Gene Expression and Micro arrays

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Lecture 12

Recap

- Sequence Alignment
- Phylogenetic Tree Analysis

Outline

- Gene Expression
- Data representation
- Microarray technology
- Applications

Statistics & Bioinformatics

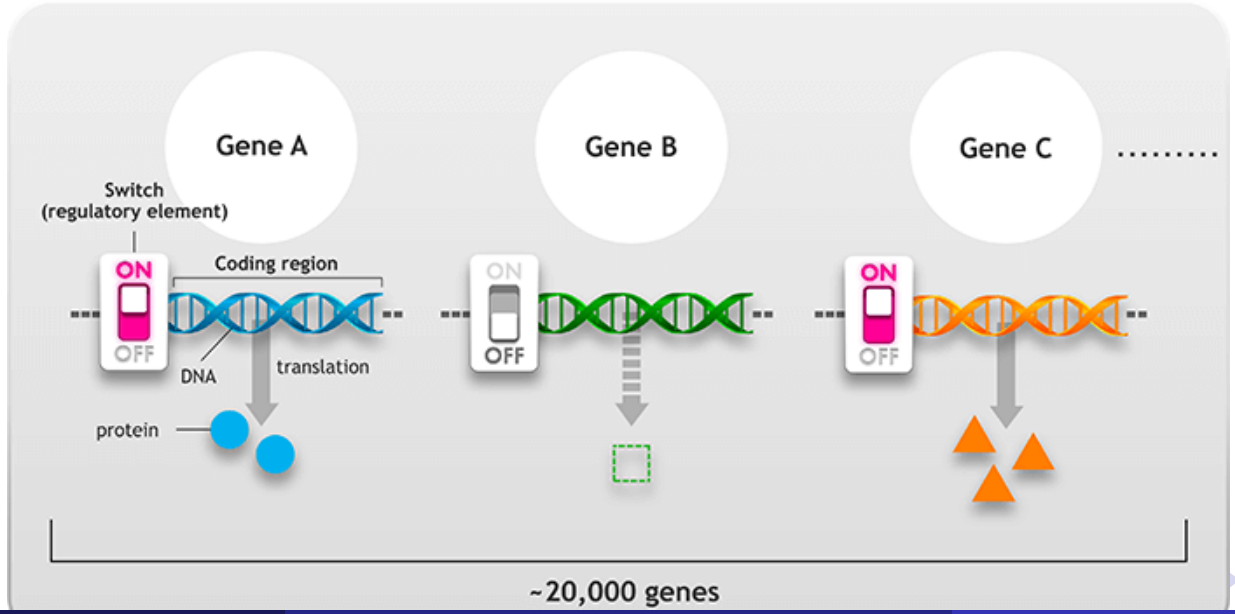
- Applications of statistics in bioinformatics range from clinical diagnosis and descriptive summaries to gene hunting and nucleotide alignment
- For example
  - assess clinical and genetic tests for the probability of a negative result, given that the condition under consideration is absent (their specificity)
  - for the probability of a positive result, given that the condition under consideration is present (their sensitivity), and for the predictive value (the probability that a condition is present, based on the results of a test)
  - The process of diagnosing patients potentially suffering from genetic disorders typically encompasses quantifying uncertainty and using statistical methods to predict long-term outcomes.

Statistics & Bioinformatics

- Mutations, chance mating, random environmental pressures, and the relative contribution of parents to the genotype of their offspring all lend themselves to statistical interpretation
- May be random in organism level, but for a large population and ecosystem, often appear as deterministic behaviors
- Mutations, chance mating, random environmental pressures, and the relative contribution of parents to the genotype of their offspring all lend themselves to statistical interpretation
- An important distinction in biological systems is that some processes or measurements are either present or absent (discrete), while others are variable within some range (continuous)
- Sequencing machines generate data on thousands of base pairs per hour, and **microarray experiments** can collect data on the expression of tens of thousands of genes in a few hours.
- There are numerous potential sources of variability in the microarray experimental process and consequently a concomitant need for statistical processing.

