## Table 4.1

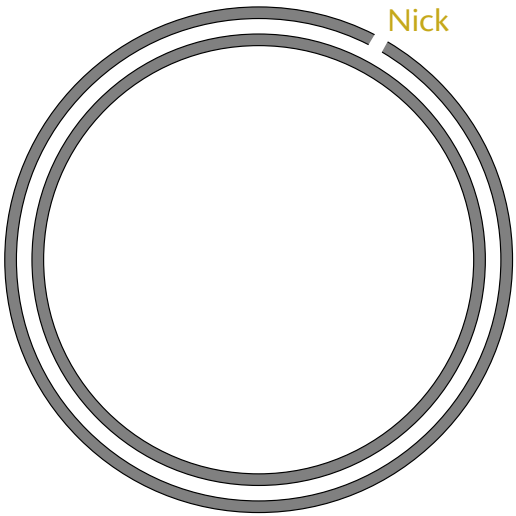
**TABLE 4.1 Some naturally occurring plasmids and the traits they carry**

| 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* |

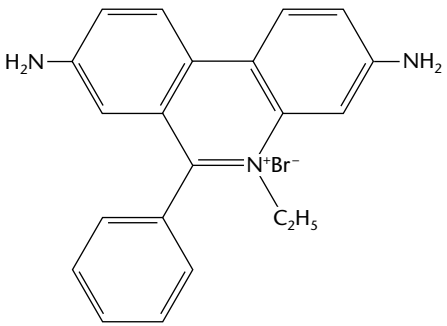
## Box 4.2


A
A T A-T-A-A-T-T-T-T-T-T-A-T-T-A-G-T-A - - - - - // - - - - - T-A-C-T-A-A-A-T-A-A-A-T-A-T-T-A-T A T
T A T-A-T-T-A-A-A-A-A-A-T-A-A-T-C-A-T - - - - // - - - - - - A-T-G-A-T-T-T-A-T-T-T-A-T-A-A-T-A T A
B
Borrelia sp.
A T A-T-A-A-T-T-T-T-T-T-A-T-T-A-G-T-A - - - - -
T A T-A-T-T-A-A-A-A-A-A-T-A-A-T-C-A-T - - - - -
African swine fever virus
T T T-A-T-A-A-T-T-T-T-A-T-A-T-A-T-A-T - - - - -
T A-T-A-T-T-A-T-A-T-G-A-A-T-A-T - - - - - - - -
A-T
Vaccinia virus
A - // -T-A-T-A-A-T-T-T-T-A-T-A-A-T-T-A-A-T - -
A
A-A - — — — — — — — — —

**Figure 4.1**


Nick
Relaxed, no supercoiling
Supercoiled, covalently
closed circular DNA

# Figure 4.2




Chemical structure of ethidium bromide

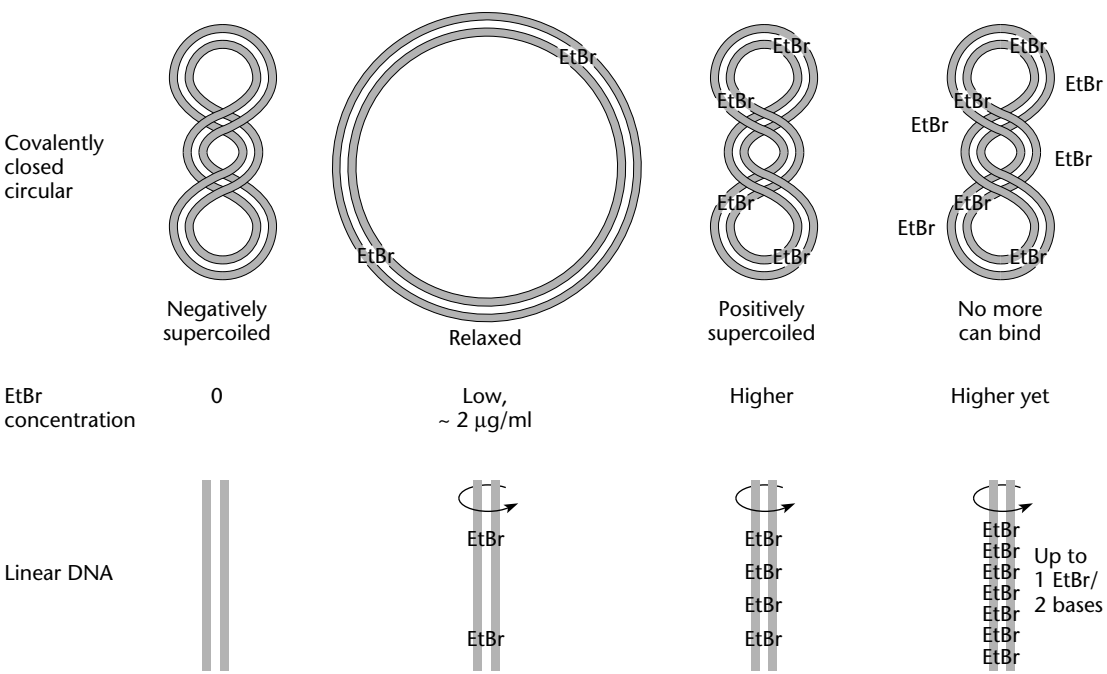
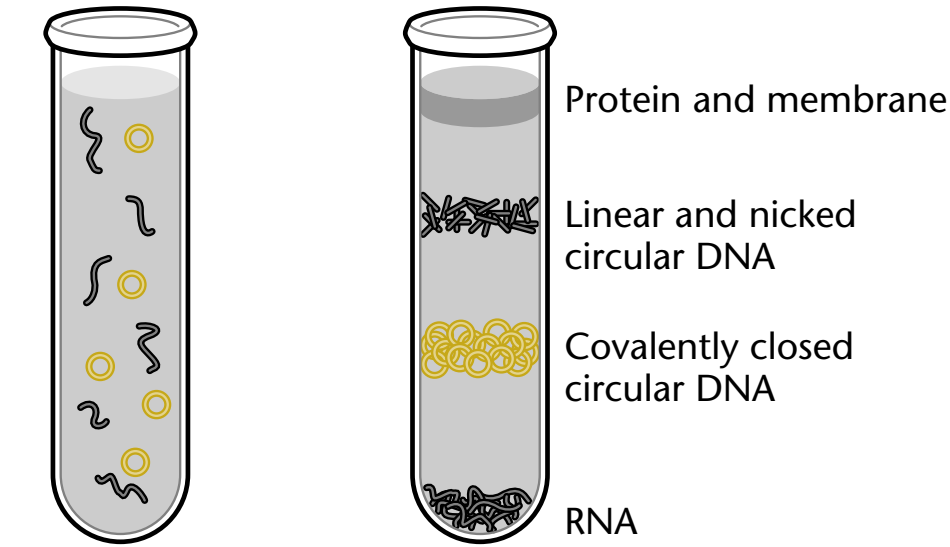


**Figure 4.3**


Protein and membrane
Linear and nicked circular DNA
Covalently closed circular DNA
RNA
Before centrifugation
After centrifugation

