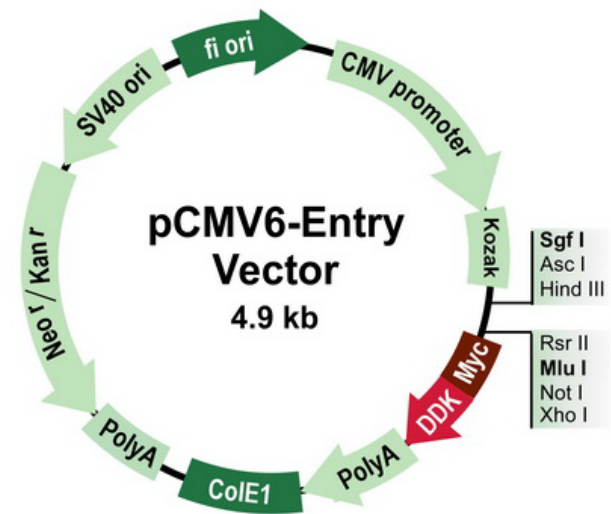
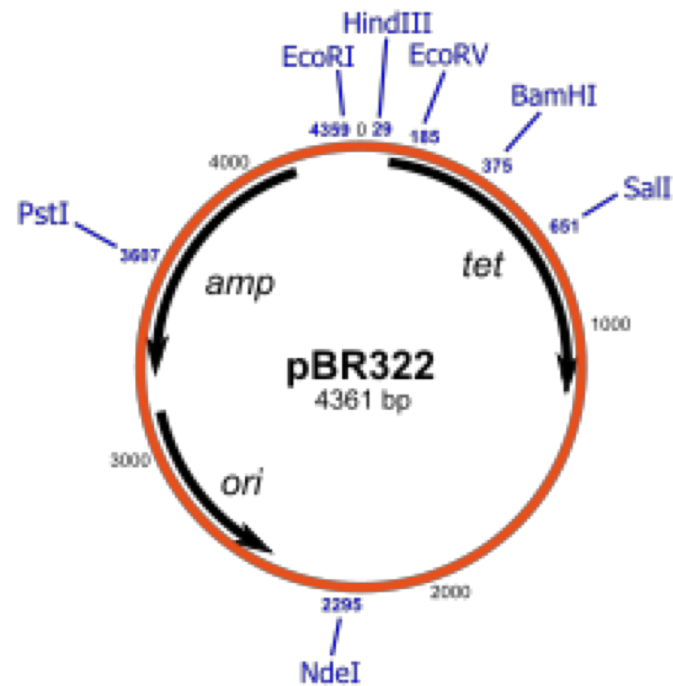
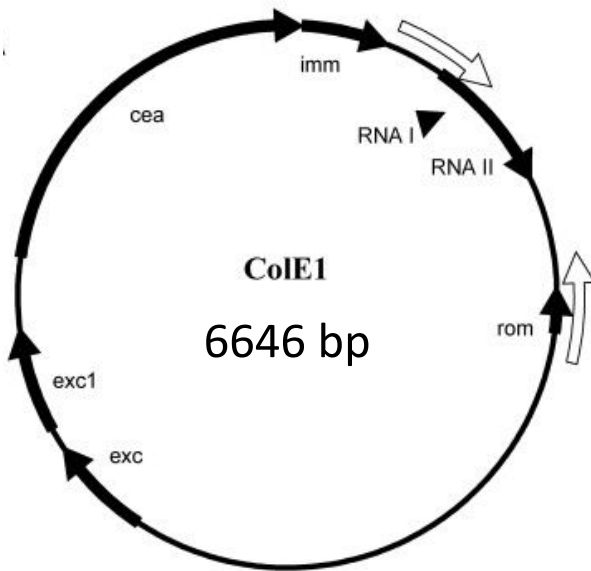
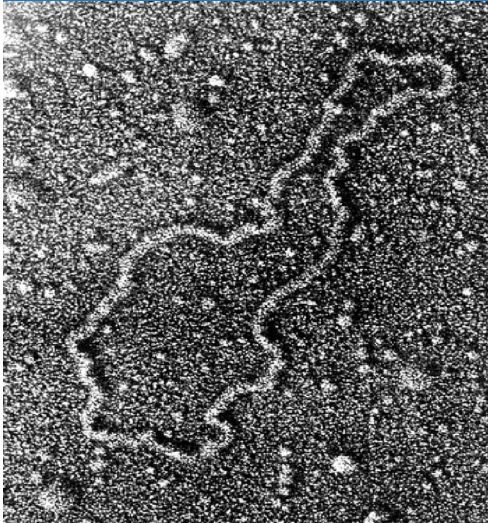
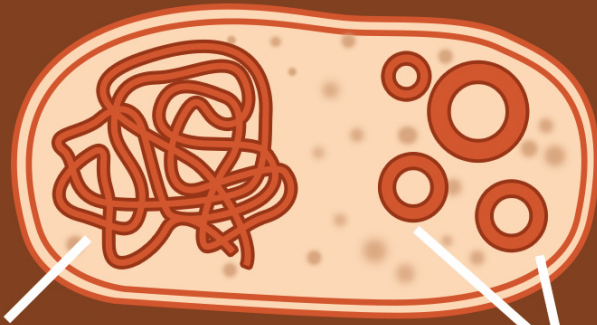


Plasmids



Plasmids



**Bacterial chromosome
(circular)**

Plasmids

**Plasmids are self-replicating
and stable extrachromosomal units of
double stranded DNA.**

Buzzle.com

A plasmid is a **small DNA molecule (1-200kb)** within a cell that is **physically separated** from a chromosomal DNA and can **replicate independently** (“**replicons**”).

Copy number:

1 – 1000nds

Shape:

Cicular, doublestrandend

Some linear plasmids exist

Present in:

Bacteria

but also sometimes in archea and eukaryotic cells (yeast)

Advantage to bacteria: - plasmid often carry genes that give a seletvie advantage
- plasmid can be passed on to other bacteria: horizontal gene transfer

What is the difference to viruses? - plasmids are not packaged into capsid
- virus does not give selective advantage

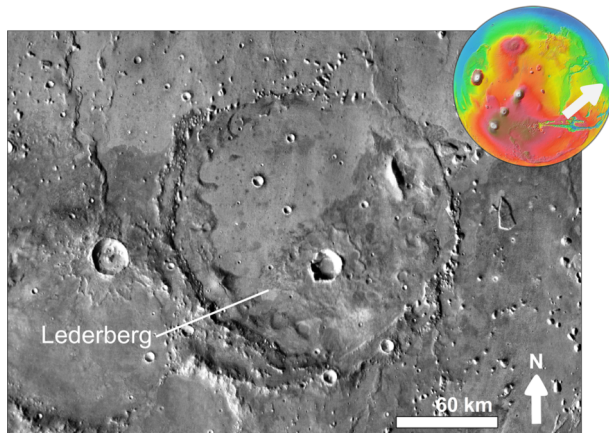
Plasmids

History

The term plasmid was introduced in 1952 by the American molecular biologist **Joshua Lederberg** to refer to "**any extrachromosomal hereditary determinant.**"

Definition also includes viruses; thus refinement:

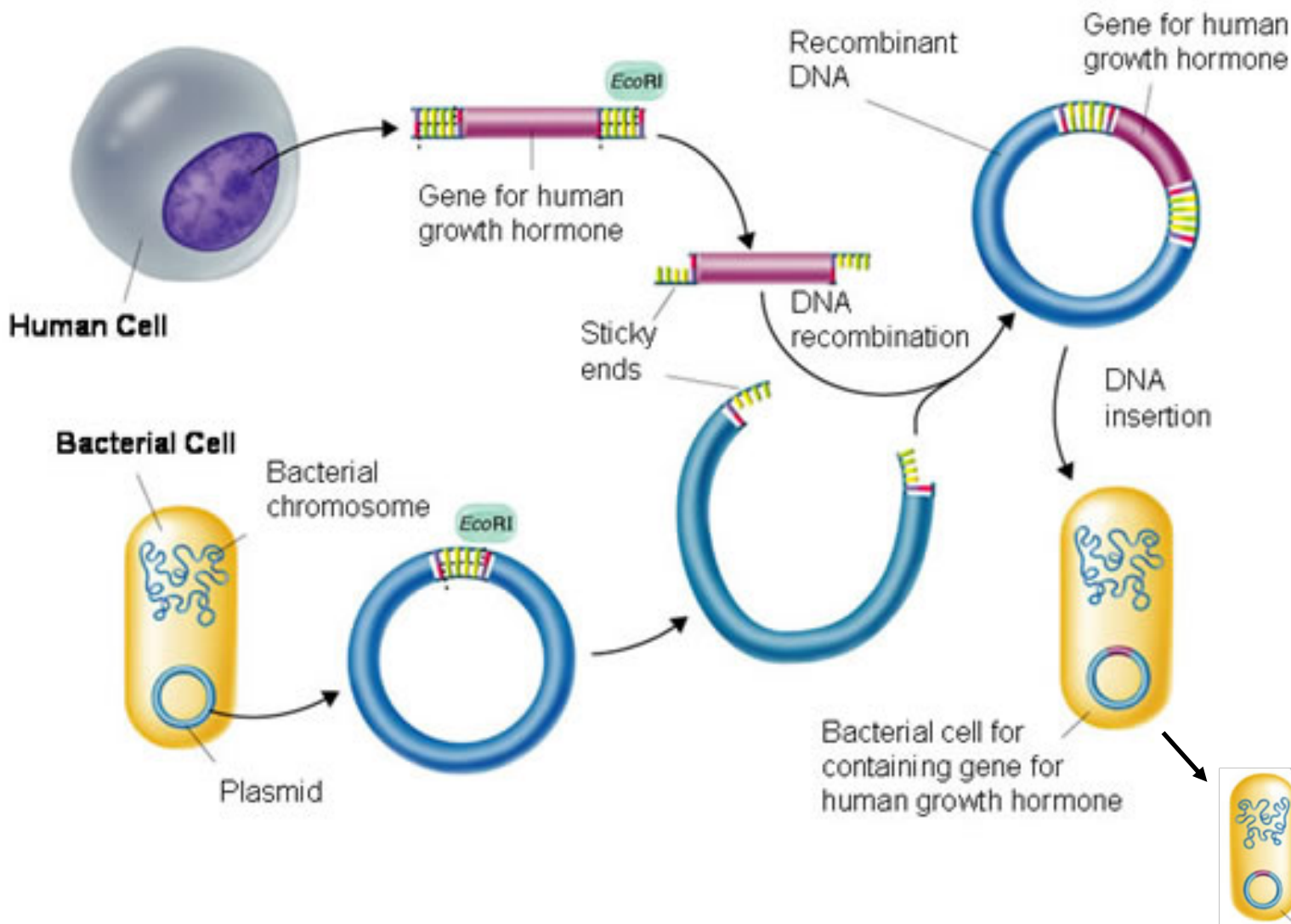
Genetic elements that exist exclusively or predominantly outside of the chromosome and can replicate autonomously.



1958 Nobel Prize in Physiology or Medicine for discovering that bacteria can mate and exchange genes (bacterial conjugation)

Plasmids

Why interesting for molecular biology? Recombinant DNA technology



Take piece of human DNA

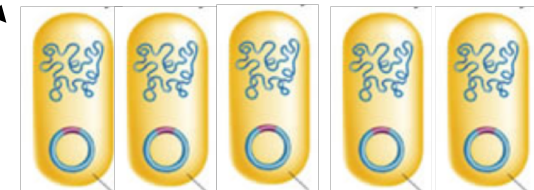
Take bacterial plasmid DNA

Insert into plasmid
="recombinant DNA"

Re-insert obtained plasmid into
bacteria that does not have own
plasmids

Bacteria proliferate
Plasmid in bacteria replicate
and can reach 1-1000 copies

Amplified human DNA can be
studied



Plasmids

Some more definitions:

Plasmid is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA.

