

Transcriptomics

GENOMICS

BIGNAUD AMAURY

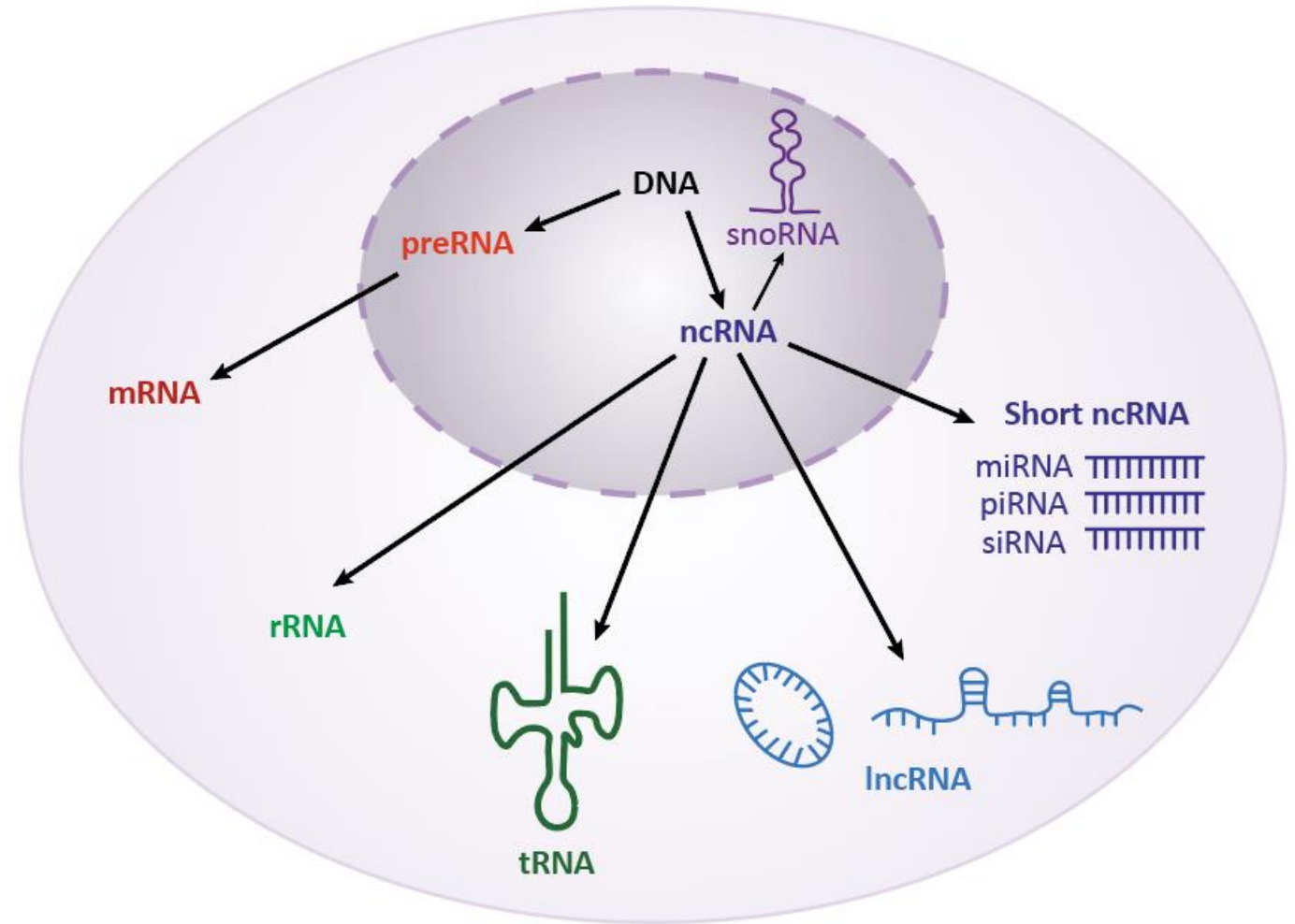
04/10/2023

What's transcriptomics ?

- It's the studied of the **transcriptome**.

