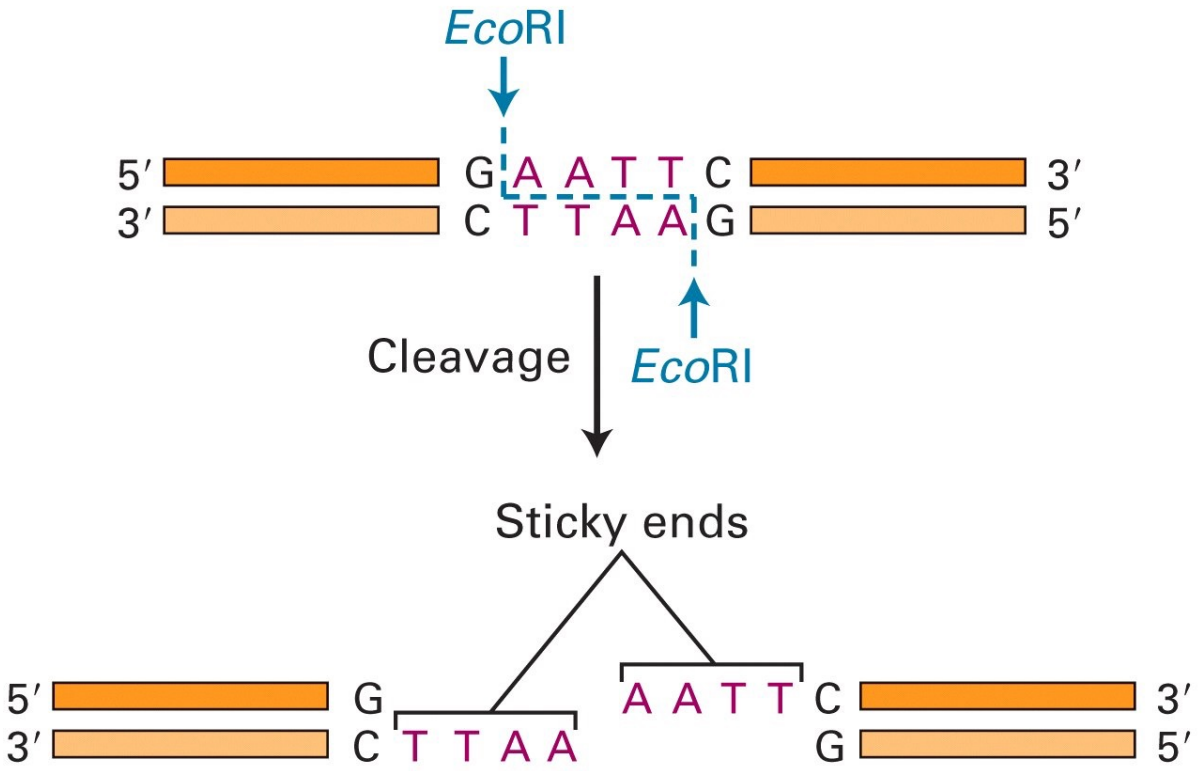
BIO 405L
Cellular and molecular biology laboratory

