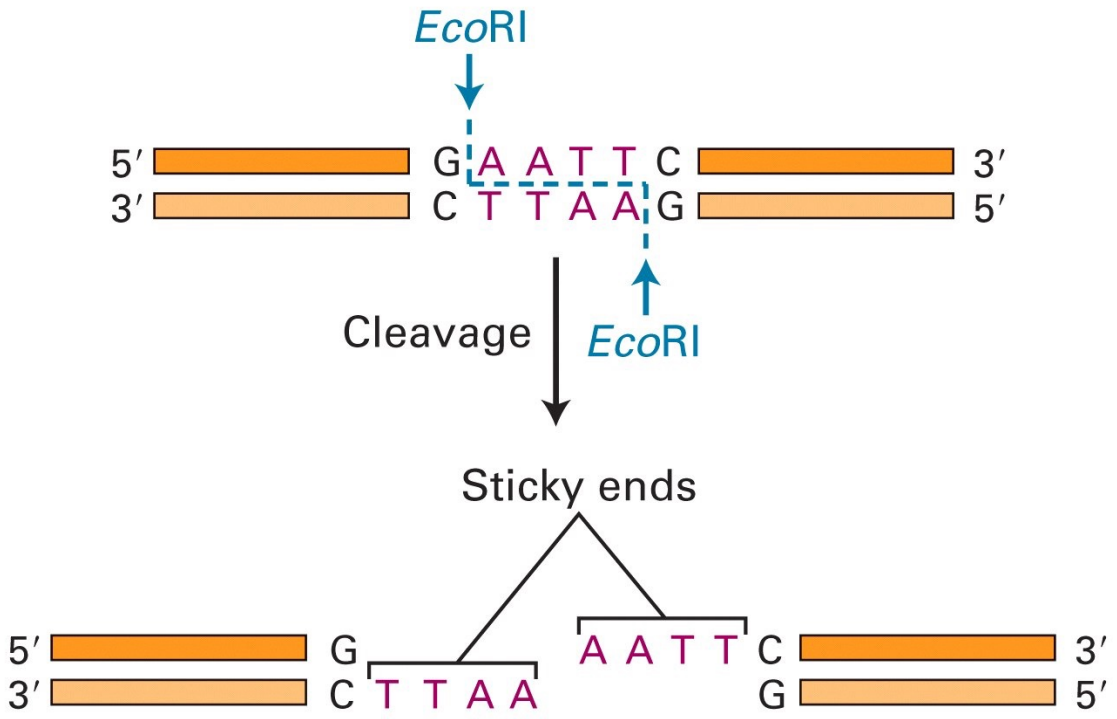
BIO 405L
Cellular and molecular biology laboratory

