Gene expression in prokaryotic and eukaryotic cells, Plasmids: types, maintenance and functions.

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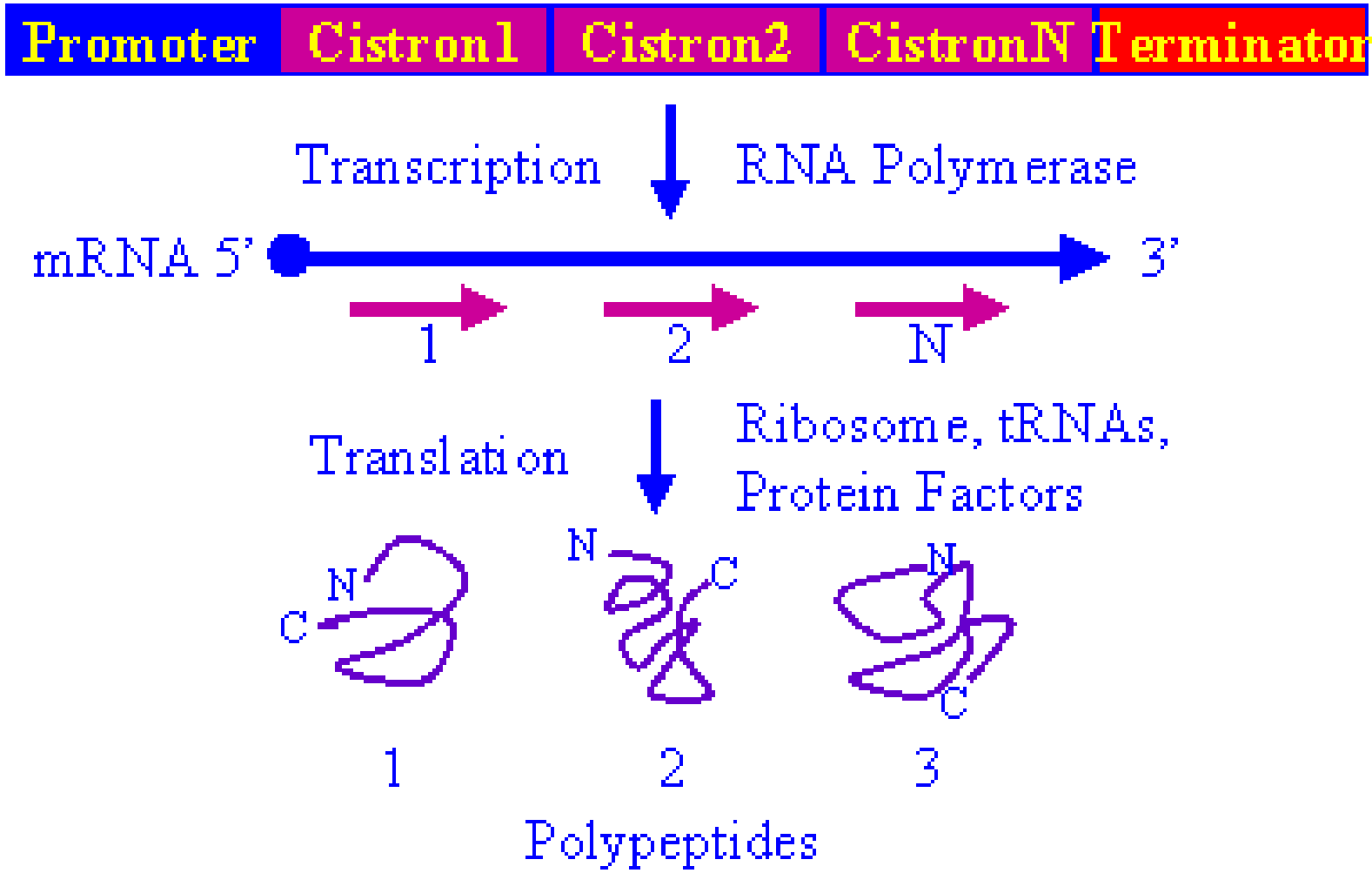
Prokaryotic Gene Expression

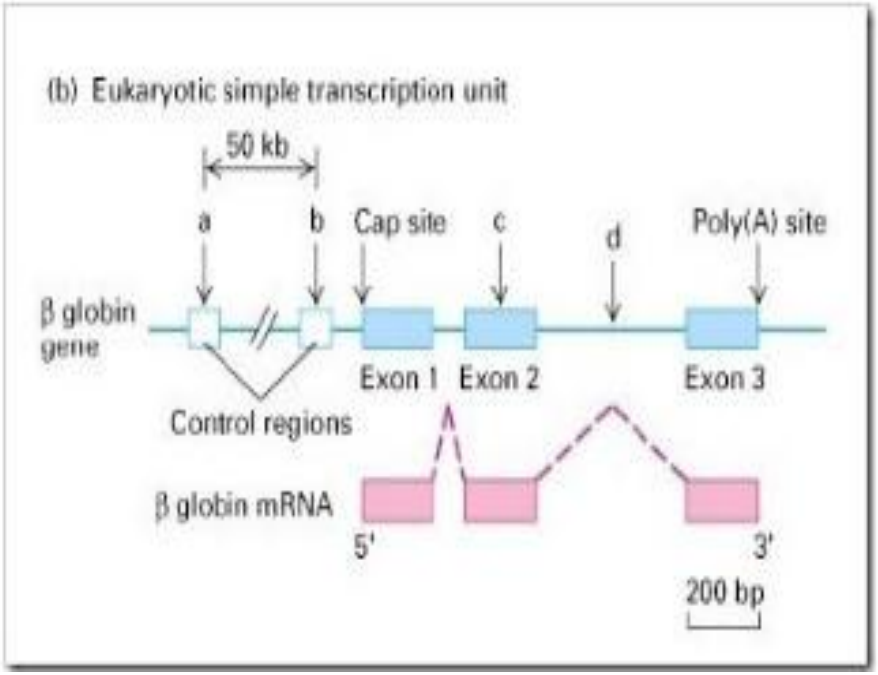


Prokaryotic Gene Expression:

