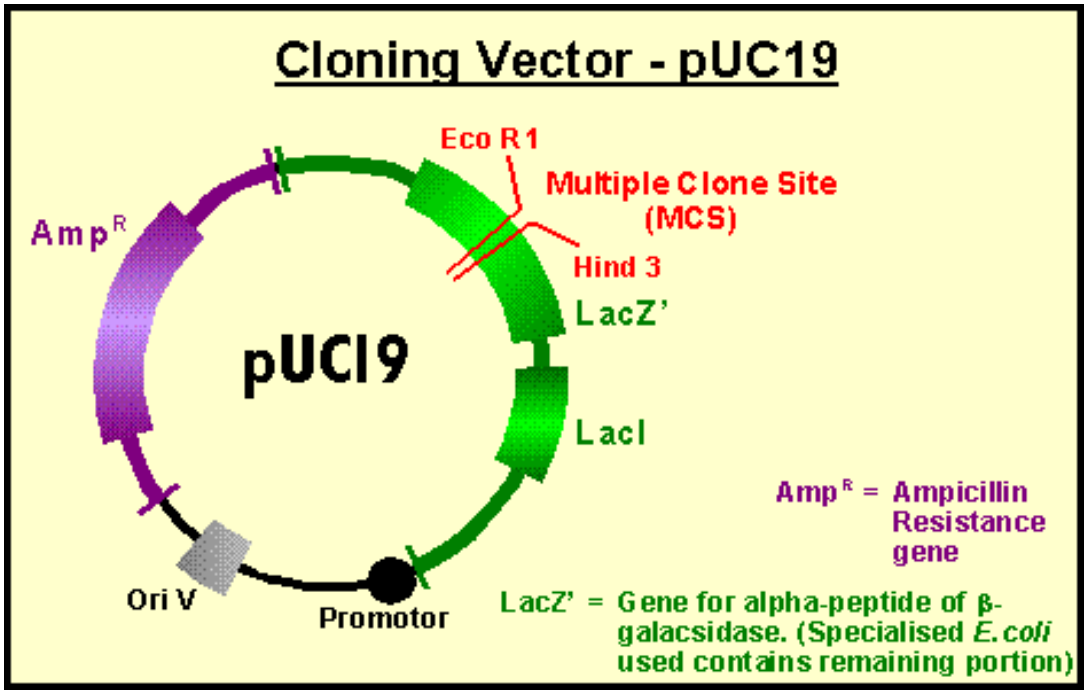
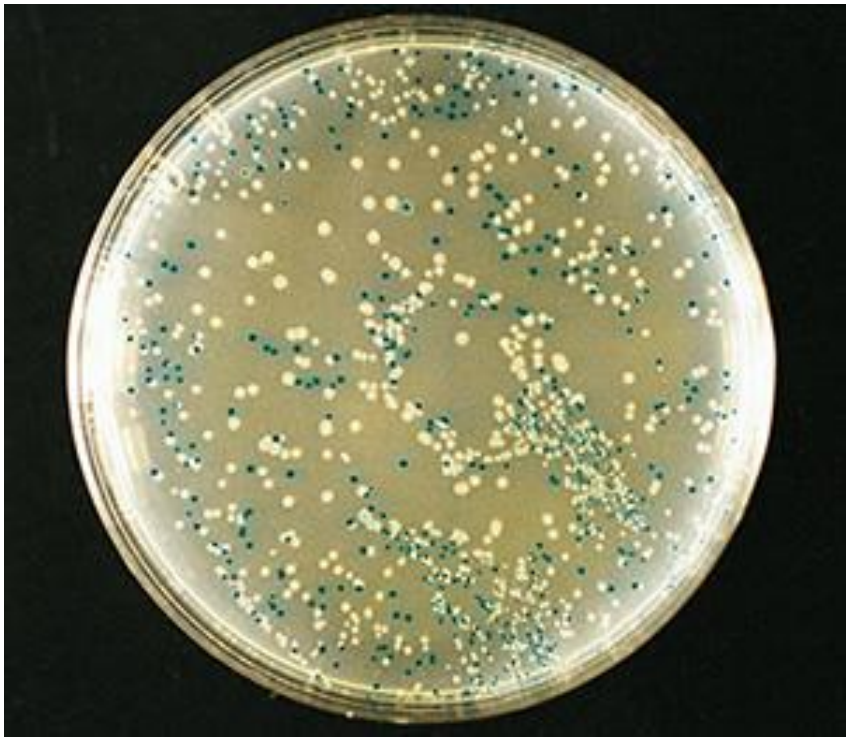
The pUC vectors also incorporate a DNA sequence that permits rapid visual detection of an insert.

