Advance Genetic Engineering

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GENETIC ENGINEERING VECTORS

PART I : PLASMIDS

Cloning Vectors

- 1. A cloning vector is a DNA molecule that has an origin of replication and is capable of replicating in a bacterial cell.
- 2. A vector is used to amplify a single molecule of DNA into many copes. A DNA fragment must be inserted into a cloning vector.
- 3. Most vectors are genetically engineered plasmids or phages.
- 4.There are also cosmid vectors, bacterial artificial chromosomes, and yeast artificial chromosomes.

Plasmid Cloning Vectors

- Plasmids are circular, double-stranded DNA molecules that exist in bacteria and in the nuclei of some eukaryotic cells. **Extrachromosomal DNA, usually circular-parasite?**
- They can replicate independently of the host cell. **Plasmid replication requires host cell functions**
- The size of plasmids ranges from a few kb to near 100 kb. **High copy plasmids are usually small; low copy plasmids can be large.**
- Can hold up to 10 kb fragments
- Plasmids have an origin of replication, antibiotic resistance genes as markers, and several unique restriction sites. **Can be essential for specific environments: virulence, antibiotics resistance, use of unusual nutrients, production of bacteriocins (colicins)**
- **Plasmids are incompatible when they cannot be stably maintained in the same cell because they interfere with each other's replication.**
- After culture growth, the clone fragment can be recovered easily.

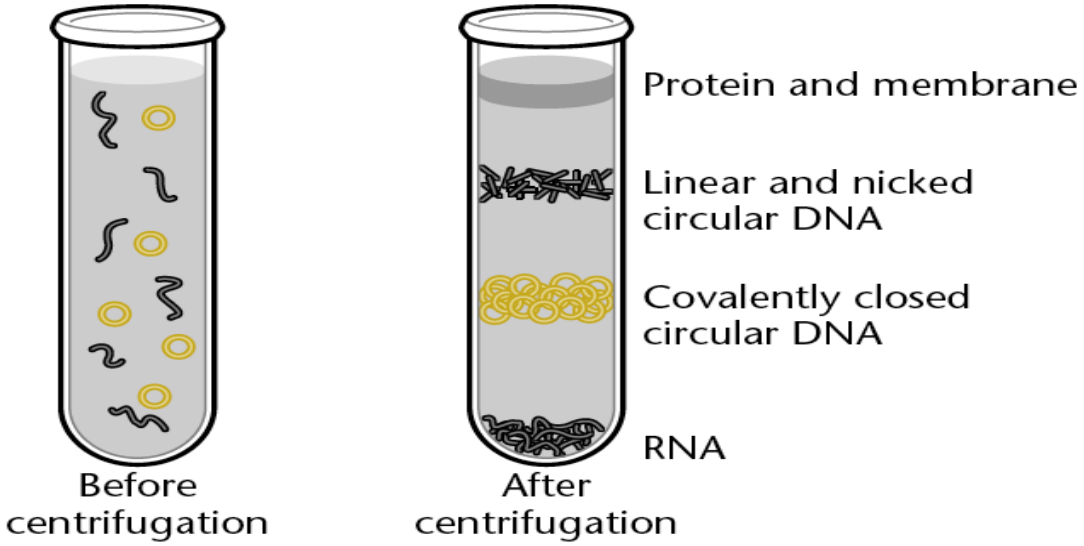
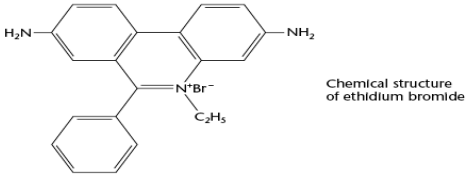
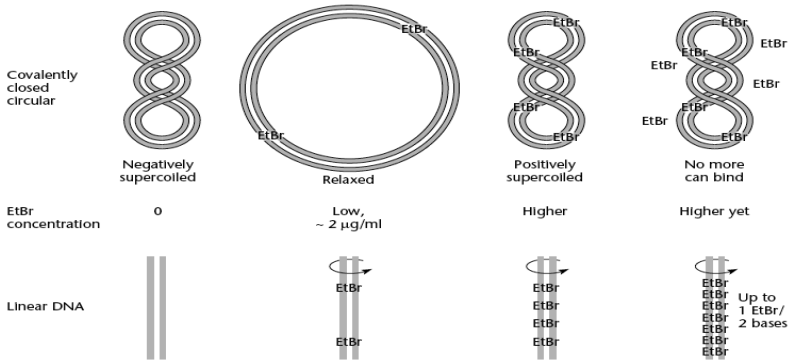
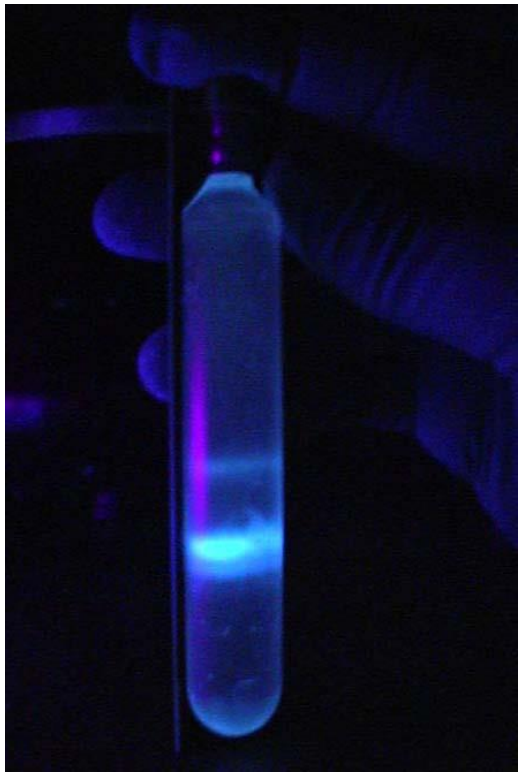


