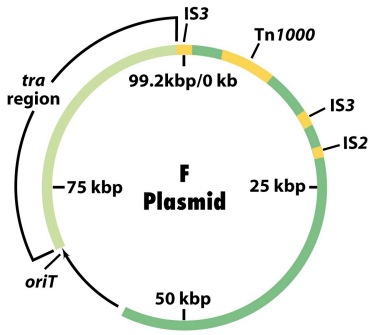
Gene movement, part III and restriction-modification

F (fertility) plasmid

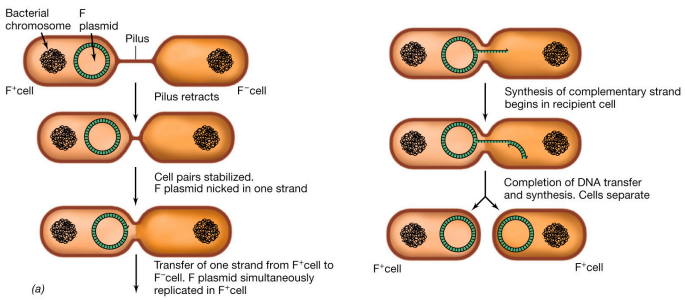
- Conjugative ability due to transfer (*tra* and F pilus genes) and adjacent *oriT*
- Rep/Par/Inc
- Plasmid with several IS/Tn elements: 1 IS3 disrupts *tra* repression system

Fig 11.19



Plasmid transfer via F conjugation

Fig. 11.21a



Representative of conjugation in many Gram neg. bacteria

DNA transfer in F conjugation

- *oriT* is nicked, 5'→3' ssDNA transfer
- DNA pol III, etc replicates (leading in donor)
- Religation of *oriT* at end by nickase

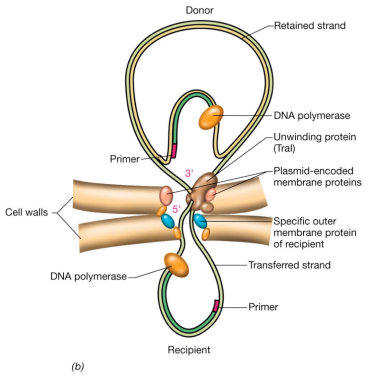
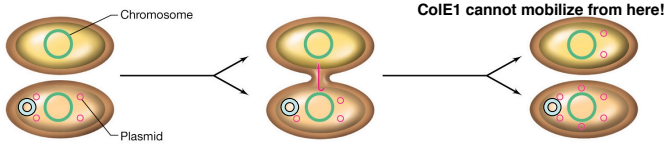


Fig. 11.21b

Mobilizable plasmids

Conjugal plasmids encode proteins for an apparatus that allows DNA movement. Mobilizable plasmids (like ColEI) can exploit the apparatus encoded by a co-resident conjugal plasmid. Usually a moblizable plasmid-specific DNA-processing function (still using ssDNA transfer process). ColE1 cannot move by itself!



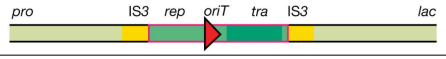
= conjugal plasmid

F plasmid can integrate into *E. coli* chromosome

Fig. 11.22

IS/Tns can allow plasmids to recombine with host chromosomes

Process forms Hfr…



F plasmid and *E. coli* Hfrs

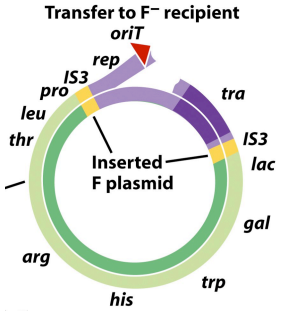


Fig. 11.23

When plasmids recombine with chromosome, conjugation functions can lead to transfer of chromosomal DNA (next to integrated plasmid) to recipient via conjugative apparatus.