Restriction enzymes

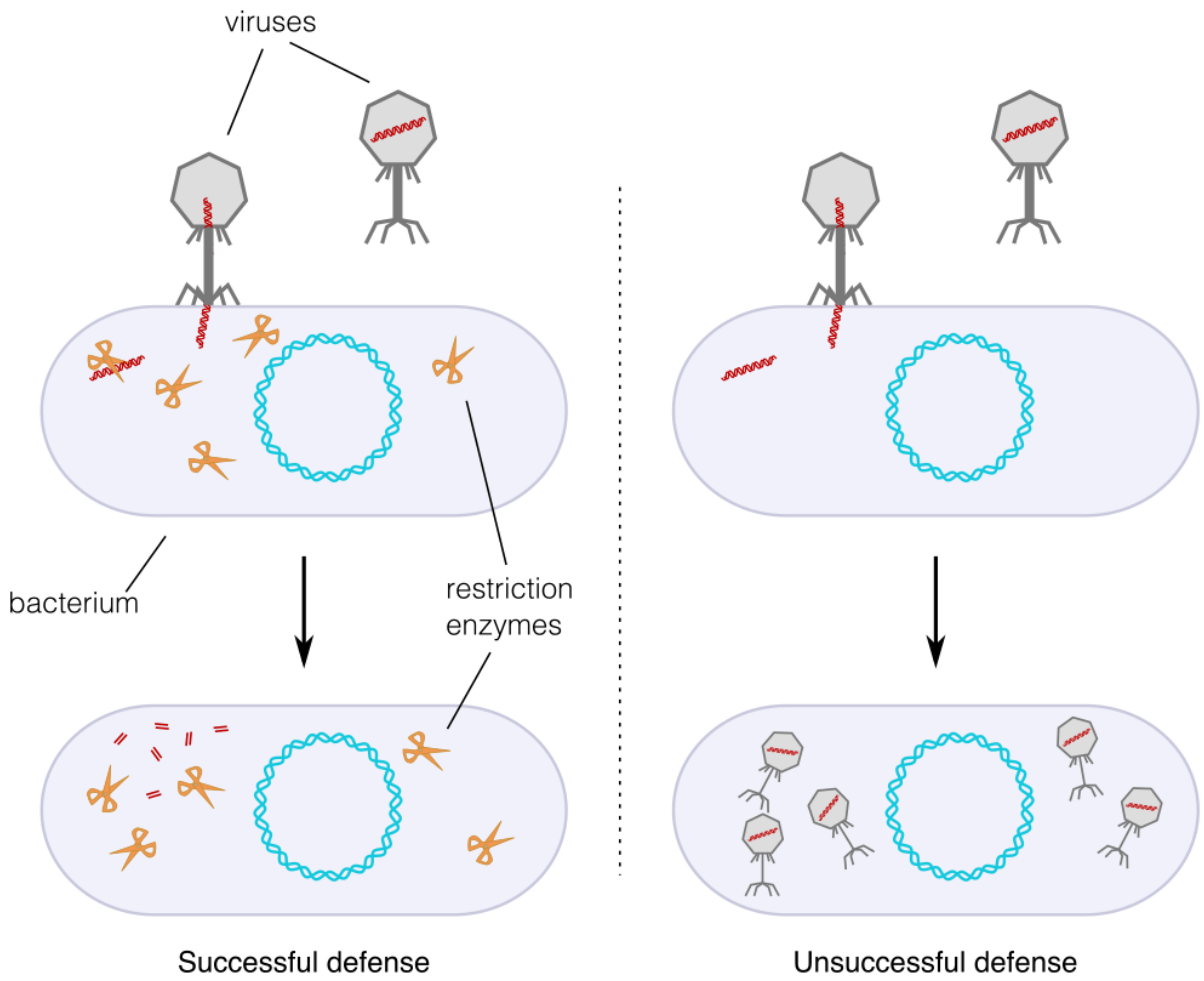
Hugo Castillo, Ph.D.

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.

