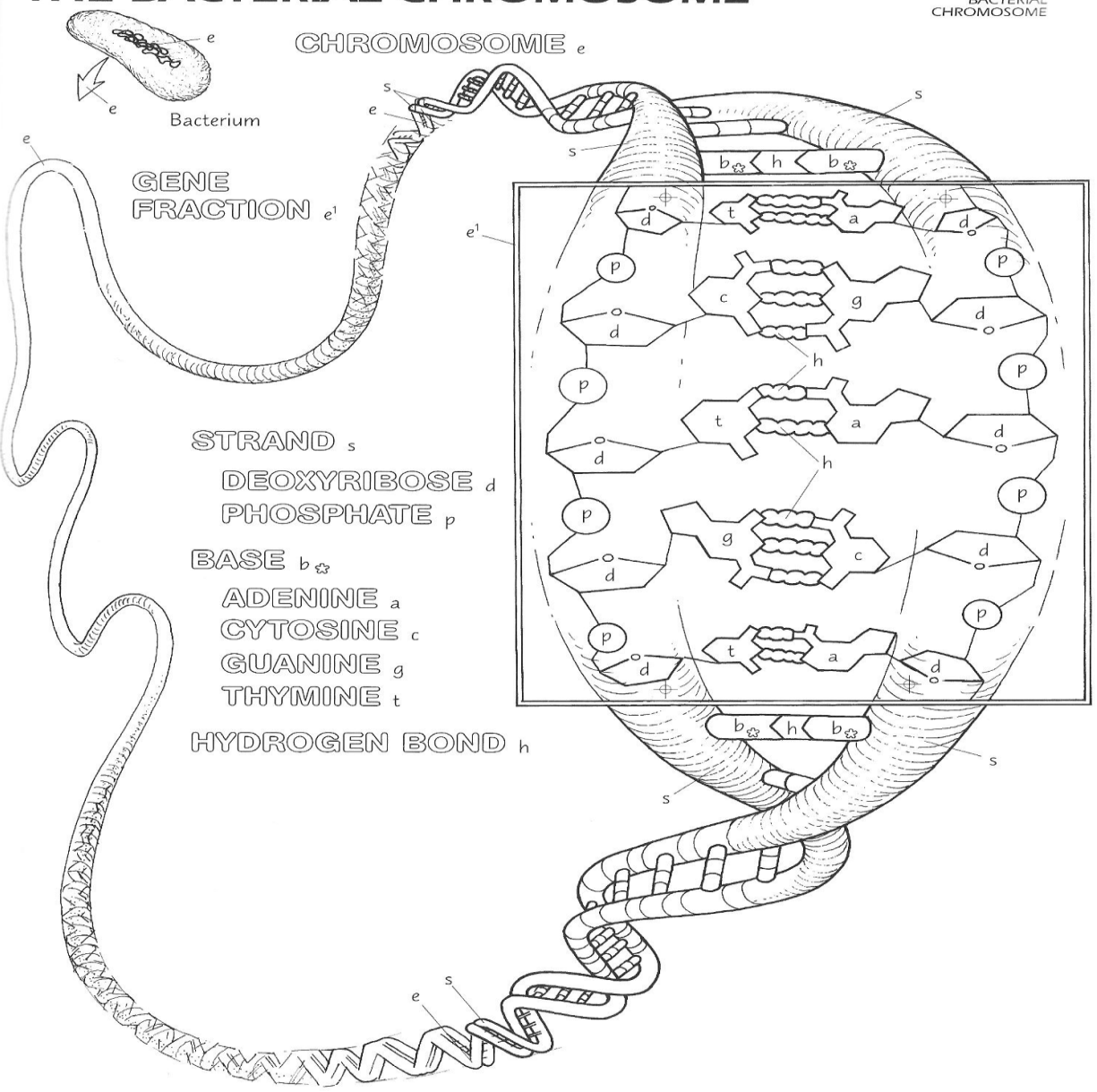
Bacterial genetics, lectures 3 ST

- Replication -DNA
- Regulation
- Change - mutation
- - gene exchange
- Genetic engineering in medecine
- Application to clinical diagnosis

DNA genetic material

- Bacterial cell - DNA - genetic information in nucleotide - circular chromosome - free of ribosomes
- Replication of DNA - bidirectionally
  - in 40 minutes
- - unidirectionally - plasmids

THE BACTERIAL CHROMOSOME



Plasmids

- Extrachromosomal genetic elements
- Autonomously replicating
- circular DNA - exept. B.burgdorferi
- do not encode essential functions - additional genetic information (phenotypic properties, atb resistance, bacteriocin and toxin production, metabolizing properties)

- Large plasmids – (fertility factor F, resistance transfer factor RTF)
  - mediate their own transfer - conjugation
- Smaller plasmids
  - not conjugative - do not encode transfer protein
  - sedentary - do not transfer
- Conjugation, transduction, incorporation