1. Transcription Enzyme: One RNA polymerase for all RNA's
2. Enhancer: Transcription enhancers are not required.
3. Primary transcript: Directly function as mRNA.
4. mRNA: Polycistronic
5. Location of transcription: Cytoplasm (Nucleoid region)
6. Coupling of transcription and translation: Occurs
7. Translation initiation factors: Three represented as IF-1, IF-2, IF-3

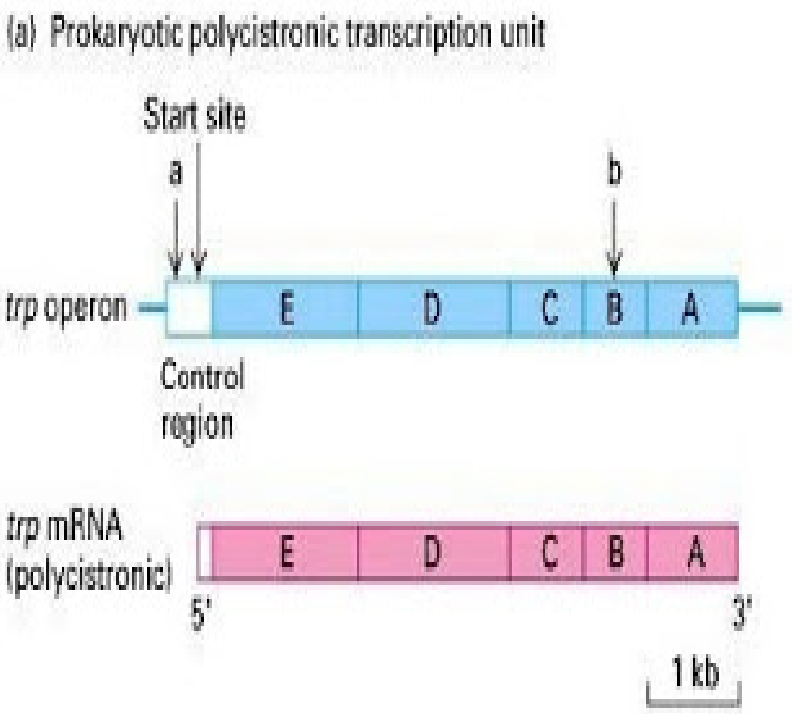
Monocistronic mRNA

1. Contains codons of a single cistron.
2. Codes only for a single protein.
3. Is transcribed from a single gene (cistron) and has one initiation and termination codon.
4. Present in eukaryotes.

