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# MEC III BASIC BIOTECHNOLOGY- 16SMBEBC3

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# *MICROARRAY*

# *INTRODUCTION*

❀ *A particular series of small spots of reporter molecules such as DNAs or proteins fixed on a solid surface to detect and screen biological molecules present in samples is called* ***Microarray.***

❀ ***Some tissues, cell types*** *or* ***chemical compounds*** *are also used as reporters in some microarrays.*

❖ *On the basis of the type of* ***reporter*** *present, microarrays are devided into the following types :*

- ❃*DNA microarrays*
- ❃*cDNA microarrays*
- ❃*Protein microarrays*
- ❃*Tissue microarrays*
- ❃*Cellular microarrays*
- ❃*Antibody microarrays*

# *DNA MICROARRAYS*

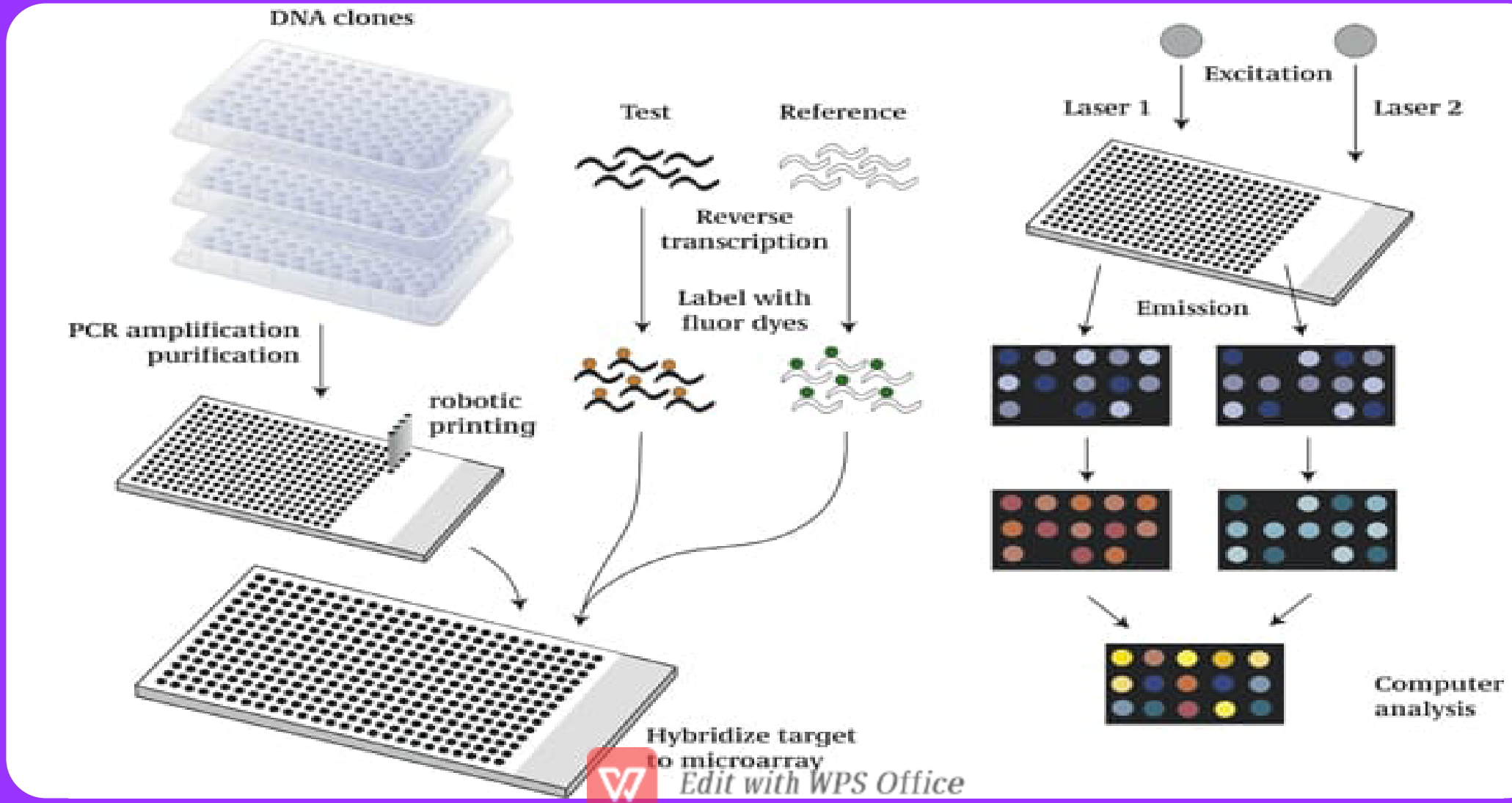
❖ *A particular series of small spots of DNAs in samples is called **DNA Microarray** or **DNA chip** or **gene chip**.*

