

TRANSCRIPTOMICS

Methods for Studying the Transcriptome

Day 01

<https://tttorres.github.io/transcriptomics/>

Transcriptomics

Definition

The word “transcriptome” was first used in the late 1990s:

The **transcriptome** is the complete set of **RNA molecules** transcribed from the genome of an organism, tissue, or cell at a specific point in time. It includes all types of RNA, such as:

- **Messenger RNA (mRNA)**: Encodes proteins.
- **Ribosomal RNA (rRNA)**: Forms part of ribosomes.
- **Transfer RNA (tRNA)**: Involved in translation.
- **Non-coding RNAs**: microRNAs (miRNAs), small interfering RNAs (siRNAs), long non-coding RNAs (lncRNAs), and others.

Piétu et al (1999) Genome Res. 1999; 9:195–209.

Velculescu et al (1997) Cell. 1997; 88:243–51.

Transcriptomics

Definition

The word “transcriptome” was first used in the late 1990s:

The transcriptome is the complete set of RNA molecules transcribed from the genome of an organism, tissue, or cell at a specific point in time. It includes all types of RNA, such as:

Key Characteristics:

- 1. Dynamic Nature:** The transcriptome varies based on cell type, developmental stage, environmental conditions, and stimuli.
- 2. Snapshot of Gene Expression:** It reflects which genes are actively being transcribed under specific conditions.

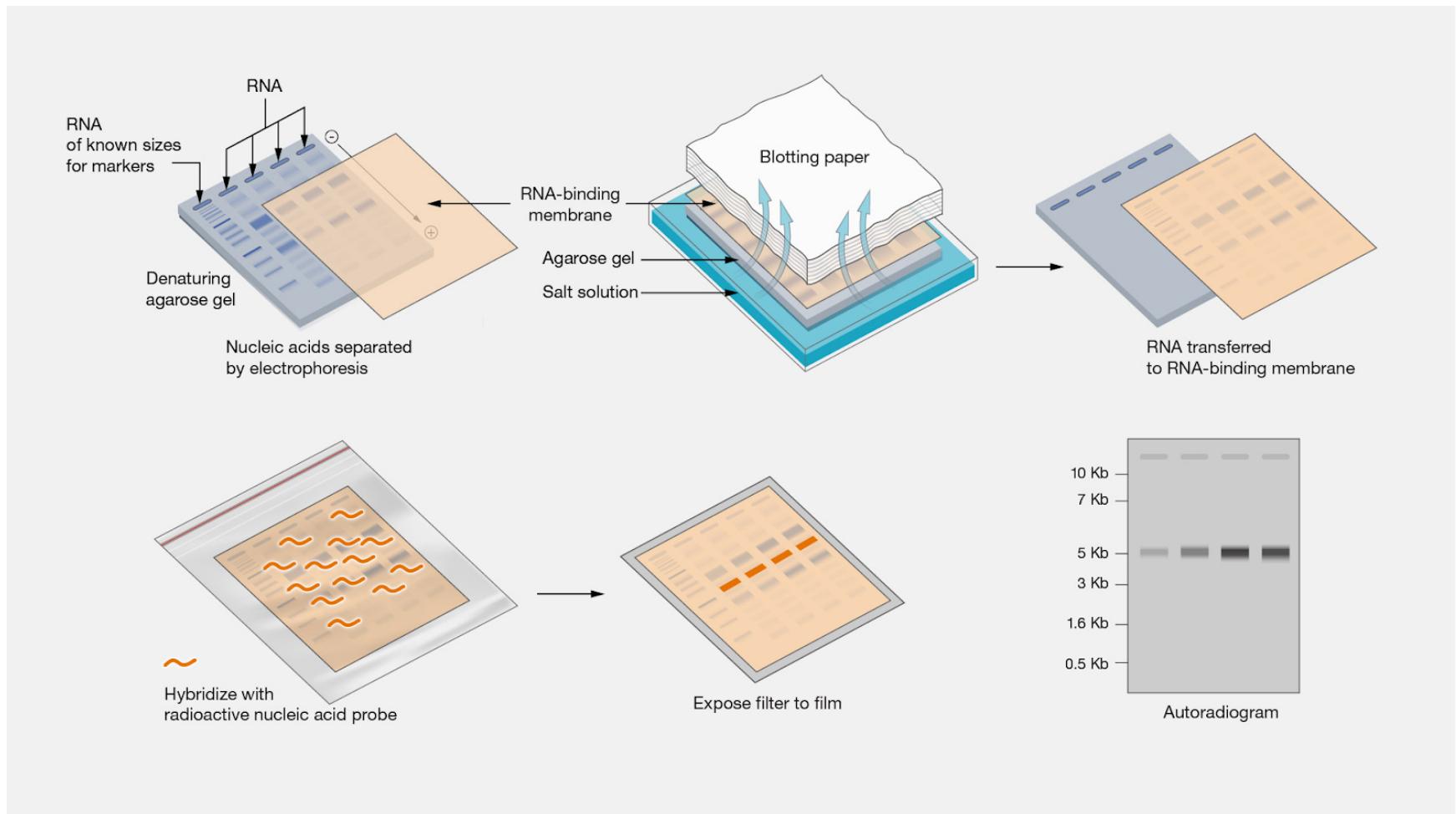
Piétu et al (1999) Genome Res. 1999; 9:195–209.

Velculescu et al (1997) Cell. 1997; 88:243–51.

Before Transcriptomics

Analysis of single genes

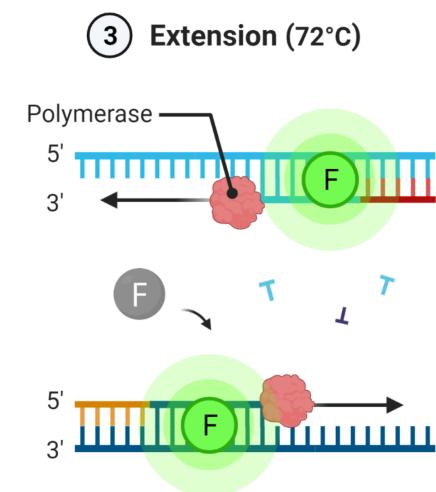
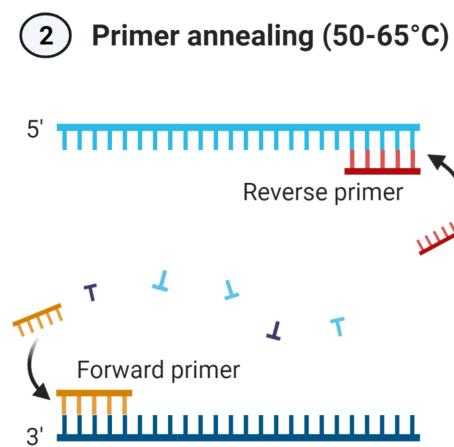
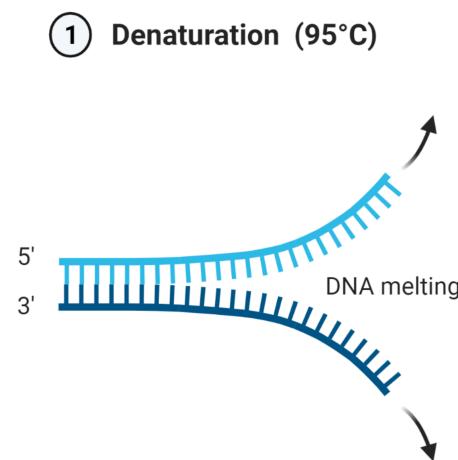
Northern blot



Before Transcriptomics

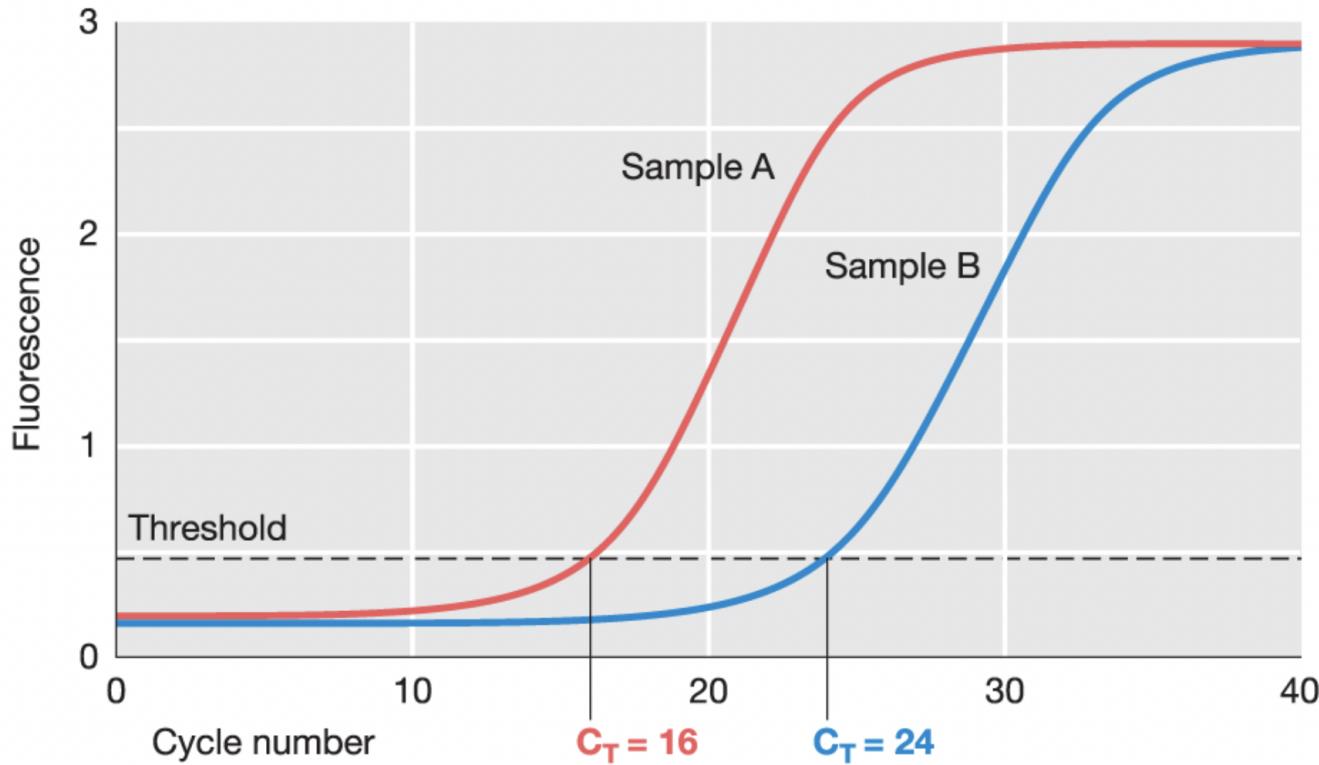
Analysis of single genes

Quantitative RT-PCR (qPCR)



Before Transcriptomics

Analysis of single genes (qPCR)



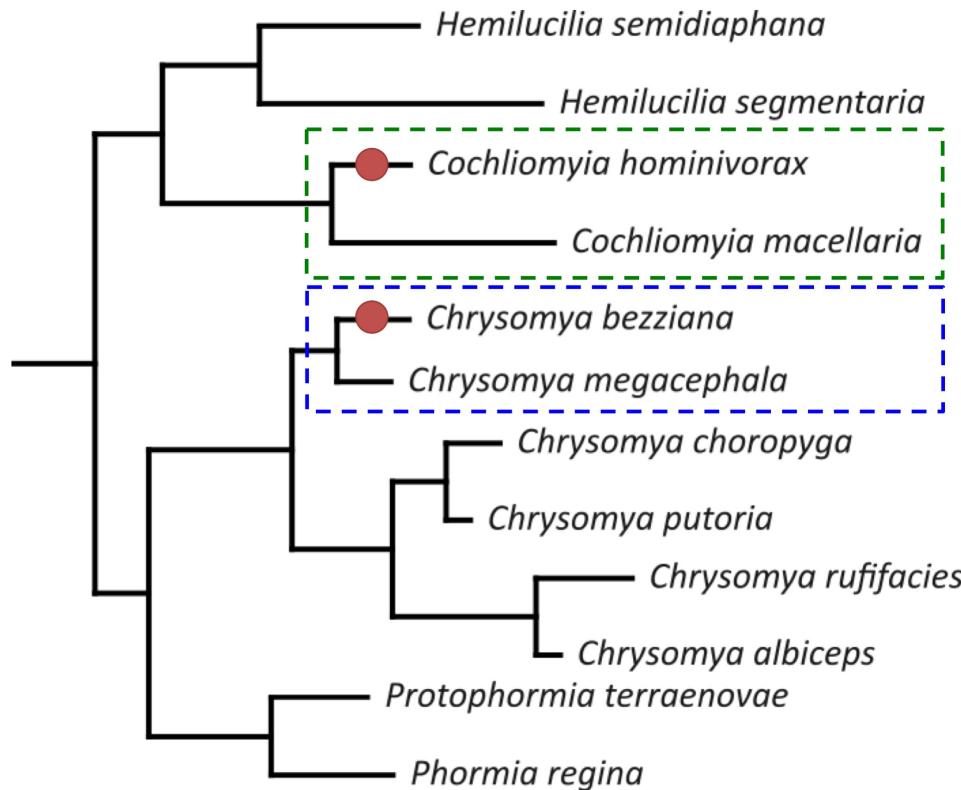
Fold difference between the two samples is $2^{-\Delta C_T}$

$$2^{-(16-24)} = 2^8 = 256''$$

Before Transcriptomics

Analysis of single genes (qPCR)

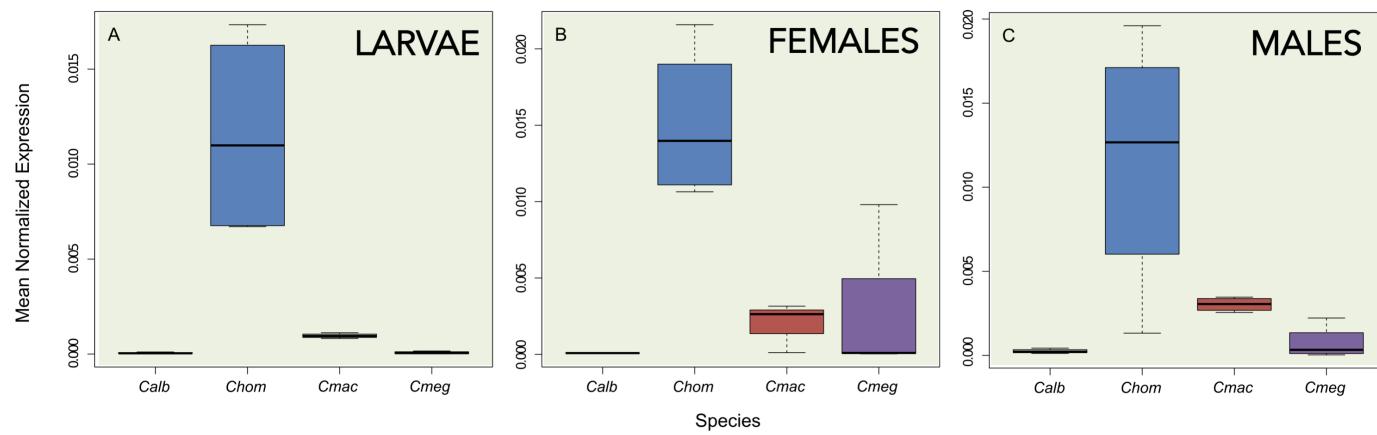
Application example



Before Transcriptomics

Analysis of single genes (qPCR)

Application example



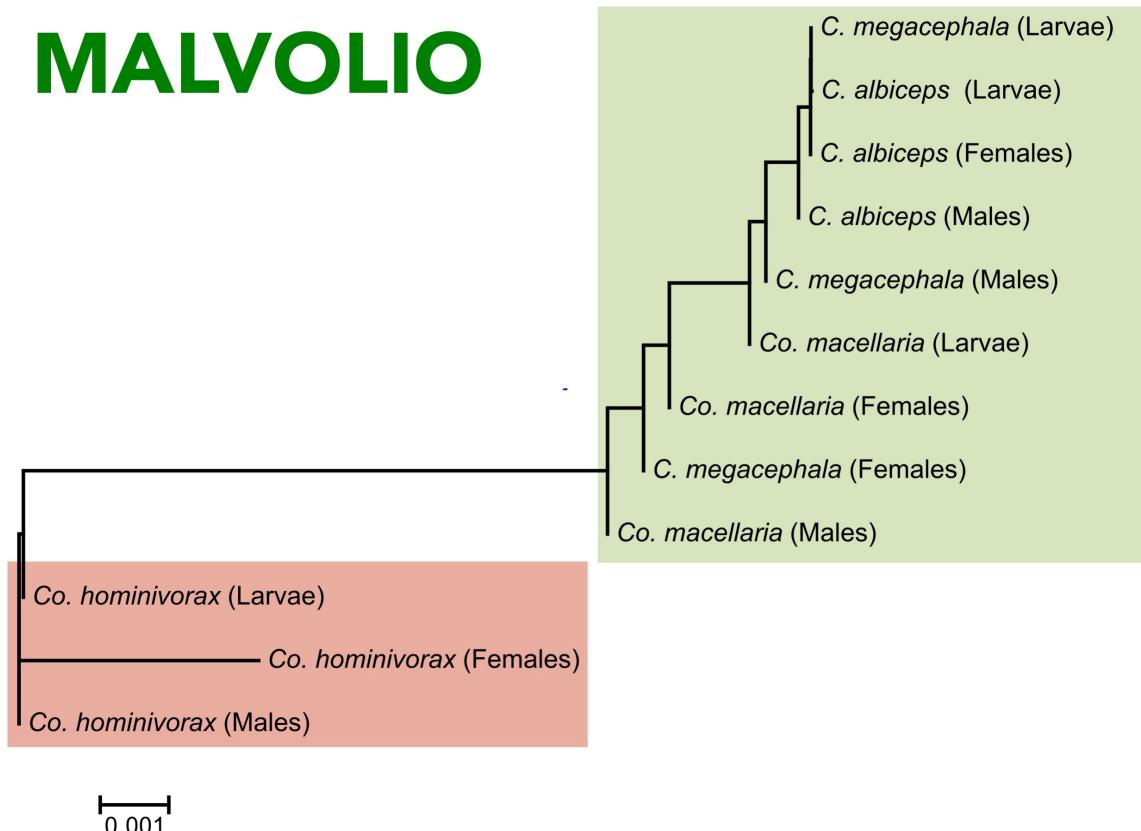
Cardoso et al (2016) PeerJ, 4, e2598

Before Transcriptomics

Analysis of single genes (qPCR)

Aplication example

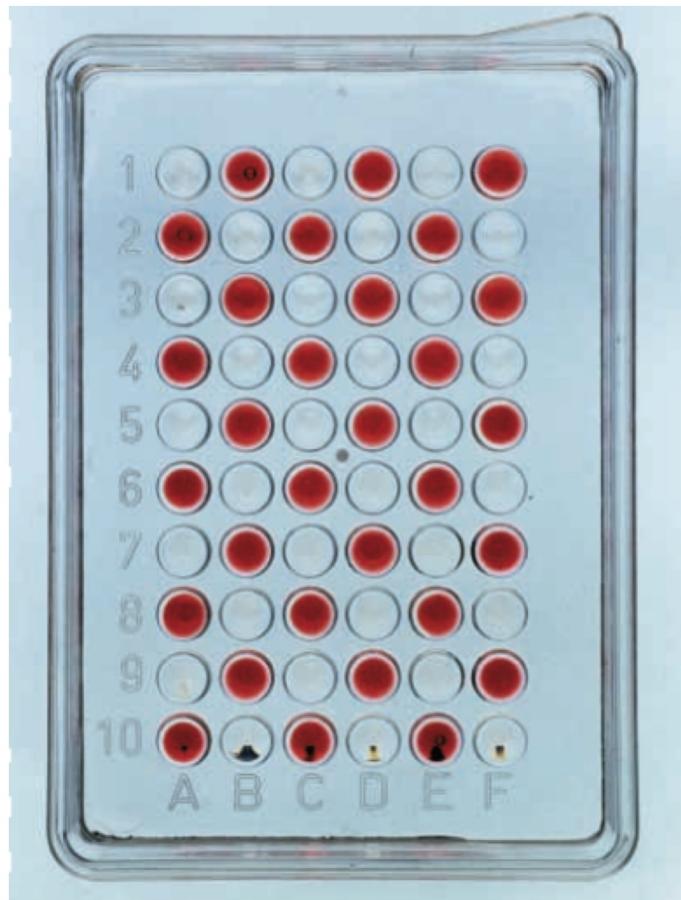
MALVOLIO



Before Transcriptomics

Analysis of single genes (qPCR)

Application example



Before Transcriptomics

Analysis of single genes (qPCR)

Aplication example



**Shakespeare's
Twelfth Night**

“taste with
distempered taste”

Transcriptomics

Why not the whole genome?

Transcriptomics

Why not the whole genome?