Figure 4.2



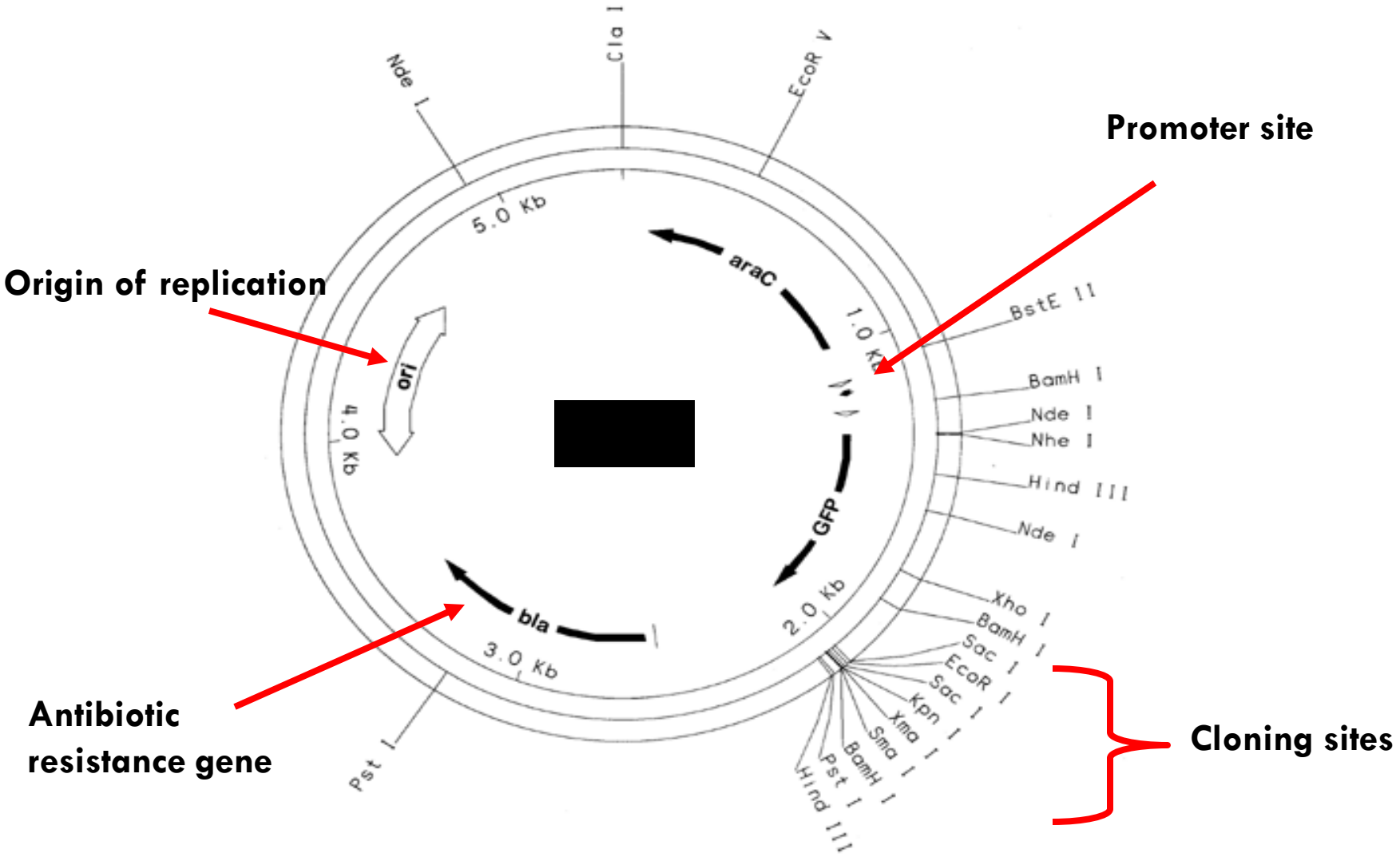


Three forms of plasmid DNA



CsCl gradient with ethidium bromide and UV light.