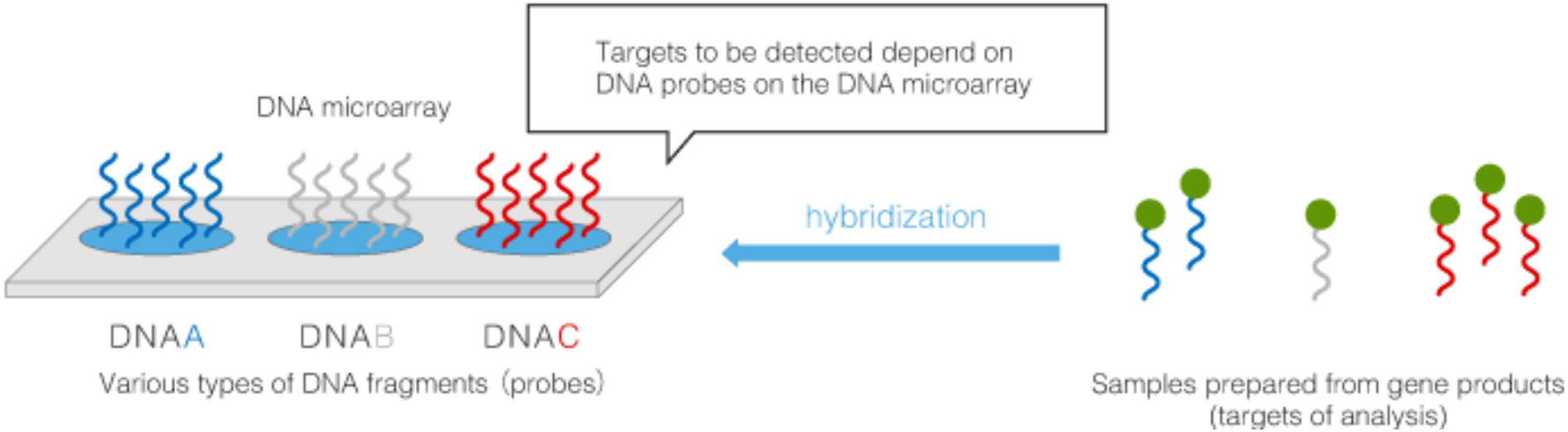
❖ *DNA microarrays are very useful to study the expression of a large number of genes simultaneously in an organism or **multiple sequences** within the genome.*

❖ *Therefore, DNA chips can be used to detect 16,000 different genes or mRNAs in the sample at a time.*

❖ *With the use of a DNA microarray, thousands of* ***disease*** *or* ***genes*** *can be determine at a time from a small volume of sample.*

❖ *After washing, the microarray can be reused for analysing other sample.*





# *PREPARATION OF DNA MICROARRAY*

❁ *The probe DNAs are isolated separately from* ***open reading frames(ORFs)*** *of the genome.*

❁ *All these DNA probes are filled in separate test tubes and the tubes are kept in a proper order.*

❁ *The chemical matrix provides free reactive groups for establishing covalent bonds with the DNA probes.*

❁ *The DNA probes are then spotted as **small dots** on the solid surface with the help of **robot**.*

❁ *The robot has an arm called **microarray**. It has a series of small syringes called microarray printing **pins**.*

# *TARGET PREPARATION*

✿ *The target is the DNA or RNA sample, genomic DNA of a source organism or a particular culture is isolated and cut into small pieces using a* ***restriction enzyme.***

✿ *The PCR products are* ***denatured*** *into* ***single strands*** *and labelled with a green or red* ***fluorescent dye.***