CACTGGCCATGGGTGCCCCCTGTAAGGGCCAATTGGCTGAGGGCTGCGAATCAGCCCCGCTTCAAGCCACAAACGGGCACCAAATTGAGCAGGCAGCTCGCCGGCAGCAGTTCTCATGAGGGCATCTCCGCCCTGAGCAA

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CAACCGAGCCACTTCGTTAGCAGAAGTGTGGTAGGCTAGGGATTCACCGCTCCAGAGACGCTCAGTTGGAGCAGGGCATCAGAACTGGAGGGGAAGGGCTGTCAGAAGAGTCCACAAATCTACC

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GACCATCATGATGCGAACGGGCCTGATCCGGCAGGGGAGTACAGCGATTTCTCAAGAGtgagtagccctccccgtatcccgtggcttccctttggaaataccctcctatcgccgcctctcgccactcttcgaagaccaga

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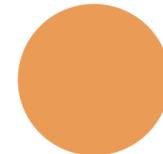
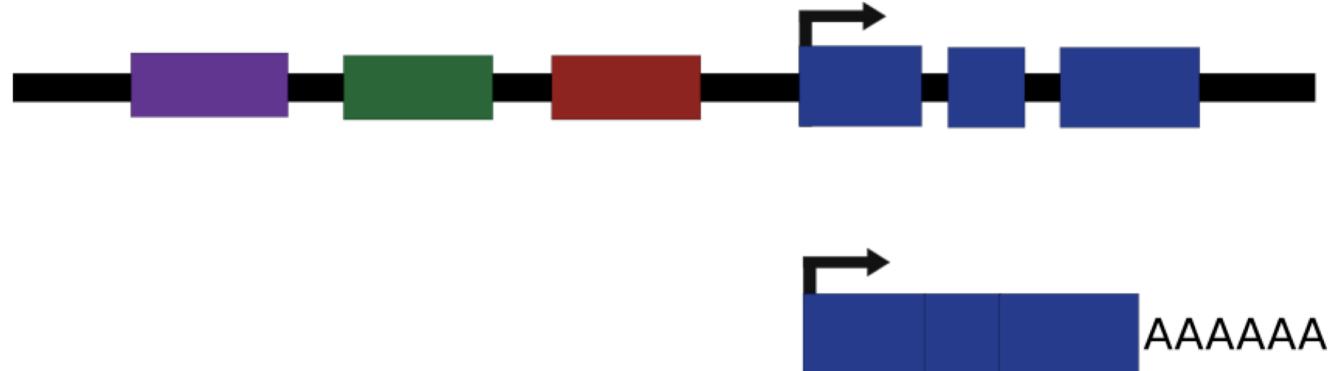
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TCA GCT AATT CCA AT CTAG CAG AGA TCA AGC AT CGCT AC GAC GAG GG GC CAT GG AA CGA AAG taa gca attt gaaaaaaaaaaaac acg aat ca aga taa gca att gag ga at taa gca at tgg aag c aagg tcaa

tgaactacctccaaatcaacaattttgttcatgtttaaatgtatattaccgatagtcaagagagggctataaaaataaccacattcgatttgactcaaactagtagtgtataatcgcgttggcaagaagatggaaaaataatt

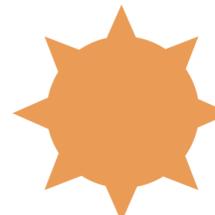
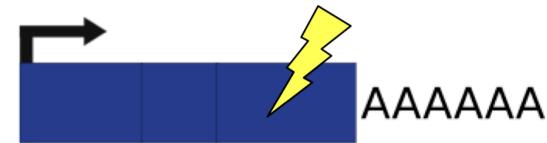
Transcriptomics

Coding regions



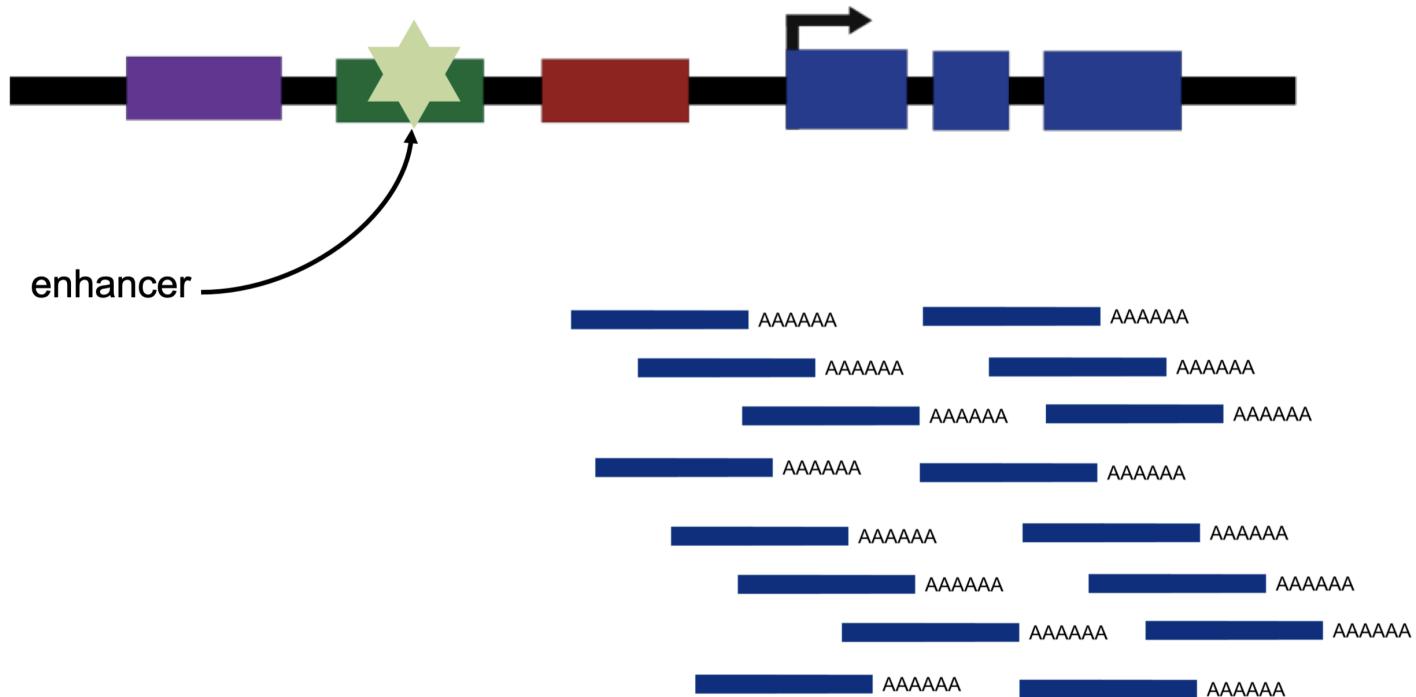
Transcriptomics

Coding regions



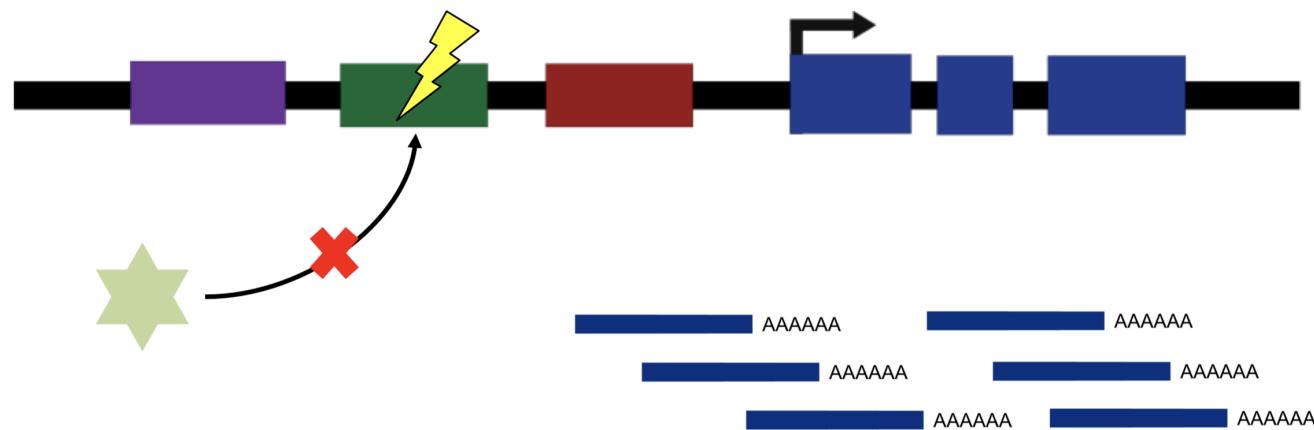
Transcriptomics

Cis-regulatory regions



Transcriptomics

Cis-regulatory regions



Transcriptomics

Cis-regulatory regions

species #1

EYTPOTHISJTIPXELSOTEXTKISYPICSEKYPHISOTPPTOIHIDDENHSIVH

JETPOJITPTGITHISSELSSOTTEXTTOPICSYPHISTAPTXPJHIDDENHSIVH

species #2

Transcriptomics

Cis-regulatory regions

species #1

EYTPOTHISJTIPXELSOTEXTKISYPICSEKYPHISOTPPTOIHIDDENHSIVH



species #2

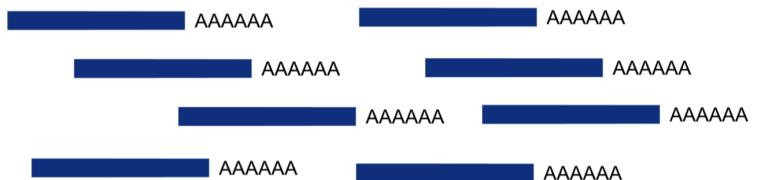
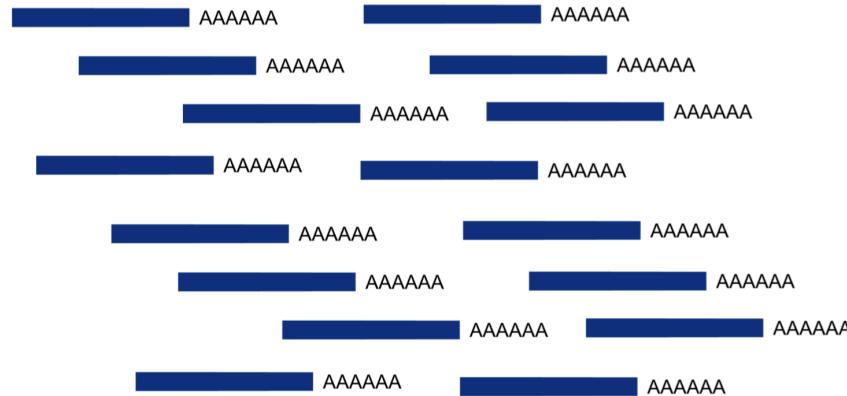
Transcriptomics