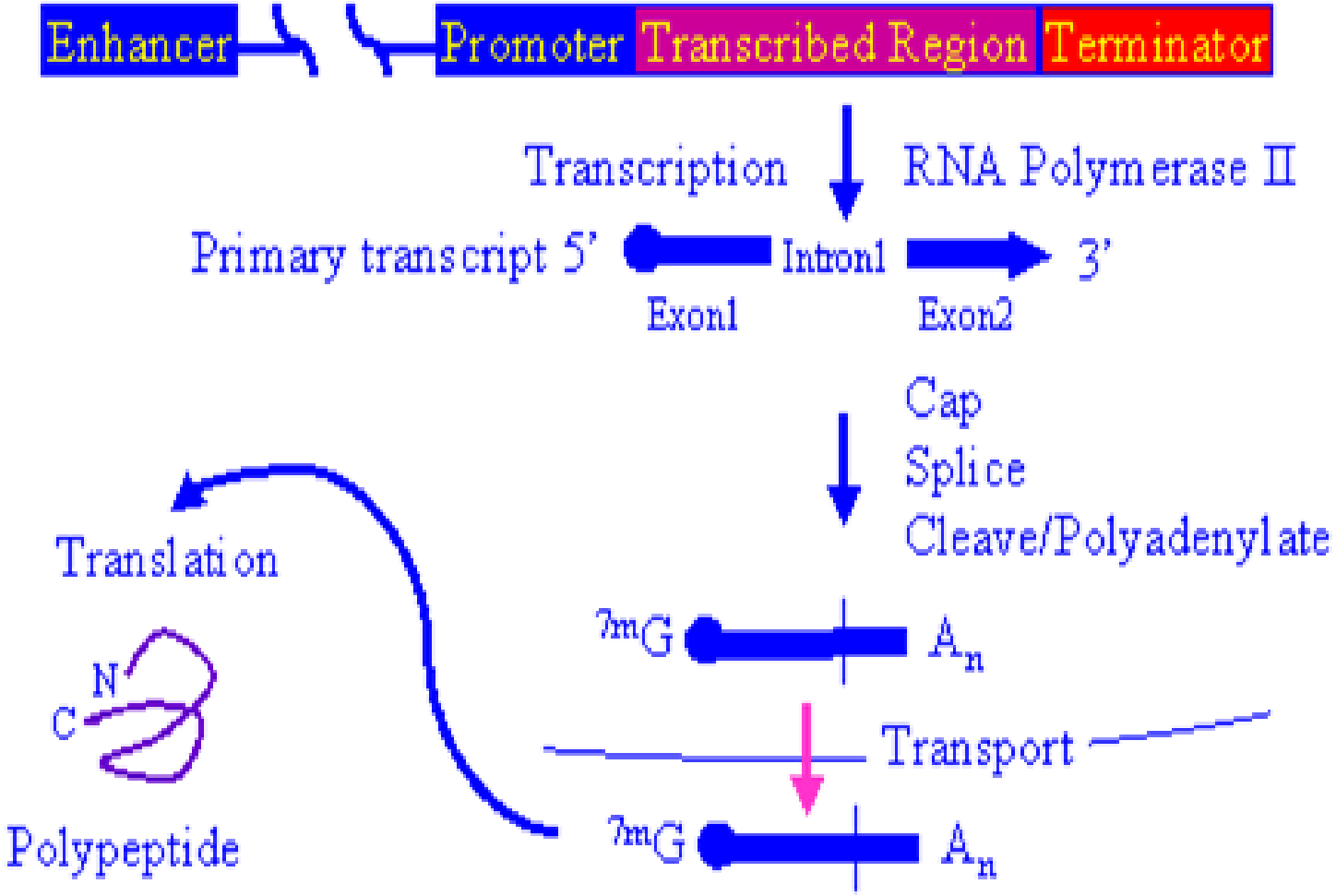


Polycistronic mRNA

1. Contains codons of a more than one cistron.
2. Codes only for more than one protein.
3. Is transcribed from a more than one gene (cistron) and has as many as initiation and termination codons.
4. Present in prokaryotes.



Eukaryotic Gene Expression



Eukaryotic Gene Expression:

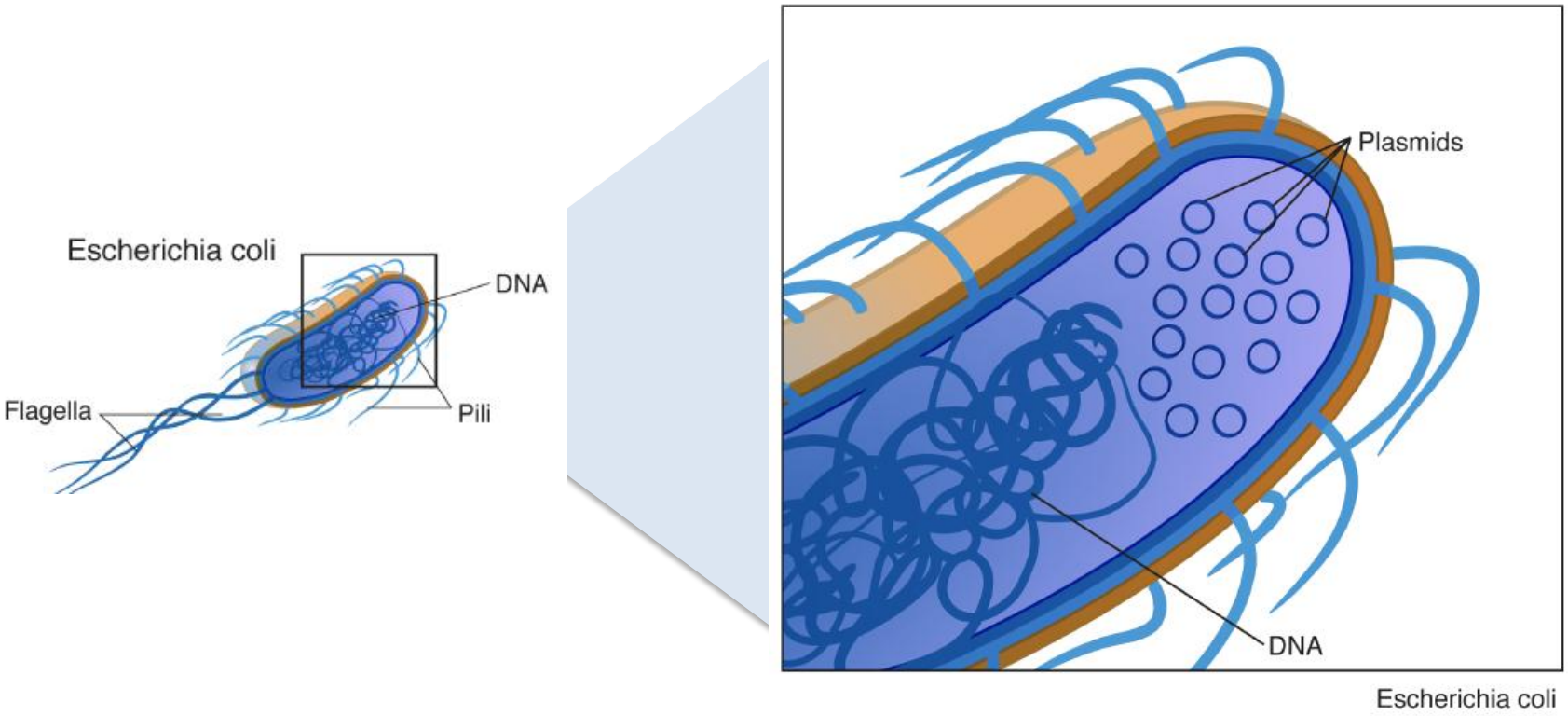
1. Transcription Enzyme: At least three (RNA polymerase I for rRNA, RNA polymerase II for hnRNA, RNA polymerase III for tRNA, 5SRNA and snRNA)
2. Enhancer: Alongwith promoter region, enhancers are also required.
3. Primary transcript: It is hnRNA that contains both introns and exons. Processing removes introns and produces functional mRNA.
4. mRNA: Monocistronic
5. Location of transcription: Nucleus
6. Coupling of transcription and translation:
7. Translation initiation factors: Nine represented as eIF where e donates eukaryotes. These are eIF2, eIF3, eIFIA, eIF4B, eIF4E, eIF4G, eIF5 & eIF6.