✿ *If the mRNAs have to be analyzed, the mRNAs are isolated from a cell type or sample and then converted into cDNAs by reverse PCR.*

# *HYBRIDIZATION*

❀ *Hybridization is performed in a device called* ***hybridization cassette.***

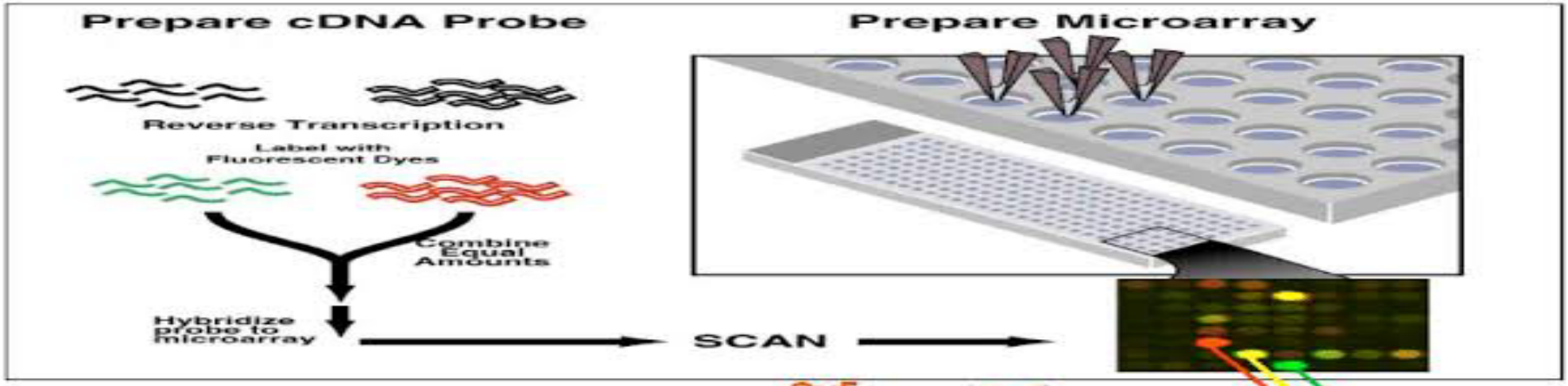
❀ *This device consists of a* ***hybridization vessel*** *and a* ***lid,*** *which are made of high quality plastics.*

❀ *A hybridization* ***buffer*** *is poured in the vessel of* ***a hybridization cassette*** *and the cDNA preparation is added to it.*

❀ *The microarray is placed in the buffer and the vessel is closed with its lid.*

❀ *ThE microarray is inserted into the slide port of the* ***microarray scanner****. Glass slide chipsbandchips and nano-well arraysarrays are in common use in laboratories.*

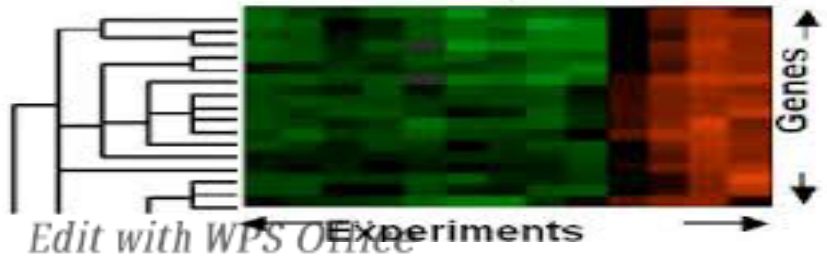
# Microarray Hybridization




## Image Analysis

| Cy3 | Cy5 | $\frac{Cy5}{Cy3}$ | $\log\left[\frac{Cy5}{Cy3}\right]_2$ |
|---|---|---|---|
| 200 | 1000 | 50.00 | 5.64 |
| 4800 | 4800 | 1.00 | 0.00 |
| 9000 | 300 | 0.03 | -4.91 |