BACTERIAL CHROMOSOME REPLICATION

PARENT CHROMOSOME e
- PARENT STRAND s
- BASE b
- HYDROGEN BOND h

ENZYME z-z²

NUCLEOTIDE n
- DEOXYRIBOSE d
- PHOSPHATE p
- BASE b
- ADENINE a
- CYTOSINE c
- GUANINE g
- THYMINE t

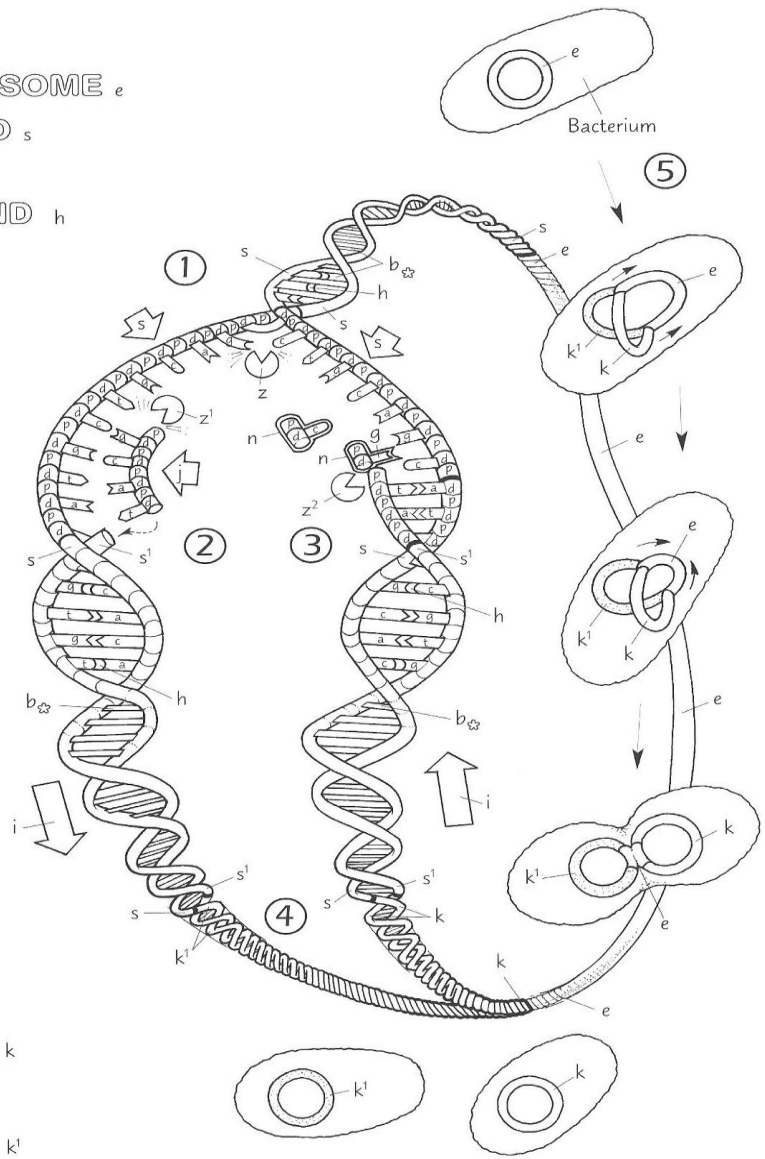
DIRECTION OF REPLICATION i

OKAZAKI FRAGMENT j

REPLICATED STRAND s¹

REPLICATED CHROMOSOME 1 k

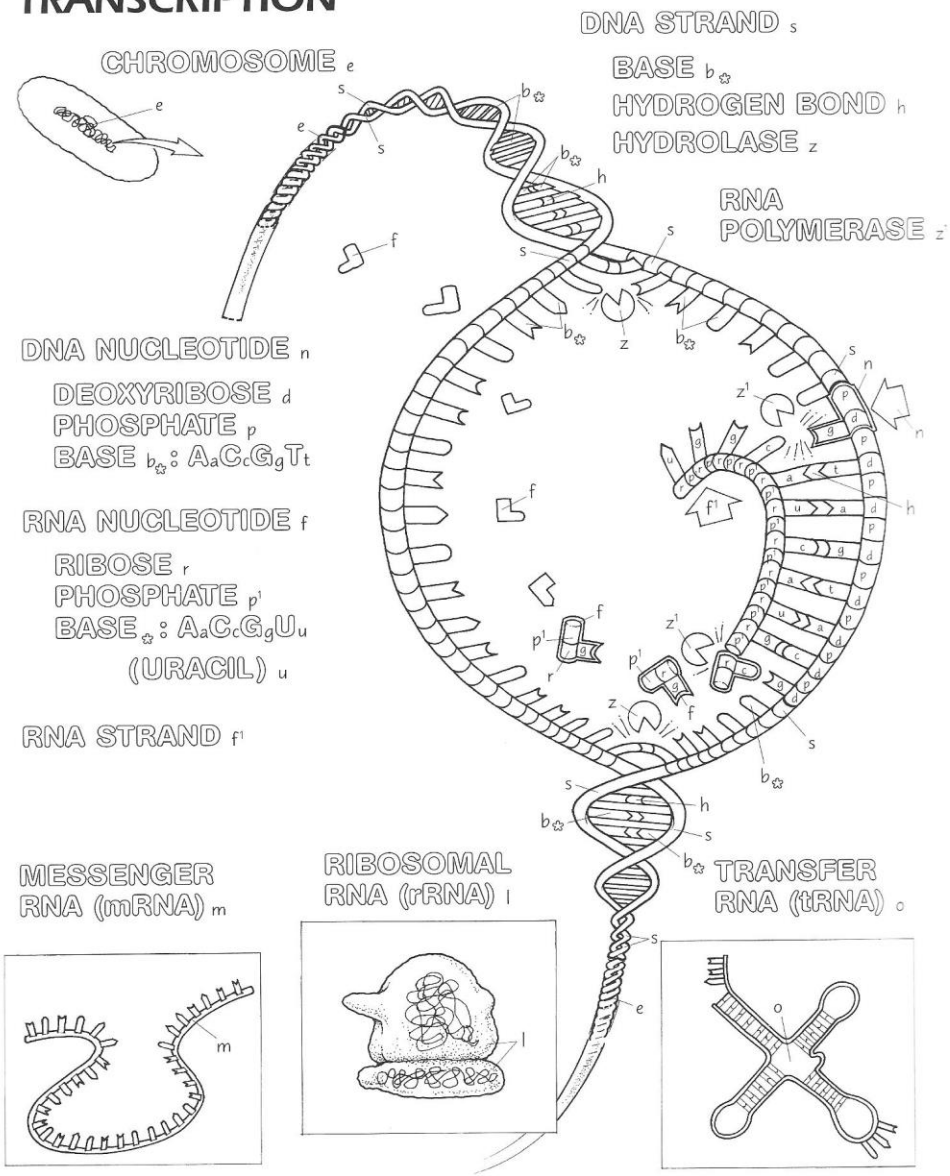
REPLICATED CHROMOSOME 2 k¹



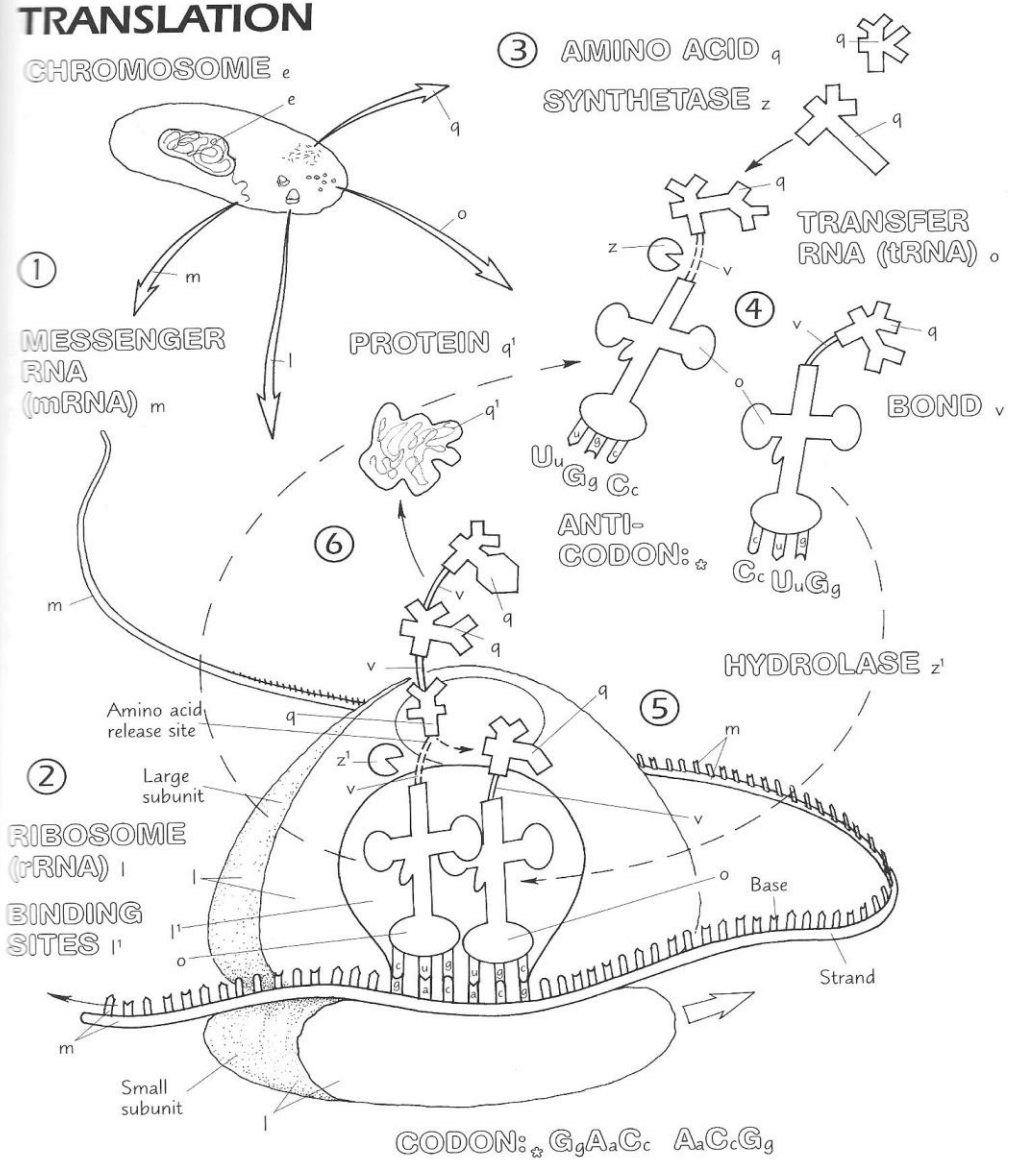
Replication of DNA

- Transcription
- Translation

PROTEIN SYNTHESIS: TRANSCRIPTION



PROTEIN SYNTHESIS: TRANSLATION



Regulation of gene expression

- Cell must adapt to the changing of conditions
  - elementary regulatory mechanisms
  - minimize requirements for energy
  - turned on/off when needed
- Grouping of genes for enzymes of a pathway
  - OPERON: promoter, genes, terminator

  coordinately regulated, transcribed, translated

Transcriptional regulation

- 1) negative control - genes are expressed unless they are switched off by repressor protein
- 2) positive control - genes will not be transcribed unless apoinducer - active regulator protein -si present

- Operons
  - a) inducible - introduction of substrate leads to expresssion of E necessary for metabolism
  - b) repressible - presence of the end-product reduces the amount of enzymes

Change of genetic information

- Damage to DNA - mutation - accidental mutation, DNA repair systems
- Exchange of genes - recombinantion

Mutation

- Any change of base sequence of DNA
  - single base mutation - insertion, deletion, transition, transversion
  - DNA is transcribed by RNA polymerase into mRNA that is translated by tRNA loaded with specific AmAc that recognize set of 3 nucleotides(codon) on mRNA and add next protein produced by ribosomes

Origin of mutanions

- Spontaneously
- Induced - heat - deamination,
  - ultraviolet light - pyrimidine dimer formation,
  - ionizing radiation - opening of the ring,
  - chemical mutagens - nucleotide base analogues - structural similarity, frameshift mutagens - addition or deletion of one base, DNA reactive chemicals - modification of the base to chemically different structure

Repair mechanisms of DNA

- Direct DNA repair - enzymatic removal
- Excision repair (excision of damaged DNA segment and synthesis od the new)
- Recombinational repair - retrieves missing information by genetic recombination
- SOS response - interruption of replication
- Error-prone repair