Plasmids

1. **Extrachromosomal DNA, usually circular-parasite**
2. **Usually encode ancillary functions for in vitro growth**
3. **Can be essential for specific environments: virulence, antibiotics resistance, use of unusual nutrients, production of bacteriocins (colicins)**
4. **Must be a replicon - self-replicating genetic unit**
5. **Plasmid DNA must replicate every time host cell divides or it will be lost**
   a. **DNA replication**
   a. **partitioning (making sure each progeny cells receives a plasmid)**
6. **High copy plasmids are usually small; low copy plasmids can be large**
7. **Partitioning is strictly controlled for low copy, but loose for high copy**
8. **Plasmid replication requires host cell functions**
9. **Copy number is regulated by initiation of plasmid replication**
10. **Plasmids are incompatible when they cannot be stably maintained in the same cell because they interfere with each other's replication.**

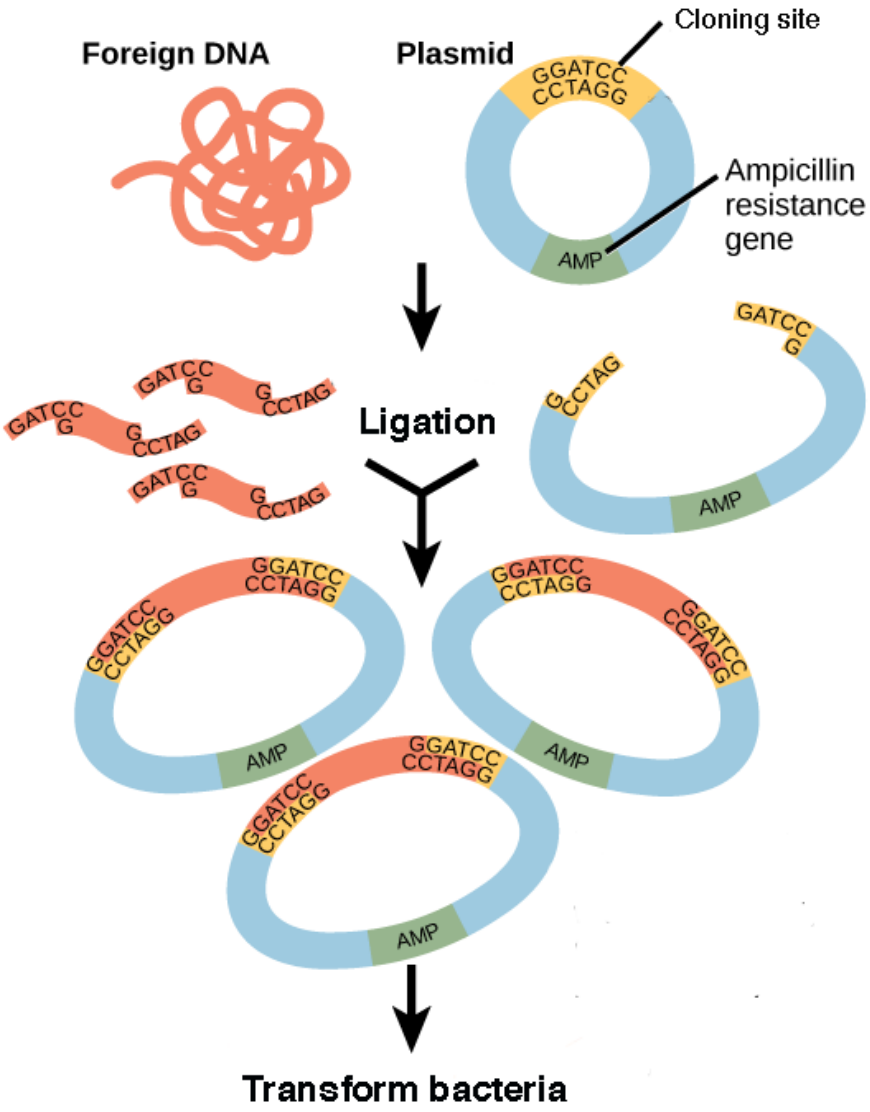
Plasmids

Small circular DNA molecules that replicate independently of the host chromosomes



Indispensable tools that allow molecular biologists to obtain essentially unlimited amounts of a DNA sequence

Plasmids used in molecular biology have been constructed in the lab



Molecular cloning

Enzymes are used to insert desired pieces of foreign DNA into plasmids

Bacterial cells are transformed with the plasmids. Copies of the plasmids are purified from bacteria.

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*

Also, virulence plasmids from *Salmonella, Shigella, Yersinia, B. anthracis, E.coli,* and others.

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
pIJ101	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

Plasmid replication

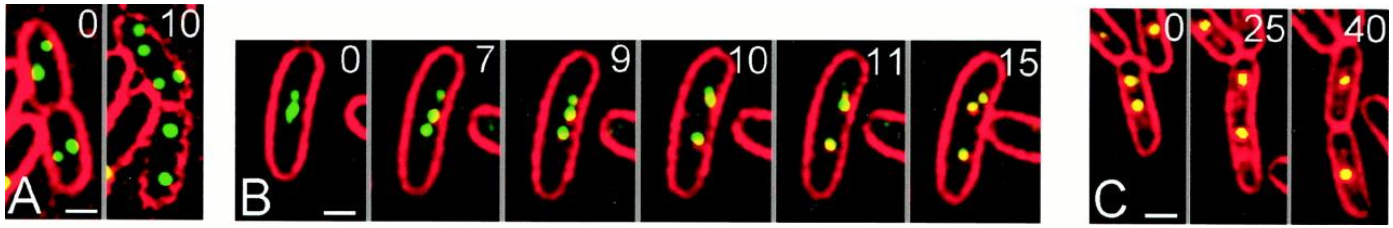
1. Plasmid replication requires host DNA replication machinery.
2. Most wild plasmids carry genes needed for transfer and copy number control.
3. All self replication plasmids have a *oriV*: origin of replication
4. Some plasmids carry and *oriT*: origin of transfer. These plasmids will also carry functions needed to be mobilized or *mob* genes.
5. 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.
6. There are 5 main “incompatibility” groups of plasmid replication. Not all plasmids can live with each other.
7. Agents that disrupt DNA replication destabilize or cure plasmids from cells.