A more detailed look at plasmids- General Model



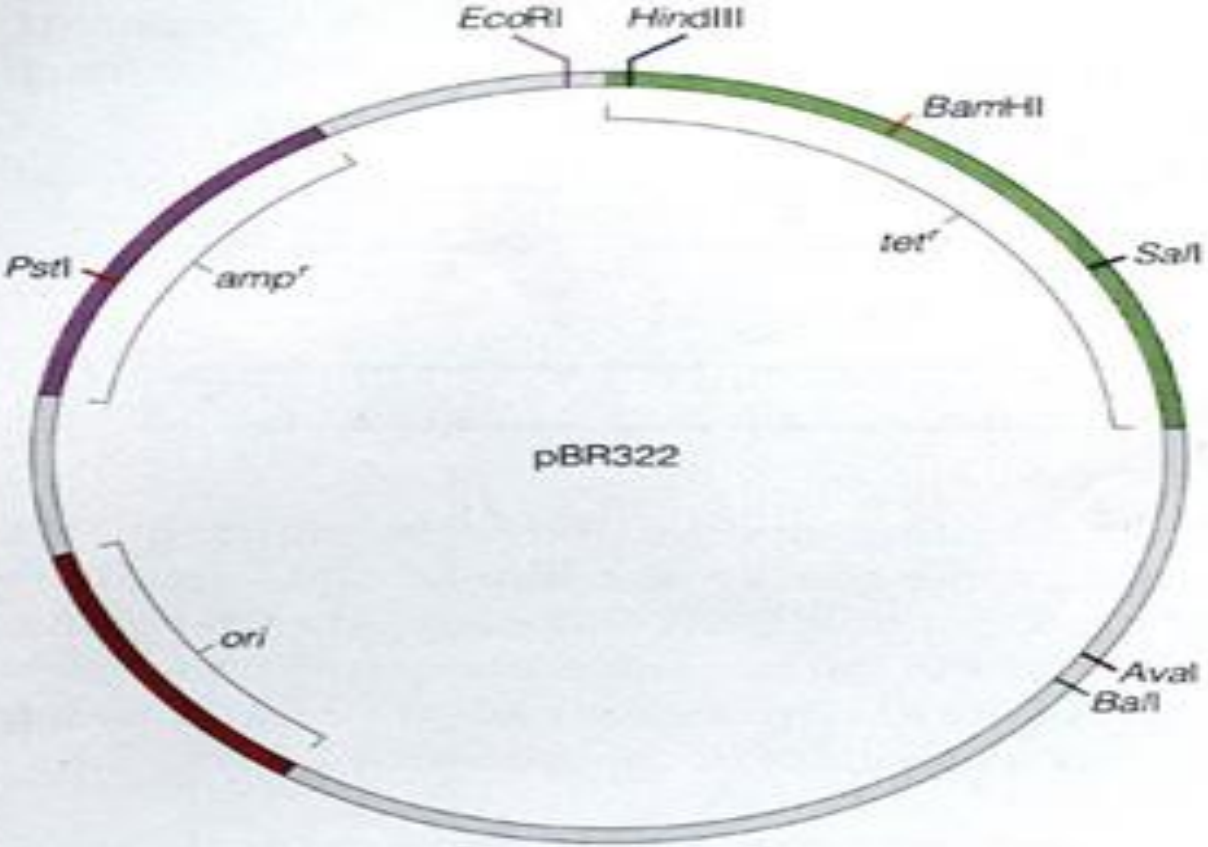


Figure 9.2 Plasmid pBR322. Seven of the unique restriction sites are shown, as well as the two selectable marker genes, *amp^r* and *tet^r*, the ampicillin and tetracycline resistance genes; *ori* represents the origin of replication. (Adapted from Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press. Used by permission)

TABLE 4.1 Some naturally occurring plasmids and the traits they carry

Plasmid	Trait	Original source
ColE1	Bacteriocin which kills *E. coli*	*E. coli*
Tol	Degradation of toluene and benzoic acid	*Pseudomonas putida*
Ti	Tumor initiation in plants	*Agrobacterium tumefaciens*
pJP4	2,4-D (dichlorophenoxyacetic acid) degradation	*Alcaligenes eutrophus*
pSym	Nodulation on roots of legume plants	*Rhizobium meliloti*
SCP1	Antibiotic methylenomycin biosynthesis	*Streptomyces coelicolor*
RK2	Resistance to ampicillin, tetracycline, and kanamycin	*Klebsiella aerogenes*

TABLE 4.2 Copy numbers of some plasmids

Plasmid	Approximate copy number
F	1
P1 prophage	1
RK2	4–7 (in *E. coli*)
pBR322	16
pUC18	~30–50
plJ101	40–300

Table 11-1 Examples of some plasmids and their properties

Plasmid	Size (Kb)	Number of copies per chromosome	Self-transmissible	Phenotypic features
Col plasmids				
ColE1	6.4	10–15	No	Colicin E1 disrupts energy gradient, host immunity to Colicin E1
ColE2	7.6	10–15	No	Colicin E2 is a DNase, host immunity to Colicin E2
ColE3	7.6	10–15	No	Colicin E3 is a ribosomal RNase, host immunity to Colicin E3
F plasmid	94.5	1–2	Yes	F-pilus, conjugation
R plasmids				
R100	106.7	1–2	Yes	Cam^r Str^r Sul^r Tet^r
RK2	56.0	5–8	Yes	Broad host range
pSC101	9.0	<5	No	Low copy number, compatible with ColE1-type plasmids, Tet^r
Phage plasmid				
λdv	6.4	50	No	λ genes *cro, cI, O, P*
Recombinant plasmids				
pBR322	4.4	20	No	Medium copy number, ColE1-type replication, Amp^r
pUC18	2.7	200–500	No	High copy number, ColE1-type replication with a mutation that increases the copy number, Amp^r
pACYC184	4.0	10–12	No	Cam^r Tet^r

