



Cloning vectors

- Propagation of DNA
 - gene of interest
 - cDNA or genomic library
- Manipulation of DNA
 - nucleotide sequencing
 - site-directed mutagenesis
- Delivery of DNA
 - expression of large quantities of protein
 - functional expression



Types of cloning vectors

- plasmids
- filamentous phages
- lambda phages
- cosmids
- bacterial artificial chromosomes (BACs)
- yeast artificial chromosomes (YACs)



Plasmids

Plasmids are double-stranded, circular, self-replicating, extra-chromosomal DNA molecules.

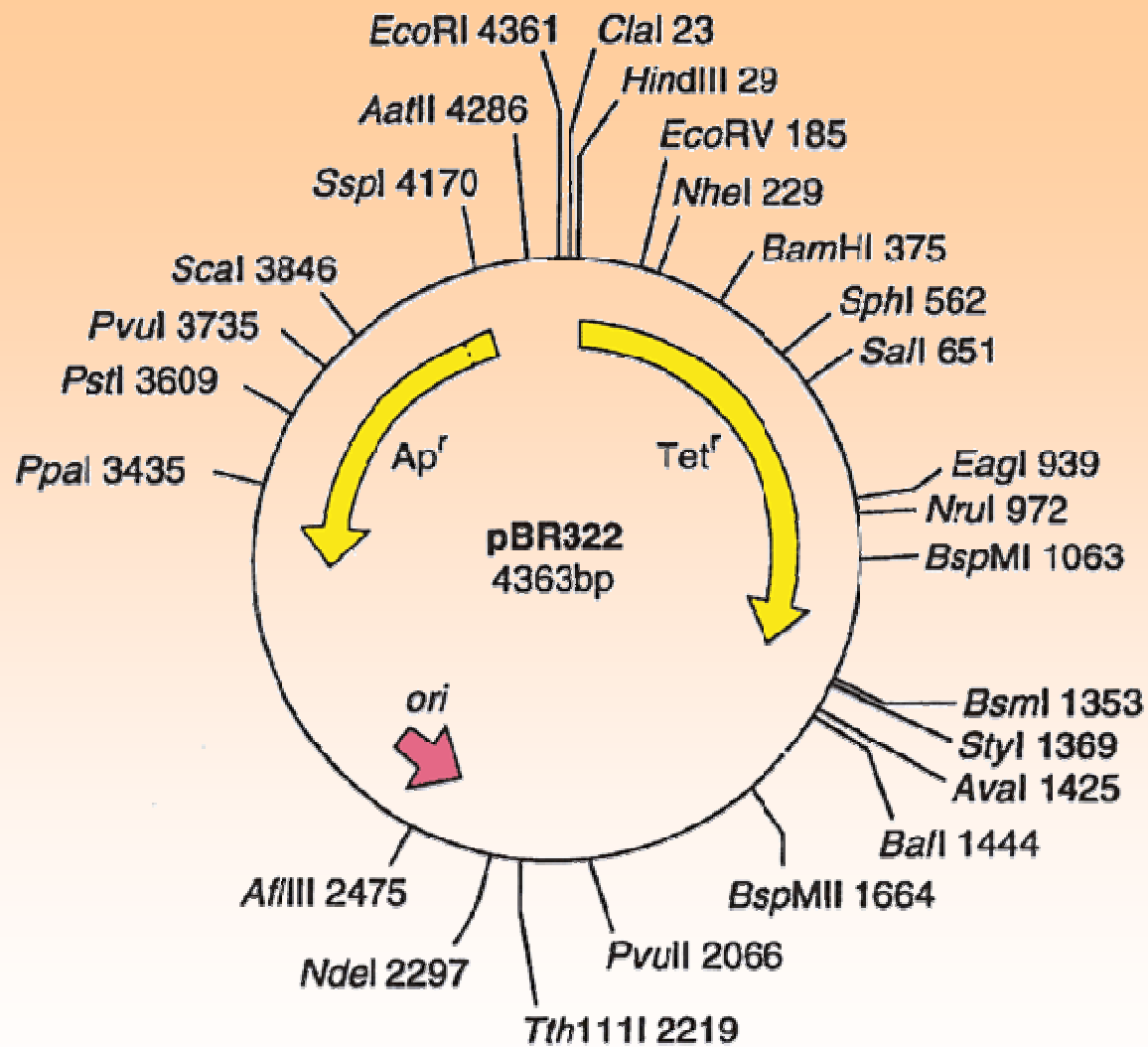
Plasmids (continued)