The MCS is inserted into the *lacZ sequence,which encodes the promoter and the a-peptide of ~-galactosidase.*

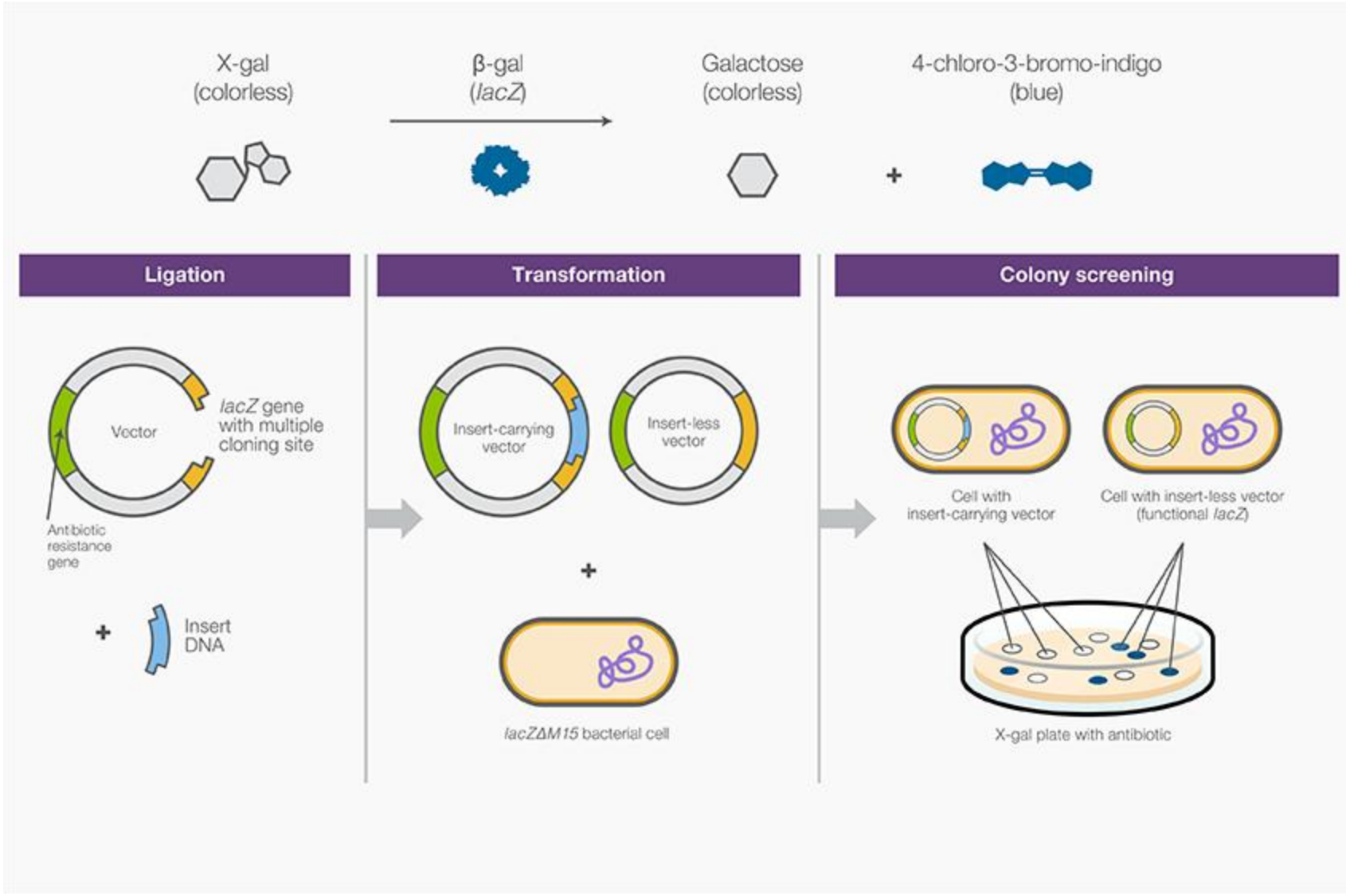
The insertion of the MCS into the lacZ' fragment does not affect the abilityof the a-peptide to mediate complementation, but cloning DNA fragments into the MCS does.