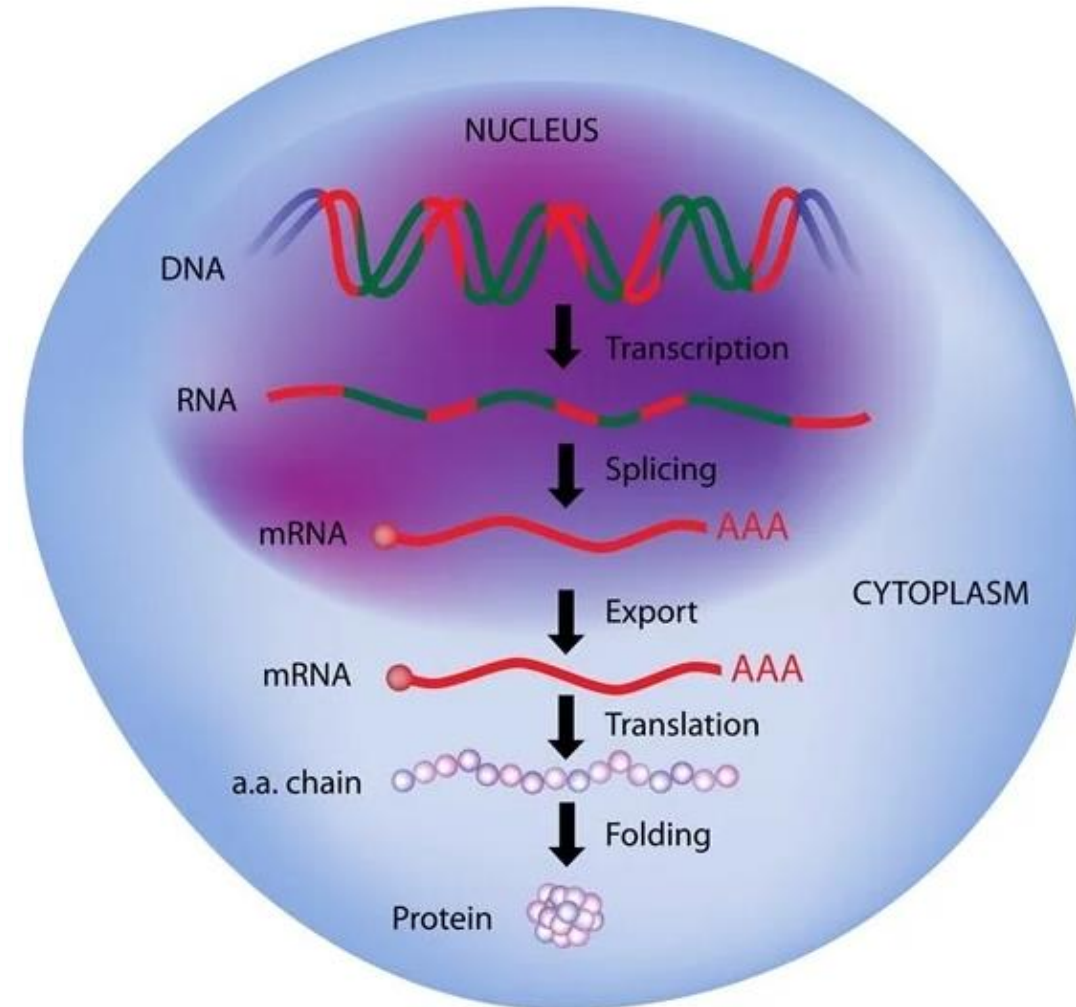
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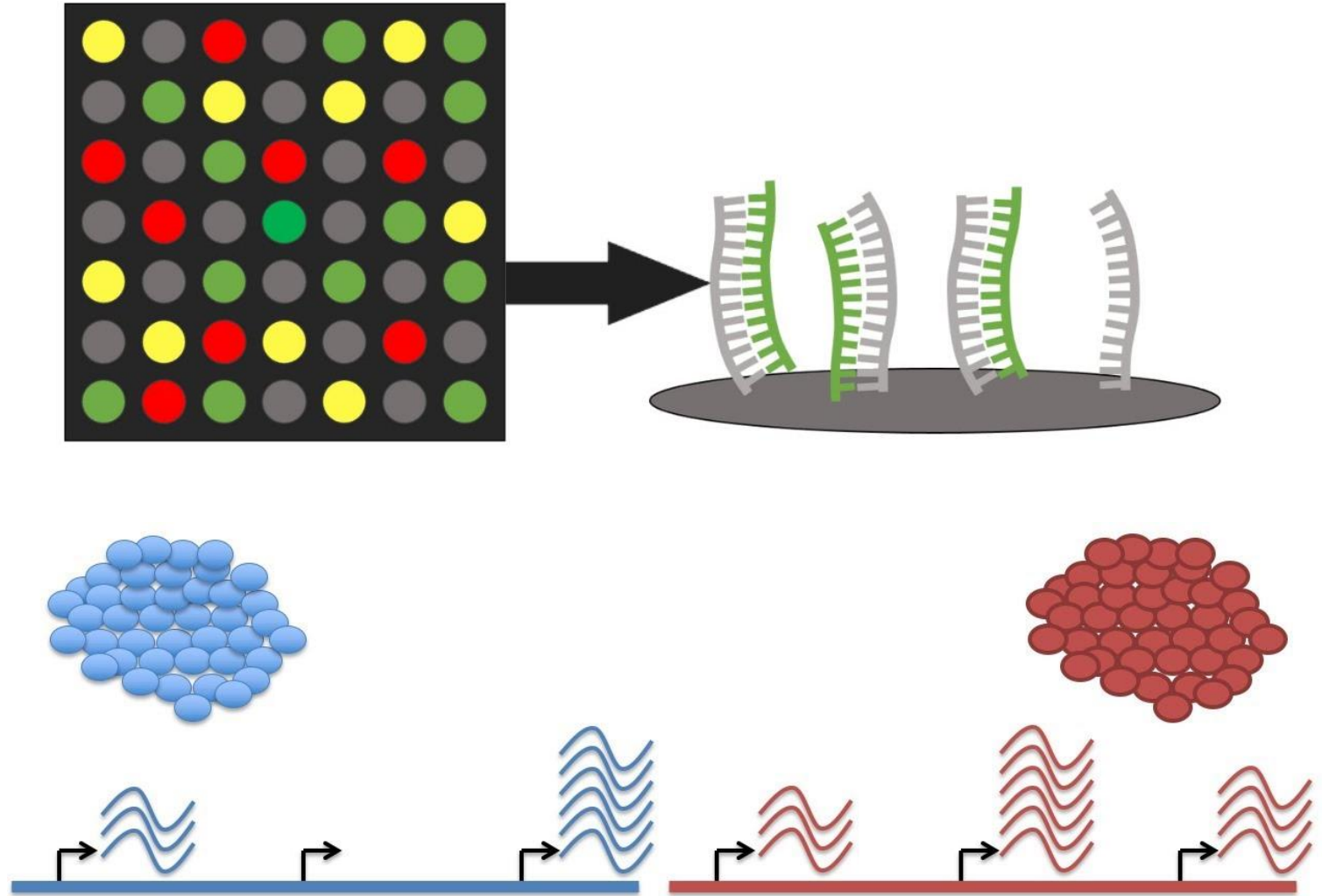
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- Generally, the goal is to study **gene expression**.



