TRANSMISSIBLE GENETIC ELEMENTS: PLASMIDS, TRANSPOSONS & INTEGRONS

MOBILE GENETIC ELEMENTS

1 PLASMIDS

-MOST OFTEN CIRCULAR MOLECULES OF DOUBLE-STRANDED DNA

-VARY WIDELY IN SIZE

-SELF-TRANSMISSIBLE ELEMENTS

- CONTAIN ANTIBIOTIC RESISTANCE GENES

- TRANSFER OTHER STRUCTURES WITH RESISTANCE GENES

2 TRANSPOSONS

-TRANSFER RESISTANCE GENES BETWEEN PLASMIDS AND CHROMOSOME

-VARIETY OF STRUCTURES WITH SIMILAR CHARACTARISTICS TO THOSE DESCRIBED FOR SELF-TRANSMISSIBLE PLASMIDS: TYPE 1 (IS, Tn5), TYPE 2 (Tn3), TYPE 3 (Bacteriophage μ), TYPE 4 (Conjugatives)

MOBILE GENETIC ELEMENTS

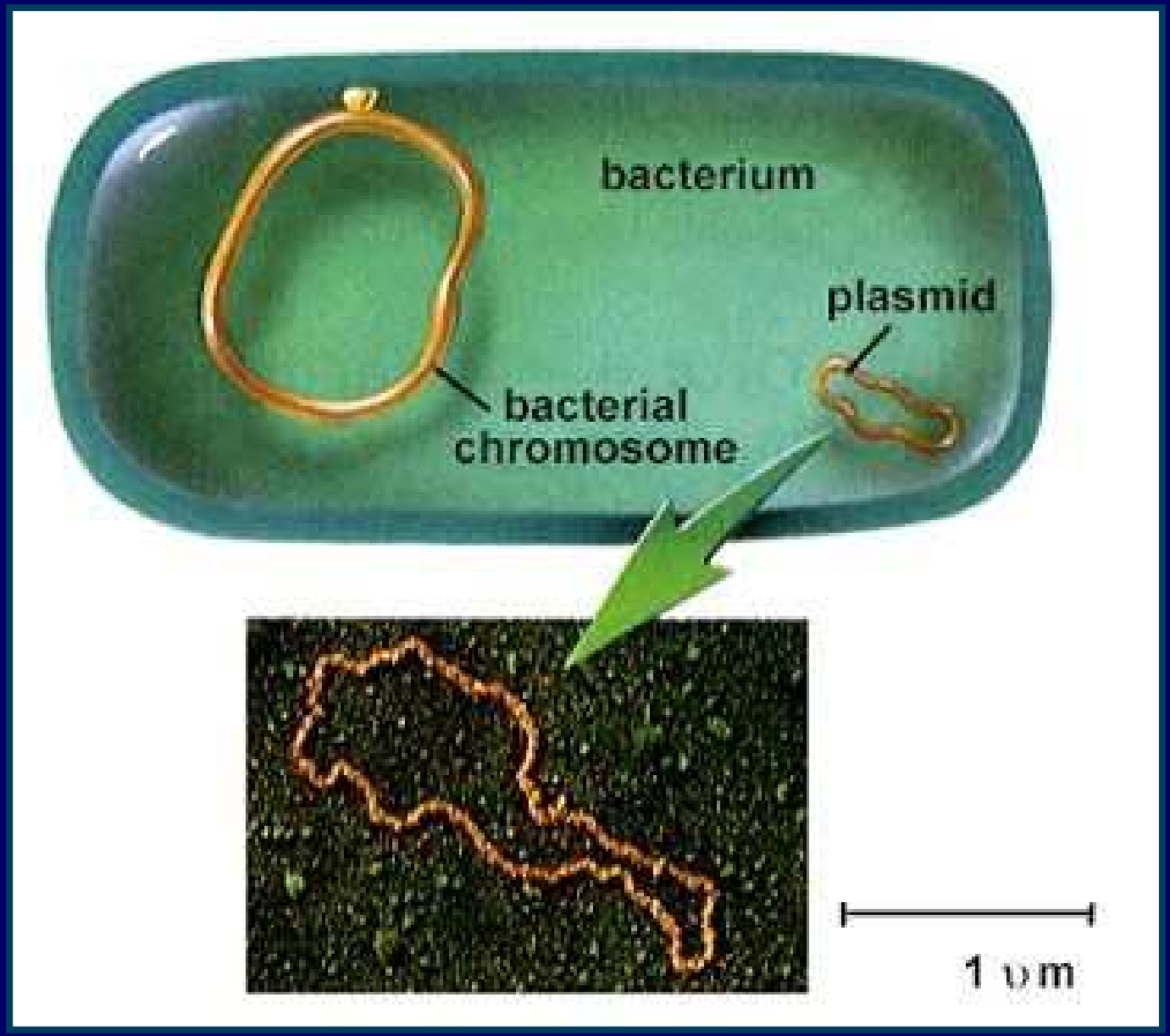
3 INTEGRONS

- DNA SEGMENT CONTAINING GENES FOR:
  - -INTEGRASE
  - -PROMOTER
  - -INTEGRATION SITE
- -PROPIERTIES: FORMS CLUSTERS OF RESISTANCE GENES (ALL UNDER THE CONTROL OF THE SAME PROMOTER)
- -SIZE: 800-3900 bp.

Contain genetic information for the expression of a protein responsable of the uptake of IS and cassettes with antibiotic resistance genes

- integration: site-specific recombination
- promoters:
  - P1
  - P2: increases the level of transcrition

PLASMIDS



PROPIERTIES ENCODED BY PLASMIDS

- CIRCULAR EXTRACHROMOSOMAL ELEMENTS
- MAY ENCODE A VARIETY OF SUPPLEMENTARY GENETIC INFORMATION, INCLUDING THE INFORMATION OF SELF-TRANSFER TO OTHER CELLS
- REPLICATE INDEPENDENTLY OF THE CHROMOSOME
- UBIQUITOUS IN BACTERIA
- BROAD RANGE OF SIZE AND NUMBER OF COPIES
- MANY ENCODE GENETIC INFORMATION FOR SUCH PROPIERTIES AS:
    - RESISTANCE TO ANTIBIOTICS
    - BACTERIOCIN PRODUCTION
    - RESISTANCE TO TOXIC METAL IONS
    - PRODUCTION OF TOXINS AND OTHER VIRULENCE FACTORS
    - REDUCED SENSITIVITY TO MUTAGENS
    - THE ABILITY TO DEGRADE COMPLEX ORGANIC MOLECULES

