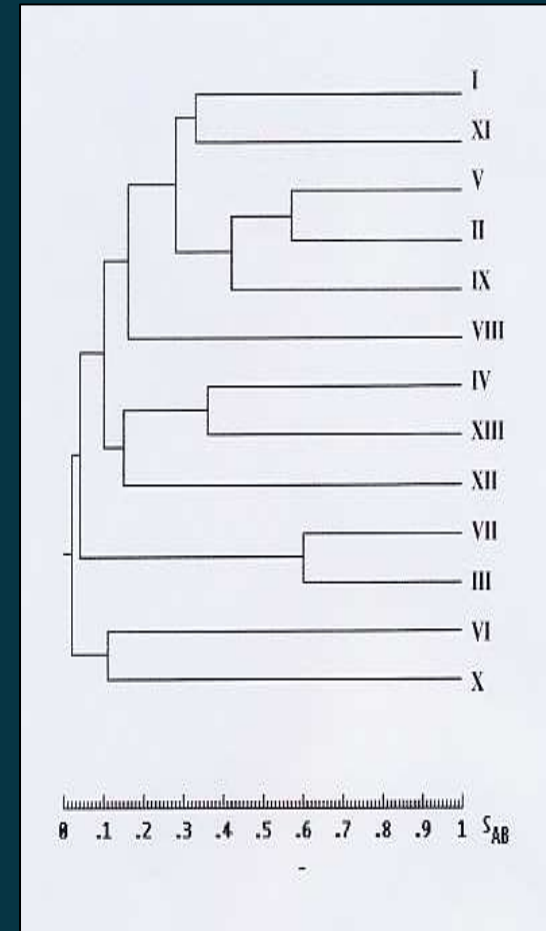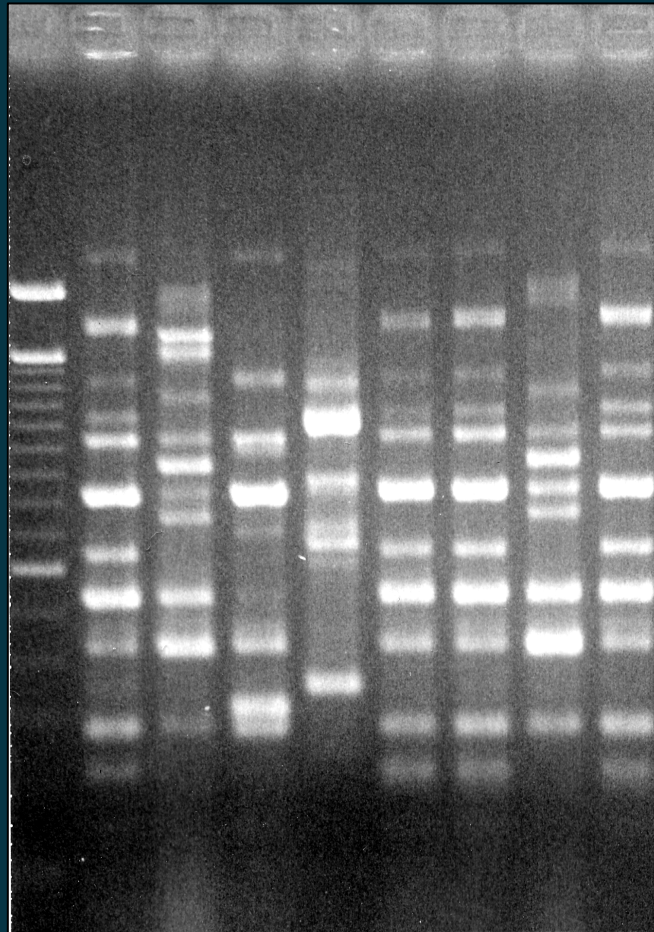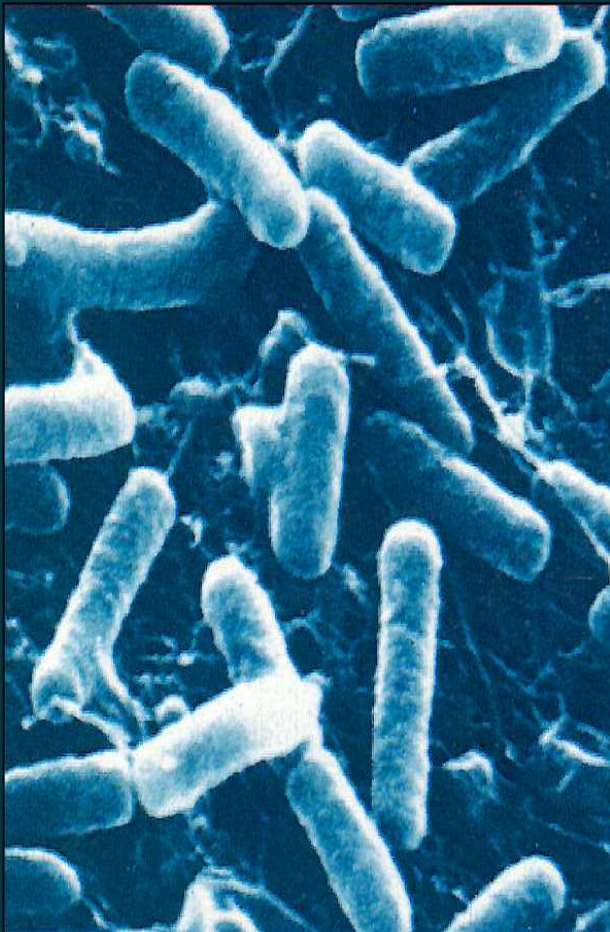# USEFUL TOOLS TO CONTROL NOSOCOMIAL INFECTIONS

NOSOCOMIAL INFECTIONS: PREVALENCE

1.   AFFECT DEVELOPING AND NON-DEVELOPING COUNTRIES
    - Highest level in Eastern Mediterranean countries and Southeast Asia

    - Prevalence in Europe: 8%

2. MAJOR CAUSE OF MORBIDITY AND MORTALITY LEADING TO AN ENORMOUS INCREASE IN THE COST OF HOSPITAL CARE

3. CLINICAL FEATURES ON NOSOCOMIAL INFECTIONS:

    -Urinary tract infections, lower tract respiratory infections and wounds infections.

