

MICROARRAYS vs. RNA-SEQ

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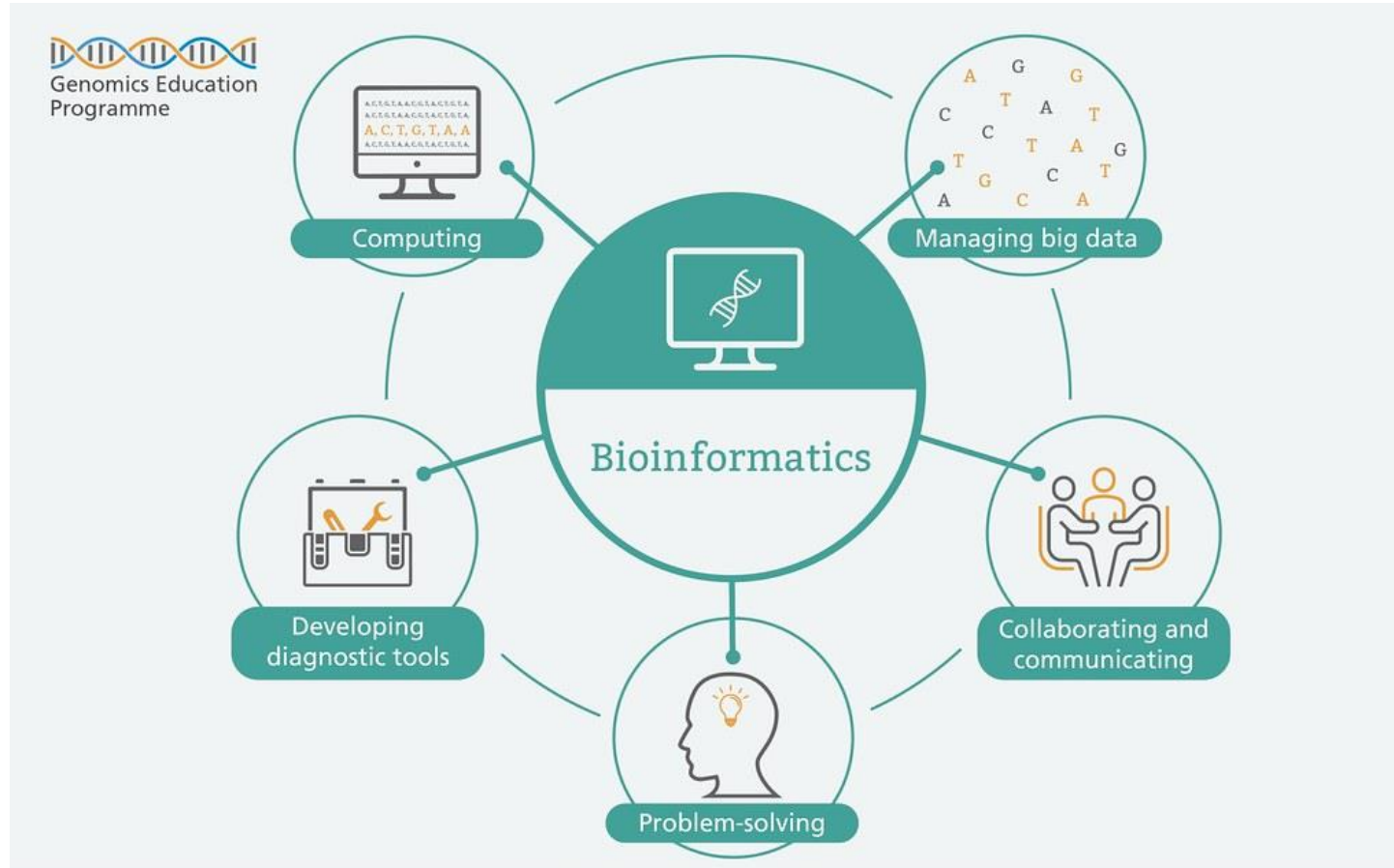