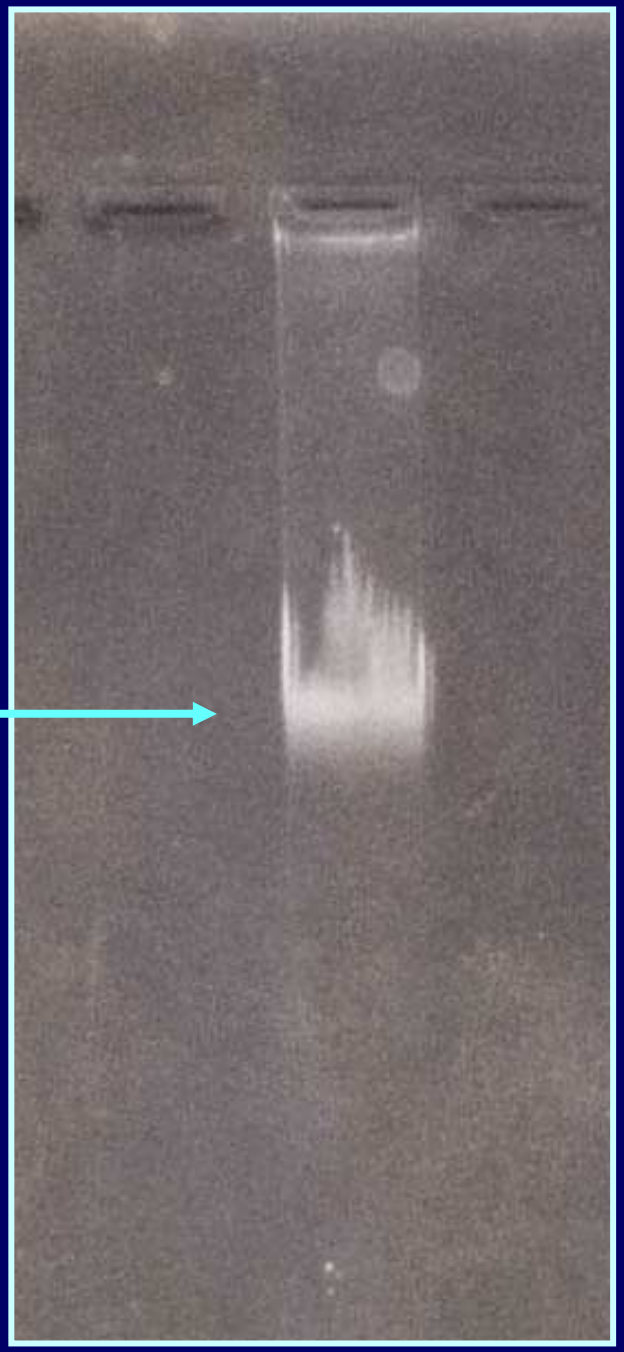
METHODS TO MAKE PLASMIDS VISIBLE

- AGAROSE GEL ELECTROPHORESIS AND PULSED FIELD GEL ELECTROFORESIS

- THE RATE OF MIGRATION OF DNA THROUGH AGAROSE DEPENDS ON THE SIZE OF THE FRAGMENT: THE SMALLER THE MOLECULE, THE FASTER IT RUNS

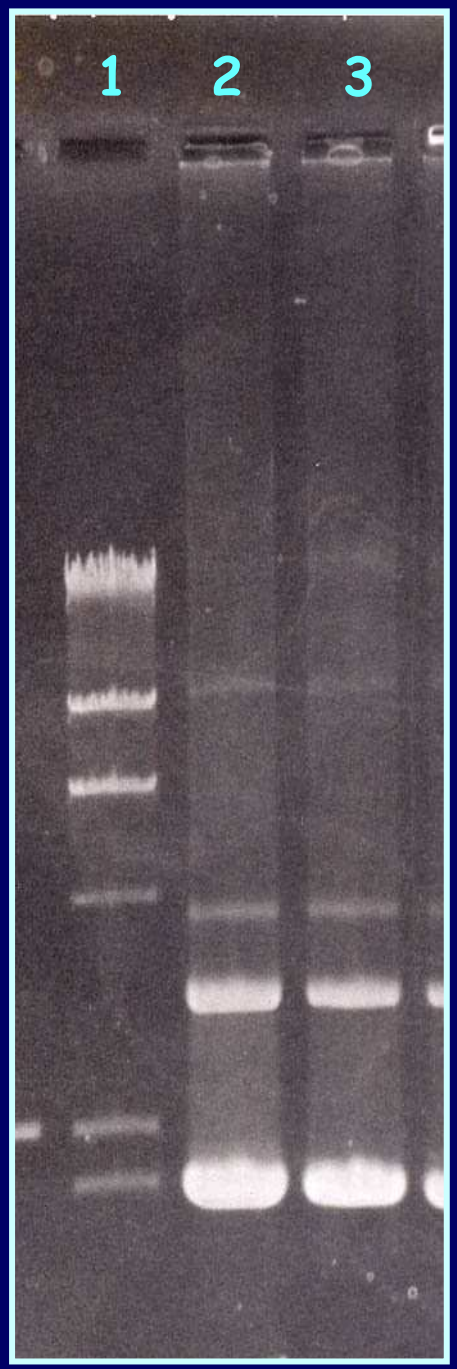
- THE RATE OF MIGRATION IS ALSO AFFECTED BY THE SHAPE OF THE DNA MOLECULE: CIRCULAR MOLECULES MIGRATE DIFFERENTLY FROM LINEAR FRAGMENTS WITH THE SAME MOLECULAR WEIGHT (CCC, OC and L: circular and covalently closed, open circular and linear forms)

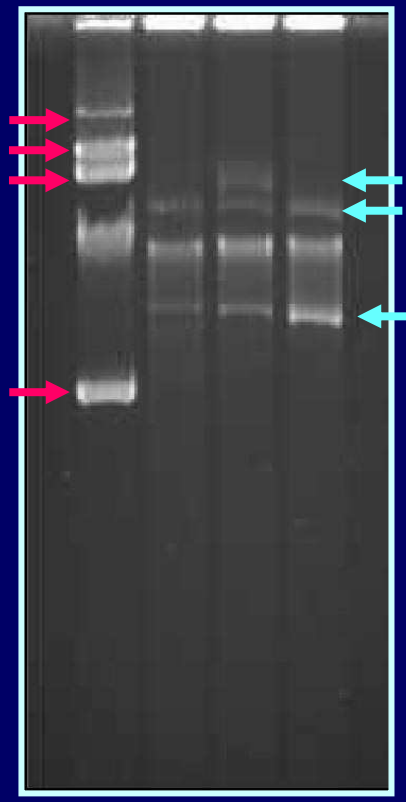
Chromosomal DNA



1. λ digested with *Hind*III endonuclease

2 &3. pSU615 plasmid DNA
(CCC, OC & L forms)