    -Intensive Care Units, Surgical wards

    -Immunocompromised patients (risk factors: age, pre-existing diseases, medical or surgical procedures used, drug treatment….)

COMMONLY OCCURRING MICRO-ORGANISMS IN HOSPITAL INFECTION

1. URINARY TRACT INFECTIONS
   - *Escherichia coli*
   - *Klebsiella, Serratia,*
   - *Proteus* spp.
   - *Pseudomonas aeruginosa*
   - *Enterococcus* spp

   - *Candida albicans*

2. RESPIRATORY INFECTIONS

   - *Haemophilus influenzae*

   - *Streptococcus pneumoniae*

   - *Staphylococcus aureus*

   - Enterobacteriaceae

   - Respiratory viruses

   - Fungi

3. WOUNDS AND SKIN SEPSIS:

   - *Staphylococcus aureus*

   - *Streptococcus pyogenes*

   - *Escherichia coli*

   - *Proteus* spp

   - Anaerobes

   - *Enterococcus* spp

   - Coagulase-negative *staphylococci*

3. GASTRO-INTESTINAL INFECTIONS:

   - *Salmonella* serotypes

   - *Clostridium difficile*

   - Viruses (Norwalk-like)

The impact of nosocomial infections

1. LEADING MORTALITY CAUSE

2. ENORMOUS INCREASE IN THE COST OF HOSPITAL CARE: extra days/extra charges (drugs, diagnostic techniques)

3. CONTINUOUS PRESSURE: elderly patients, prevalence of chronic disease, increase of invasive techniques and treatments

4. EMERGENCE OF NEW HEALTH HAZARDS FOR THE COMMUNITY

RESERVOIRS OF NOSOCOMIAL INFECTION

1. **PATIENT:** SELF-INFECTION FROM THE PATIENT´S OWN FLORA

2. **OTHER PATIENTS, MEDICAL STAFF:** CROSS-INFECTION PATIENT TO PATIENT (CAUSED BY "HOSPITAL" STRAINS)

3. **EQUIPMENT AND MATERIALS IN USE IN HOSPITALS** (WATER, DISINFECTANTS, BEDS, FOOD, DUST…..)

ROUTES OF TRANSMISSION

1. AIR-BORNE

2. CONTACT SPREAD

3. FOOD-BORNE SPREAD

4. BLOOD-BORNE SPREAD

5. SELF-INFECTION AND CROSS-INFECTION

FACTORS

1. **MICROORGANISM**

2. **PATIENT-SUSCEPTIBILITY**

   AGE
   IMMUNE DEFENSES
   UNDERLYING DISEASE
   ANTIBIOTIC, IMMUNOSUPPRESSOR TREATMENTS
   MALNUTRITION

3. **ENVIRONMENT (WATER, AIR, FOOD)**

4. **RESISTANCE TO ANTIBIOTICS**

DEFINITIONS

ISOLATE: PURE CULTURE OF BACTERIA OBTAINED BY SUBCULTURE OF A SINGLE COLONY FROM A PRIMARY ISOLATION PLATE, PRESUMED TO BE DERIVED FROM A SINGLE ORGANISM.

EPIDEMIOLOGICALLY RELATED ISOLATES: DERIVED FROM A COMMON SOURCE AND CULTURED FROM SPECIMENS COLLECTED FROM PATIENTS, FOMITES, OR THE ENVIRONMENT DURING A DISCRETE TIME FRAME OR FROM A WELL-DEFINED AREA AS PART OF AN EPIDEMIOLOGICAL INVESTIGATION.

GENETICALLY RELATED ISOLATES (CLONES): INDISTINGISHABLE FROM EACH OTHER BY A VARIETY OF GENETIC TESTS OR THAT ARE SO SIMILAR THAT THEY ARE PRESUMED TO BE DERIVED FROM A COMMON PARENT

DEFINITIONS

OUTBREAK: INCREASED INCIDENCE OF AN INFECTIOUS DISEASE IN A SPECIFIC PLACE DURING A GIVEN PERIOD THAT IS ABOVE THE BASELINE RATE FOR THAT PLACE AND TIME FRAME

STRAIN: ISOLATE OR GROUP OF ISOLATES THAT CAN BE DISTINGUISHED FROM OTHER ISOLATES OF THE SAME GENUS AND SPECIES BY PHENOTYPIC OR GENOTYPIC CHARACTERISTICS OR BOTH.

* A STRAIN IS A DESCRIPTIVE SUBDIVISION OF A SPECIES

DEFINITIONS

OUTBREAK STRAINS :

* ISOLATES OF THE SAME SPECIES THAT ARE BOTH EPIDEMIOLOGICALLY RELATED AND GENETICALLY RELATED

* PRESUMED TO BE CLONALLY RELATED

ENDEMIC STRAINS:

* ISOLATES RECOVERED FREQUENTLY FROM INFECTED PATIENTS IN A PARTICULAR HEALTH CARE SETTING OR COMMUNITY INDISTINGUISHABLE OR CLOSELY RELATED TO EACH OTHER