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LABORATORY 19



MICROARRAYS VS. RNA-SEQ



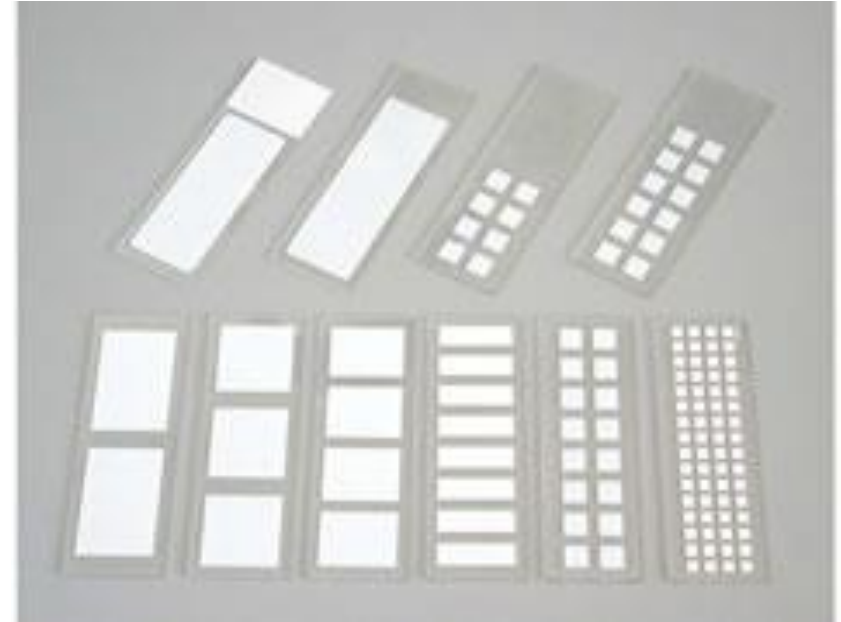
- What I want to search and what genome information is available for my samples of interest?
- How much data analysis expertise do I have or have access to?
- Which is my budget?

**EXPERIMENTAL
DESIGN**



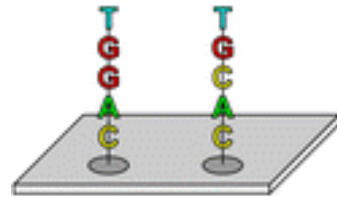
MICROARRAYS

- Microarrays are a collection of DNA **probes** (oligonucleotides) that are usually bound in defined positions to a solid surface, to which **sample** DNA fragments can be hybridized

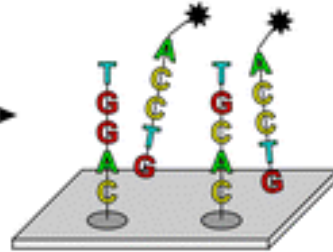


MICROARRAYS FLOW CHART

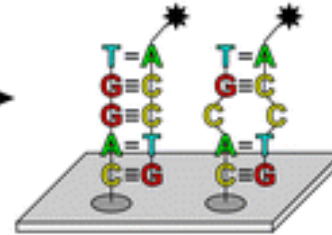
1. Two oligonucleotides with different sequences are immobilized on a DNA chip.



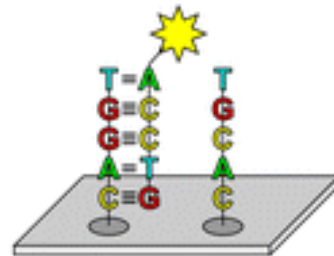
2. Hybridization of the fluorophore-labelled DNA with the immobilized oligonucleotides.



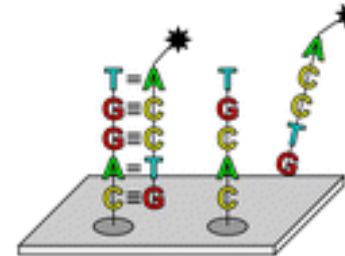
3. One oligonucleotide is perfectly bound while the other forms a mismatch.



