Molecular identification and Troubleshooting

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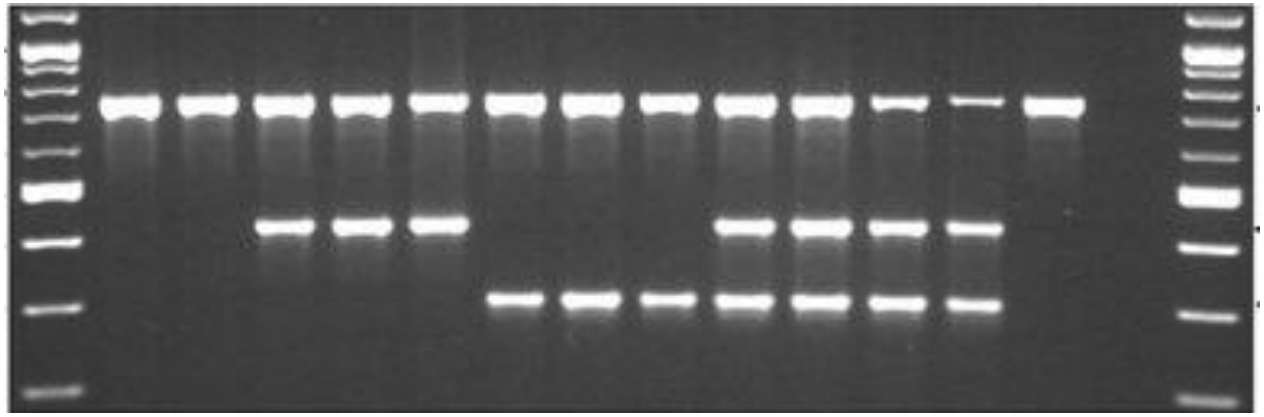
Nov. 2018