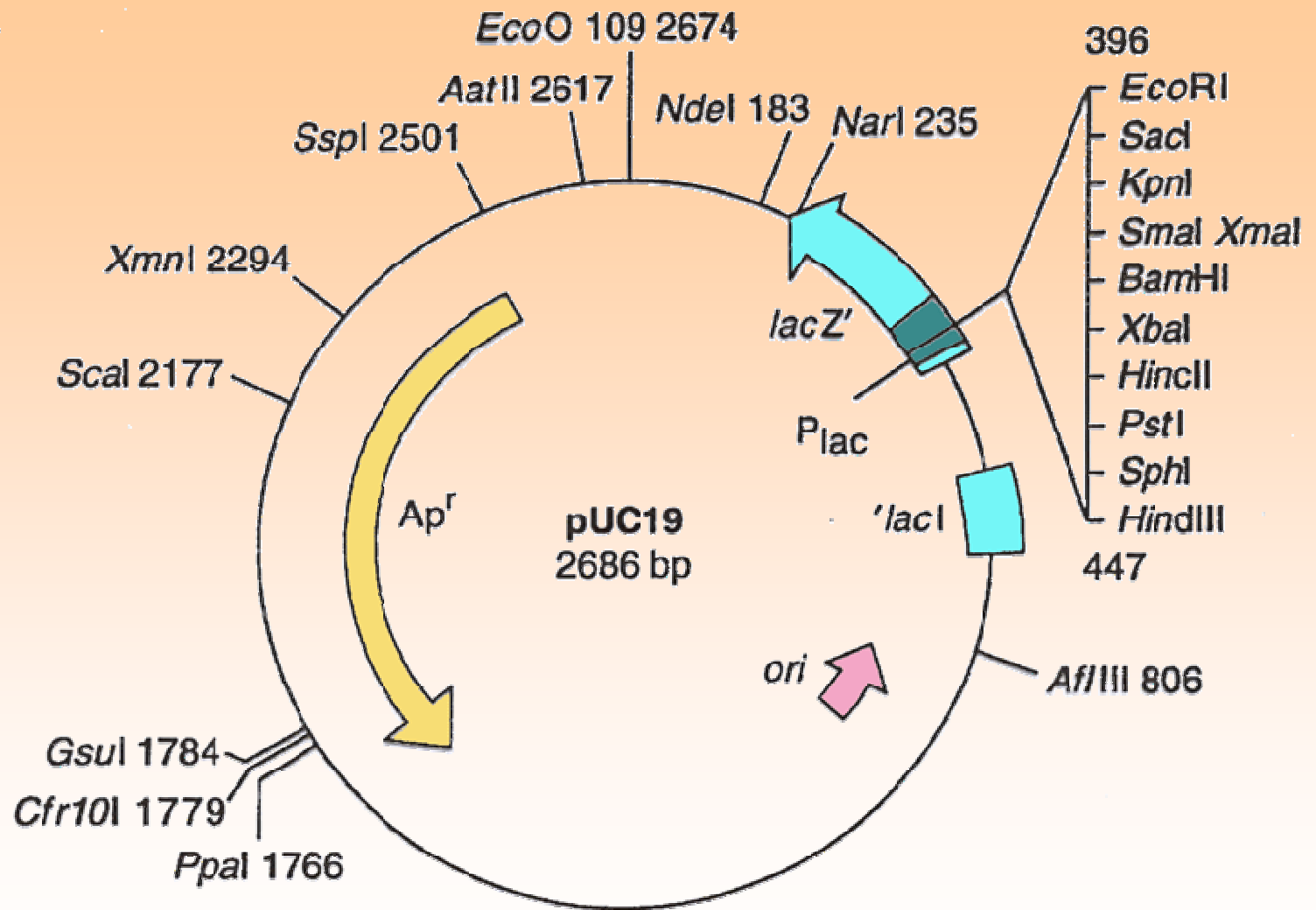
- a replicator
 - ColE1, pMB1
 - copy-number
- a selectable marker
 - dominant (e.g. Amp, Tet, Kan)
 - recessive (e.g. LeuB)
- a multiple cloning site (MCS)
 - restriction sites

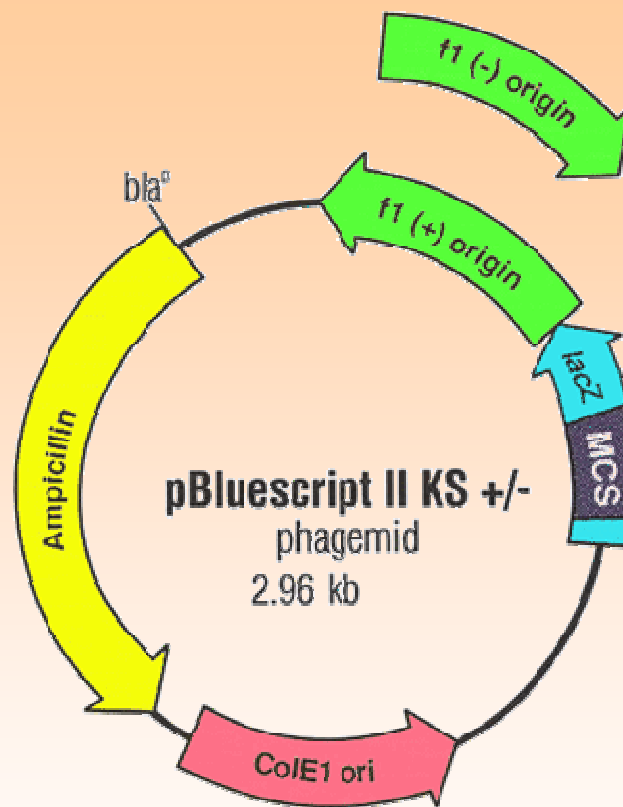


Plasmids (continued)