# Figure 4.4

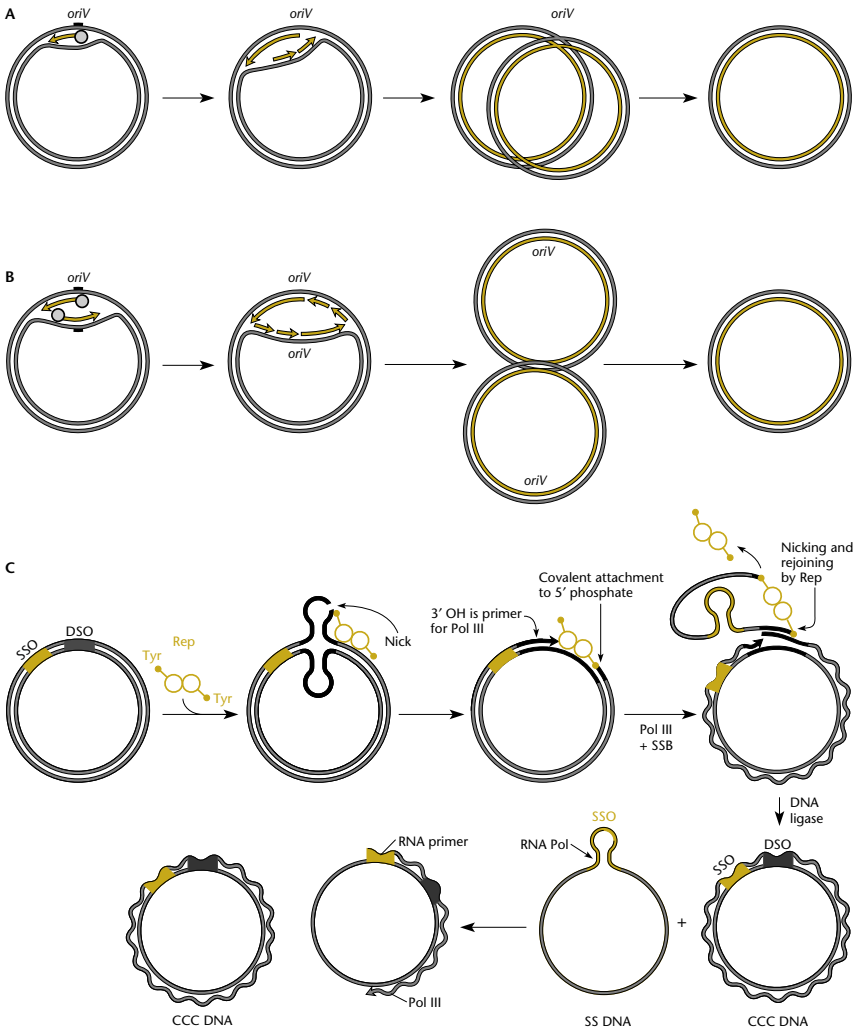

A
oriV
oriV
oriV
B
oriV
oriV
oriV
oriV
C
DSO
SSO
Rep
Tyr
Tyr
Nick
3′ OH is primer
for Pol III
Covalent attachment
to 5′ phosphate
Nicking and
rejoining
by Rep
Pol III
+ SSB
DNA
ligase
SSO
RNA Pol
DSO
SSO
RNA primer
Pol III
CCC DNA
SS DNA
CCC DNA

**Table 4.2**

| 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 |

**Figure 4.5**

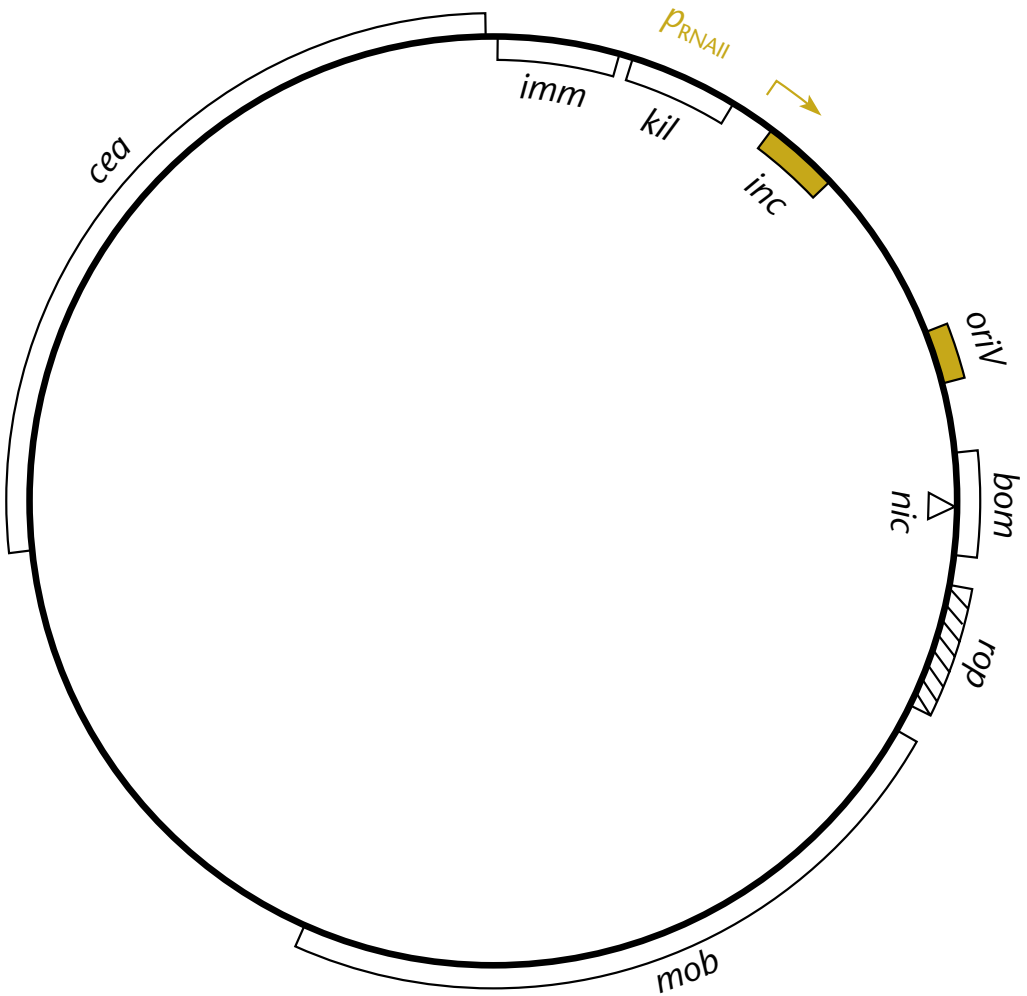
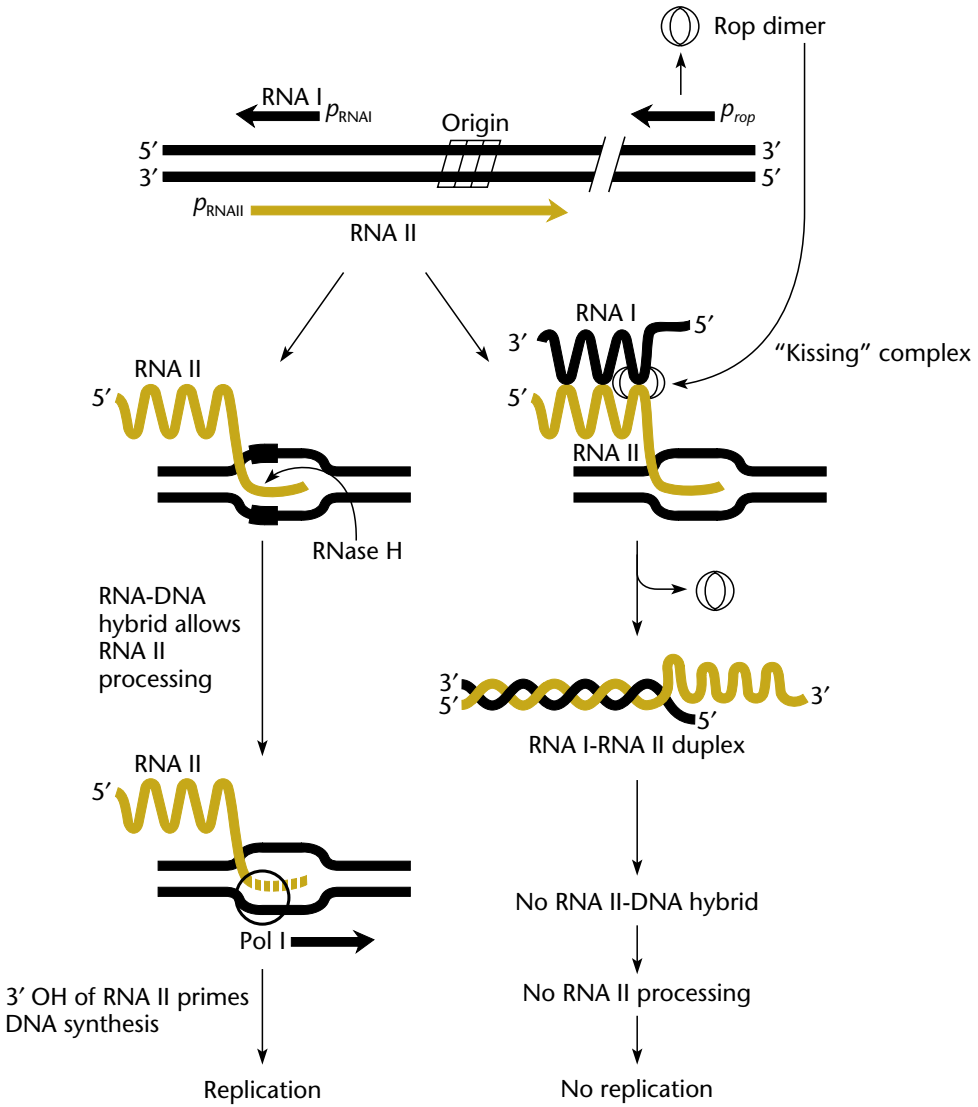
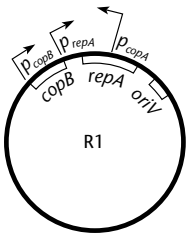