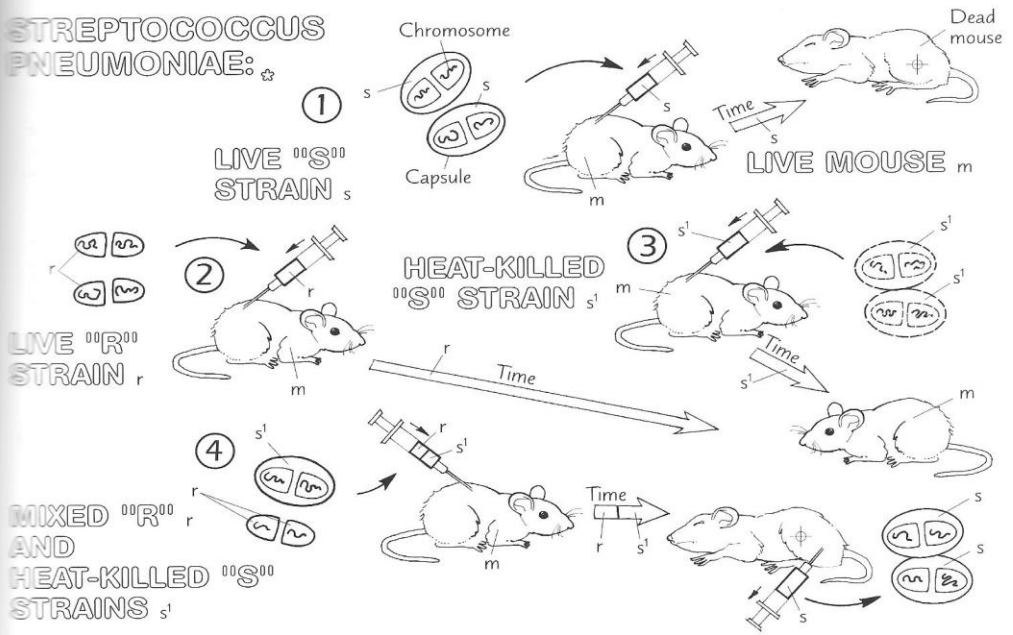
Gene exchange

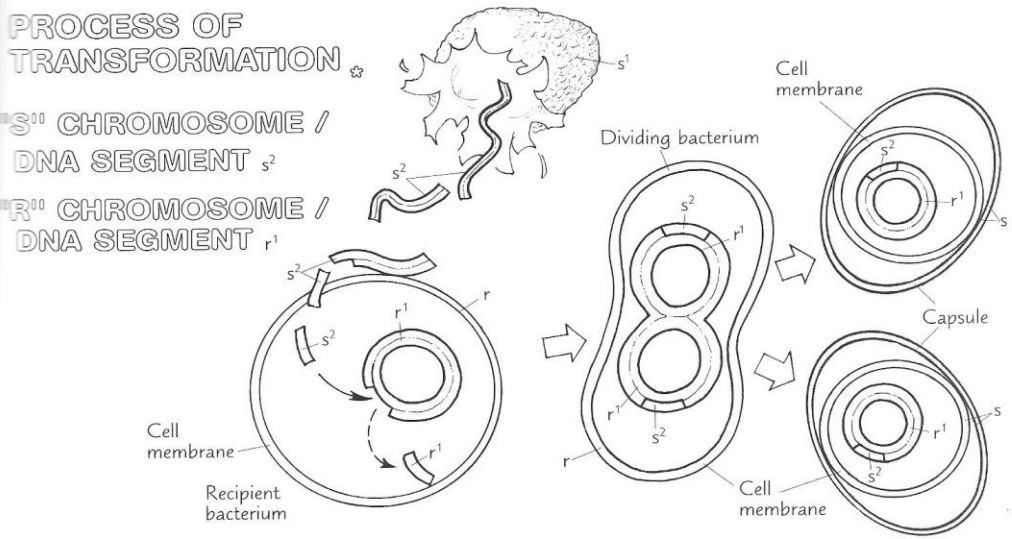
- Between bacterial cells
  1) transformation - acquisition by incorporation of exogenous or foreign DNA
  2) transduction - transfer from one bacterium to another by bacteriophage
  3) conjugation - quasi sexual exchance
- Bacteria frequently exchange DNA that is then integrated into chromosome or in plasmids and passed on to daughter cell

Transformation

- Griffiths - colonies of encapsulated and non-encapsulated pnemococcus
- take up and stably maintain exogenous DNA
  1) natural competence - ability of a cell to interact with exogenous DNA, not permanent feature, toward the end of logarithmic growth - (H.i., Str.pn.,Bacillus)
  2) chemical methods, electroporation