* PRESUMED TO BE CLONALLY RELATED

TYPING TECHNIQUES/OBJETIVES

1. DETERMINATION OF THE ORIGIN AND EXTENSION OF AN INFECTIOUS OUTBREAK

2. ESTABLISHMENT OF CROSS-INFECTIONS (PATIENT TO PATIENT)

3. STUDY EVOLUTION OF INFECTION ALONG TIME

4. EVALUATION OF ANTIBIOTIC TREATMENT EFICACY, LEVELS OF RESISTANCE AND PATIENT´S IMMUNE RESPONSE

QUALITY ASPECTS OF MICROBIAL TYPING

1. TYPABILITY
2. REPRODUCIBILITY
3. DISCRIMINATORY CAPACITY
4. APPROPIATE COST
5. STANDARDISATION

PHENOTYPIC TECHNIQUES

BIOTYPING
ANTIBIOGRAM TYPING
SEROTYPING
PHAGE-TYPING
PROTEIN TYPING

DISADVANTAGES
REPRODUCIBILITY
DISCRIMINATORY CAPACITY
NON-TYPEABLE ISOLATES
COST

GENOTYPIC TYPING METHODS: ADVANTAGES

1. RAPIDITY

2. SENSITIVITY

3. SPECIFICITY

4. RESULTS ARE NON-DEPENDENT ON PHENOTYPIC EXPRESSION

GENOTYPIC TYPING METHODS

1.RESTRICTION ENZYMES
- -REA/RFLP
- -RIBOTIPING
- -PFGE

2.PLASMID ANALYSIS

3.POLYMERASE CHAIN REACTION
- -MAAP (AP-PCR, RAPD, DAF-PCR)
- - REPETITIVE SEQUENCES (ERIC & REP-PCR)
- -PCR-RIBOTIPING
- -MULTIPLEX-PCR
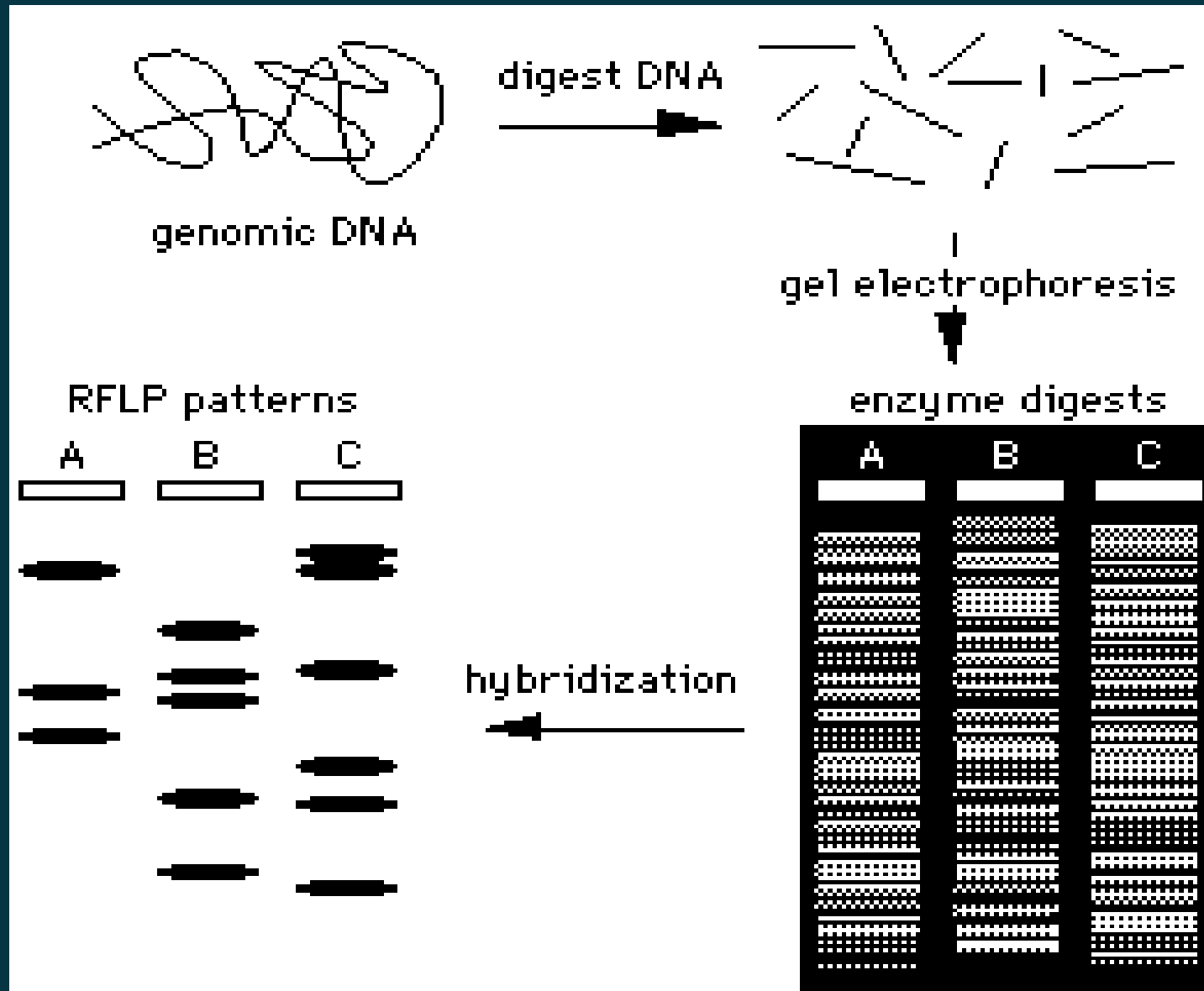- -NESTED-PCR