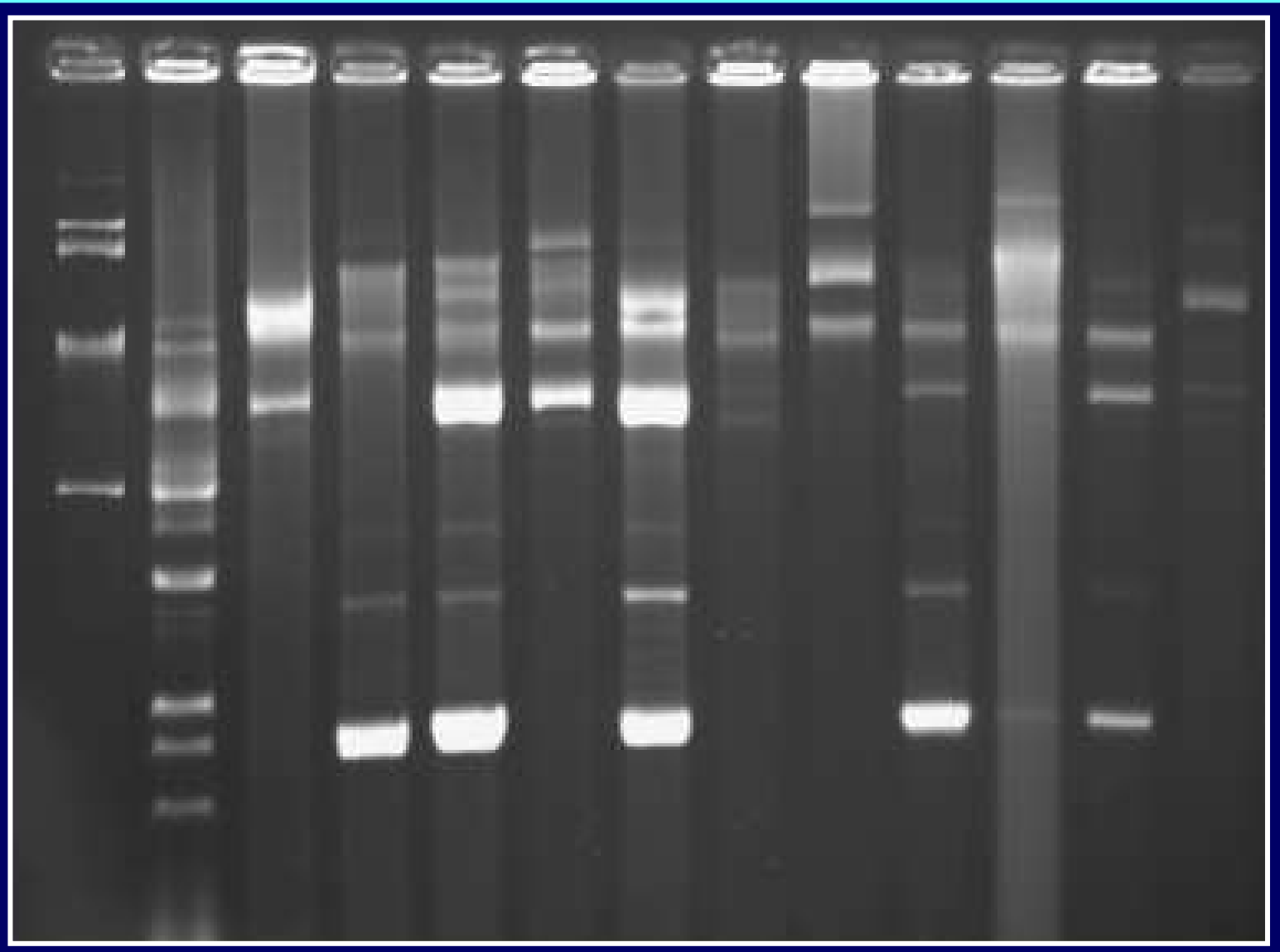


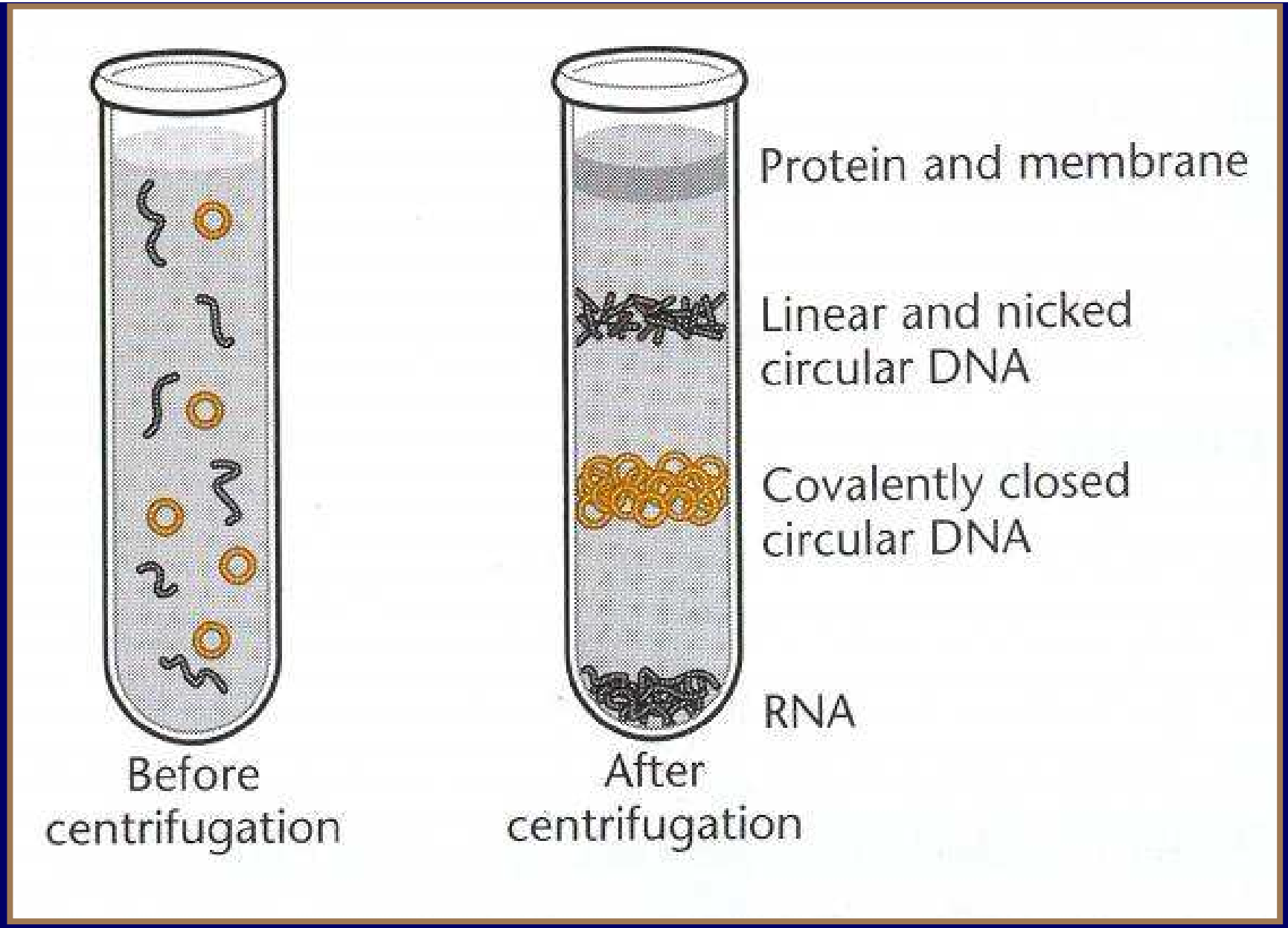


→ *E. coli* control strain plasmids

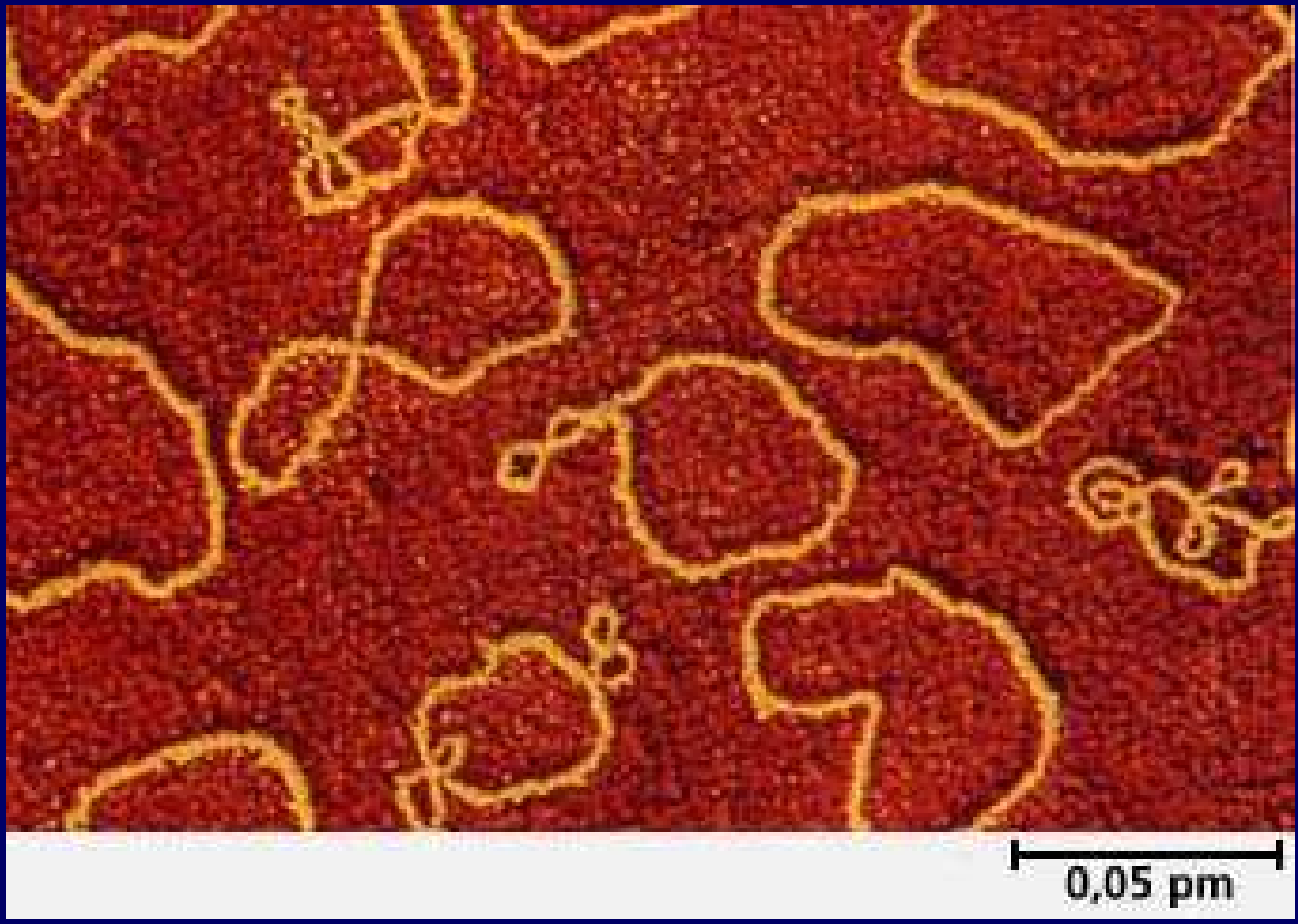
→ *A. baumannii* plasmids

PLASMID PROFILES FROM DIFFERENT *A baumannii* ISOLATES AND THEIR CORRESPONDING SIZE (Kb)





PURIFICATION OF PLASMID DNA BY CAESIUM CHLORIDE-ETHIDIUM BROMIDE DENSITY GRADIENT CENTRIFUGATION


0,05 pm

PROPIERTIES OF PLASMIDS:REPLICATION

- INDEPENDENT OF THE CHROMOSOME REPLICATION

- THEY ARE REPLICONS: HAVE AT LEAST ONE ORIGIN OF REPLICATION, OR *ori* SITE

- EACH TYPE OF PLASMIDS REPLICATES BY ONE OF TWO GENERAL MECHANISMS DETERMINED BY ITS *ori* REGION :

  - *oriV*: plasmid replication origin

  - *oriT*: site at which DNA transfer initiates in plasmid conjugation

  *THETA (θ) REPLICATION:

    - THE MOST COMMON IN GRAM-NEGATIVE BACTERIA
    - USED BY ColE1, RK2, F, P1, AND THE CROMOSOME

  * ROLLING-CIRCLE REPLICATION:

    - GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA
    - RC PLASMIDS

ori REGION

1. HOST RANGE:

   -ColE1: NARROW HOST RANGE (*E. coli* & closely related bacteria)

   -RK2: BROAD HOST RANGE (the most common in gram-negative bacteria)

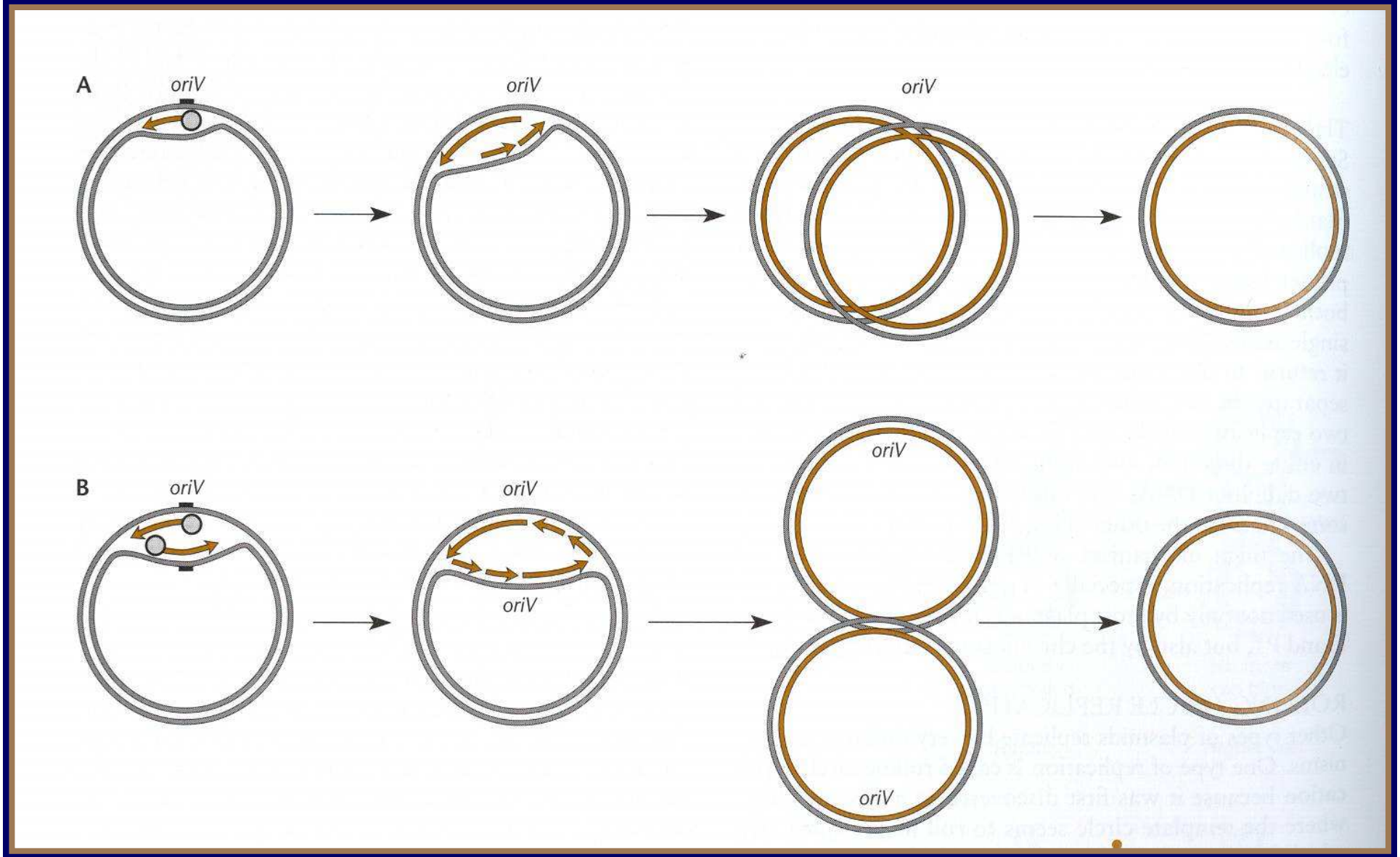
   -RSF1010:gram-negative and gram-positive bacteria

   -PLASMIDS FROM GRAM-NEGATIVE BACTERIA DO NOT REPLICATE IN GRAM-POSITIVE ONES

2. REGULATION OF COPY NUMBER:

   RELAXED PLASMIDS: HIGH-COPY NUMBER (eg. ColE1)

   STRINGENT PLASMIDS: LOW-COPY NUMBER (eg. F)


A
oriV
oriV
oriV
B
oriV
oriV
oriV
oriV

PLASMID INCOMPATIBILITY

• MANY BACTERIA CONTAIN MORE THAN ONE TYPE OF PLASMID BUT NOT ALL TYPES OF PLASMIDS CAN STABLY COEXIST IN A BACTERIA

•SOME TYPES WILL INTERFIERE WITH EACH OTHER´S REPLICATION OR PARTITIONING:if two such plasmids are introduced into the same cell, one or the other will be lost at a higher than normal rate when the cell divides.