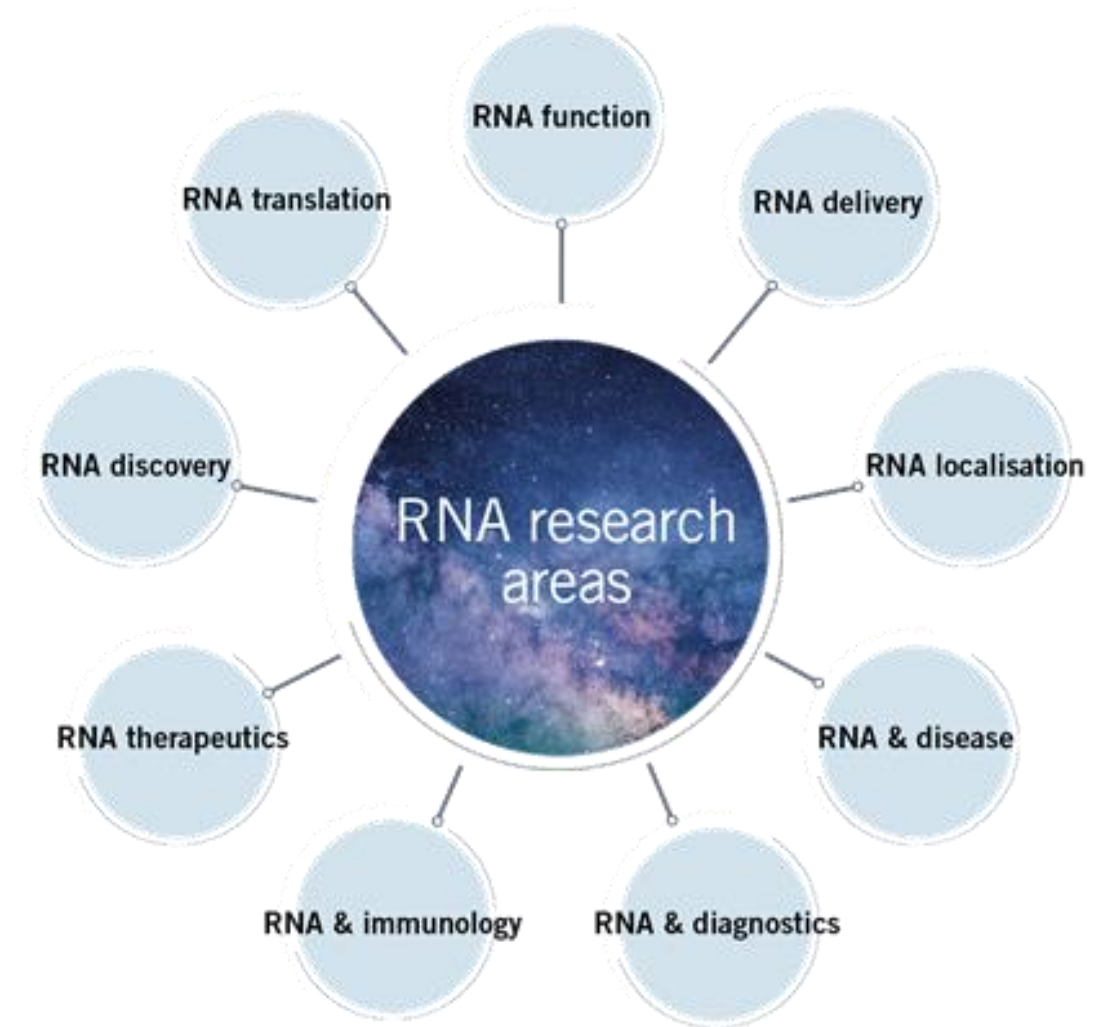
What's transcriptomics ?

- It's the studied of the **transcriptome**.
- Generally, the goal is to study **gene expression**.
- Based on two main techniques:
 - Microarrays
 - RNA-seq