4.DNA SEQUENCING: SLST & MLST

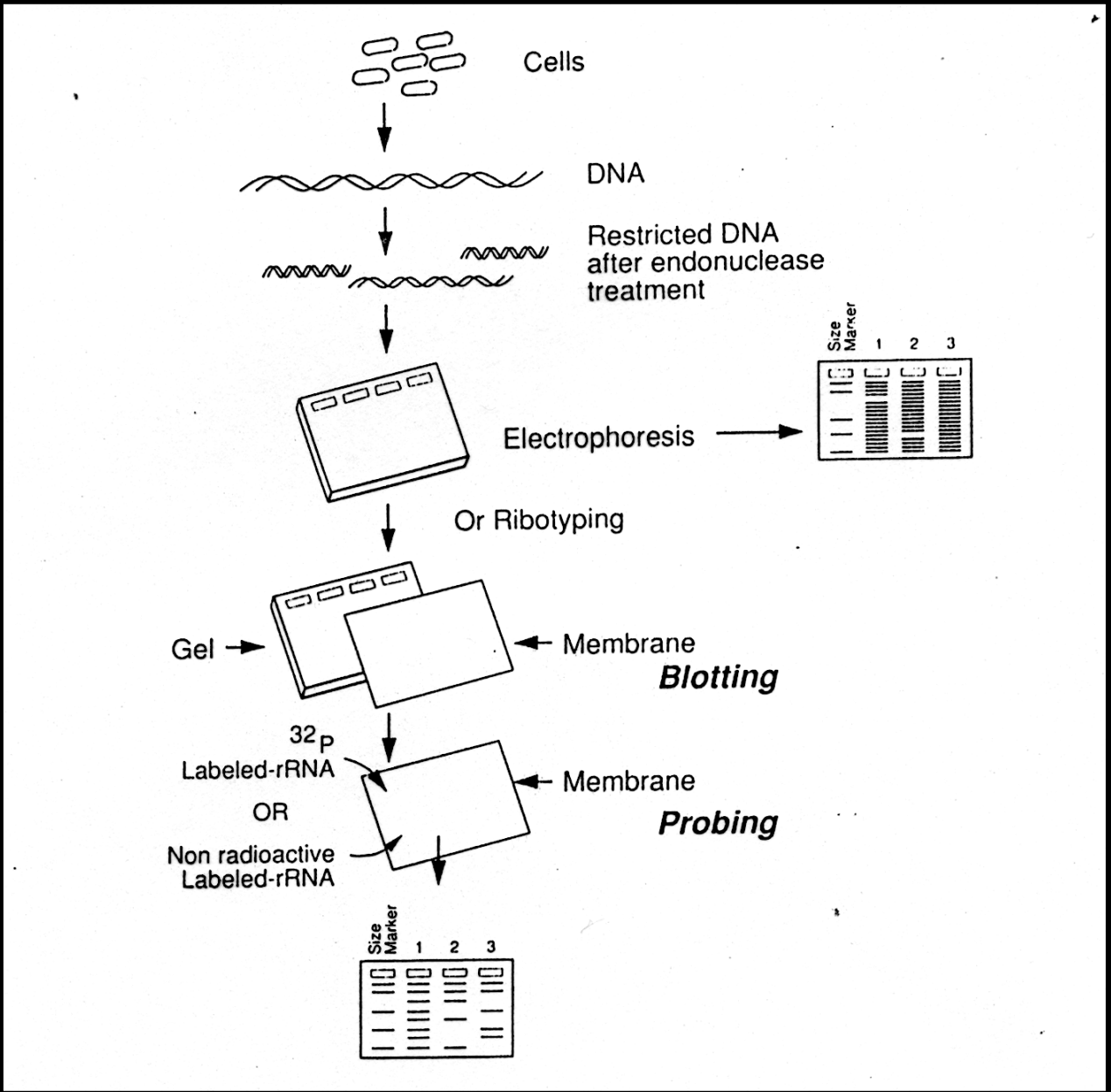# 1. RESTRICTION ENZYMES
   -REA/RFLP
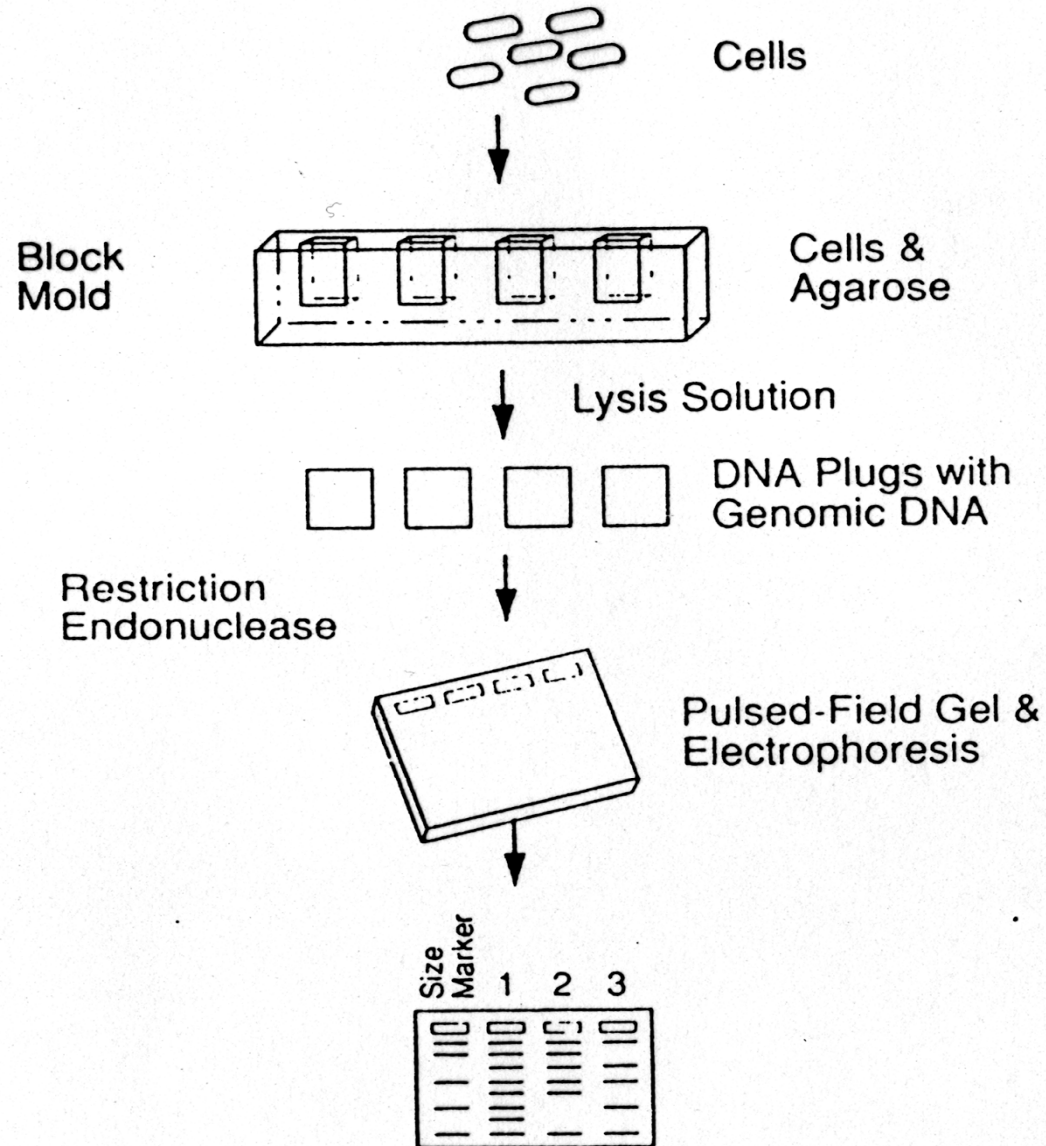   -RIBOTYPING
   -PFGE