Classification of plasmids

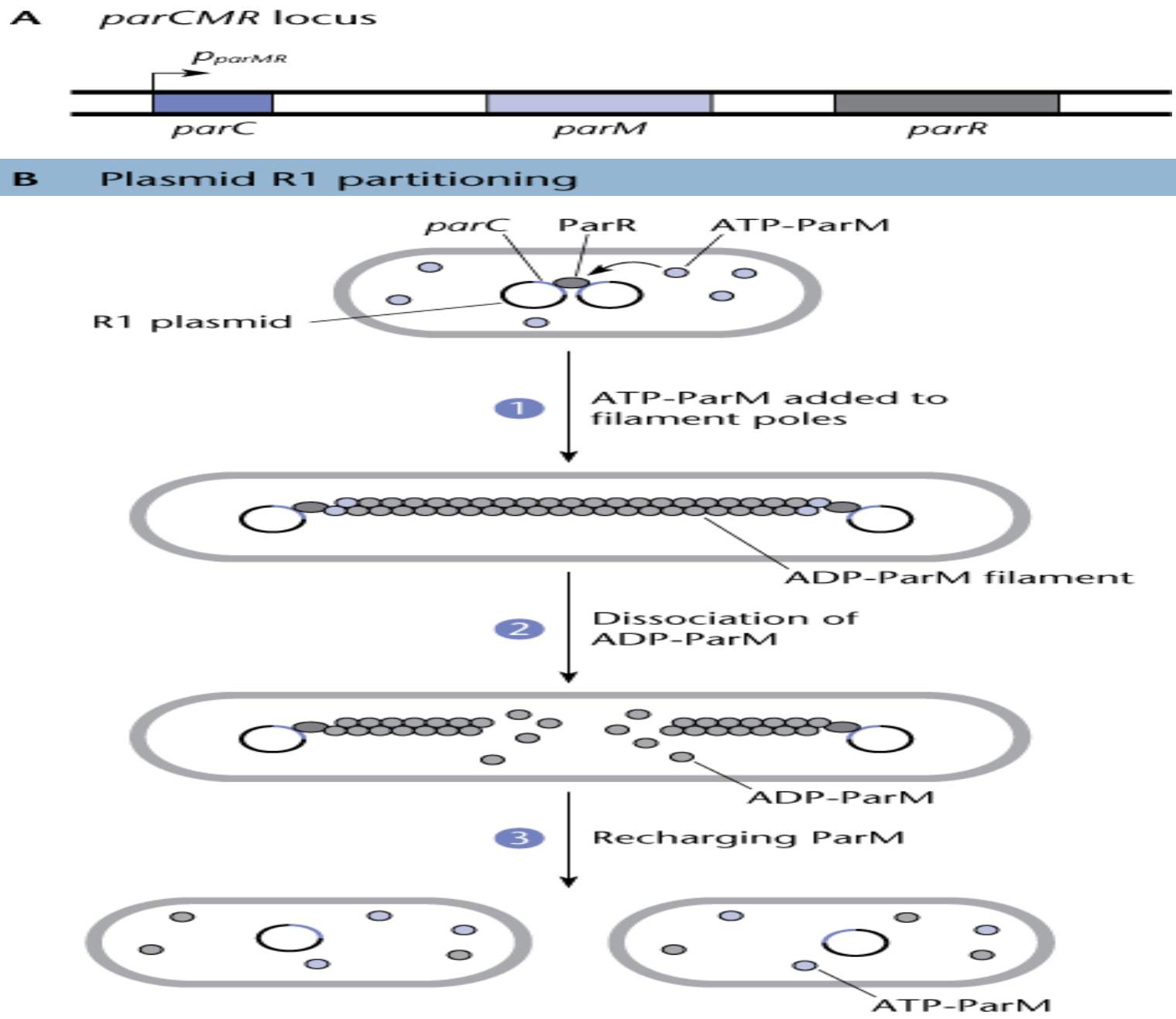
1. F-plasmids
2. R-plasmids
3. Col-plasmids
4. Degradative plasmids
5. Virulence plasmids

Characterization of cloning plasmids

1. With good size
2. With defined genetic map
3. With selective marker
4. With ability to replicate
5. Stable
6. With many sites for different restriction enzymes

Plasmid replication

1. Plasmid replication requires host DNA replication machinery. Large plasmids usually have their own enzymes.
2. Some plasmids are integrate to host genome-Episomes others replicate inside host.
3. Most wild plasmids carry genes needed for transfer and copy number control.
4. All self replication plasmids have a *oriV*: origin of replication
5. Some plasmids carry and *oriT*: origin of transfer. These plasmids will also carry functions needed to be mobilized or *mob* genes.
6. Plasmid segregation is maintained by a *par* locus-a partition locus that ensures each daughter cells gets on plasmid. Not all plasmids have such sequences.