**Figure 4.6**


Rop dimer
RNA I
$p_{RNAI}$
Origin
$p_{rop}$
5′
3′
3′
5′
$p_{RNAII}$
RNA II
RNA I
3′
5′
"Kissing" complex
RNA II
5′
5′
RNA II
RNase H
RNA-DNA hybrid allows RNA II processing
3′
5′
3′
5′
RNA I-RNA II duplex
RNA II
5′
No RNA II-DNA hybrid
Pol I
3′ OH of RNA II primes DNA synthesis
No RNA II processing
Replication
No replication

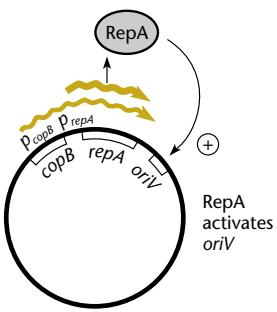
# Figure 4.7

**A Plasmid genetic organization**




| Promoter | Gene products expressed |
|---|---|
| $p_{copB}$ | RepA and CopB |
| $p_{repA}$ | RepA |
| $p_{copA}$ | CopA antisense RNA |

**B Replication occurs after plasmid enters cells**




**C Replication shutdown**

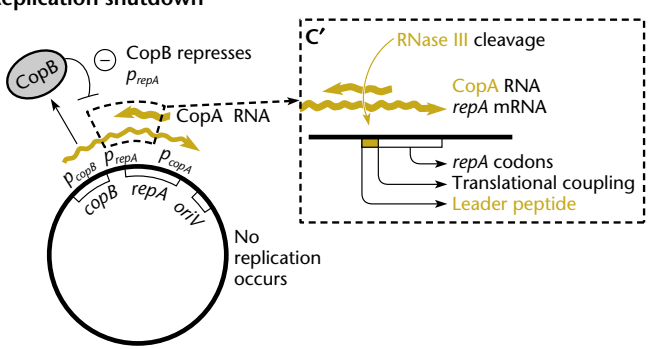


**Figure 4.8**

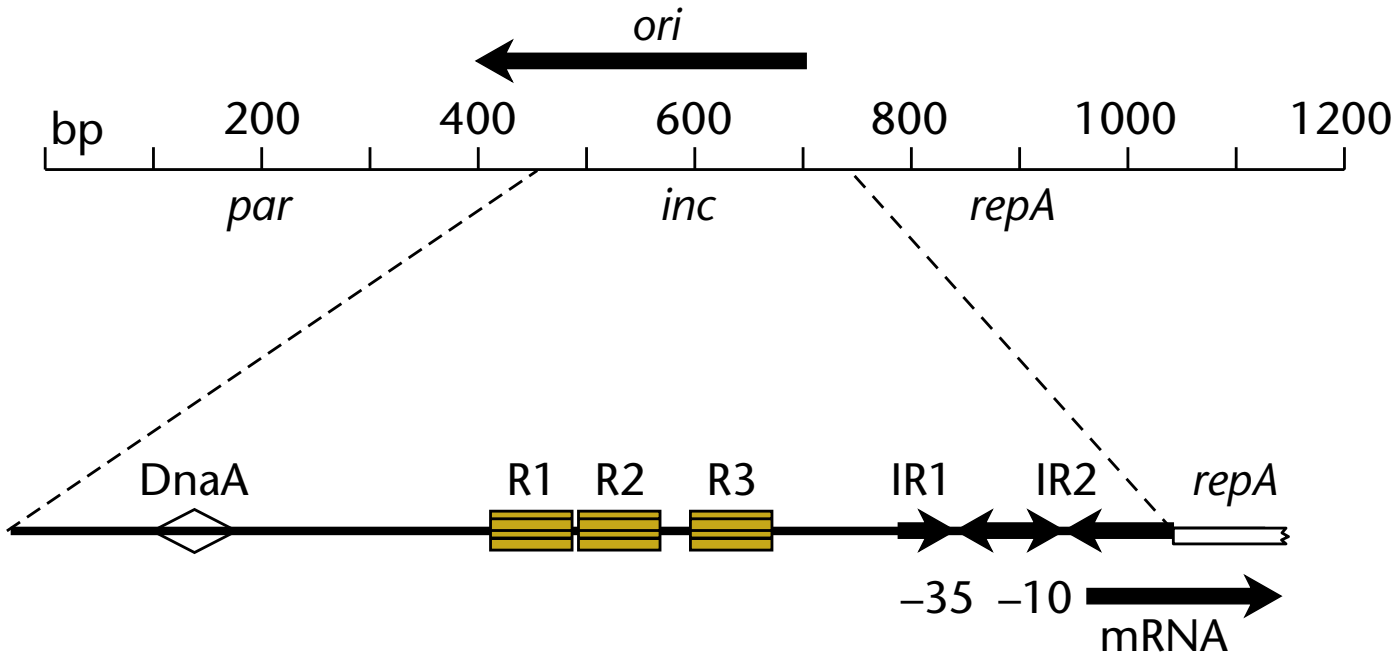

ori
bp
200
400
600
800
1000
1200
par
inc
repA
DnaA
R1
R2
R3
IR1
IR2
repA
–35
–10
mRNA

**Figure 4.9**

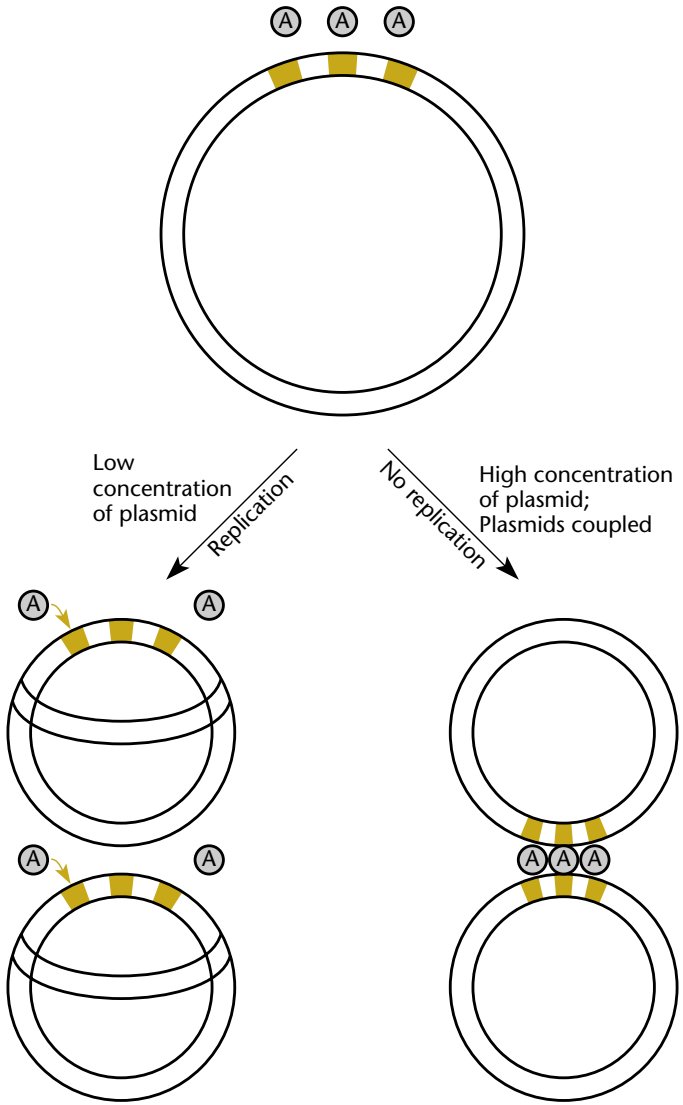

A
A
A
Low concentration of plasmid
Replication
No replication
High concentration of plasmid; Plasmids coupled
A
A
A
A
A
A
A