Cis-regulatory regions

species #1

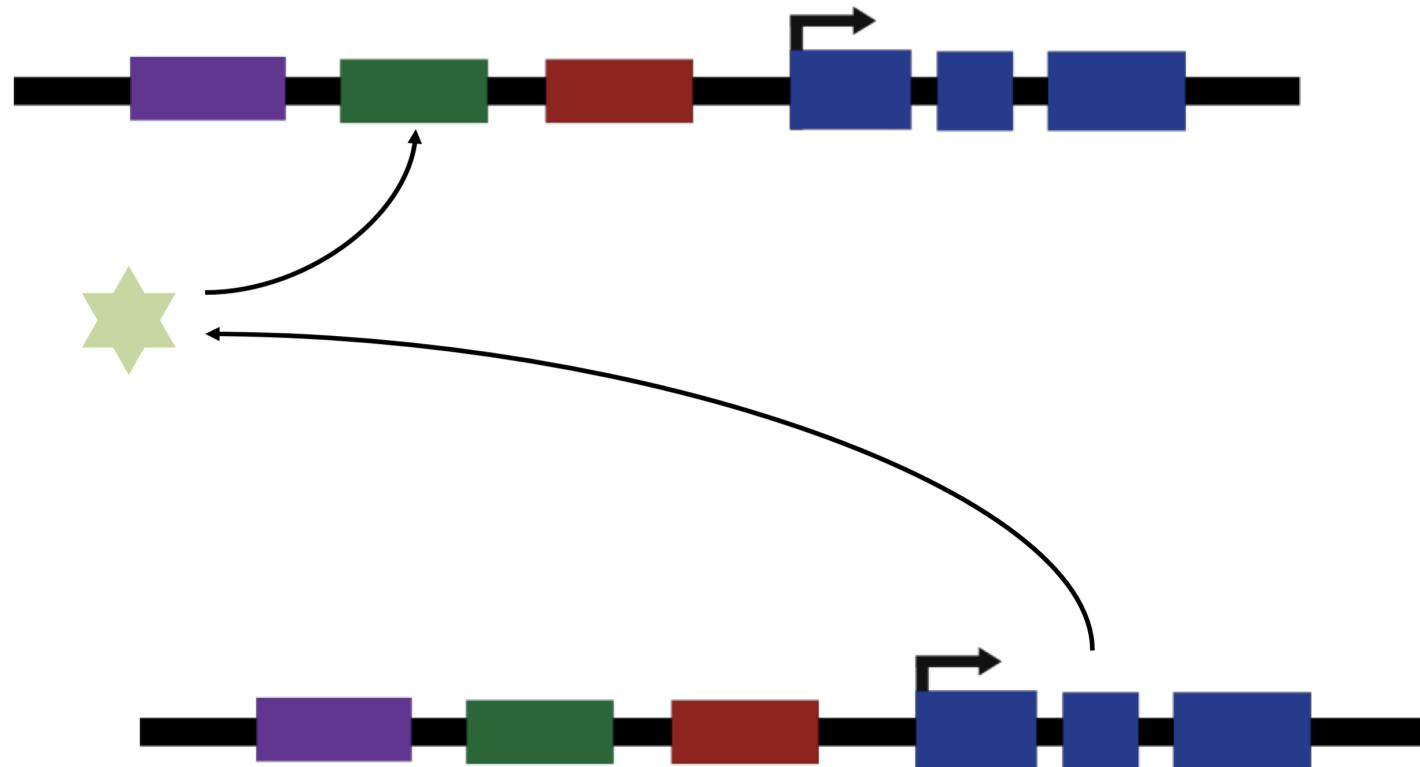


species #2



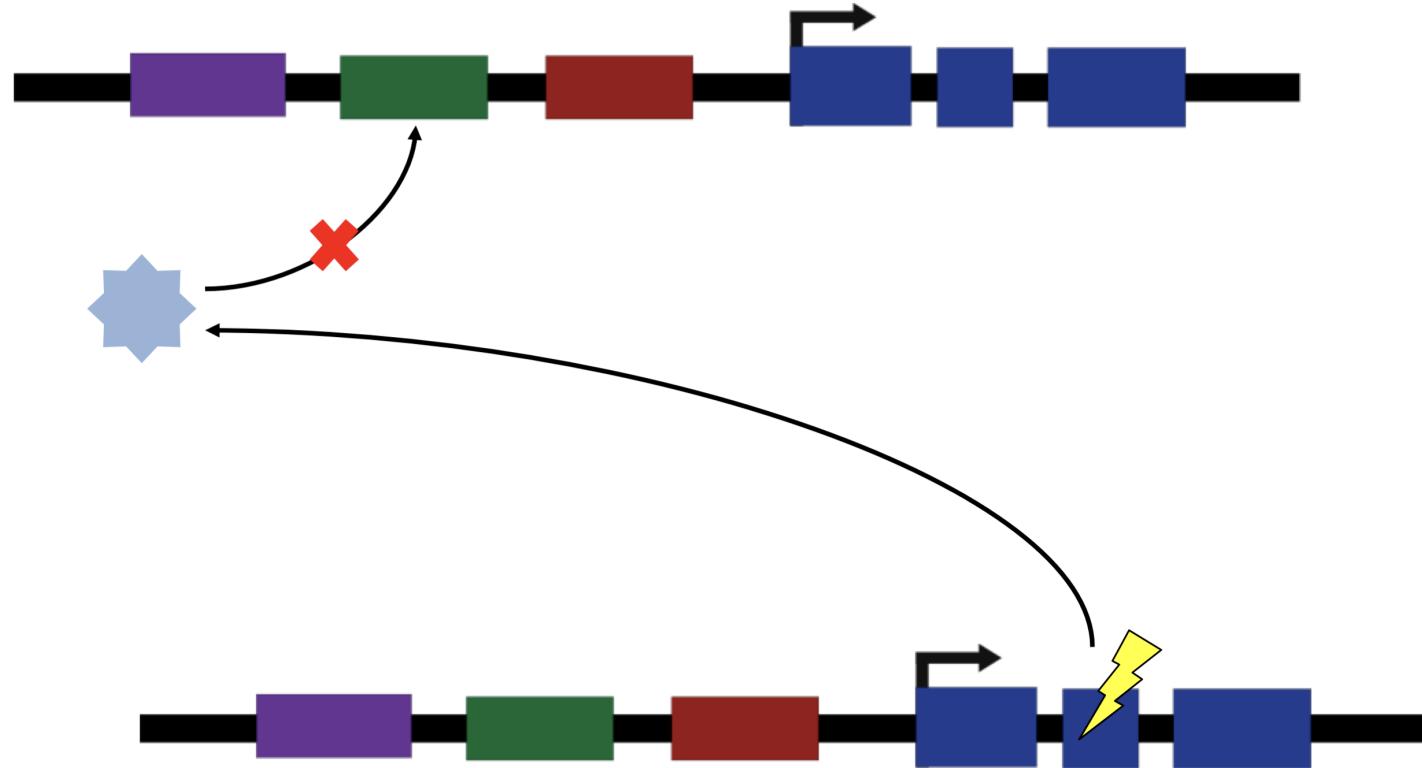
Transcriptomics

Trans-regulatory regions



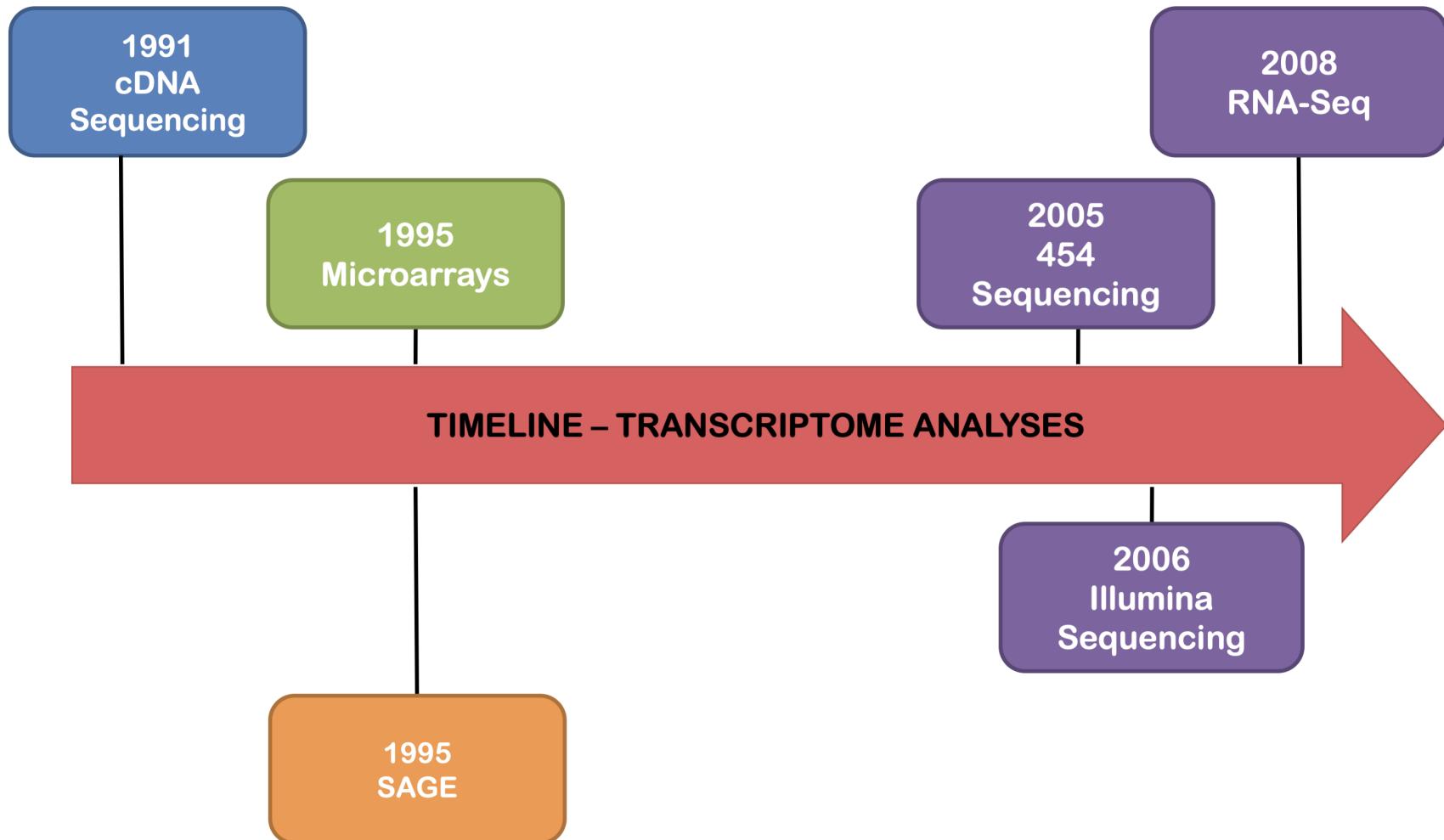
Transcriptomics

Trans-regulatory regions



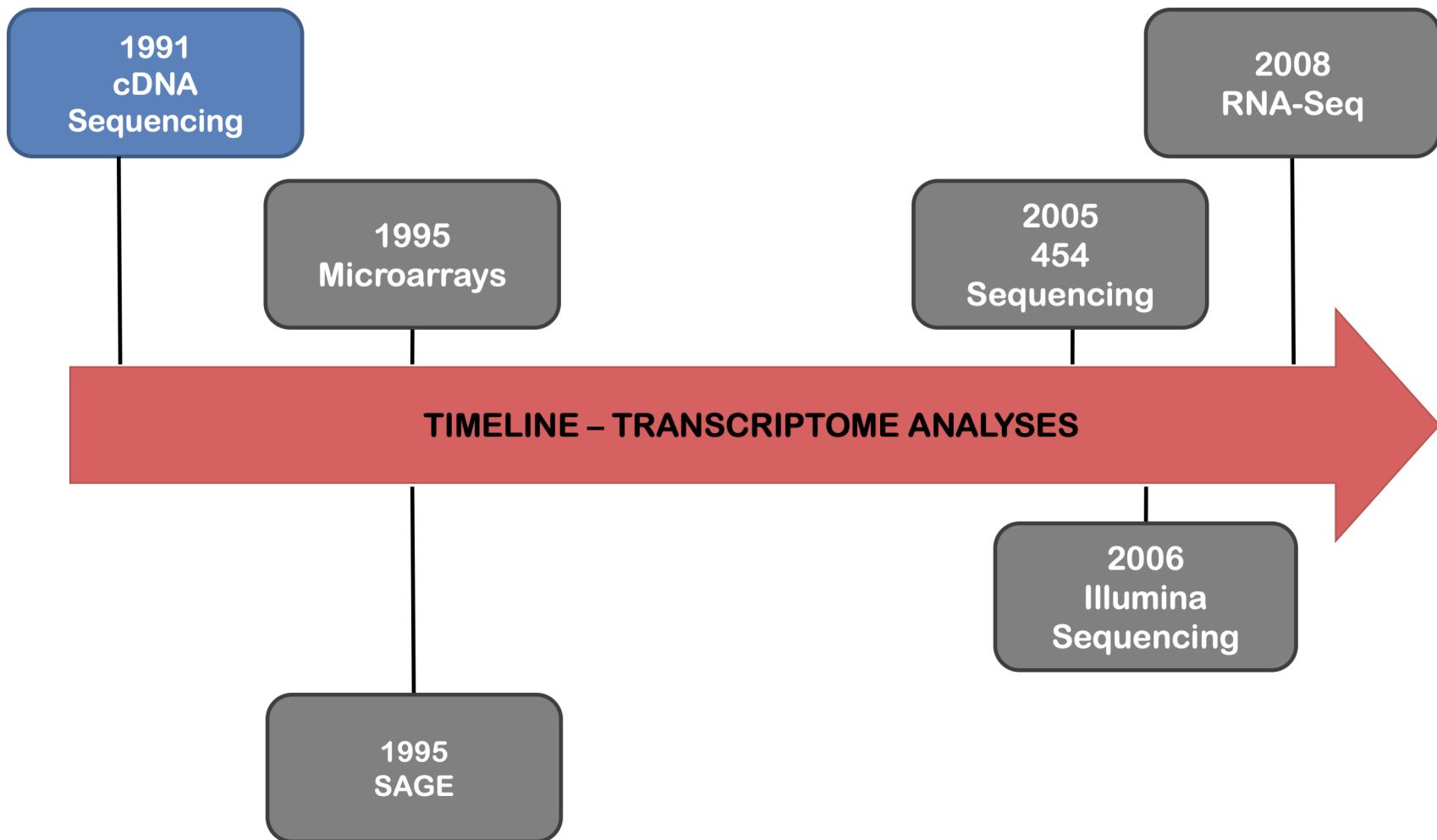
Transcriptomics

The evolution of the methods



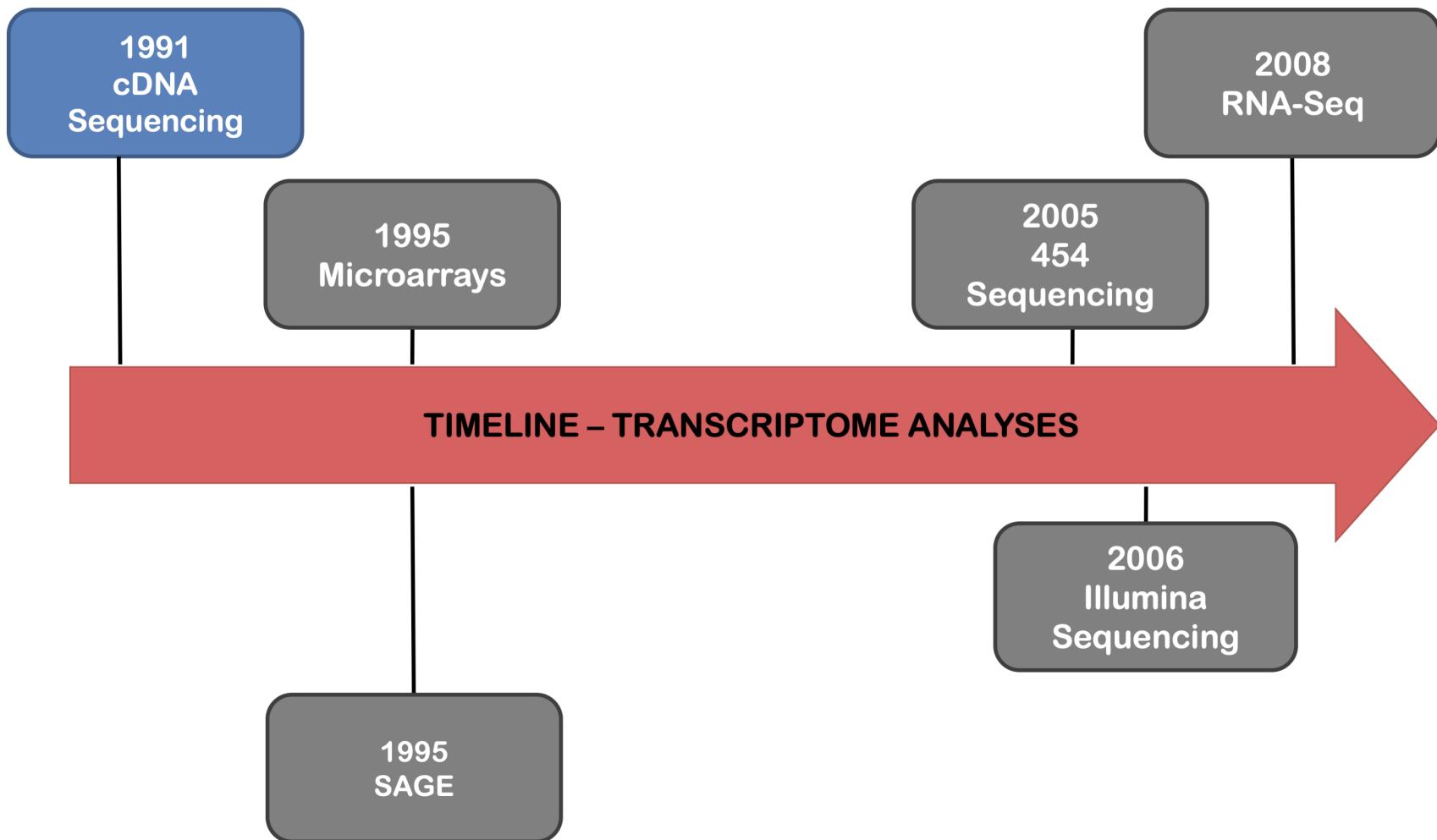
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The evolution of the methods



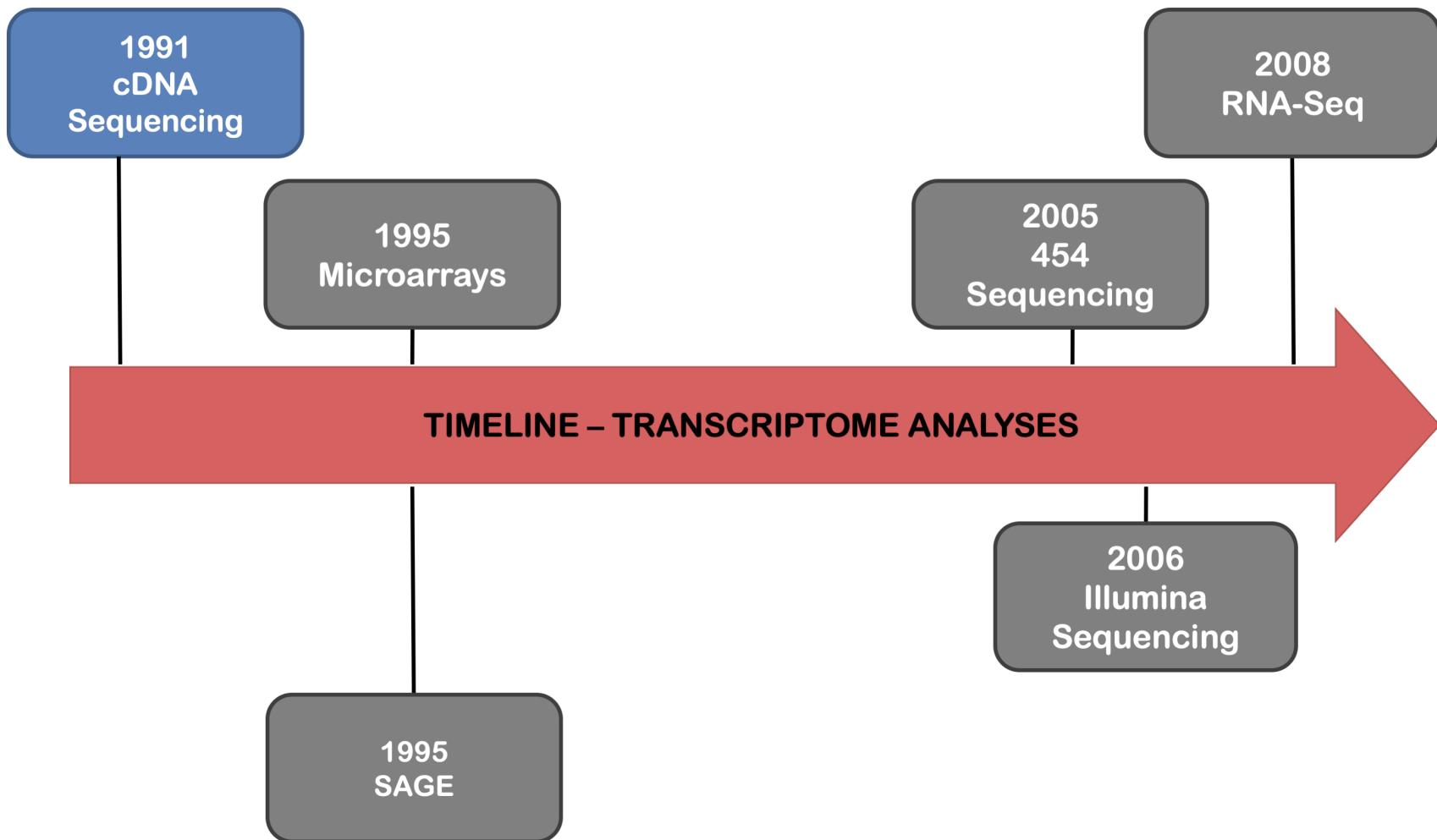
Transcriptomics

The evolution of the methods



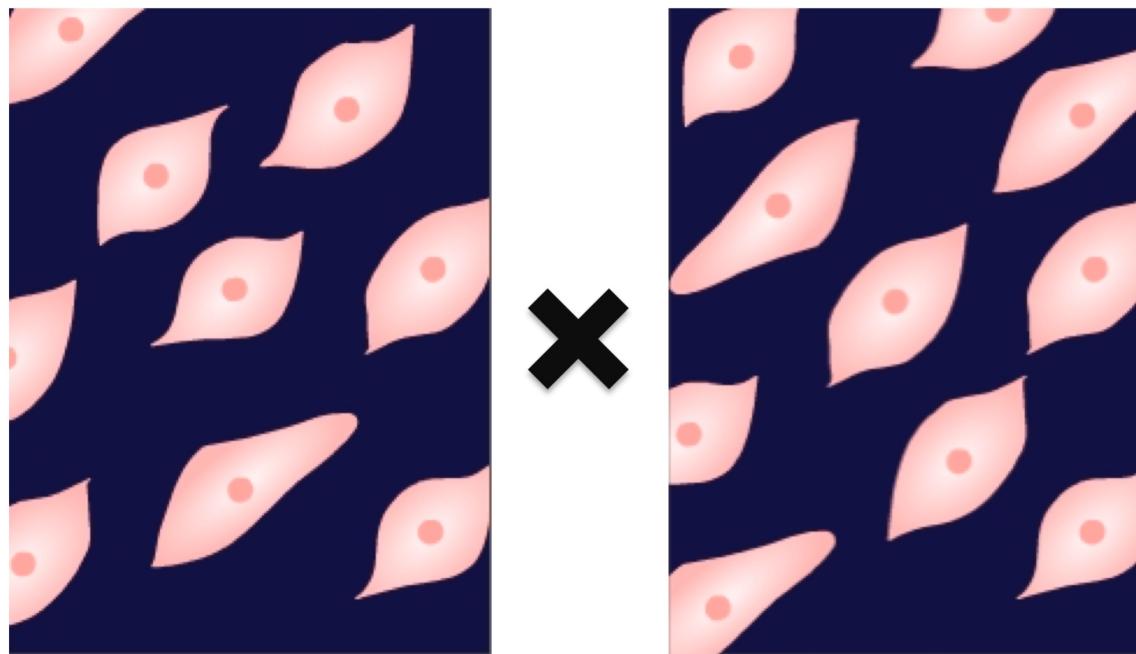
Transcriptomics

The evolution of the methods



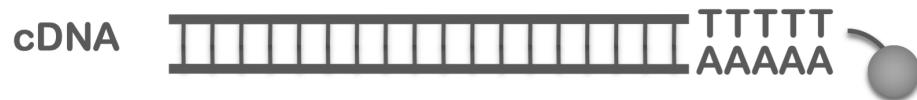
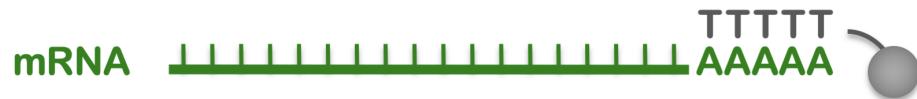
Transcriptomics

cDNA sequencing



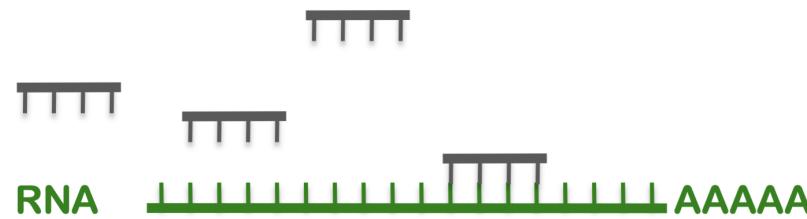
Transcriptomics

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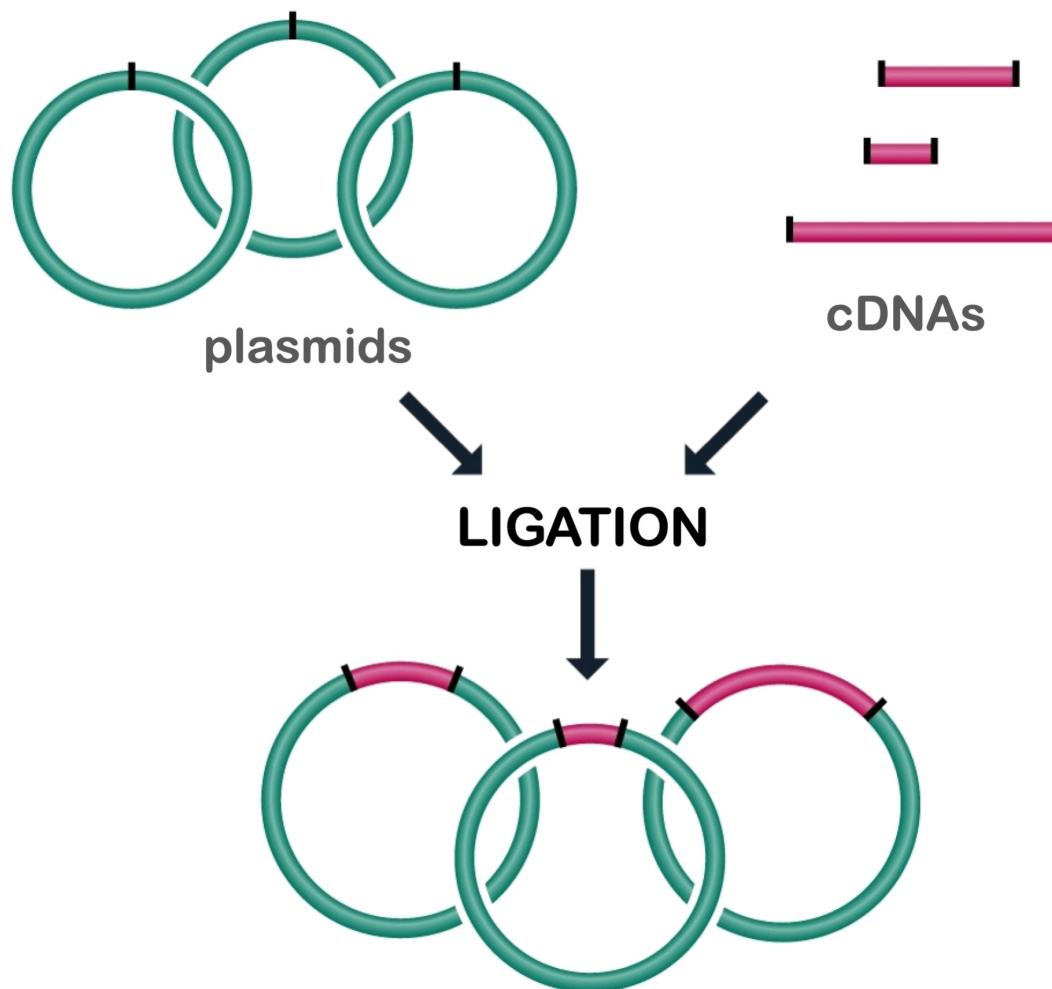
Transcriptomics

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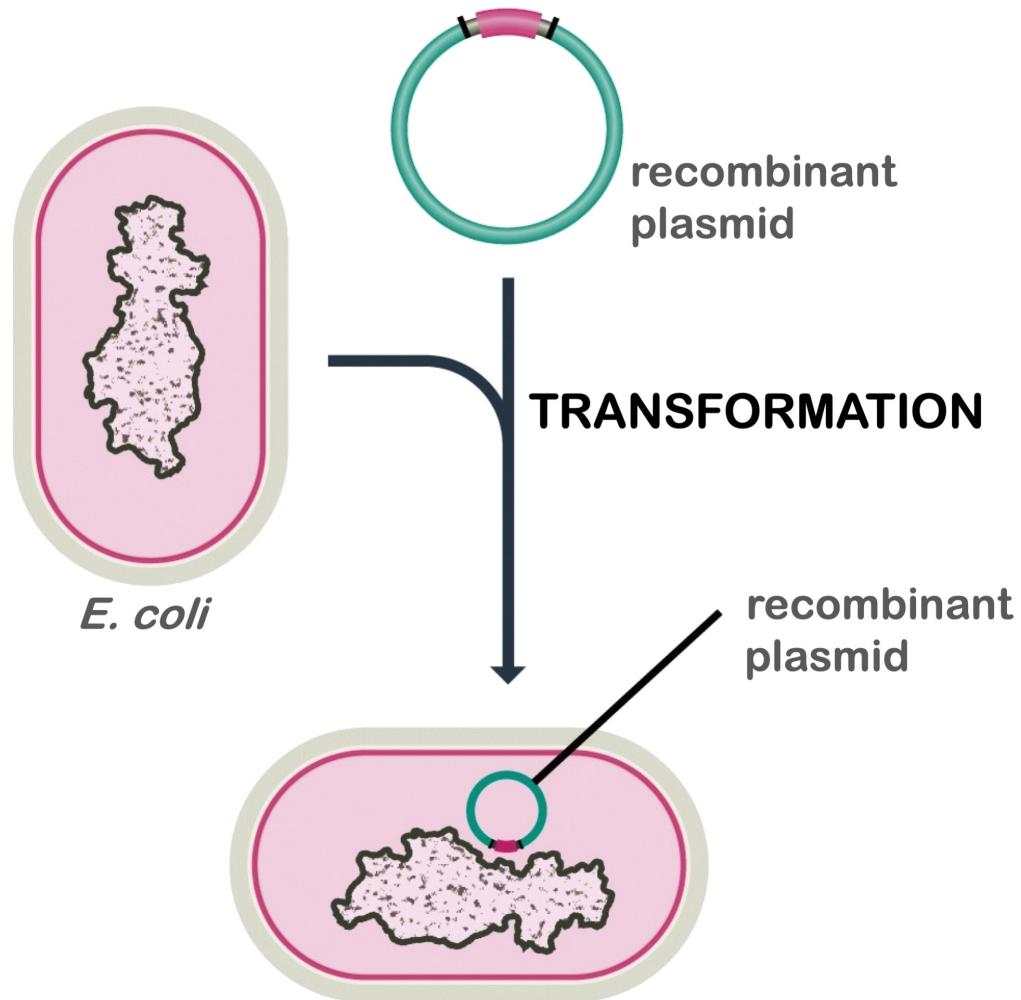
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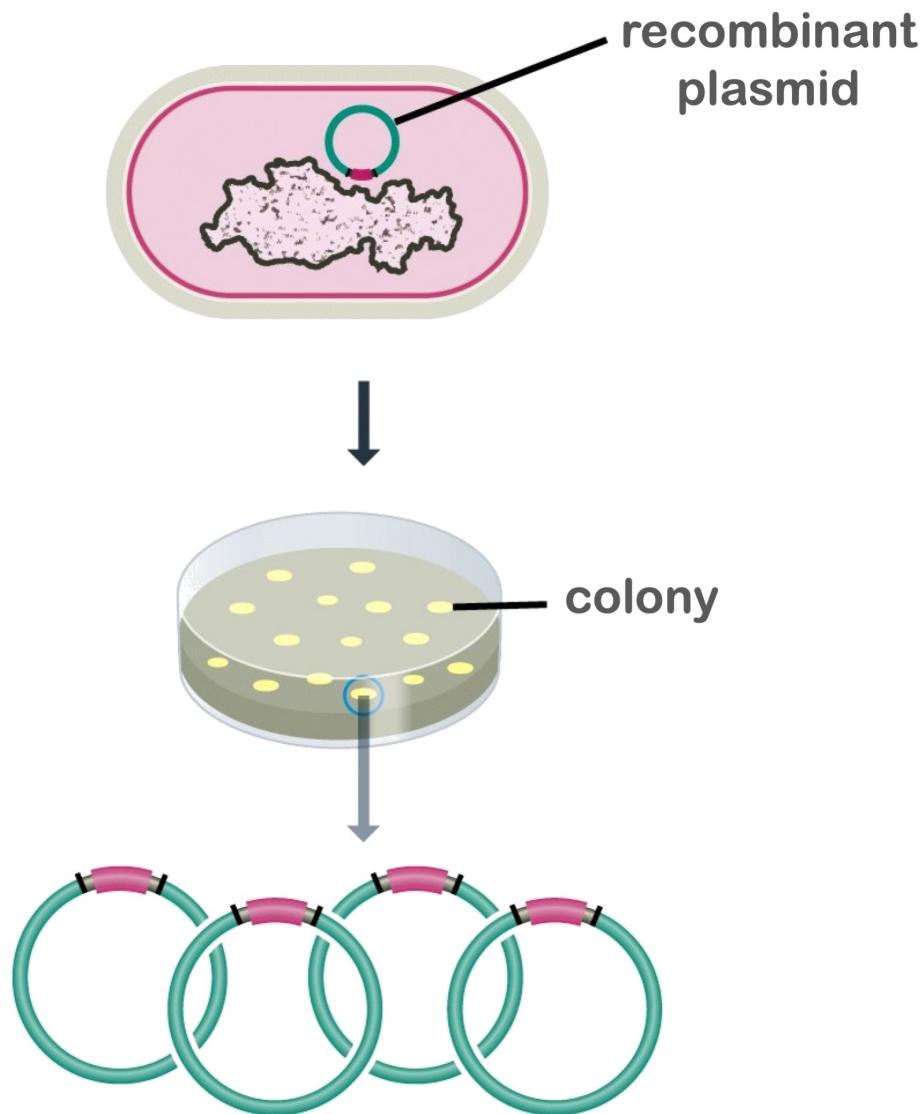
Transcriptomics