Vector – is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed (e.g.- **plasmid**, cosmid, Lambda phages, virus)

| Sono disponibili vari tipi di vettori di clonaggio | | | |
|--|---------------------------------|--|--------------------------|
| Vettore | Caratteristiche | Isolamento del DNA | Contenuto massimo di DNA |
| Plasmide | Alto numero di copie | Fisico | 10 kb |
| Fago | Infetta batteri | Attraverso l'impacchettamento nel fago | 20 kb |
| Cosmide | Alto numero di copie | Attraverso l'impacchettamento nel fago | 48 kb |
| BAC | Basato sul plasmide F | Fisico | 300 kb |
| YAC | Origine + centromero + telomero | Fisico | >1 Mb |

Natural, engineered

Natural, engineered

Engineered

Engineered

Engineered

Lenti-, Adeno, Retroviruses

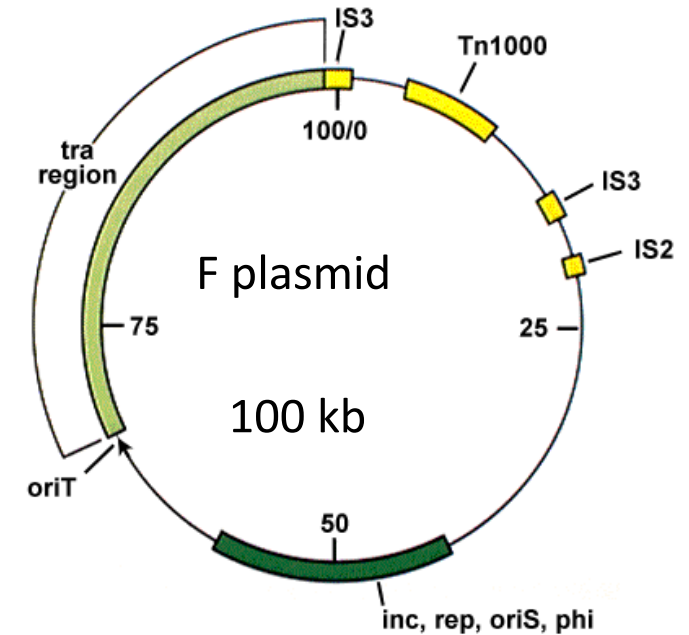
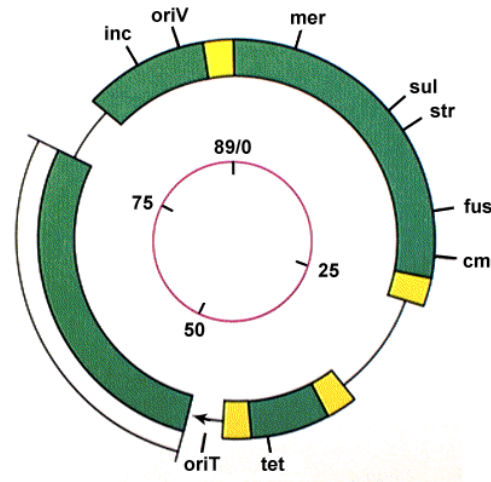
Natural, engineered

Plasmids

Natural Plasmids - Grouped after their properties

- **F-plasmids:** encode tra region for horizontal gene transfer (conjugation), (transFer); F⁺ (plasmid donor); F⁻ plasmid recipient

- **R- plasmid:** Encode genes for resistance against antibiotics and/or heavy metals. (Ampicilin, Kanamycin)



- **Col – plasmids:** - produces colicins (antibacterial)
- **Catabolic plasmids:** -have properties to use odd carbon/ energy source (many *Pseudomonas* have such plasmids)
- **Virulent plasmids:** - Encode toxins, pathogenic.
- **Cryptic plasmids:** - no known property

Plasmids

Natural Plasmids – other useful terms of classification

Classification based on possibility to do horizontal gene transfer

- Conjugative plasmids (F plasmids): able to do horizontal gene transfer (geni *tra*)
- Non-conjugative plasmids
 - Plasmidi R, • Plasmidi Col, • Plasmidi degradativi, • Plasmidi della virulenza:

Classification based on copy number

- High copy number plasmids (relaxed plasmids); Plasmidi ad alto numero di copie (rilassati)
- Low copy number plasmids (stringent plasmids); Plasmidi a basso numero di copie (stringenti)

Plasmids

Natural Plasmids - Grouped after their essential genes:

1) **Essential** genes for keeping the plasmid within the cell

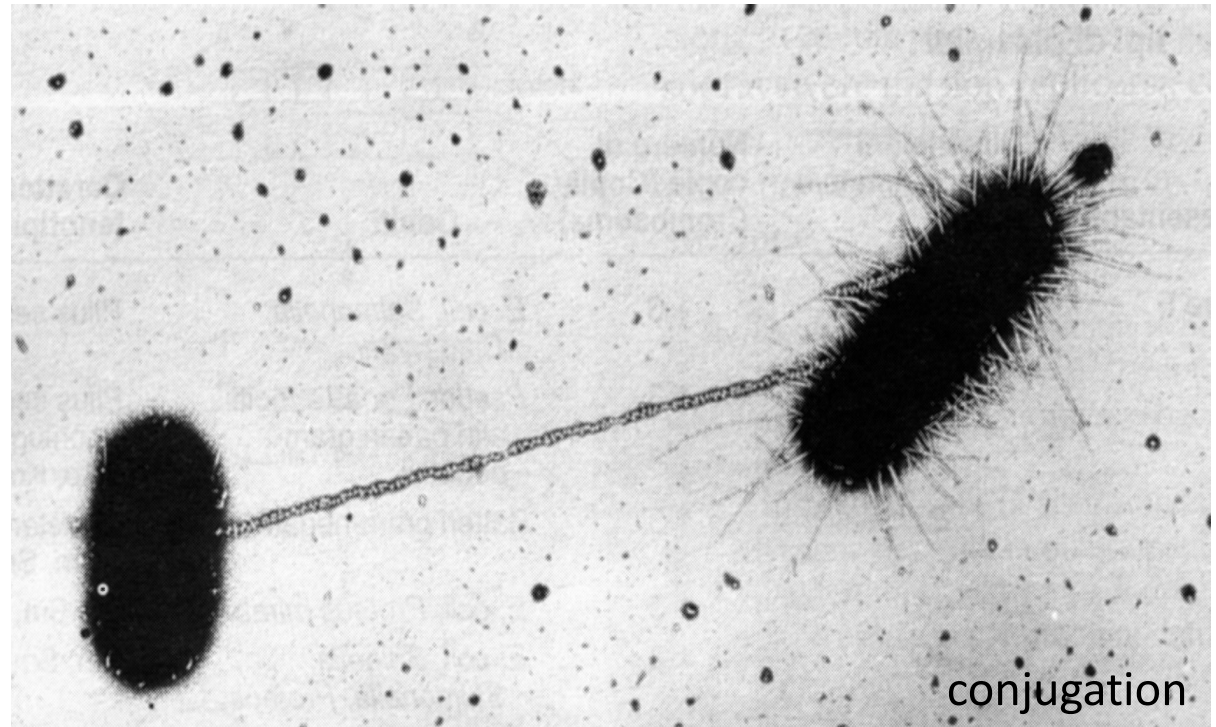
- Replication:
 - uses the replication system of the host cell
 - have its own initiation, elongation and termination
 - occurs during the entire cell cycle
 - All plasmids contain the “**ori**” **region** that encodes information for the replication of the plasmid
- Copy number:
 - a certain amount of copies present per cell
 - controlled by the initiation frequency
 - low (1-4) to high (10-100)
- Partitioning:
 - only a problem for low and medium copy number
 - genes that control the passage of plasmid to daughter cells
- Host specificity/range: - low to broad

Plasmids

Natural Plasmids - Grouped after their essential genes:

2) Non-essential –important for horizontal transfer

- Important genes
 - *pili*-genes
 - *oriT*
 - *tra/ mob* genes



Pili sessuali: presenti in numero di 1-10 per cellula, sono spessi 9-10 nm

Plasmids

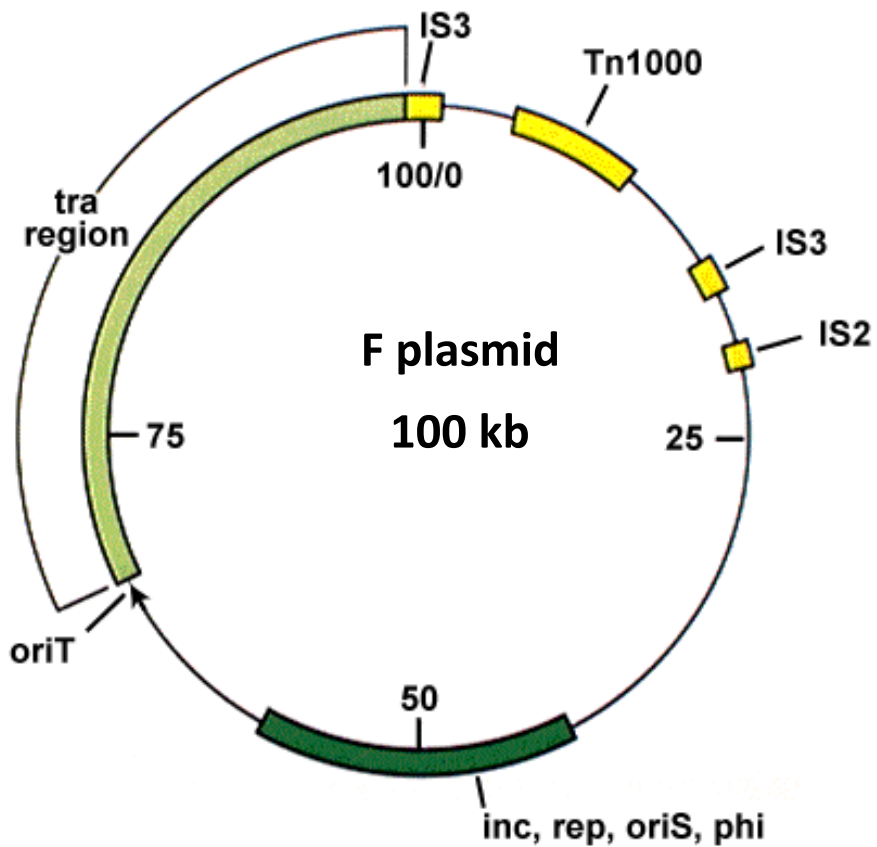
Natural Plasmids - Grouped after their essential genes:

3) Non-essential –with surviving value

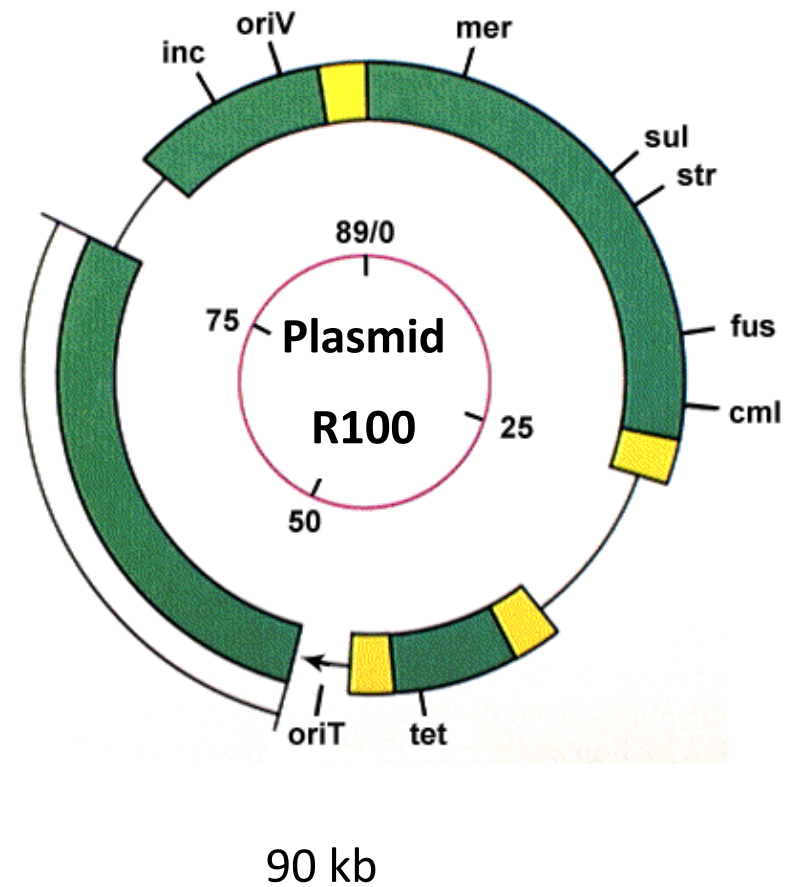
- Resistance against antibiotics
- Host defence against foreign DNA
- Production of antibacterial substances (colicins)
- genes for pathogenesis/virulence
- genes to be able to use special energy/carbon sources, e.g. phenol

Plasmid Maps

Natural plasmids



Note: F plasmid can also integrate into host genome = primitive transposon (IS2, IS3 sites)



Note: Plasmids that can integrate into genome are also called episomes

Esempi di fenotipi conferiti da plasmidi

- Produzione di antibiotico ▶ SCP1 ▶ *Streptomyces coelicolor*
- Antibiotico-resistenza ▶ RP4 ▶ *Pseudomonas aeruginosa*
- Resistenza al batteriofago ▶ pNP40 ▶ *Lactococcus lactis*
- Produzione di batteriocina ▶ p9B4-6 ▶ *Lactococcus lactis*
- Trasferimento coniugale ▶ F ▶ *Escherichia coli*
- Cristallo proteico insetticida ▶ pHD2 ▶ *Bacillus thuringiensis*
- Competenza ecologica nel suolo ▶ pRtrW14-2c ▶ *Rhizobium leguminosarum*
- Produzione di emolisina ▶ pJH1 ▶ *Enterococcus faecalis*
- Degradazione dell'erbicida ▶ 2,4-D pJP4 ▶ *Alcaligenes eutrophus*
- Fermentazione del lattosio ▶ pLM3601 ▶ *Lactococcus lactis* subsp. *cremoris*
- Resistenza ai metalli pesanti ▶ pMERPH ▶ *Pseudomonas* sp.
- Fissazione dell'azoto ▶ pIJ1007 ▶ *Rhizobium leguminosarum*
- Nodulazione ▶ pPN1 ▶ *Rhizobium trifoli*
- Degradazione di alcaloidi ▶ pRme41a ▶ *Rhizobium meliloti*
- Formazione di tumori ▶ Ti plasmid ▶ *Agrobacterium*
- Produzione di proteasi ▶ pLM3001 ▶ *Lactococcus lactis*
- Produzione di feromoni ▶ pAD1 ▶ *Enterococcus faecalis*
- Produzione di sideroforo ▶ pDEP10 ▶ *Escherichia coli*
- Tolleranza a NaCl ▶ pRtrW14-2b ▶ *Rhizobium leguminosarum*
- Degradazione del toluene ▶ Tol plasmids ▶ *Pseudomonas putida*

NIH Guidelines for use of bacteria and recombinant DNA

○ BASIC RULE

- Specified handling and construction processes
- Microorganisms containing recombinant DNA were prohibited outside of the laboratory
- Vectors that sexually move to “unsafe” bacteria was prohibited
- Tra region and mob region must be non-functional
- Nic/bom region must be non-functional (nic/bom containig plasmids can be mobilized by mob encoding palsmids)

| The roles of some tra-gene encoded proteins: ^[4] | |
|---|--|
| Pili Assembly and Production | <i>traA, traB, traE, traC, traF, traG, traH, traK, traL, traQ, traU, traV, traW,</i> |
| Inner Membrane Proteins | <i>traB, traE, traG, traL, traP</i> |
| Periplasmic Proteins | <i>traC, traF, traH traK, traU, traW</i> |
| DNA transfer | <i>traC, traD, traI, traM, traY</i> |
| Surface Exclusion Proteins | <i>traS, traT</i> |
| Mating Pair Stabilization | <i>traN, traG</i> |

Replication of Plasmids

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.