Gene Expression

- Gene expression is the process by which the instructions in our DNA are converted into a functional product, such as a protein or non-coding RNA
- It is a tightly regulated process that allows a cell to respond to its changing environment
- It acts as both an on/off switch to control when proteins are made and also a volume control that increases or decreases the amount of proteins made



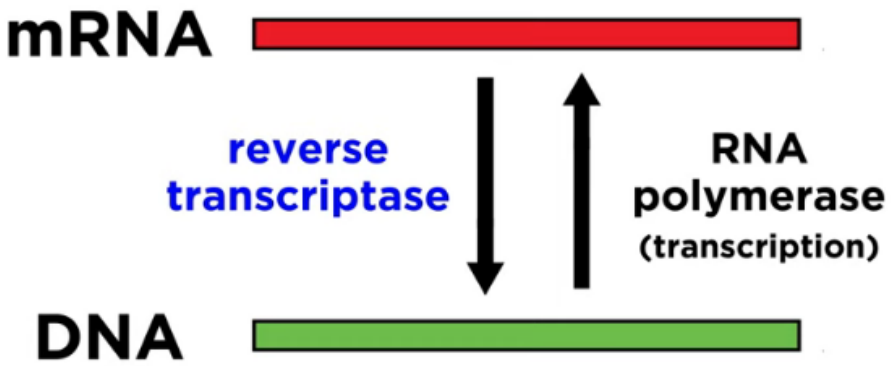
Gene Expression- Analysis

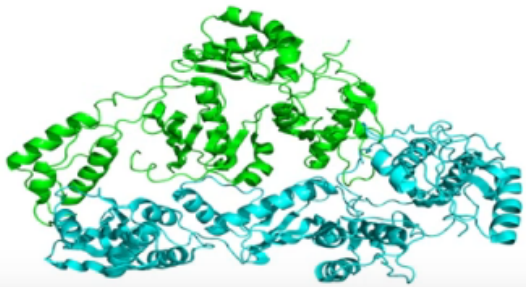
- What genes are present in the genome?
- What proteins are produced when genes are expressed?
- What is happening in a particular cell?
- How gene expression altered in cancer cell?
- .....

For answering these questions, we must identify the mRNA being transcribed by the cell