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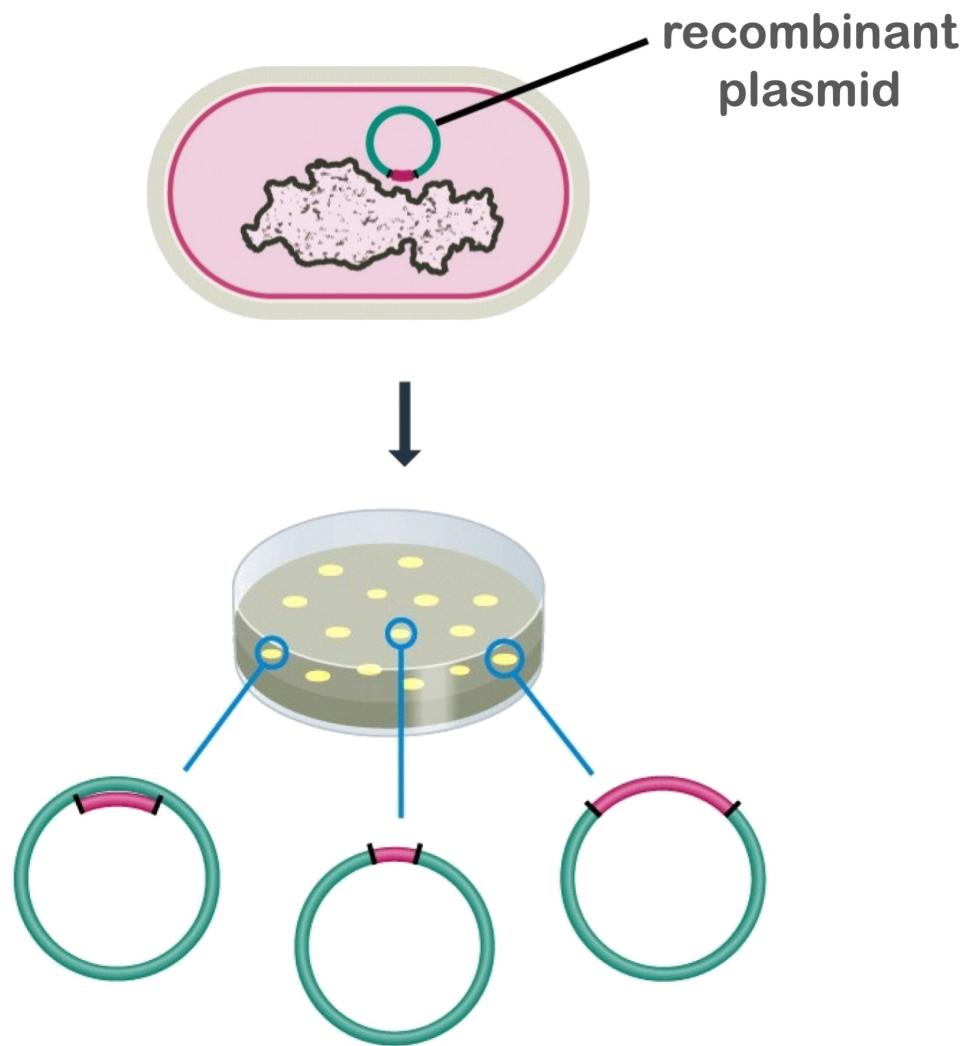
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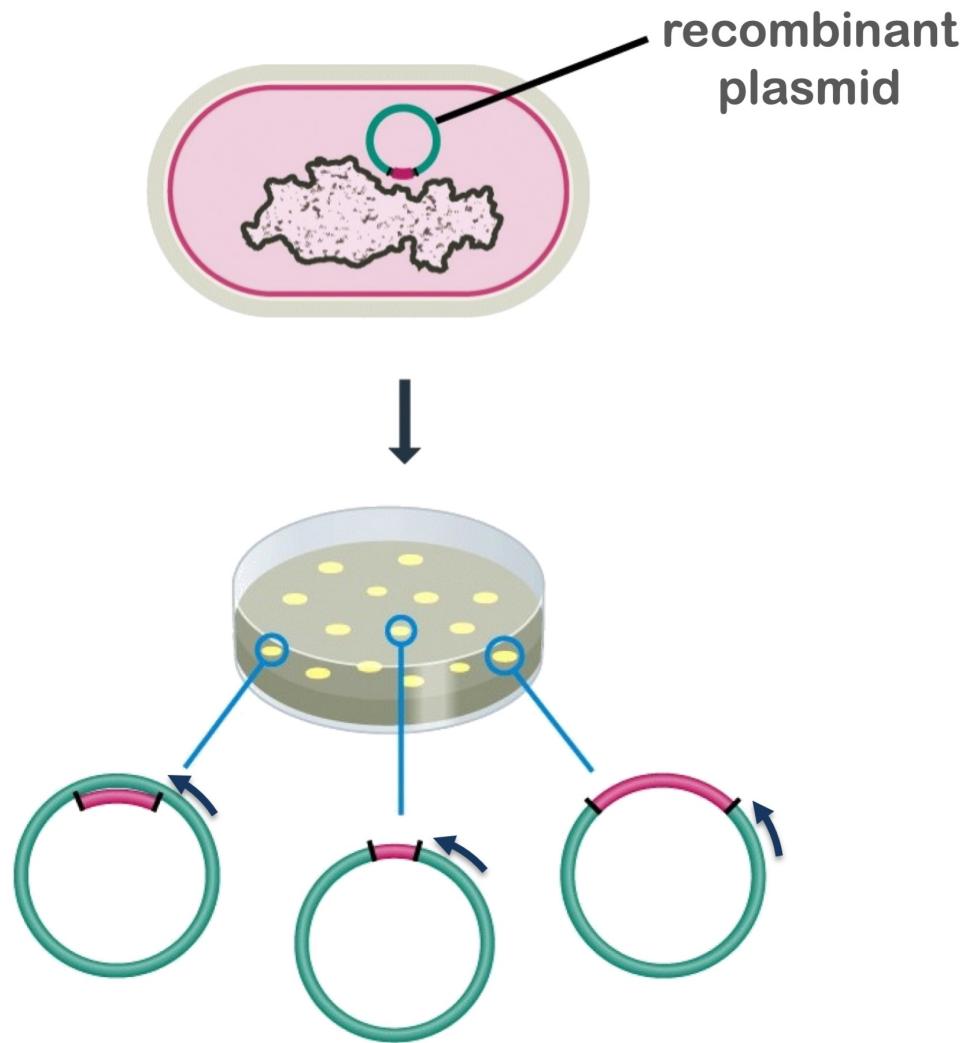
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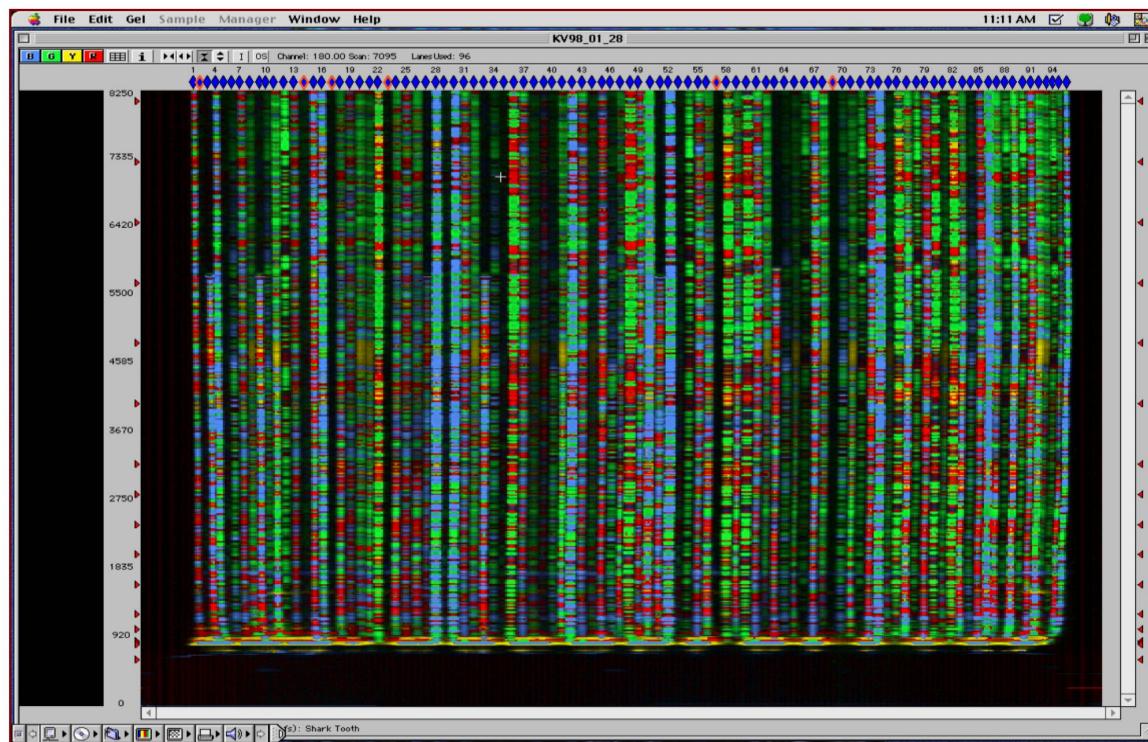
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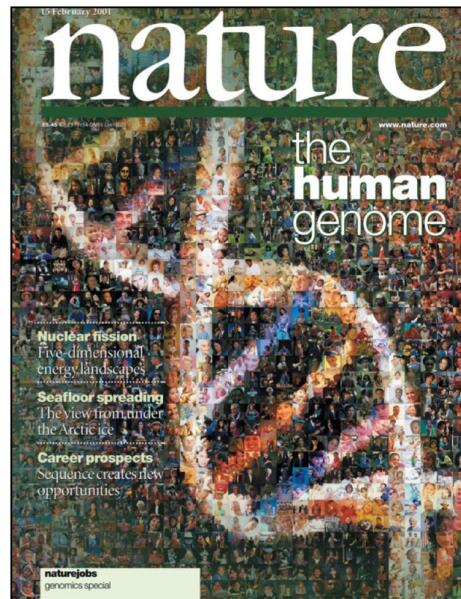
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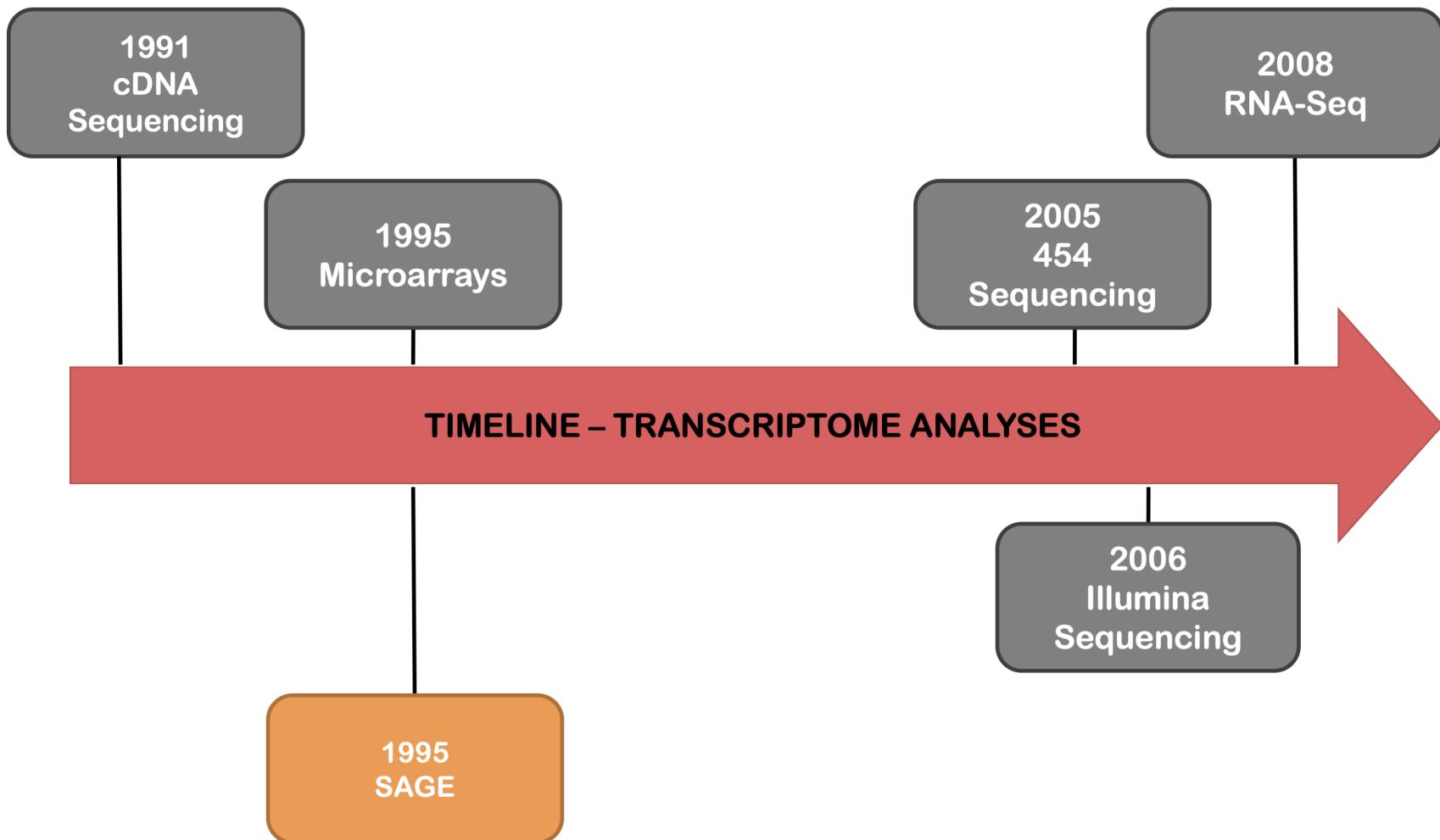
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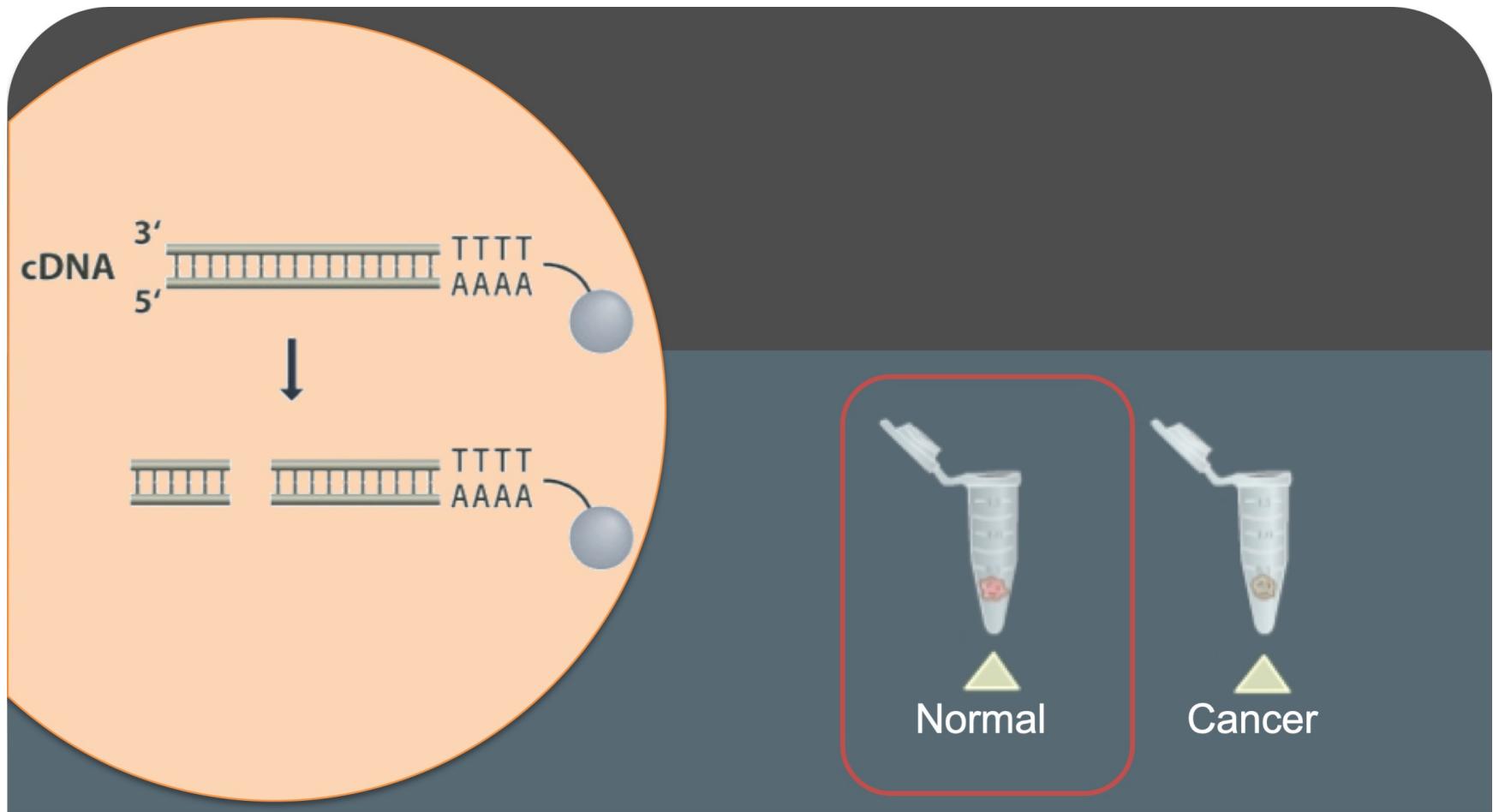
Transcriptomics

The evolution of the methods



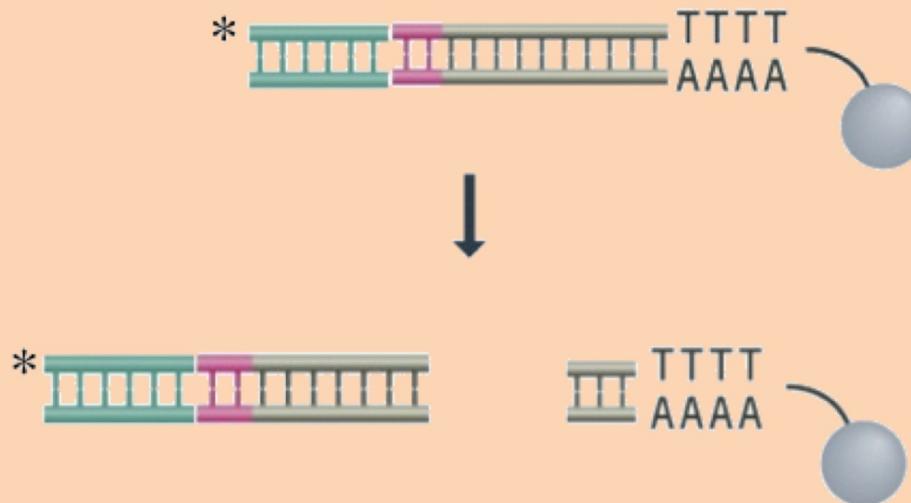
Transcriptomics

Serial Analysis of Gene Expression (SAGE)



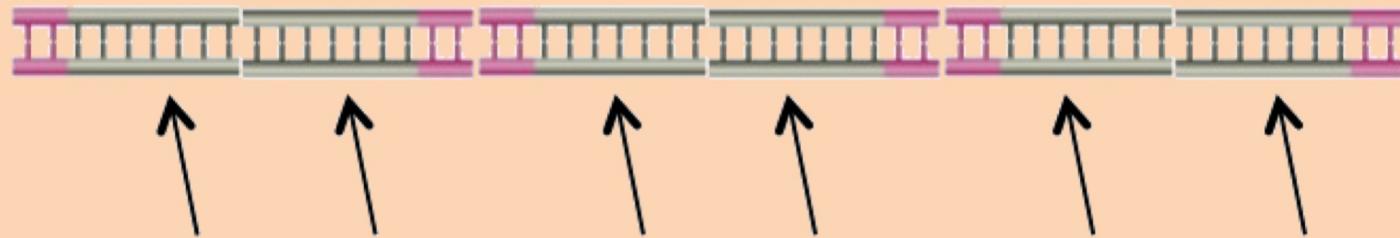
Transcriptomics

Serial Analysis of Gene Expression (SAGE)



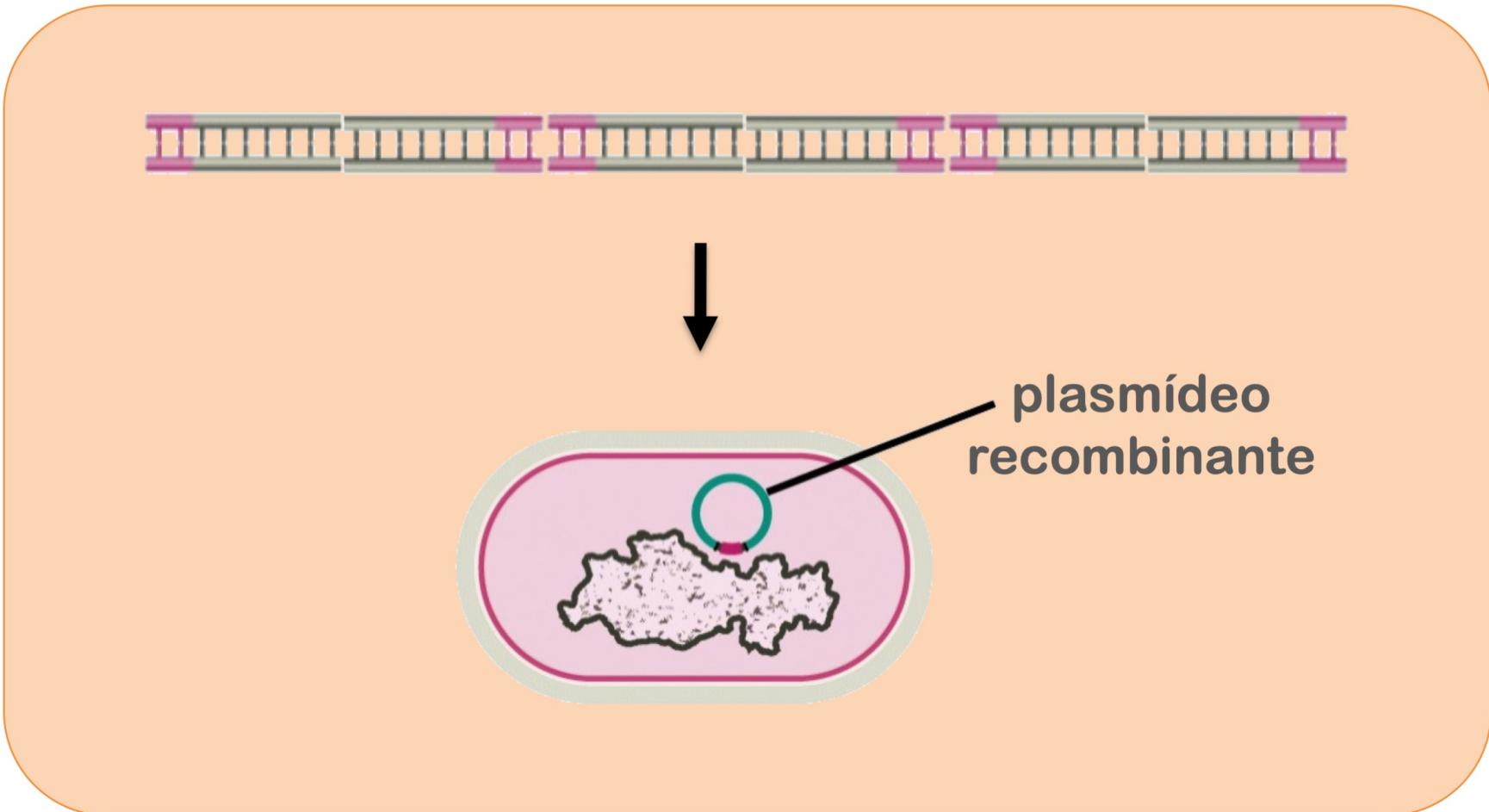
Transcriptomics

Serial Analysis of Gene Expression (SAGE)