Incompatibility Groups

1. Not all plasmids can live together.
2. Plasmids that are able to coexist in the same cell do not interfere with each other's replication
3. A single cell can have as many plasmids as it can tolerate and replicate!

Partion Locus: a region on broad host range plasmids that binds to a structure on the inner membrane of the cell to ensure proper segregation. Plasmids labeled with fluorescent protein

-move to each daughter cell during division.



Pogliano, Joe et al. (2001) Proc. Natl. Acad. Sci. USA 98, 4486-4491

F-plasmid

1. large (100 kb)
2. low copy (1-2 copies/cell)
3. self transmissible
4. requires protein synthesis (chloramphenicol-sensitive)
5. *repE* gene encodes RepE protein
6. RepE protein binds to origin of replication (*oriS*) and initiates DNA replication
7. RepE binds to the *repE* promoter and activates transcription
8. RepE binds to the *copA/incC* locus binding copies of F together via RepE – inhibiting replication (coupling)

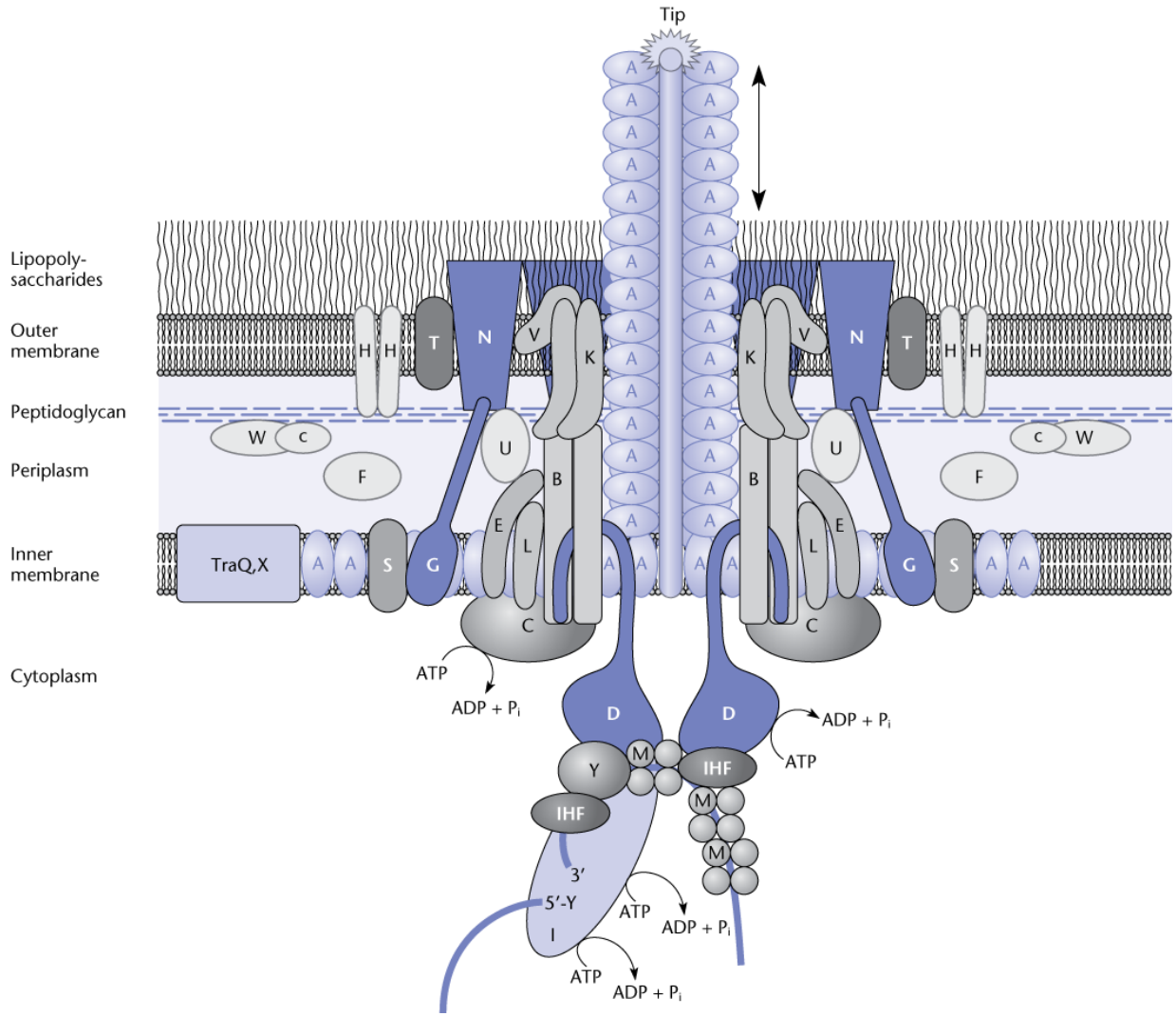
Table 5.1

TABLE 5.1 Some F-plasmid genes and sites

Symbol	Function
ccdAB	Inhibition of host cell division
incBCE	Incompatibility
oriT	Site of initiation of conjugal DNA transfer
oriV	Origin of bidirectional replication
sopAB	Partitioning
traABCEFGHKLQUVWX	Pilus biosynthesis, assembly
traGN	Mating-pair stabilization
traD	Coupling protein
traI	Relaxase
traYM	Accessories for relaxosome
traJ, finOP	Regulation of transfer
traST	Entry exclusion