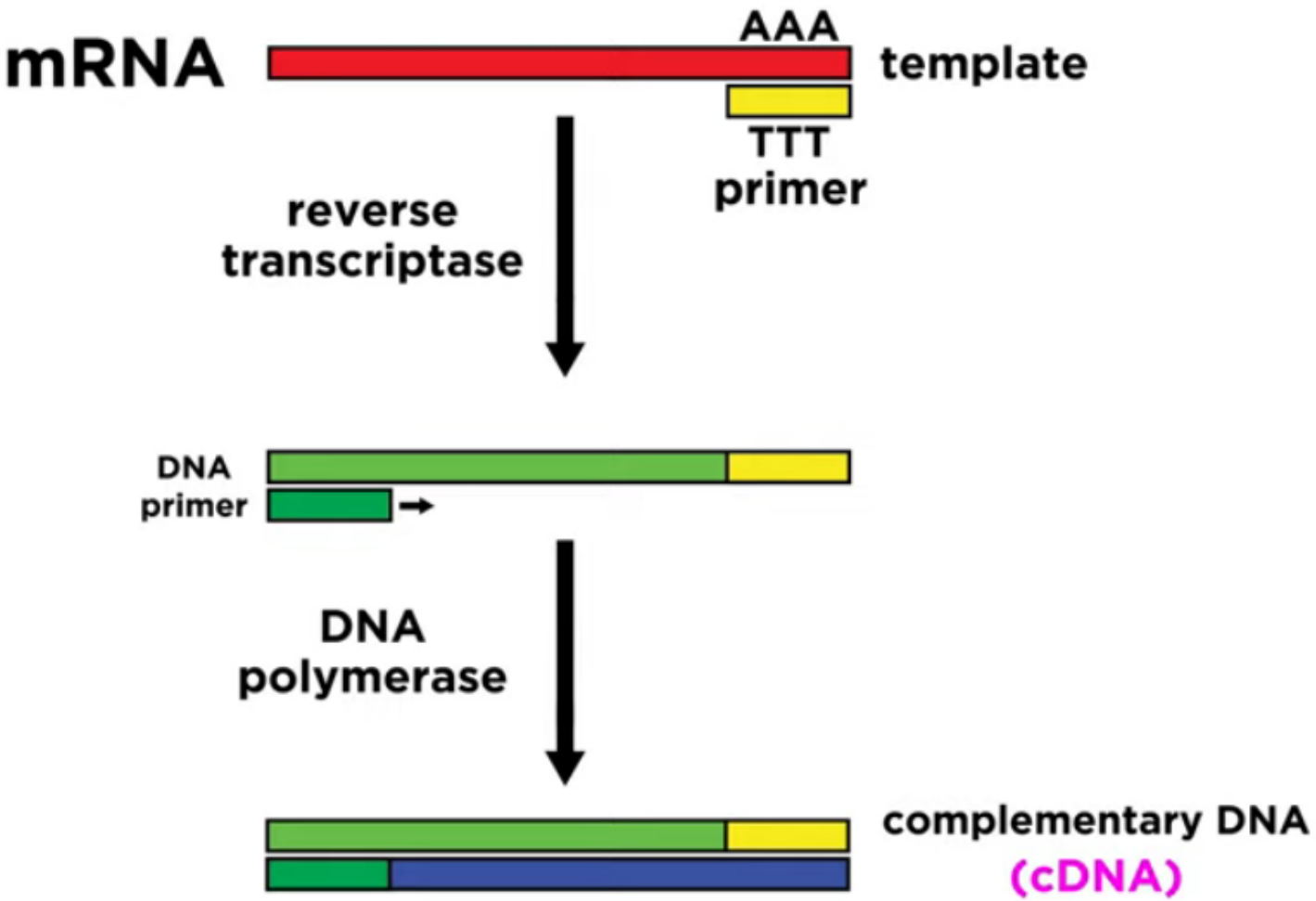
Gene Expression- Analysis

- Isolate the mRNA from the cell
- Perform reverse transcript polymerase chain reaction (RT-PCR) to identify the complementary DNA
- i.e Reverse engineering the DNA template that would have generated the mRNA