Restriction enzymes

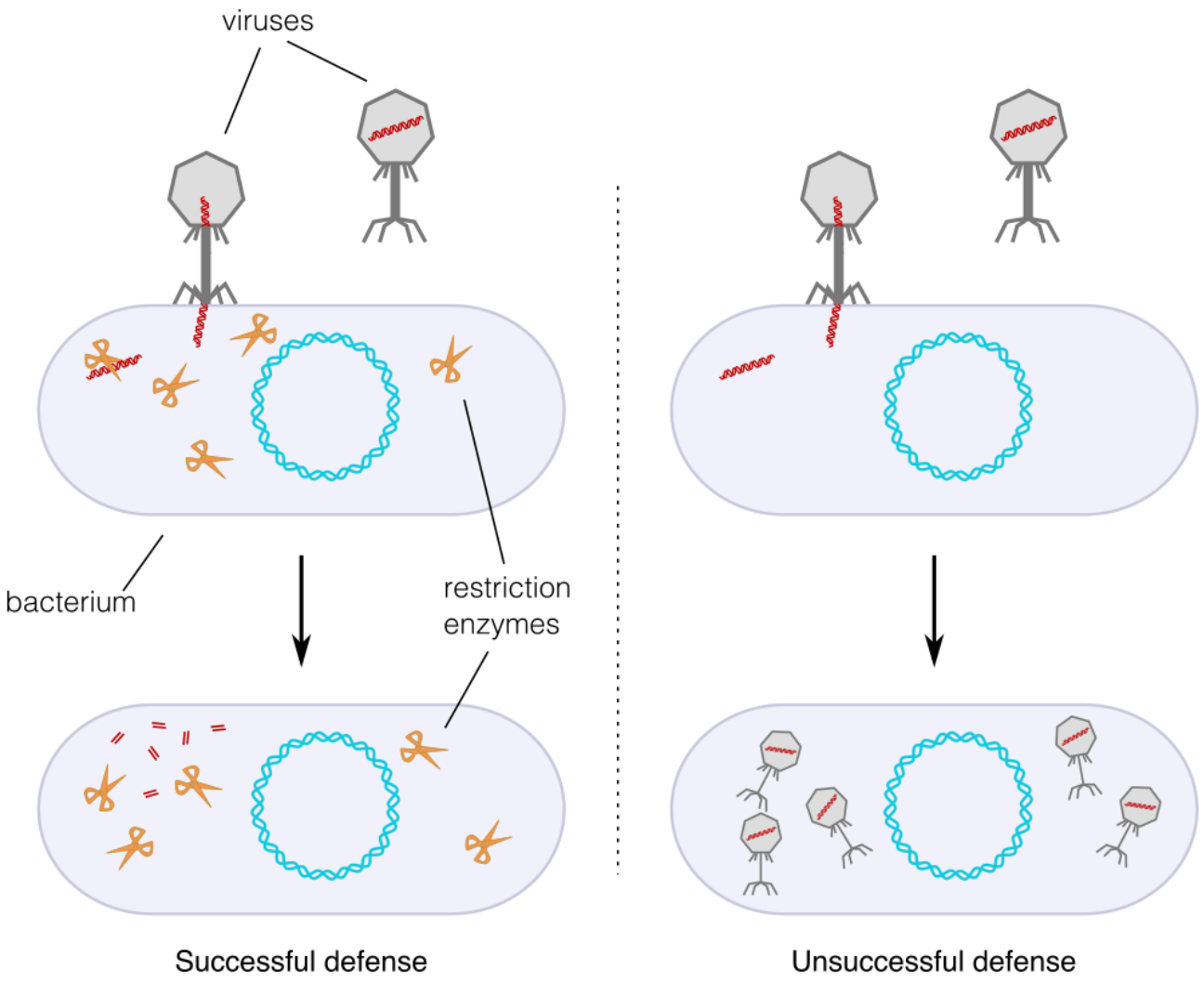
Hugo Castillo, Ph.D.

A **restriction enzyme**, **restriction endonuclease**, or ***restrictase*** is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules known as restriction sites.