F Pilus assembly

Figure 5.3

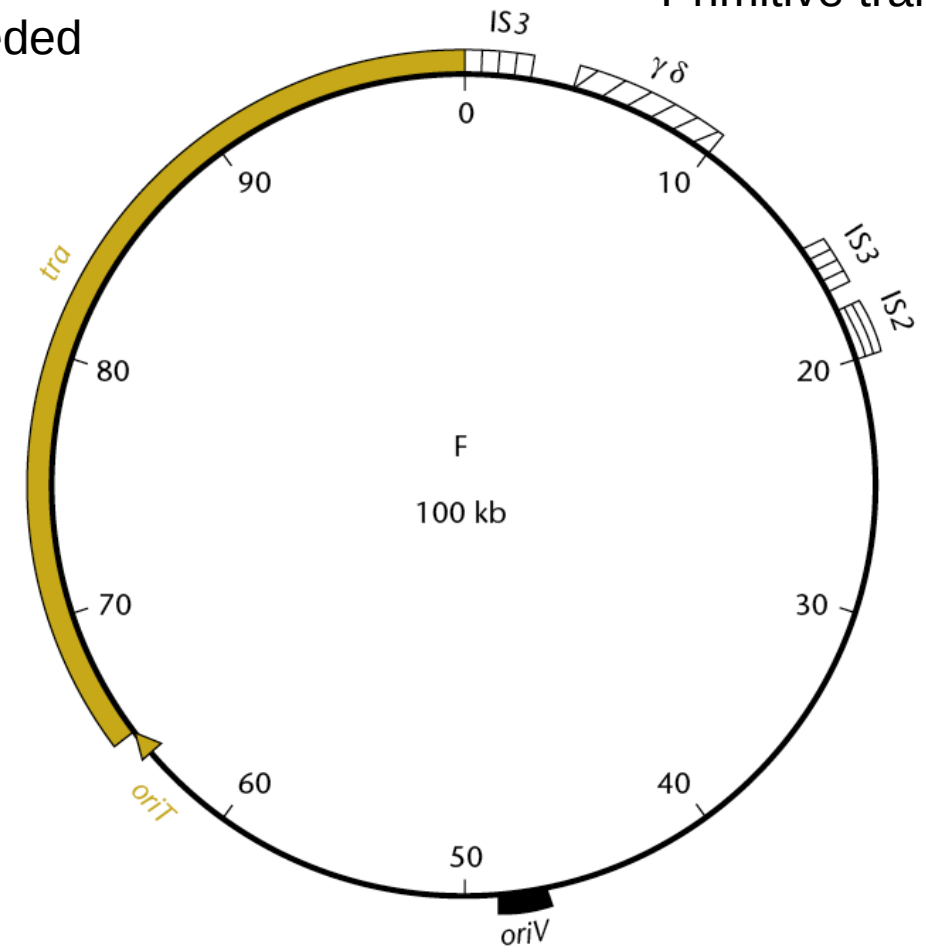


Genetic organization of F

Figure 5.6

Primitive transposon

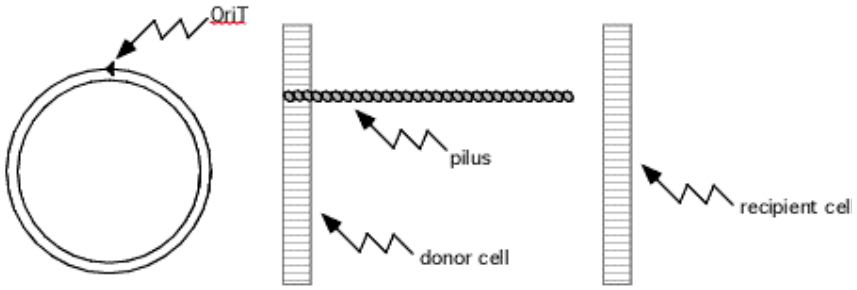
30+ genes needed
For transfer



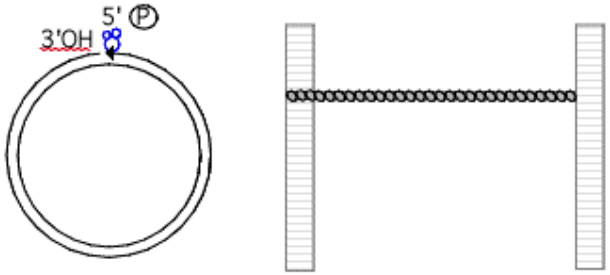
IS elements

replication

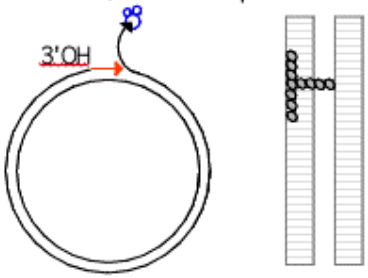
F-transfer at fine detail



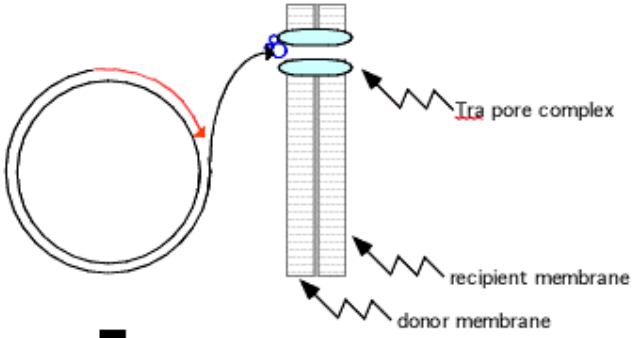
Contact between donor and recipient cells.
DNA relaxase ( ) nicks at oriT and covalently binds to 5' Ⓟ