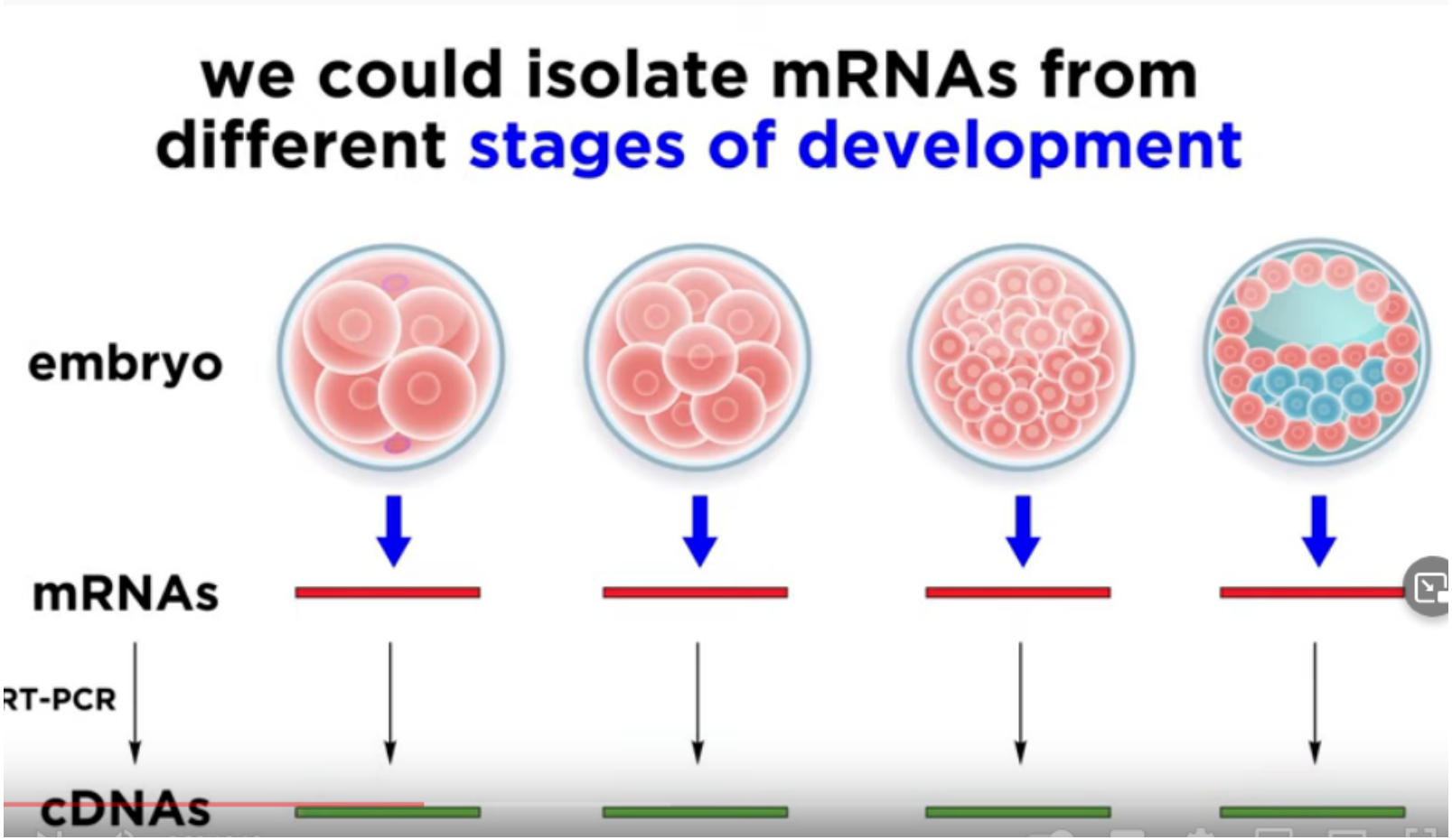




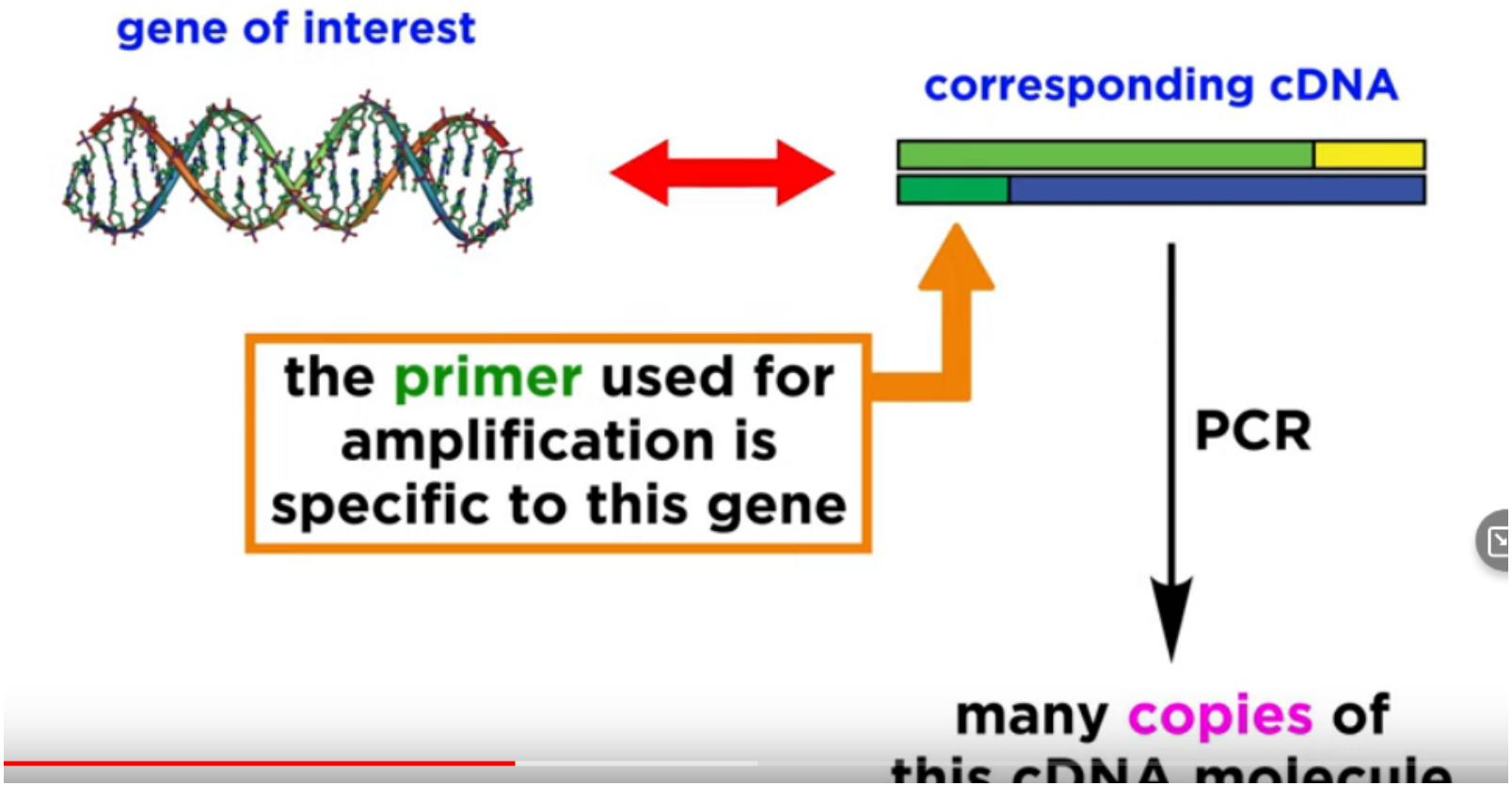
Gene Expression- Analysis



Gene Expression- Analysis



Gene Expression- Analysis



Gene Expression- Analysis

- Gene level: Identify the gene of interest, find the complementary DNA and analyse the expression
- We could identify which tissue is expressing particular gene for particular function
- **We need Genome-wide analysis!**- how different gene interact and express together for particular biological function
- **Microarrays**

Microarray Technology

- A microarray is a laboratory tool used to detect the expression of thousands of genes at the same time
- Microscope slides that are printed with thousands of tiny spots in defined positions, with each spot containing a known DNA sequence or gene. Often, these slides are referred to as **gene chips or DNA chips**.
- The DNA molecules attached to each slide act as **probes to detect gene expression**, which is also known as the transcriptome or the set of messenger RNA (mRNA) transcripts expressed by a group of genes