- 8-bp-cutter sites for insert excission
- oligonucleotide complementary sequences
- RNA promoters for transcription of cRNA
- LacZ gene for insert selection
- Trc promoter for regulated expression in *E. coli*
- propagation, selection and expression outside *E. coli*
- transcriptional / translational fusion products







*Bss*H II

T7 ↓

*Sac*I

*Bsf*X I

*Sac*II

*Eag*I

*Not*I

*Xba*I

*Spe*I

*Bam*HI

*Sma*I

*Pst*I

*Eco*R I

*Eco*R V

*Hind*III

*Bsp*106 I (*Cla*I)

*Sal*I/*Hinc*II/*Acc*I

*Xho*I

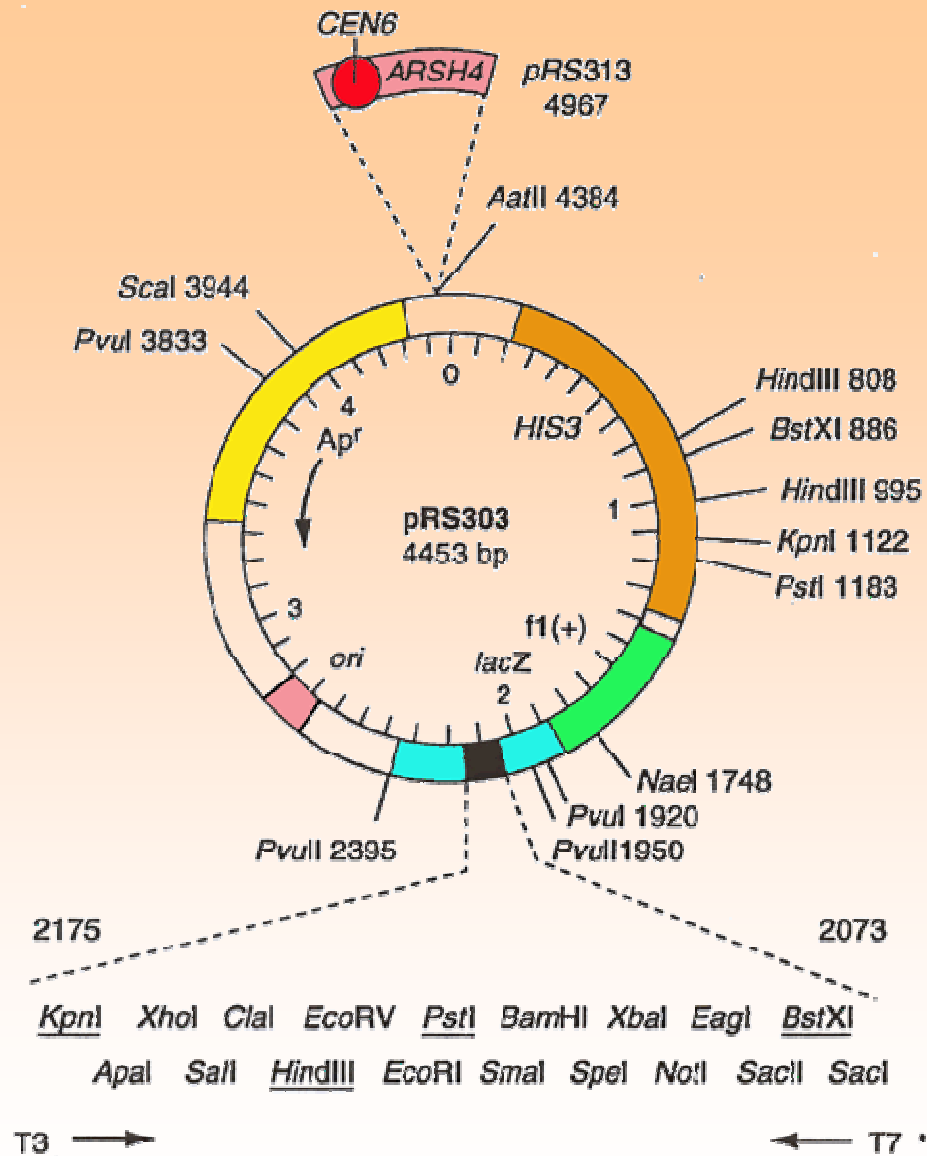
*Eco*0109 I (*Dra*II)