Replication of Plasmids

Natural plasmid

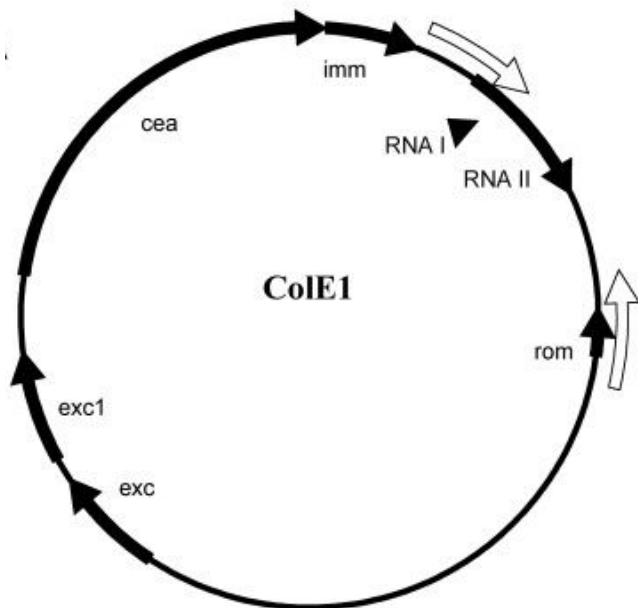


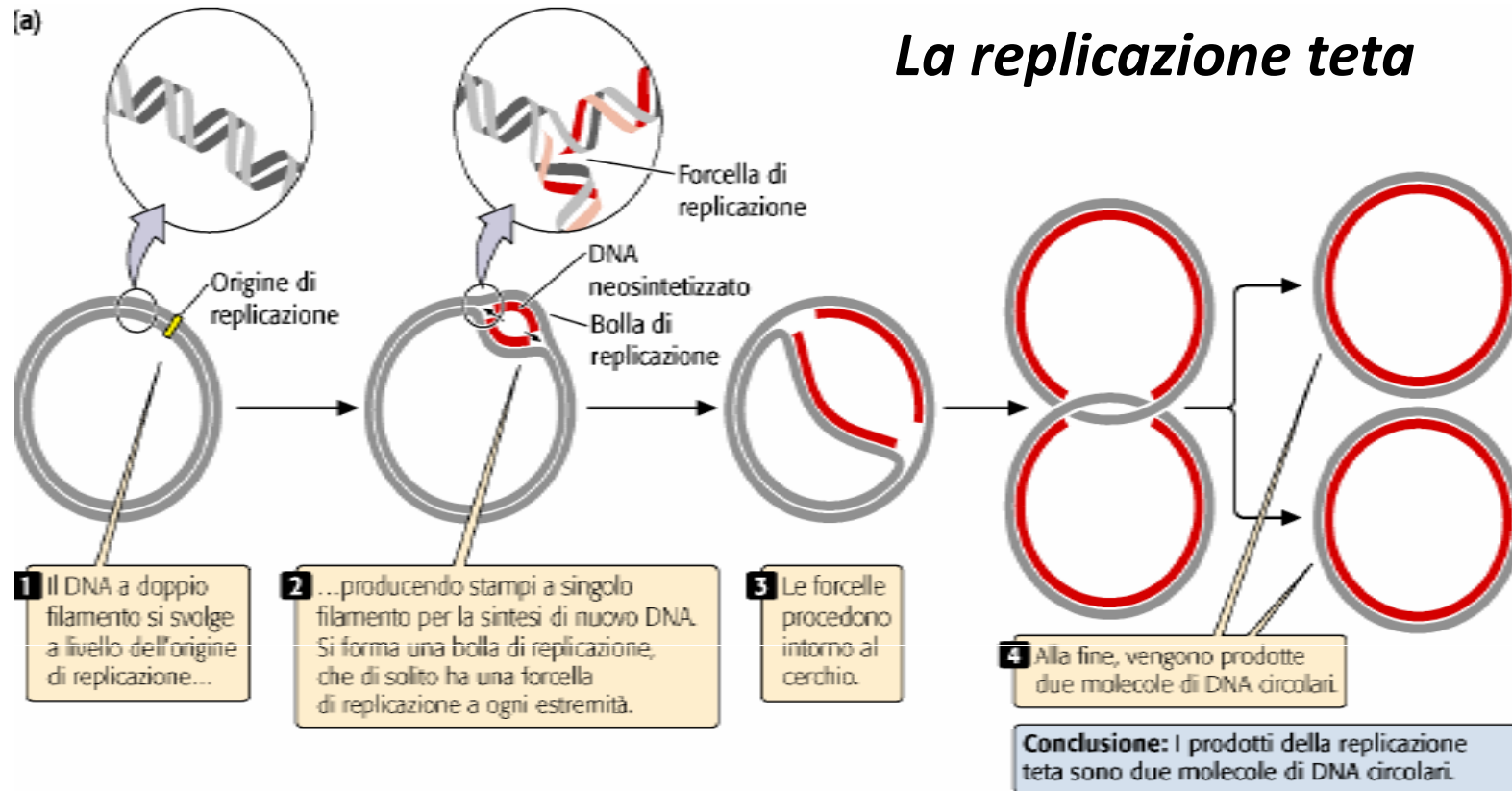
Table 11-1 Examples of some plasmids and their properties

| Plasmid | Size (Kb) | Number of copies per chromosome | Self-transmissible | Phenotypic features |
|-----------------------------|-----------|---------------------------------|--------------------|---|
| <i>Col plasmids</i> | | | | |
| 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 |
| <i>F plasmid</i> | 94.5 | 1–2 | Yes | F-pilus, conjugation |
| <i>R plasmids</i> | | | | |
| 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 |
| <i>Phage plasmid</i> | | | | |
| λdv | 6.4 | 50 | No | λ genes <i>cro</i> , <i>ci</i> , <i>O</i> , <i>P</i> |
| <i>Recombinant plasmids</i> | | | | |
| 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 |

Replication origins of plasmids control:

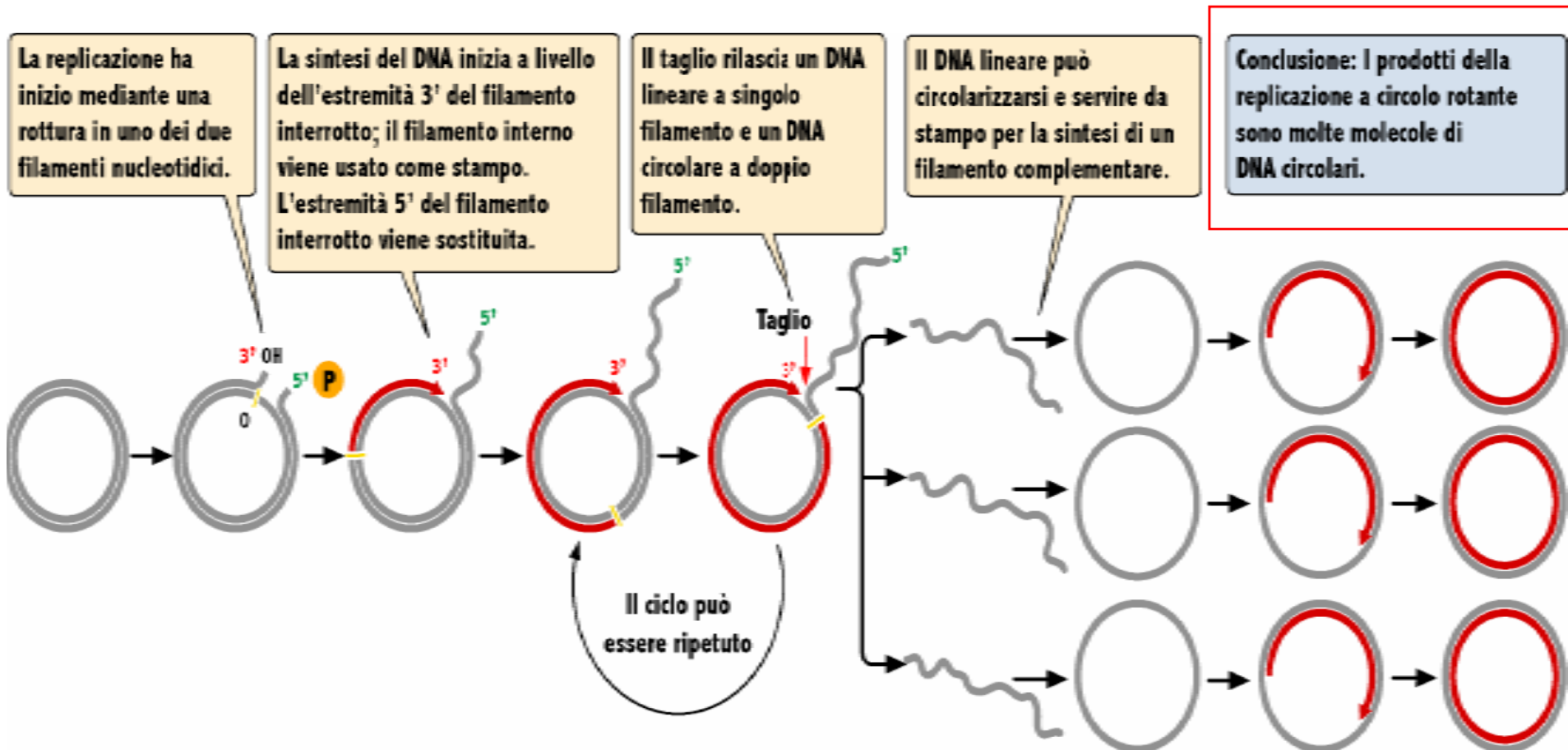
- ❖ **Il numero di copie** / Copy number (High/low copynumber plasmids)
- ❖ **Lo spettro d'ospite** / Host spectrum (Broad/Narrow host spectrum)
- ❖ **I gruppi di incompatibilità** / Incompatibility group (some plasmids cannot co-exist in bacteria)

Replication of Plasmids



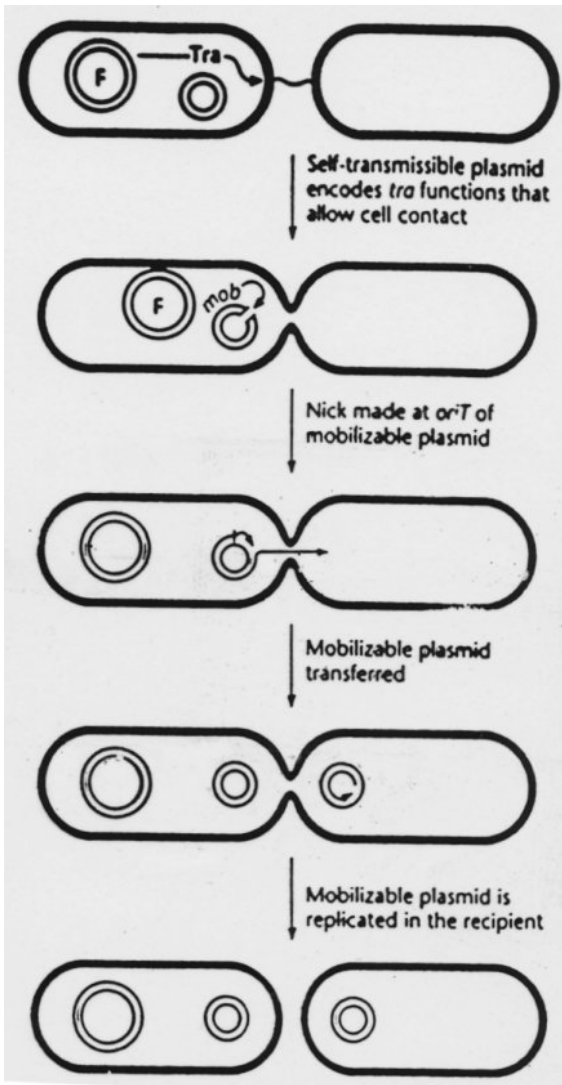
Replication of Plasmids

La replicazione a circolo rotante (rolling circle)



Replication of Plasmids

La replicazione a circolo rotante (rolling circle)



Rolling circle
DNA replication is
linked with
horizontal transfer
of plasmids
(mobility)

Replication of Plasmids

| Common Vectors | Copy Number ⁺ | ORI | Incompatibility Group | Control |
|----------------|--------------------------|-----------------------------|-----------------------|-----------|
| pUC | ~500-700 | pMB1 (derivative) | A | Relaxed |
| pBR322 | ~15-20 | pMB1 | A | Relaxed |
| pET | ~15-20 | pBR322 | A | Relaxed |
| pGEX | ~15-20 | pBR322 | A | Relaxed |
| pColE1 | ~15-20 | ColE1 | A | Relaxed |
| pR6K | ~15-20 | R6K* | C | Stringent |
| pACYC | ~10 | p15A | B | Relaxed |
| pSC101 | ~5 | pSC101 | C | Stringent |
| pBluescript | ~300-500 | ColE1 (derivative) and F1** | A | Relaxed |
| pGEM | ~300-500 | pUC and F1** | A | Relaxed |

Plasmids (vectors) commonly used in the laboratory contain oriV from native plasmids.