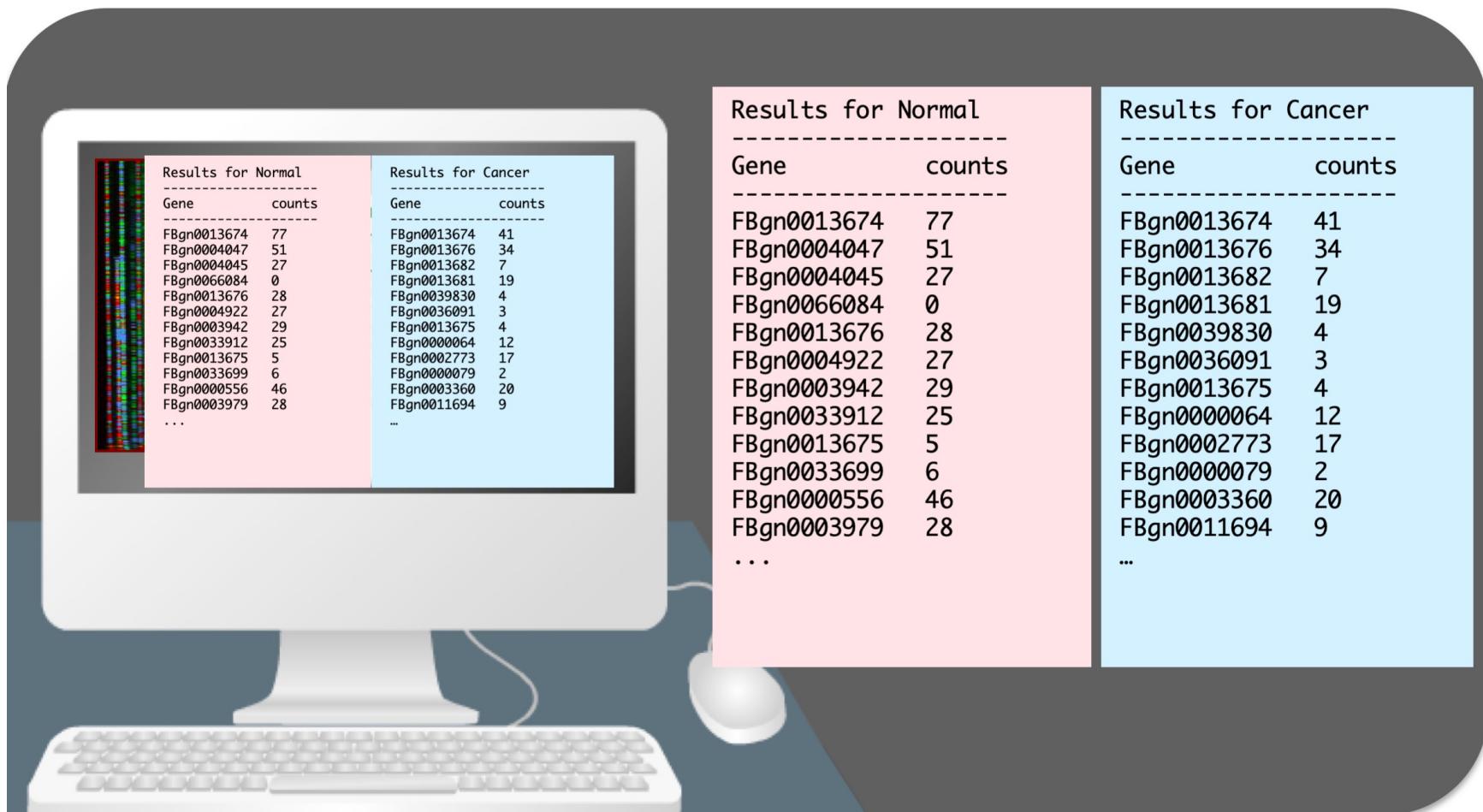
Transcriptomics

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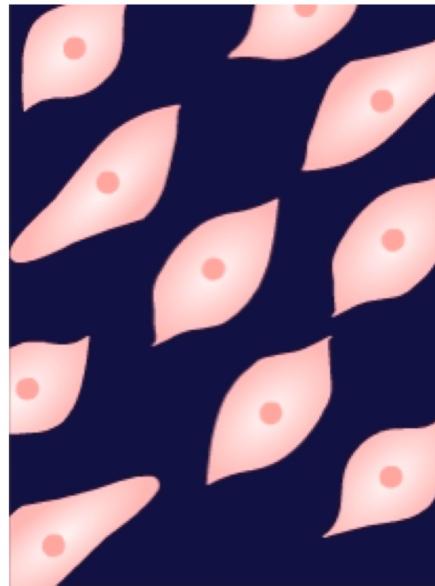
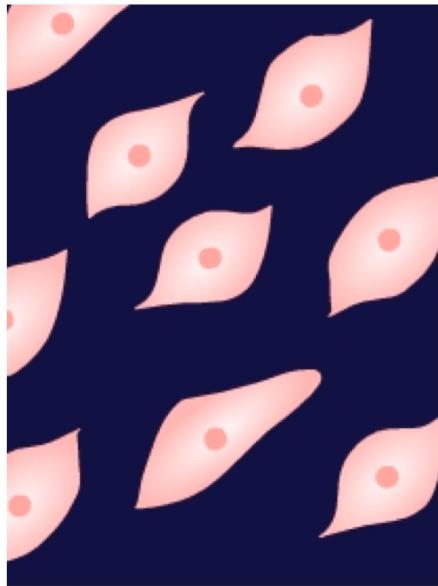
Transcriptomics

Serial Analysis of Gene Expression (SAGE)



Transcriptomics

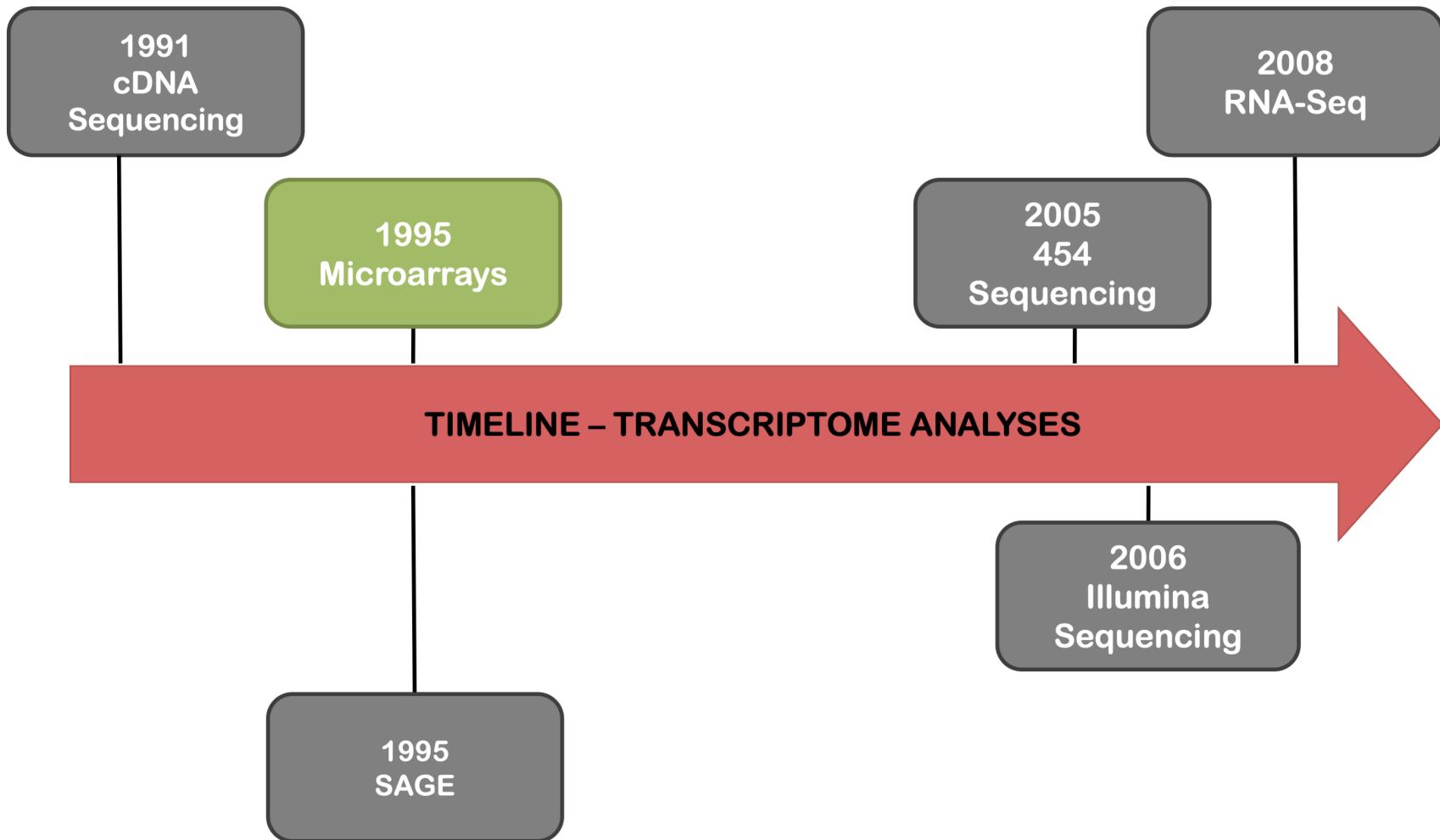
Serial Analysis of Gene Expression (SAGE)