**Figure 4.10**

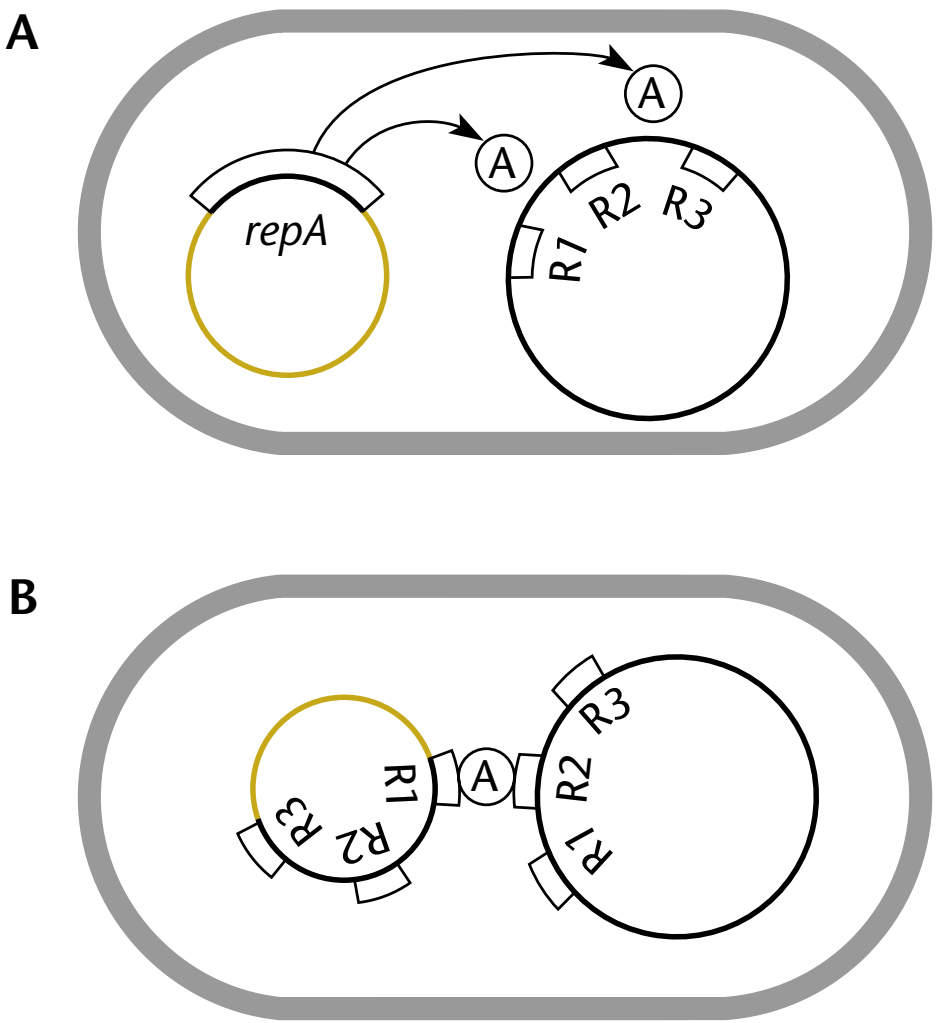


**Figure 4.11**

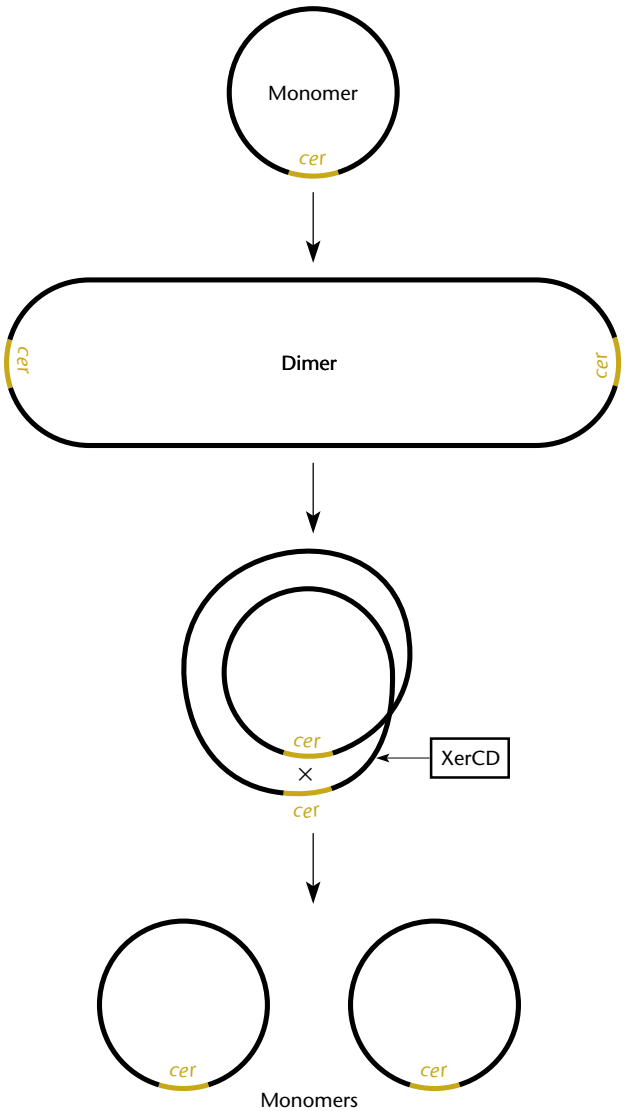