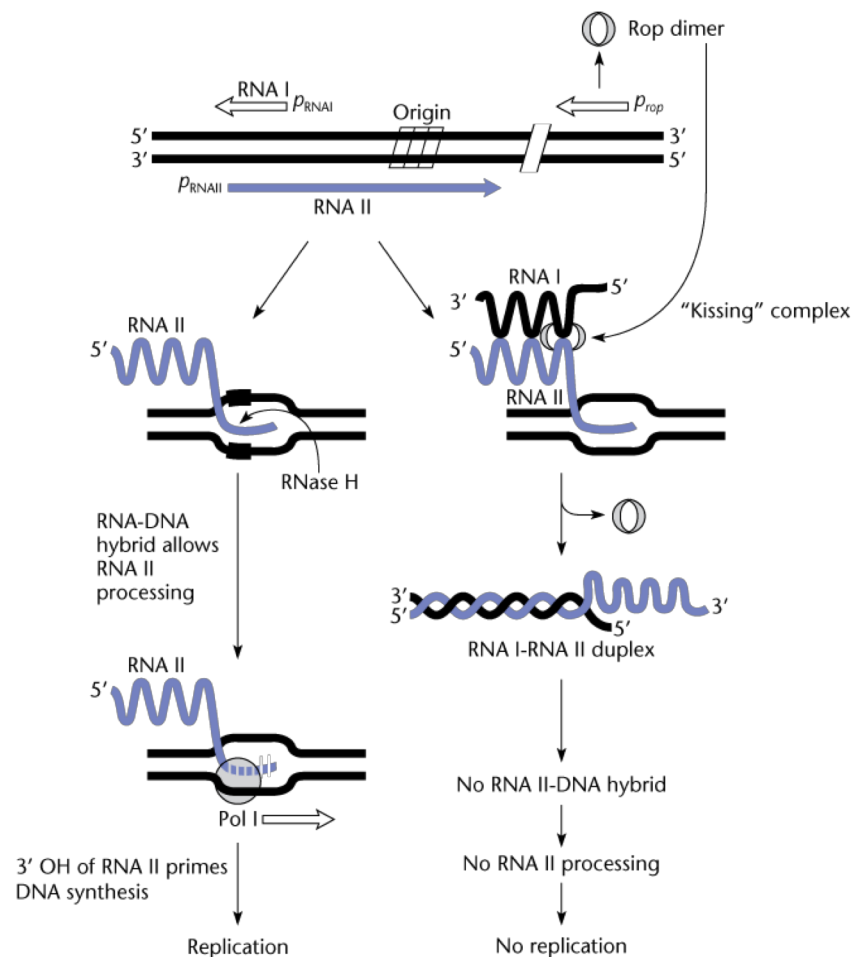
OriV sequences can be improved by mutation (pUC contains pMB1 oriV with some 1 or 2 mutations)

Plasmid oriV use cell proteins to replicate

Replication and copy number control of plasmids

Example: ColEI found in bacteria. Its name derives from the fact that it carries a gene for colicin E1 (the *cea* gene). It also codes for immunity from this product with the *imm* gene. In addition, the plasmid has a series of mobility (*mob*) genes.

ColEI oriV is used for many laboratory plasmids



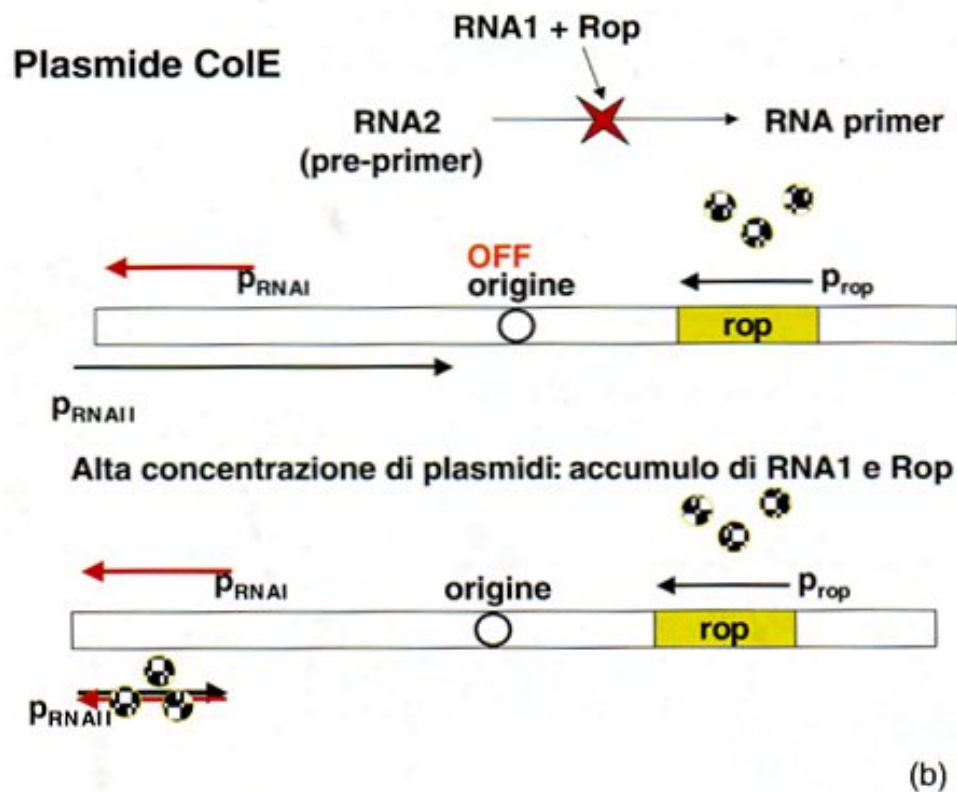
ColEI Replication Control-an example of primer control of replication

1. RNA II will serve as a primer for the replication fork.
2. The 3' end is processed by host RNase H to allow efficient RNA-DNA hybrid to form
3. The hybrid acts as a primer for host Pol I
4. As the concentration of plasmid increases, Rop also increases
5. Rop stabilizes the RNA I-II complex
6. No RNA for replication priming.
- 7. Copy number controlled**

Replication and copy number control of plasmids

How to increase copy number of laboratory plasmids??

INTRODUCTION OF MUTATIONS IN Rop



La funzione di Rop

Rop controlla negativamente la replicazione plasmidica:
→ aumenta il tasso di legame di RNAI ad RNAII.

Rop assente → Replicazione plasmidica più frequente

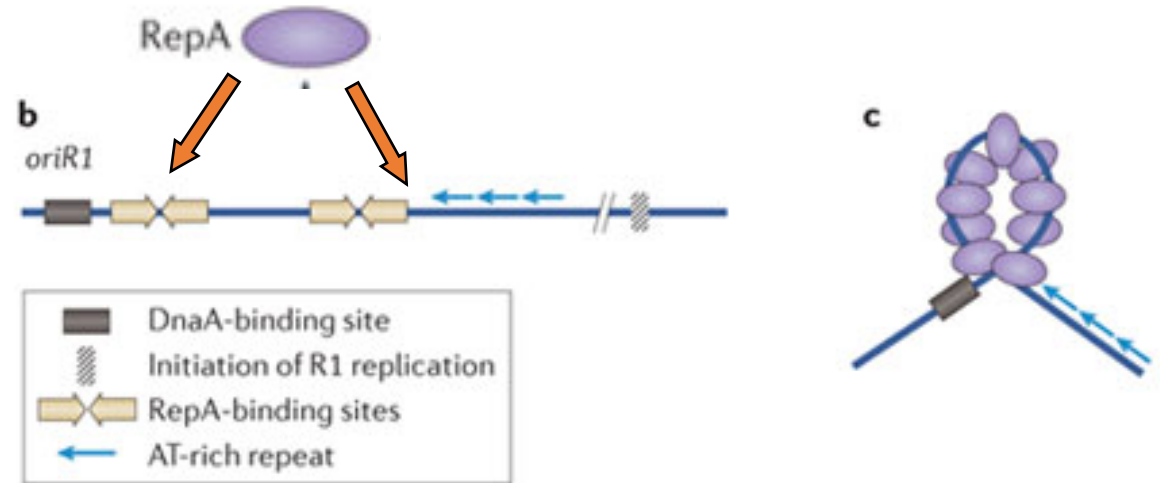
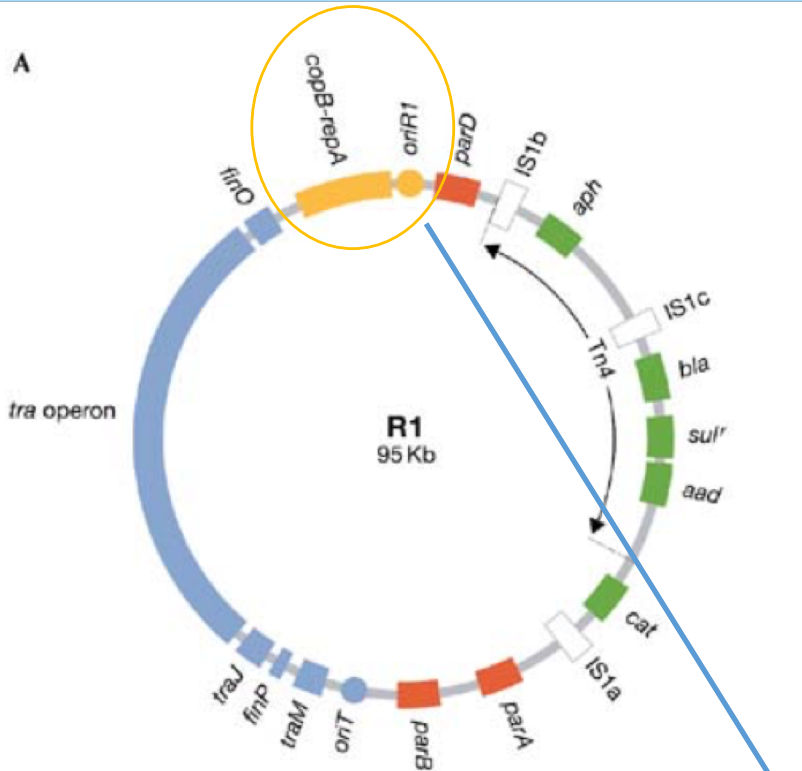
pBR322 rop^+ 15 copie/cellula

derivativo di pBR322
costruito per delezione

pUC18 Δrop 50-100 copie/cellula

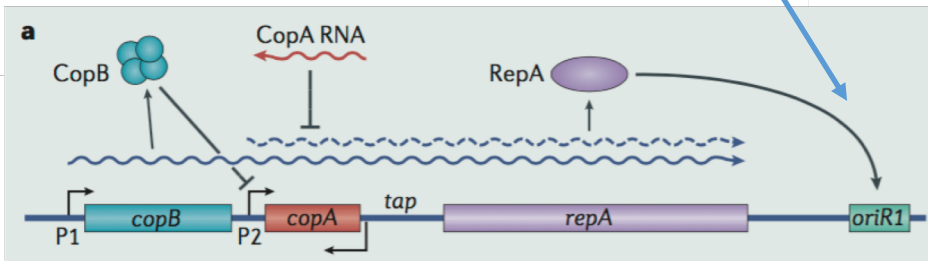
Note: pMB1 (a plasmid in the ColE1 compatibility group)

Replication and copy number control of plasmids



R1 plasmid: replication controlled by the plasmid encoded RepA protein

Plasmid R1 provides a well-studied model for replication systems of enteric plasmids. In this plasmid, the replication initiator RepA binds to the origin site, oriR1, which lies downstream of repA (see the figure, part a). This oriR1 site contains binding sites for RepA flanked by a DnaA box at one end and three AT-rich repeats at the other (see the figure, part b). DnaA is not essential for replication of this plasmid, but seems to have an accessory role. DNA loop formation, mediated by RepA (see the figure, part c), is thought to drive DNA melting at the AT-rich region, which allows DnaC to load the replicative DNA helicase, DnaB. Replication initiates 400 nucleotides downstream of this site.