Microarray Technology

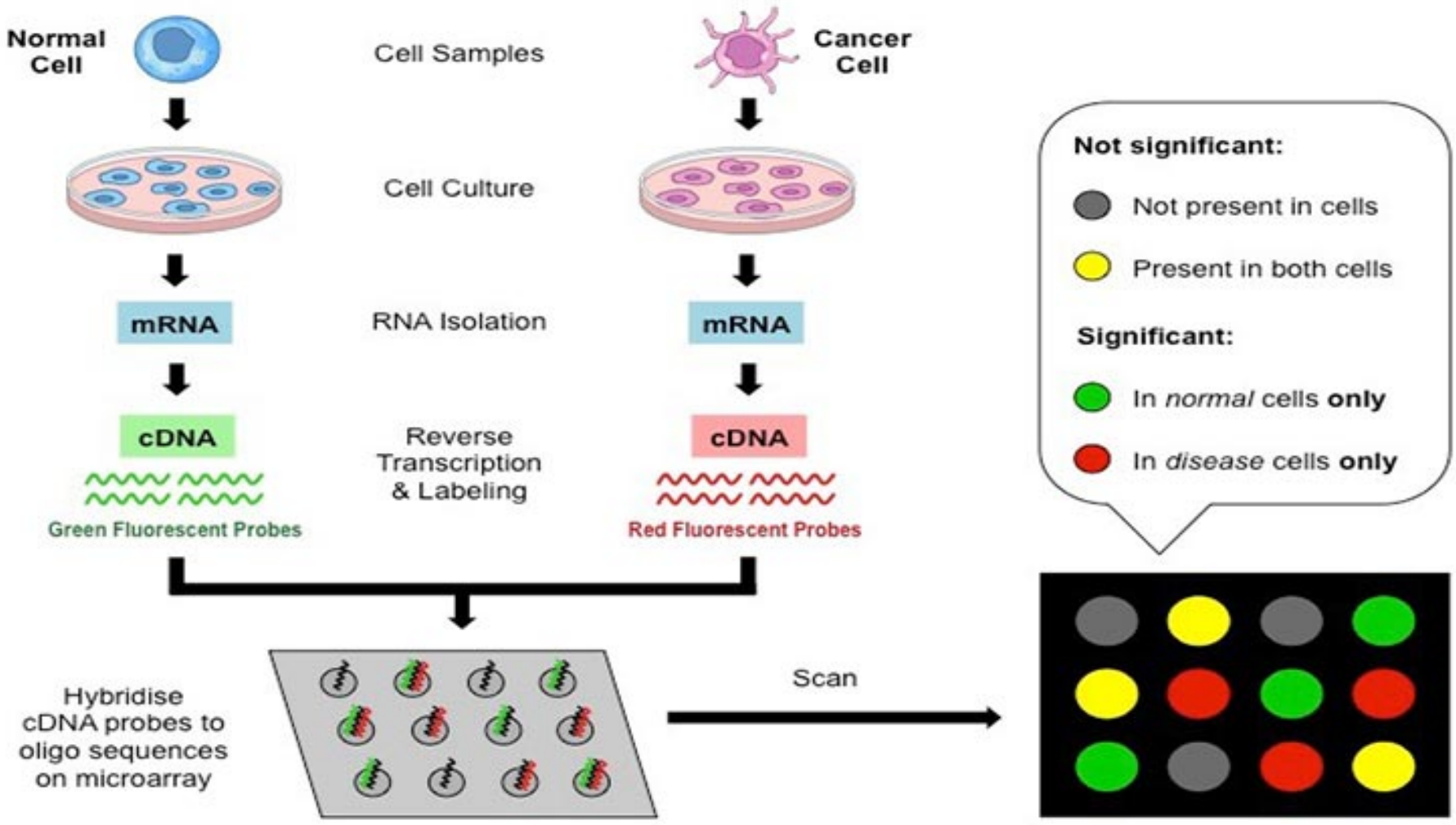
- Microarray allows rapid measurement and visualisation of differential expression of the whole genome scale
- RNA sample from any cell or tissue type can be analysed for changes in transcript levels
- Allow a complete analysis of genetic material and the monitoring of expression changes occurring in a biological sample under various conditions.
- Microarrays have been used successfully in various research areas including sequencing, single nucleotide polymorphism detection, characterization of protein-DNA interactions, DNA computing, mRNA profiling, and many more.
- Applications of microarrays also include gene expression studies, disease diagnosis, pharmacogenomics, drug screening, pathogen detection, and genotyping.