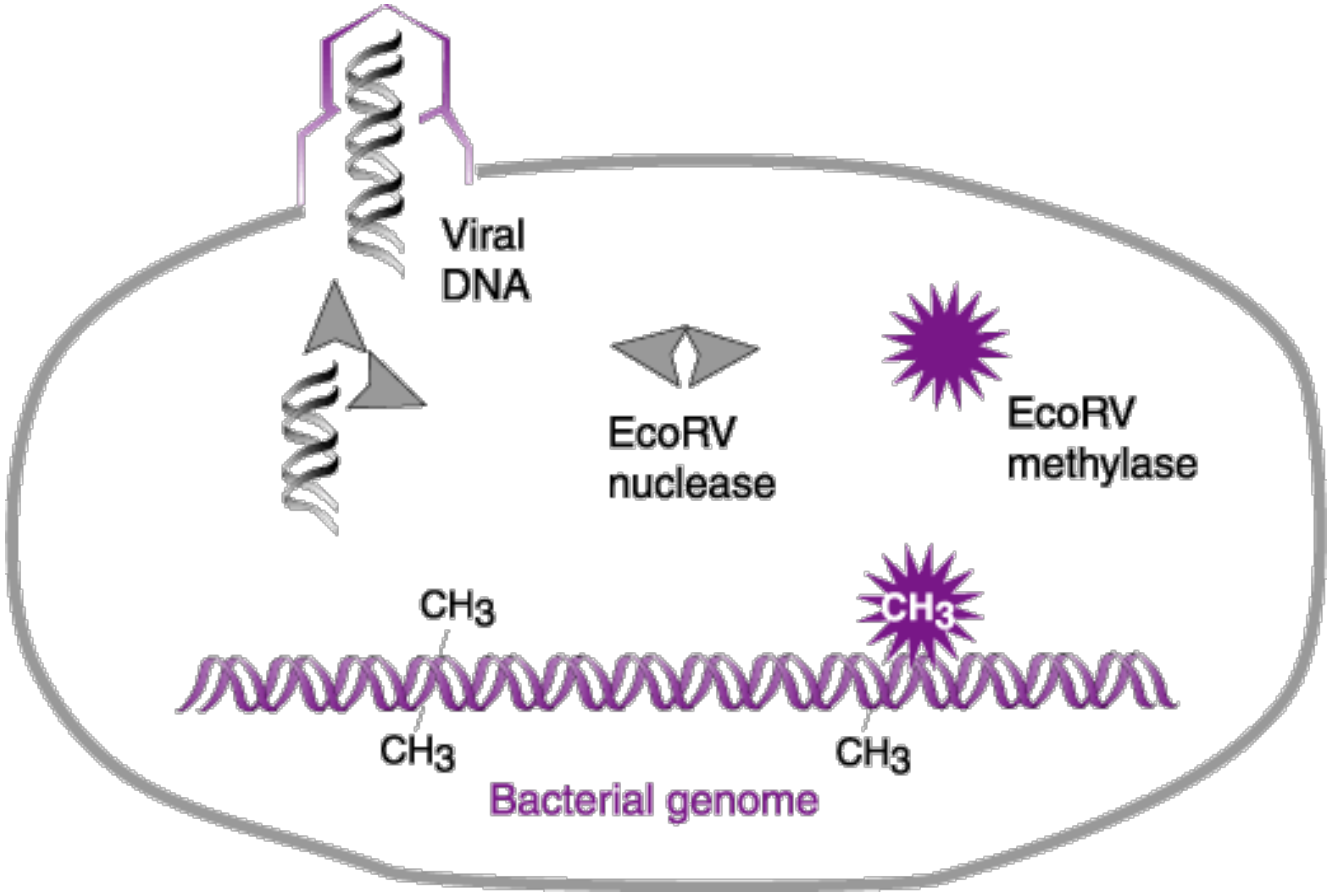


These enzymes are produced by bacteria and archaea

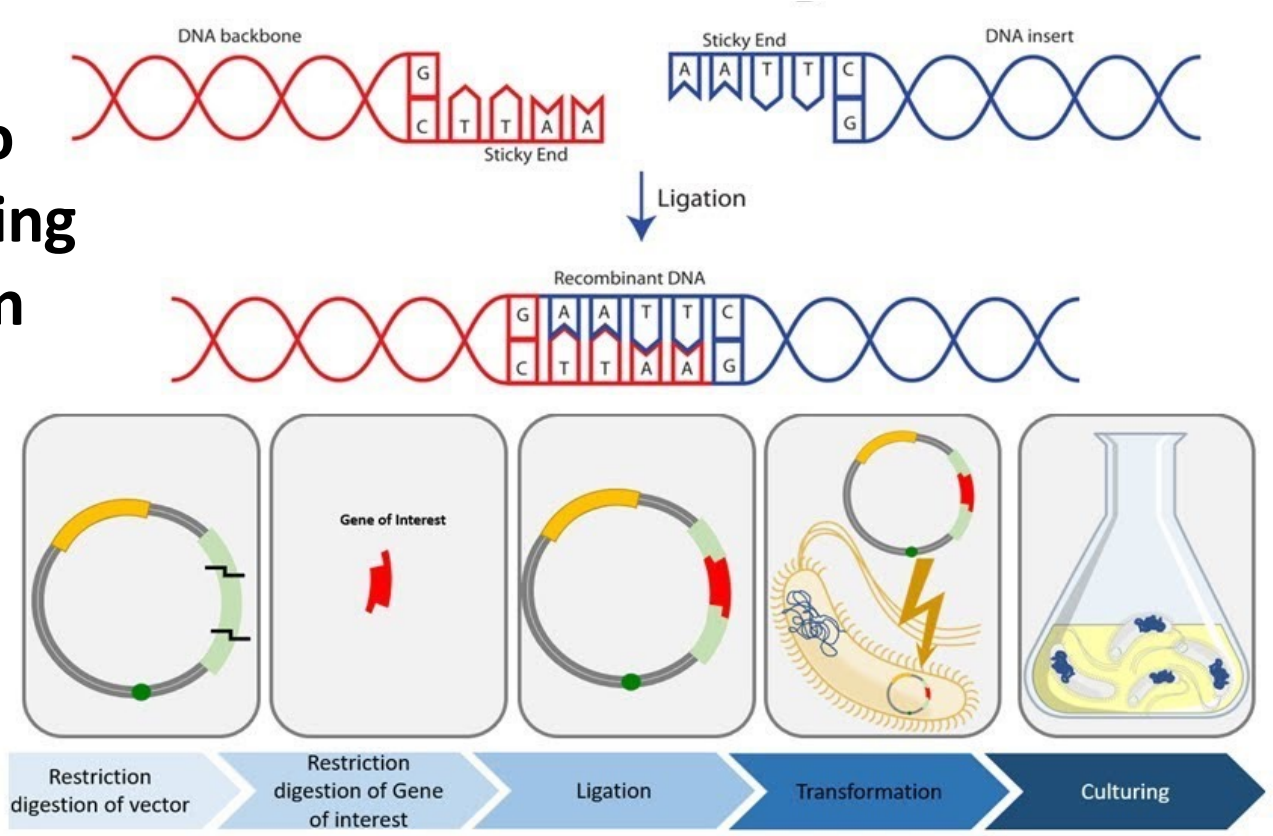
Name of the enzyme	Source	Recognition site and cleavage site	Nature of cut ends
Eco R1	*E. coli* RY13	5'–G|AATTC–3' 3'–CTTAA|G–5'	Sticky
Hind III	*Haemophilus influenzae* Rd	5'–A|AGCTT–3' 3'–TTCGA|A–5'	Sticky
Bam HI	*Bacillus amyloliquifaciens* H	5'–G|GATCC–3' 3'–CCTAG|G–5'	Sticky
Hae III	*Haemophilus aegiptius*	5'–GG|CC–3' 3'–CC|GG–5'	Blunt