PCR based methods

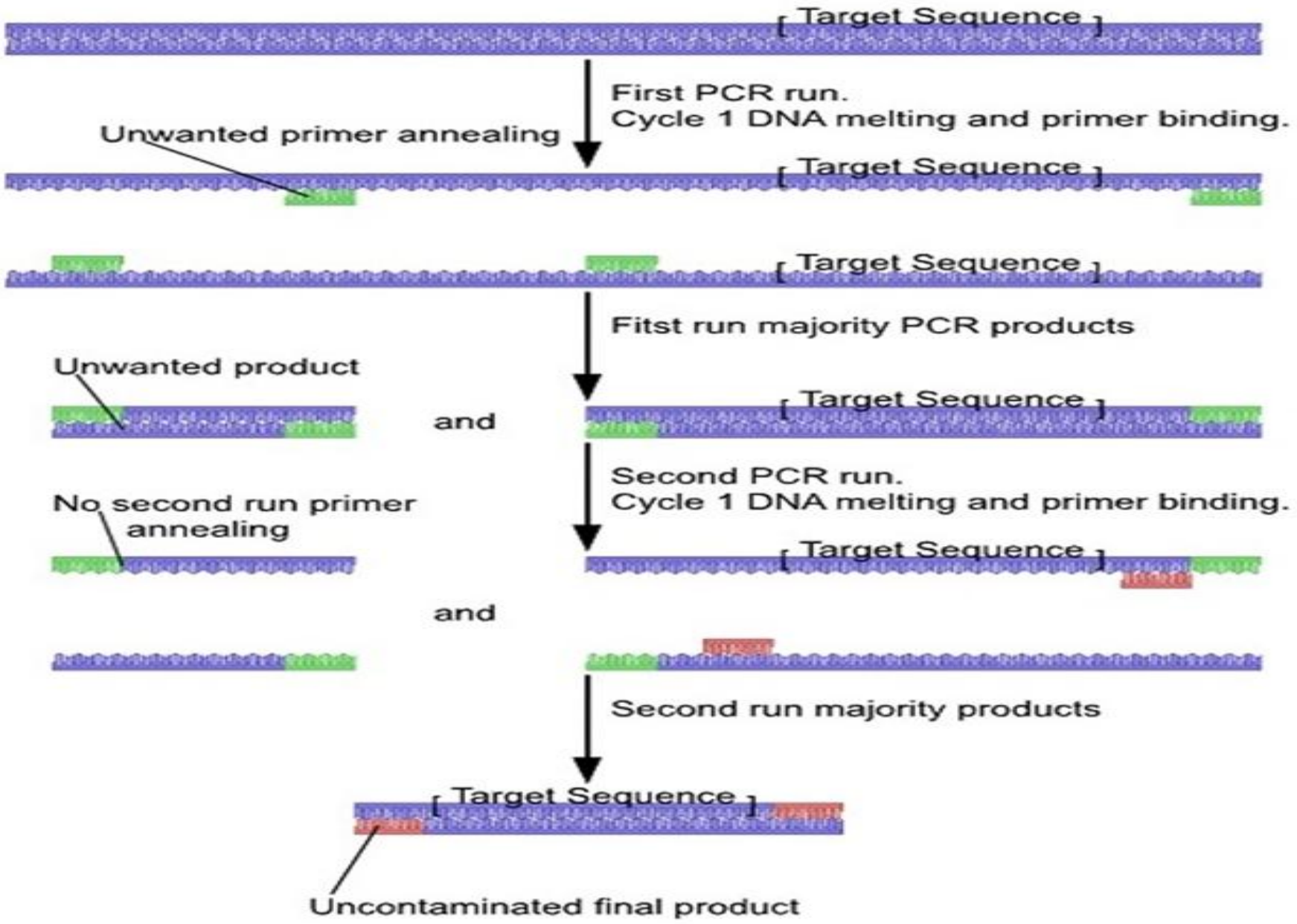
- Conventional PCR
- multiplex PCR

  Detection multiple organisms simultaneously
- reverse-transcription PCR



Nested PCR

- Reduce non-specific binding in products due to the amplification of unexpected primer binding sites
- More use to detect virus


Target Sequence
First PCR run.
Cycle 1 DNA melting and primer binding.
Unwanted primer annealing
Target Sequence
Target Sequence
Fitst run majority PCR products
Unwanted product
and
Target Sequence
Second PCR run.
Cycle 1 DNA melting and primer binding.
No second run primer annealing
Target Sequence
and
Second run majority products
Target Sequence
Uncontaminated final product

Transcription-mediated amplification [TMA]

* Transcription-mediated amplification (TMA) is an isothermal, single-tube nucleic acid amplification system utilizing two enzymes, RNA polymerase and reverse transcriptase, to rapidly amplify the target RNA, enabling the simultaneous detection of multiple pathogenic organisms in a single tube.

Transcription-mediated amplification [TMA]

* Transcription-mediated amplification has several advantages compared to other amplification methods including:
* TMA is isothermal; a water bath or heat block is used instead of a thermal cycler.
* TMA produces RNA/DNA amplicon rather than DNA amplicon. Since RNA is more labile in a laboratory environment, this reduces the possibility of carry-over contamination.
* TMA produces 100–1000 copies per cycle (PCR) exponentially doubles each cycle). This results in a 10 billion fold increase of DNA (or RNA) copies within about 15–30 minutes.