Microarray Technology-Steps

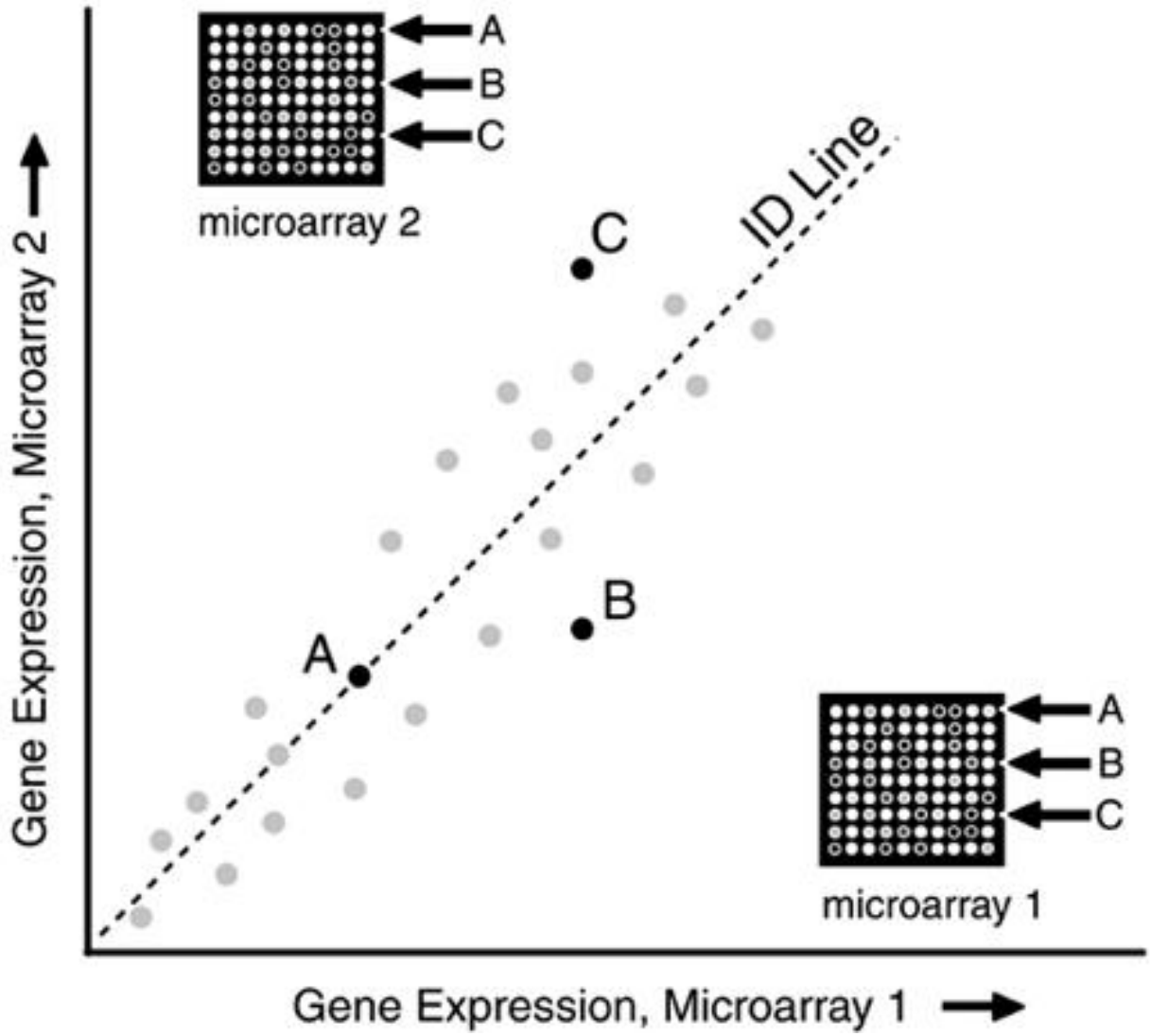
- mRNA is isolated from the cells of interest and converted into labeled cDNA cDNA is then washed over a microarray carrying features representing all the genes that could possibly be expressed in those cells
- If hybridization occurs to a certain feature, it means the gene is expressed.
- Signal intensity at that feature/spot indicates how strongly the gene is expressed (as it is a sign of how much mRNA was present in the original sample).

Microarray: Steps

https://www.youtube.com/watch?v=6ZzFihESjp0


Normal Cell
Cell Samples
Cancer Cell
Cell Culture
mRNA
RNA Isolation
mRNA
cDNA
Reverse Transcription & Labeling
cDNA
Green Fluorescent Probes
Red Fluorescent Probes
Hybridise cDNA probes to oligo sequences on microarray
Scan
Not significant:
Not present in cells
Present in both cells
Significant:
In normal cells only
In disease cells only

Microarray: Comparison



Microarray: Analysis

- Classification
- Clustering
- Identifying differentially expressed genes
- Regulatory genes
- Patterns in expression