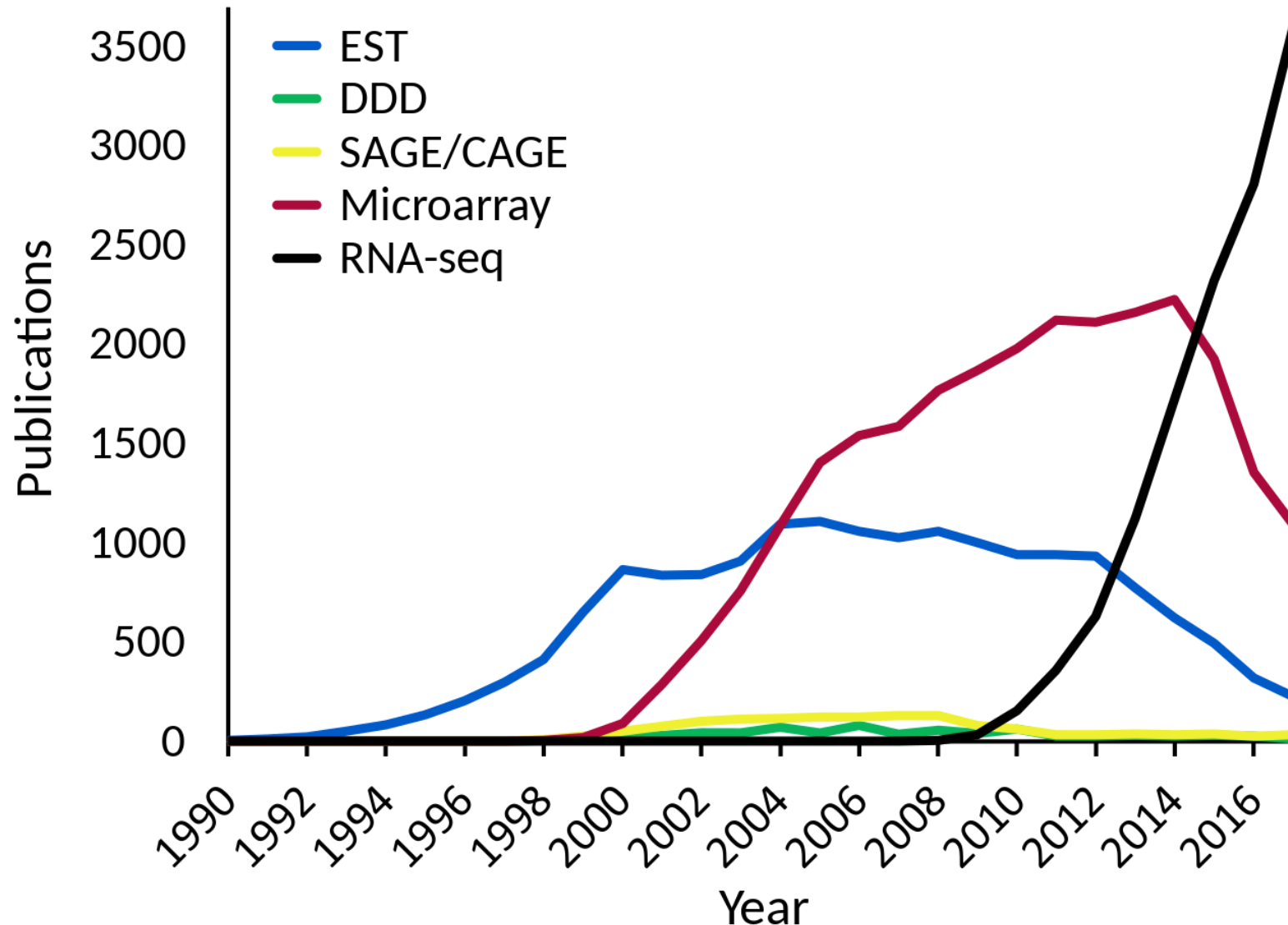


What's transcriptomics ?

- It's the study of the **transcriptome**.
- Generally, the goal is to study **gene expression**.
- Based on two main techniques:
 - Microarrays
 - **RNA-seq**
- Goals:
 - Measure the gene expression in various conditions (development stage, healthy vs diseased tissues...).
 - Measure the difference of expression between conditions.
 - Understand functions of genes based on detected variations.
 - Understand diseases.

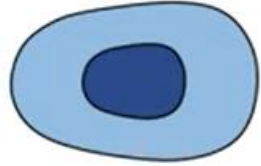


Transcriptomics methods

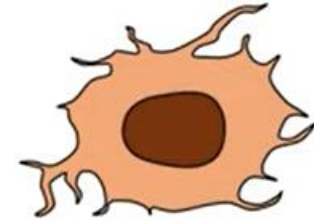


- First method to do transcriptomics was EST (Expressed sequence tag) invented in 1979.
- The DDD (Differential Display cDNA), SAGE/CAGE and microarrays arrived later.
- Now RNA-seq is the most used method with the apparition of the NGS (Next Generation Sequencing) technologies.