5. Fluorescence detection of the hybrid on the chip surface.



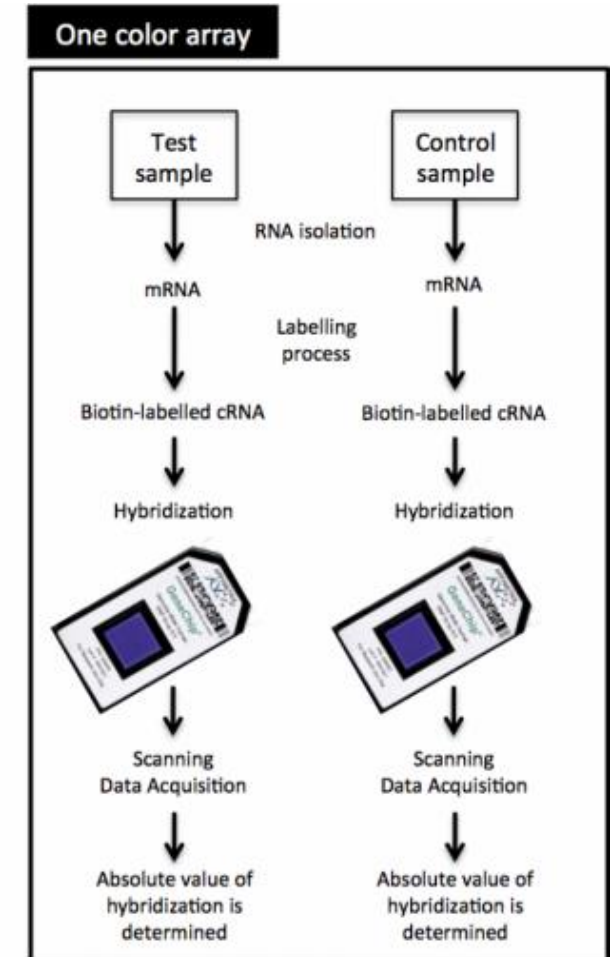
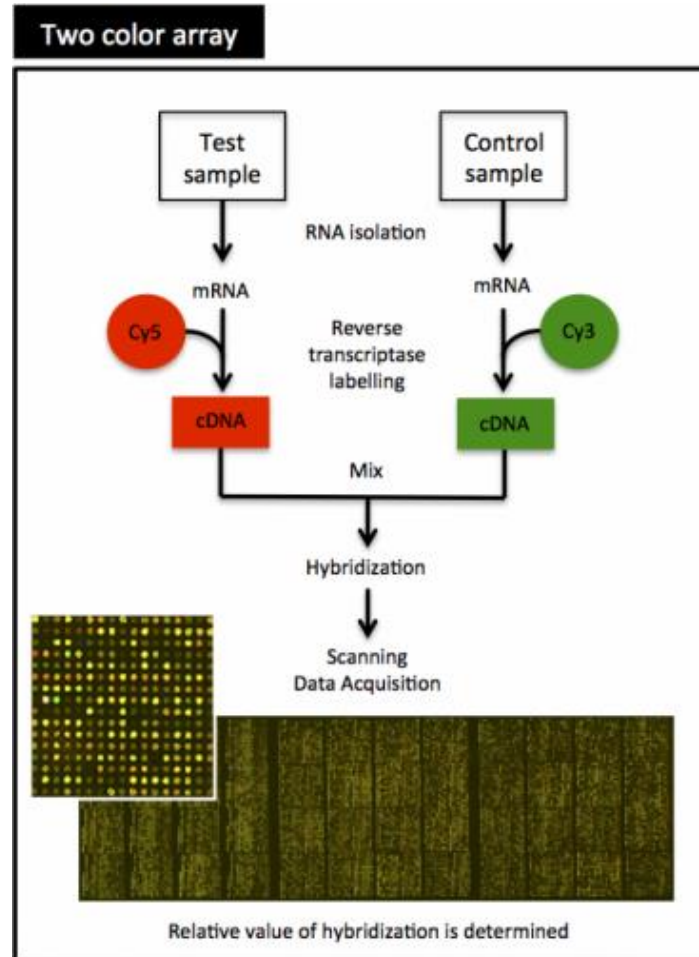
4. Stringent wash of the chip.



MICROARRAYS FLOW CHART

1. RNA extraction
2. labeled with a detectable marker
3. hybridize to to complementary gene
4. laser scanning
5. Calculation of relative level of expression

Analyses are expression measures for
each gene in each sample



MICROARRAYS

Advantages	Disadvantages
<ul style="list-style-type: none">✓ Robust, reliable method✓ Streamlined handling-can be easily automated✓ Straightforward data analysis✓ Short turn- around time (5 days)✓ Lower cost	<ul style="list-style-type: none">✗ Dependent on prior sequence knowledge✗ Cannot detect structural variations✗ Cannot detect isoforms✗ Hybridization and sample labeling biases✗ Not an absolute quantification method