TWO PLASMIDS THAT CANNOT STABLY COEXIST ARE MEMBERS OF THE SAME INCOMPATIBILITY (Inc) GROUP

- INCOMPATIBILITY IS DUE TO REPLICATION CONTROL (*par*):

two plasmids that share the same mechanism of replication control will be incompatible.

PLASMIDS AS CLONING VECTORS IN RECOMBINANT DNA TECHNOLOGY

CLONING VECTOR: AUTONOMOUSLY REPLICATING DNA (REPLICON) INTO WHICH OTHER DNAs CAN BE INSERTED

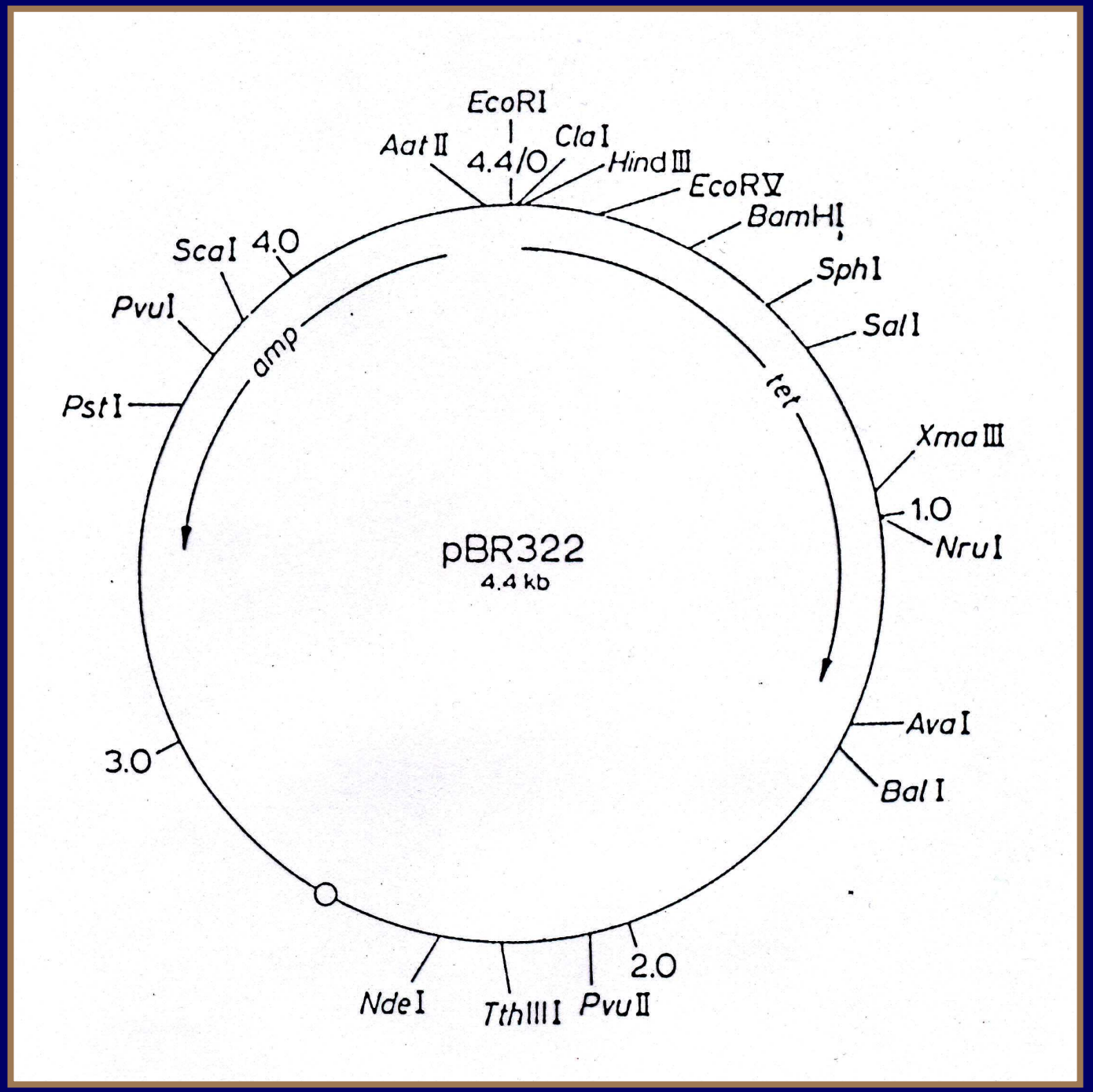
ADVANTAGES AS CLONING VECTORS:

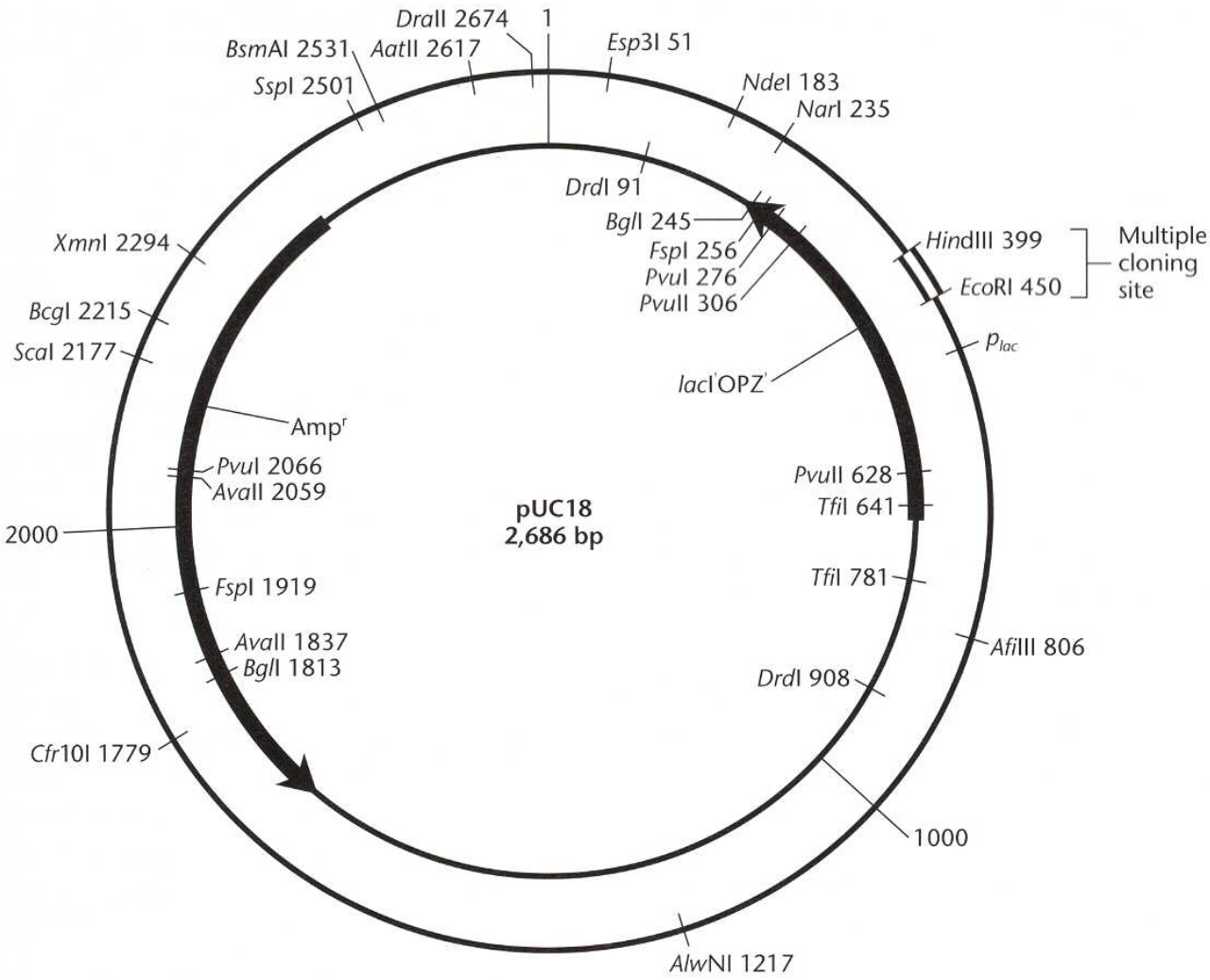
-THEY ARE "MADE TO ORDER"