BACTERIAL TRANSFORMATION





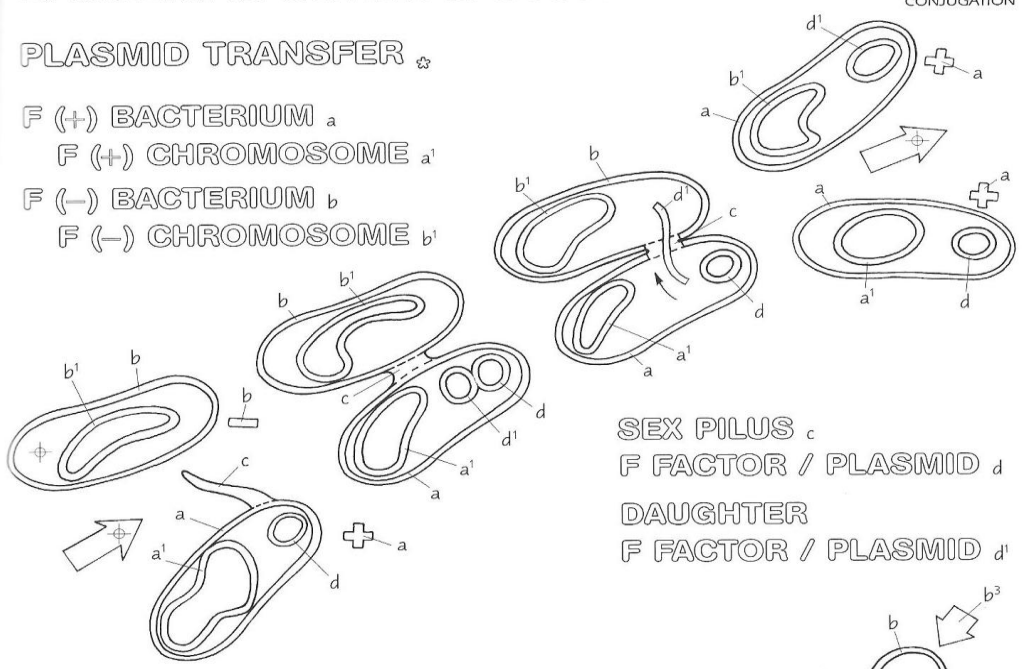
Conjugation

- Cell to cell interaction, unidirectional from „male“ to „female“
- Different conjugative plasmids:
  1)Fertility factor:contact between F+ and F-
  F+responsible for sex specific pilli synthesis
  -wall to wall contact by cytoplasmatic bridge, - contact initiate plasmid replication and transfer
  2) Atb resistance-R: in G+, adhesin on the surface of the donor

BACTERIAL CONJUGATION

PLASMID TRANSFER ✿

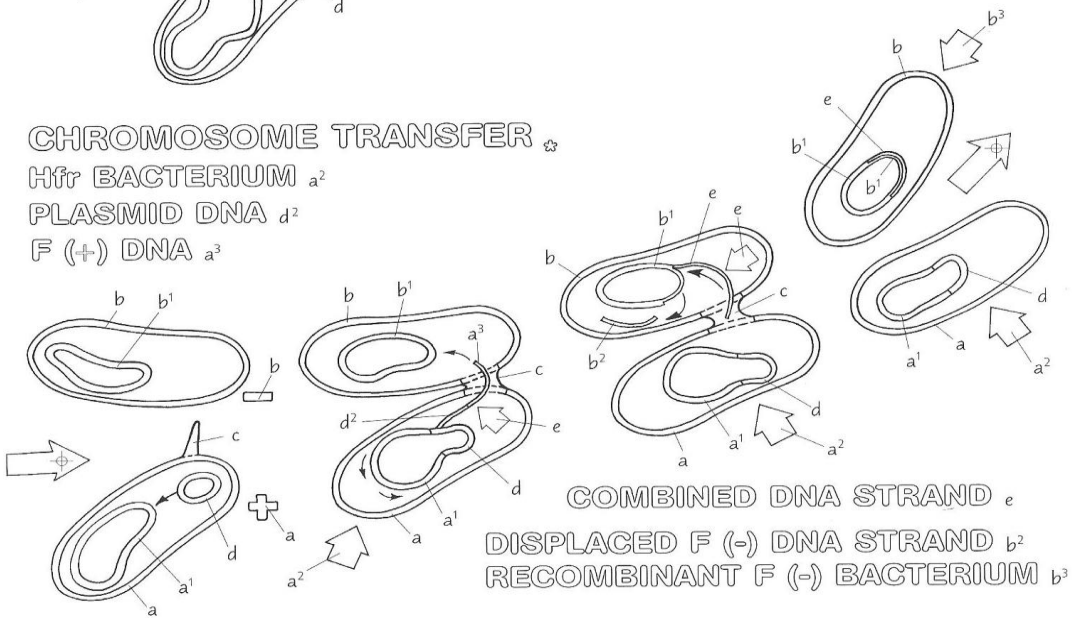
F (+) BACTERIUM a
F (+) CHROMOSOME a^1
F (–) BACTERIUM b
F (–) CHROMOSOME b^1



SEX PILUS c
F FACTOR / PLASMID d
DAUGHTER
F FACTOR / PLASMID d^1

CHROMOSOME TRANSFER ✿

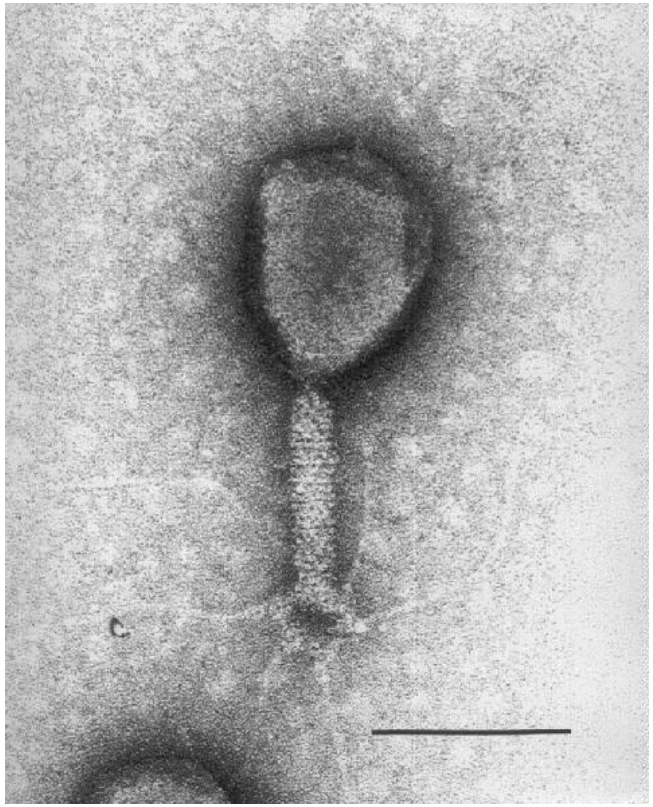
Hfr BACTERIUM a^2
PLASMID DNA d^2
F (+) DNA a^3