Directionality of process leads to rare transfer of *tra* genes

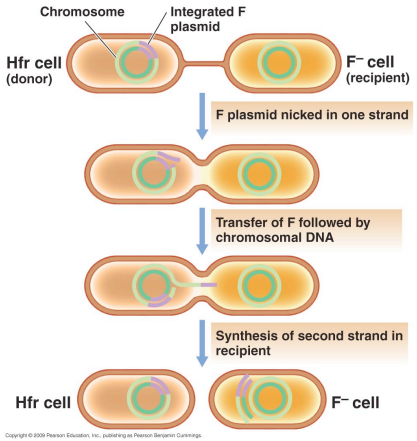
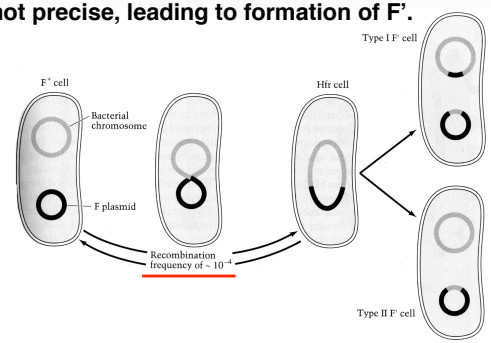


Fig. 11.24

F plasmid: Hfrs and F primes

Hfr formation is reversible. Sometimes, the reversal is not precise, leading to formation of F'.

Fig. 4



Only host genes next to integrated F can be picked up by plasmid

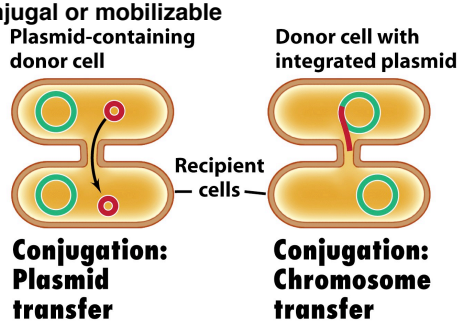
Plasmids and chromosomes

While formation of Hfrs and F prime plasmids only well described in *E. coli*, process goes on in other bacteria with other plasmids

- R prime plasmids will form (*e.g.*, IncP plasmid with *R. meliloti cys* genes)
- Hfrs have been found in *Pseudomonas*

Conjugal plasmids can mediate two distinct DNA transfer events

Conjugal or mobilizable



In both cases, ssDNA transferred

DNA must recombine with recipient; recipient rarely becomes a donor; 10-100 kb transferred

Gene movement: the bacterium fights back…

While many mechanisms to move DNA from one cell to another exist, the bacterial cell is not necessarily a "passive" recipient. Some incoming DNA can obviously have negative impact on cell (Phage infection/sensitivity).

Bacteria have developed one important strategy to combat the flow of DNA into a cell: Restriction-Modification (R-M) systems

Discovery of R-M systems

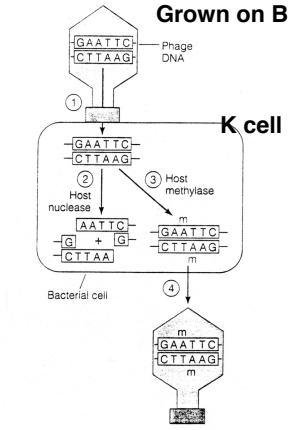
Work from several phage groups (50's-60's):

λ infects both B and K strains of *E. coli*, but…

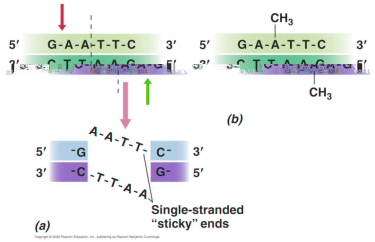
- λ preps grown on *E. coli* B strain with 10000x lower titer on K strain than on B
- λ preps grown on *E. coli* K strain with 10000x lower titer on B strain than on K
- Discovered that reduction in "efficiency" of infection due to strain-specific nucleases
- Demonstrated that the R-M enzymes act indiscrimantly on dsDNA in cell; normal host DNA is protected due to its modification

Discovery of R-M systems

- Upon entering *E. coli* K, DNA from λ grown on B strain could either be degraded (restricted) or modified
- If modified, subsequent infections of phage in K strains would not be subject to K-specific restriction



Recognition sequence for *E. coli* Type II R-M enzyme



See Fig. 12.1

The EcoRI restriction enzyme makes staggered cuts on both strands of DNA, leaving ss "sticky ends". The modifying enzyme adds -CH_3 to 1 base of each strand in recognition sequence and prevents cleavage. R-M systems widespread in Bacteria and Archaea (rare in euks).