RNA-SEQ

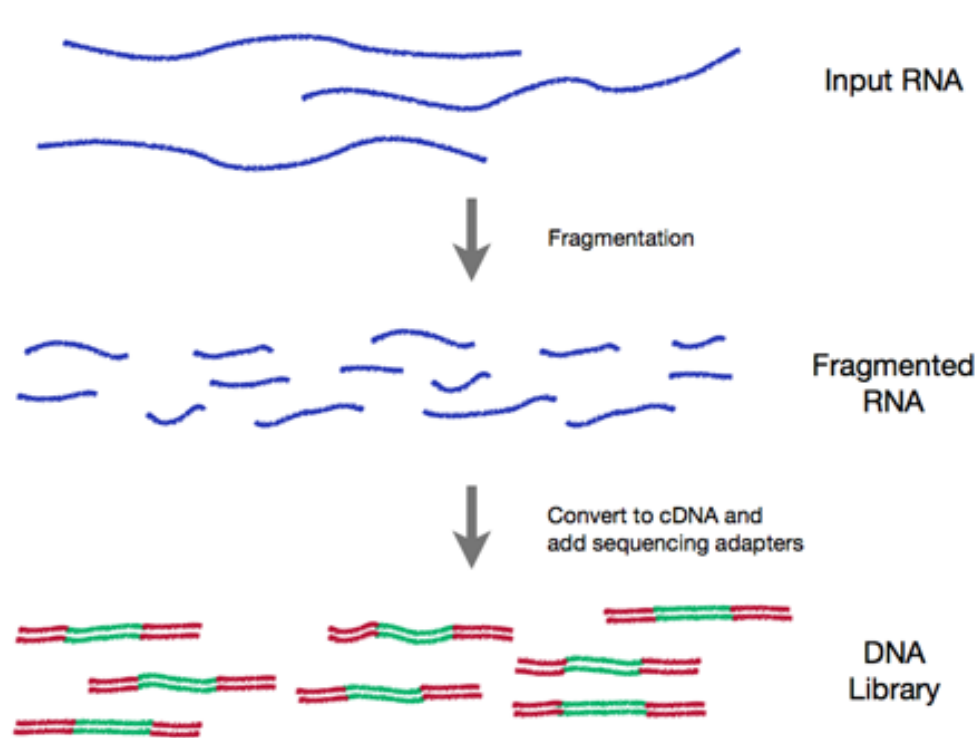
- **RNA-sequencing** is a technique that can examine the quantity and sequences of RNA in a sample using **next generation sequencing** (NGS)