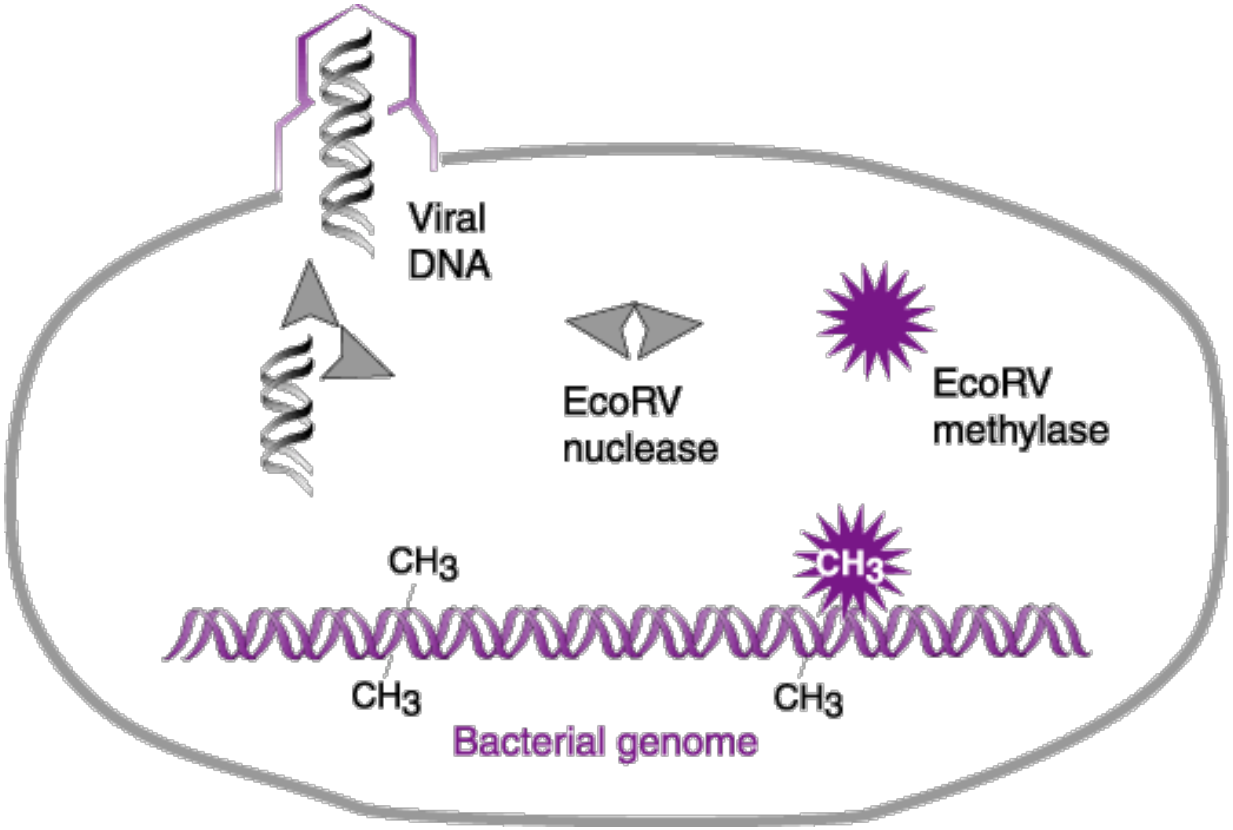


These enzymes are produced by bacteria and archaea

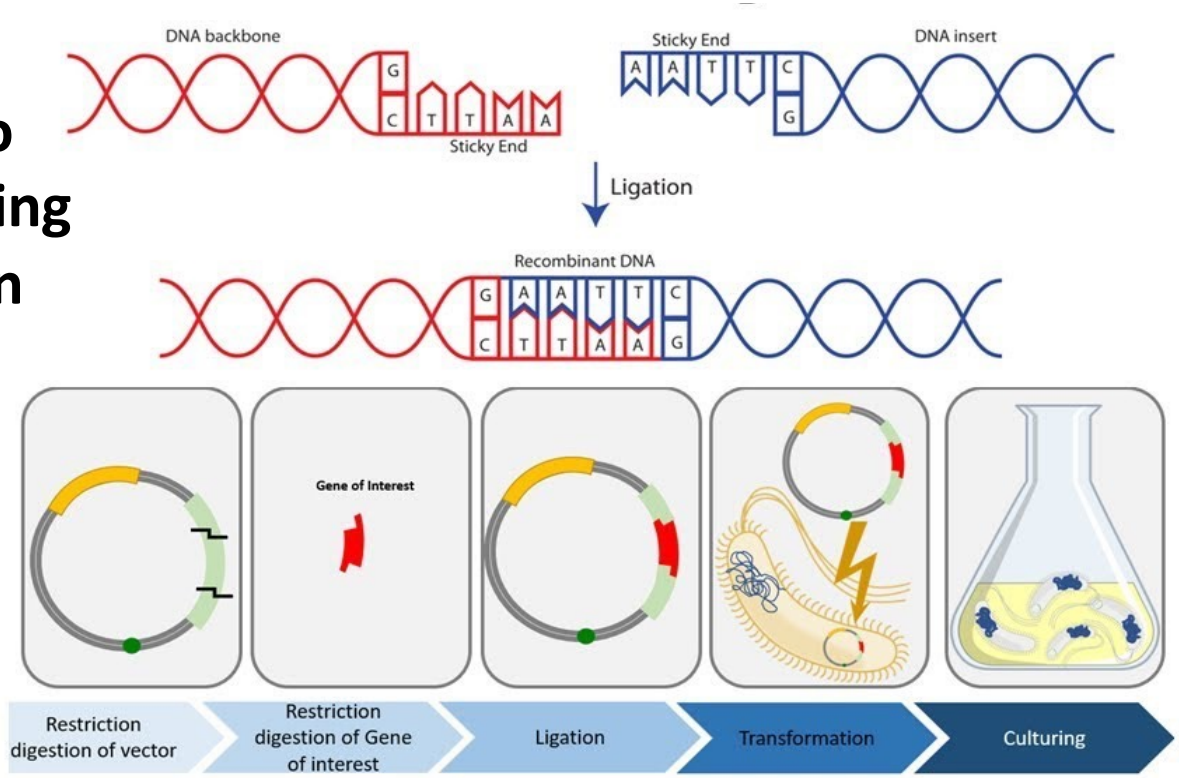
Name of the enzyme	Source	Recognition site and cleavage site	Nature of cut ends
Eco R1	*E. coli* RY13	5'–G|AATTC–3' 3'–CTTAA|G–5'	Sticky
Hind III	*Haemophilus influenzae* Rd	5'–A|AGCTT–3' 3'–TTCGA|A–5'	Sticky
Bam HI	*Bacillus amyloliquifaciens* H	5'–G|GATCC–3' 3'–CCTAG|G–5'	Sticky
Hae III	*Haemophilus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