Three types of R-M systems

	Type I	**Type II**	**Type III**
Example	*Eco*B	*Eco*RI	*Eco*PI
Recognition site	**TGAN$_8$TGCT**	GAATTC	AGACC
Cleavage site	*ca* 1 Kb away (distant)	Between G and A (in sequence)	24-26 bp on 3' side (closeby)
Joint Nuclease/ Methylase?	Yes	No	Yes
ATP-dependent	Yes	No	No

Type II R-M enzymes

Because they have separate R/M enzymes and they cleave in recognition sequence, the restriction endonucleases of Type II systems are useful for molecular biology.

Restriction enzymes with different recognition sequences have been isolated from wide variety of bacteria.

Type II systems most common but Type I systems widely distributed; Type III systems rare

Type II R-M enzymes

Organism	Enzyme	Recognition sequence
B. subtilis	*Bsu*RI	GG↓C*C
H. influenzae	*Hind*II	GTPy↓PuAC*
H. influenzae	*Hind*III	A↓AGCTT
Nocardia otitidis	*Not*I	GC↓GGC*CGC

Generally, restriction endonucleases with larger recognition Sequences (6-8 bp) are most useful for molecular biology.

Antagonizing R-M enzymes

Restriction-modification systems obviously widely distributed

Phages and plasmids have developed ways to circumvent this protection:

- Modification of T4 DNA allows it to escape the *E. coli* R-M system
- Several plasmids have anti-restriction systems or will reduce frequency of recognition site in the plasmid sequence.

Restriction enzymes and biotechnology

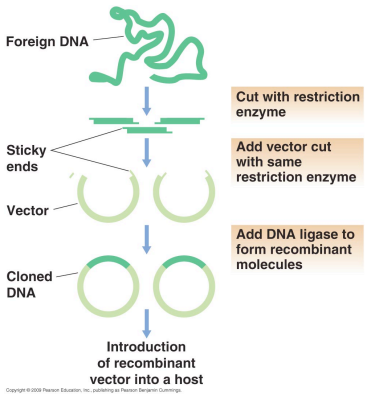
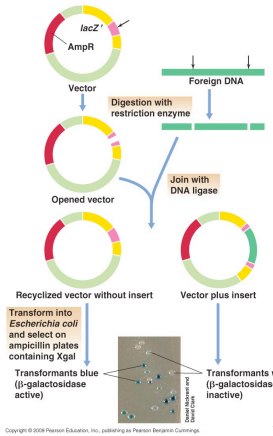


Fig. 12.5

DNA can be cleaved *in vitro* with restriction enzymes and ligated together.

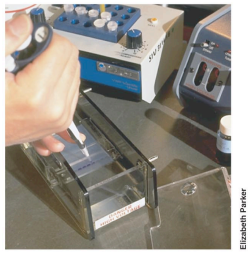
A practical example of cloning

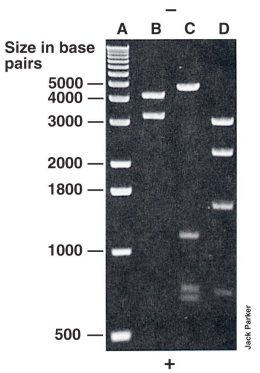


Steps:
1.Cut vector and insert DNA
2.Join with ligase
3.Introduce into E. coli
4.Screen on selective medium

Fig. 12.7

How do we verify that our clone is good?





(a) (b)

Fig. 12.2

Cloning & recombinant DNA

- Over last 30 years a wide variety of cloning strategies have been exploited, including plasmid and viral “vectors”
- Can manipulate interesting DNA and move it into variety of bacterial or eukaryotic cells
- One strategy exploits natural ability of *Agrobacterium tumefaciens* to move plasmid DNA into plants (trans-kingdom conjugation).