

Molecular methods for microbial diagnosis (Genotyping)

Learning outcome

You should be able to:

- Explain each methods used for diagnosis
- Compare between probe and primer
- Understand the principle, steps, components, and applications of PCR

Genotyping methods

Genotyping methods includes:

1. Nucleic acid probes
2. PCR
3. Nucleic acid sequence analysis
4. 16s rRNA analysis

Nucleic acid probes

- Nucleic acid hybridization detect a specific DNA sequence within microorganism
- The process uses nucleic acid probe specific for that microorganism
- The target DNA is attached to the solid matrix such as nitrocellulose membrane or nylon phase.

Nucleic acid probes

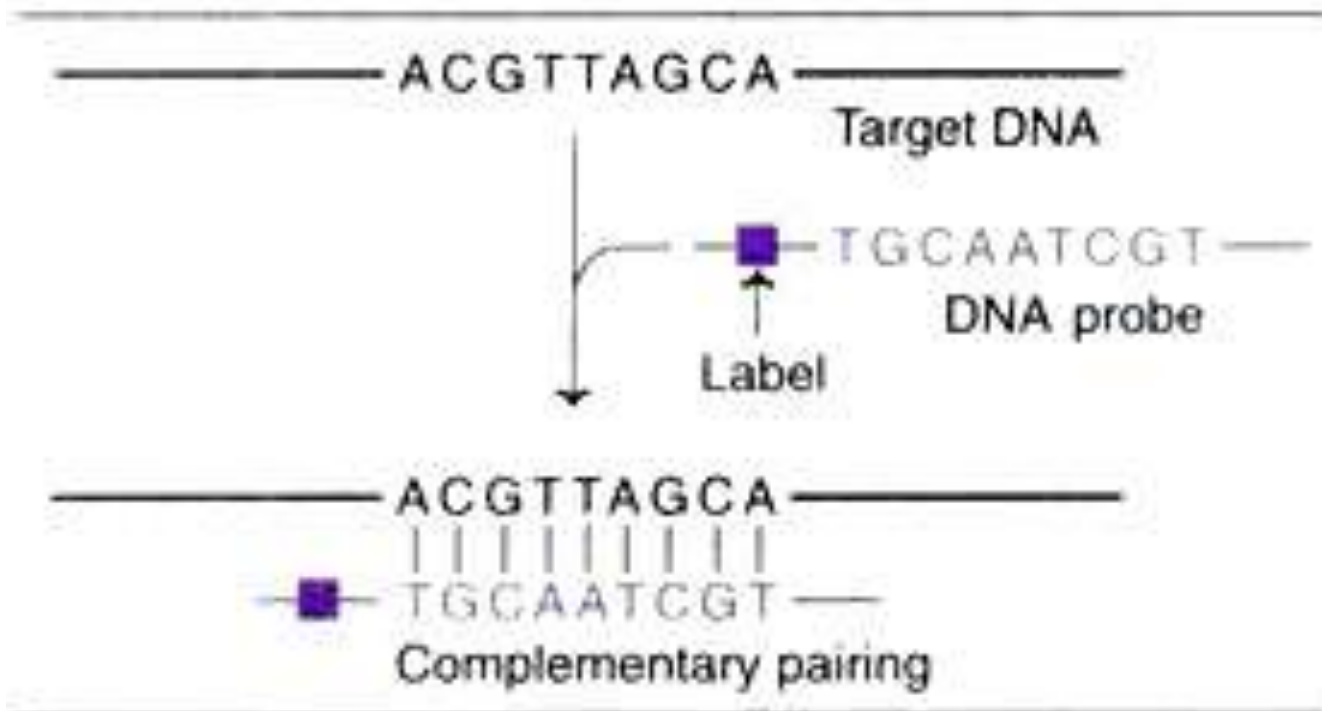


Fig. 14.1 : Hybridization of target DNA with DNA probe (with radioactive isotope label).