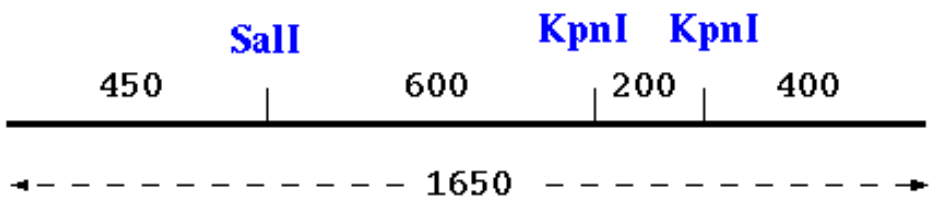
Insertion of genes into plasmid vectors for cloning and protein production



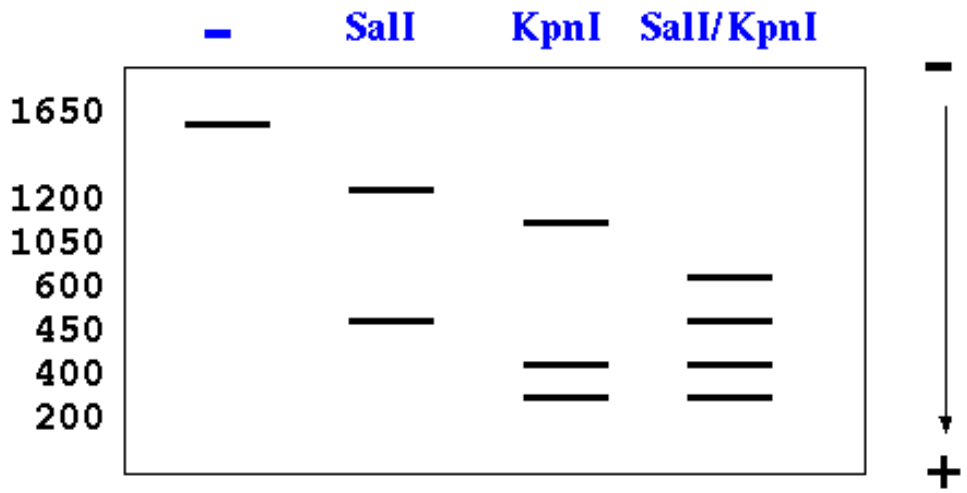
Reagent	EcoRI	BamHI	HindIII	E+B+H	Control
Water	14	14	14	14	16
Buffer	2	2	2	2	2
Lambda DNA	2	2	2	2	2
Enzyme	2	2	2	2*	0