Microarray

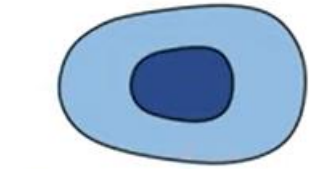


Sample 1



Sample 2

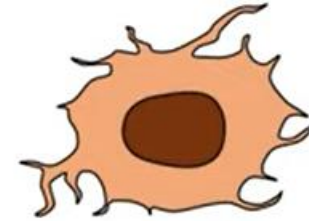
Microarray



Sample 1



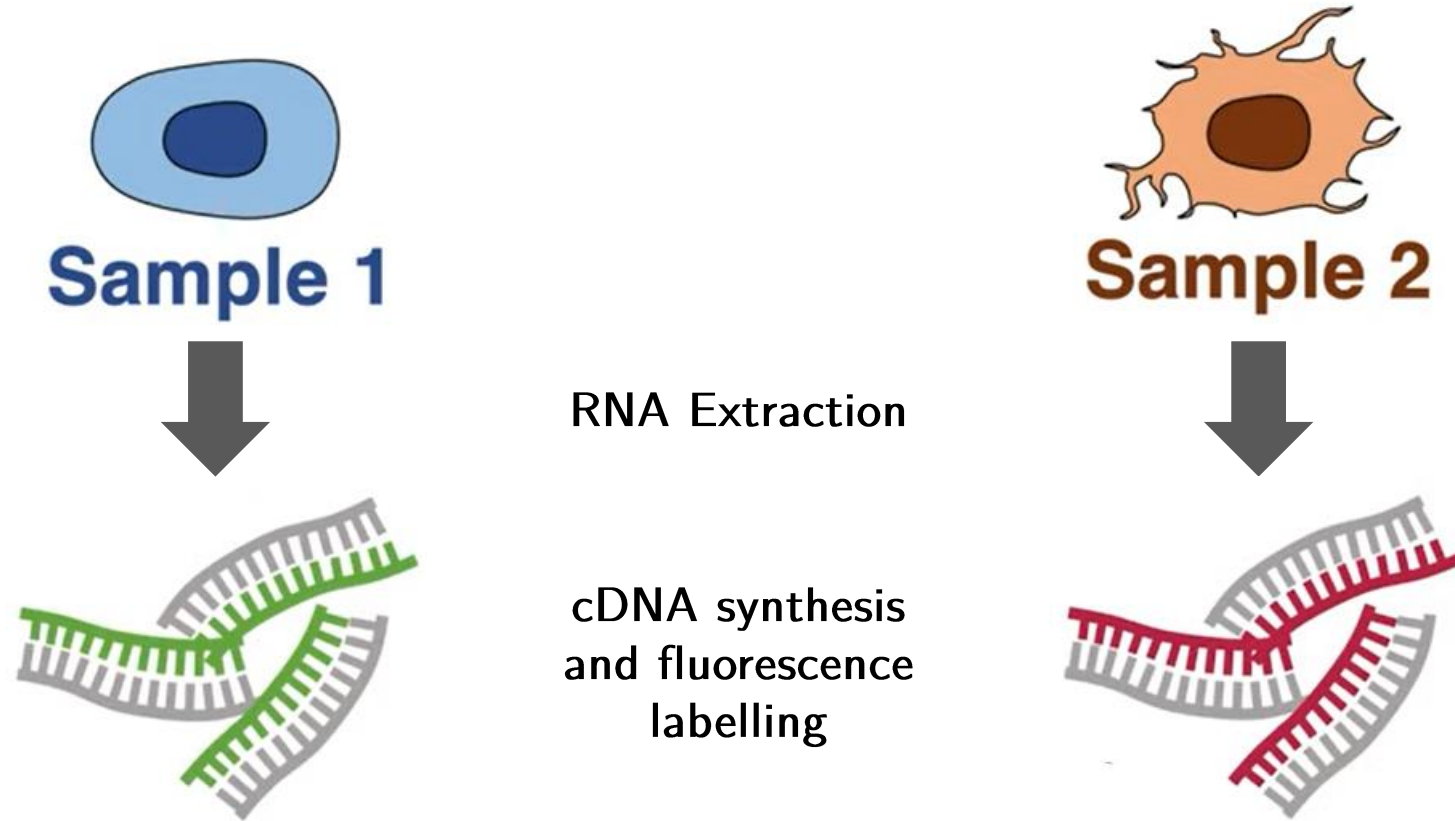
RNA Extraction