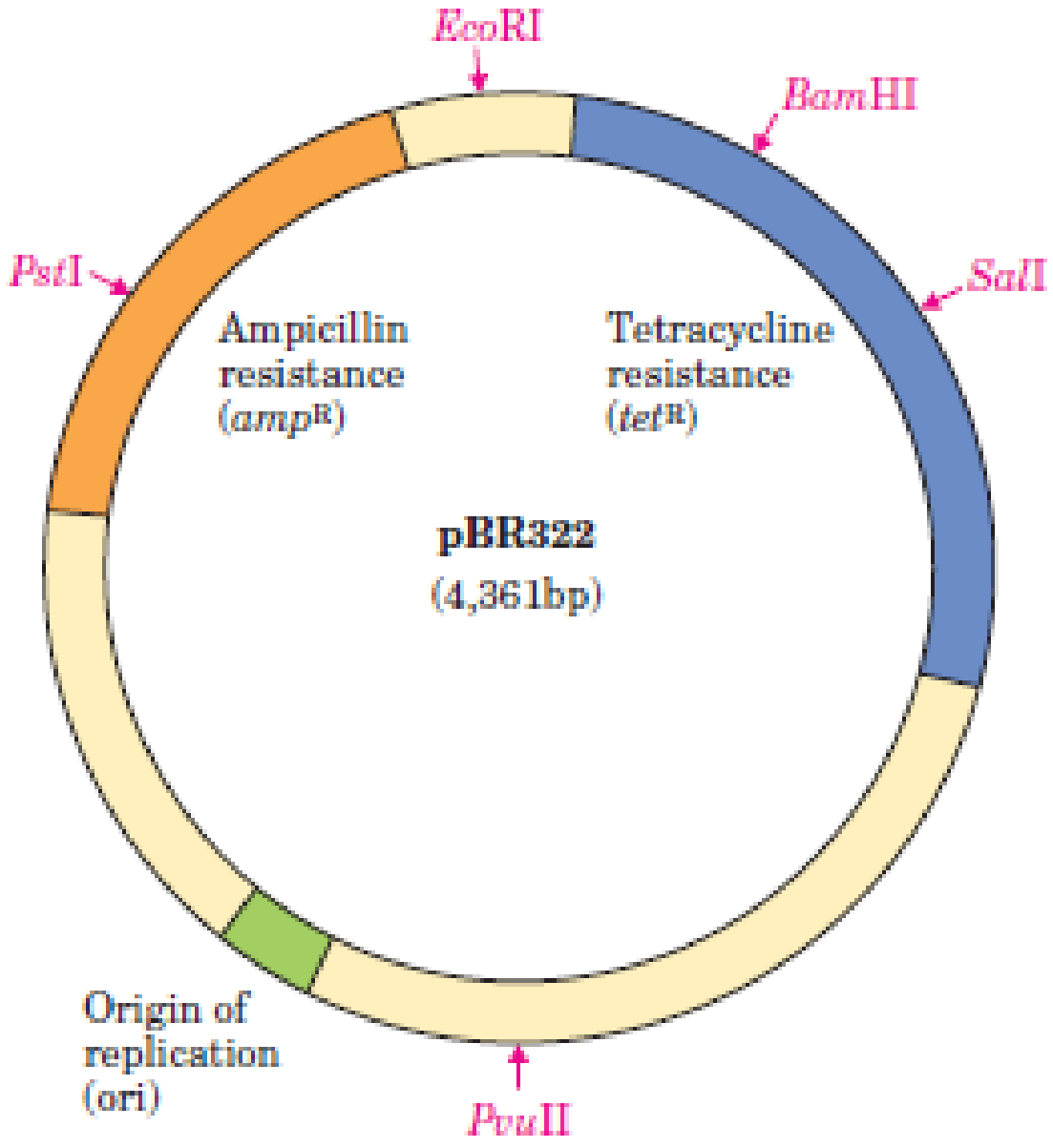
Therefore,recombinants can be detected by blue/white screening on growth medium containing Xgal.