TFF3	Colorectal
Sox9	
HoxA10	
CDH17	Gastric
Claudin-18	

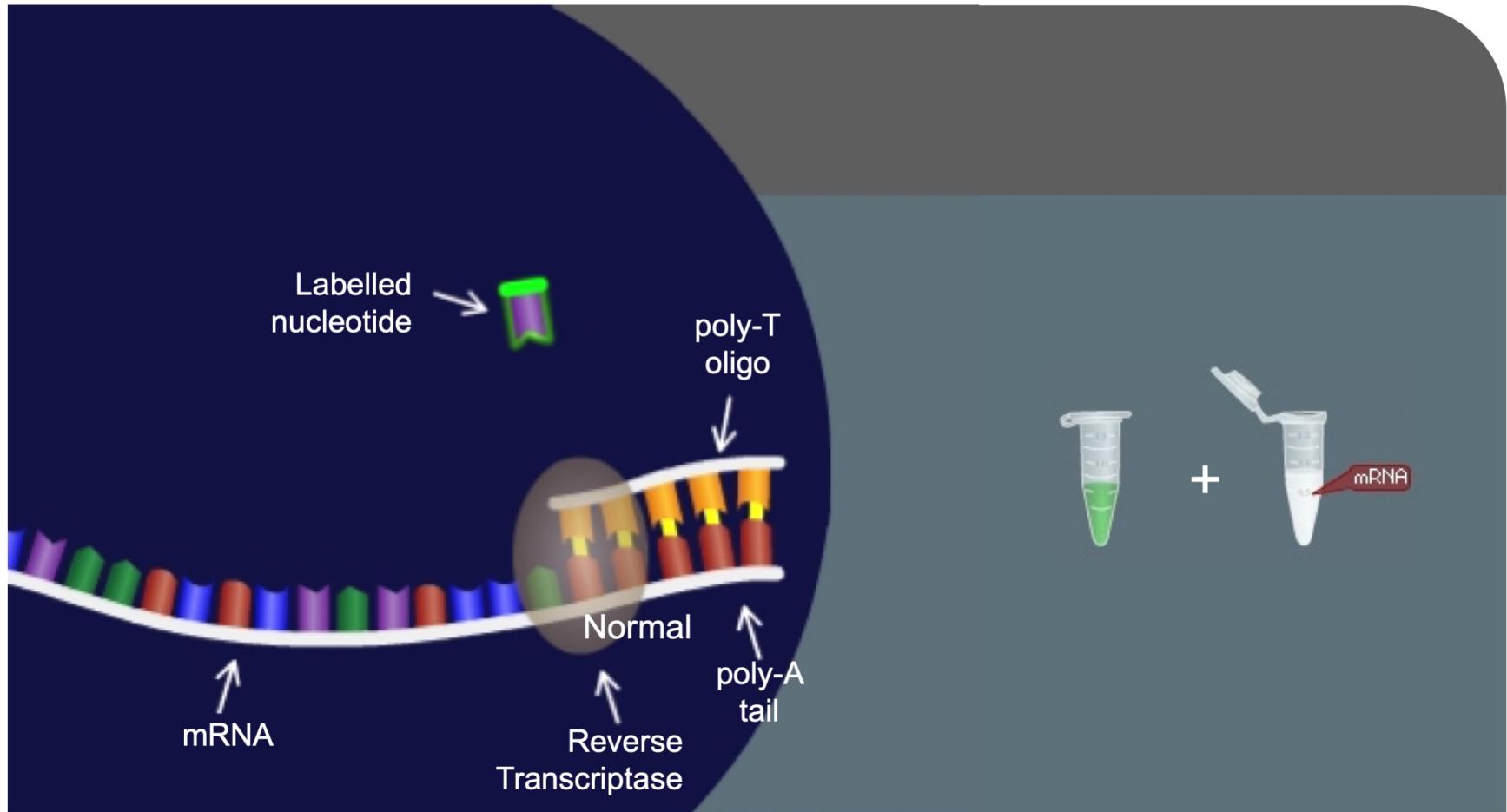
Transcriptomics

The evolution of the methods



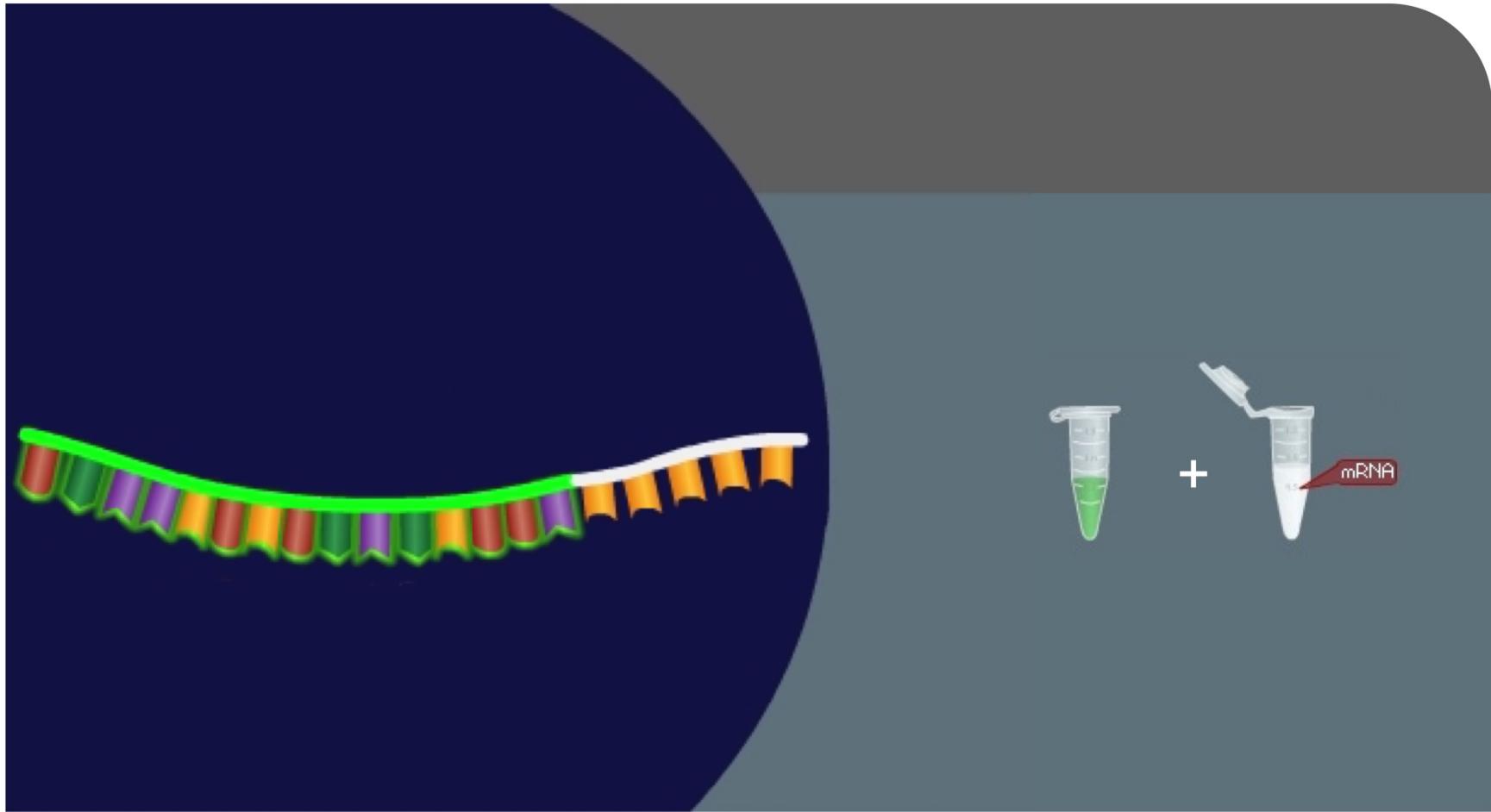
Transcriptomics

Microarrays



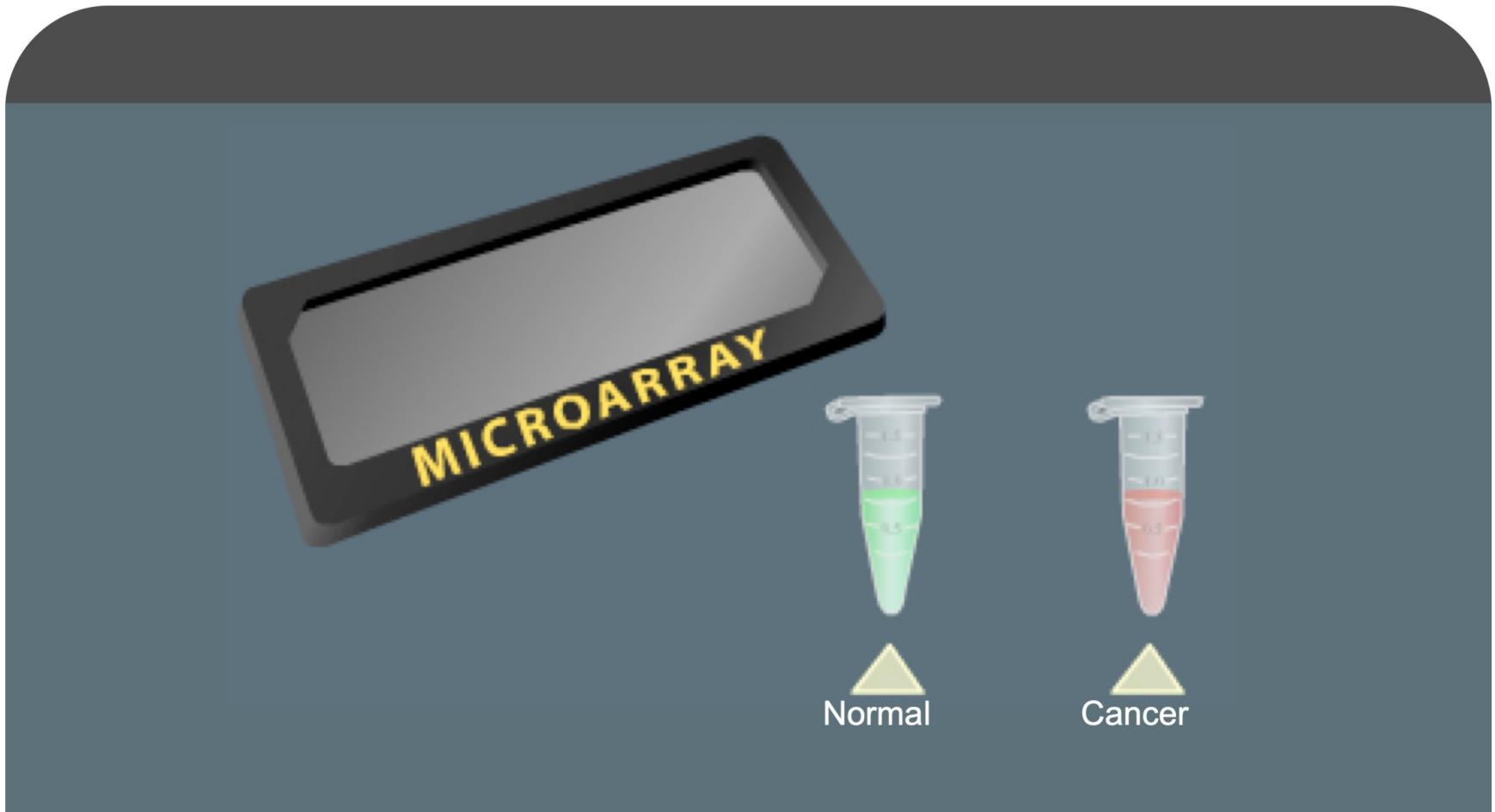
Transcriptomics

Microarrays



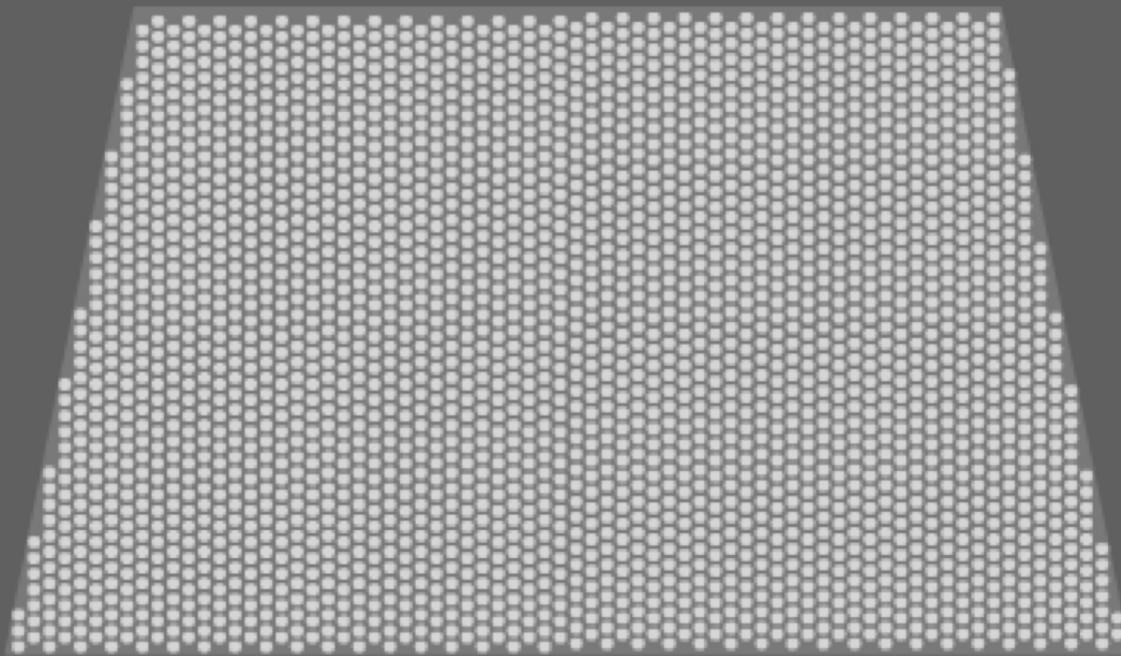
Transcriptomics

Microarrays



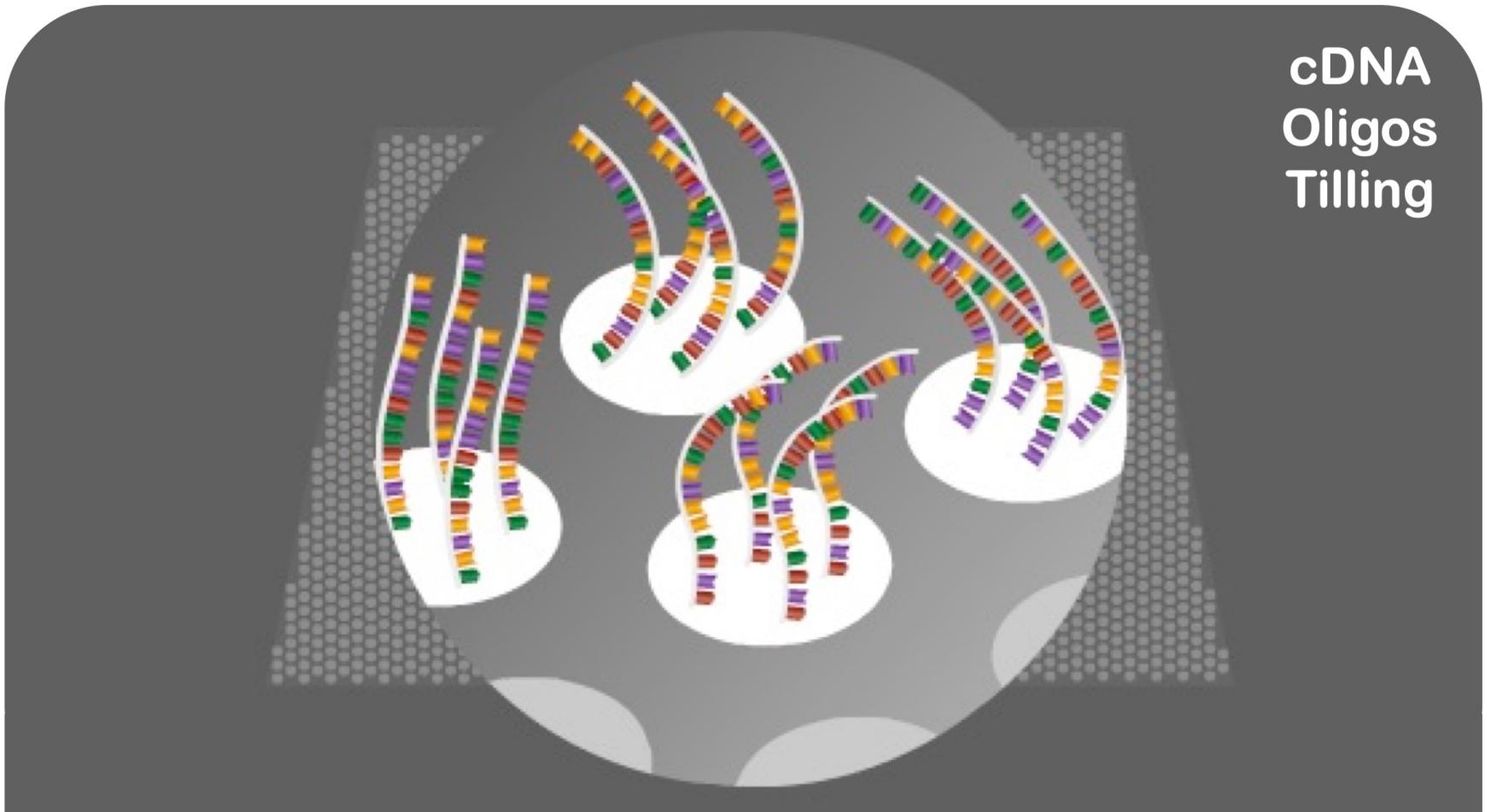
Transcriptomics

Microarrays



Transcriptomics

Microarrays



cDNA
Oligos
Tilling

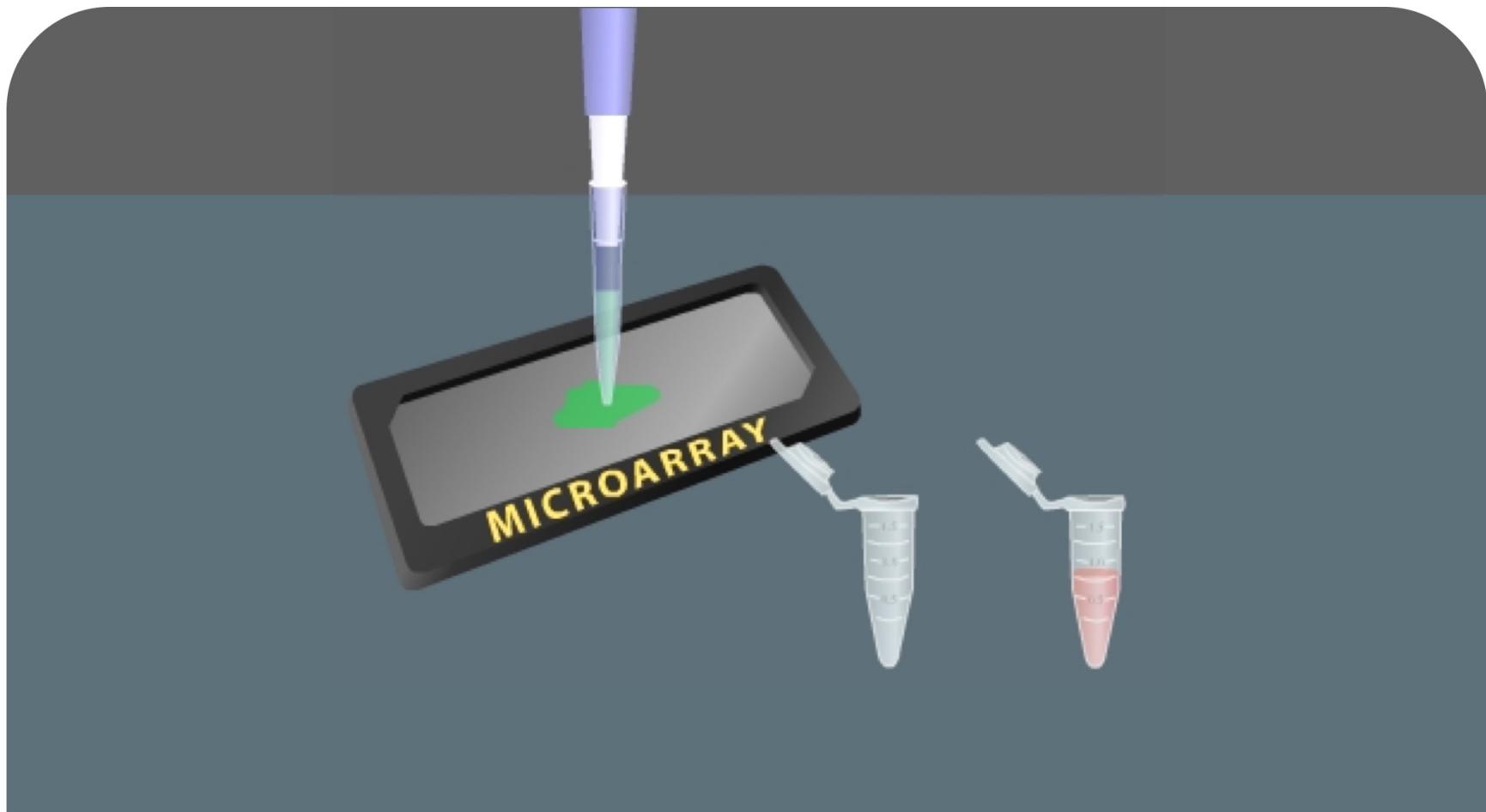
Transcriptomics

Microarrays



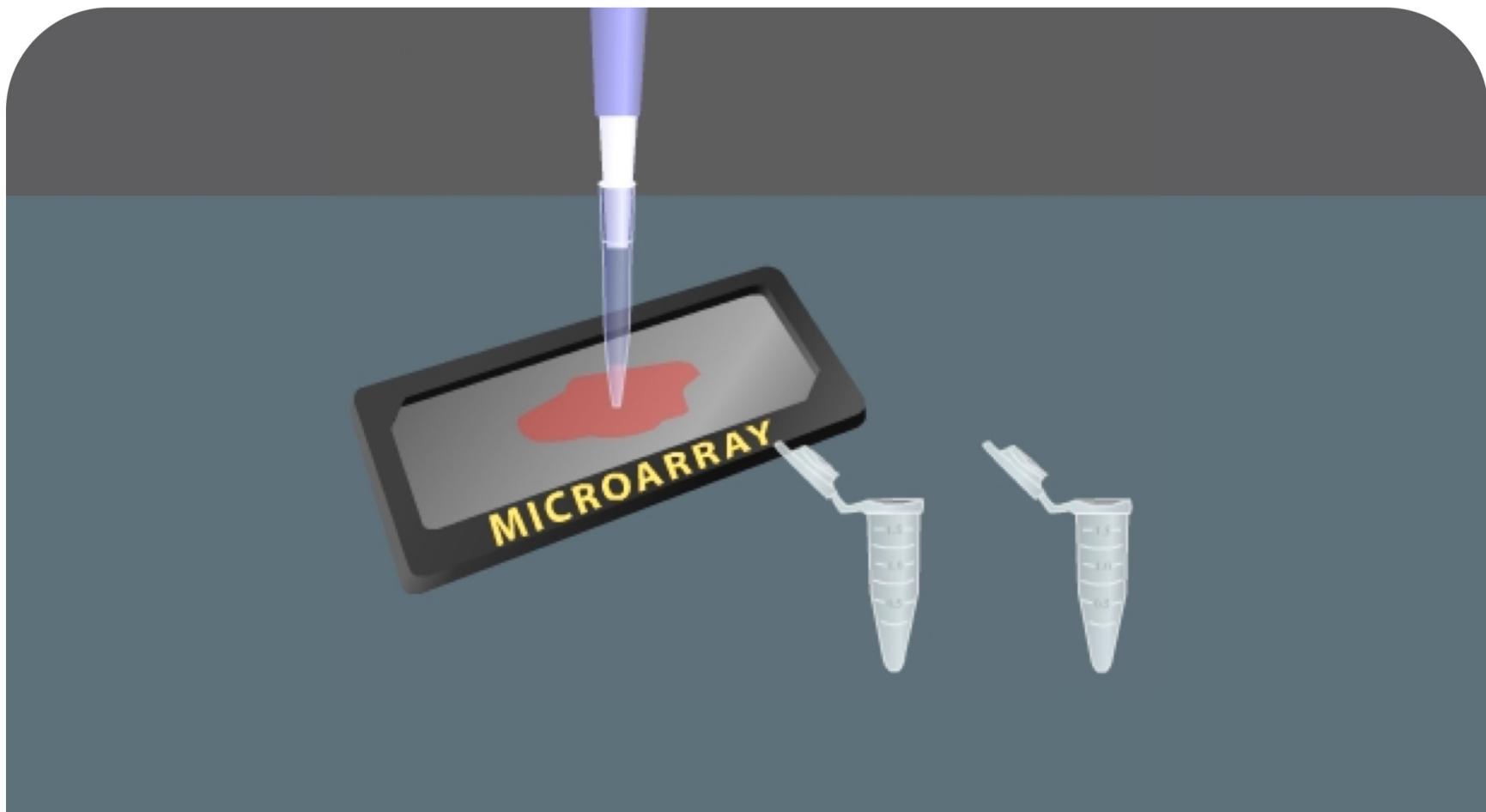
Transcriptomics

Microarrays



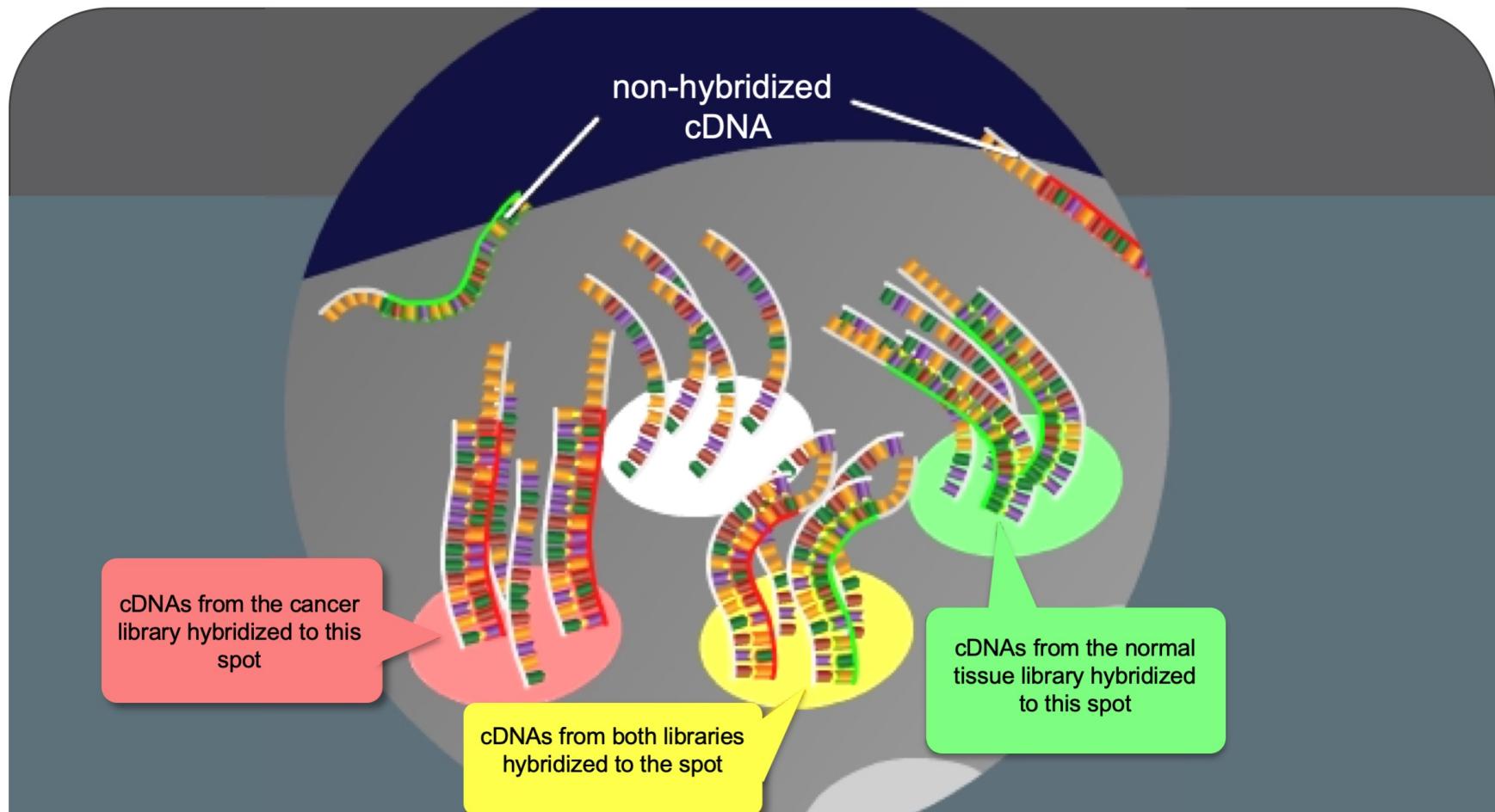
Transcriptomics

Microarrays



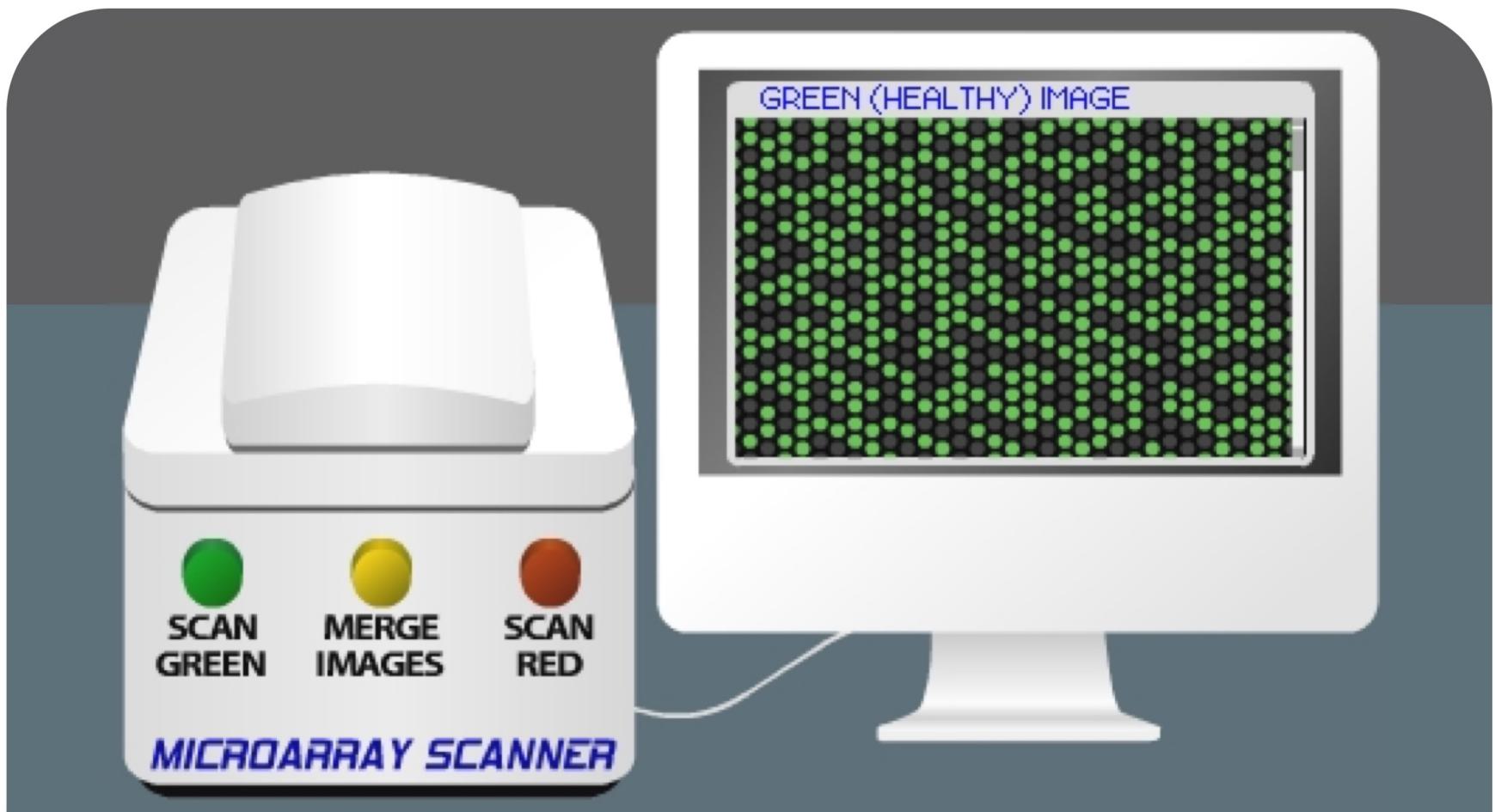
Transcriptomics

Microarrays