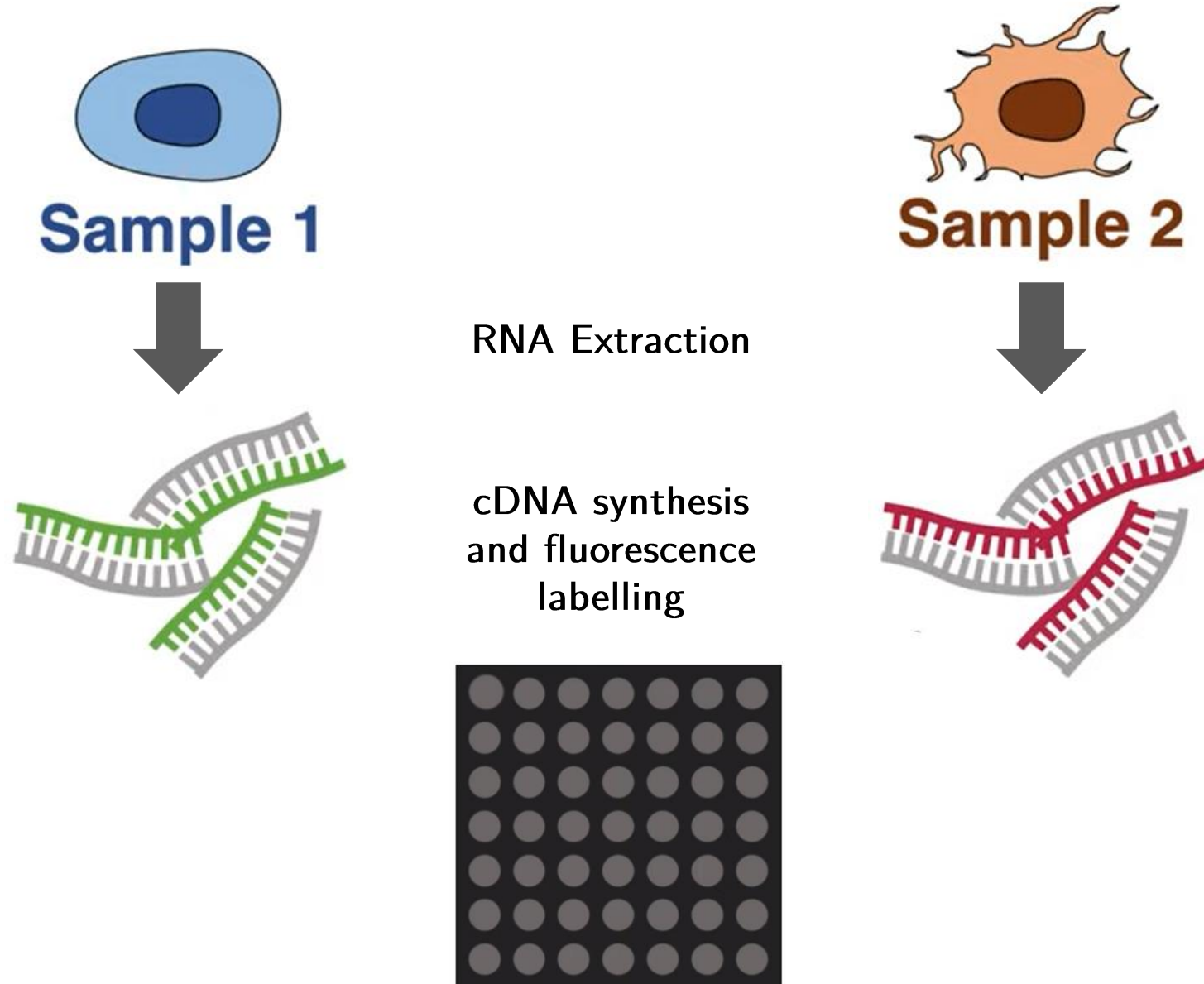
Sample 2



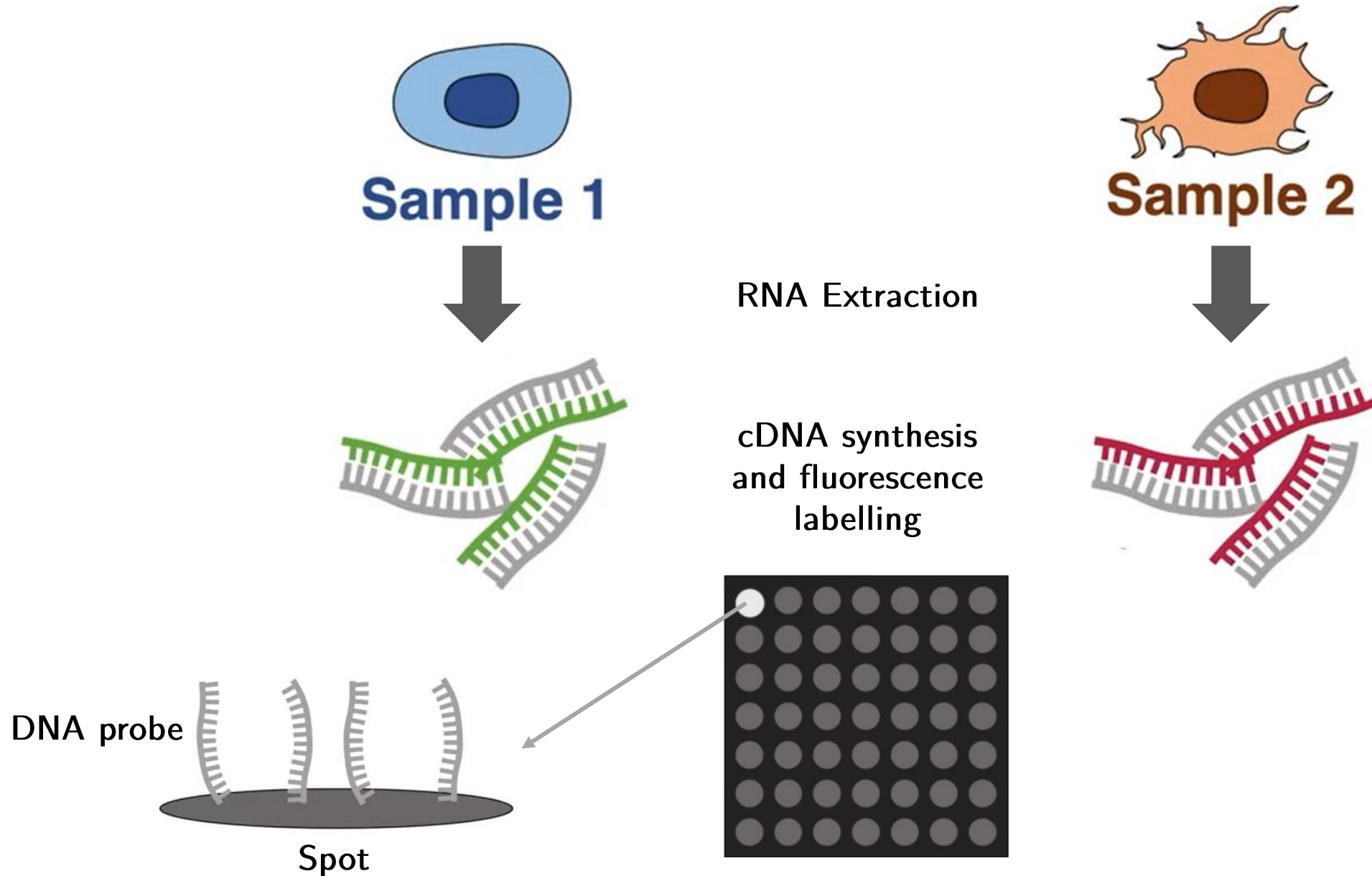
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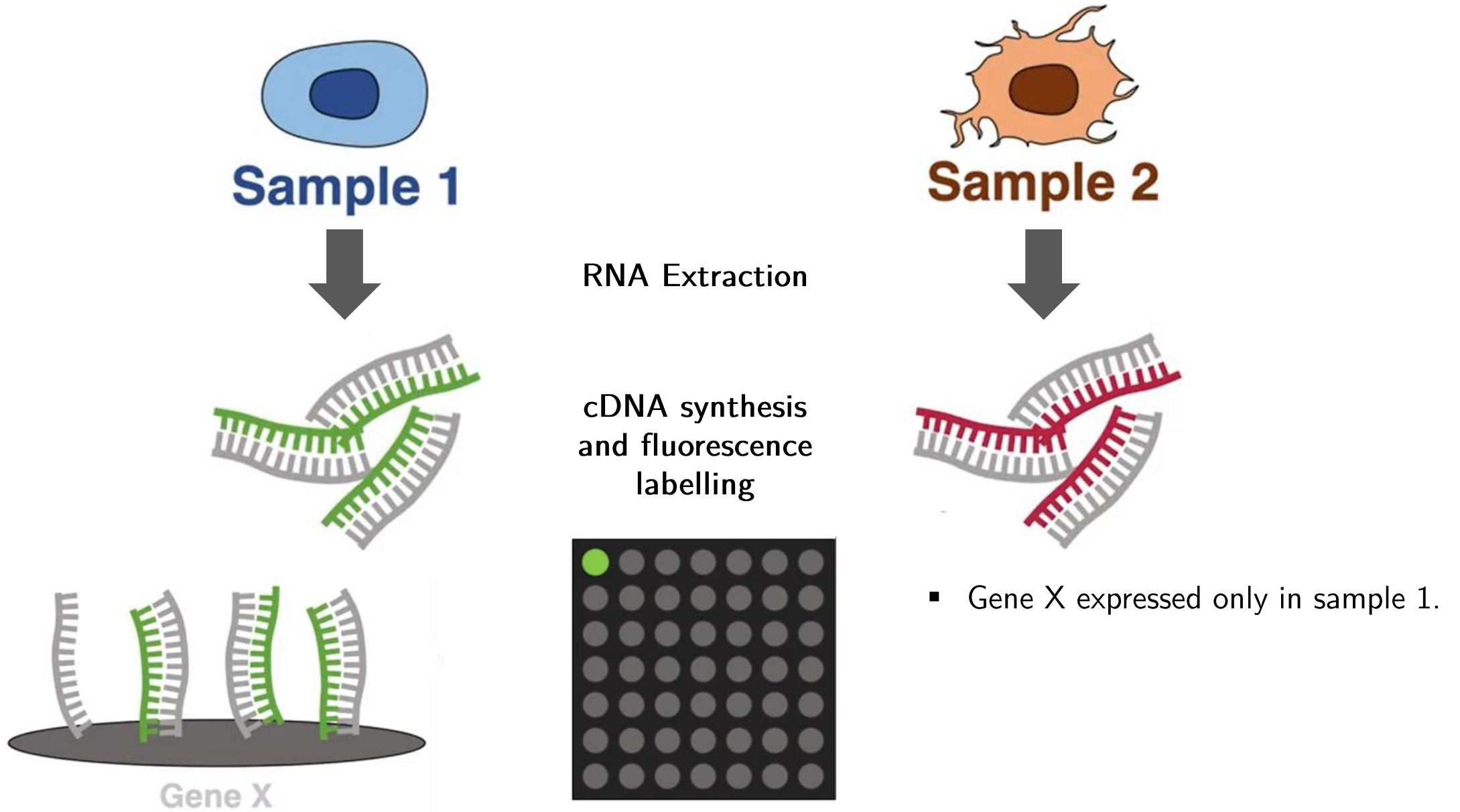