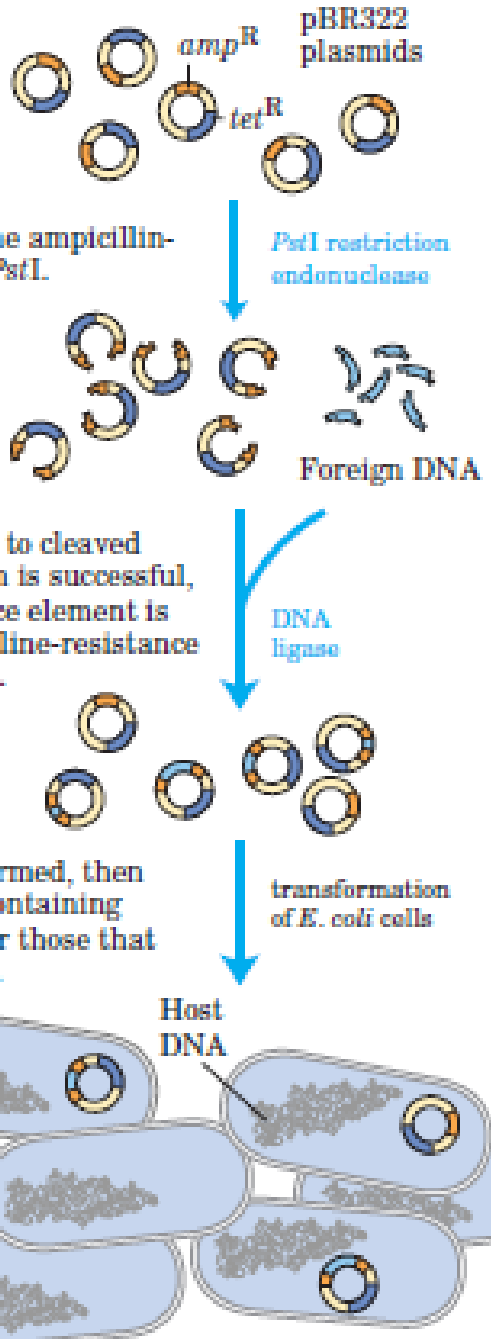




To identify whether the transformed colonies contain an insert, a number of methods can be employed, of which the most common are “blue/white” screening and positive selection. Blue/white screening relies on transforming a bacterial strain that expresses a mutant *lacZ* gene (*lacZ*ΔM15), which can be complemented with the alpha peptide of beta-galactosidase, encoded on the vector (**alpha complementation**). Transformed cells are plated on a growth medium that includes a transcriptional inducer for *lacZ* expression, IPTG, and a chromogenic substrate of LacZ, X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). In blue/white screening, LacZ will hydrolyze the X-gal, producing a blue dye and hence a blue colony. When a DNA insert disrupts the vector-encoded lacZα gene, no functional LacZ is formed, and transformed colonies are white.


X-gal
(colorless)
β-gal
(lacZ)
Galactose
(colorless)
4-chloro-3-bromo-indigo
(blue)
Ligation
Transformation
Colony screening
Vector
lacZ gene
with multiple
cloning site
Antibiotic
resistance
gene
Insert
DNA
Insert-carrying
vector
Insert-less
vector
lacZΔM15 bacterial cell
Cell with
insert-carrying vector
Cell with insert-less vector
(functional lacZ)
X-gal plate with antibiotic

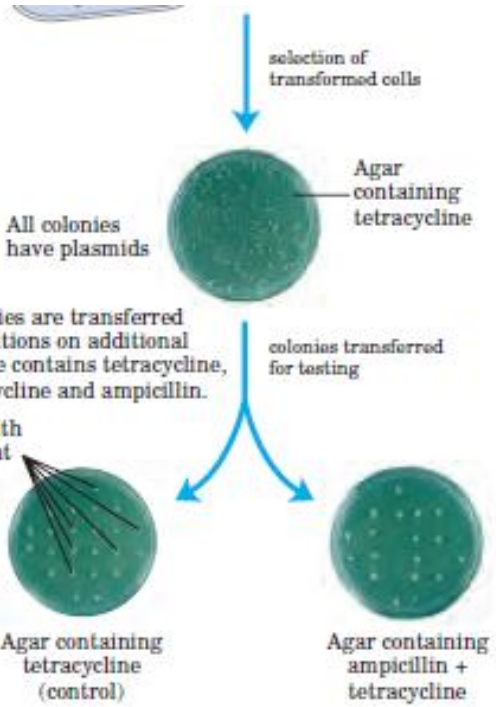

EcoRI
BamHI
SalI
PstI
Ampicillin resistance (ampR)
Tetracycline resistance (tetR)
pBR322
(4,361bp)
Origin of replication (ori)
PvuII



① pBR322 is cleaved at the ampicillin-resistance element by *Pst*I.

② Foreign DNA is ligated to cleaved pBR322. Where ligation is successful, the ampicillin-resistance element is disrupted. The tetracycline-resistance element remains intact.