Real- Time PCR

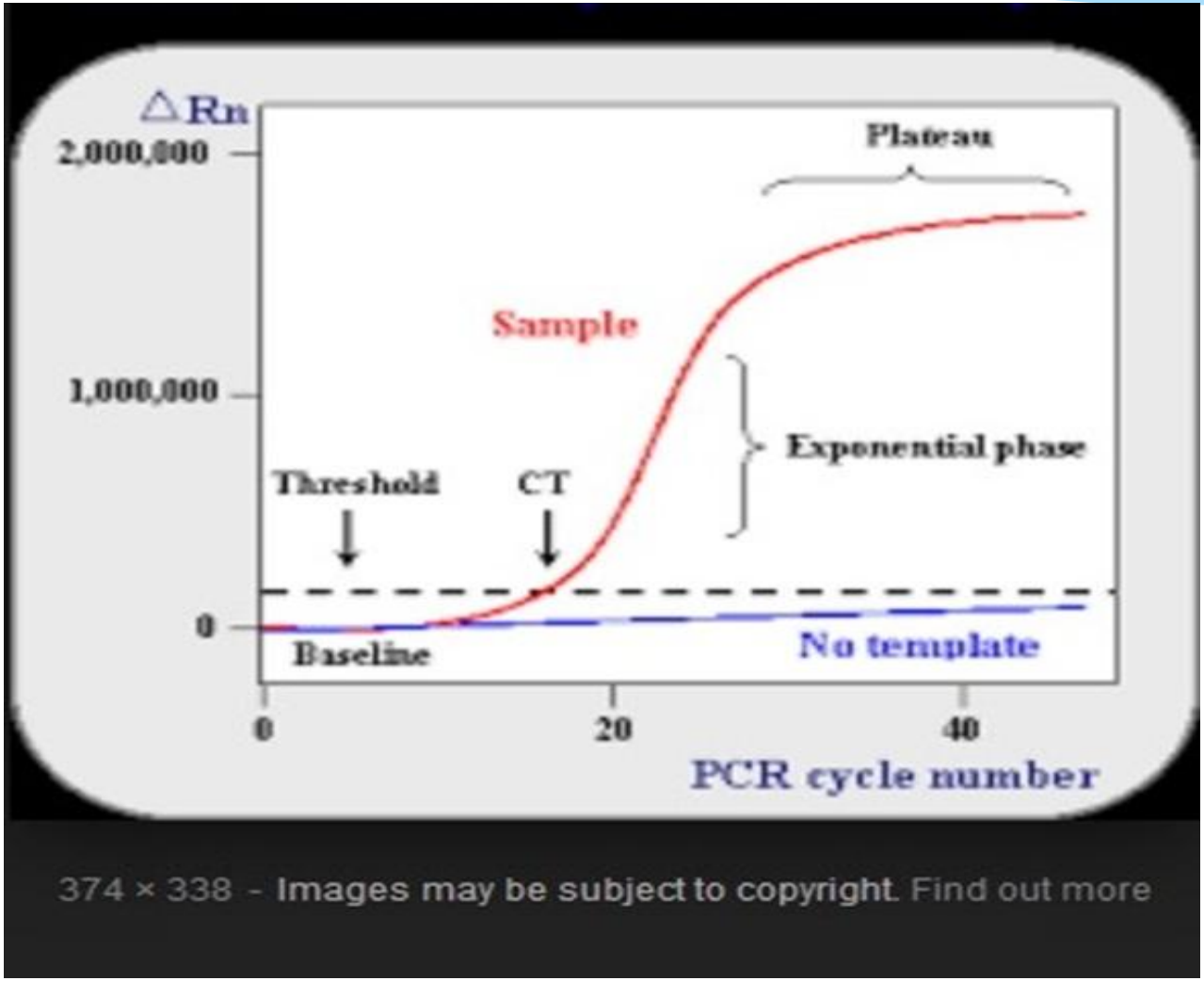
- It monitors the amplification of a targeted DNA molecule during the PCR, i.e. in real-time, and not at its end, as in conventional PCR.