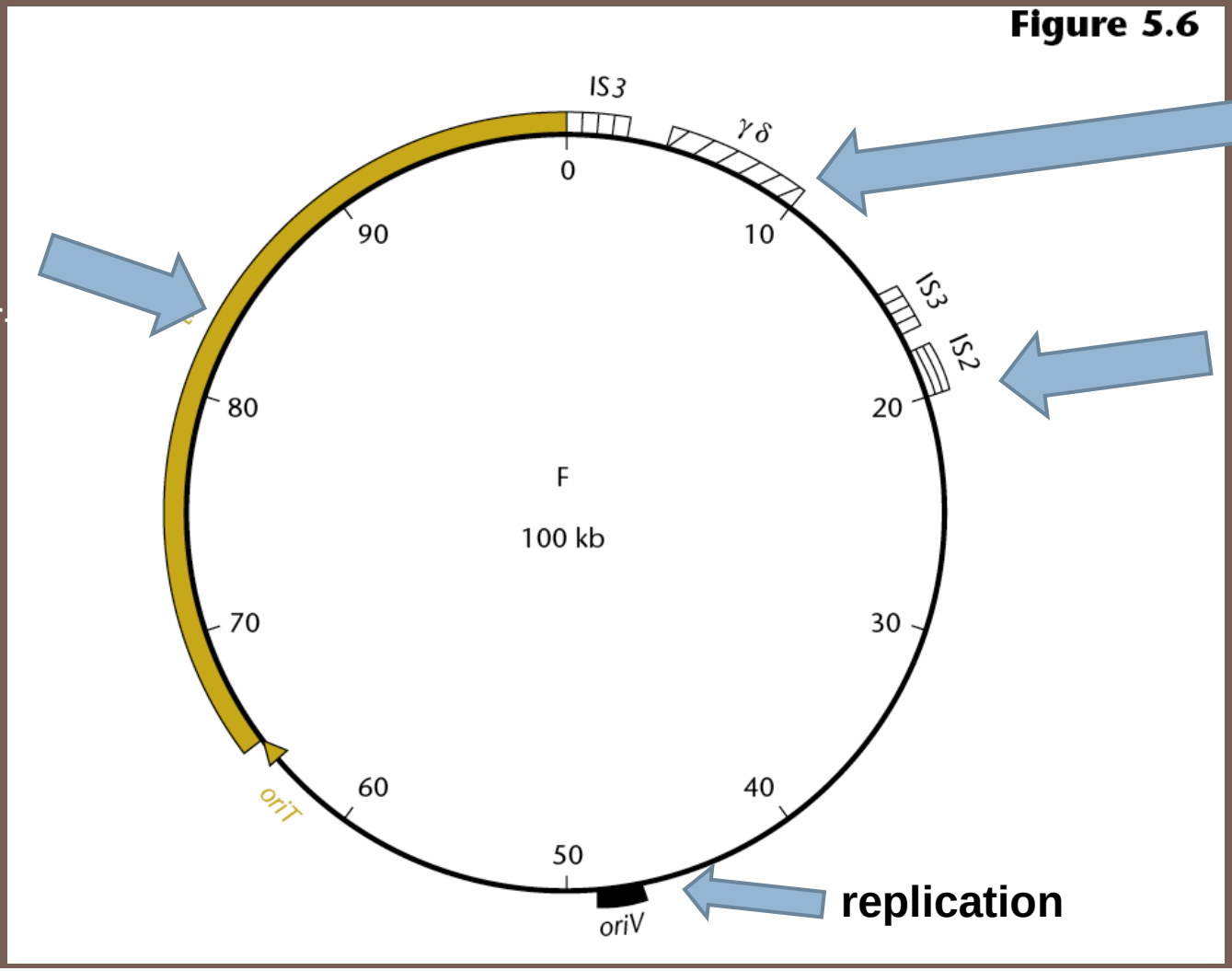

A parCMR locus
p_{parMR}
parC
parM
parR
B Plasmid R1 partitioning
parC
ParR
ATP-ParM
R1 plasmid
1
ATP-ParM added to filament poles
ADP-ParM filament
2
Dissociation of ADP-ParM
ADP-ParM
3
Recharging ParM
ATP-ParM

Conjugative and Non-conjugative plasmids

- Cells can be transfer their plasmids to cells without plasmids- Conjugative plasmids or Fertility plasmids or Sex plasmids.

-Conjugative Process controlled by a group of genes called transfer genes or tra genes-F+

