King Saud University
Department of Botany &
Microbiology

Microbial Diagnostic
320 MIC

Lecture 6

Genotypic methods

- **Genetic methods generally seek to detect at the level of nucleic acid**
- **Genotypes are more specific, more easily, quantified and standardized among the different organisms**
- **The genome is unique in individual**
- **Several molecular methods in a chosen genetic marker are commonly used to target the genome or the organism**

Looking for mutations

Molecular Diagnostics (Molecular Microbiology) Why?

- very powerful tools
- Qualitative Testing
- need for rapid answer for Rapid Detection and Diagnosis of human pathogens (presence or absence)
- Genetic methods can much more rapidly determine the species of a particular isolate, often without the need to isolate a pure culture or unculturable or difficult to culture. These tests can provide information as to whether a particular gene is present which is important in determining if a particular isolation is pathogenic) .
- inadequacy of phenotypic methods (biochemical)
- Prognosis and management of infected individuals
- need for quantitative information (viral)
- susceptibility testing (drug resistance testing) without culture
- Molecular resistance testing to detect mutations associated with resistance
- Molecular Epidemiology - molecular strain identification to examine disease for strain typing for epidemiological investigations (Outbreaks)
- Genetic tests can also provide increased differentiation between isolates within the same species. This subtyping is useful in the study of outbreaks of disease: are the isolates from the patients all the same and is there a common source from which the same subtype can be isolated.

Genotypic Methods

- Genotypic methods involve examin the genetic material of the organism and microbial identification and classification.

Genotypic Characteristics include:

Nucleic acid probe (Hybridyzation)

- **DNA amplification (Polymerase chain reaction (PCR) – Real time PCR (RT-PCR)- RAPD-PCR.**
- **rRNA sequencing**
- **pulsed Field Gel Electrophoresis PFLP**
- **plasmid fingerprinting**

- Increasingly genotypic technique are becoming the sole means of identifying many microorganisms because of its speed and accuracy.

Molecular Biology Techniques

A- Hybridization - Genetic probes (DNA or RNA probes):

*** Detection of a segment of DNA sequence (gene) in unknown organism using a labeled probe**

***The identification of microbe by DNA probe hybridization methods is based on the presence or absence of particular genes. This is in contrast to most biochemical and immunological tests that are based on the detection of gene products such as antigens or chemical end products of a metabolic pathway**

Probe: consists of specific short sequence of labeled single stranded DNA or RNA that form strong covalently bonded hybrid with specific complementary strand of nucleic acid of organism.