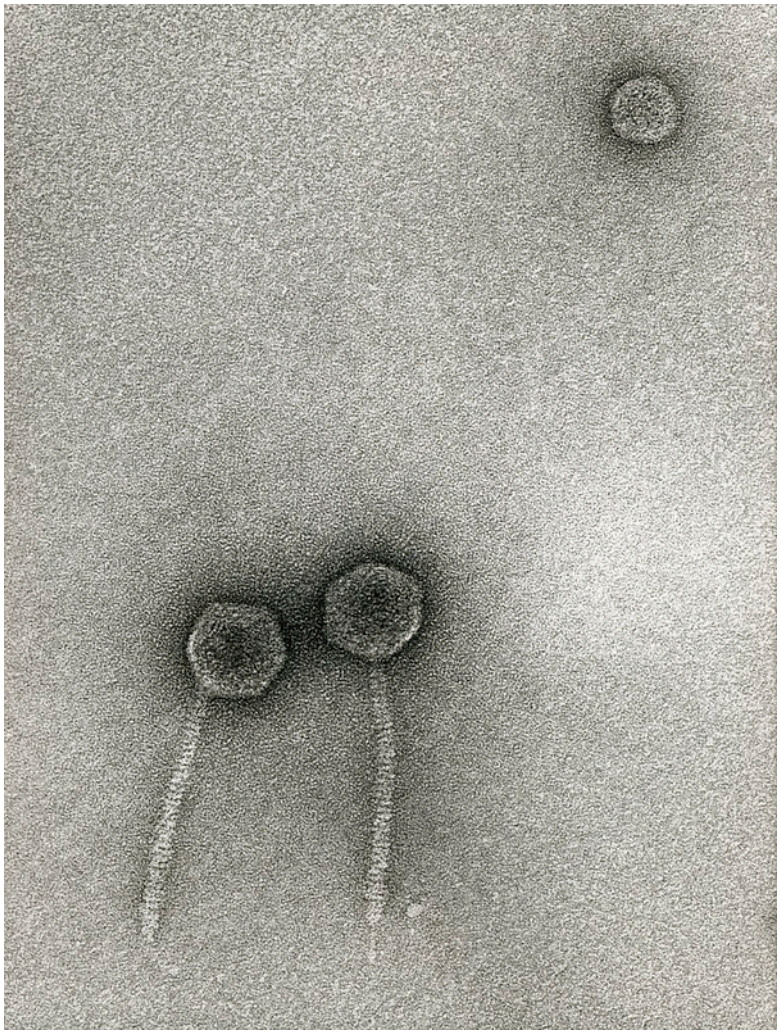
Incubation temperature: 37 C/ 2 h
Inactivation: 80 C/15 min
*Enzyme mixture, already prepared