Genetic organization of F



30+ genes needed For transfer-tra genes

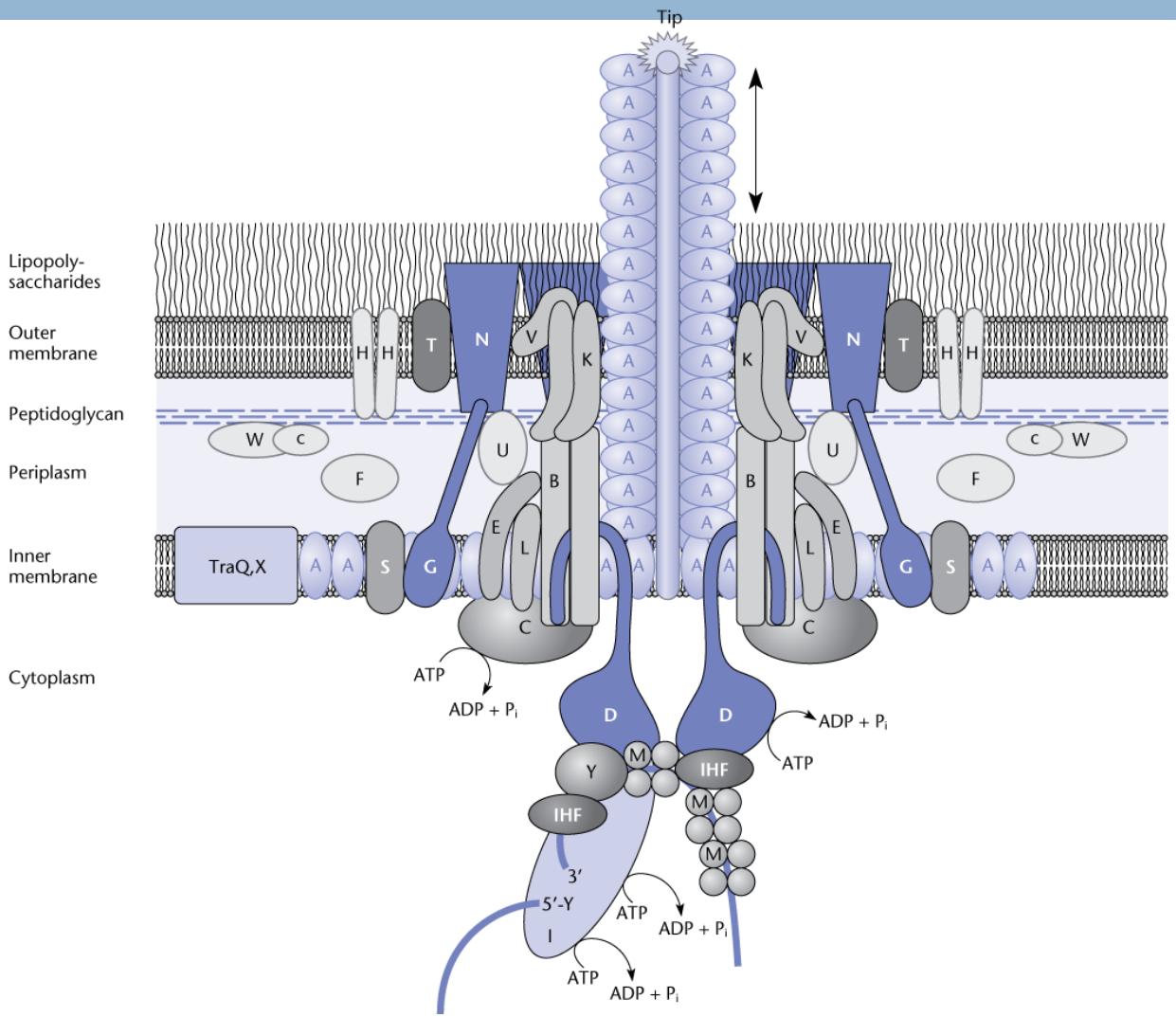
Primitive transposon

Transposable elements or insertion elements-IS elements

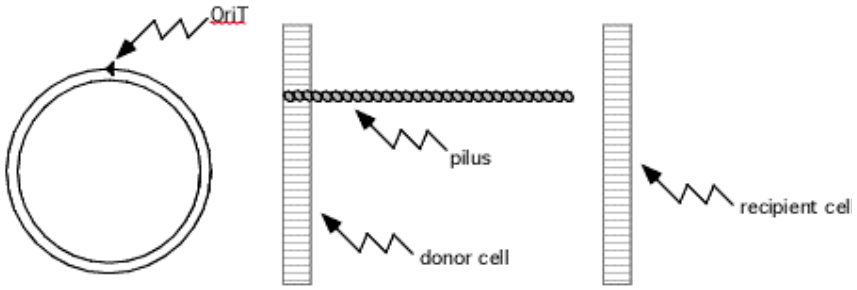
replication

F Pilus assembly

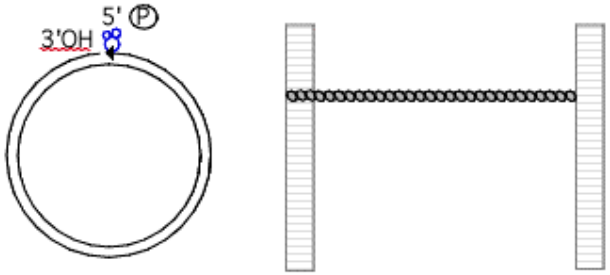
Figure 5.3



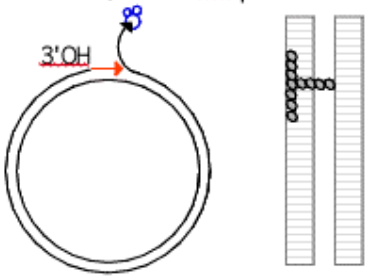
F-transfer at fine detail



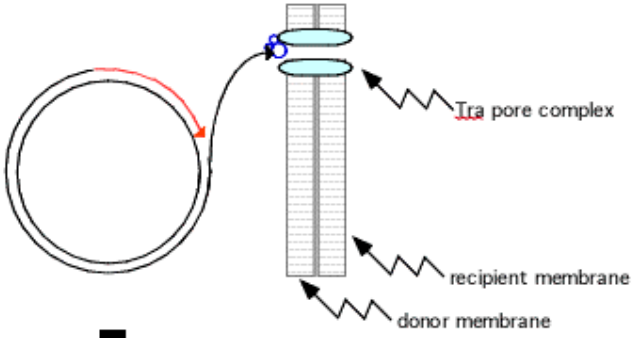
Contact between donor and recipient cells.
DNA relaxase ( ) nicks at oriT and covalently binds to 5' Ⓟ