**Figure 4.12**

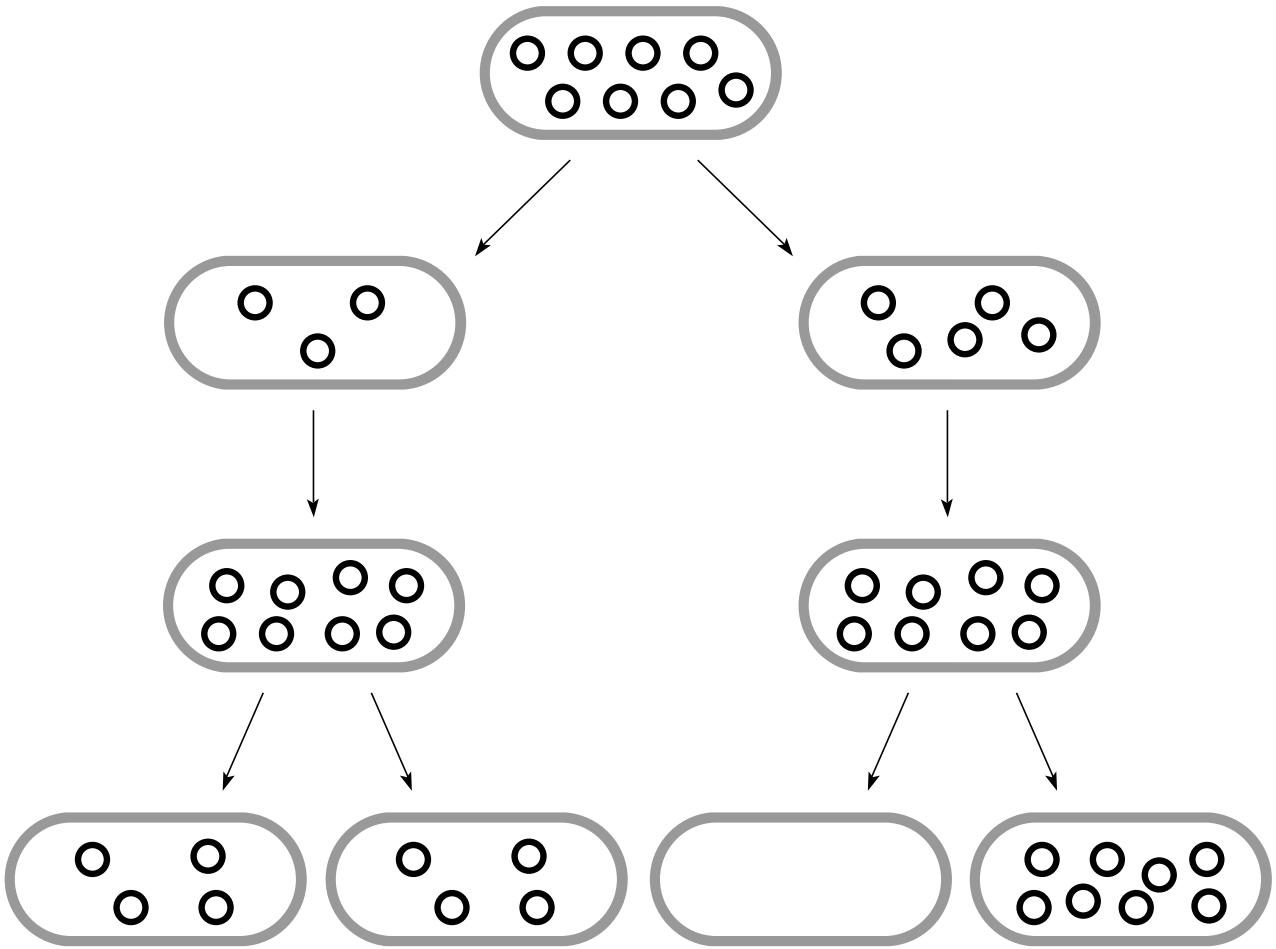
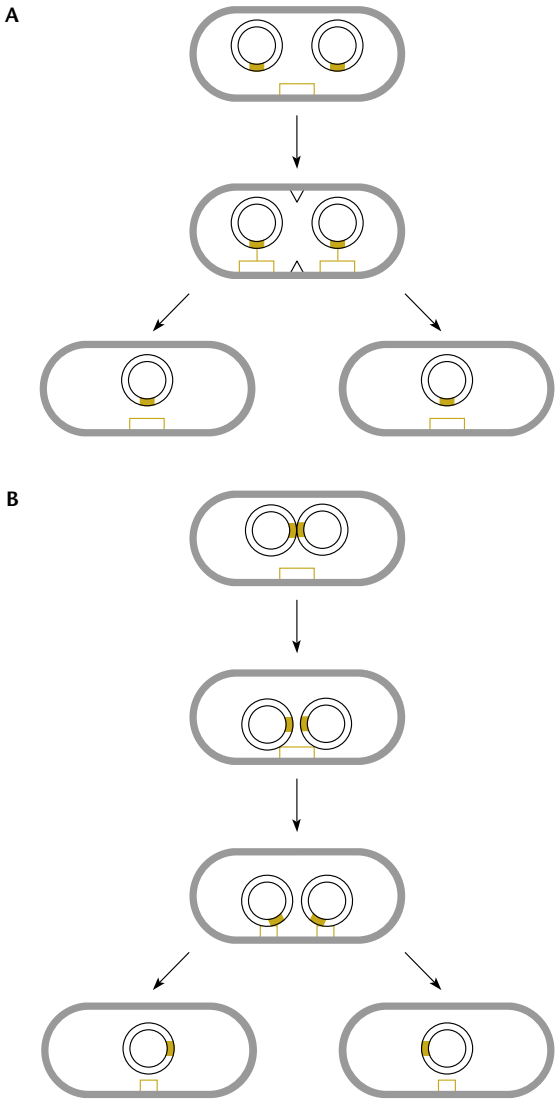