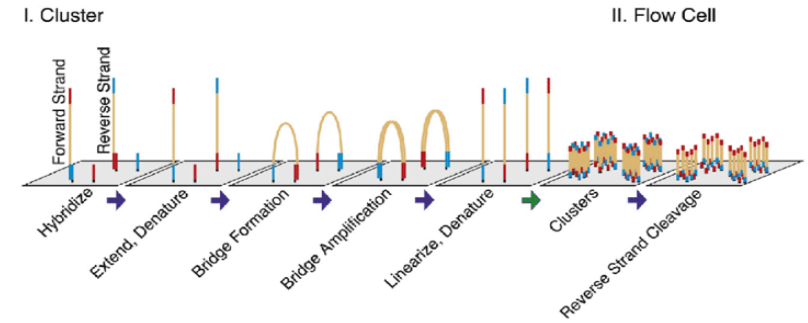
Illumina HiSeq



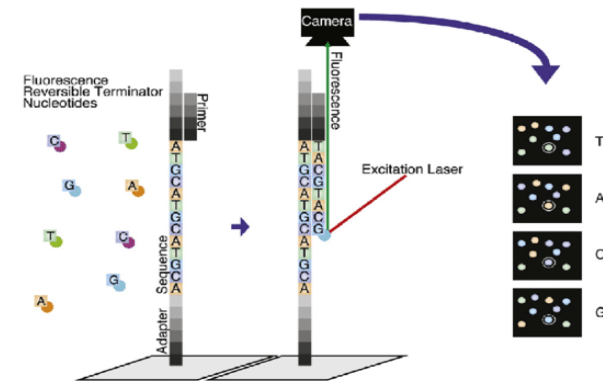
RNA-SEQ FLOW CHART



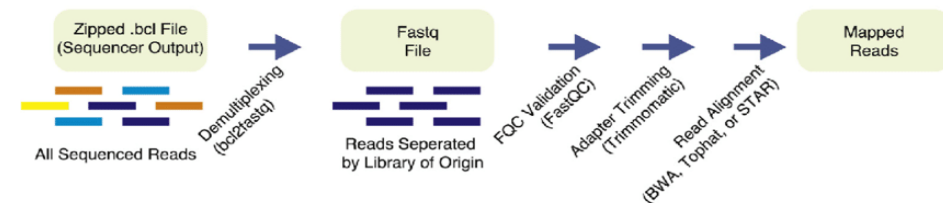
A. Clustering



B. High-throughput sequencing



C. Demultiplexing samples and read mapping



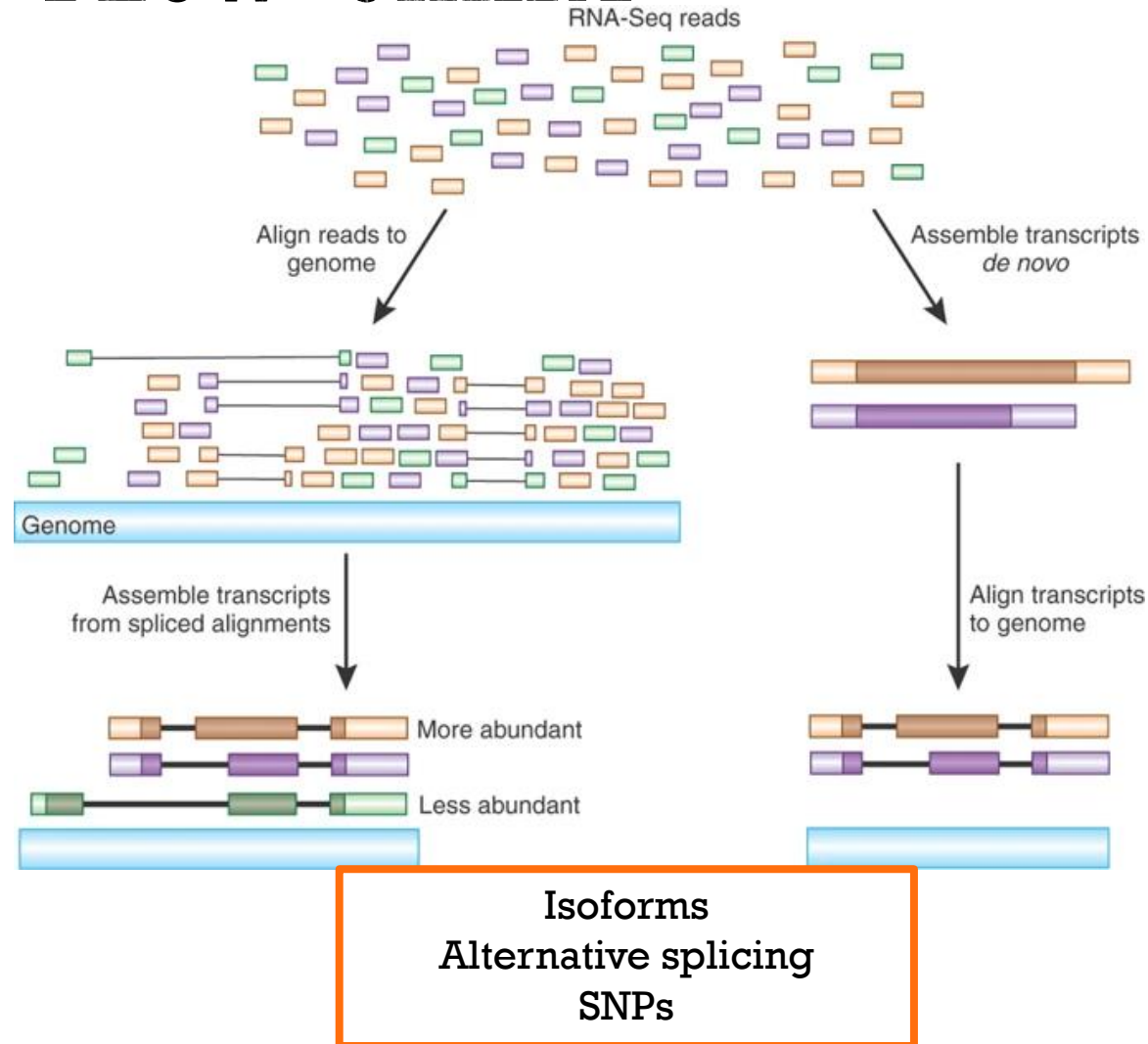
Sequencing



RNA-SEQ FLOW CHART

With Reference Genome

- No knowledge of the set of transcribed regions or which exons are spliced together
- Unannotated transcripts



Without Reference Genome

- reads need to be assembled first into longer → **contig**
- **contigs** are re-mapped for quantification



RNA-SEQ

Advantages	Disadvantages
<ul style="list-style-type: none">✓ Provides a comprehensive view of the transcriptome✓ Not dependent on any prior sequence knowledge✓ Tuneable sensitivity✓ Can detect structural variations as gene fusions or alternative splicing✓ A truly digital solution (absolute vs. Relative abundance)	<ul style="list-style-type: none">✗ Technology is new to most researchers✗ Data storage is more challenging✗ Analysis is more complex (not standard protocol)✗ Specialized computing infrastructure and personel are required✗ More expensive