Advantages of using nucleic acid probe

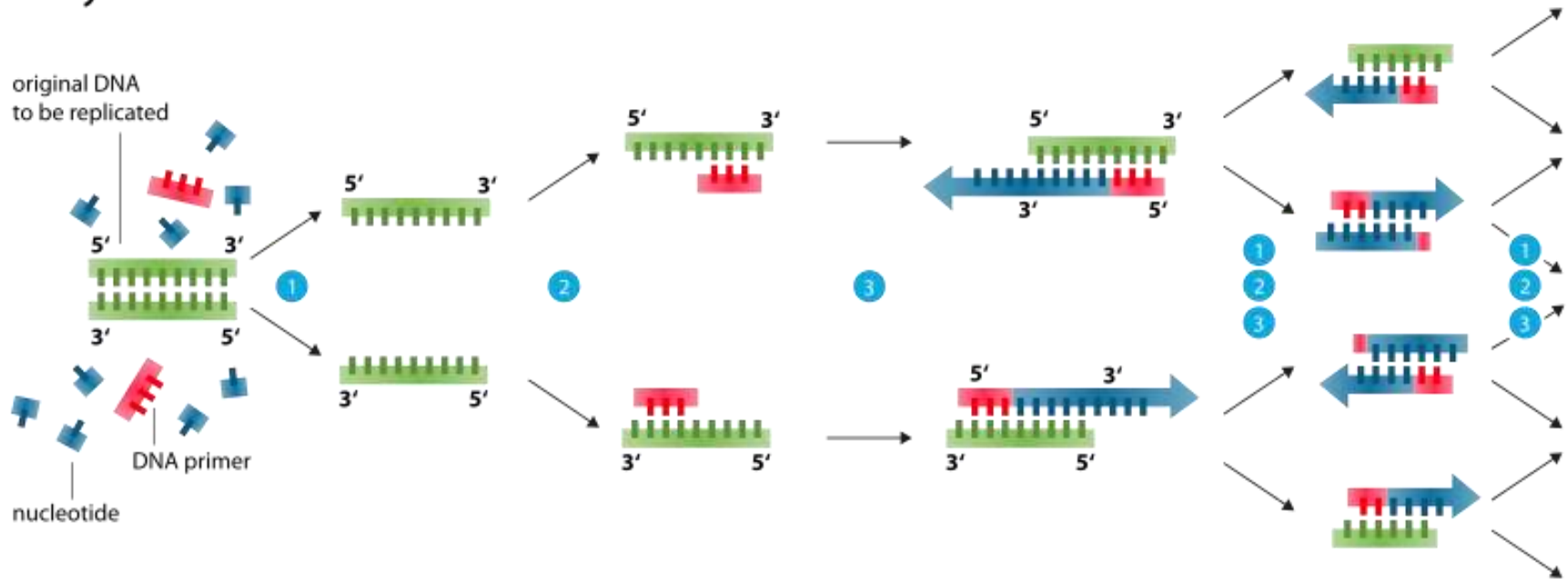
1. More specific than antibodies
2. More Stable (temperature, pH, organic solvents....)
3. Detection of no longer alive microorganism

Polymerase chain reaction (PCR)

1. Used widely for microorganisms identification
2. Sequence specific primer is used for DNA or RNA amplification of specific pathogen
3. Detection of few cells are present and viable non-culturable
4. Amplified DNA shows positive confirmation of gene existence of particular pathogen

PCR Steps

Polymerase chain reaction - PCR



- 1 **Denaturation** at 94-96°C
- 2 **Annealing** at ~68°C
- 3 **Elongation** at ca. 72 °C

PCR-Types

- Quantitative PCR
- Real-Time PCR
- Nested PCR
- Multiplex PCR
- Reverse transcriptase (rtPCR)

PCR Cycles	Target Copies
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024
15	32,768
20	1,048,576
25	33,554,432
30	1,073,741,842

PCR Components

1. DNA template (the sample DNA that contains the target sequence)
2. Deoxyribonucleoside triphosphates (dNTPs)
3. Primers (forward and reverse)
4. Taq polymerase
5. PCR buffer (Mg^{+2})

PCR Applications

- To analyze clinical specimens for the presence of infectious agents, including HIV, hepatitis, malaria, anthrax, etc.
- Providing information on a patient's prognosis, and predict response or resistance to therapy..

PCR Applications Cont.

- Detection of mutations that occur in many genetic diseases (e.g. cystic fibrosis, sickle cell anaemia, and muscular dystrophy).
- Forensics laboratories
- Cloning procedure
- The Human Genome Project

Thank you