viruses
bacterium
restriction
enzymes
Successful defense
Unsuccessful defense

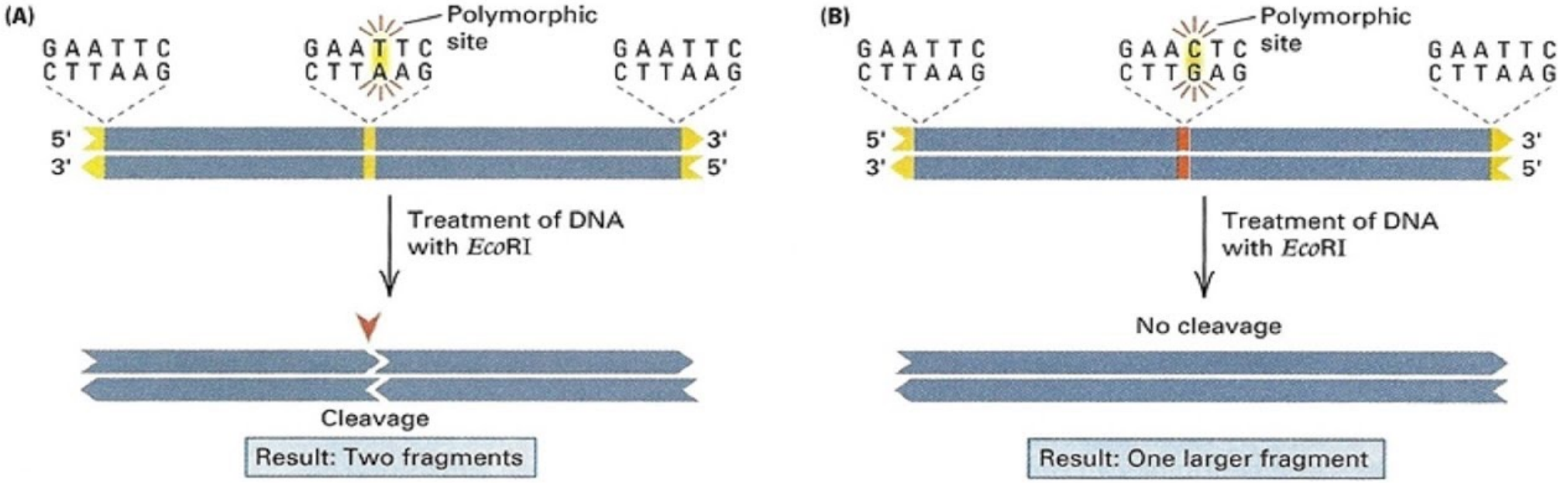
RE provide a defense mechanism against invading viruses. Inside bacteria, the RE selectively cut foreign DNA while evading the host DNA protected by methylation.