MICROARRAYS VS. RNA-SEQ

SIMILARITIES

- Reproducible
- High correlation
- Background noise
- Statistical principles
- Biases
- Detect genomic information

DIFFERENCES

- Technique
- Sensivity
- Accuracy
- Detection of SNPs
- New DNA sequences
- Prize



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Group CIC-IBMCC

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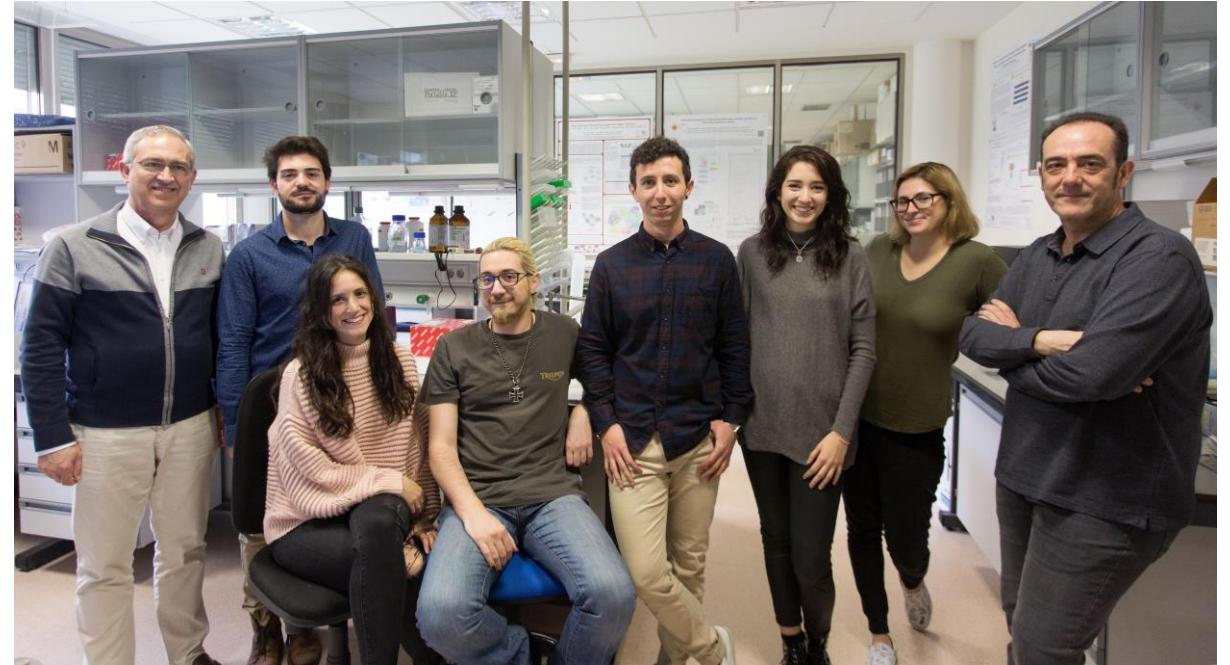
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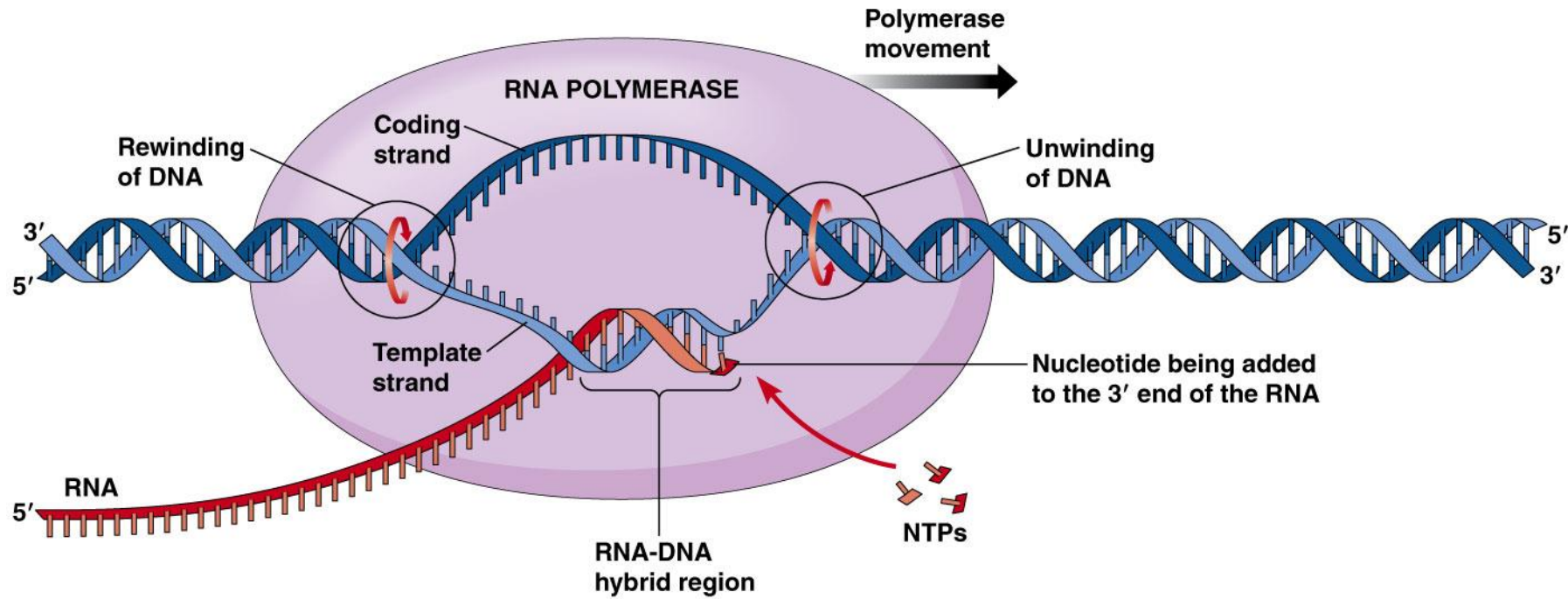
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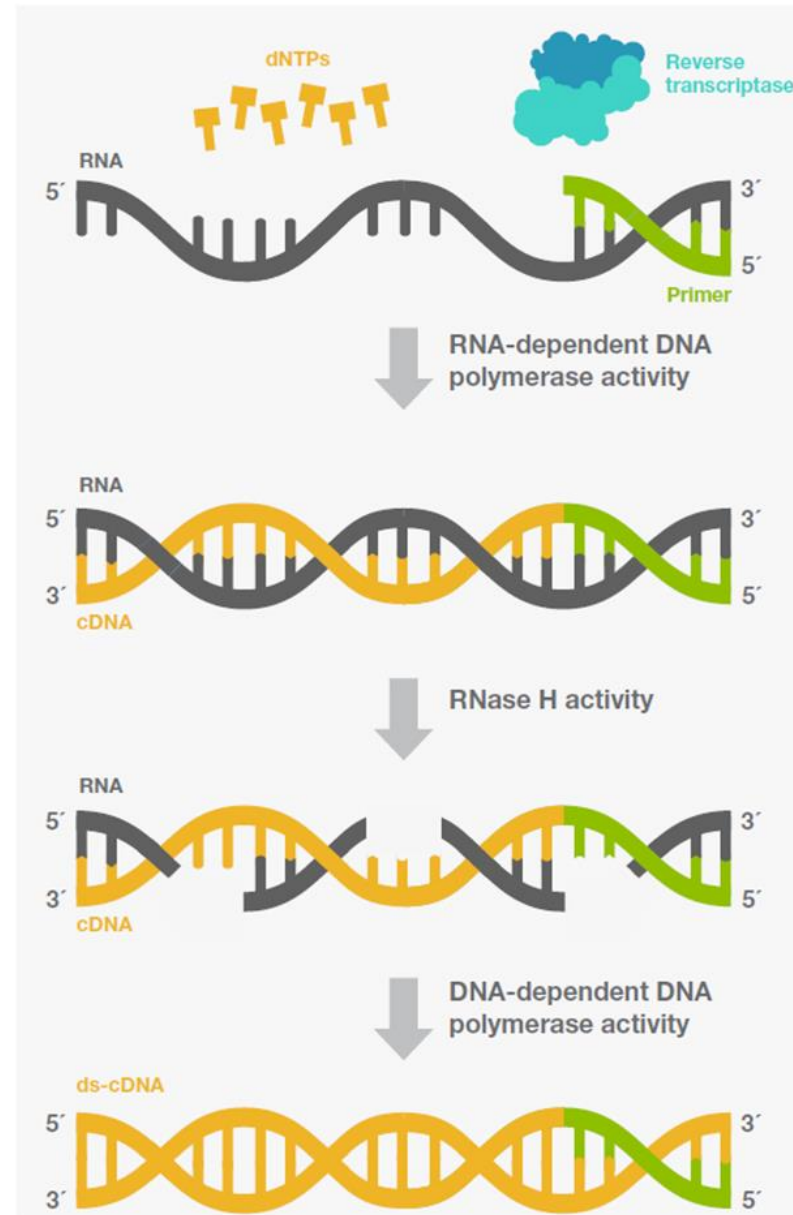
TRANSCRIPTION: DNA → RNA