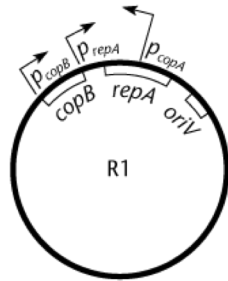


(A) A map of R1 showing antibiotic resistance genes (green), insertion sequences (white), its basic replicon (yellow), conjugation genes (blue) and stability systems (red).

Replication and copy number control of plasmids

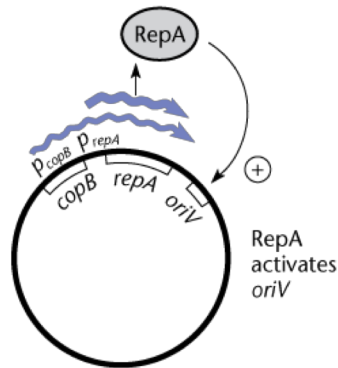
A Plasmid genetic organization

Example: R1 plasmid

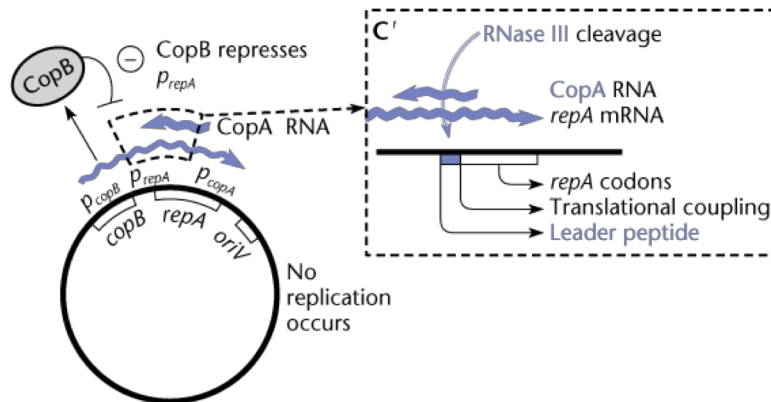


| Promoter | Gene products expressed |
|------------|----------------------------------|
| P_{copB} | RepA and CopB |
| P_{repA} | RepA |
| P_{copA} | 90-nucleotide CopA antisense RNA |

B Replication occurs after plasmid enters cells



C Replication shutdown



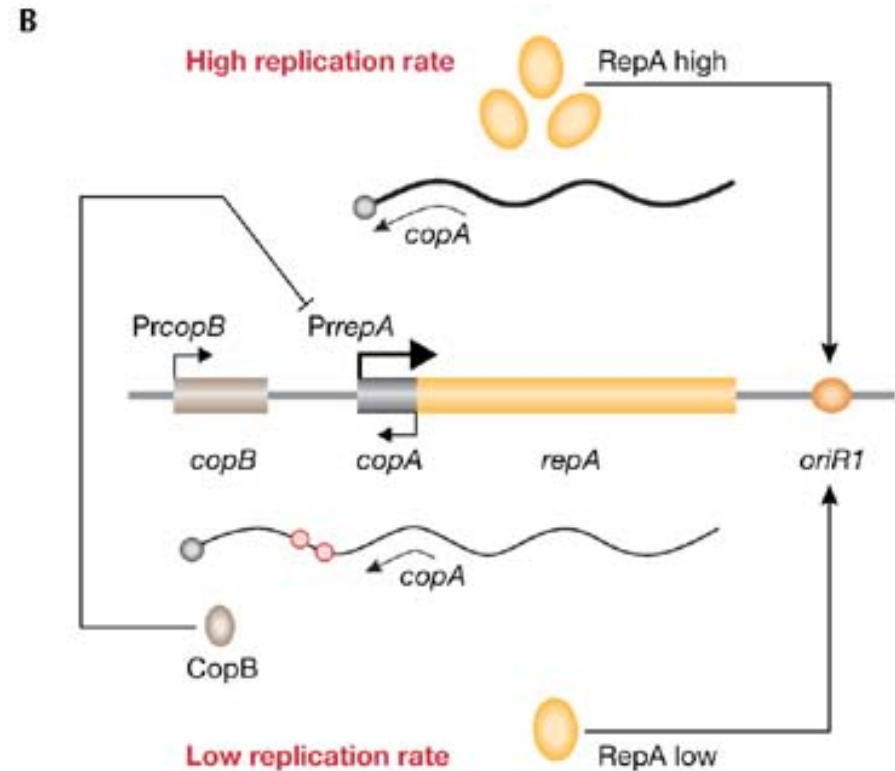
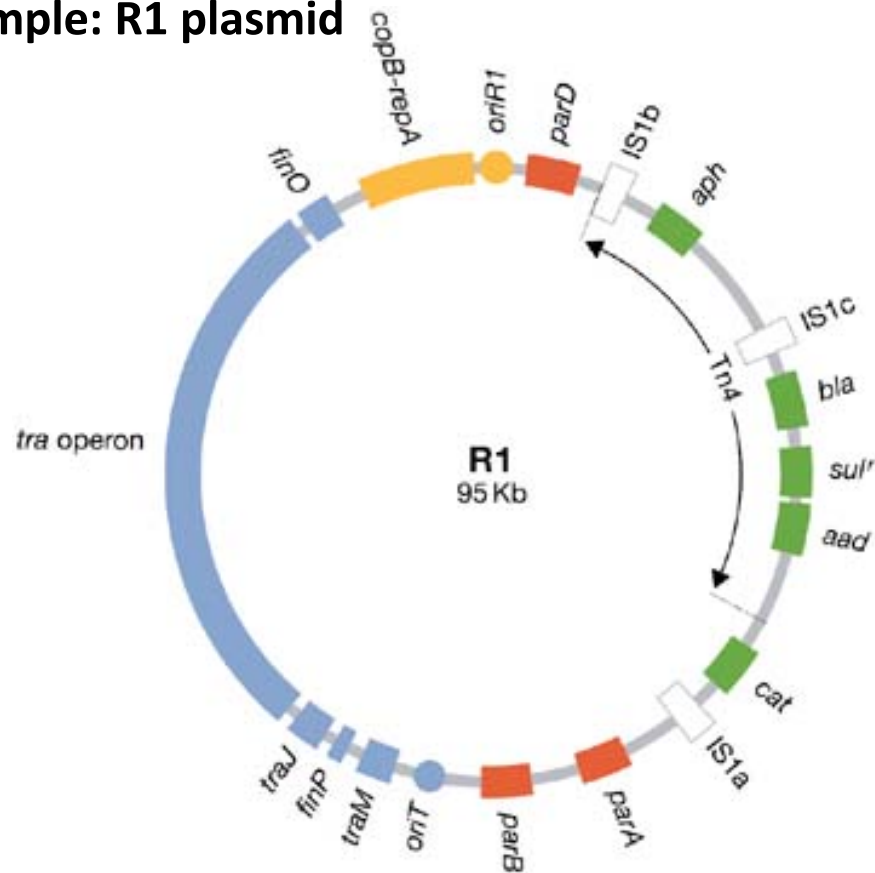
The events upon entry into a cell

1. RepA mRNA is made from P_{repA} + P_{copB} until copy number becomes high
2. CopB expression increase and CopB represses RepA expression at P_{repA}
3. CopA now is made-a 90base antisense RNA
4. short RNA CopA binds to 5-end of the RepA mRNA, forming dsRNA
5. This is recognized by host RNaseIII and degraded.

Thus concentration of RepA protein is maintained by rate of RNA-RNA hybrid formation.

Replication and copy number control of plasmids

Example: R1 plasmid



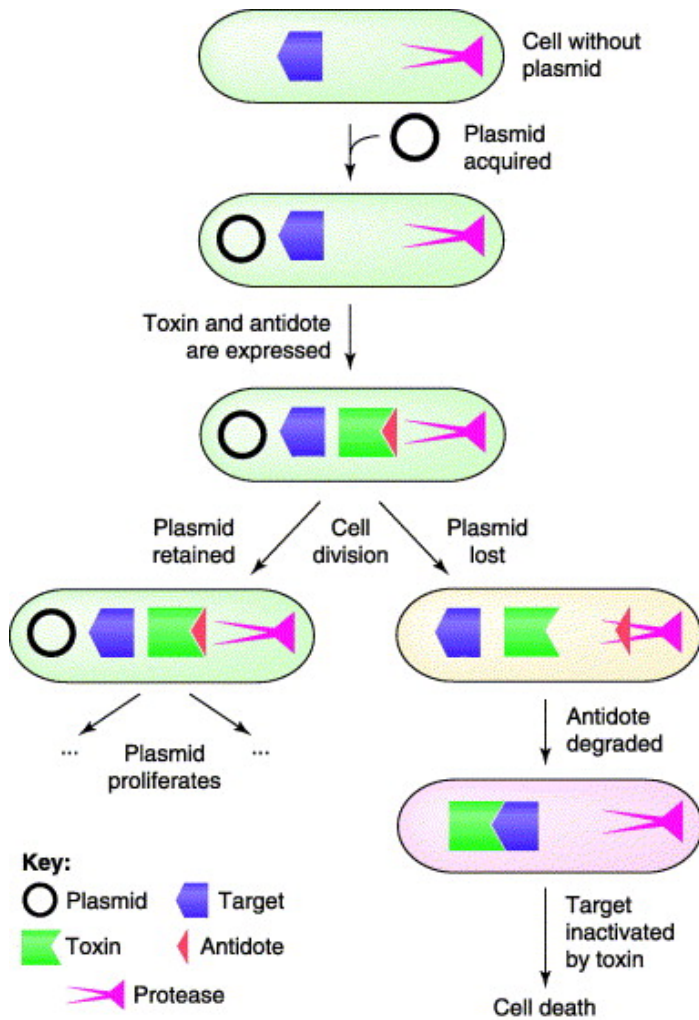
Guillermo de la Cueva-Méndez, and Belén Pimentel EMBO Rep. 2007;8:458-464

R1 and copy-number control. (A) A map of R1 showing antibiotic resistance genes (green), insertion sequences (white), its basic replicon (yellow), conjugation genes (blue) and stability systems (red). (B) *PrcopB* produces some RepA as well as CopB, a repressor of *PrrepA*, which keeps R1 copy number low. In the absence of CopB, stronger *PrrepA* increases RepA and R1 copy number. Antisense RNA *copA* limits translation of RepA and is less effective when *PrrepA* is active. Red circles on RNA denote **UUACU sites**. Cop, copy-number control gene; ori, origin of replication; Pr, promoter; Rep, replication initiation factor.
UUACU sites: can be cleaved by RNase (additional mechanisms of regulation; not relevant for our lecture)

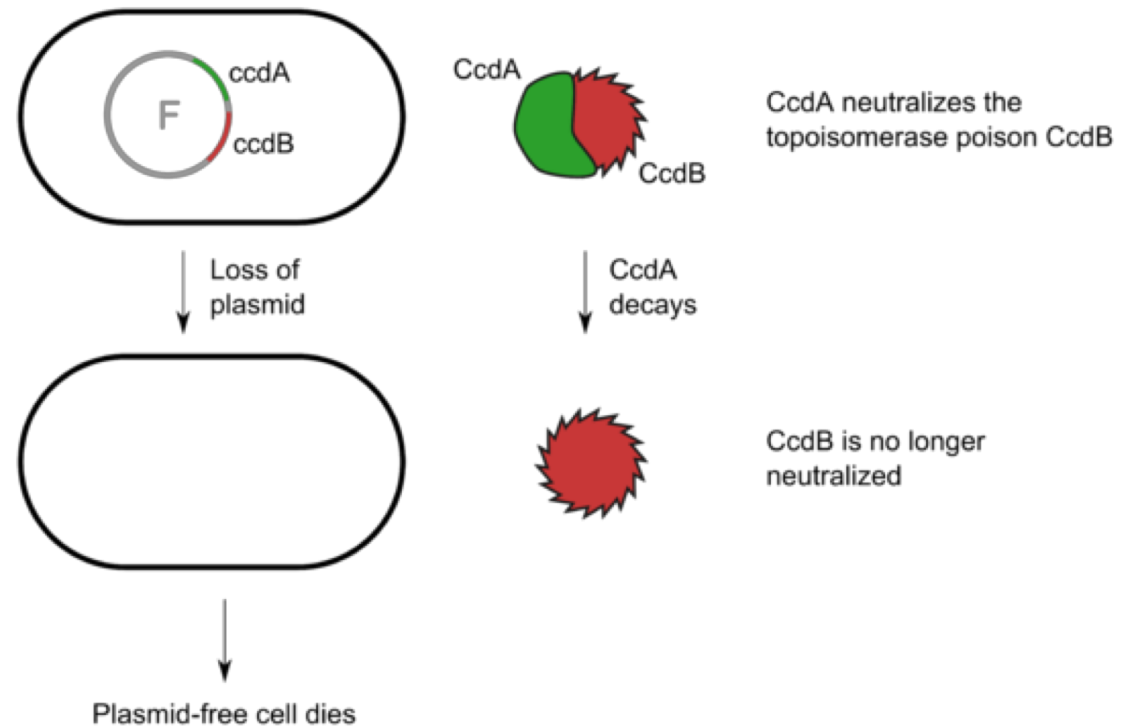
Maintenance of plasmids in bacteria

A. Plasmid partition systems

B. Toxin – Antitoxin systems



F1 plasmid



Il plasmide F sintetizza un sistema basato su tossina-antitossina in grado di eliminare le cellule che, in seguito ad un errore nella divisione cellulare non hanno ricevuto almeno una copia del plasmide F. La proteina **CcdB è una tossina stabile (con bersaglio la DNA girasi)** la cui funzione viene bloccata dal legame con un **antitossina CcdA più facilmente degradabile**. Se il plasmide è presente la continua sintesi di CcdA inibisce CcdB. Se non vi è plasmide invece CcdA verrà degradata + velocemente di CcdB che rimarrà quindi libera e potrà inibire la girasi provocando la morte delle cellule