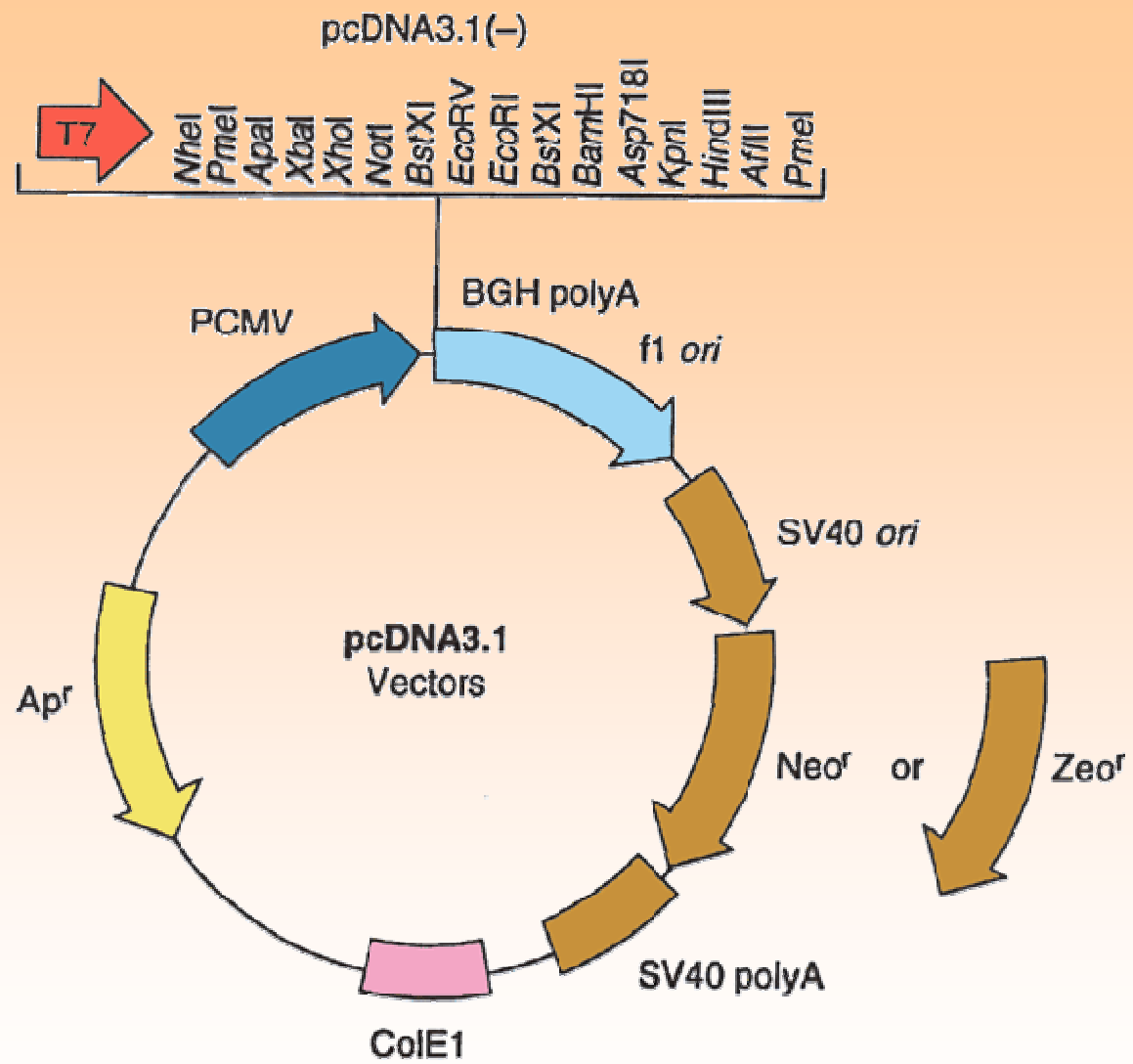
*Apa*I

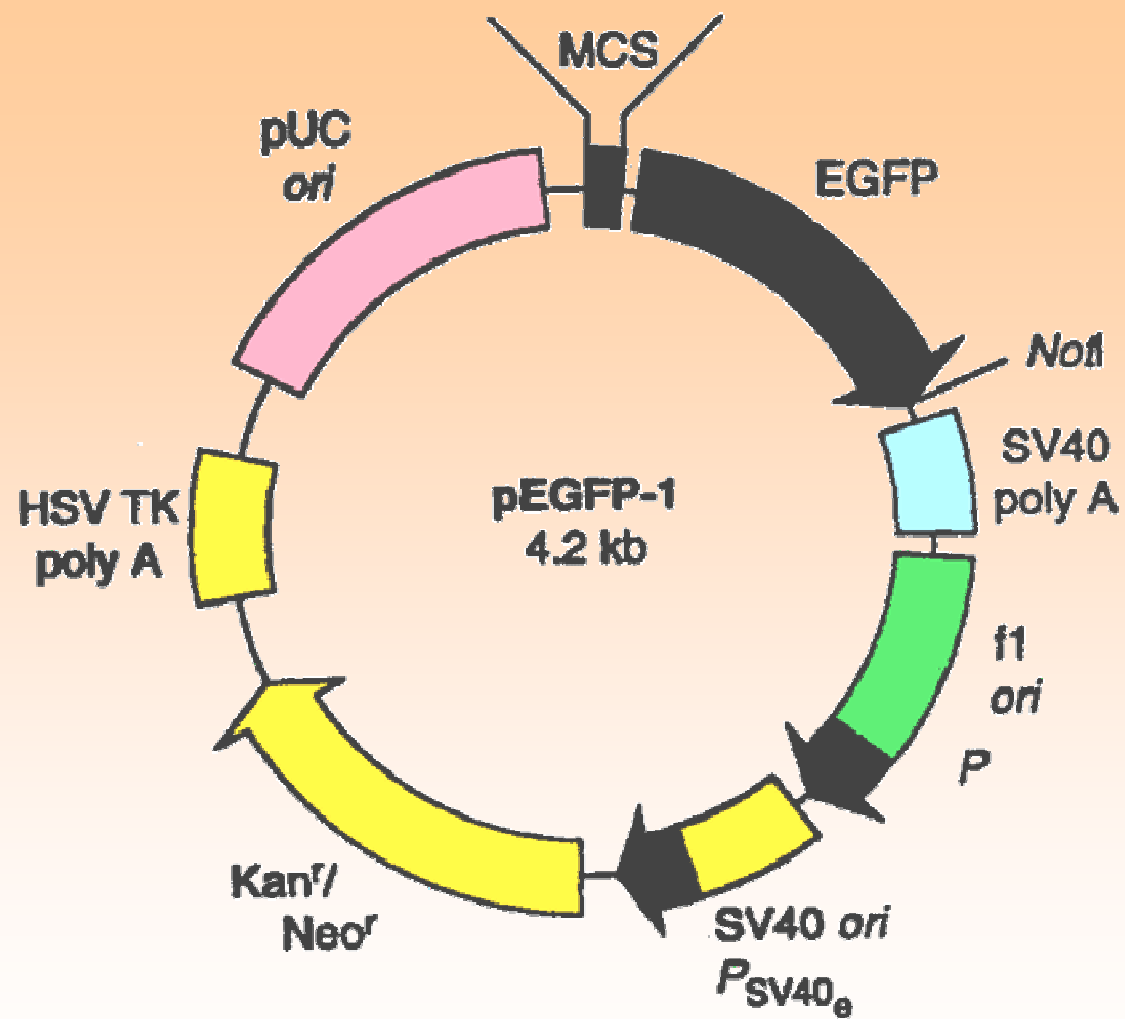
*Kpn*I

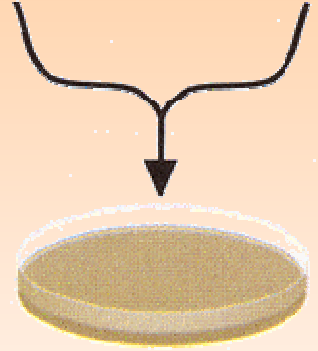
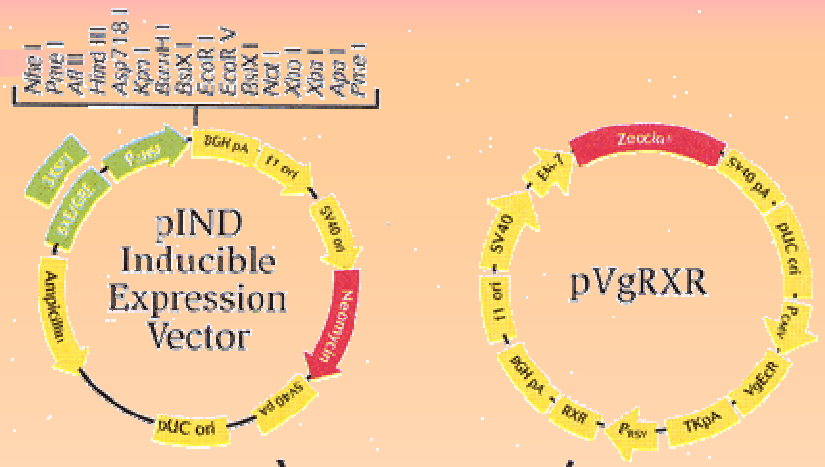