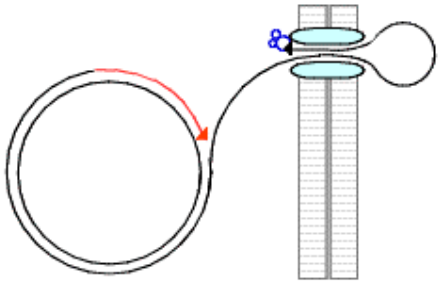
Pilus retracks, bringing donor and recipient into close proximity
and Tra proteins form a pore complex that spans the membranes



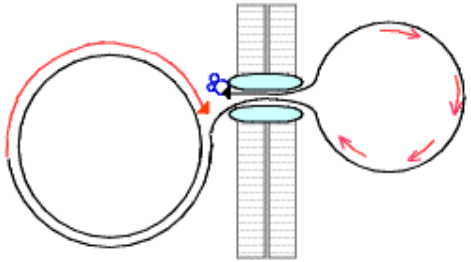
Rolling circle DNA replication initiates at 3'OH and proceeds 5' to
Membranes brought into close proximity to form mating bridge.
Relaxase interacts with membrane Tra pore complex



DNA replication pushes the ssDNA into the recipient cell



Lagging strand DNA replication in recipient cell converts ssDNA to dsDNA



Upon complete replication of plasmid, the old and new oriT sites
"collide", and nicking between oriT sites occurs

Insertional inactivation assay
Plasmid Polylinkers and Marker Genes for Blue-White screening

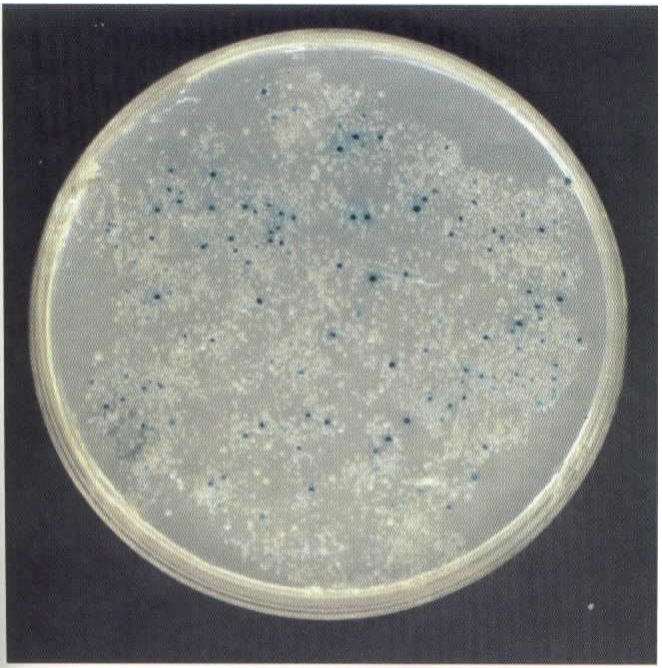


Figure 9.8 Blue-white screening on medium with ampicillin, X-gal, and IPTG. Blue colonies contain nonrecombinant plasmids. White colonies contain recombinant plasmids and can be isolated directly from this plate.

- A vector usually contains a sequence (polylinker) which can recognize several restriction enzymes so that the vector can be used for cloning a variety of DNA samples.
- Colonies with recombinant plasmids are white, and colonies with nonrecombinant plasmids are blue.
- **Example: pUC19**
- Resistant to ampicillin, has (amp^r gene)
- Contains portion of the lac operon which codes for beta-galactosidase.
- X-gal is a substrate of beta-galactosidase and **turns blue** in the presence of functional beta-galactosidase is added to the medium.
- Insertion of foreign DNA into the polylinker disrupts the lac operon, beta-galactosidase becomes non-functional and the colonies fail to turn blue, but **appear white.**