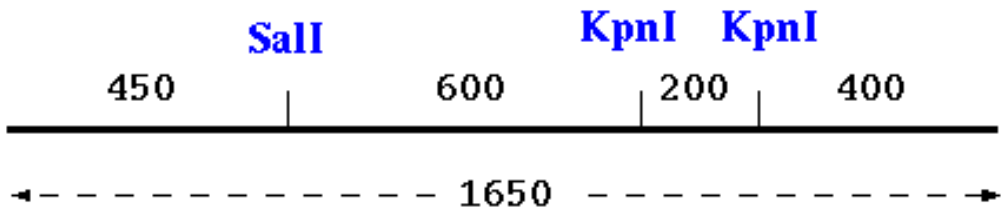
Insertion of genes into plasmid vectors for cloning and protein production



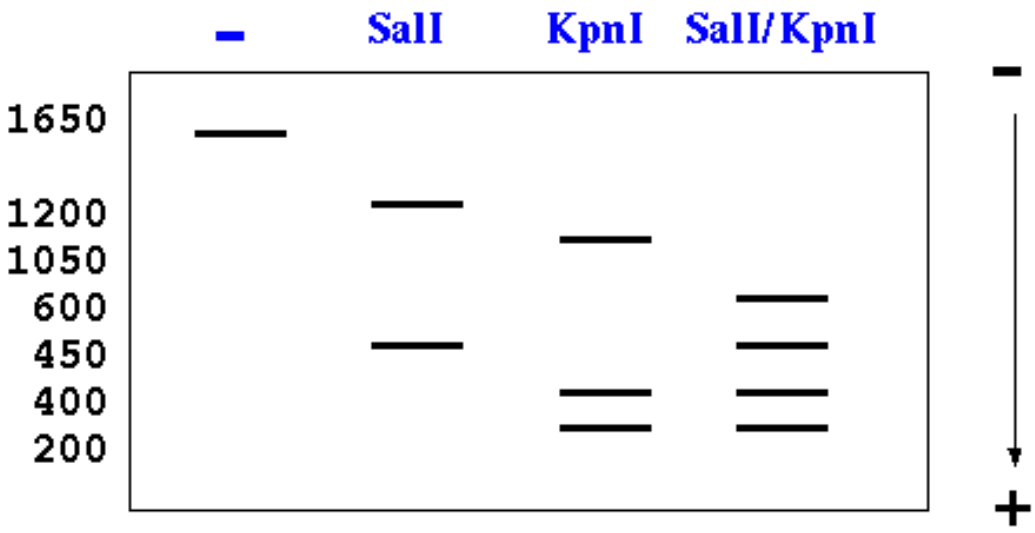
Restriction fragment length polymorphism (RFLP)