  Quantitative

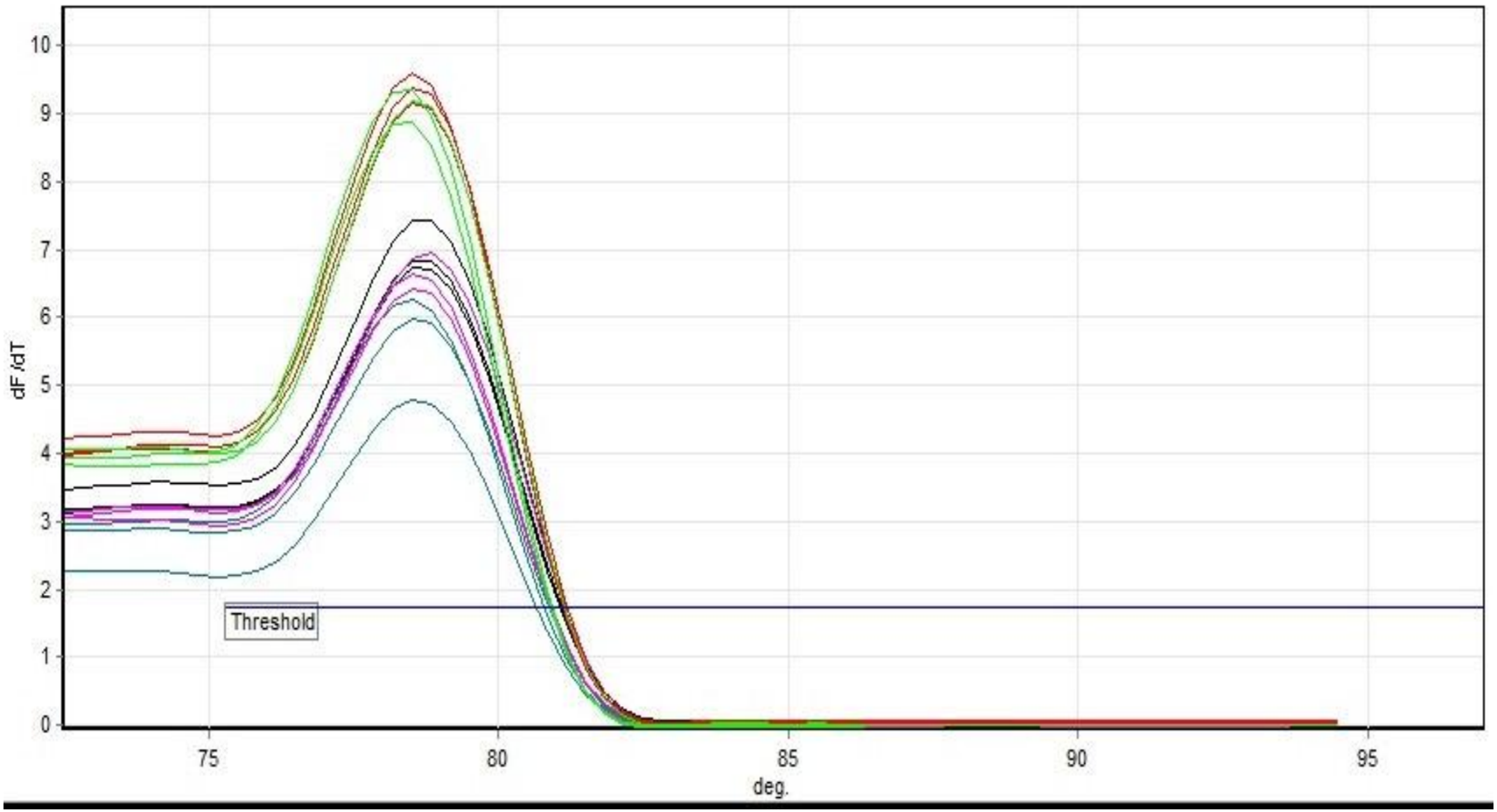
  Semi quantitative

  Qualitative

- With Primer
- With Primer and probe (more specific)



374 × 338 - Images may be subject to copyright. Find out more


10
9
8
7
6
5
4
3
2
1
0
dF /dT
Threshold
75
80
85
90
95
deg.

Trouble shutting

- Non- specific band
- Do not observed band in the positive control
- observed ban in negative control
- Unclear specific band
- Primer dimer in Real- Time PCR
- Omit specific band in the next PCR assay

Non- specific band

- Decrease primer concentration
- Decrease number of PCR cycles
- Increase primer annealing temperature
- Decrease DNA concentration

Do not observed band in the positive control

- PCR program
- Primer annealing temperature
- Number of PCR cycles
- Non- specific primer

observed ban in negative control

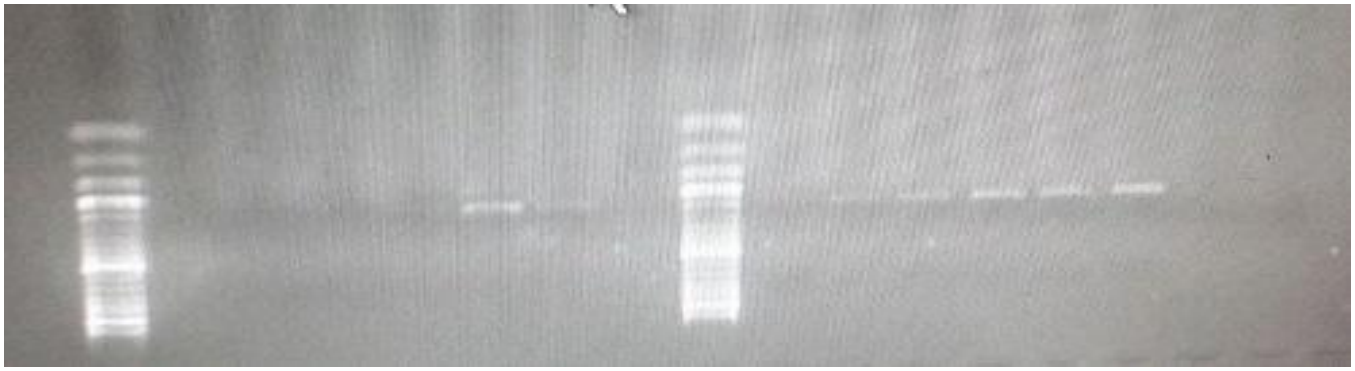
- Control water
- Control master mix
- Control other materials such as sampler tips