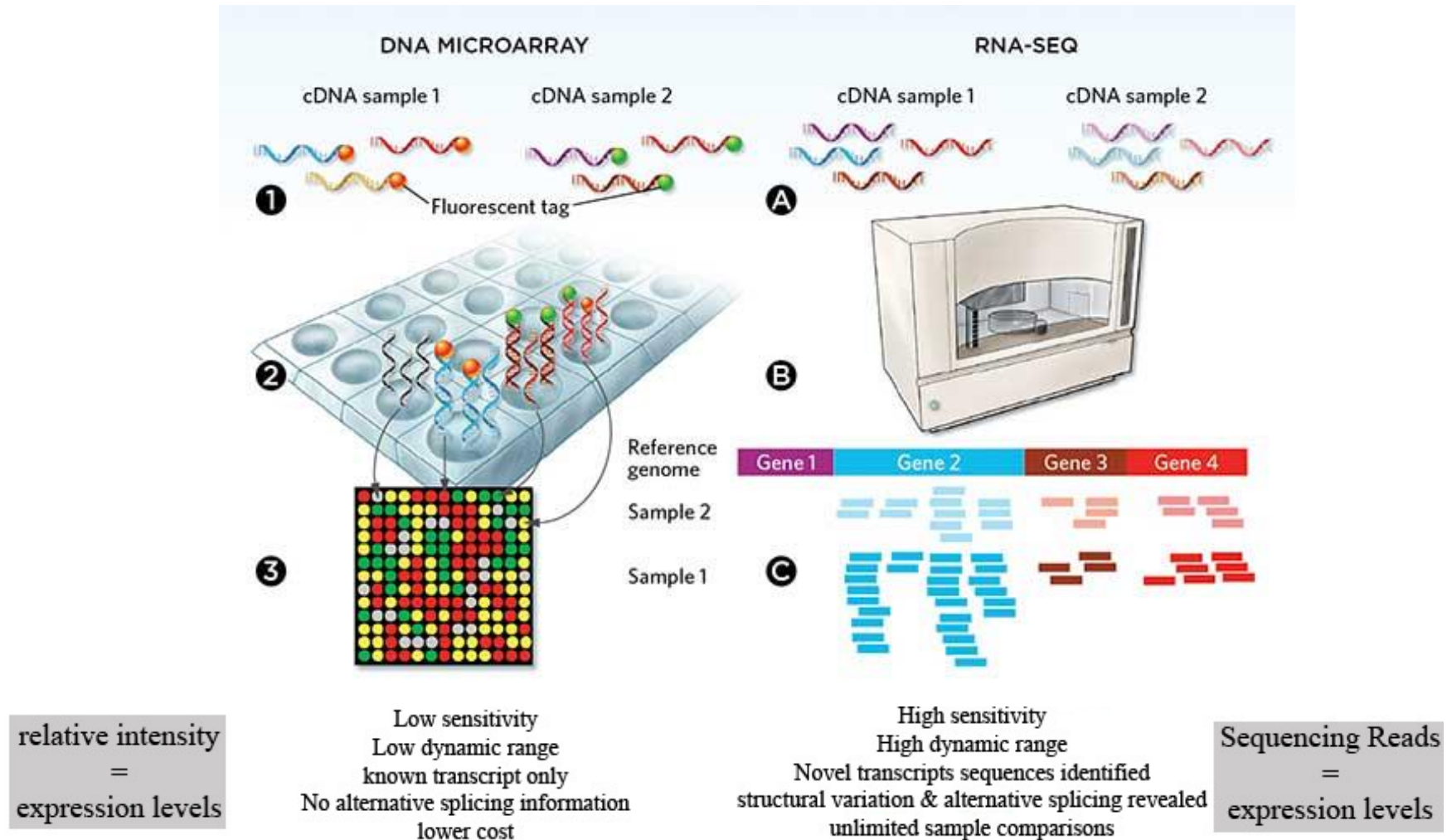
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RNA → cDNA



MICROARRAYS VS. RNA-SEQ



SUPP1

Comparisons of microarrays