**Figure 4.13**



# Figure 4.14

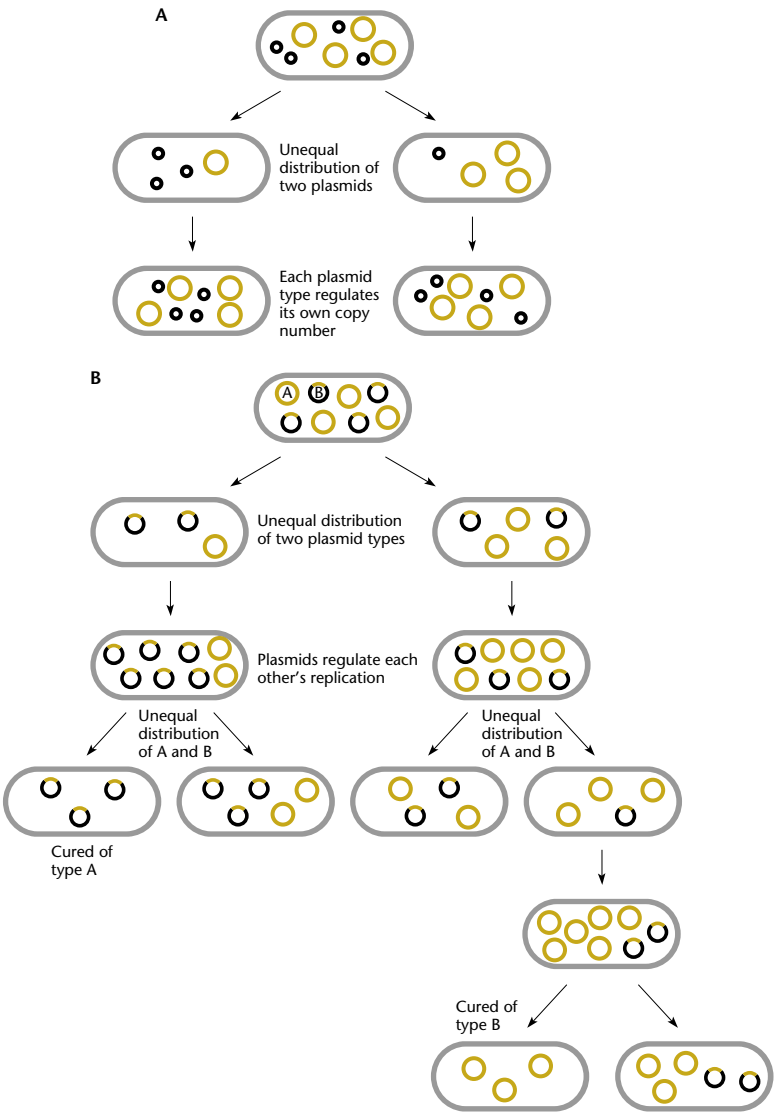

A
Unequal distribution of two plasmids
Each plasmid type regulates its own copy number
B
A
B
Unequal distribution of two plasmid types
Plasmids regulate each other's replication
Unequal distribution of A and B
Unequal distribution of A and B
Cured of type A
Cured of type B

**Figure 4.15**

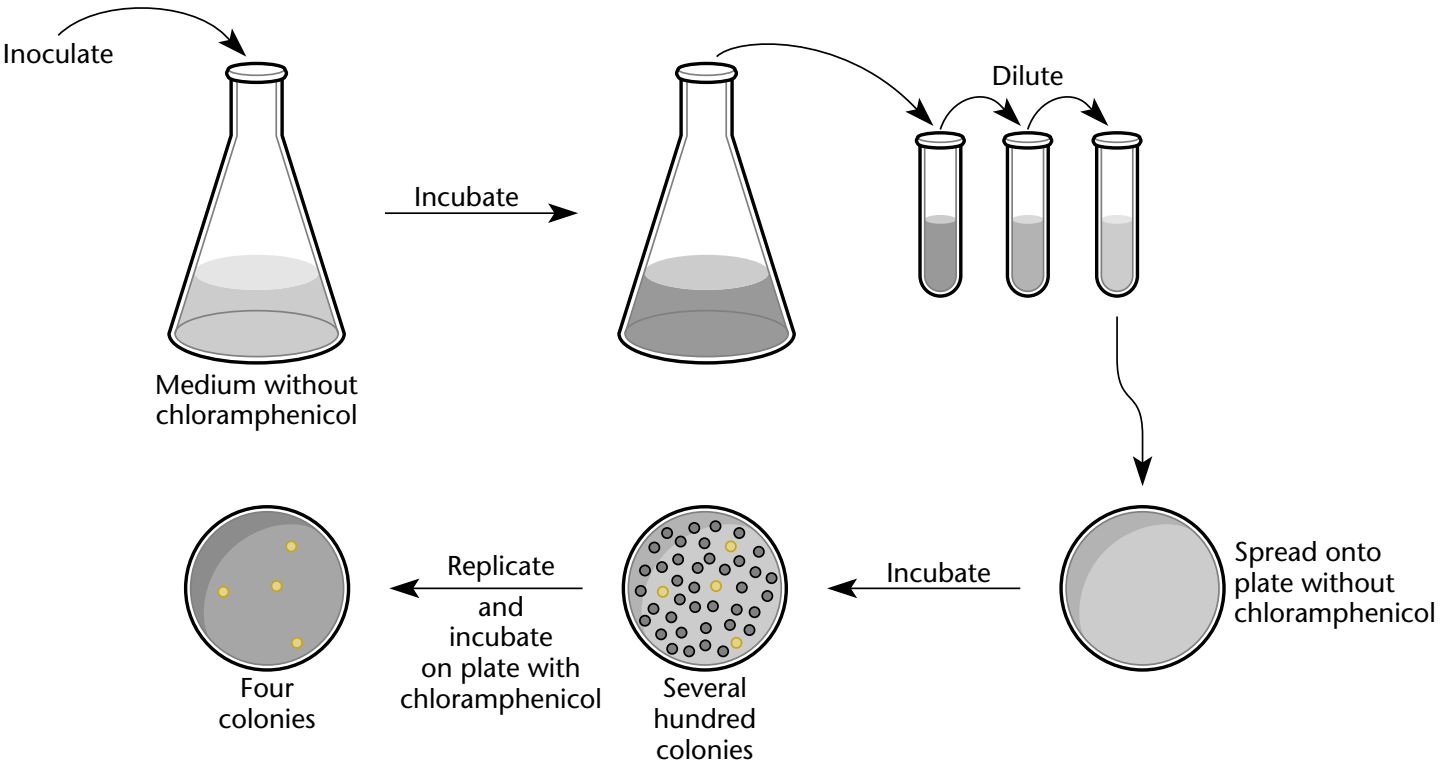

Inoculate
Medium without chloramphenicol
Incubate
Dilute
Spread onto plate without chloramphenicol
Incubate
Several hundred colonies
Replicate and incubate on plate with chloramphenicol
Four colonies