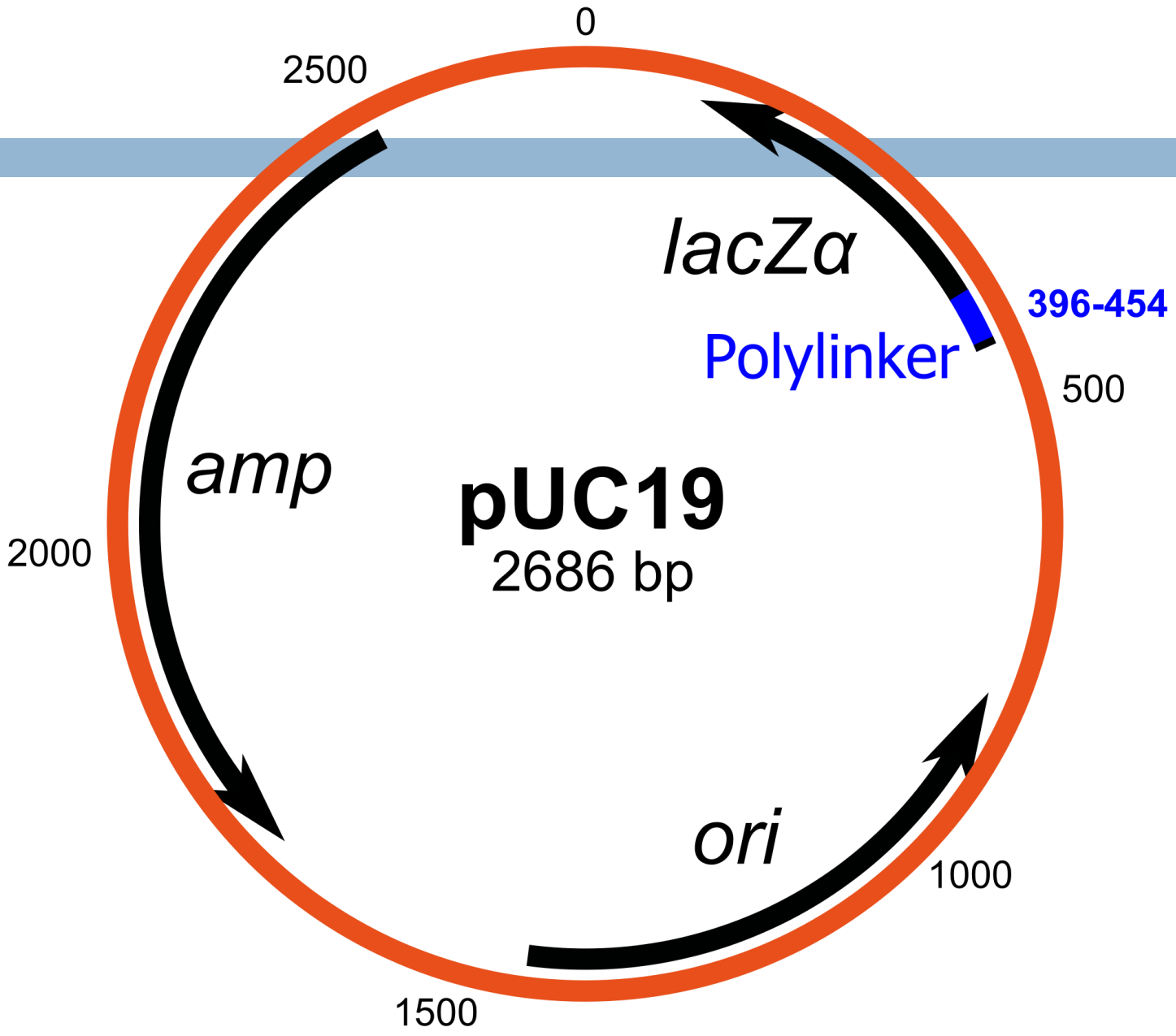

0
500
1000
1500
2000
2500
lacZα
396-454
Polylinker
amp
pUC19
2686 bp
ori

Fig 23.3 Plasmid vector pBR322

- pBR322 has 4361 base pairs
- Origin of replication (*ori*)
- Antibiotic resistance genes *amp* and *tet*
- *Rop* gene regulates replication for ~20 copies of the plasmid per cell

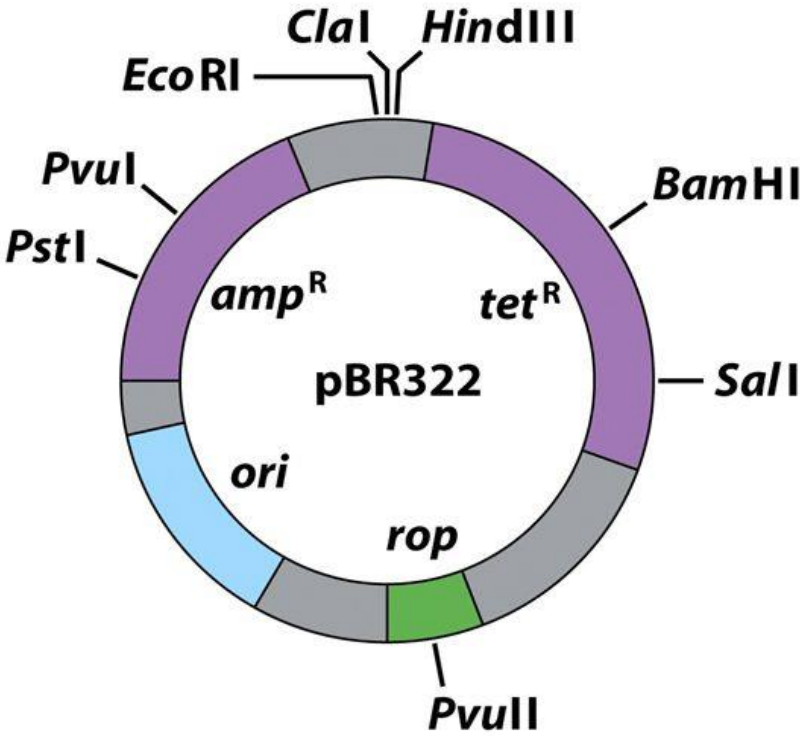


Figure 23-3 Principles of Biochemistry, 4/e

The Major Limitation of Cloning in Plasmids

- Upper limit for clone DNA size is 12 kb
- Requires the preparation of "competent" host cells
- Inefficient for generating genomic libraries as overlapping regions needed to place in proper sequence
- Preference for smaller clones to be transformed
- If it is an expression vector there are often limitations regarding eukaryotic protein expression