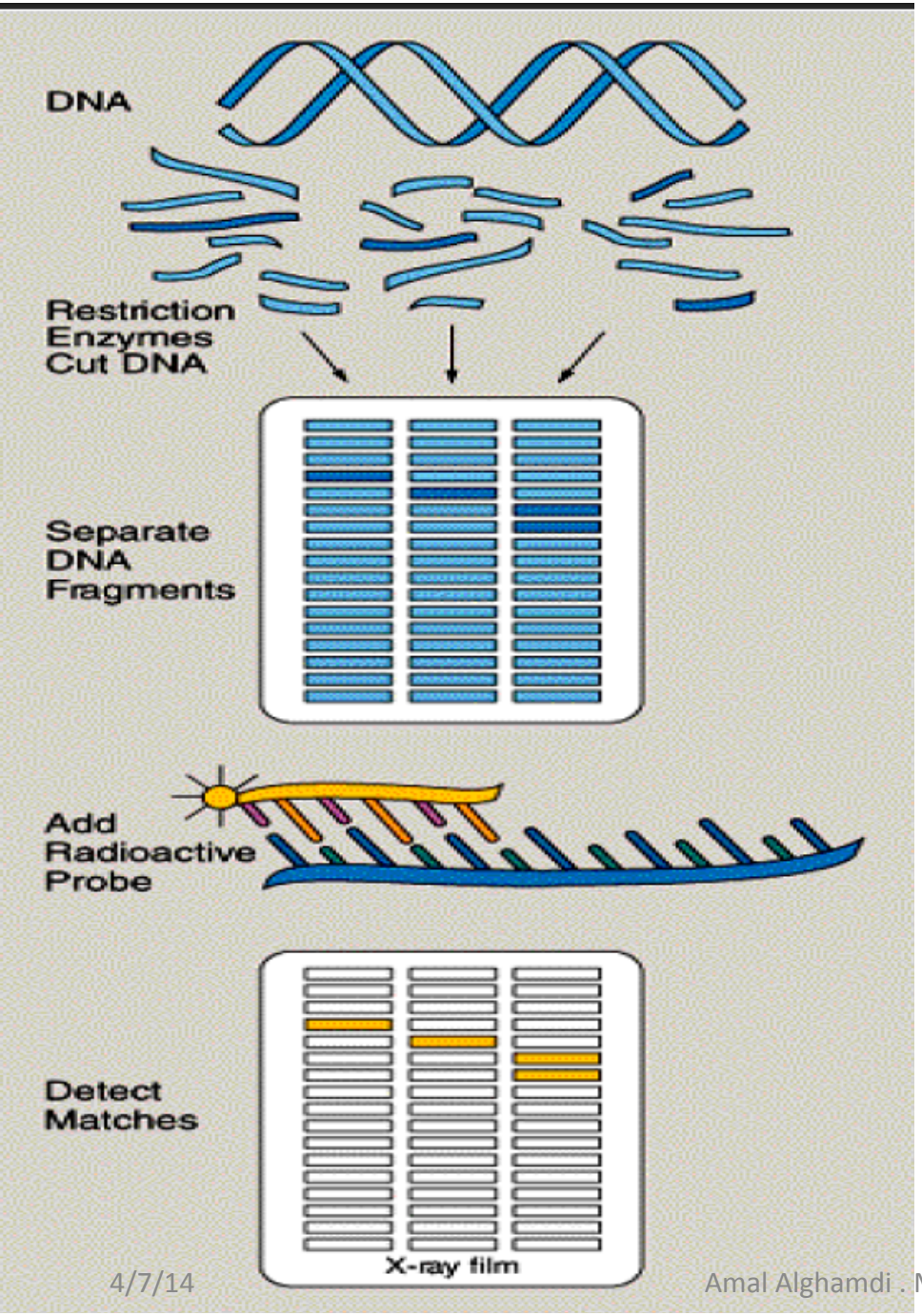
(DNA/RNA Hybridization- Colony Hybridization)

B- Polymerase chain reaction (PCR):

Amplification of a short sequence of target DNA or RNA

C- Plasmid profile analysis (plasmidtyping):