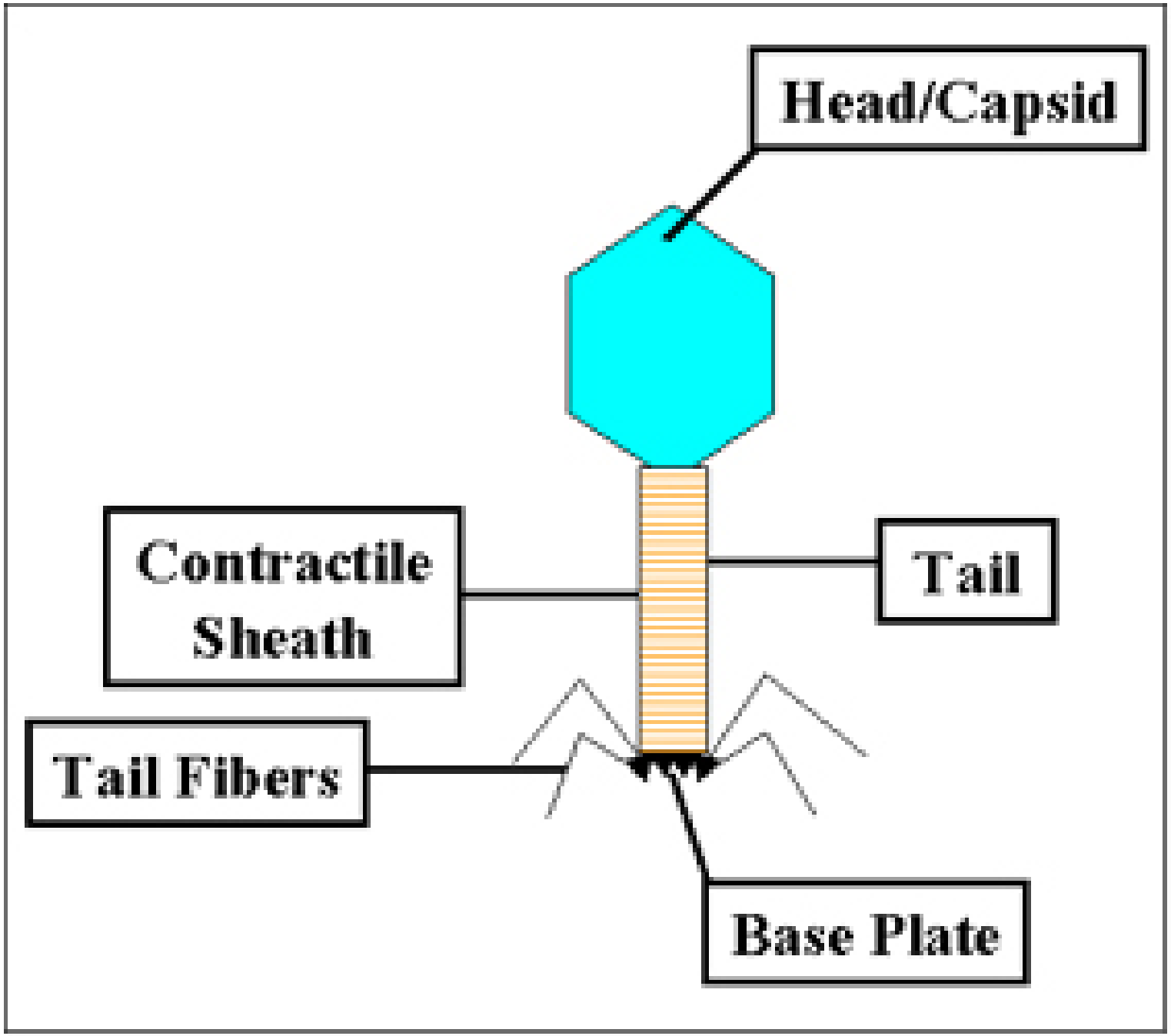


COMBINED DNA STRAND e
DISPLACED F (-) DNA STRAND b^2
RECOMBINANT F (-) BACTERIUM b^3

Transduction-bacteriophage

- Bacteriophage - parasitic virus of bacterial cell using their energy systém and protein synthesizing factors.
  DNA or RNA.
  Infection of bacterium
  - only nucleic acid

