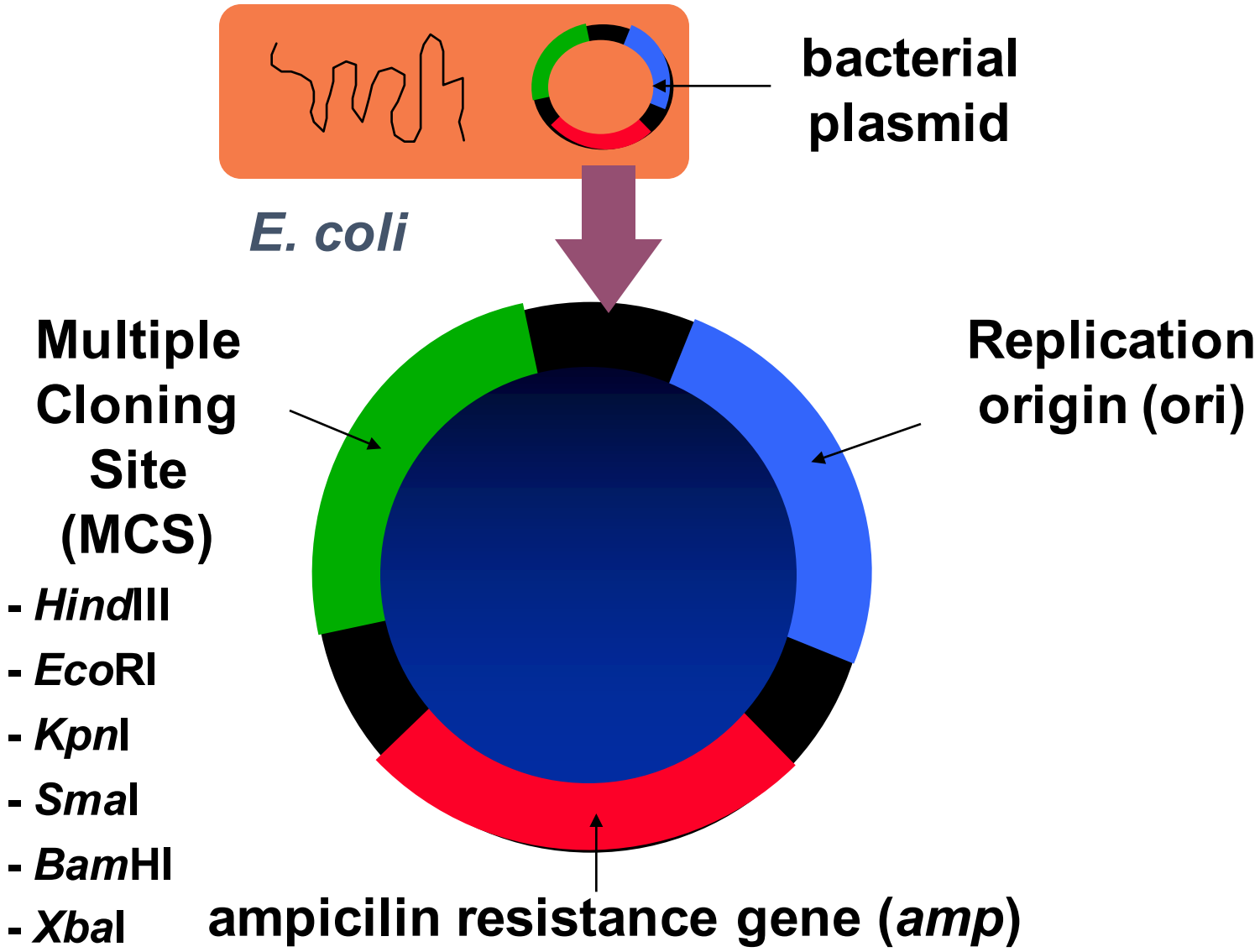
VECTORS

DNA LIGASE ENZYME

COMPETENT BACTERIAL CELLS

ANTIBIOTICS

DNA CLONING: plasmid vectors



DNA CLONING: TRANSFORMATION

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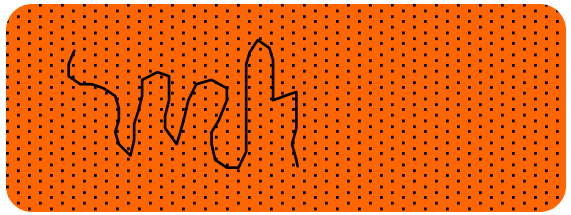

E. coli

“VECTOR”

+

“COMPETENT CELLS”
Chemically treated cells for easy DNA intake into the cell

“TRANSFORMED” BACTERIA