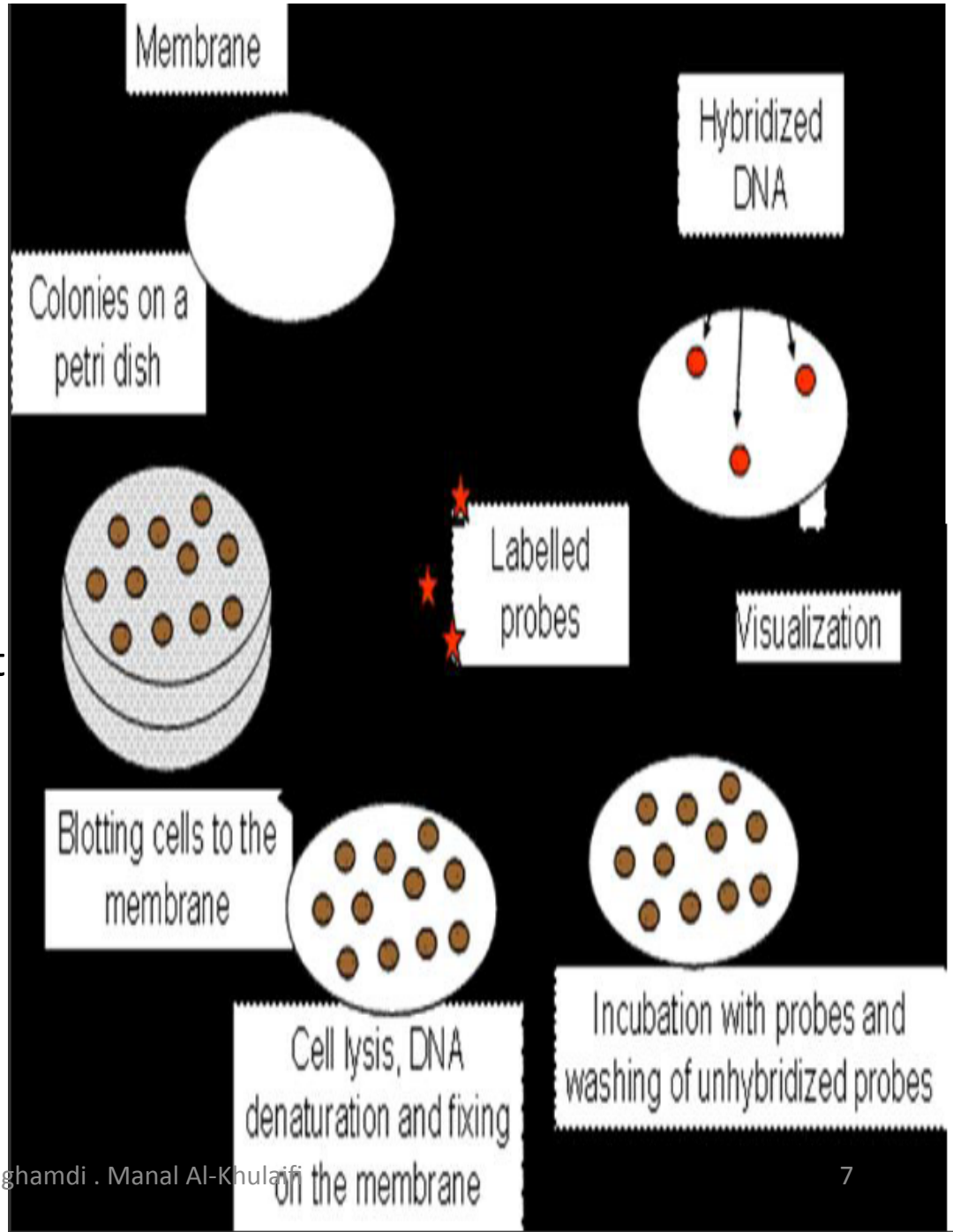
Isolation of plasmids from bacteria and determination of their size and number compared with standard strains by agarose gel electrophoresi.



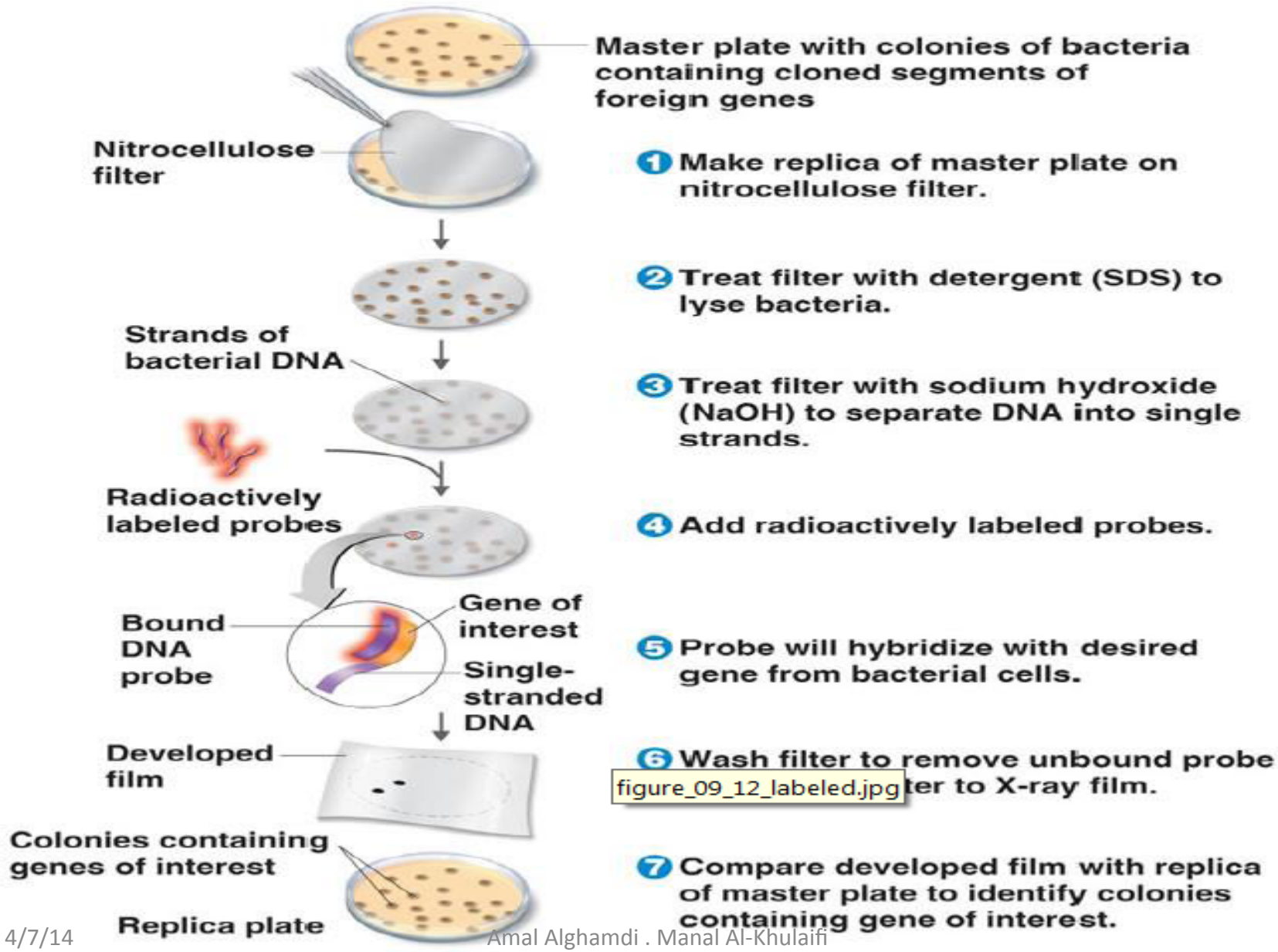
DNA probe - a length of single-stranded DNA that matches part of the gene and is linked to a radioactive atom. The single-stranded probe binds to the gene. Radioactive signals from the probe are then made visible on x-ray film, showing where the probe and gene matched.