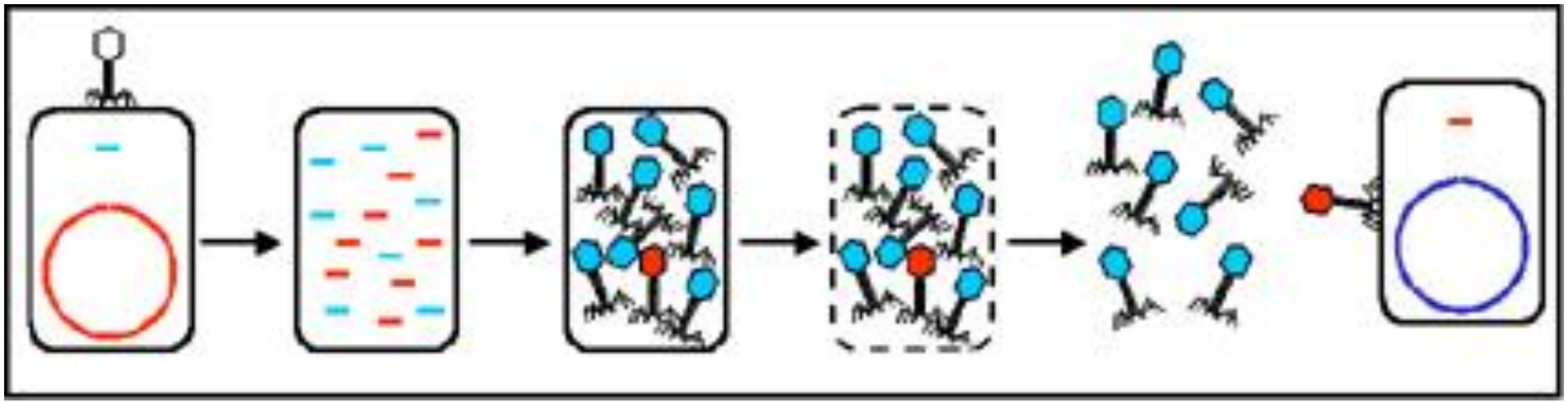



Head/Capsid
Contractile Sheath
Tail
Tail Fibers
Base Plate

Transduction-bacteriophage

Life cycles

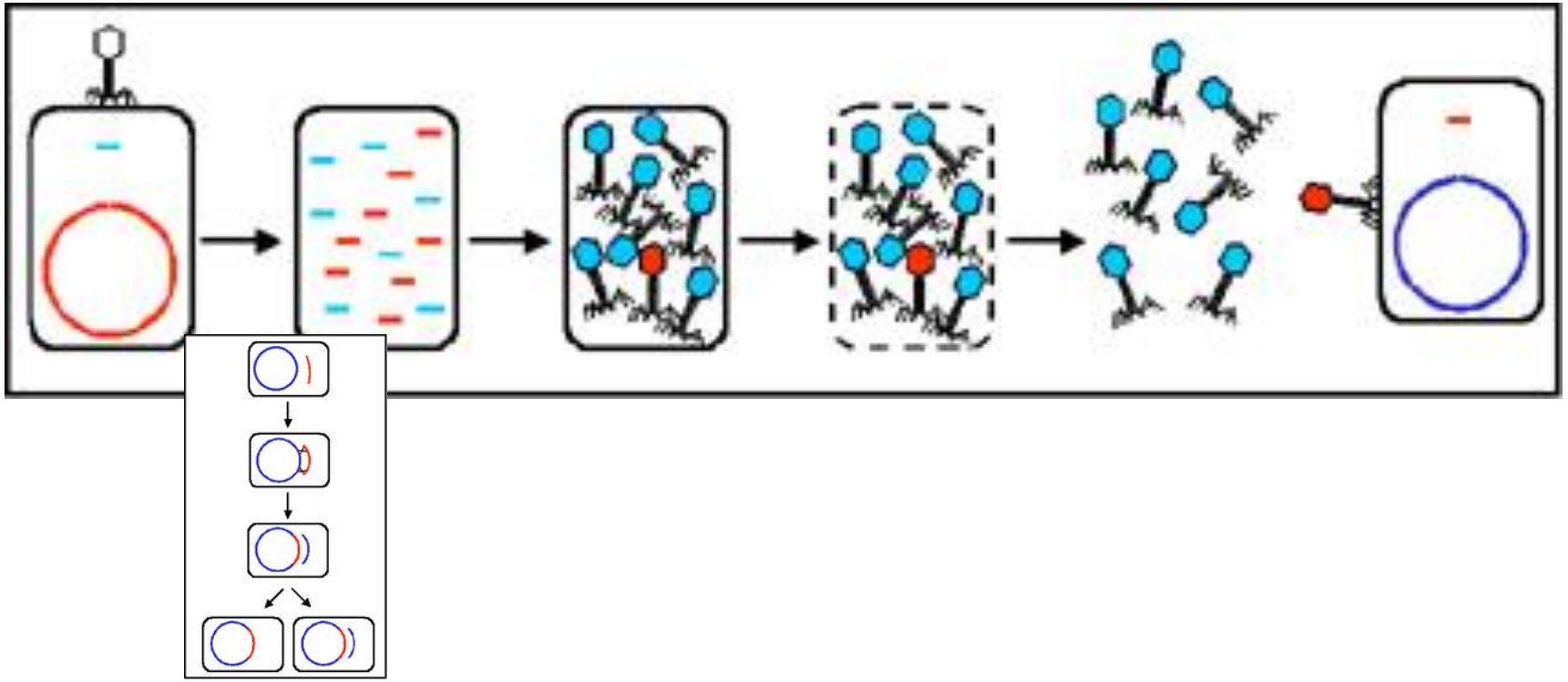
- lytic - lysis of the cell- virulent phage