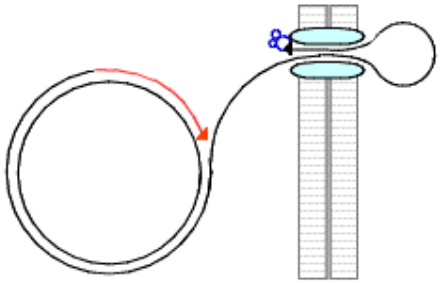
Pilus retracks, bringing donor and recipient into close proximity
and Tra proteins form a pore complex that spans the membranes



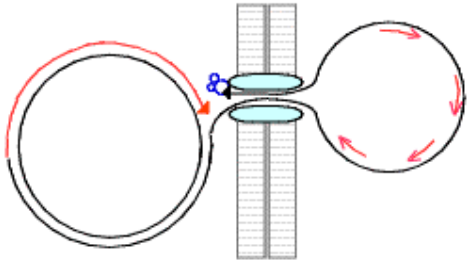
Rolling circle DNA replication initiates at 3'OH and proceeds 5' to
Membranes brought into close proximity to form mating bridge.
Relaxase interacts with membrane Tra pore complex



DNA replication pushes the ssDNA into the recipient cell



Lagging strand DNA replication in recipient cell converts ssDNA to dsDNA

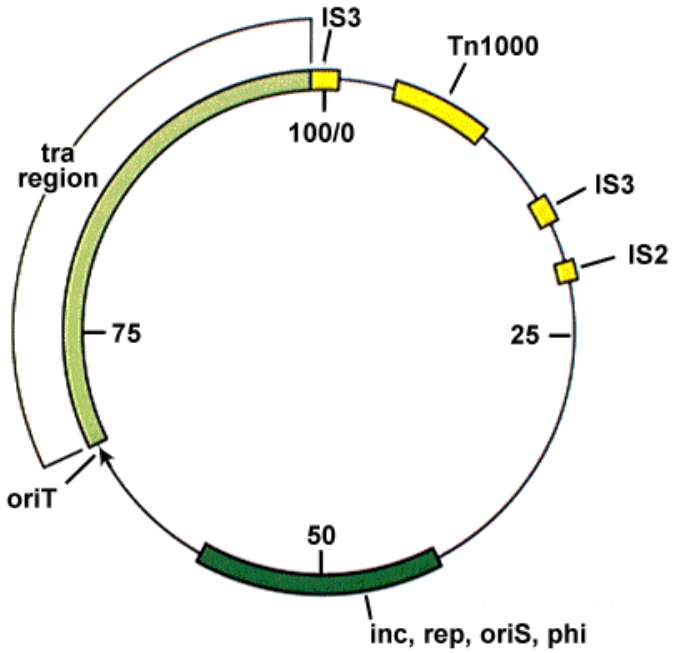


Upon complete replication of plasmid, the old and new oriT sites
"collide", and nicking between oriT sites occurs

Different plasmids

Grouped after their properties:

- F-plasmid /factor: ~ 100 kb

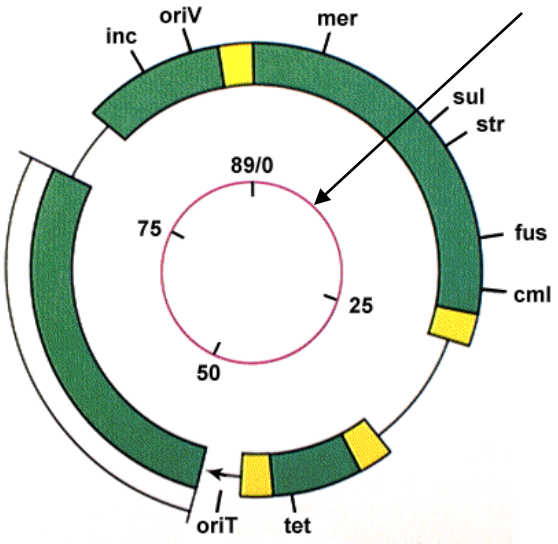


Different plasmids, cont.

- R- plasmid: - have **genes for resistance** against antibiotics and/or heavy metals