Colony blot hybridization

Colony blot hybridization is applied to DNA or RNA released from blotted microbial colonies. The microbial colonies are transferred (blotted) to a membrane. The cells are lysed in place to release the nucleic acids. The RNA or DNA (after denaturation) is fixed to the filter and hybridized with a labeled probe. Blocking reagent may be added prior to the probe to prevent unspecific binding. Excess probe is washed away and the membrane is visualized by UV or autoradiography. Colony blot hybridization can be used for screening clones or bacterial isolates.

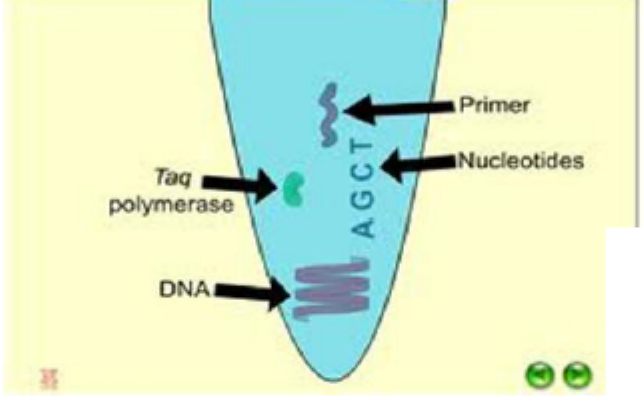


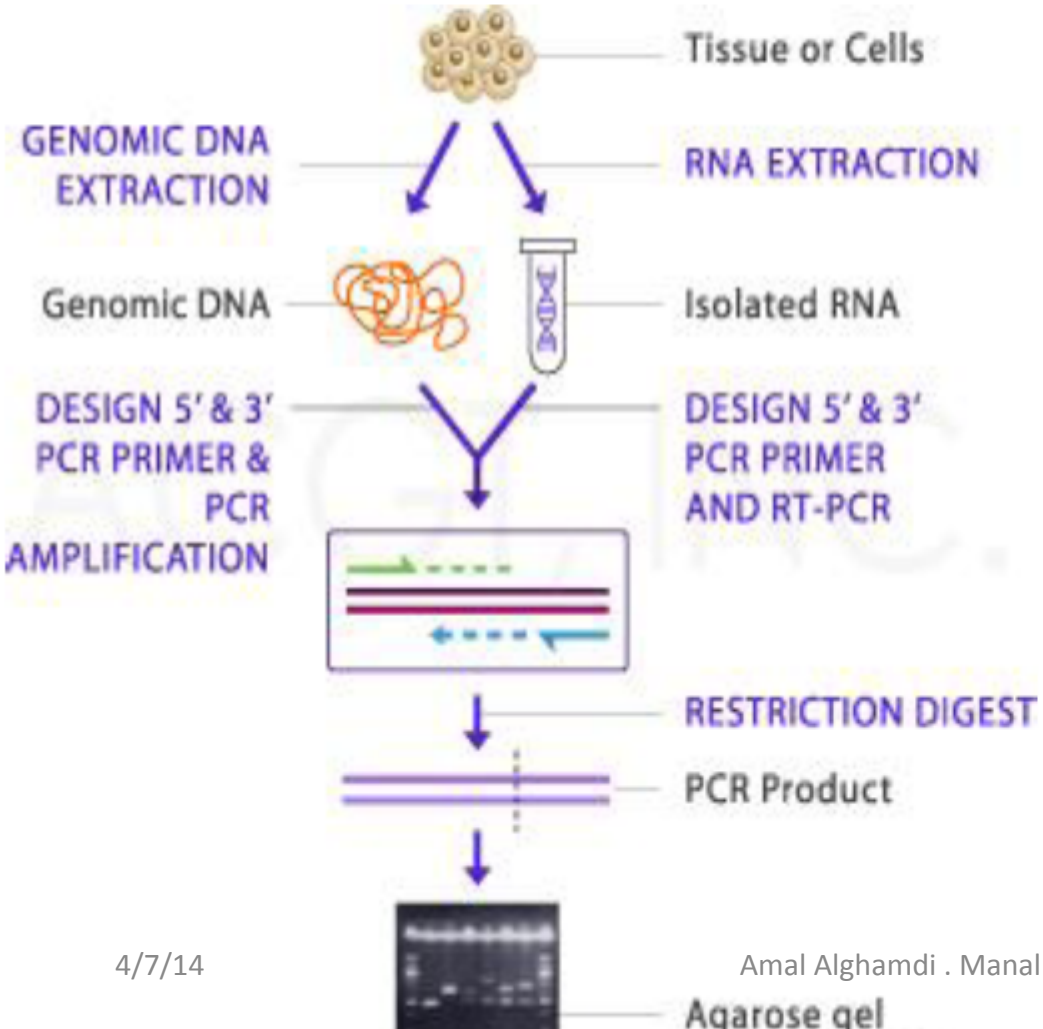
Colony Hybridyzation

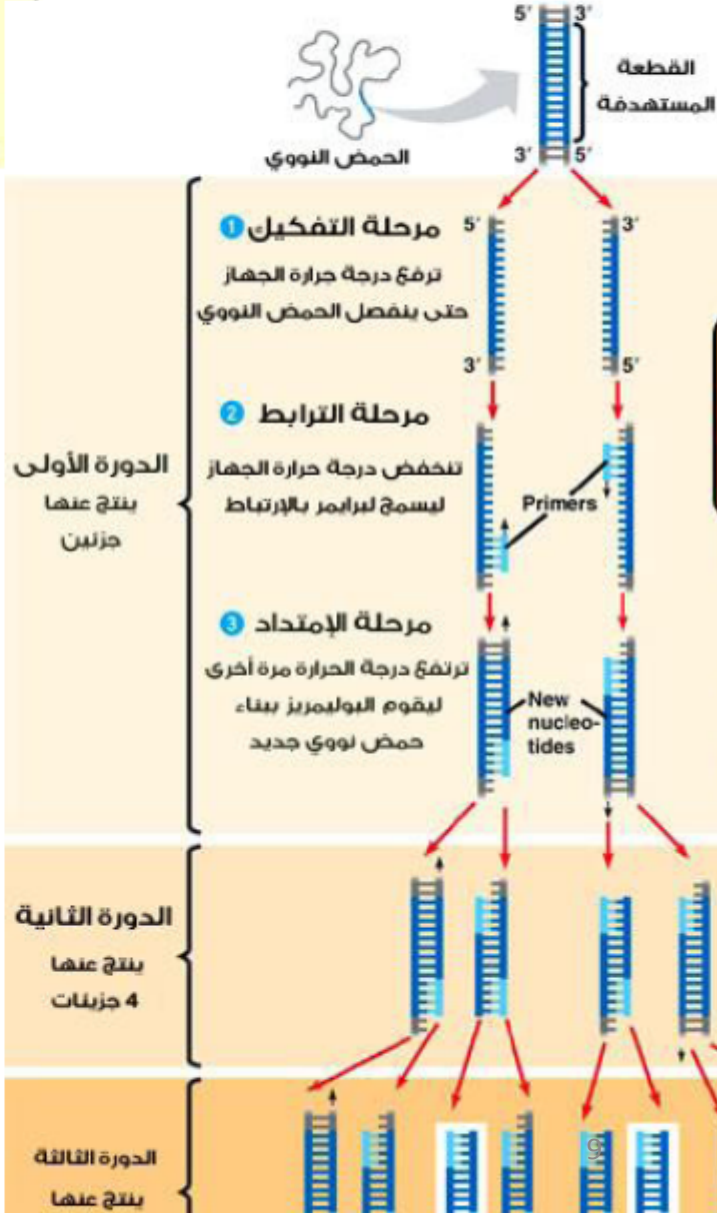


Genotyping by PCR

Helps rapid detection

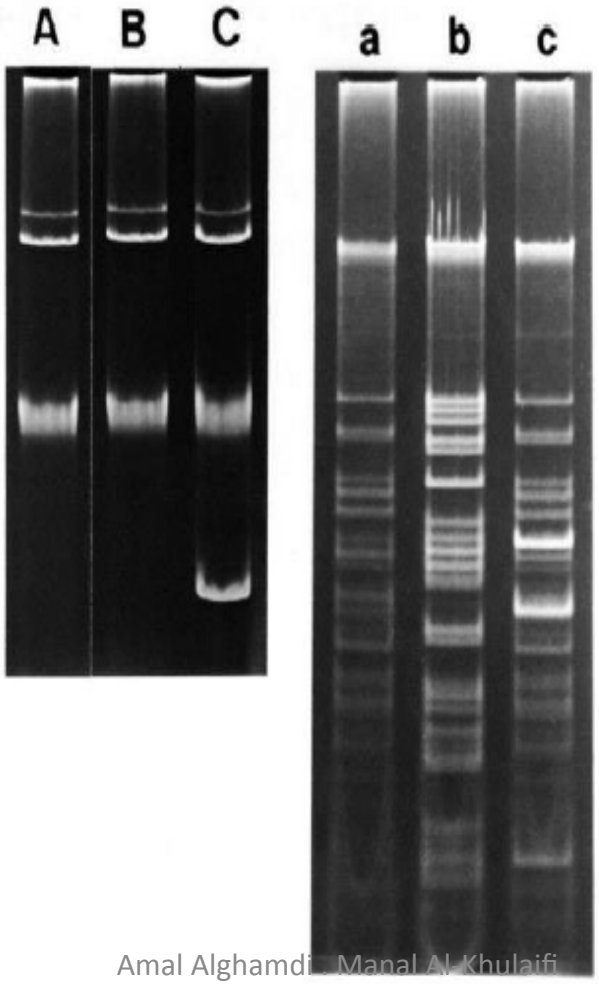


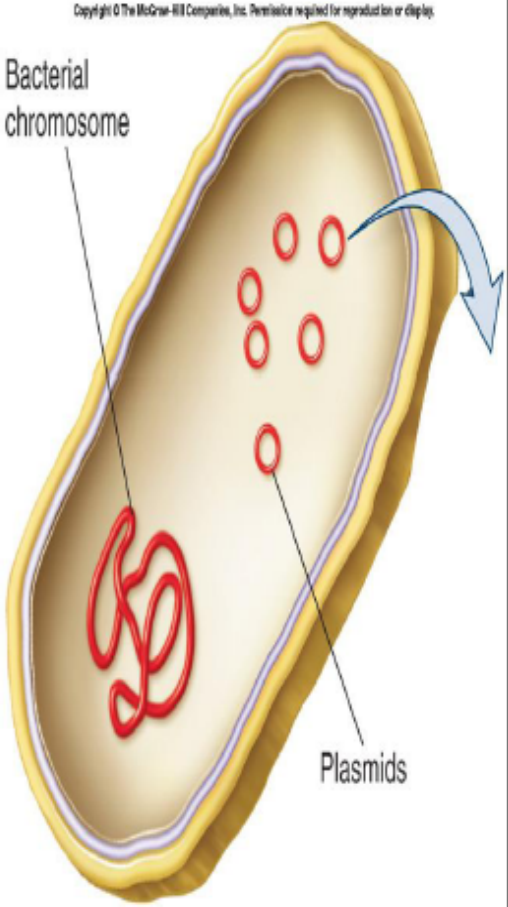




Plasmid fingerprinting

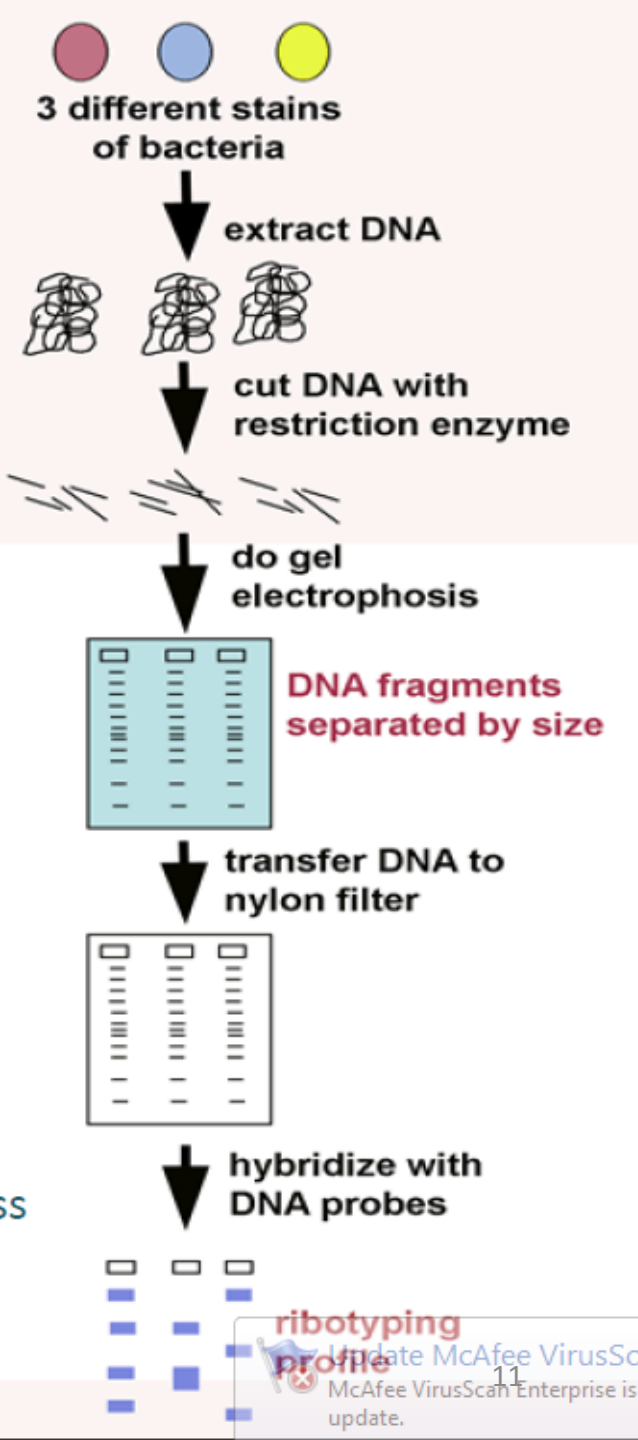
- characterizes bacteria based on number of plasmids and their molecular weight





Ribotyping

- used to identify bacterial genera
- based on high level of 16S rRNA conservation among bacteria
- rRNA encoding genes or fragments are amplified by PCR
- the nucleotide sequence of the amplified DNA is determined and compared with those in the National Center for Biotechnology (NCBI)



Advantages: allows to differentiate different strains of bacteria in a very sensitive manner

Disadvantages: Must carefully choose probe so there is no cross reactivity