**TABLE 4.3** Replication origins of several *E. coli* plasmid vectors

| Plasmid | *ori* | Copy number |
|---|---|---|
| pBR322 | pMB1 | 15–20 |
| pUC vectors | pMB1 mutant | 100s |
| pET vectors | pMB1 mutant | 100s |
| pBluescript | pMB1 mutant | 100s |
| pACYC184 | p15A | 10–12 |
| pSC101 | pSC101 | 5 |

**Figure 4.16**

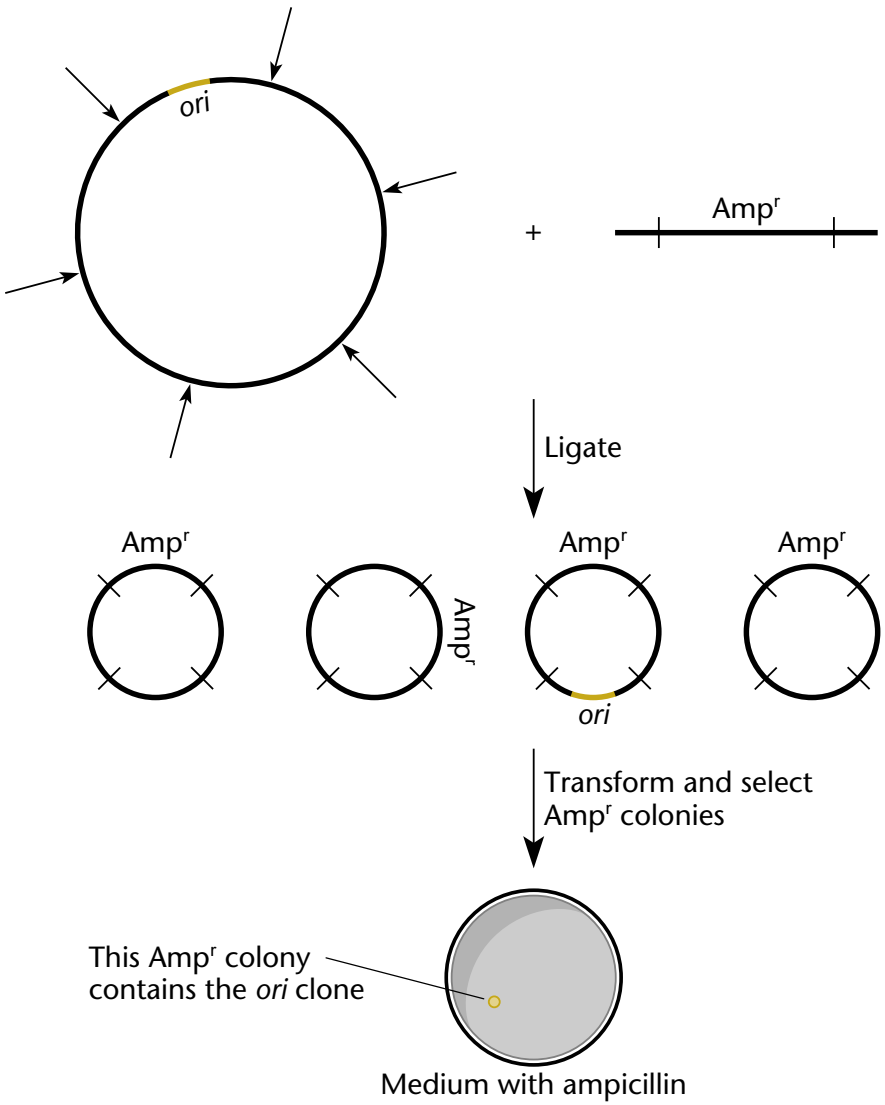
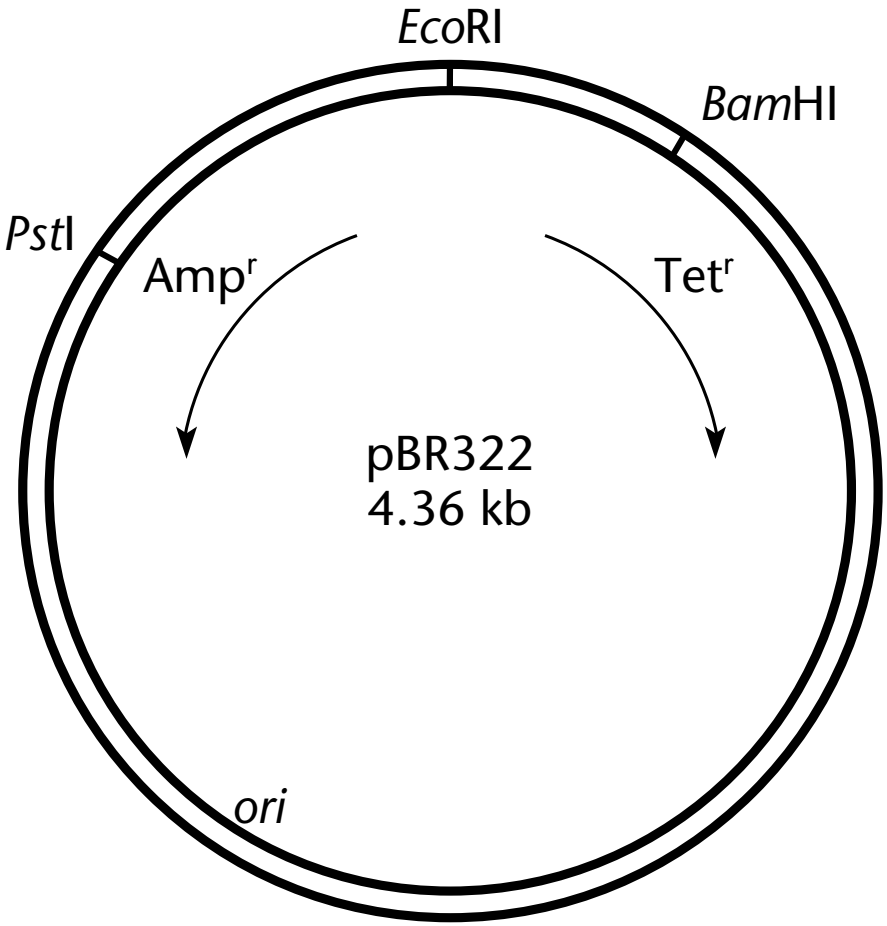
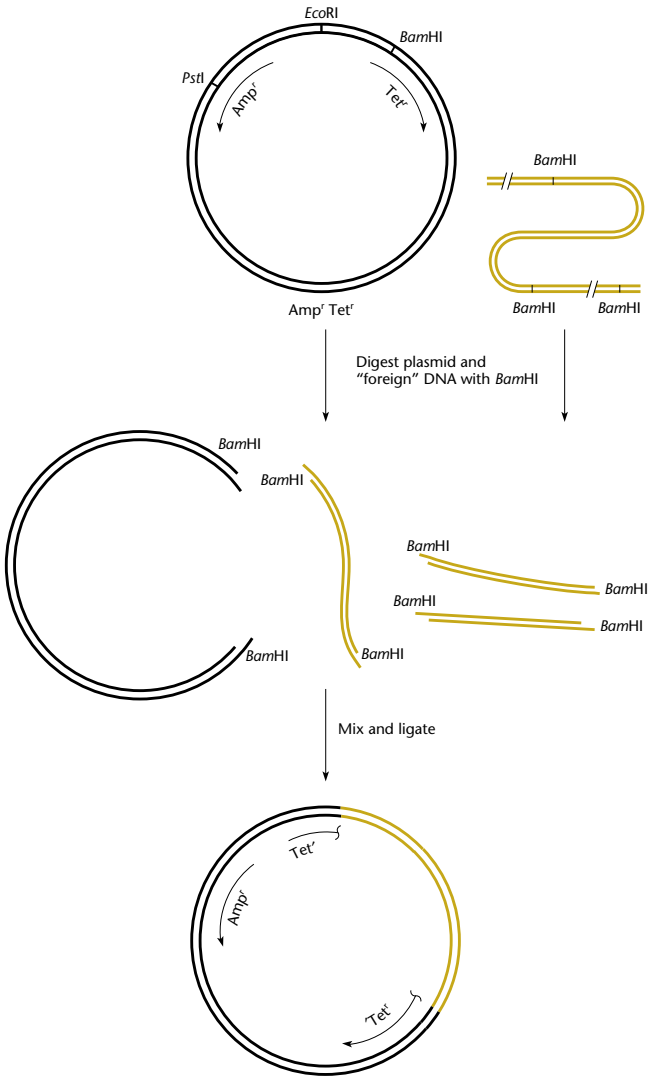