T3 ↑

*Bss*H II

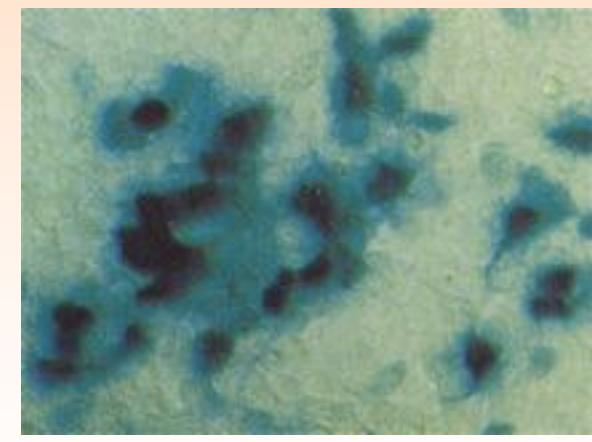
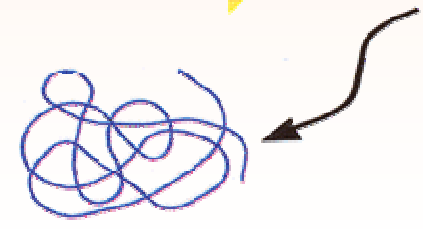
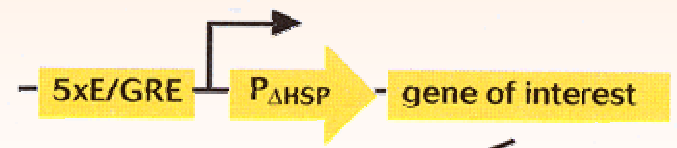