-DO NOT KILL THE HOST CELL

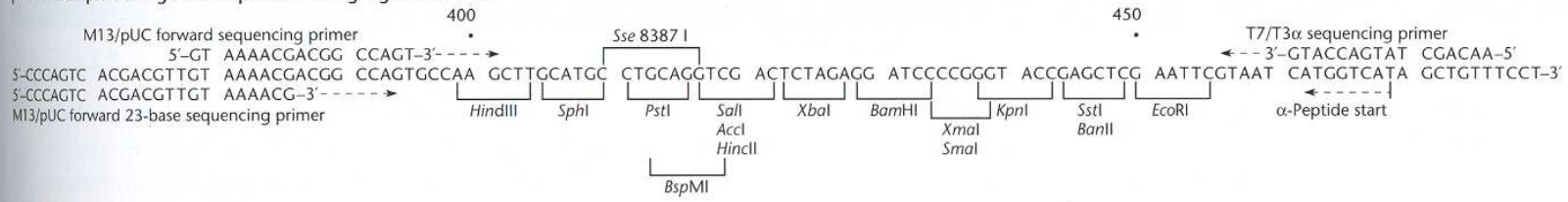
-EASY TO PURIFY TO OBTAIN THE CLONED DNA

-CAN BE MADE RELATIVELY SMALL


EcoRI
AatII
4.4/0
ClaI
HindIII
EcoRV
BamHI
SphI
SalI
XmaIII
1.0
NruI
AvaI
BalI
2.0
PvuII
TthIIII
NdeI
3.0
PstI
PvuI
ScaI
4.0
amp
tet
pBR322
4.4 kb



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



TRANSPOSONS

PROPIERTIES

- DNA ELEMENTS THAT HOP, OR TRANSPOSE, FROM ONE PLACE IN DNA TO ANOTHER ("JUMPING GENES")

- KNOWN TO EXIST IN ALL ORGANISMS ON EARTH

- TRANSPOSITION: THE MOVEMENT BY A TRANSPOSON
- TRANSPOSASES: THE ENZYMES THAT PROMOTE TRANSPOSITION

- MOVE AMONG DIFFERENT GENERA OF BACTERIA

- TYPES:
  - TRUE TRANSPOSONS
  - RETROTRANSPOSONS (BEHAVE LIKE RNA RETROVIRUSES)

PROPIERTIES

STRUCTURE:

- SIZE:
  - SMALL (ABOUT 1000 bp LONG, CARRY ONLY GENES FOR THE TRANSPOSASES)
  - LARGE (CARRY ANTIBIOTIC RESISTANCE GENES): CONJUGATIVE TRANSPOSONS ARE VERY LARGE AS THEY CARRY *tra* GENES AS WELL AS TRANSPOSITION FUNCTIONS
- ALL CONTAIN INVERTED REPEATS AT THEIR ENDS
- PRESENCE OF SHORT DIRECT REPEATS OF THE TARGET DNA THAT BRACKET THE TRANSPOSONS

- TYPES OF BACTERIAL TRANSPOSONS:
  - INSERTION SEQUENCE (IS) ELEMENTS
  - COMPOSITE TRANSPOSONS: FORMED BY TWO IS ELEMENTS OF THE SAME TYPE
  - NONCOMPOSITE TRANSPOSONS

INTEGRONS

DESCRIPTION

5' END

-LENGHT: 1,3 Kb

- STRUCTURE:
  - -INTEGRASE PROMOTER
  - - *int* GENE CODING FOR AN INTEGRASE OR RECOMBINASE (SITE-SPECIFIC RECOMBINATION)
  - - *attI* SITE (TARGET FOR INSERTION SEQUENCES)
  - -PROMOTER (1/2) FOR THE RESISTANCE GENES

DESCRIPTION

3' END

-LENGHT: FROM 2 Kb

- STRUCTURE:
  - *qacEAI* GENE (RESISTANCE TO QUATERNARY AMONIUM COMPOUNDS)
  - *sulI* GENE (RESISTANCE TO SULPHONAMIDES)
  - -OPER READING FRAME *orf 5* (UNKNOWN FUNTION)

DESCRIPTION

INSERTED SEQUENCES / GENETIC CASSETTES

- SIZE: FEW HUNDRED BASE PAIRS

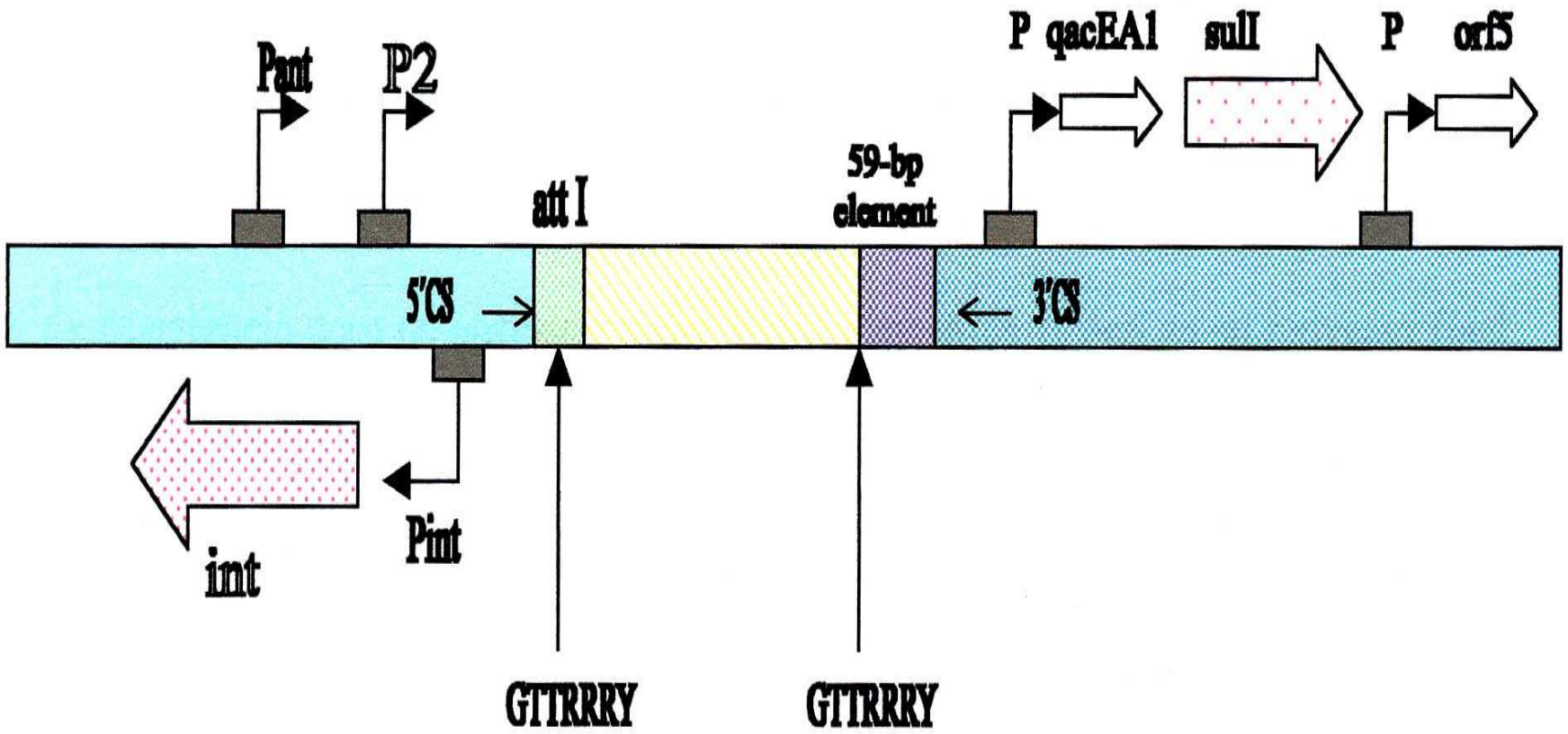
- attC SITE :3´END, 59 bp, ONE RECOMBINATION SITE BUT NO PROMOTER

- EXIST AS FREE MOLECULES: CIRCULAR DNA WITHOUT REPLICATION/TRANSPOSITION CAPABILITY.