## Cluster Analysis




R/G ratio represents relative abundance of transcripts

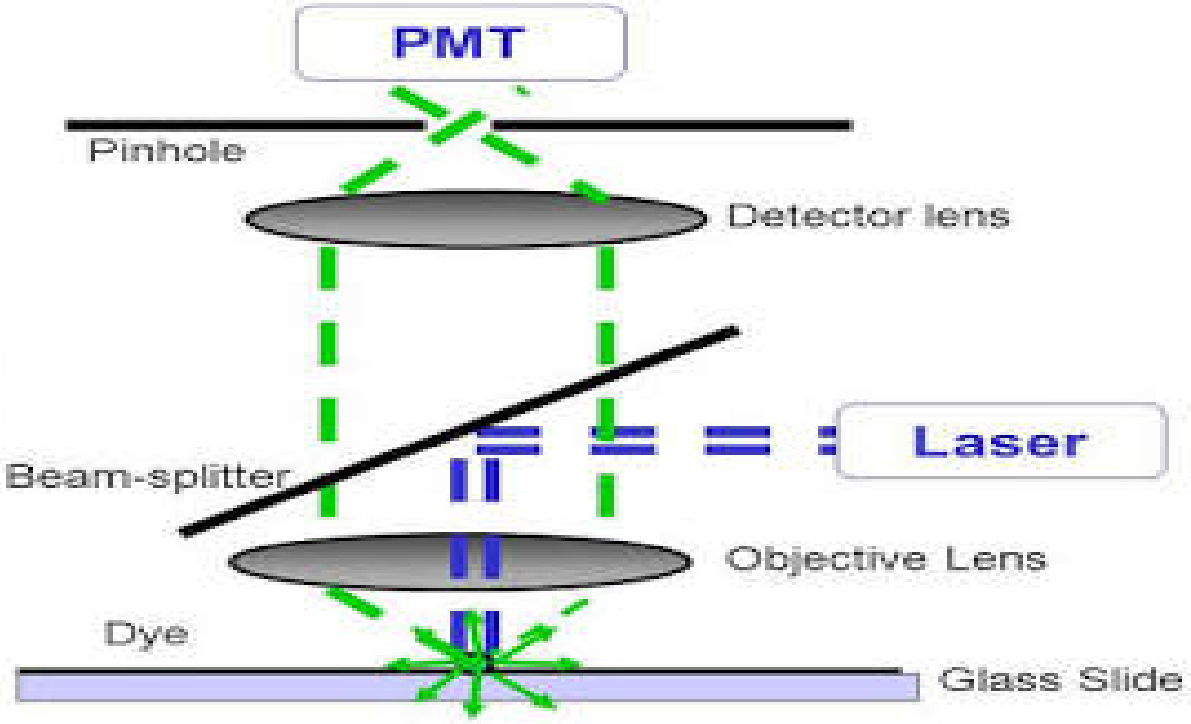
# *SCANNING*

❃ *The scanner emits a **laser beam** on the microarray spots and captures the fluorescence emitted from the spots with the **PMT** coupled with a **confocal microscope.***

❃ *The scanned image of the microarray is captured in the **computer screen.***

❃ *Fluorescent spots indicate the presence of corresponding mRNAs or DNAs in the sample.*

# Microarray Scanning



❃ *In a protein microarray, different* ***protein molecules*** *are fixed as separate spots in a proper order. When the protein chip is kept dipped in a solution containing a green or red dye labelled proteins, the protein emit green or red fluorescence from the appropriate spots.*

❃ *The fluorescence is captured with a scanner to identify the proteins in the sample.*

❃ *There are several types of protein microarrays depending on the solid surface on which the protein molecules are affixed.*

❃ *The bound proteins emit green or red fluorescence from the appropriate spots.*

❃ *Glass* ***slide chip*** *and* ***nano-well arrays*** *are in common use in laboratories.*

# *USES OF DNA MICROARRAYS*

✿ *They are very important to determine the gene content of closely related cells or organism. The apporoch is called* ***comparative genomic hybrization.***

✿ *DNA microarrays are now used to identify GEMOs and pathogenic organisms in the food materials.*

❖ *DNA sequence closely bound to proteins are isolated and allowed to hybridize with proteins to determine* ***DNA binding sites*** *in the genome.*

❖ *DNA microarrays are used in the* ***genotyping*** *of genomes through* ***single nucleotide polymorphism*** *analysis.*

❖ *Species specific DNA microarrays can be used to identify different species of microbes and others.*

# *Thank you...*