← **Ponasterone A**





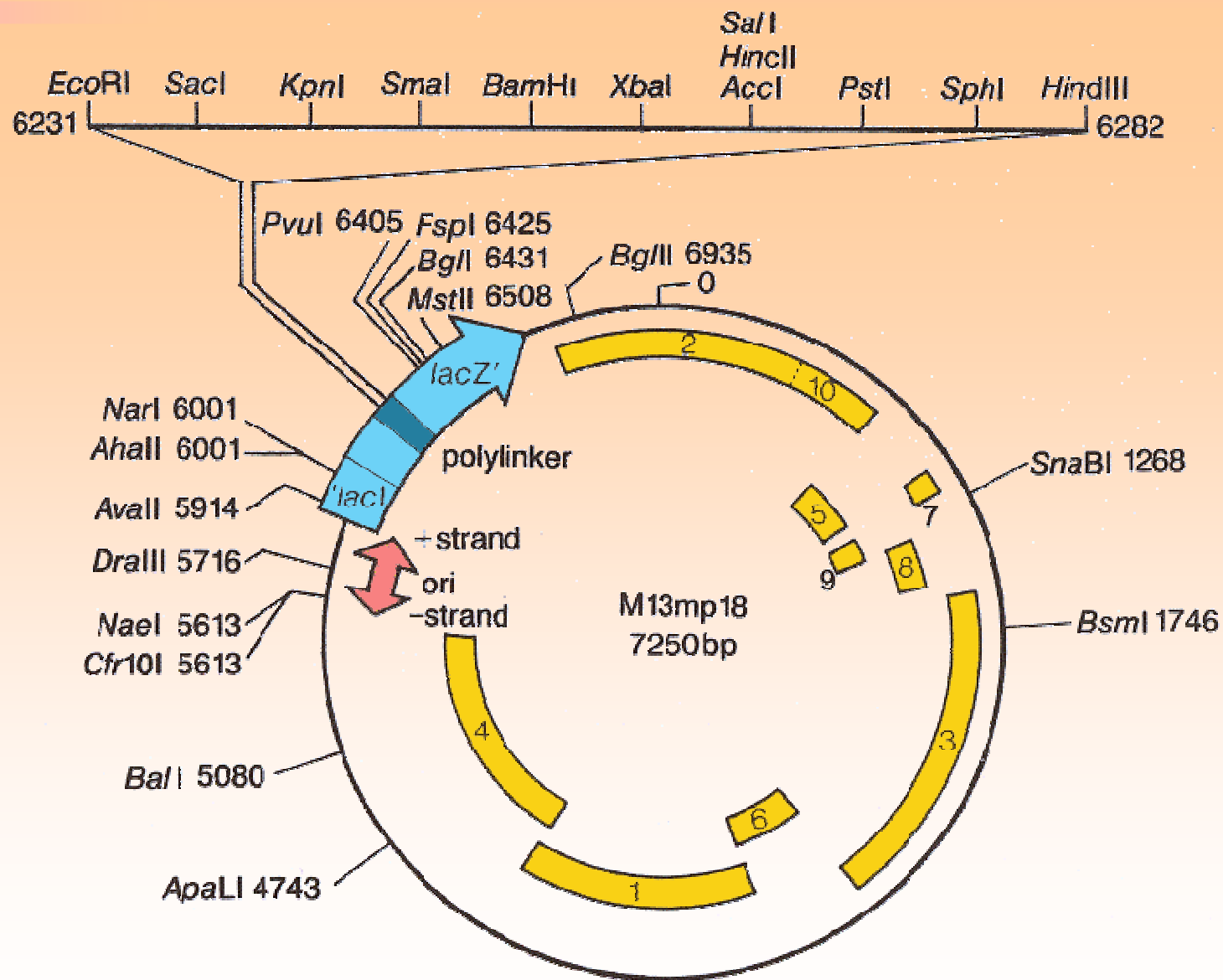
Philamentous phages

Philamentous phages are single-stranded DNA phages that infect F-factor containing E. coli, and can be recovered in both single-stranded (phage) and double-stranded (plasmid) form.



Philamentous phages (continued)

- examples: f1, M13, fd
- advantages: very useful for sequencing and mutagenesis
- some plasmids can propagate as “phagemids” when co-transfected with helper phages