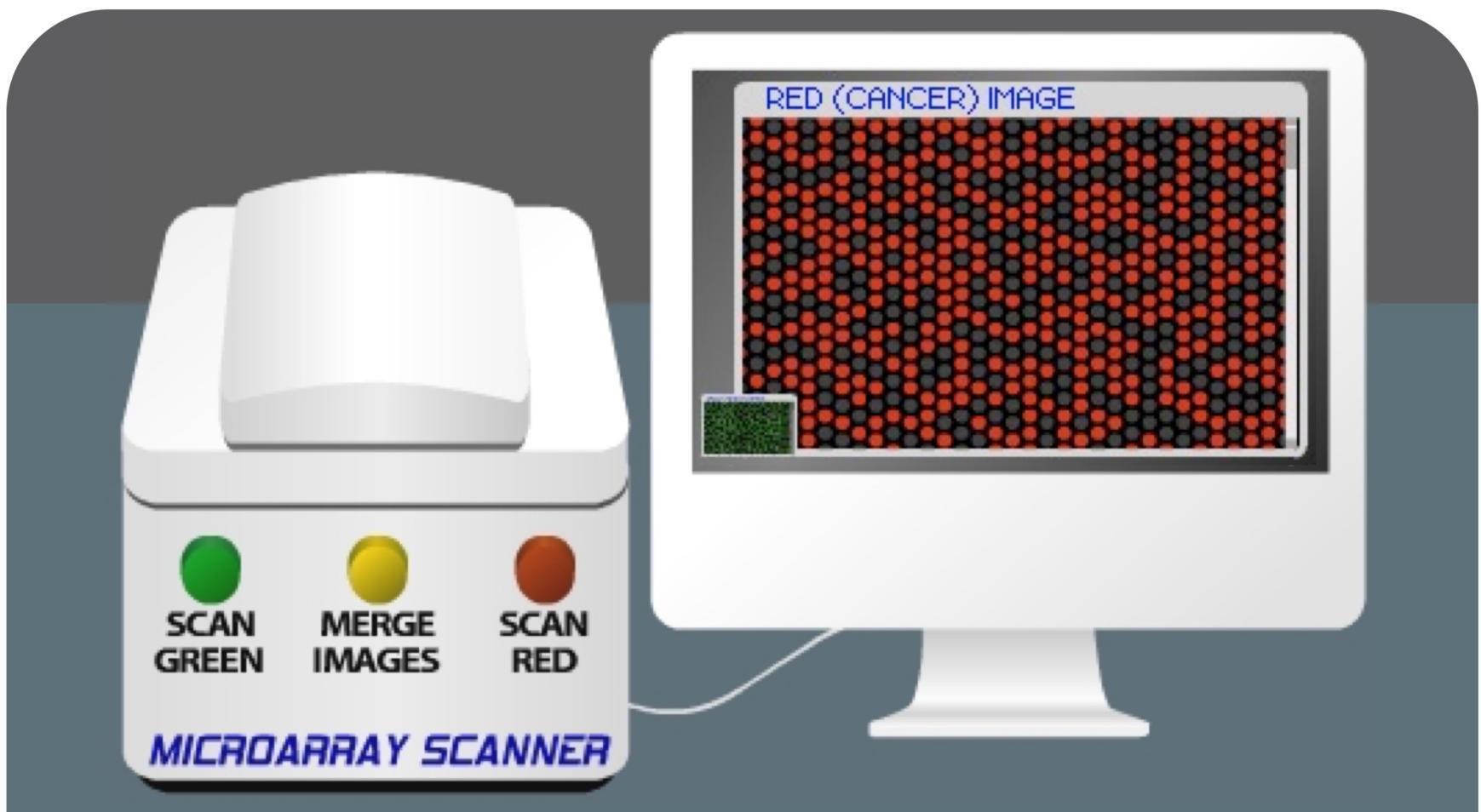
Transcriptomics

Microarrays



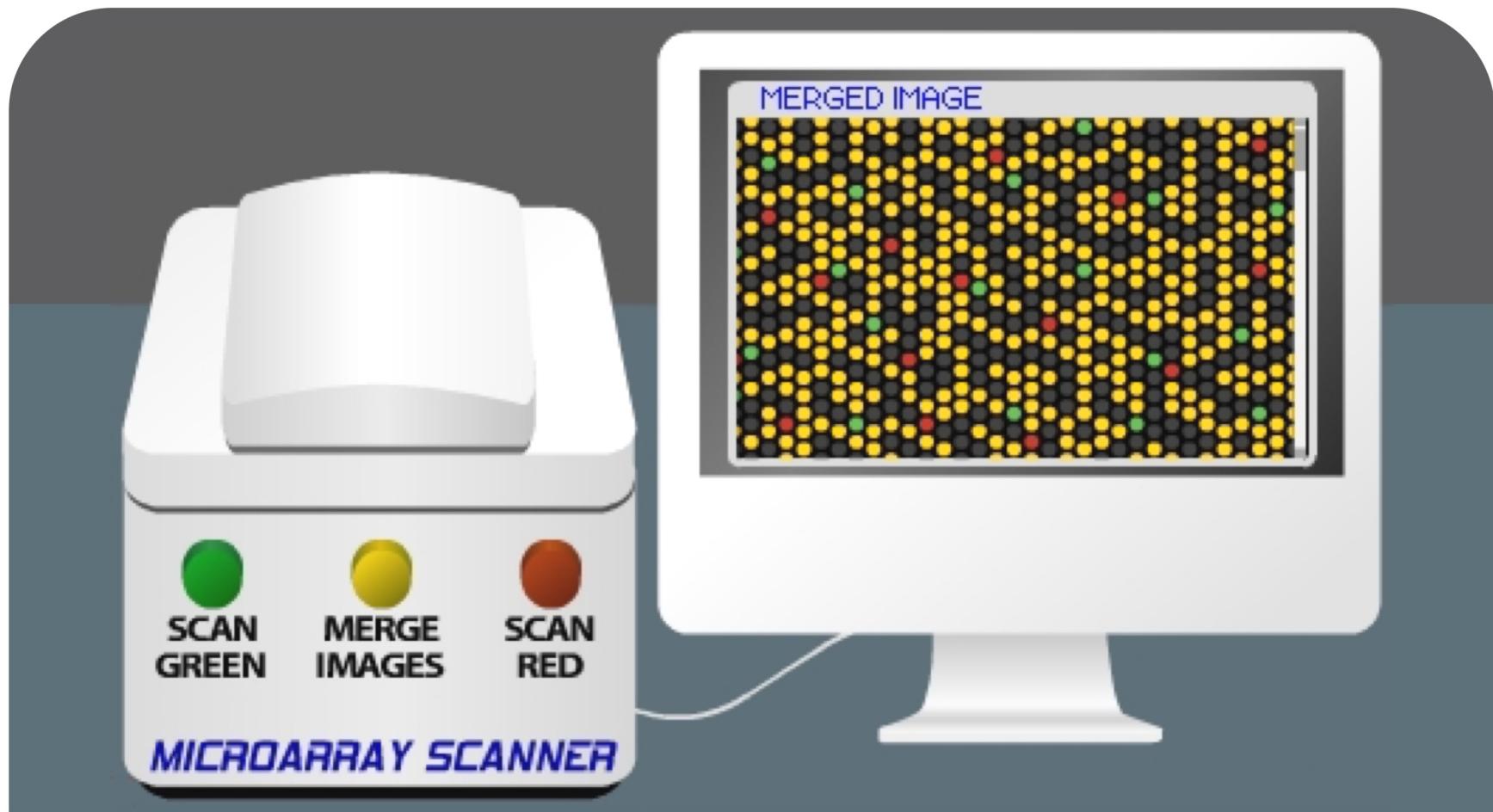
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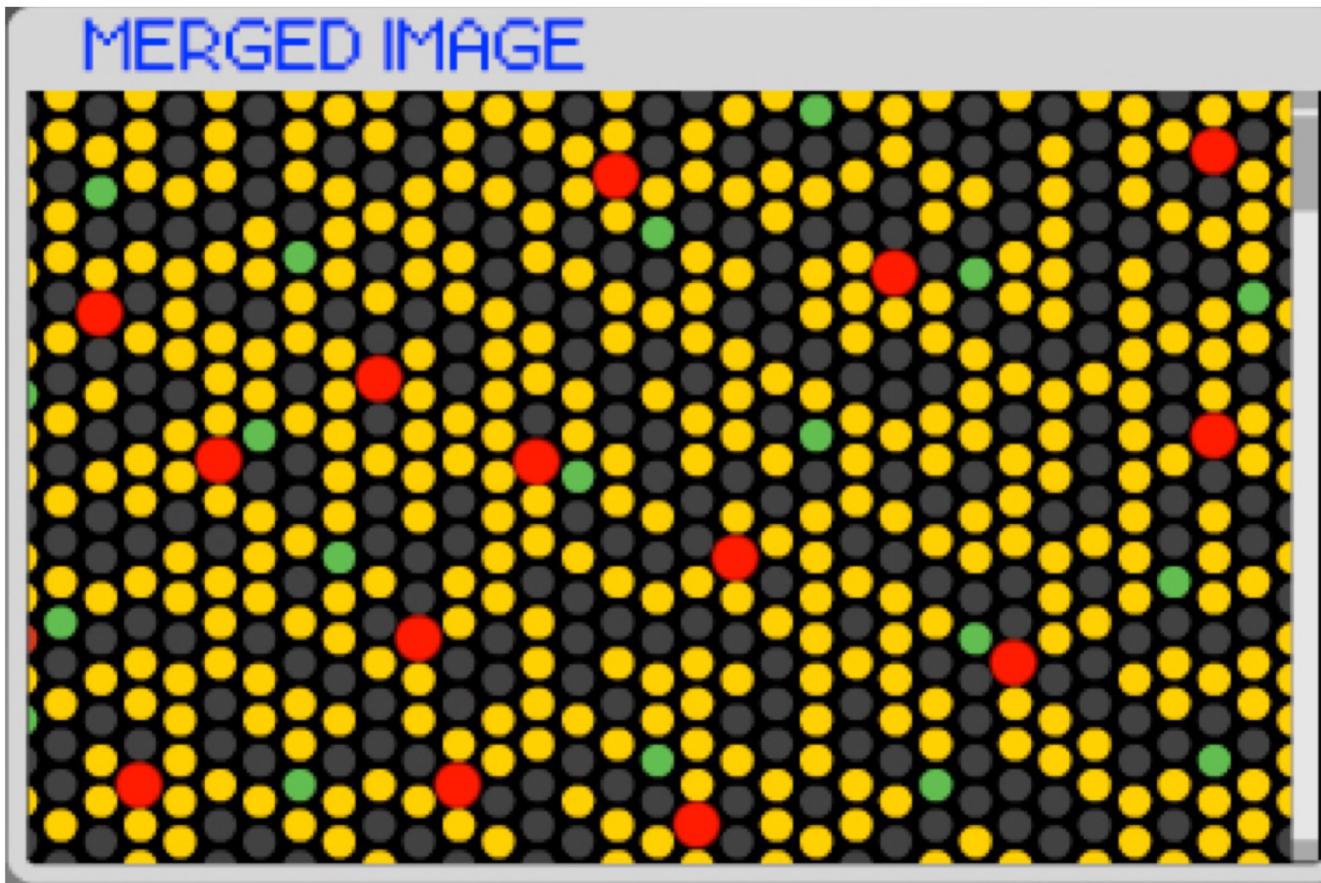
Transcriptomics

Microarrays



Transcriptomics

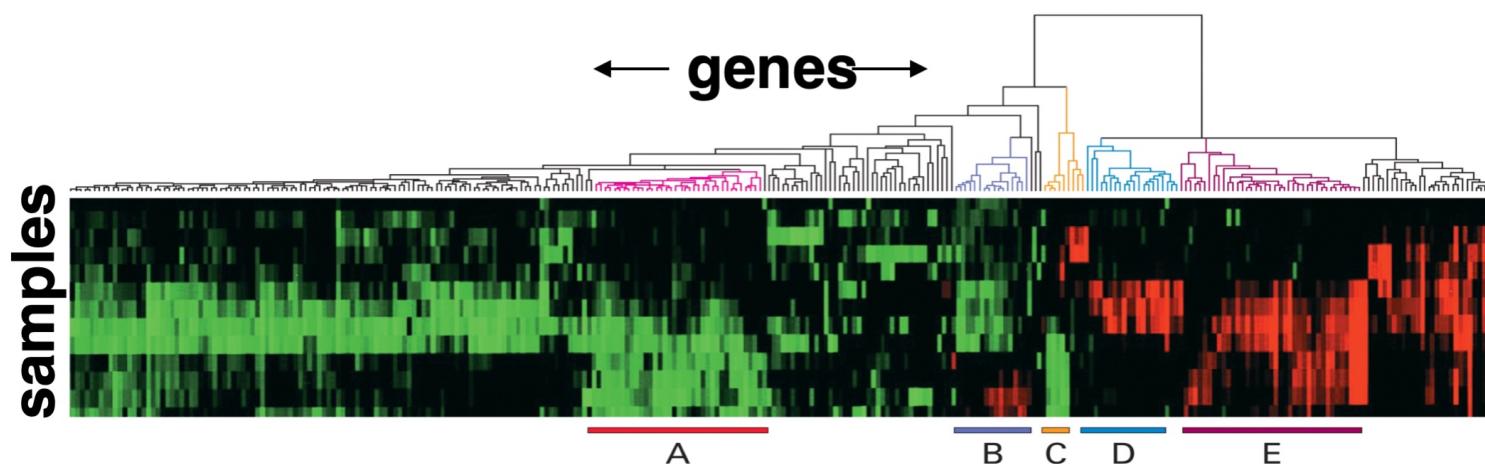
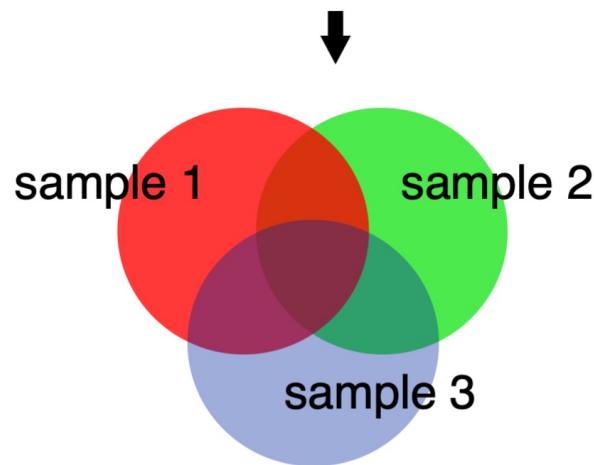
Microarrays



Transcriptomics

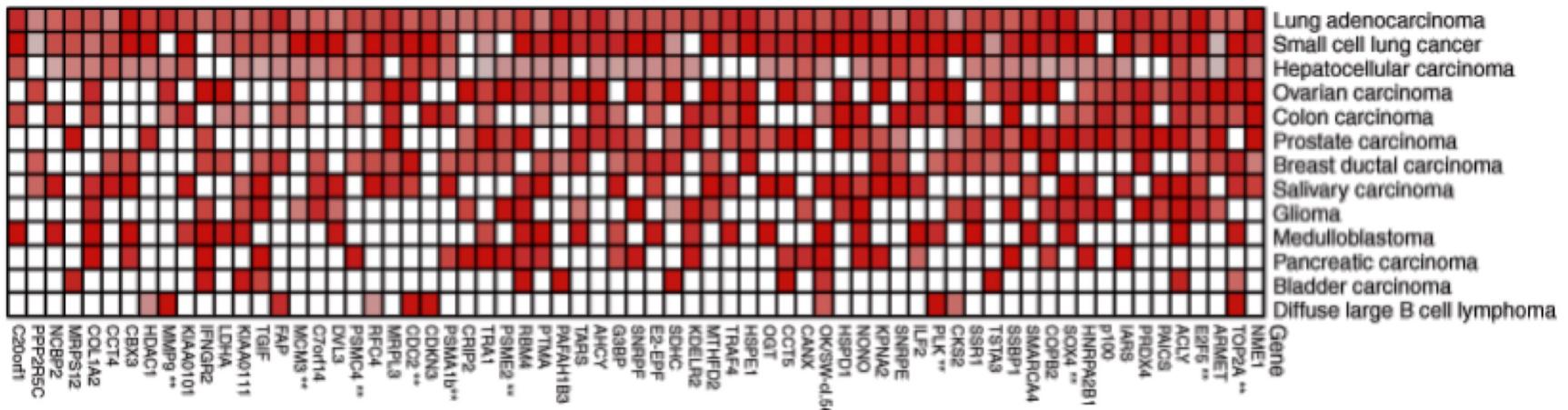
Microarrays

sample processing and statistics



Transcriptomics

Microarrays



Rhodes e Chinnaiyan (2005) *Nature Genetics* 37, S31 - S37

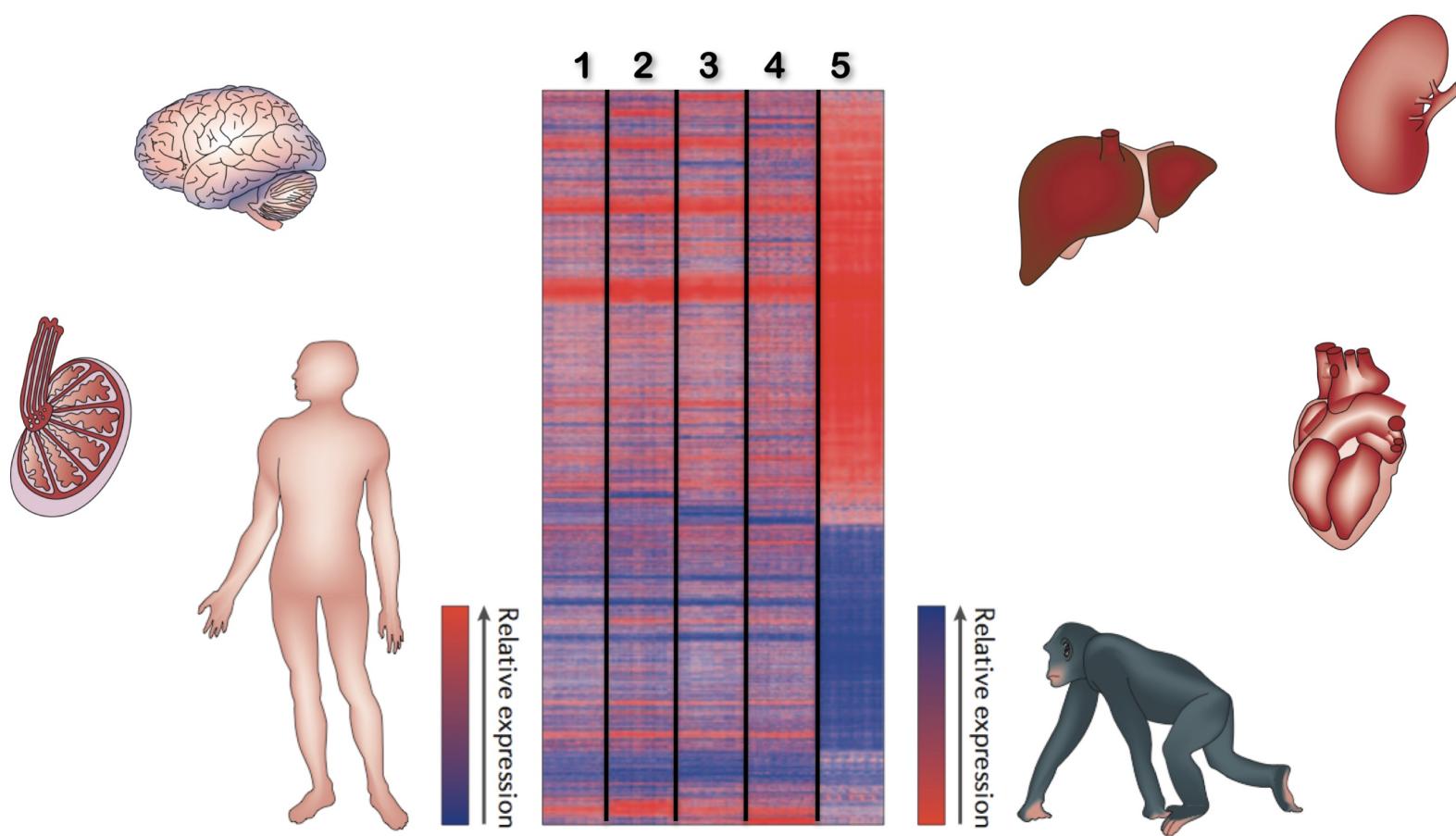
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Microarrays



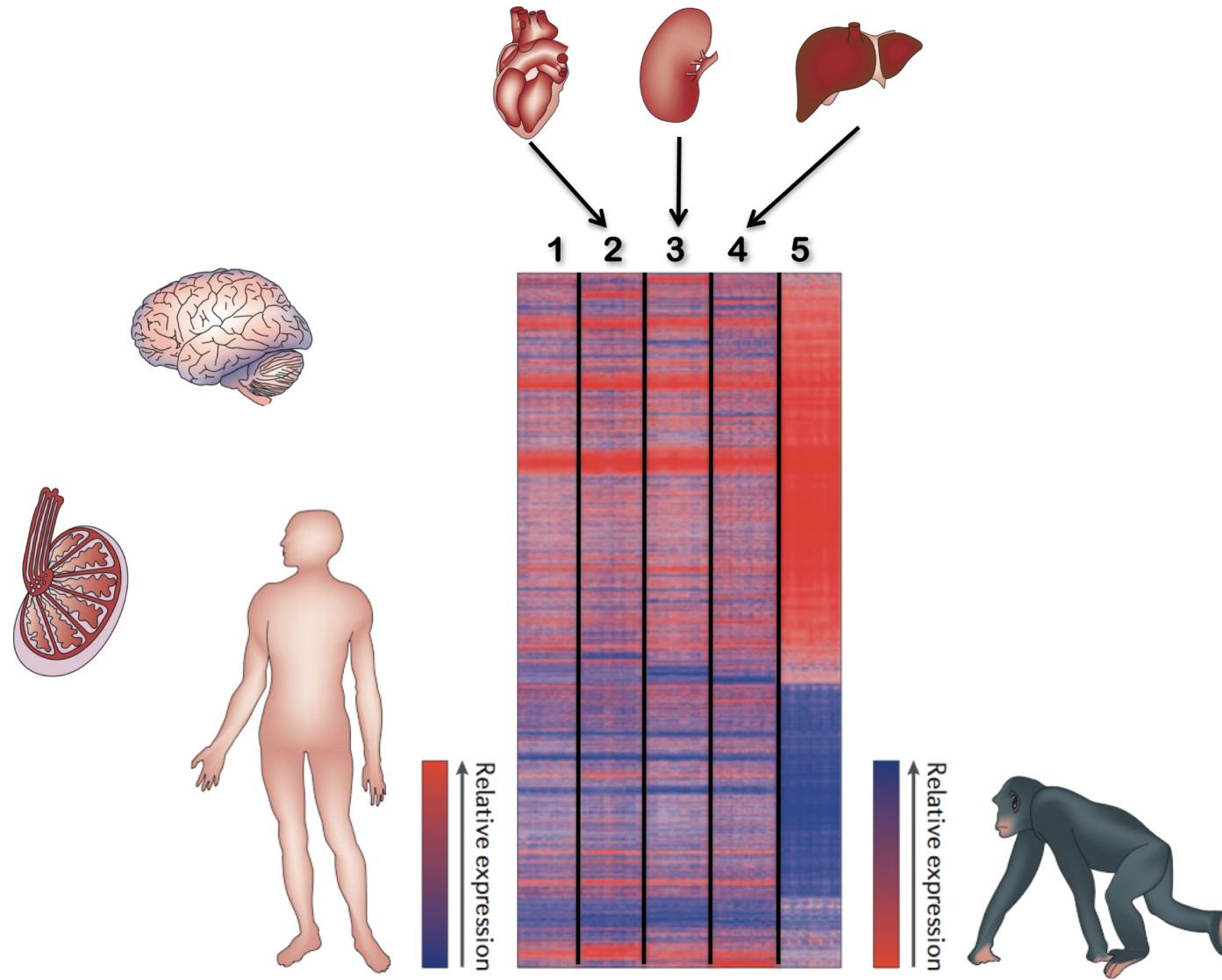
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Microarrays