ori
+
Amp$^r$
Ligate
Amp$^r$
Amp$^r$
Amp$^r$
Amp$^r$
ori
Transform and select
Amp$^r$ colonies
This Amp$^r$ colony
contains the *ori* clone
Medium with ampicillin

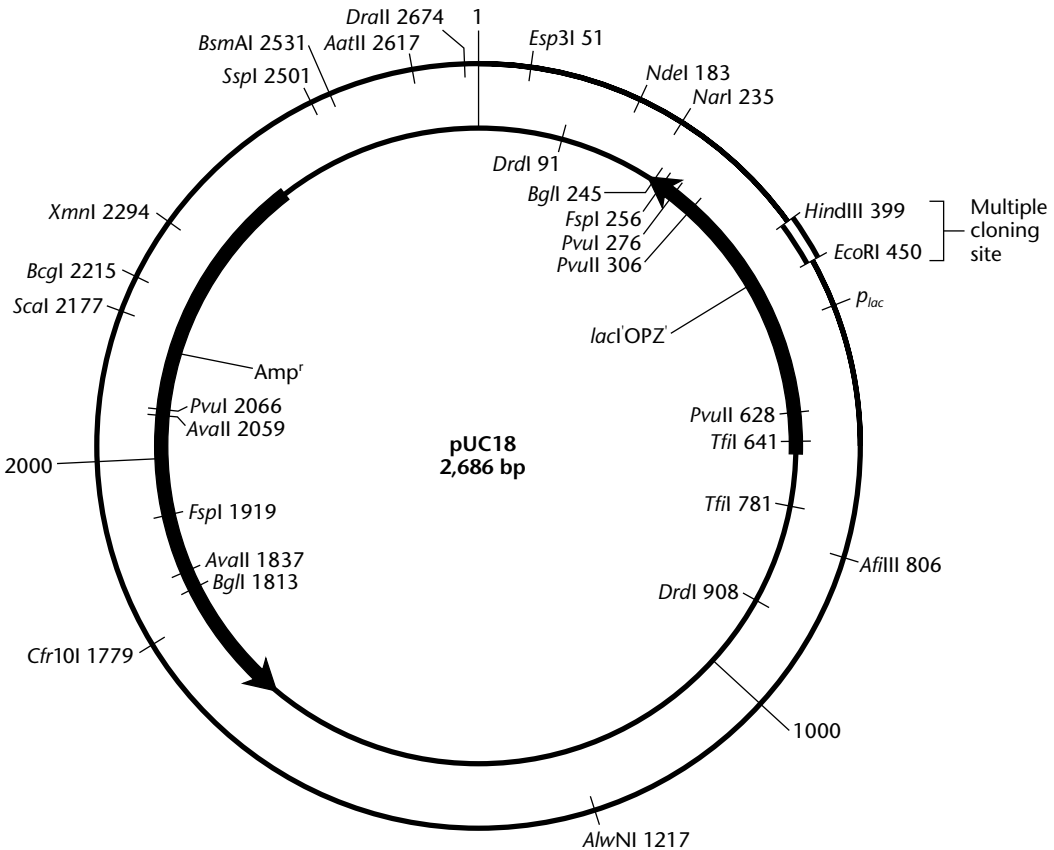
**Figure 4.17**



# Figure 4.18


EcoRI
BamHI
PstI
Amp^r
Tet^r
Amp^r Tet^r
BamHI
BamHI
BamHI
Digest plasmid and
"foreign" DNA with BamHI
BamHI
BamHI
BamHI
BamHI
BamHI
BamHI
BamHI
Mix and ligate
Tet^r
Amp^r
Tet^r
Amp^r

# Figure 4.19




**pUC18 multiple cloning site and primer binding regions:** 364–480

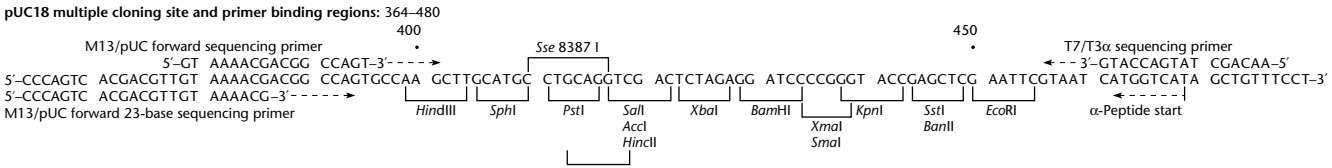


**Figure 4.20**