Lambda phages

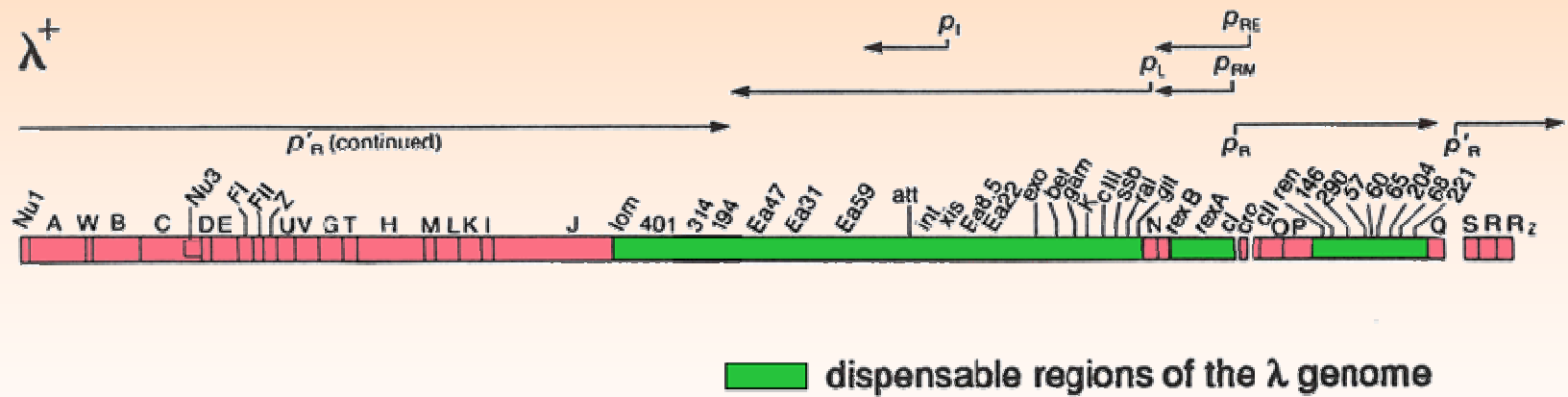
Lambda phages are ~50 kb double-stranded DNA phages that replicate in *E. coli* either in the lytic or in the lysogenic mode.



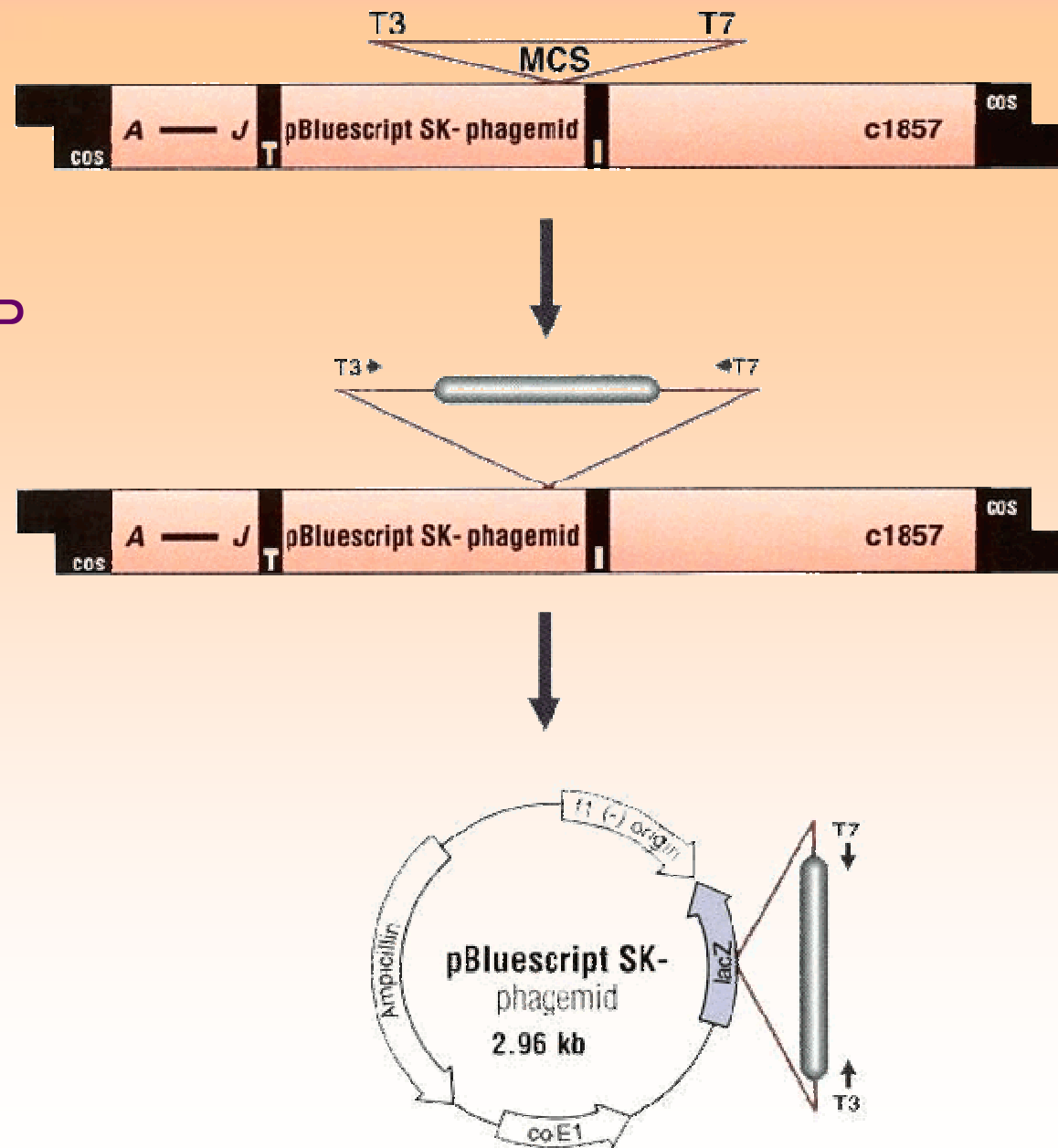
Lambda phages (continued)