Size in kb pairs

Plasmid R100

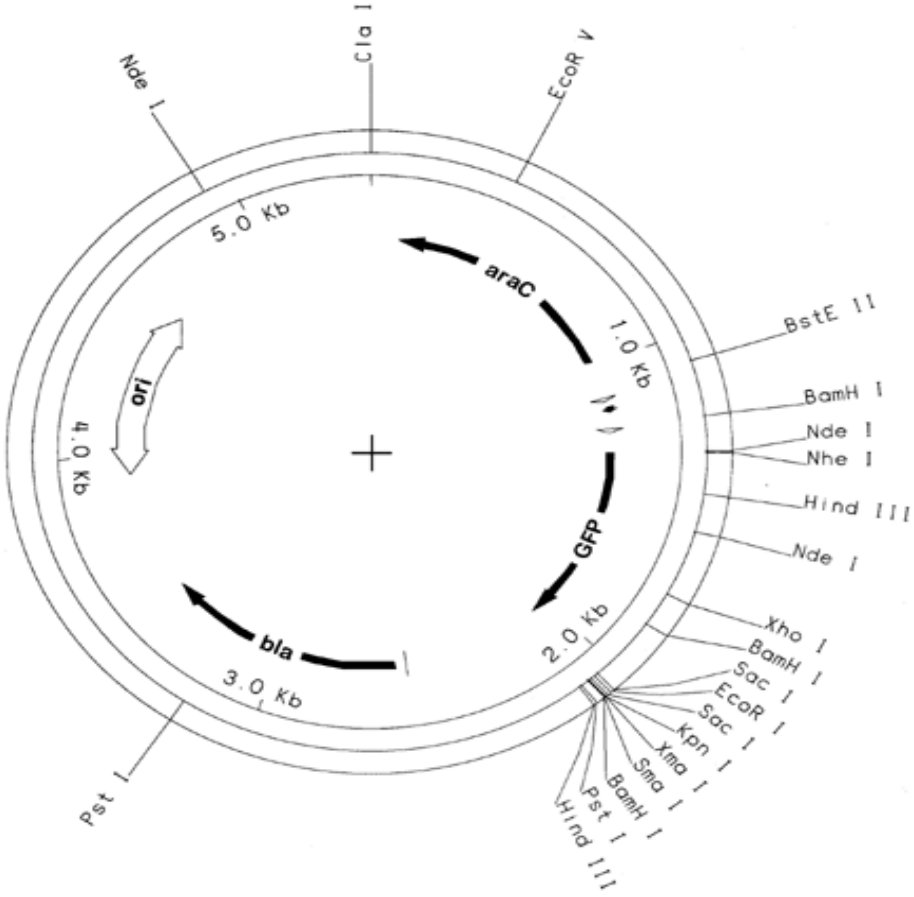


Different plasmids, cont.

- Col – plasmids:
  - **produces colicins**
  - antibacterial
- Catabolic plasmids:
  - have properties to **use odd carbon/ energy sources**
  - many *Psuedomonas* have such plasmids
- Cryptic plasmids:
  - **no known** property

Next Major Advance in Plasmid

- The inclusion of polylinkers into plasmid vectors
- Polylinker is a tandem array of restriction endonuclease sites in a very short expanse of DNA
- For example, pUC18's polylinker
  - Sites for 13 RE's
  - Region spans the equivalent of 20 amino acids or 60 nucleotides



Source: Bio-Rad Laboratories

The Polylinker Advantage

- Unique sites (usually)
- Insert excision facilitated
- Restriction endonuclease mapping and Subcloning made easier

Figure 4.6

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Another Major Advance: Blue-White Screening

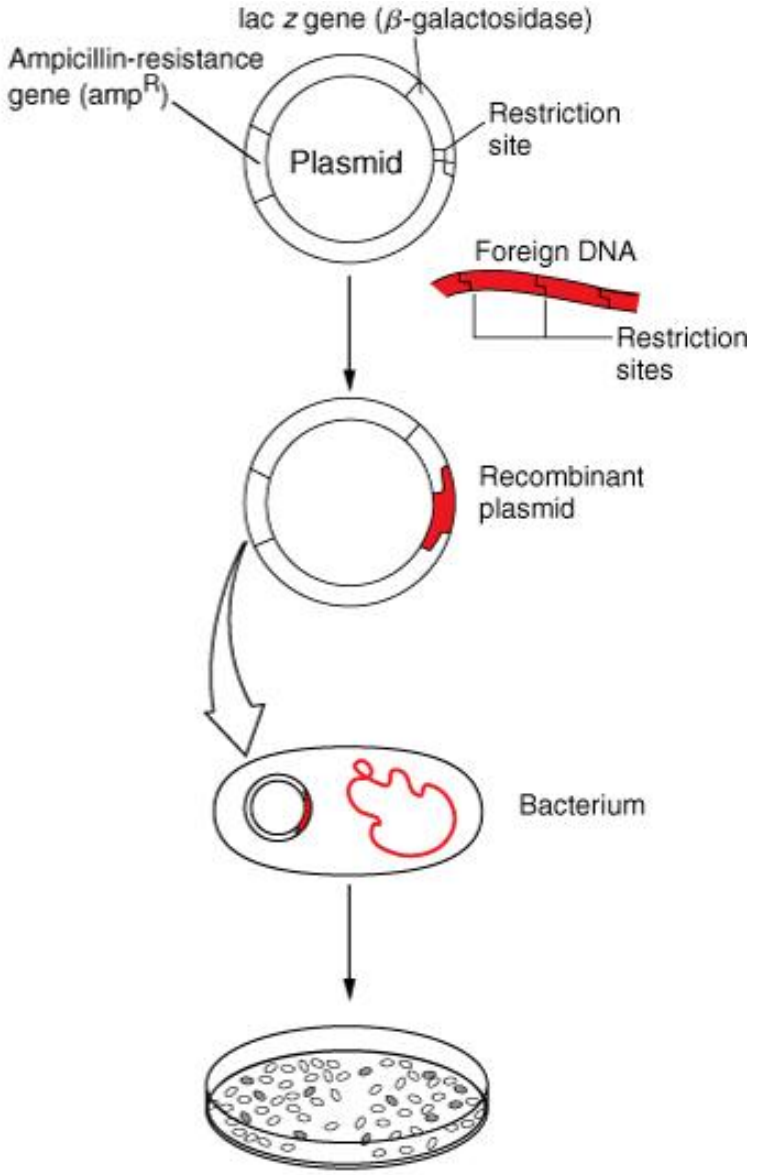
1) Plasmid DNA and foreign DNA are both cut with the same restriction enzyme.

2) Foreign DNA is inserted into the plasmid, where it inactivates the lac z gene.

3) The recombinant plasmid is introduced into a bacterium, which becomes ampicillin-resistant.

4) All treated bacteria are spread on a nutrient agar plate containing ampicillin and β-galactosidase substrate and incubated.

5) White colonies that appear must contain foreign DNA. Blue (gray in this illustration) colonies do not contain foreign DNA.



Features of many modern Plasmids

- Small size
- Origin of replication
- Multiple cloning site (MCS)
- Selectable marker genes
- Some are expression vectors and have sequences that allow RNA polymerase to transcribe genes
- DNA sequencing primers

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

Plasmid vectors