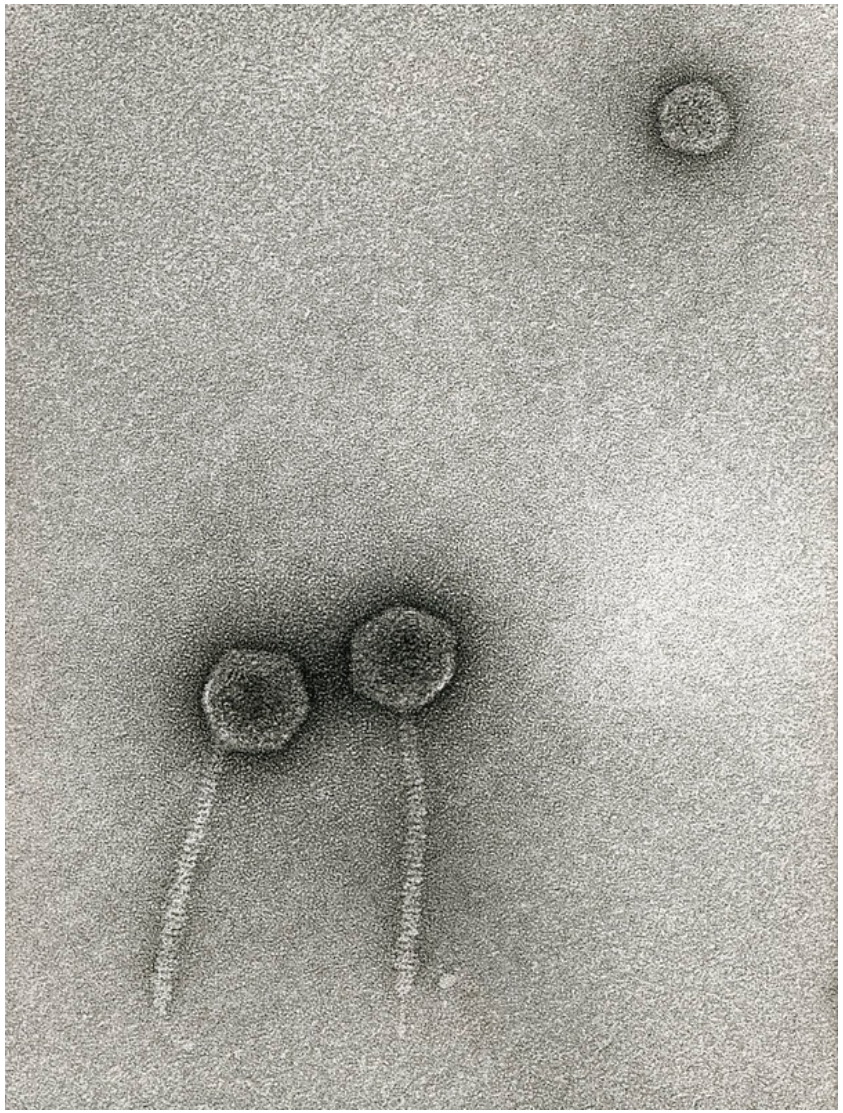


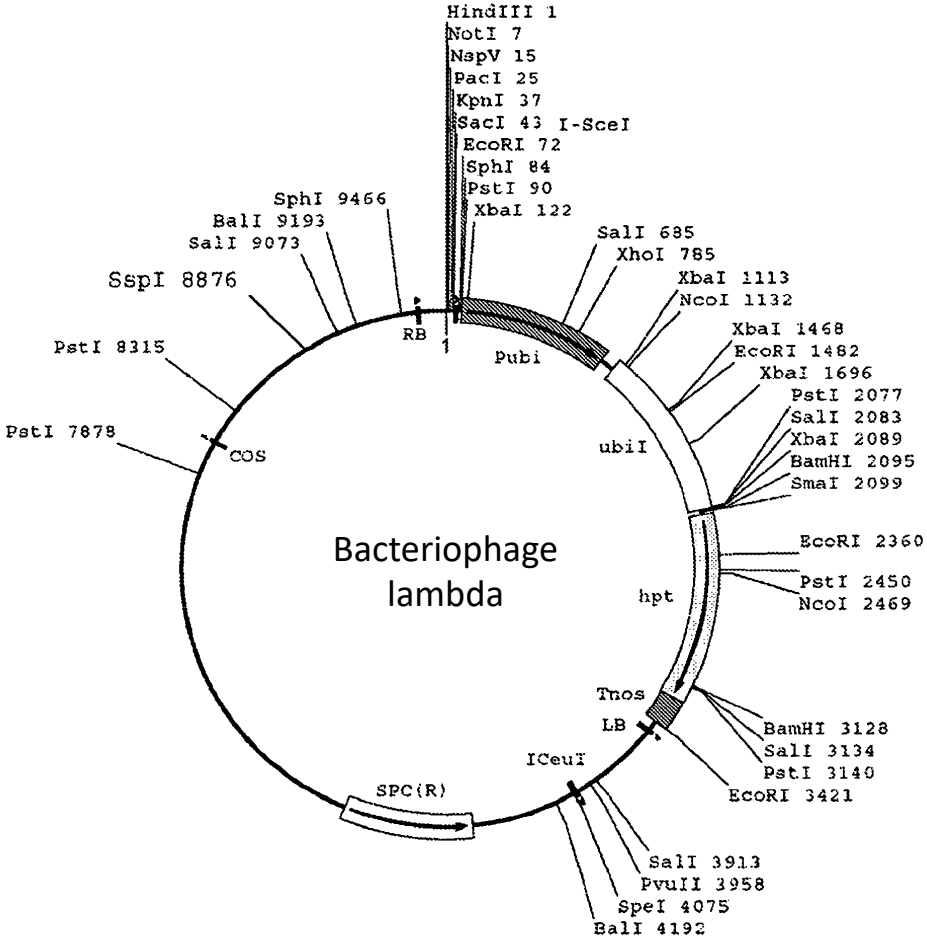
Restriction map

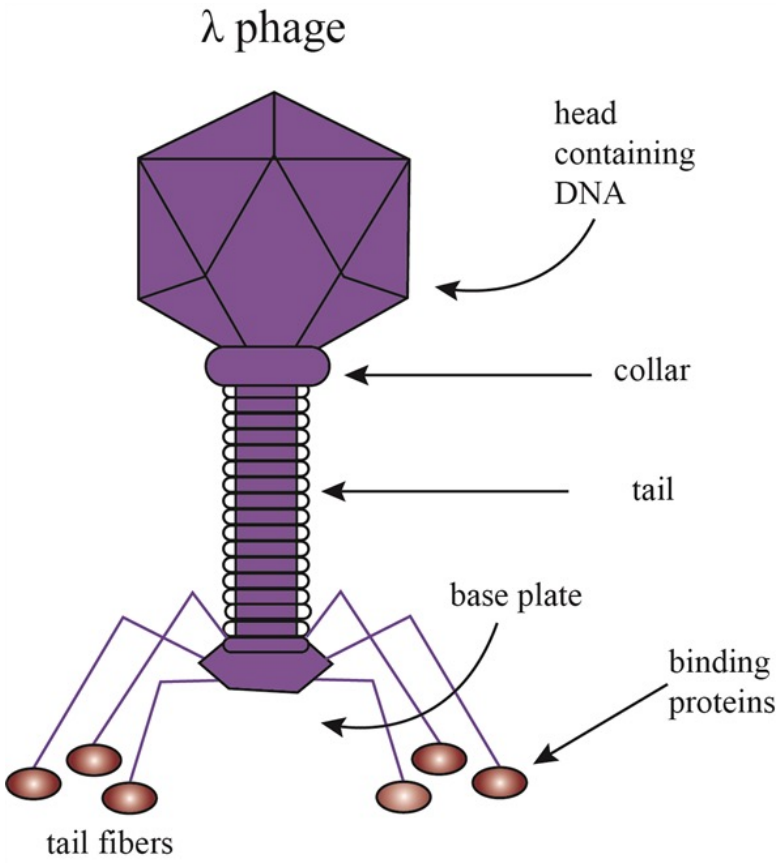


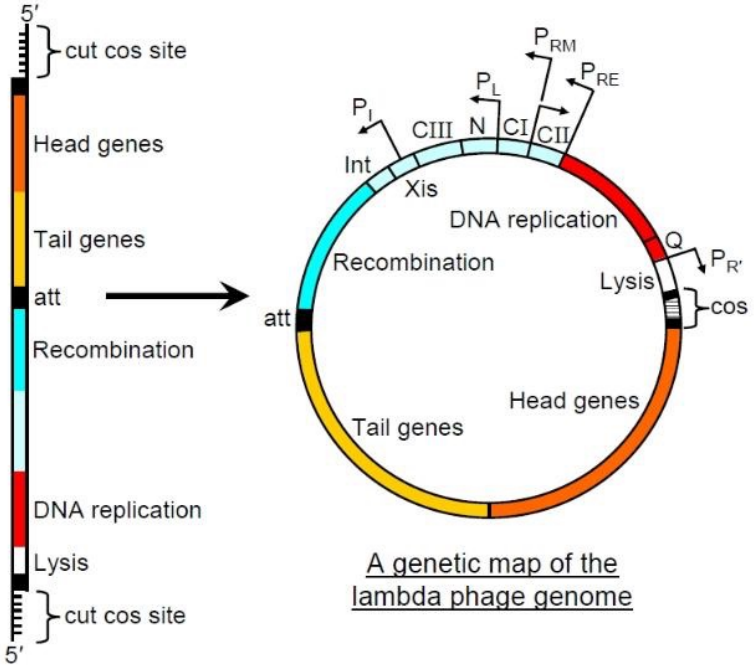
Predicted digest fragments






HindIII 1
NotI 7
NspV 15
PacI 25
KpnI 37
SacI 43 I-SceI
EcoRI 72
SphI 84
PstI 90
XbaI 122
SphI 9466
BalI 9193
SalI 9073
SspI 8876
PstI 8315
PstI 7878
RB
COS
Pubi
SalI 685
XhoI 785
XbaI 1113
NcoI 1132
XbaI 1468
EcoRI 1482
XbaI 1696
PstI 2077
SalI 2083
XbaI 2089
BamHI 2095
SmaI 2099
ubiI
Bacteriophage
lambda
EcoRI 2360
PstI 2450
NcoI 2469
hpt
Tnos
LB
BamHI 3128
SalI 3134
PstI 3140
EcoRI 3421
ICeuI
SPC(R)
SalI 3913
PvuII 3958
SpeI 4075
BalI 4192