RFLP

RIBOTYPING

PFGE

PFGE APPLICATIONS

1.- IDENTIFYING RESTRICTION FRAGMENT LENGHT POLYMORPHISMS

(USING LOW-FREQUENCY CUTTING ENZYMES, TYPICALLY WITH LESS THAN 30 CLEAVAGE SITES PER GENOME)

2.- CONSTRUCTION OF PHYSICAL MAPS

3.-DETERMINING THE NUMBER AND SIZE OF CHROMOSOMES (ELECTROPHORETIC KARYOTYPE)

4.- STUDY OF HIGH MOLECULAR WEIGHT PLASMIDS

5.- OTHERS: CLONING LARGE DNA USING ARTIFICIAL CHROMOSOMES; DETECTING "IN VIVO" CHROMOSOME BREAKAGE AND DEGRADATION

PFGE:ANALYSIS AND INTERPRETATION OF DATA

RESTRICTION PATTERNS:

RELATED ISOLATES: SAME PATTERNS

NON RELATED ISOLATES: DIFFERENT PATTERNS

MINOR PATTERNS DIFFERENCES ARISE FROM:

* POINT MUTATIONS

* INSERTIONS

* DELECTIONS

CRITERIA FOR INTERPRETING PFGE PATTERNS

CATEGORY	No. GENETIC DIFFERENCES	No. DIFFERENT FRAGMENTS	INTERPRETATION
INDISTINGUISHABLE	0	0	PART OF THE OUTBREAK
CLOSELY RELATED	1	2-3	PROBABLY PART OF THE OUTBREAK
POSSIBLY RELATED	2	4-6	POSSIBLY PART OF THE OUTBREAK
DIFFERENT	≥3	≥ 7	NOT PART OF THE OUTBREAK