- examples: EMBL3, λ gt10, λ ZAP
- suitable for either small (0-5 kb) or large (10-20 kb) inserts
- efficient cloning and easy maintenance (cDNA and genomic libraries)
- easy screening of large amounts of recombinant clones (libraries)

wild-type lambda DNA



λ ZAP



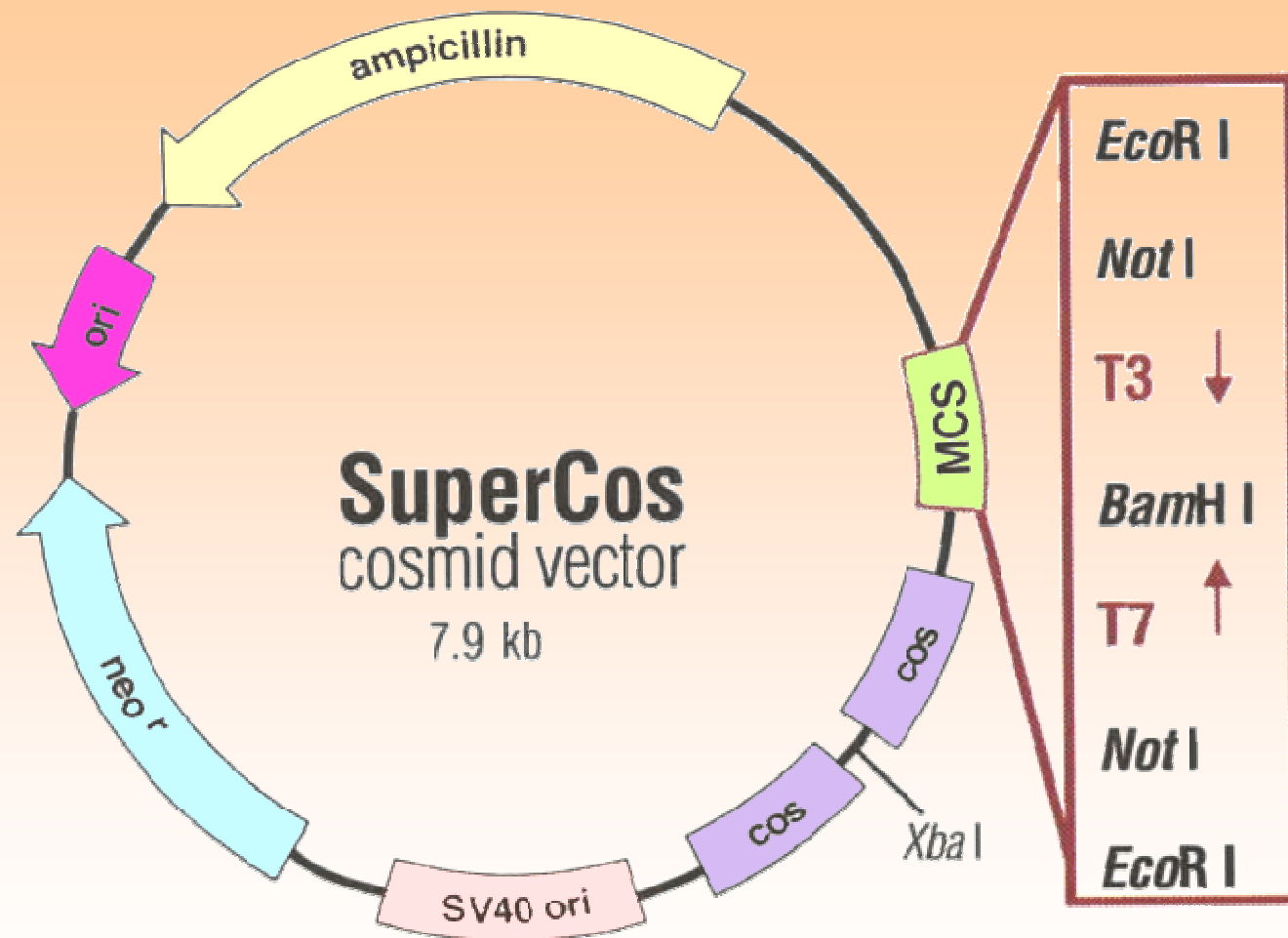
λ DASH





Cosmids, BACs and YACs

These vectors can host extremely large inserts (upto 50 kb for cosmids, and 100-500 kb for BACs and YACs), and are primarily used in genome sequencing projects.





What determines the choice vector?

- insert size
- vector size
- restriction sites
- copy number
- cloning efficiency
- ability to screen for inserts
- **what down-stream experiments do you plan?**