Omit specific band in the next PCR assay

- DNA should be keep in room temperature before PCR

Unclear specific band

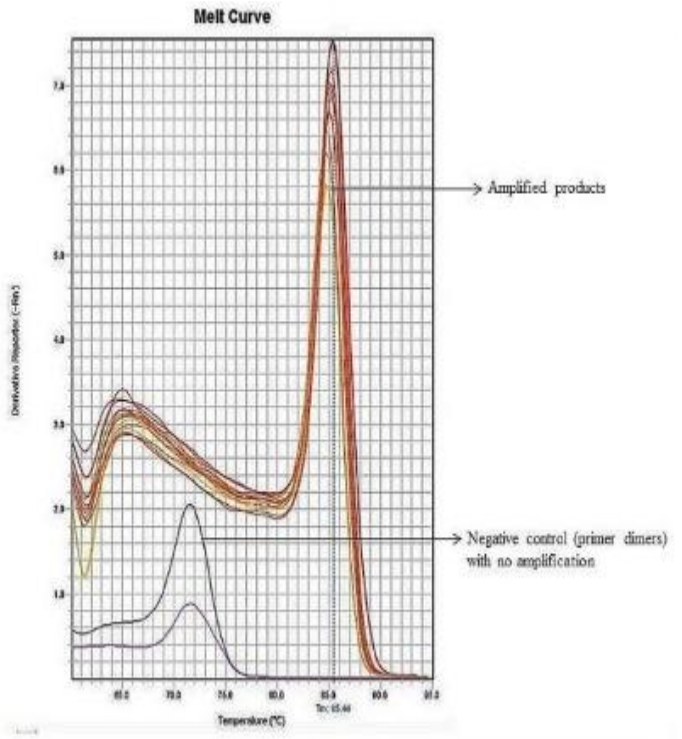
* Increase number of PCR cycles
* Increase DNA concentration



Primer dimer in Real- Time PCR

- Decrease primer concentration
- Check the primer oligonucleotide

by Gene Runner or primer blast software.



Some problems

- Neisseria meningitidis (Gram-negative bacterium)
- Very susceptible DNA
- Sampling before maximum 18 hours after first dose of antibiotics
- Immediately frizzing the CSF or blood after sampling

Cautions

- Don not freeze and defreeze several time
- Aliquoted the DNA or RNA extraction
- Use UV and Sodium hypochlorite for sterilization
- Use sampler tips with filter
- Use gloves and mask

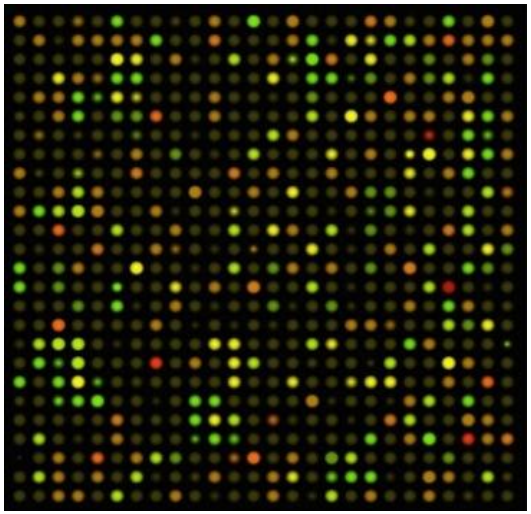
Cautions

- Separate place use for extraction, master mix preparation and add the DNA
- Use ice rake to prepare master mix and add DNA to inhibit polymerase enzyme

Other molecular methods

Microarray

- A set of DNA sequences representing the entire set of genes of an organism, arranged in a grid pattern for use in genetic testing.
- It is a collection of microscopic DNA spots attached to a solid surface.



Southern and northern blotting

- Southern blot is a method used in molecular biology for the detection of a specific DNA sequence in DNA samples.
- Southern blotting combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization.
- Northern blotting is the same but with RNA
- Can use for detection of some viruses

Molecular microbiology

* Human genetic problem
* (ARMS PCR, RFLP, rapid test)
* Antibiotic resistant mechanisms
* Gene cloning
* Proteomics
* Sequencing
* Next generation sequencing (NGS)
* Molecular epidemiology
* Wester blotting

Proteomics

In proteomics, there are multiple methods to study proteins;

using either antibodies (immunoassays)

Enzyme-linked immunosorbent assay (ELISA)

Wester blotting

SDS-PAGE

Mass spectrometry

Mass spectrometry

It is an analytical technique that ionizes chemical species and The differences in masses of the fragments allows the mass analyzer to sorts the ions based on their mass-to-charge ratio.

Use for identifying unknown microorganisms.

The role of molecular microbiologist

- Clarify the aim
- Select the best molecular method by considering

  Time

  Sensitivity

  Specificity

  Cost effectiveness


Thank you for your attention