2. PLASMID ANALYSIS
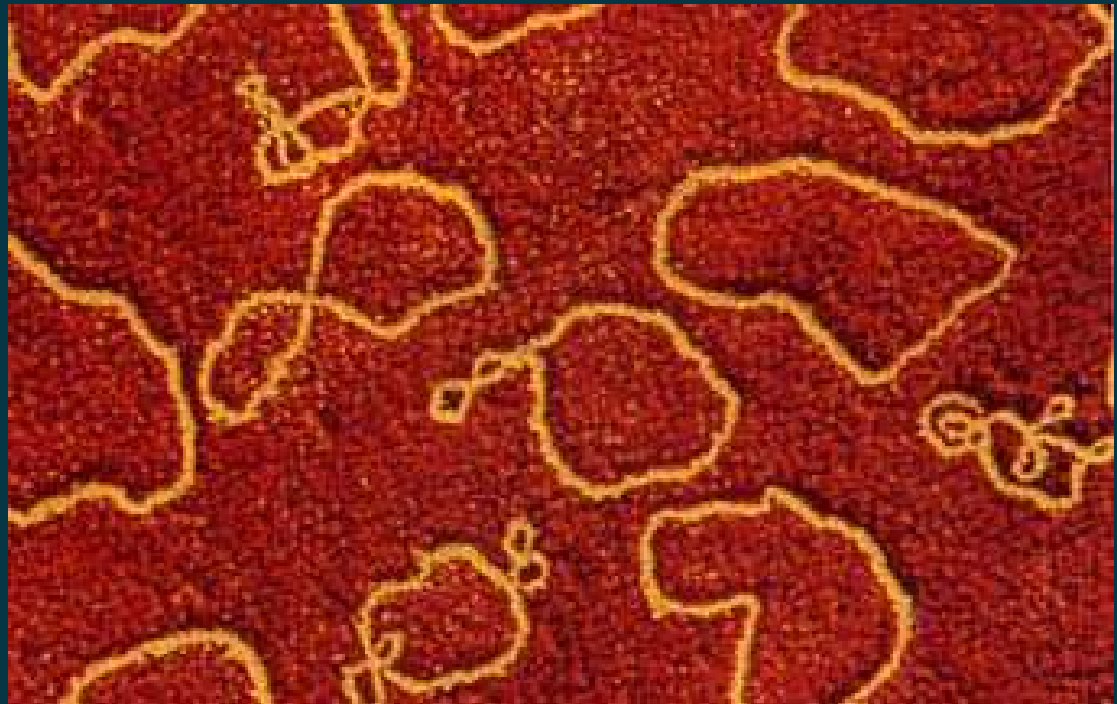
PROPIERTIES ENCODED BY PLASMIDS

- 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 FOR PLASMID ANALYSIS

1. CONVENTIONAL LYSIS METHODS (BY ALKALY, SDS, PROTEINASE K.....)

2. COMMERCIAL KITS
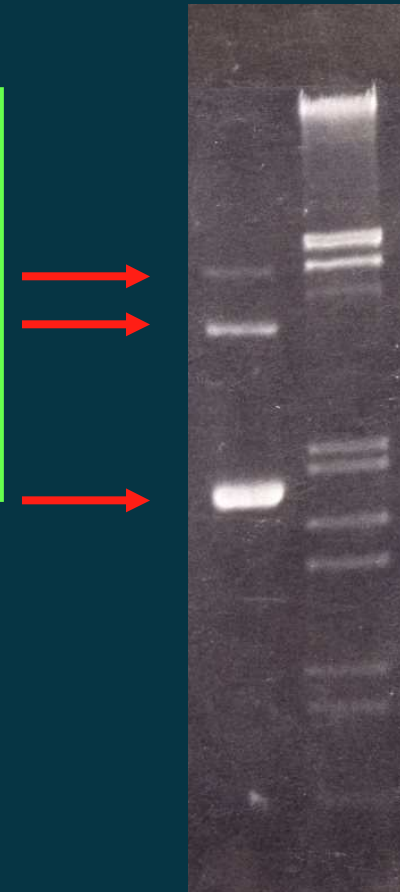
DISADVANTAGES

- NOT VERY USEFUL VERY CLINICAL ISOLATES
- REPRODUCIBILITY
- YIELD
- PLASMID INSTABILITY

LIMITATIONS OF CONVENTIONAL TECHNIQUES

1. DETERMINATION OF THE EXACT SIZE OF THE PLASMID: PLASMID CONFORMATION AFFECTS ELECTROPHORETIC MOBILITY

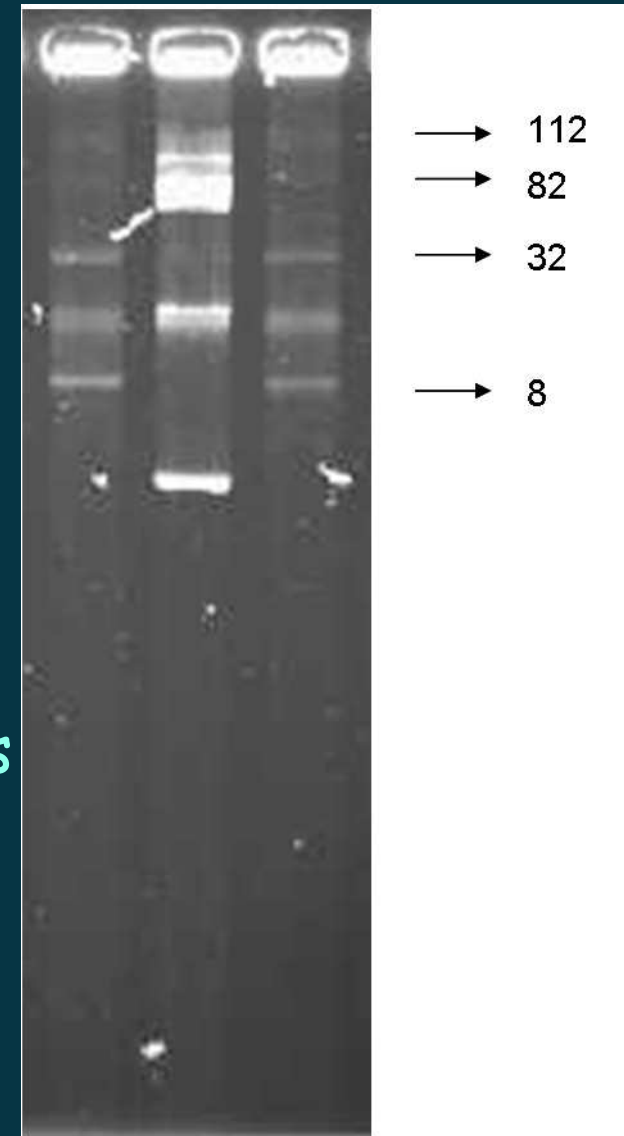**1st- CCC** (CIRCULAR COVALENTLY CLOSED)

2nd- OC/ L (OPEN CIRCULAR /LINEAR)

LIMITATION OF CONVENTIONAL TECHNIQUES

2-MEGAPLASMIDS:
- NOT VISIBLE
- EASY BREAKAGE

Conventional agarose gel electrophoresis

* Arrows indicate the size (in Kb) of visible plasmids

112
82
32
8

PFGE/ S1 NUCLEASE DIGESTION TECHNIQUE TO MAKE MEGAPLASMIDS VISIBLE

1-INTACT PLASMIDIC DNA IS OBTAINED

2-S1 DIGESTION
(ONLY L FORMS ARE VISIBLE)

3-PFGE
(MEGAPLASMIDS ARE VISIBLE)