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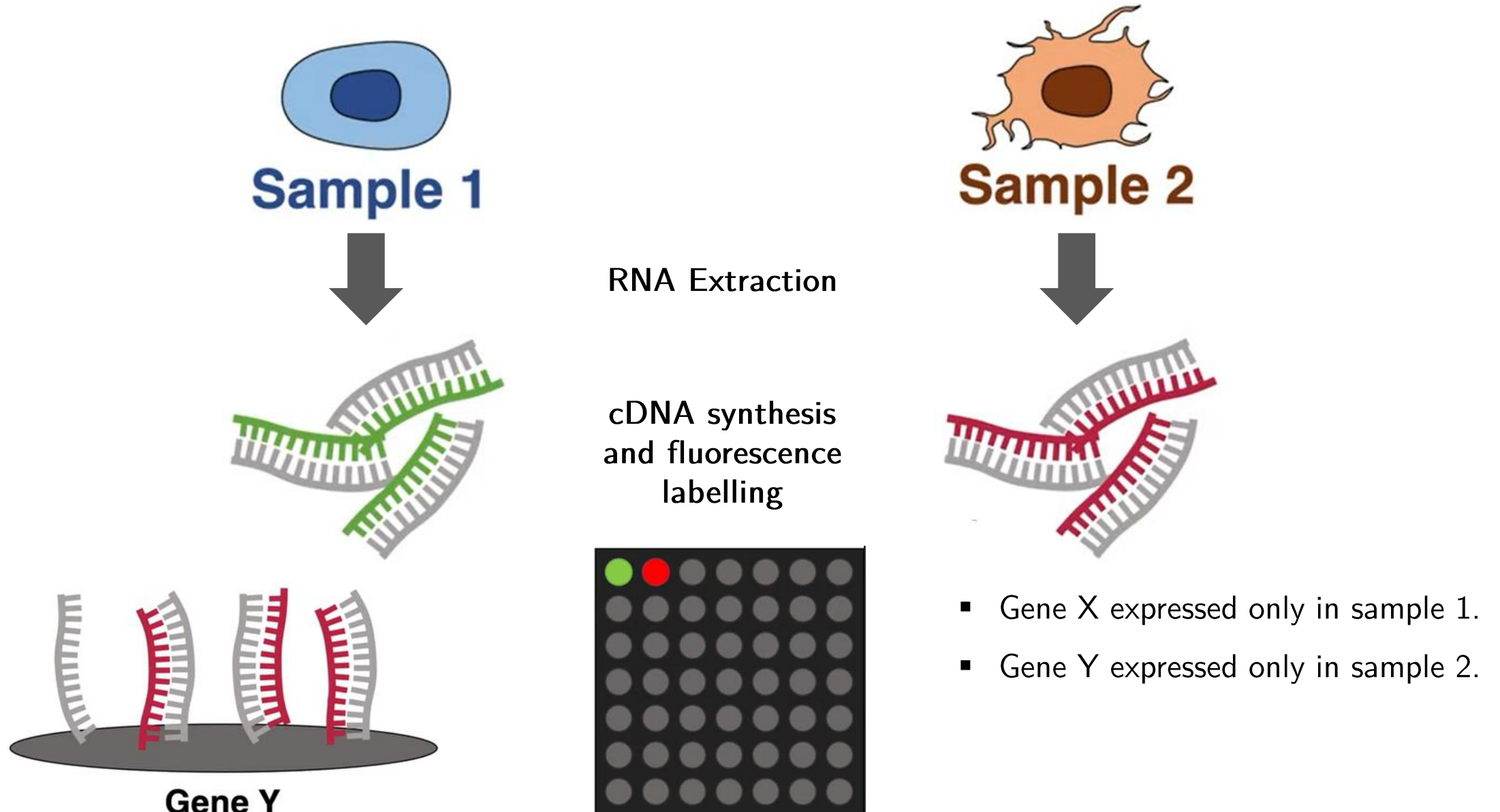
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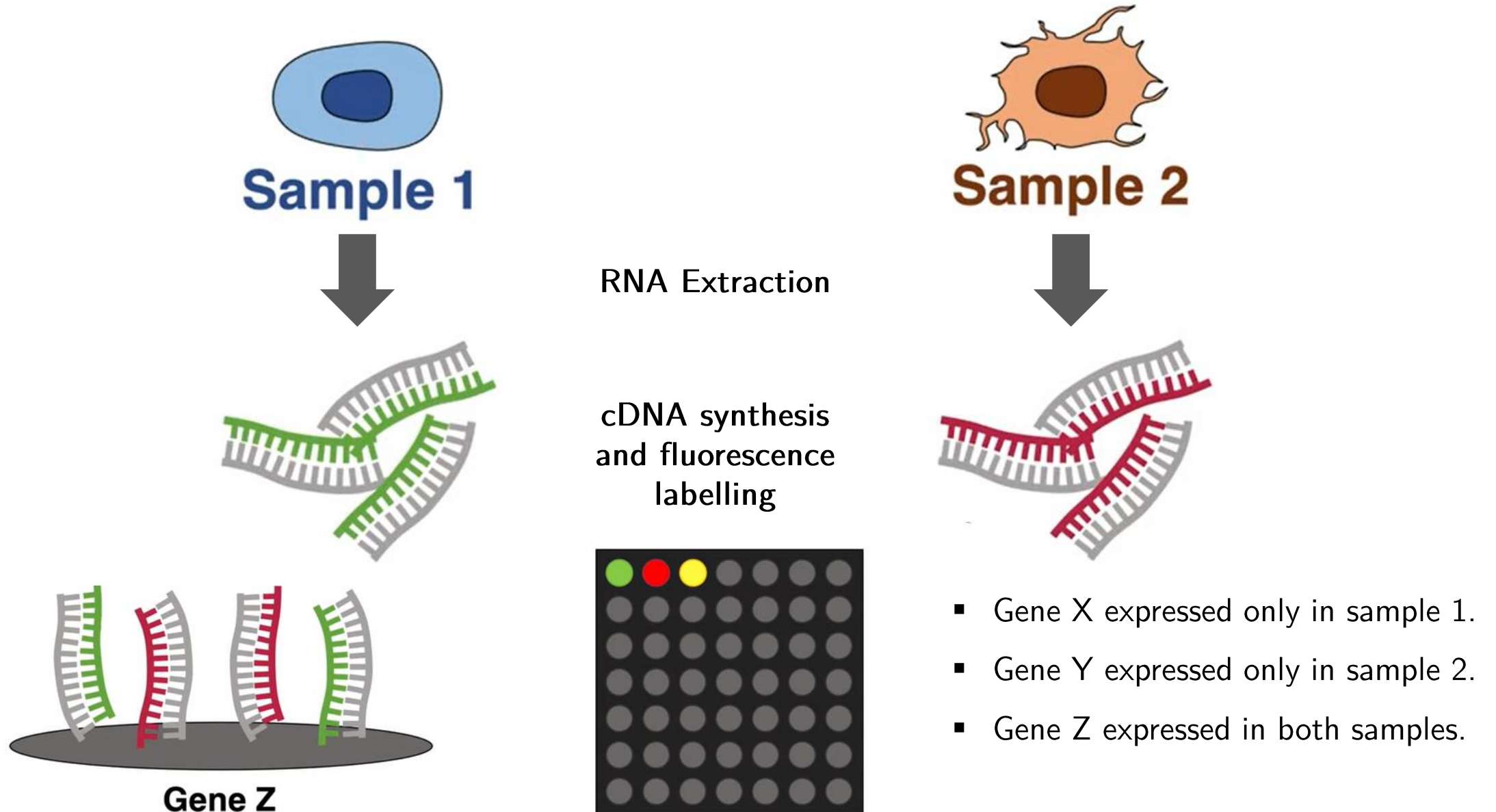
Microarray