- INSERTION:
    * ALWAYS IN THE SAME DIRECTION
    * NEW GENES ALWAYS ARE INSERTED AT THE BEGINNING
    * ONCE INSERTED THEY CAN CHANGE ITS POSITION

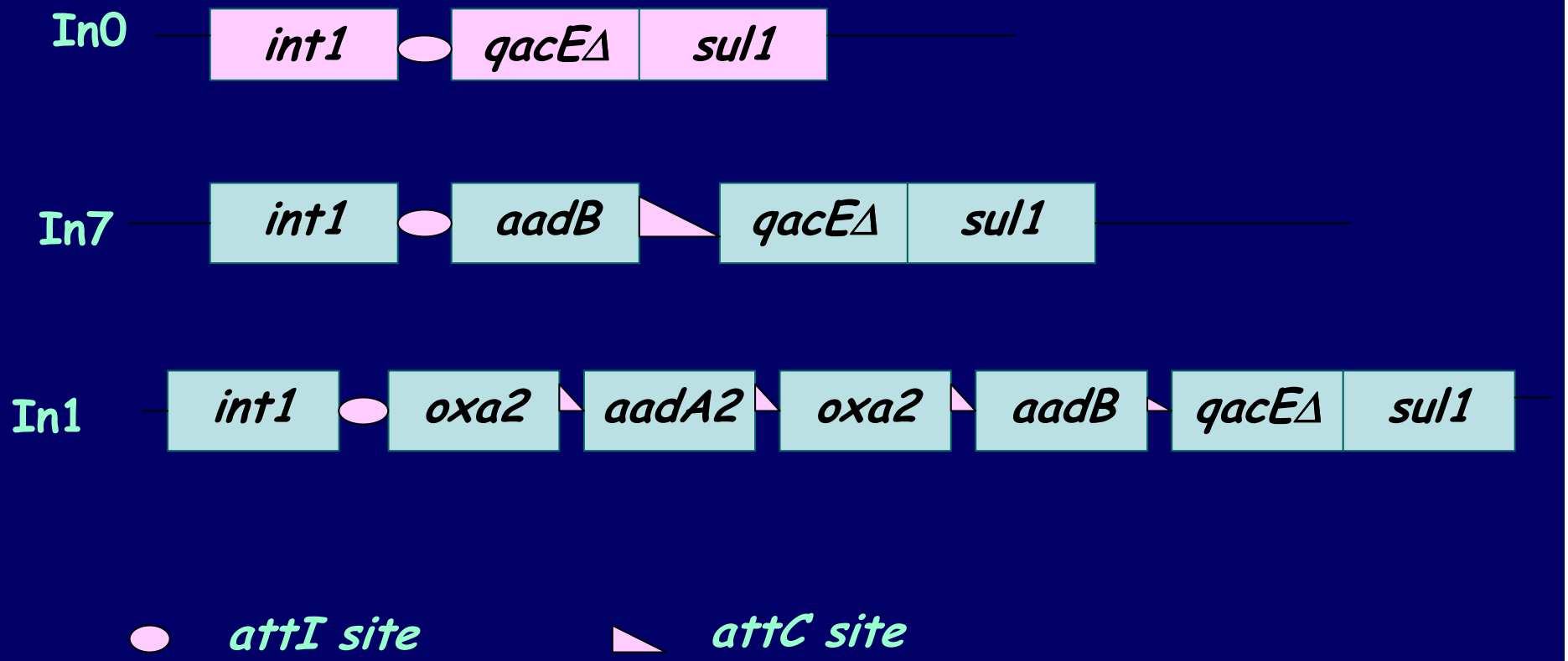
- GENES AT ENDING POSITIONS HAVE LOWER LEVEL OF EXPRESSION THAN PREVIOUS GENES

STRUCTURE

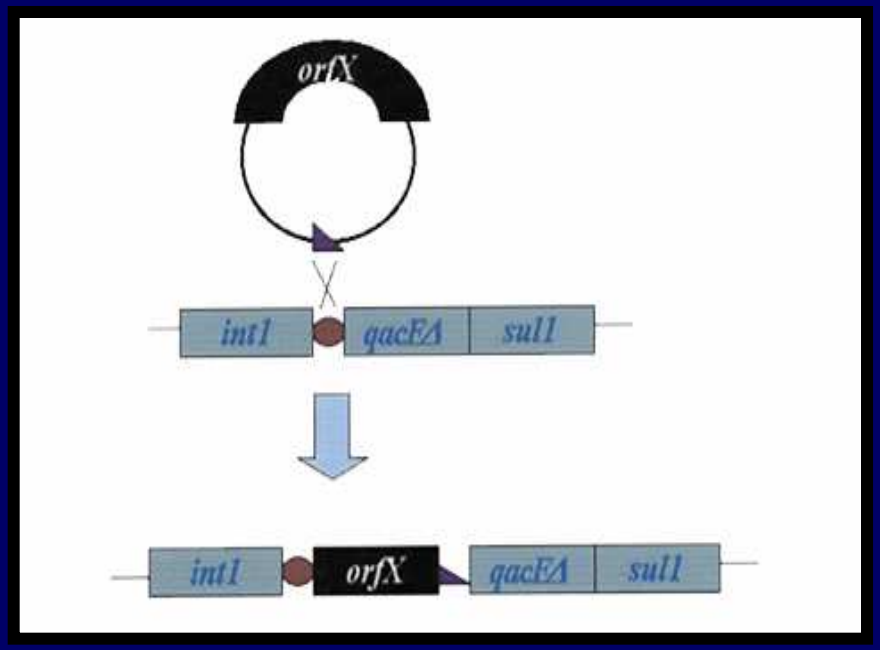


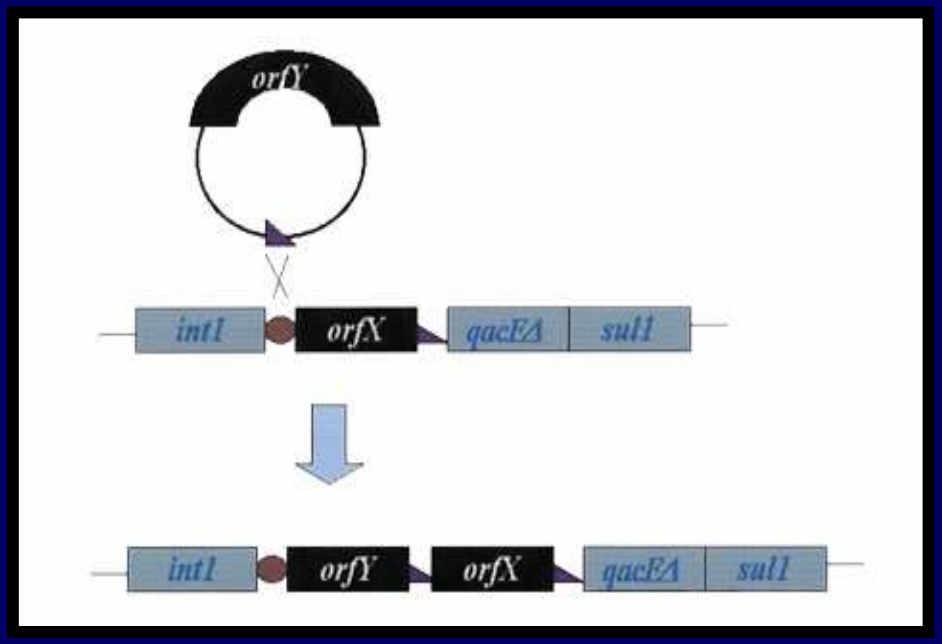
5´ END | CASSETTE | 3´ END

INTEGRONS


In0
int1
qacEΔ
sul1
In7
int1
aadB
qacEΔ
sul1
In1
int1
oxa2
aadA2
oxa2
aadB
qacEΔ
sul1
attI site
attC site