3. POLYMERASE CHAIN REACTION

- -MAAP (AP-PCR, RAPD, DAF-PCR)
- - REPETITIVE SEQUENCES (ERIC & REP-PCR)
- -PCR-RIBOTYPING
- -MULTIPLEX-PCR
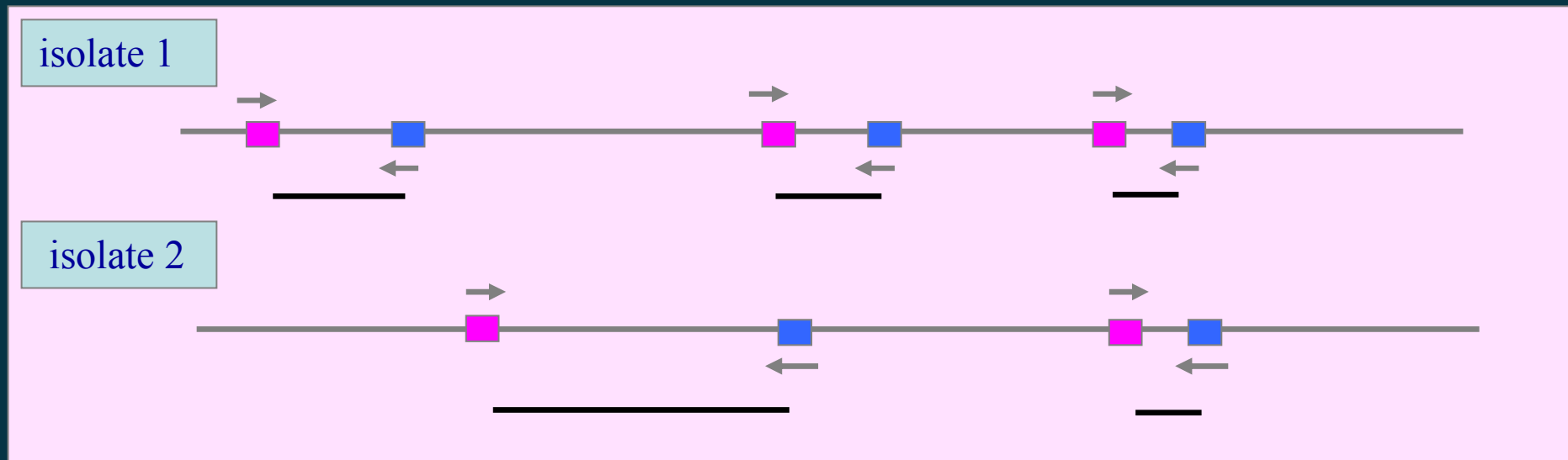- -NESTED-PCR
- -AFLP

POLYMERASE CHAIN REACTION

ADVANTAGES

- SENSITIVITY
- RAPID
- DETECTION OF FASTIDIOUS ORGANISMS
- NO NEED OF VIABLE CELLS
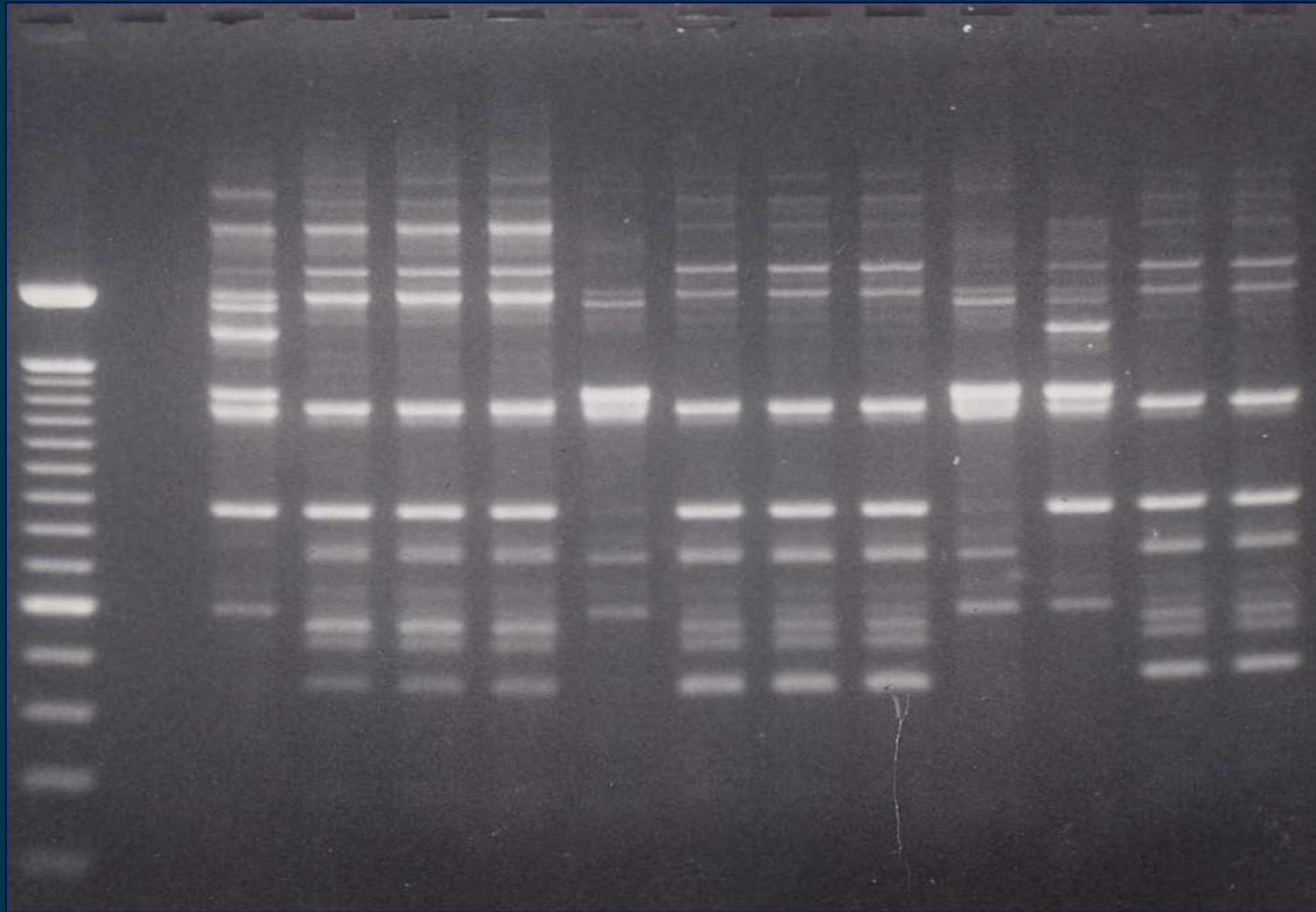- DETECTION OF UNKNOWN SEQUENCES

DISADVANTAGES

- FALSE-POSITIVE/NEGATIVE RESULTS
- REPRODUCIBILITY
- INTERLABORATORY VALIDATION
- CLINICAL INTERPRETATION

PCR fingerprinting

-MAAP (AP-PCR, RAPD, DAF-PCR)
- REPETITIVE SEQUENCES (ERIC & REP-PCR)