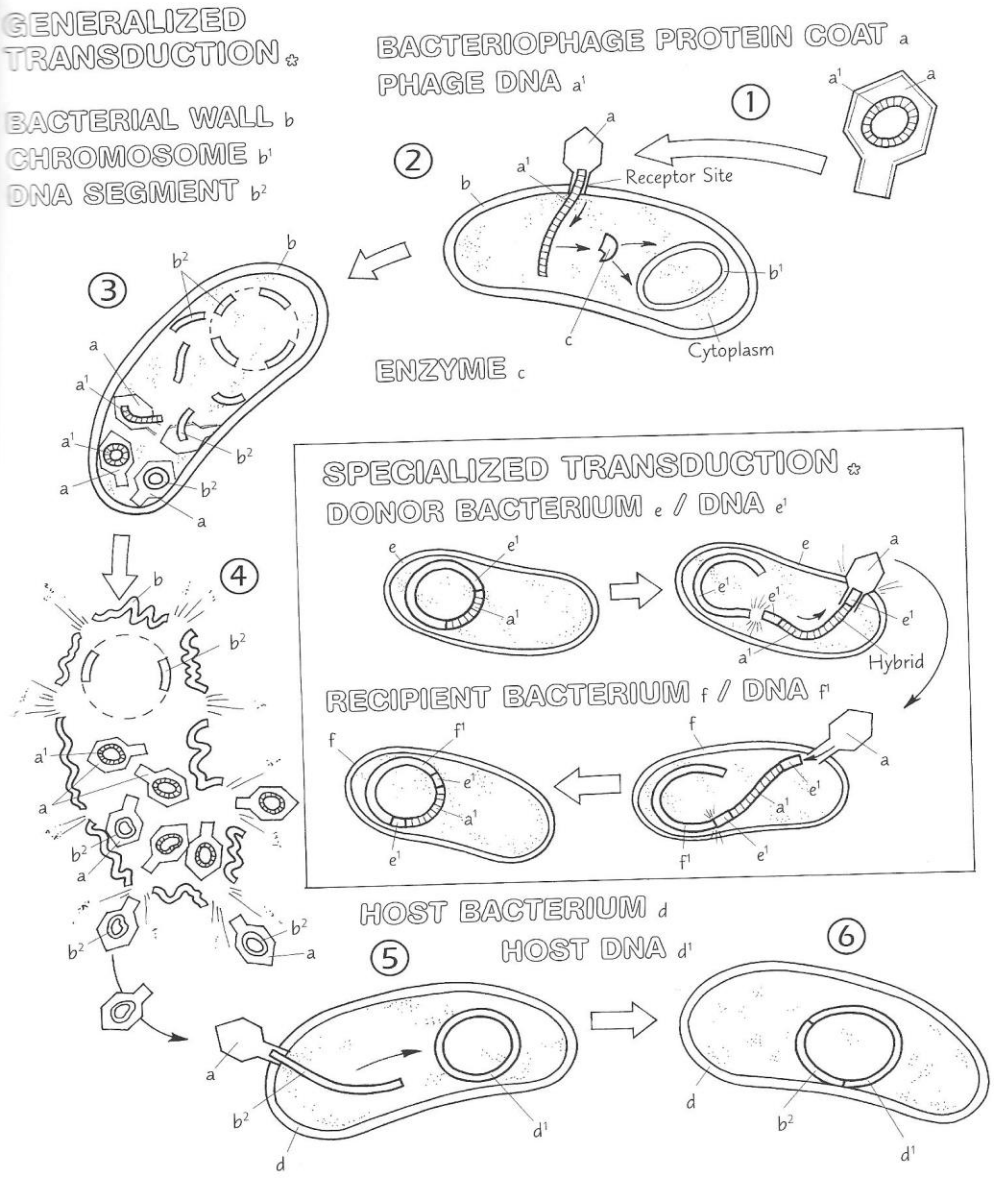
Transduction-bacteriophage

Life cycles

- lysogenic - not lysis - phage DNA is integrated - temperate phage - after many generation - induction, conversion



BACTERIAL TRANSDUCTION



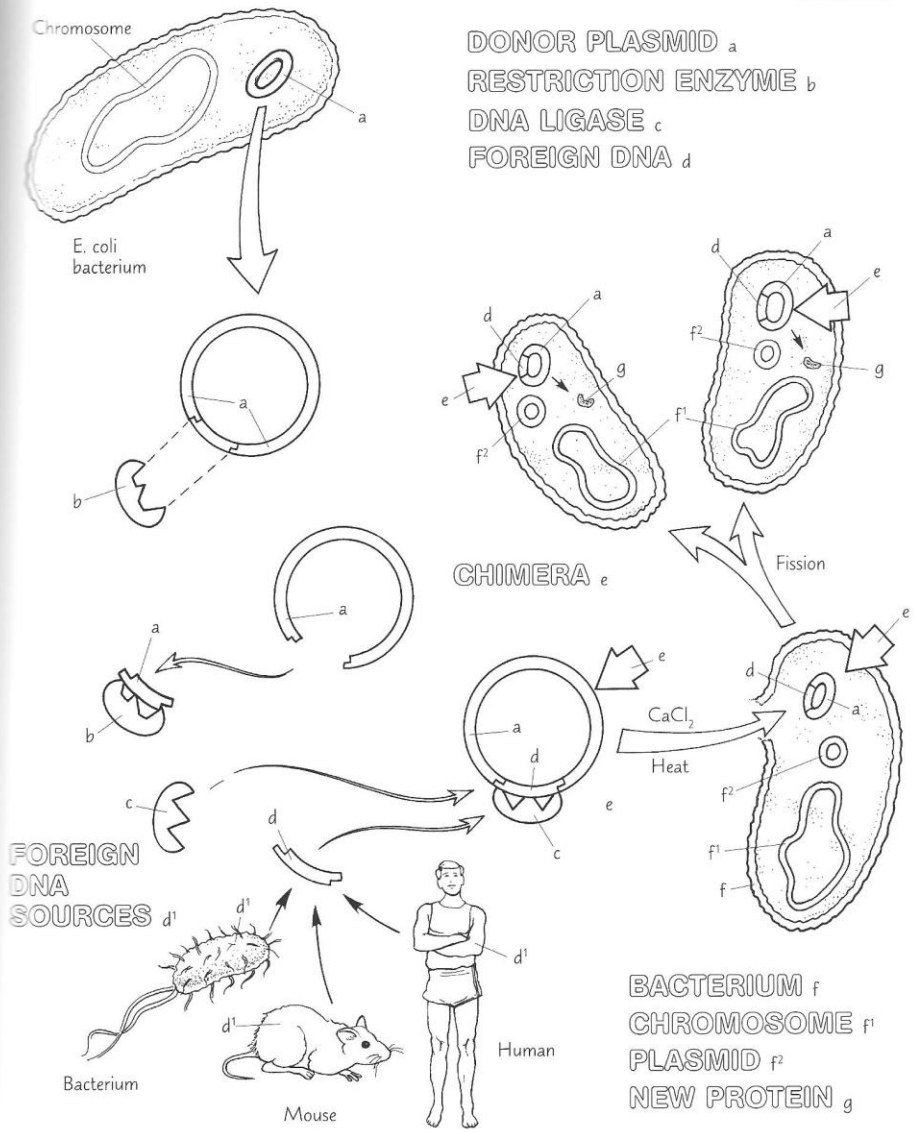
Transposons

- Segments of DNA able to move from one position to another in the genome or from chormosomal DNA to plasmid and v.v.:
  - insertion sequences - genetic information for their own transfer
  - complex trasposons - genes for various kind of resistances, part of R plasmids - resistance transfer factor
  - phage-associated transposons -

Genetic engineering in medicine

- Development of vectors or vehicles allowing the cloning of any DNA sequences
- Eucaryotic genes may be expressed in procaryotic systems
- Many genetic diseases are caused by lack of protein
- Production in bacteria of recombinant vaccines
- Replacement therapy - bacterial interference

GENETIC ENGINEERING



Molecular technologies in diagnosis

- Use of nucleic acid (DNA) probes to diagnose and study diseases
- DNA of interest is inserted to bacterium and amplified to high copy numbers and labeled - in situ hybridization
- PCR - generation of millions copies of specific pieces of nucleic acid of suspected microorganism