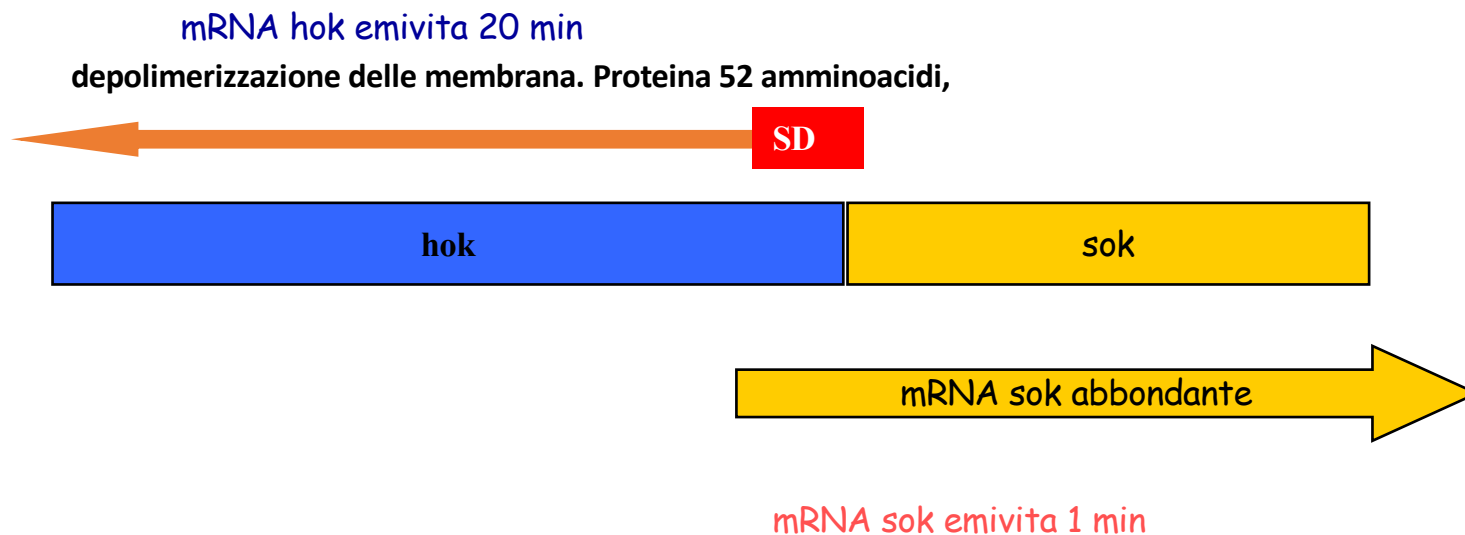
Maintenance of plasmids in bacteria

B. Toxin – Antitoxin systems

hok -sok system

Il plasmide R1(o R100) porta un gene letale *hok* (host cell killing) che codifica per una tossina in grado di provocare depolimerizzazione delle membrana.

Sull'elica complementare del DNA di *hok* viene trascritta il mRNA del gene *sok* che ha una regione di 128 nt complementare con la regione SD di *hok*. I 2 RNA hanno diversa emivita 20 min e 1 min. Hok non viene mai tradotto per azione del mRNA di sok e la cellula con R1 rimane pertanto vitale. **Se una cellula non eredita R1 in seguito a divisione allora mRNA_{sok} che ha una lunga emivita verrà tradotto perchè mRNA sok avendo un emivita più breve non sarà più presente.**



Maintenance of plasmids in bacteria

A. Plasmid partition systems – for low copy plasmids

B. Toxin – Antitoxin systems

Plasmid copies are paired around a centromere-like site and then separated in the two daughter cells. Partition systems involve three elements, organized in an auto-regulated operon:

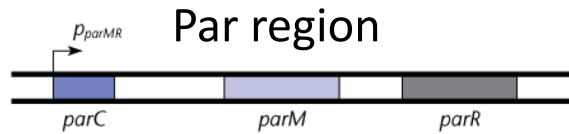
1. A centromere-like DNA site
2. Centromere binding proteins (CBP)
3. The motor protein

The **centromere-like DNA site is required in cis for plasmid stability**. It often contains one or more inverted repeats which are recognized by multiple CBPs. This forms a nucleoprotein complex termed the **partition complex**. This complex recruits the **motor protein**, which is a nucleotide triphosphatase (NTPase). The **NTPase** uses energy from NTP binding and hydrolysis to directly or indirectly **move and attach plasmids to specific host location** (e.g. opposite bacterial cell poles).

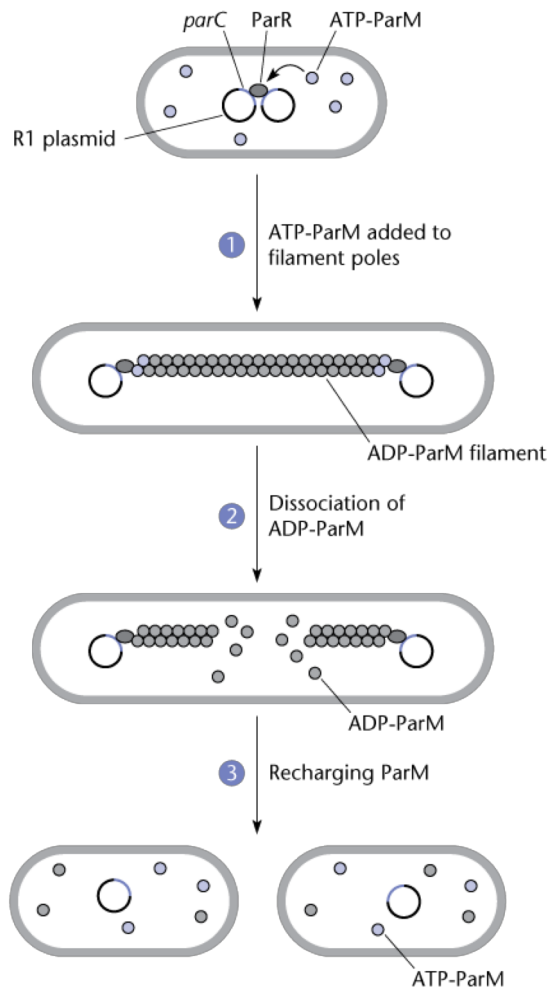
Maintenance of plasmids in bacteria

A. Plasmid partition systems – for low copy plasmids

A *parCMR* locus



B Plasmid R1 partitioning



Stabilità segregativa (funzione *par*)

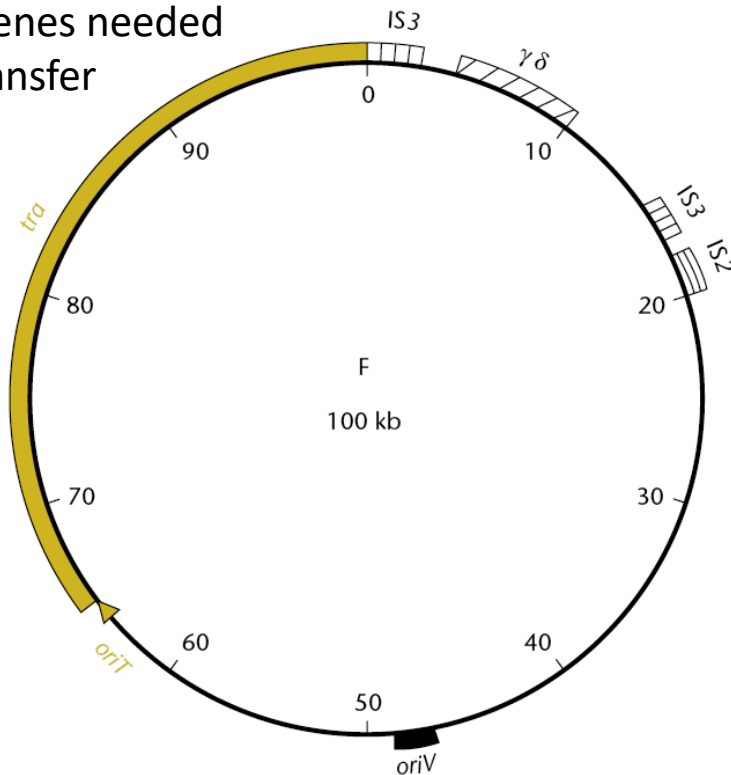
ParM binds to DNA-binding proteins, called ParR that bind centromer like DNA sequences on plasmid (*parC*)

Sister plasmid segregation is achieved through bidirectional insertional polymerization of the ParM filaments.

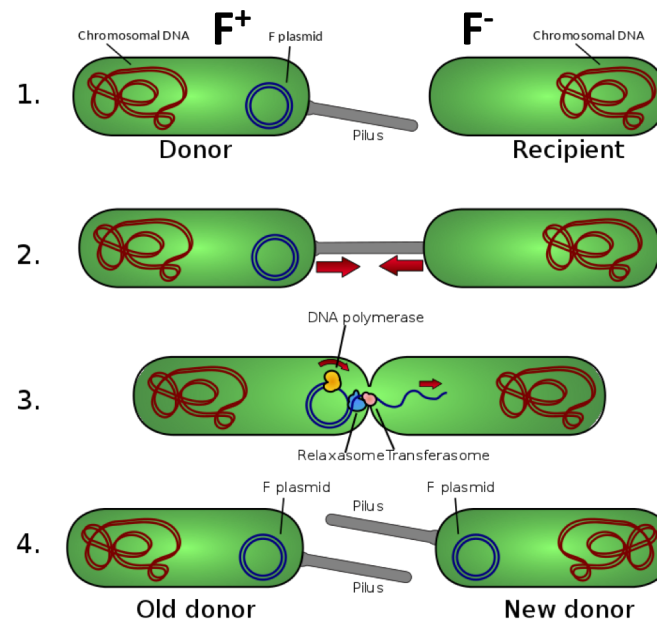
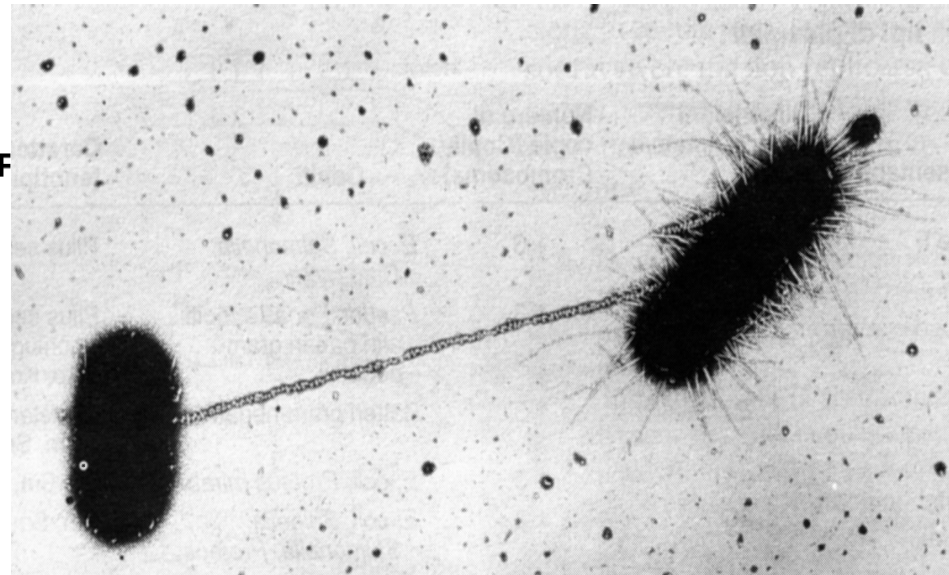
Horizontal transfer of genetic information

F plasmid

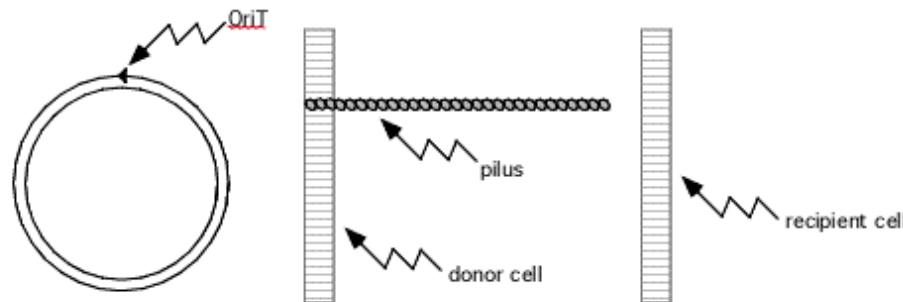
tra-region:
30+ genes needed
for transfer