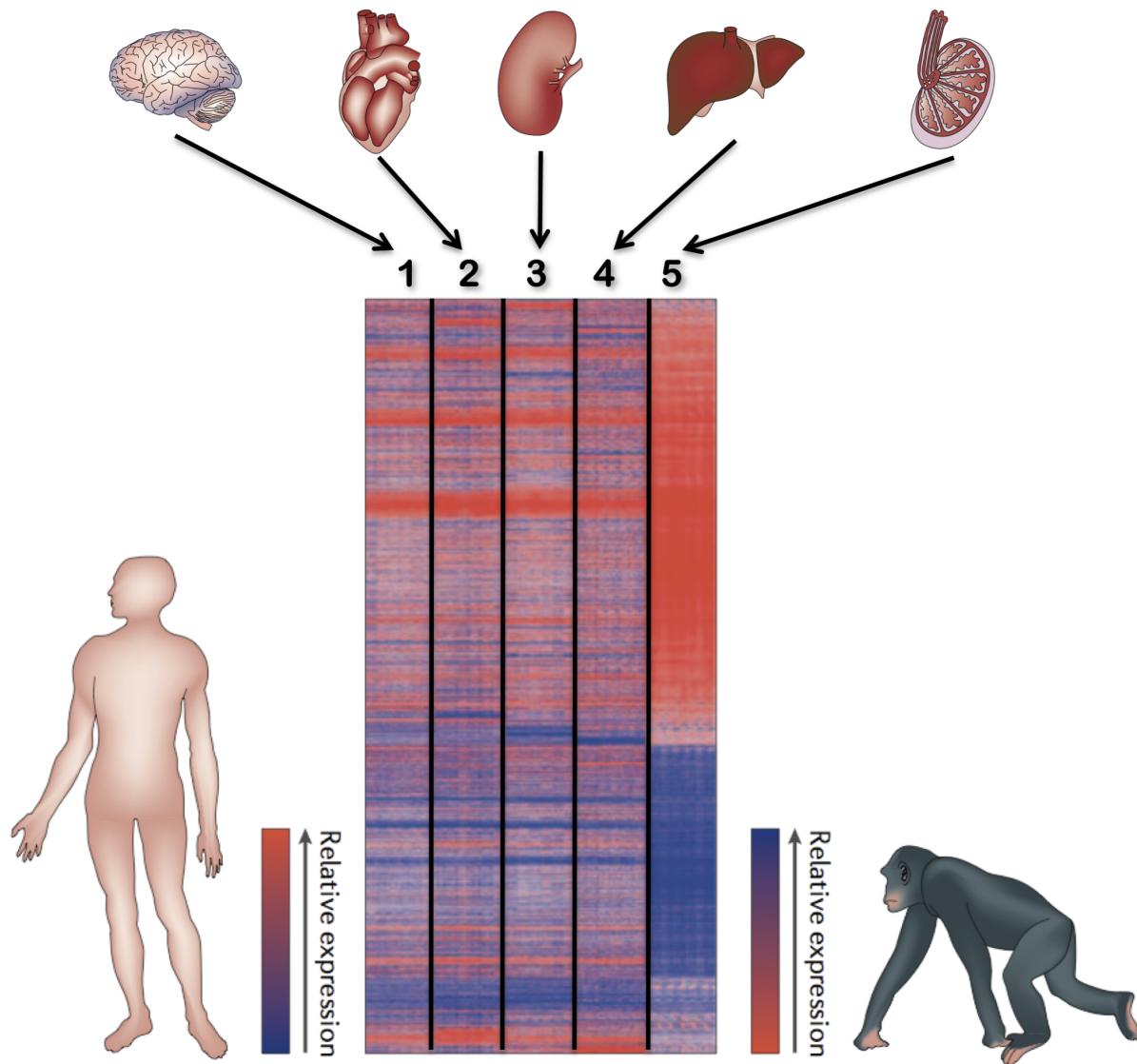
Transcriptomics

Microarrays



Transcriptomics

Microarrays



Transcriptomics

Microarrays

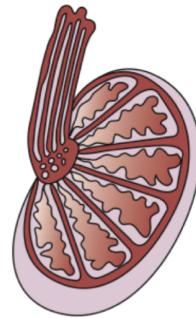
$$\omega_{\text{brain}} < \omega_{\text{heart}} \approx \omega_{\text{kidney}} < \omega_{\text{liver}} < \omega_{\text{kidney}}$$

GREATER SELECTIVE PRESSURE ON BRAIN GENES

PURIFYING SELECTION

Transcriptomics

Microarrays



SELEÇÃO DIVERSIFICADORA

Excess of genes on the X chromosome

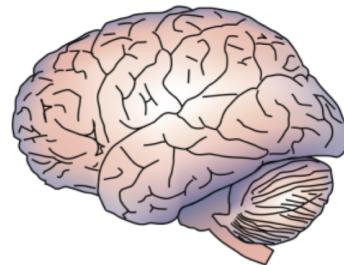
Greater representation of genes involved in spermatogenesis: sperm competition

Different mechanisms of expression regulation: alternative splicing, use of non-canonical start codons

Sperm fitness determined by their haploid genome

Transcriptomics

Microarrays



PURIFYING SELECTION

Parts of the brain involved in species-specific traits do not show differentiation in expression levels

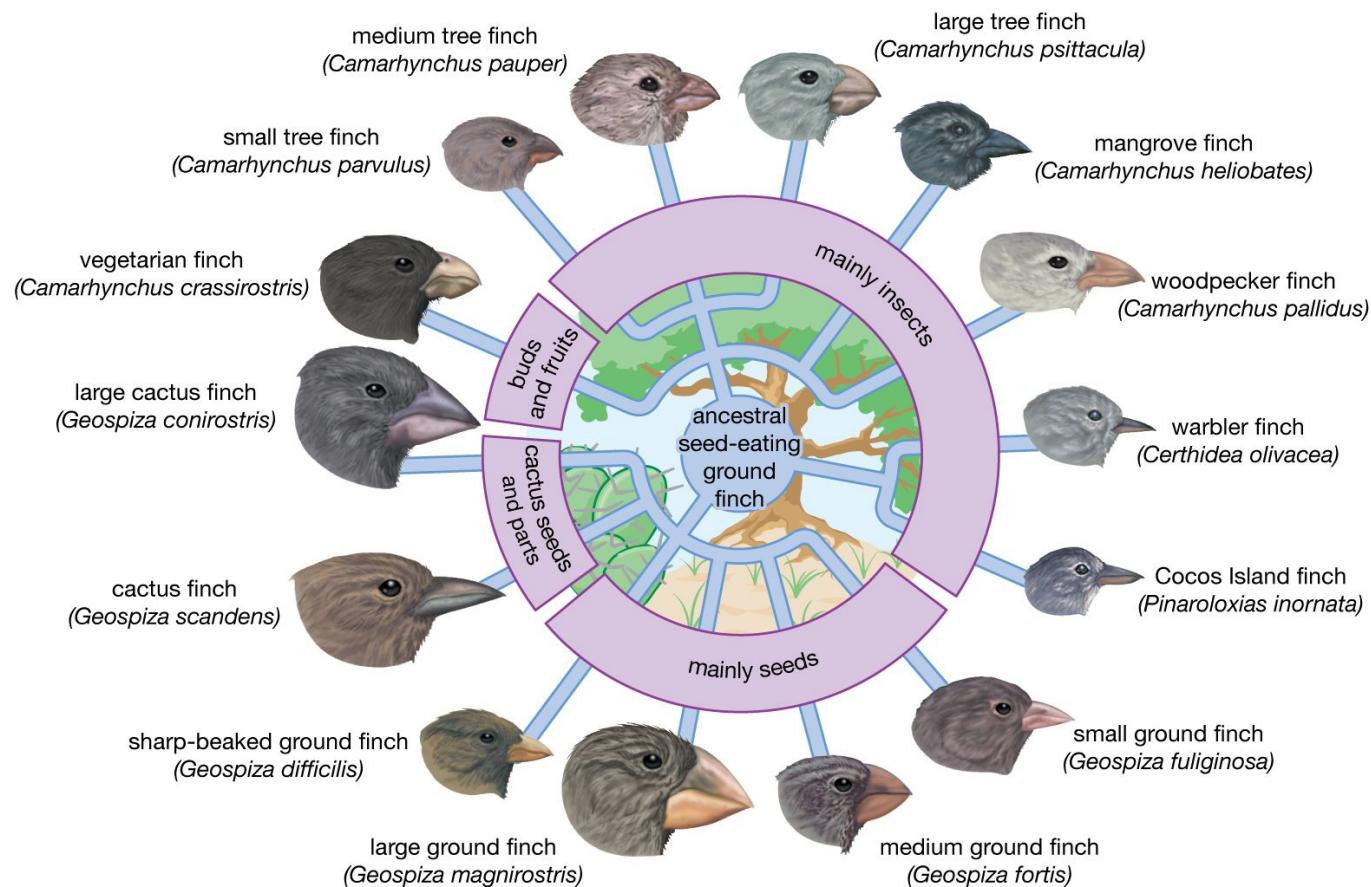
Among the few changes that occurred in the brain, most happened in the human lineage

Among all tissues, the brain harbors genes involved in regions of linkage disequilibrium. Most of these genes are related to energy production

Transcriptomics

Microarrays

Adaptive radiation in Galapagos finches



Transcriptomics

Microarrays



Vol 442 | 3 August 2006 | doi:10.1038/nature04843

nature

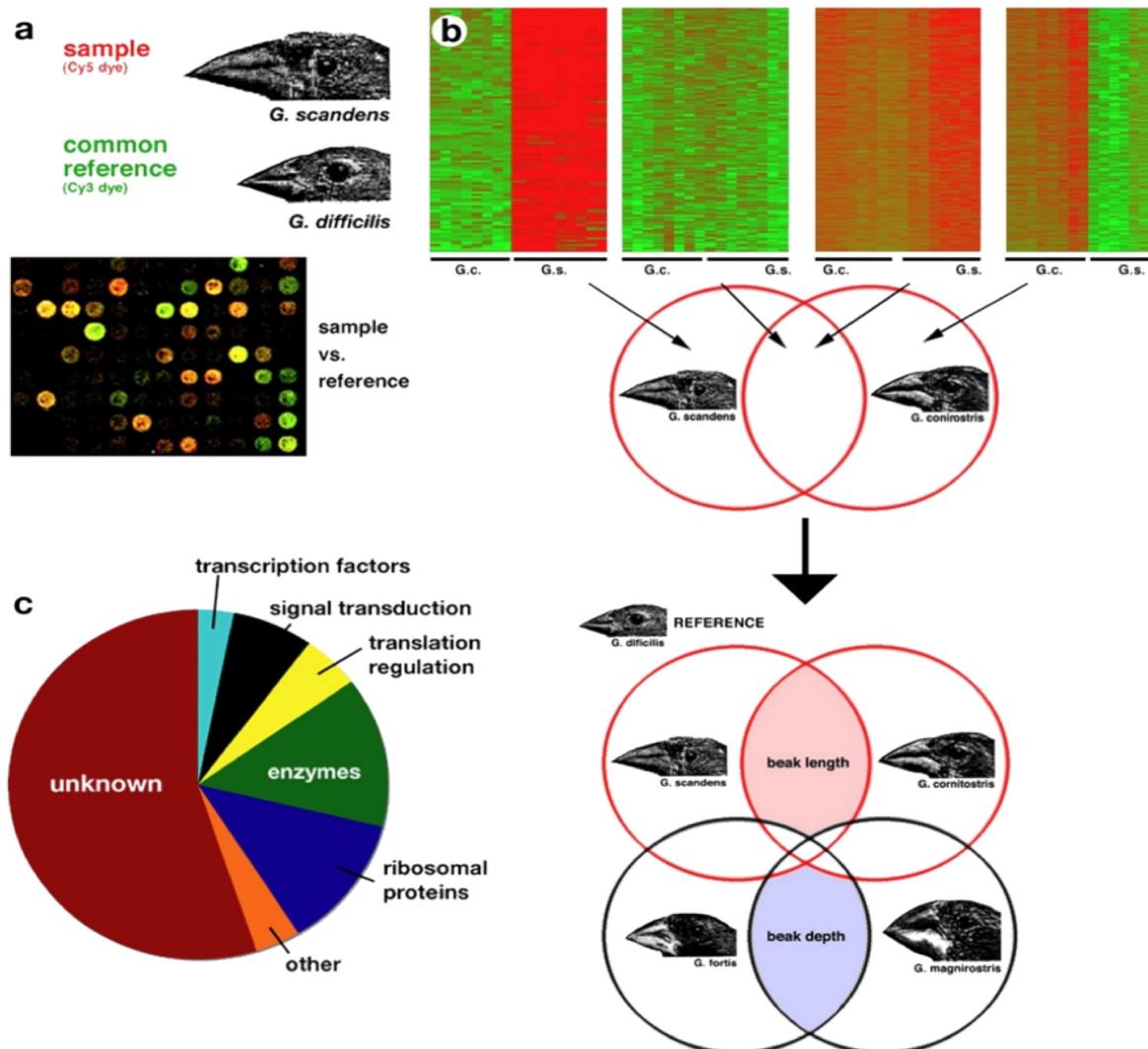
LETTERS

The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches

Arhat Abzhanov^{1†}, Winston P. Kuo^{1,2,3†}, Christine Hartmann⁴, B. Rosemary Grant⁵, Peter R. Grant⁵
& Clifford J. Tabin¹

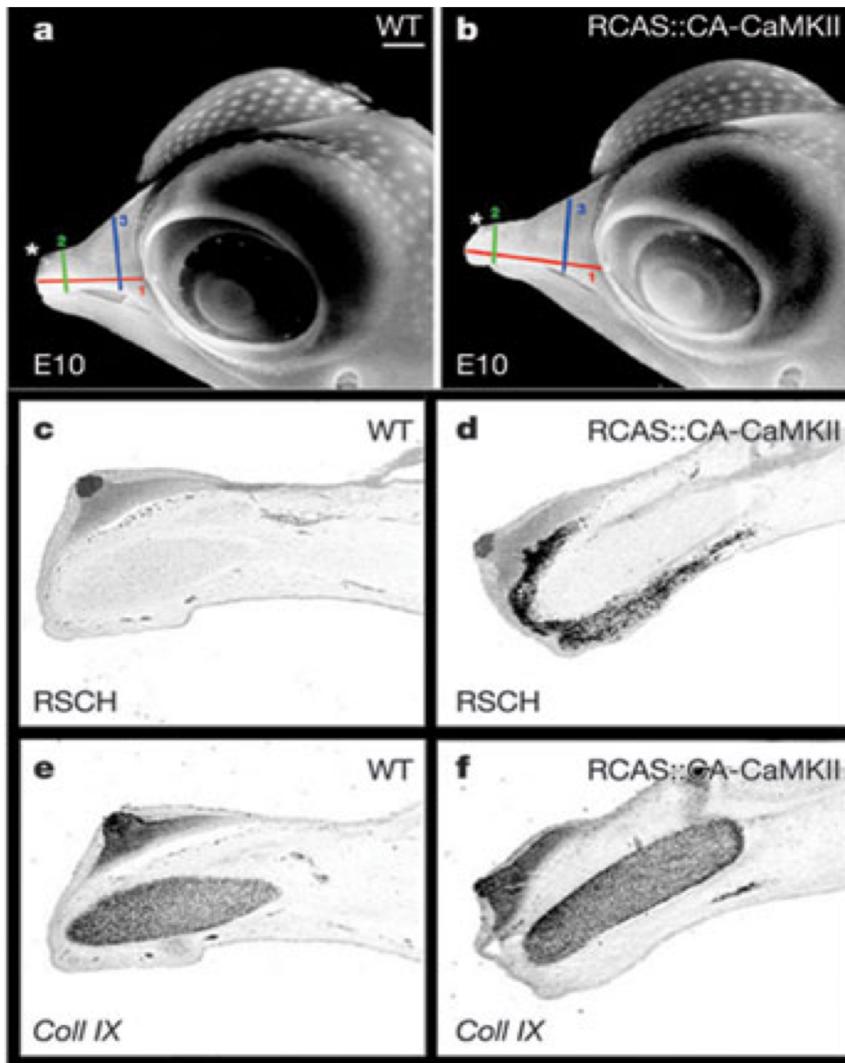
Transcriptomics

Microarrays



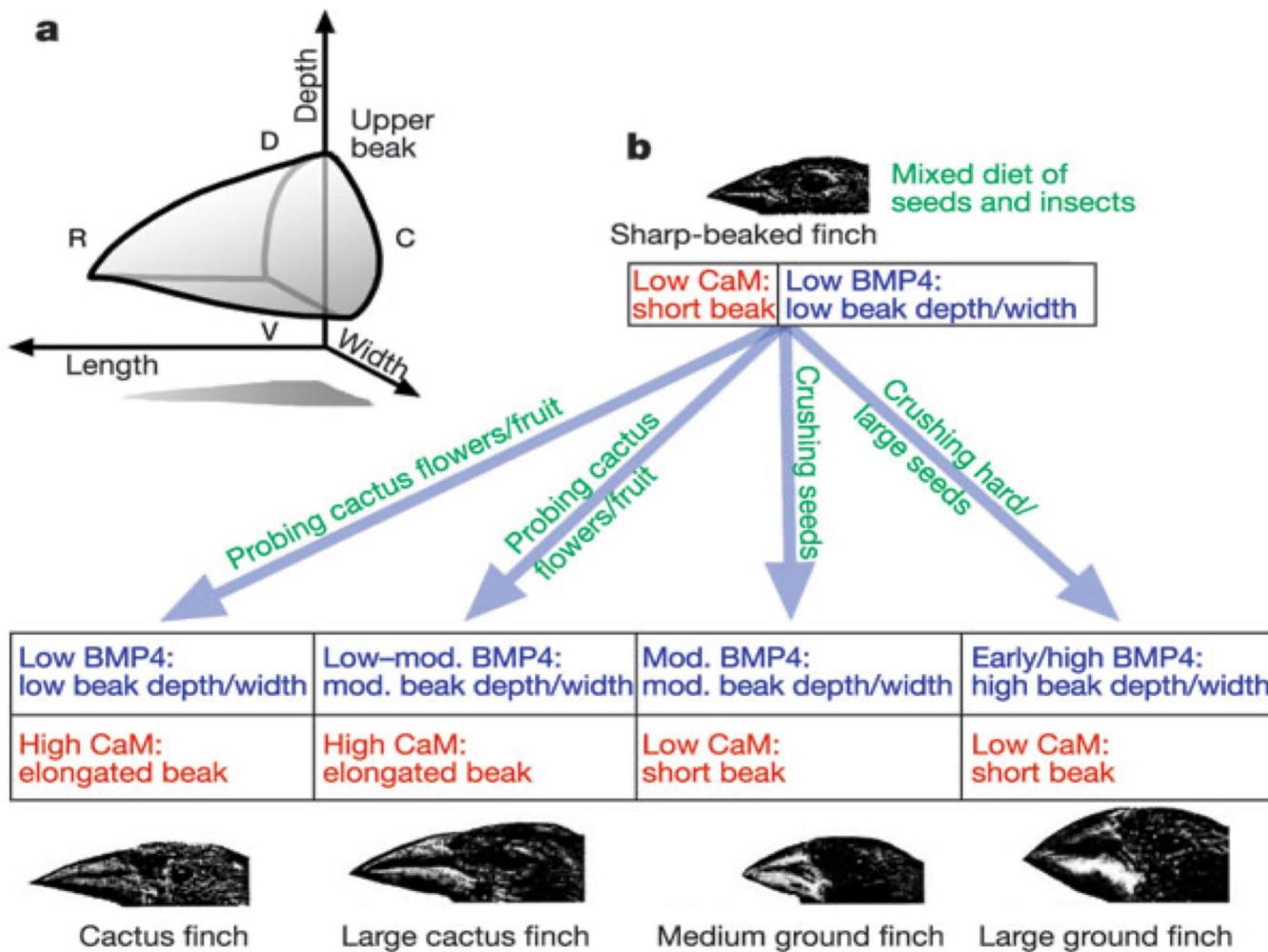
Transcriptomics

Microarrays



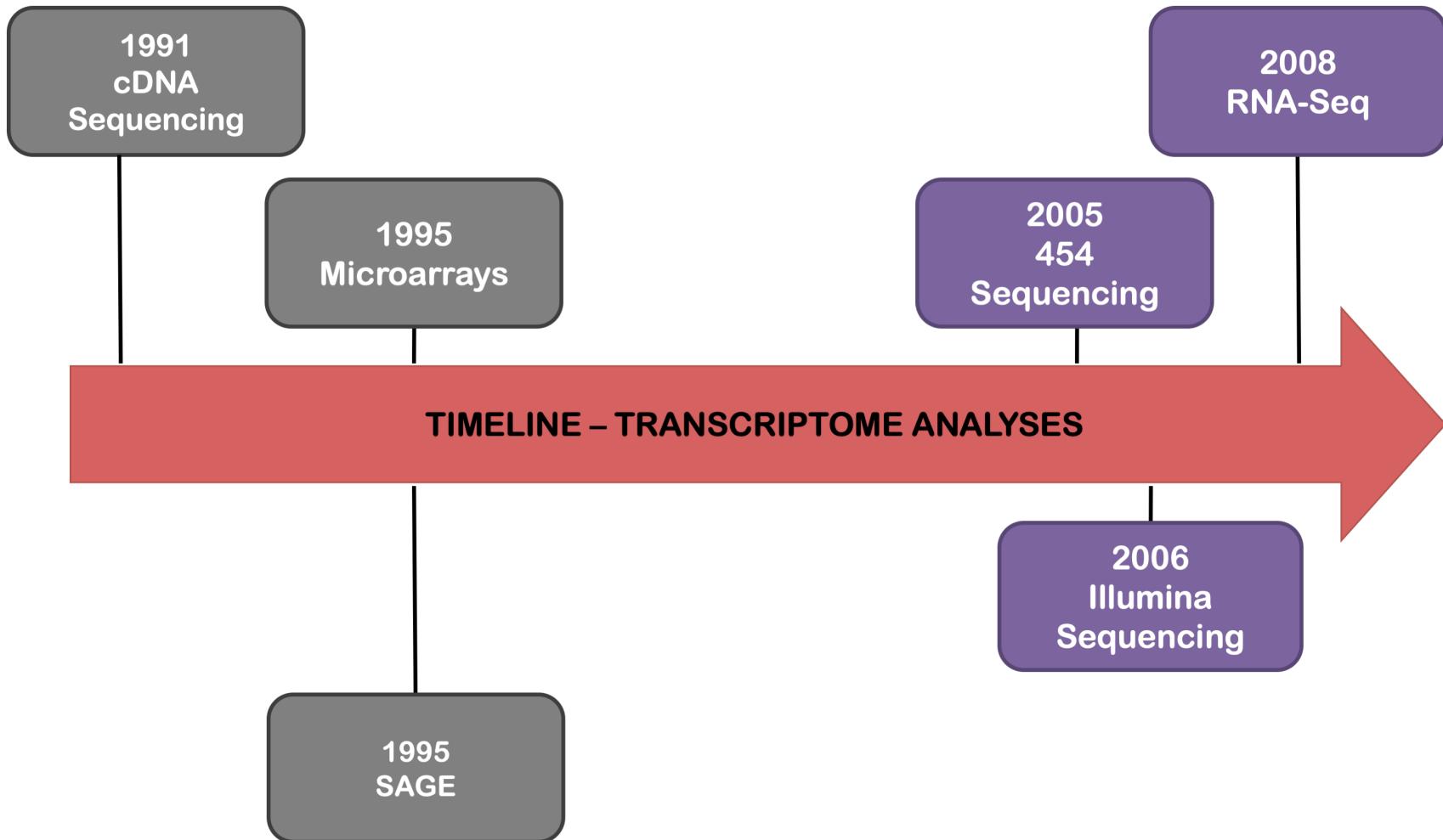
Transcriptomics

Microarrays



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The evolution of the methods



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The evolution of the methods