Different amplification profiles corresponding to different clones

PCR fingerprinting

ADVANTAGES:

100% TIPABILITY

RAPIDITY
MÍNIMUM SAMPLE NEEDED
COST
USEFUL FOR COMPARISON

ADVANTAGES:

REPRODUCIBILITY
CONTAMINATION
FALSE-POSITIVE RESULTS
LABORATORY
EASY TRAINING

PCR fingerprinting: RESULT DEPENDS ON

1. LABORATORY

2. QUALITY OF DNA

3. REACTION PARAMETERS:
   Enzyme
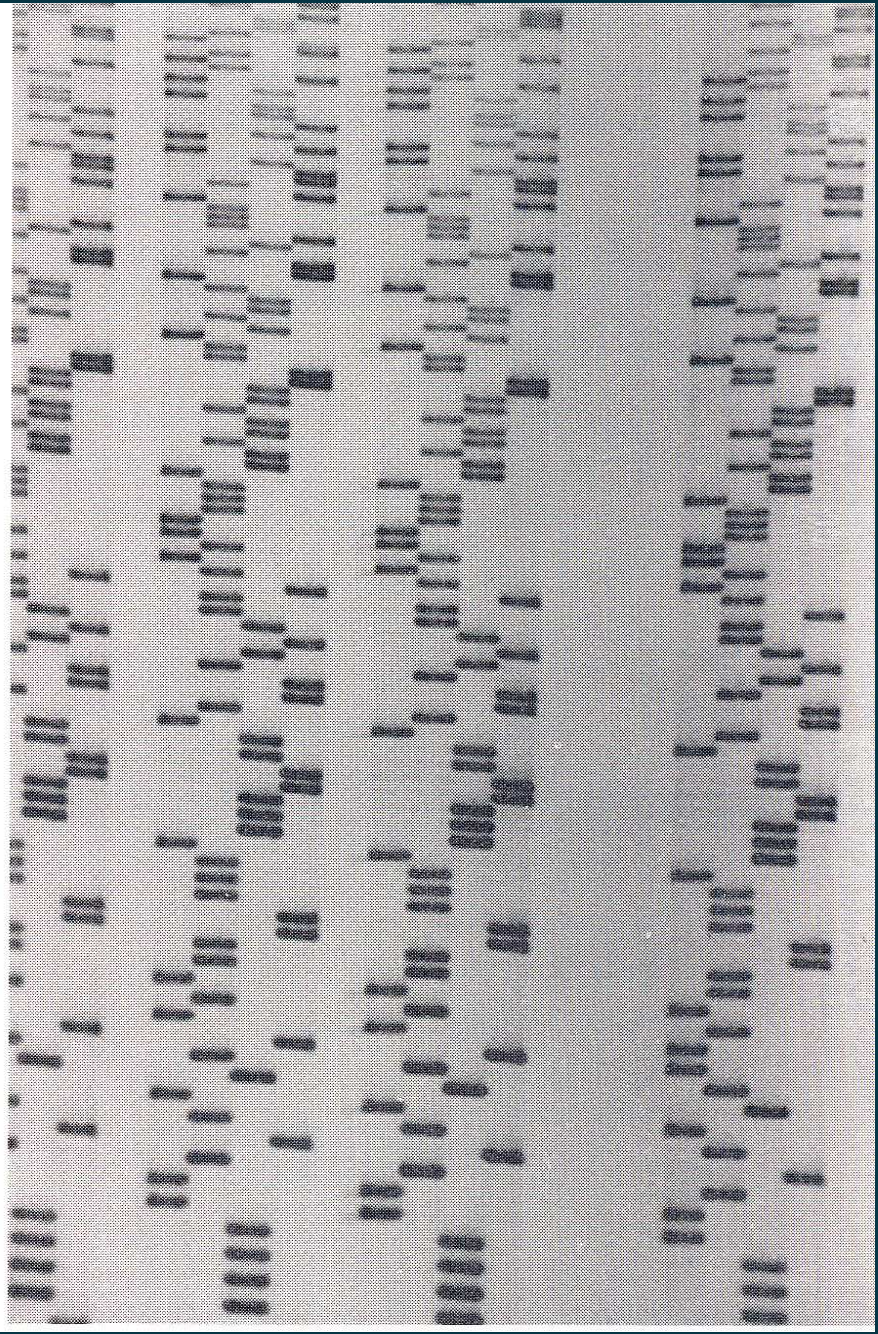   Primers
   $ClMg_2$
   Cycling conditions

4. CONTROLS

4. DNA SEQUENCING:
- SLST
- MLST

SINGLE-LOCUS SEQUENCE TYPING

-BASED ON INDIVIDUAL NUCLEOTIDE DIFFERENCES
IN GENES CODING FOR:
- VIRULENCE,
- PATOGENICITY,
- ANTIBIOTIC RESISTANCE.....

-ANALYSIS OF :
- POLIMORPHISMS OF A SINGLE NUCLEOTIDE
- REPETITIVE-SEQUENCE AREAS

MULTILOCUS SEQUENCE TYPING

-ANALYSIS OF A MAJOR PORTION OF GENOME COMPARING REGIONS OF 400-500 bp CORRESPONDING TO "HOUSEKEEPING" GENES (>7)

-POLYMORPHISMS OF SEQUENCES ARE CONSIDERED ALLELES

-ISOLATES ARE DEFINED BY ITS ALLELE PROFILES CORRESPONDING TO SEQUENCED LOCUS

CONCLUSIONS

1. NOSOCOMIAL INFECTIONS ARE EMERGING INFECTIOUS DISEASES

2. RESISTANCE TO ANTIBIOTICS IS A HEALTH HAZARD FOR THE COMMUNITY

3. GENETIC TECHNOLOGY
IS A VERY USEFUL TOOL
TO CONTROL THE SPREAD
OF MICROORGANISMS
IN THE HOSPITAL
ENVIRONMENT