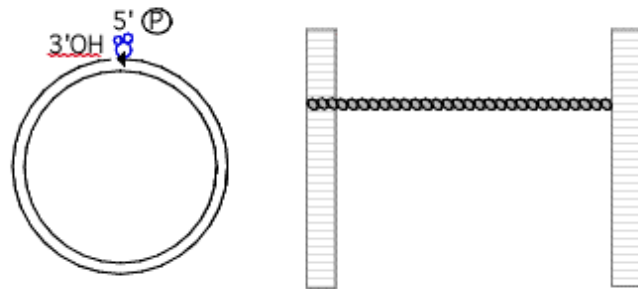
tra and trb locus encode proteins required for **conjugation** such as the pilin gene and regulatory genes, which together form pili on the cell surface



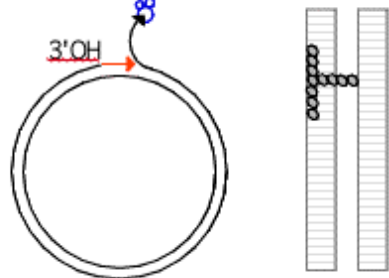
Horizontal transfer of genetic information



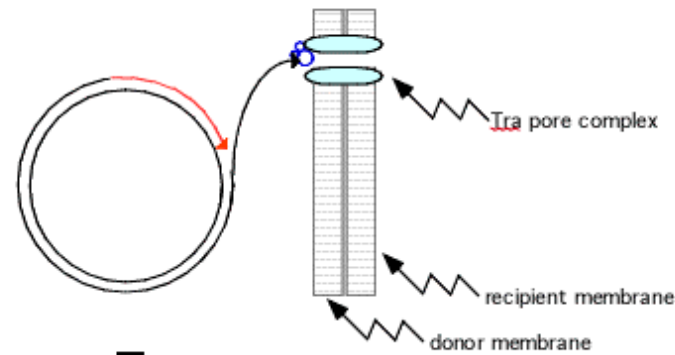
Contact between donor and recipient cells.
DNA relaxase (⊗) nicks at oriT and covalently binds to 5' (P)



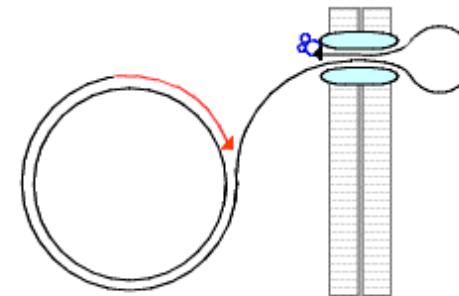
Pilus retracts, bringing donor and recipient into close proximity and Tra proteins form a pore complex that spans the membranes



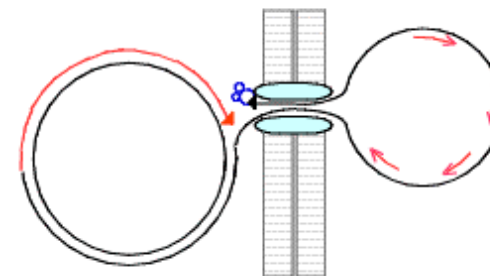
Rolling circle DNA replication initiates at 3'OH and proceeds 5' to
Membranes brought into close proximity to form mating bridge.
Relaxase interacts with membrane Tra pore complex



DNA replication pushes the ssDNA into the recipient cell



Lagging strand DNA replication in recipient cell converts ssDNA to dsDNA

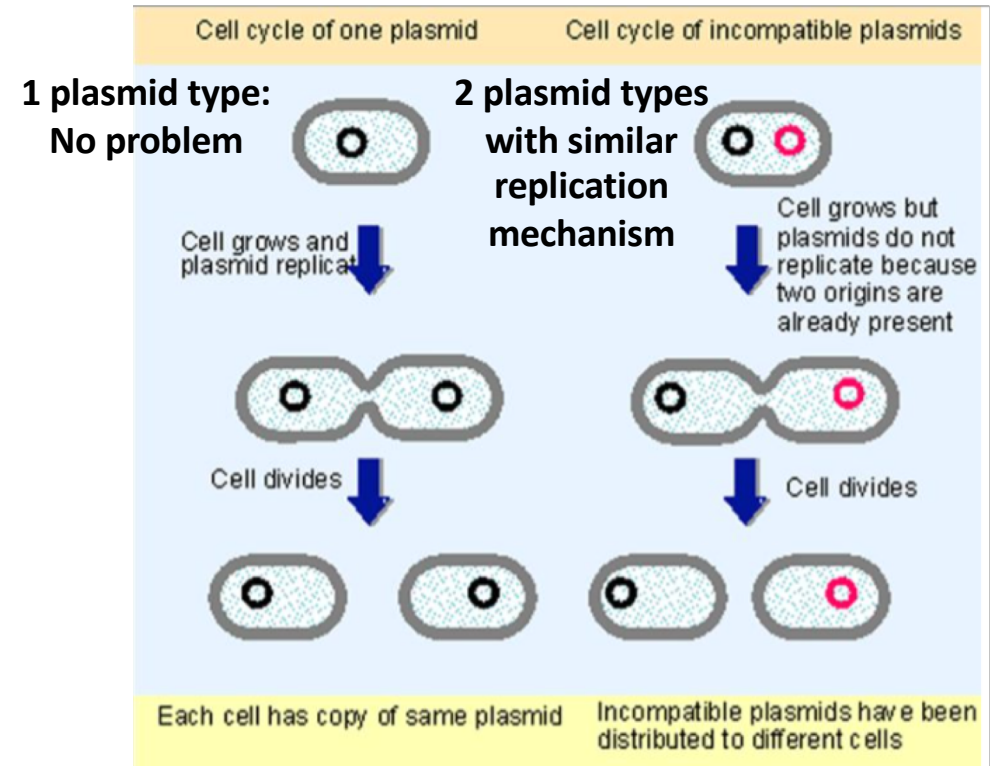


Upon complete replication of plasmid, the old and new oriT sites
"collide", and nicking between oriT sites occurs

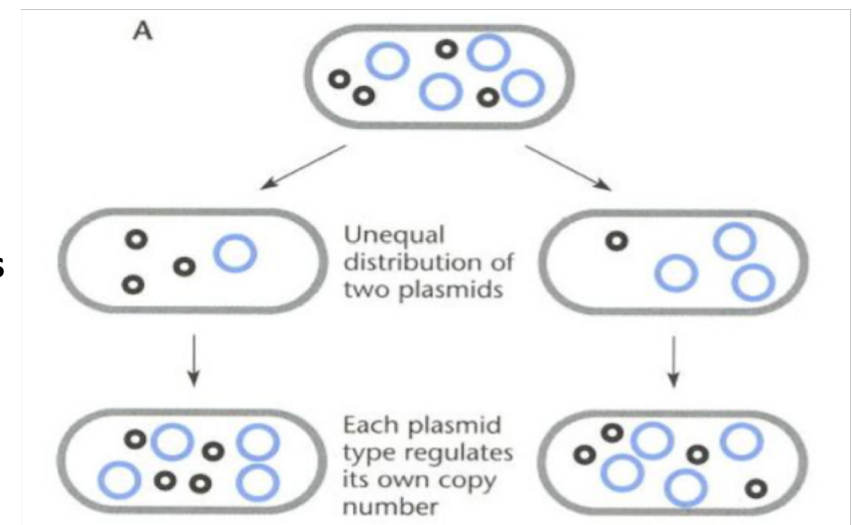
Plasmid incompatibility

Incompatibilità tra plasmidi

1. Not all plasmids can live together. → plasmids are subdivided in **incompatibility groups**
2. Plasmids that are able to coexist in the same cell do not interfere with each other's replication
3. Plasmids that have different par region can coexist
4. A single cell can have as many Inc group plasmids as it can tolerate and replicate!



2 plasmid types
with different
replication
mechanism



Plasmid incompatibility

La tabella riporta i gruppi d'incompatibilità tra plasmidi (designati con la sigla Inc), i plasmidi appartenenti allo stesso gruppo e il loro ospite batterico. Al gruppo IncFI, ad esempio, appartengono i plasmidi F, ColV e R453. Questi plasmidi non possono coesistere nello stesso batterio, ma ognuno di questi può coesistere con plasmidi del gruppo IncFII oppure IncA-c oppure IncP.

| GRUPPO INCOMPATIBILITÀ | PLASMIDI | OSPITI |
|------------------------|----------|---|
| IncFI | F | <i>Escherichia coli</i> , <i>Proteus morganii</i> , <i>Salmonella typhimurium</i> , <i>Rhizobium lupini</i> |
| | ColV-K94 | <i>Escherichia coli</i> |
| | R453 | <i>Enterobacter cloacae</i> , <i>Proteus mirabilis</i> |
| IncFII | R100 | <i>Shigella flexneri</i> |
| IncA-c | R480 | <i>Providencia</i> |
| IncP | RP1 | Batteri Gram negativi |

Maintenance of high copy plasmids

Typically used in laboraotry (in E. coli)

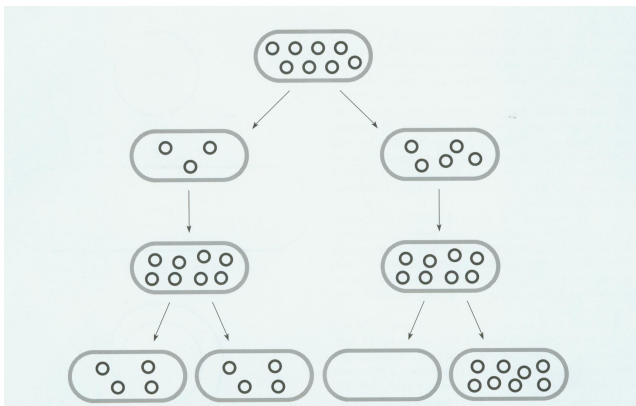
I plasmidi ad alto numero di copie si ripartiscono secondo due modalità:

1. **STOCAISTICA** o casuale
2. **ATTIVA**

1. RIPARTAZIONE ATTIVA: Nel caso della ripartizione attiva i plasmidi vengono riconosciuti da una proteina che dimerizzando forma delle coppie di plasmidi.

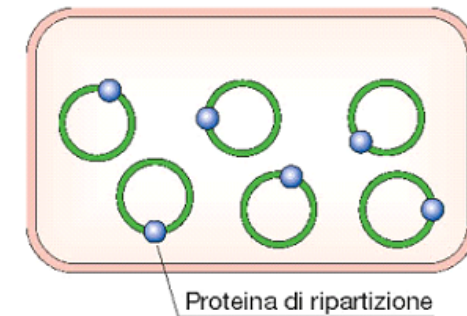
La struttura DNA –proteina-DNA si localizzerà a livello del sito di divisione garantendo così la corretta divisione tra le cellule

2. RIPARTAZIONE STOCAISTICA

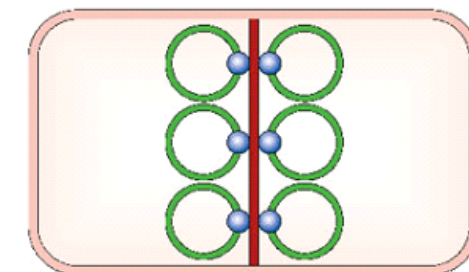
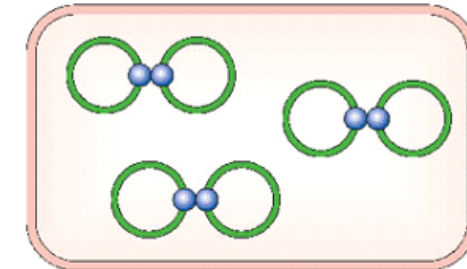


Plasmids contain
Antibiotics resistance genes!!

b) Ripartizione attiva

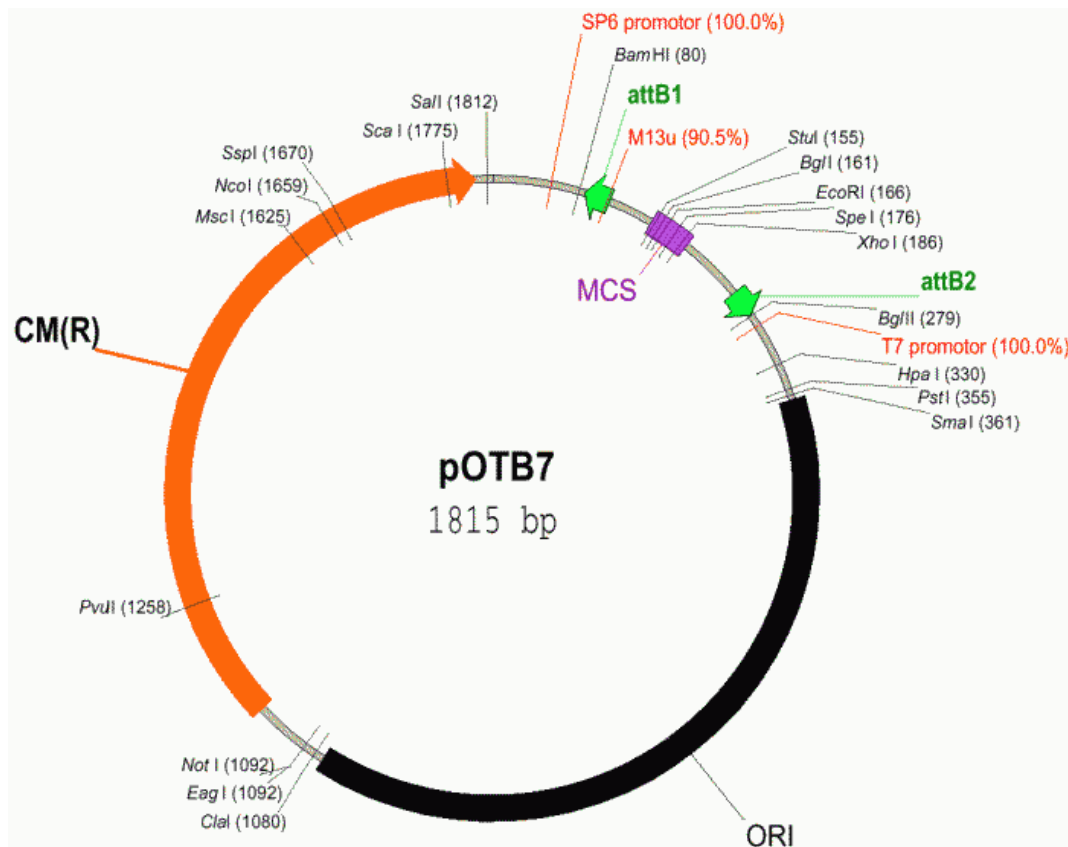


Modello di pre-accoppiamento



LABORATORY PLASMIDS = VECTORS

- ⊕ Origin of replication
- ⊕ Antibiotic resistance gene (Amp, Kan, Tet, Chl)
- ⊕ (Multiple cloning site)