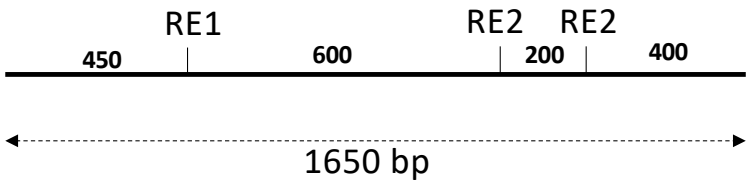




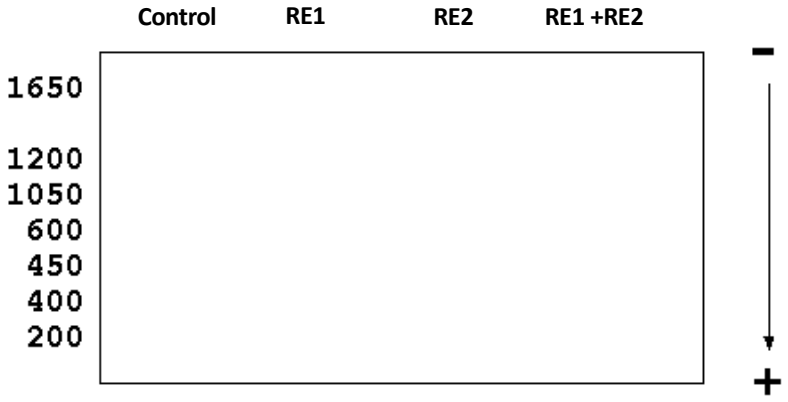
A genetic map of the lambda phage genome

When packaged in the phage head the dsDNA is linear with single-stranded complementary overhangs of single-stranded DNA at the *cos* site at each end. In the host these 'sticky-ends' join together (and are sealed by the host enzyme ligase) to form a circular dsDNA molecule protected from degradation by host exonucleases. The host enzyme DNA gyrase also supercoils the cdsDNA.

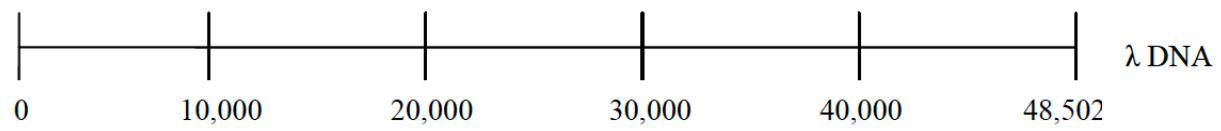
Restriction map

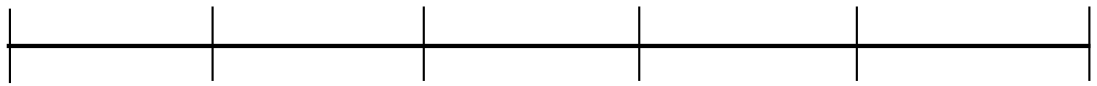


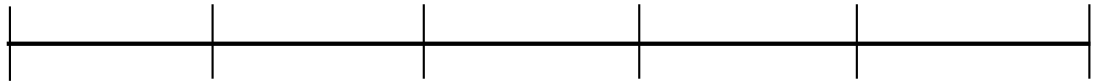
Predicted gel

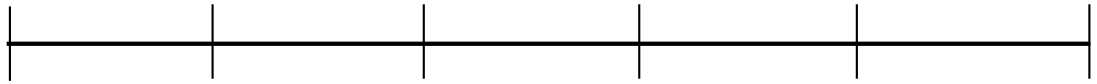


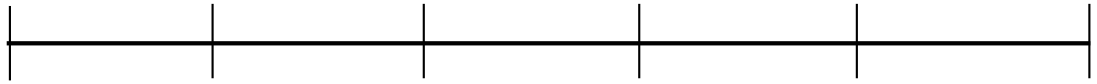
Derivation of the EcoRI name		
Abbreviation	Meaning	Description
E	*Escherichia*	genus
co	*coli*	specific species
R	RY13	strain
I	First identified	order of identification in the bacterium


λ DNA
0
10,000
20,000
30,000
40,000
48,502


EcoRI


BamHI


HindIII


AluI

Reagents	BamHI	Control
10X Tango buffer	2	2
Lambda DNA	2	2
Enzyme	2	0
Water	14	16

Reagents	EcoRI	HindIII
10X R buffer	2	2
Lambda DNA	2	2
Enzyme	2	2
Water	14	14

All enzymes and control:
Incubate at 37 C/3 h

Then:

inactivate at 80 C/20 min

Reagent	EcoRI	BamHI	HindIII	AluI	Control
Water	14	14	14	14	16
Buffer	2	2	2	2	2
Lambda DNA	2	2	2	2	2
Enzyme	2	2	2	2	0

Incubation temperature: 37 C/ 2 h
Inactivation: 80 C/15 min