③ *E. coli* cells are transformed, then grown on agar plates containing tetracycline to select for those that have taken up plasmid.



④ Individual colonies are transferred to matching positions on additional plates. One plate contains tetracycline, the other tetracycline and ampicillin.

⑤ Cells that grow on tetracycline but not on tetracycline + ampicillin contain recombinant plasmids with disrupted ampicillin resistance, hence the foreign DNA. Cells with pBR322 without foreign DNA retain ampicillin resistance and grow on both plates.

Phage were first described in 1915 by Frederick Twort and 1917 by Felix d'Herelle.

They observed that broth cultures of certain intestinal bacteria could be dissolved by addition of a bacteria-free filtrate obtained from sewage.

The lysis of the bacterial cells was said to be brought about by a virus which meant a "filterable poison" ("virus" is Latin for "poison").

The agent responsible for the lysis was transferable from culture to culture, invisible by light microscopy, and would go through the smallest filter they had.

d'Herelle coined the term "bacteriophage", signifying an entity that eats bacteria.

In the early 1950s, following some initial studies on Bacillus megaterium, Andre Lwoff and his colleagues at the Institut Pasteur described the phenomenon of **lysogeny in E. coli.**

It became clear that certain strains of E. coli were lysogenized by phage, that is to say, these bacteria harbored phage >-. **in a dormant form, called a prophage.**

The lysogenic bacteria grew normally and might easily not have been recognized as lysogenic.

However, when Lwoff exposed the bacteria to a moderate dose of ultraviolet light, the bacteria stopped growing, and after about 90 min of incubation the bacteria lysed, releasing a crop of phage into the medium

.

The released phage were incapable of infecting more E.coli that had been lysogenized by phage >-. (this is called immunity to superinfection), but nonlysogenic bacteria could be infected to yield another crop of virus.

- **Bacteriophage or phage for short are viruses that infect only bacteria.**

- **Viruses are nucleic** acid molecules surrounded by a protective coating.
- The nucleic acid inside the coating, called the **phage genome in a bacteriophage, encodes most of the gene products needed** for making more phage.

- The phage genome can be made of either double- or single-stranded DNA or RNA, depending on the bacteriophage in question. The genome can be circular or linear.

- The protective coating or **capsid surrounding the** phage genome is composed of phage-encoded proteins.

- Bacterial cells can undergo one of two types of infections by viruses termed **lytic infections** and **lysogenic (temperate)** infections.

- In *E. coli*, lytic infections are caused by a group seven phages known as the T-phages, while lysogenic infections are caused by the phage lambda

A landmark in molecular biology was reached when the entire sequence of the phage lambda genome, 48,502 nucleotide pairs, was determined by Fred Sanger and his colleagues (Sanger *et al. 1982).*

Bacteriophage vectors

Both single-stranded (filamentous) and double-stranded E. coli phages have been exploited as cloning vectors.

Filamentous phages. Filamentous phages are not lytic. They coexist with the infected cells for several generations and are convenient for cloning genes which produce toxic products.

Among the filamentous phages, fd, fl, and **M13** have been well characterized and their genomes have been sequenced.

Discovered in 1963, M13 bacteriophage was first isolated from Escherichia coli bacteria.

M13 is a single stranded DNA virus that belongs to the **Inoviridae** family of filamentous bacteriophages, which infect gram- negative bacteria.

Single-stranded circular DNA genome (6407 bp long) – packaged inside rod-shaped protein capsid

- It is a member of the Ff (F-specific filamentous phage) class of phages and is one of the smallest filamentous bacteriophages known.

- M13 phage has the advantage of being able to reproduce within the infected host without causing cell lysis.

- Phage M13 is widely used in nucleotide sequencing and site-directed mutagenesis since its genome can exist either in a single-stranded form inside a phage coat or as a double stranded replicative form within the infected cell.

- During replication, only the plus strand of the replicative form is selectively packaged by the phage proteins. The replicative form is a covalently closed circular molecule and hence can be used as a plasmid vector and transformed into the host by the usual transformation procedures.