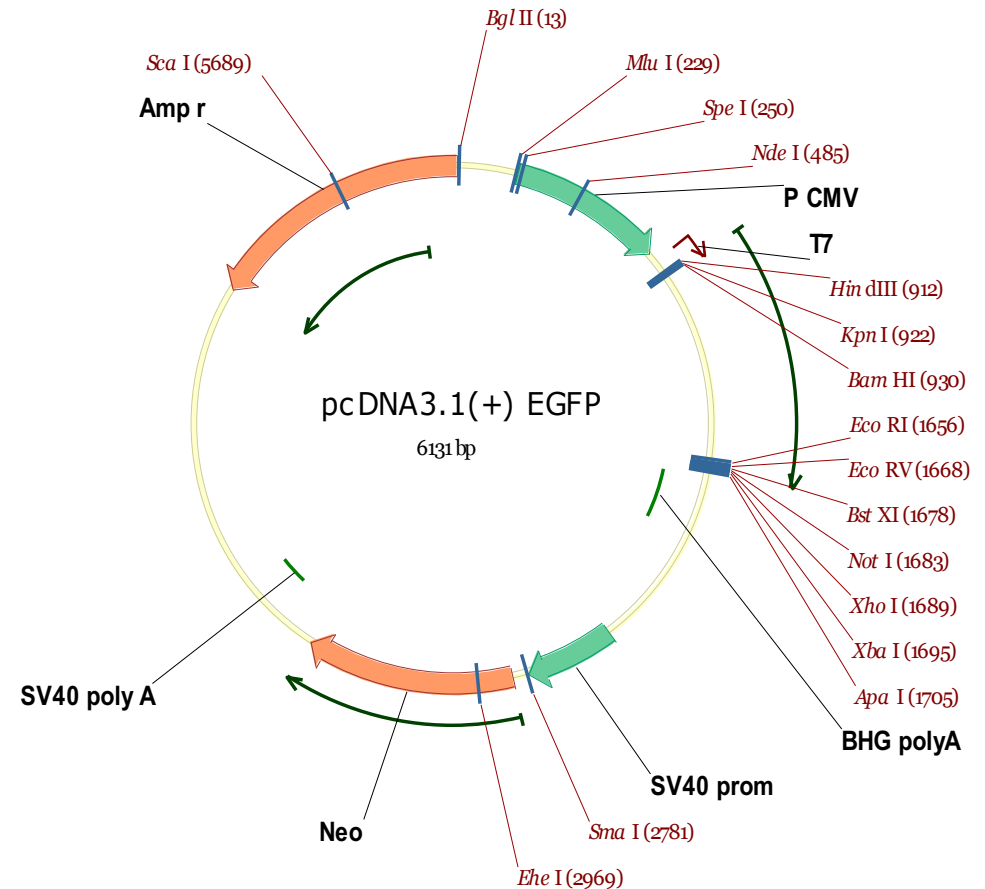
Map of pOTB7 vector showing Chloramphenicol resistance gene (CMR), replication origin (ORI) and multiple cloning site (MCS)

TO MAINTAIN PLASMID IN BACTERIA, CELLS ARE GROWN ON AGAR CONTAINING CHLORAMPHENICOL ONLY BACTERIA THAT CARRY PLASMID CAN SURVIVE

LABORATORY PLASMIDS = VECTORS

Optional plasmids elements

- ⊕ Multiple cloning site
- ⊕ Promoter for cloned sequence
- ⊕ Reporter gene
- ⊕ Tag
- ⊕ Regulatory sequences
- ⊕ Cassette for blue-white colony selection (*lacZ*)

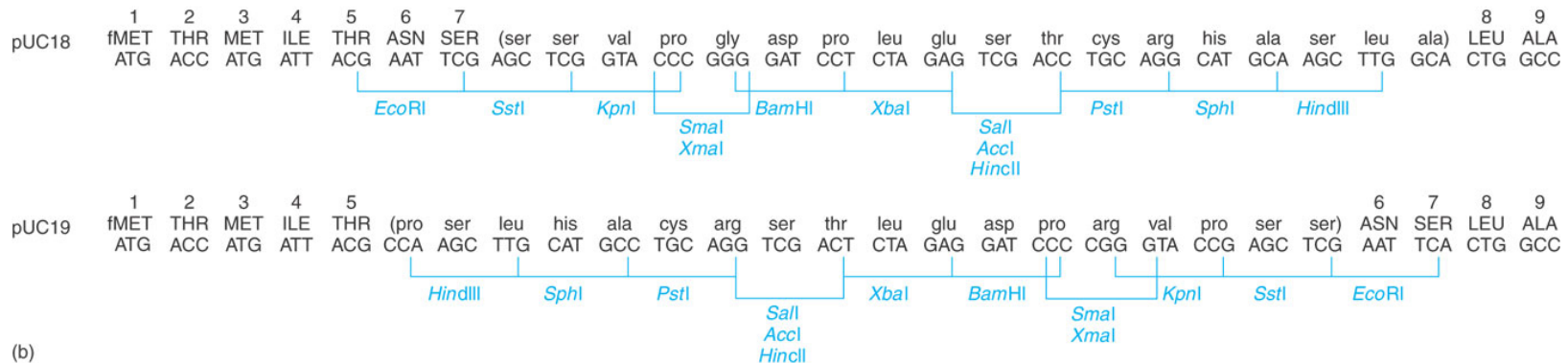
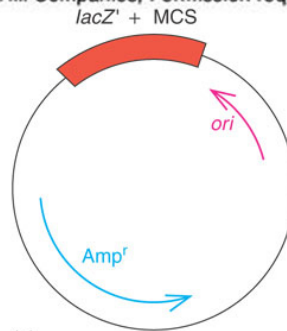


MULTIPLE CLONING SITE ADVANTAGE

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

Figure 4.6

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CLONING AND BLUE - WHITE SELECTION

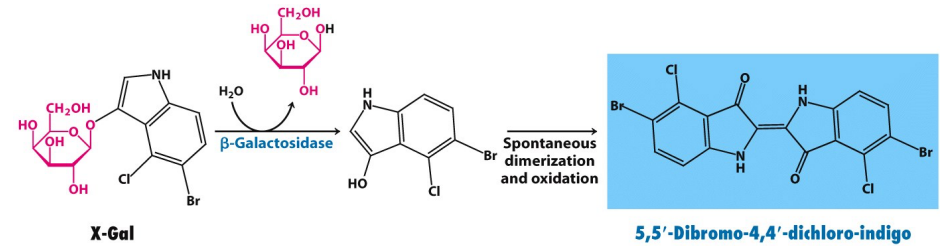
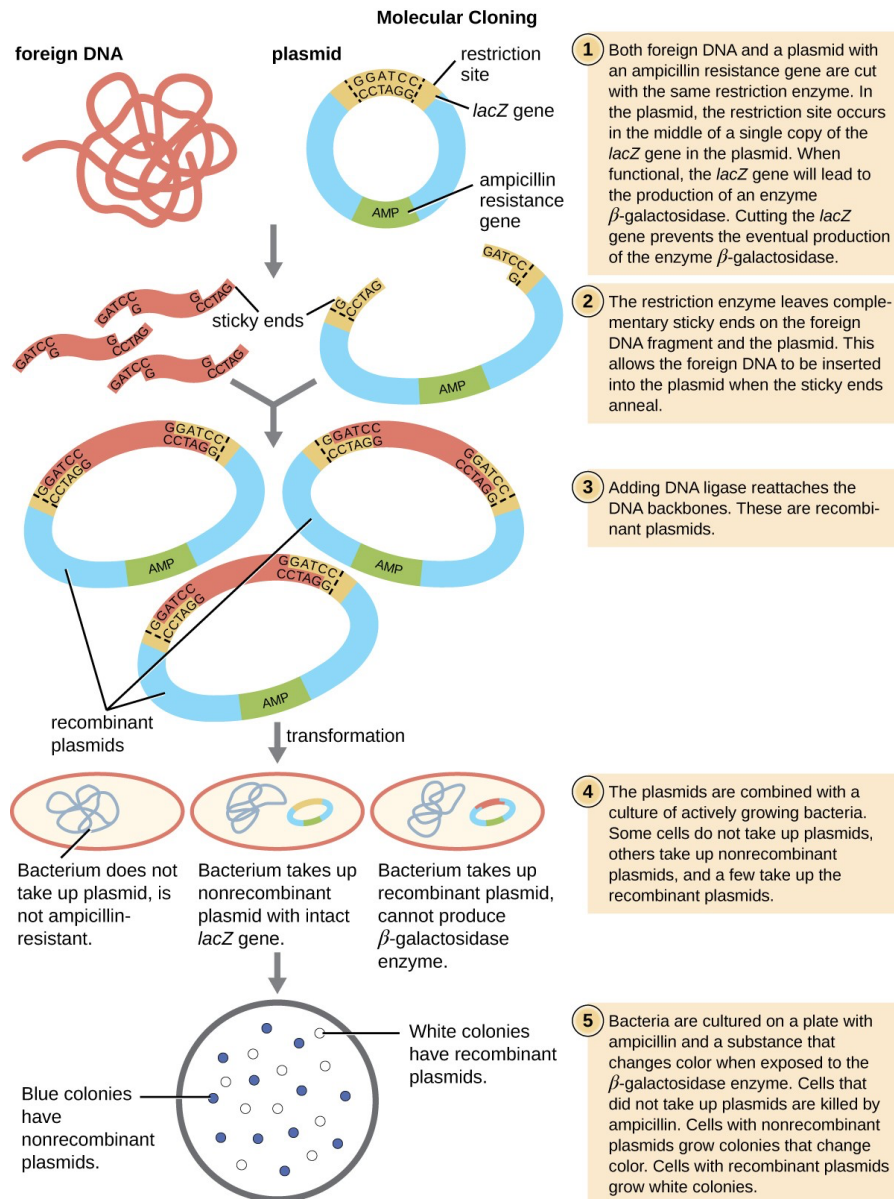
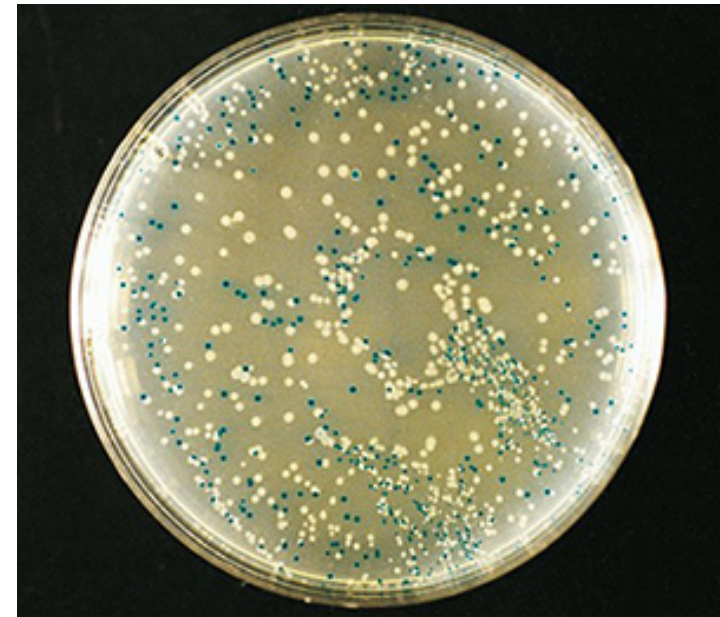


Figure 31.5
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Agar containing Ampicillin and X-GAL
 Blue: no insert
 White: insert

A DEFINED VECTOR FOR EACH APPLICATION

- ⊕ Cloning and sequencing of DNA and cDNA fragments
- ⊕ Generation of genomic and cDNA libraries
- ⊕ Expression of recombinant proteins
- ⊕ Generation of mutant proteins
- ⊕ Analysis of regulatory sequences
- ⊕ Gene targeting