INTEGRATION OF CASSETTES





TYPES OF INTEGRONS

CLASS 1

-MOST FREQUENTLY FOUND IN CLINICAL ISOLATES
-TYPE 1 INTEGRASE

CLASS 2

-TYPE 2 INTEGRASE (*intI* 2)
-40% HOMOLOGY WITH *intI* 1 GENE
-Tn7 AND DERIVATIVES

CLASS 3

-TYPE 3 (*intI* 3)
-61% HOMOLOGY WITH *intI* 1 GENE

RESISTANCE GENES DETECTED IN INTEGRONS

1.AMINOGLYCOSIDES	*aadA* (SPECTINOMYCIN & STREPTOMYCIN) *aadB* (KANAMICIN, GENTAMICIN & TOBRAMICIN) *aacA7* (AMIKACIN & TOBRAMICIN)
2.BETALACTAMS	OXA, IMP & VIM-TYPE β-LACTAMASES
3.ERITROMYCIN	*ereA* GENE
4.TRIMETHOPRIM	*dhfrV* GENE
5.CHLORANPHENICOL	*catB3* GENE
6.ANTISEPTICS, DISINFECTANTS	

BACTERIAL INTEGRONS

GRAM-NEGATIVE

-ENTEROBACTERIA: *E.coli, Klebsiella spp.,Proteus spp...*
-NON-FERMENTING RDOS: *P. aeruginosa & A. baumannii*

GRAM-POSITIVE

Staphylococcus aureus
Enterococcus

MYCOBACTERIA: *Mycobacterium fortuitum*

DETECTION OF INTEGRONS BY PCR

CLASS 1

5'CS (-GGCATCCAAGCAGCAAG-)
3'CS (-AAAGCAGACTTGACCTGA-)
(conserved sequences)

CLASS 2

INT72 (-GCACTCCATGGAATATCCAGGGCCATTCCCG-)
INT74 (-GCTTGCTTGCAGGGATATAATCAATATCGC-)
(dihydropholate reductase)

CLASS 3

INT3L (-GCAGGGTGTGGACGAATACG-)
INT3R (-ACAGACCGAGAAGGCTTATG-)
(*intI3*)

CARACTERIZATION OF INTEGRONS FROM AMPLIFIED DNA

1. ENDONUCLEASE DIGESTION

TARGET DNA: 5µl AMPLIFICATED DNA (final volume, 25 µl)
ENZYMES: *Hinf I, HaeIII, BstEII …….*

2. DNA SEQUENCING

1.PURIFICATION OF THE AMPLIFICATION FRAGMENT
2.SEQUENCE DETERMINATION

DETECTION OF INTEGRONS: HIBRIDATION WITH DNA PROBES

CLASS 1

*int*21 PROBE (specific for *intI1*)
(-CCTGGCTTCAGGAGATCGGAAGACCTCGGC-)

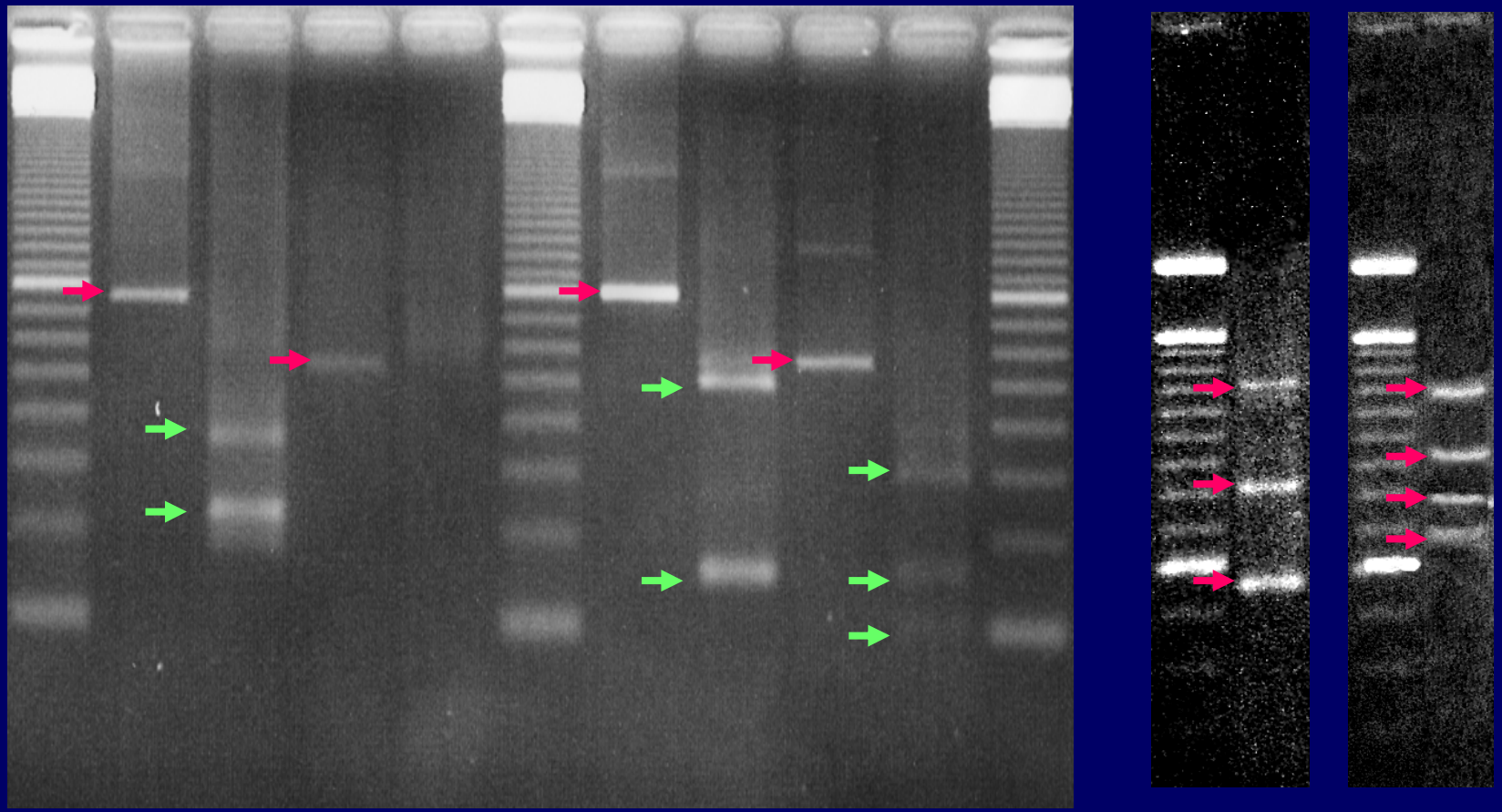
CLASS 2

inf7 PROBE (specific for *intI2)*
(-GCGATATTGATTATATCCCTGCAAGCAAGC-)

INTEGRONS DETECTED IN CLINICAL ISOLATES OF *A. baumannii*

1999 YEAR

2002-8 YEARS



→ INTEGRONS

→ ENDONUCLEASE DIGESTIONS