Phage particles bind to F pilus

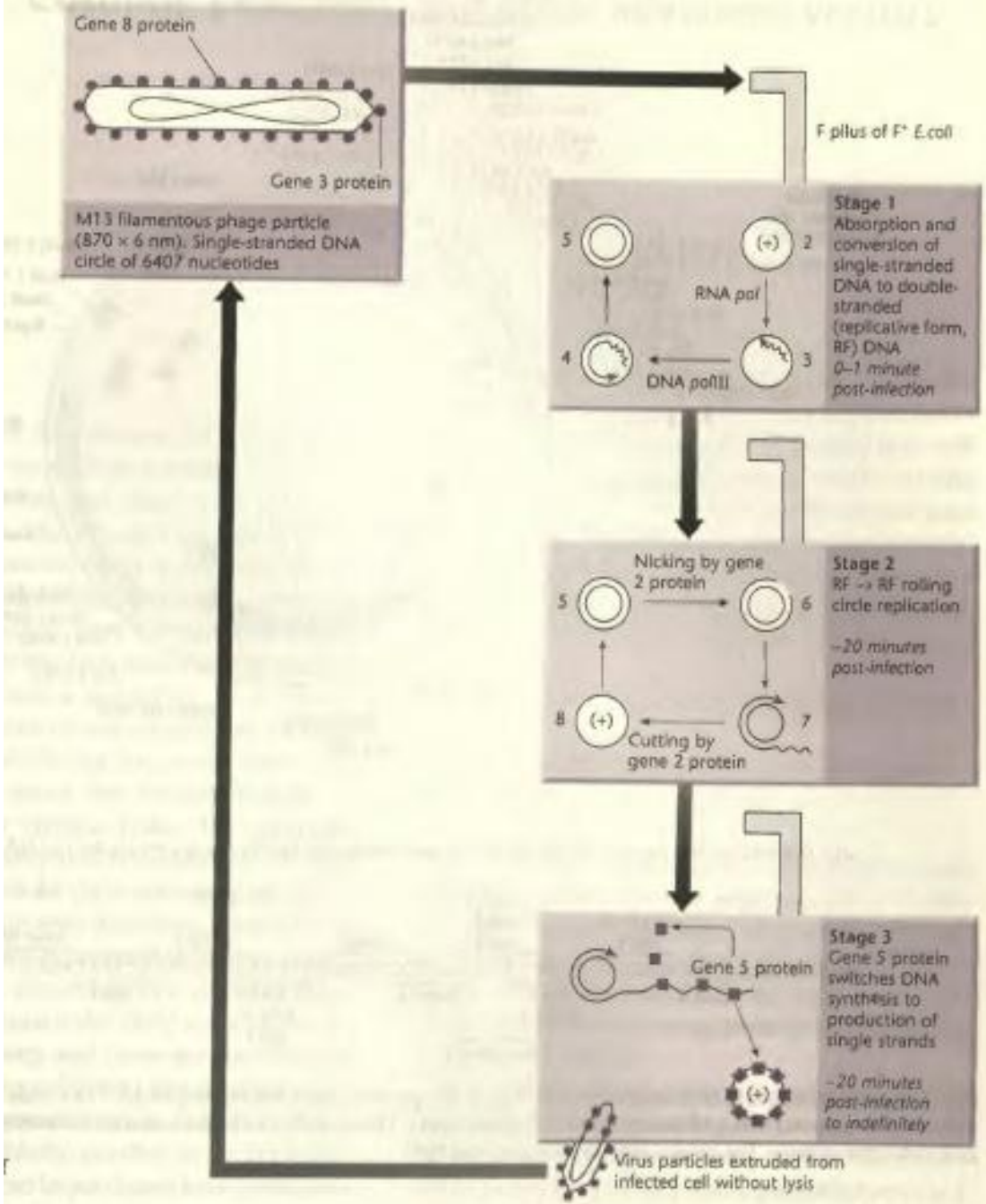
single-stranded DNA genome enters cell designated as “+” strand

“+” strand repaired – double-stranded **replicative form (RF)**

RF contains “+” and “–” strands

“–” strand is template – for mRNA synthesis – for production of new “+” strands – by rolling circle replication

“+” strands are packaged in phage coat protein – exit cell as phage particle


Gene 8 protein
Gene 3 protein
M13 filamentous phage particle (870 × 6 nm). Single-stranded DNA circle of 6407 nucleotides
F pilus of F+ E.coli
Stage 1
Absorption and conversion of single-stranded DNA to double-stranded (replicative form, RF) DNA
0–1 minute post-infection
RNA pol
DNA pol III
Stage 2
RF → RF rolling circle replication
~20 minutes post-infection
Nicking by gene 2 protein
Cutting by gene 2 protein
Stage 3
Gene 5 protein switches DNA synthesis to production of single strands
~20 minutes post-infection to indefinitely
Gene 5 protein
Virus particles extruded from infected cell without lysis