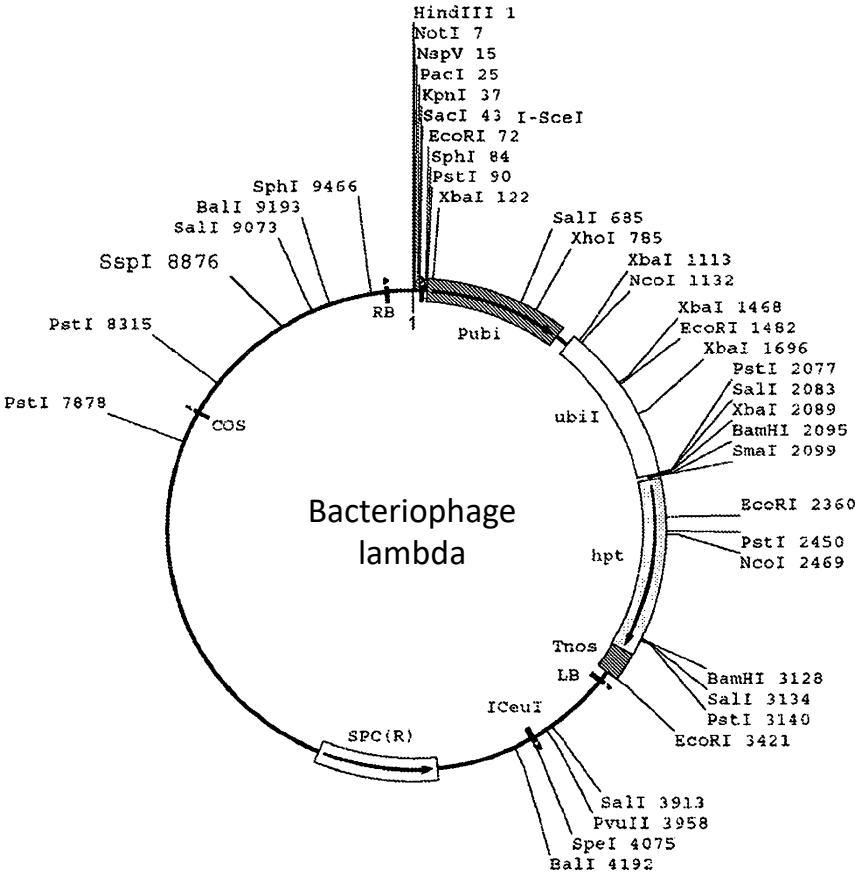
Restriction map

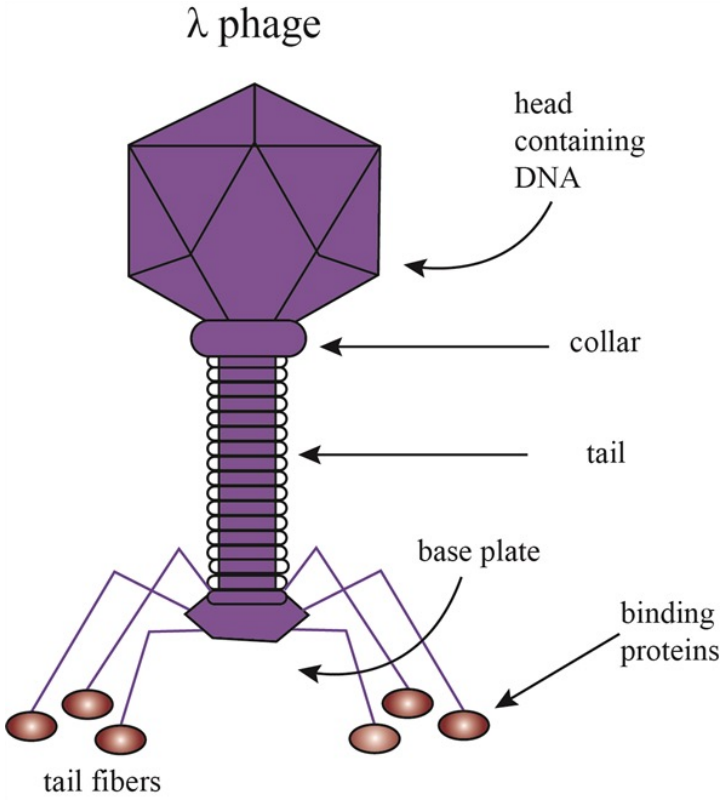


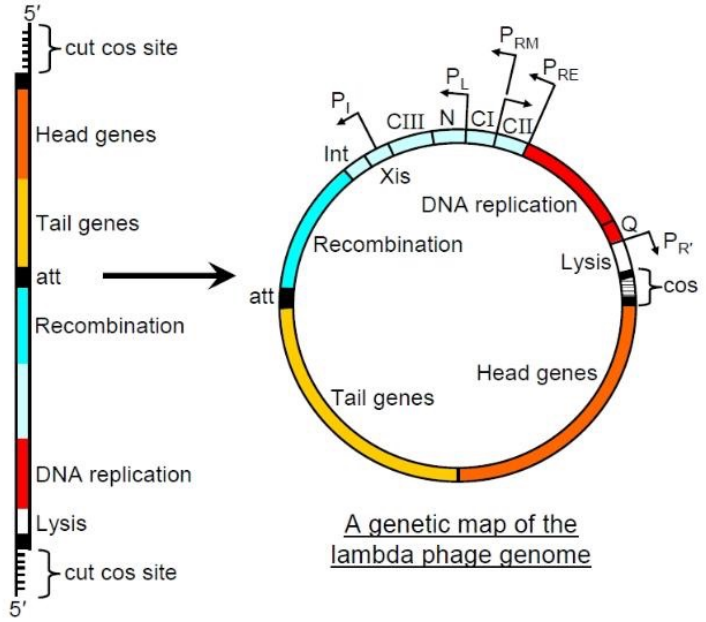
Predicted digest fragments






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





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.