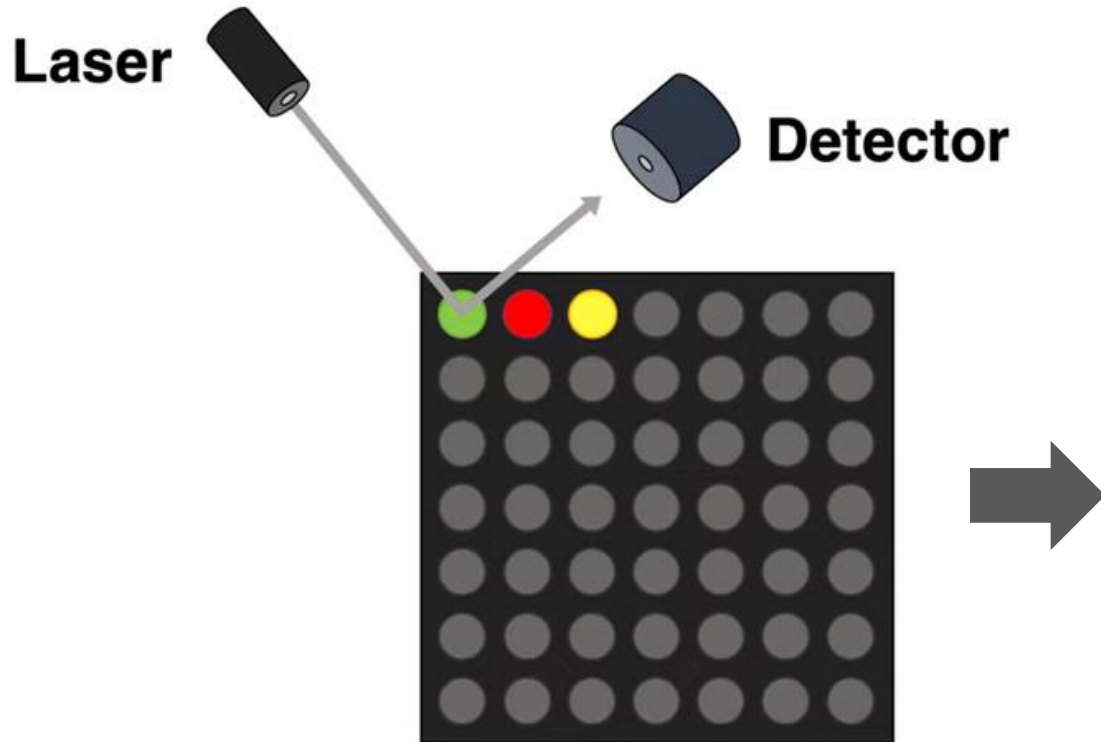
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Microarray



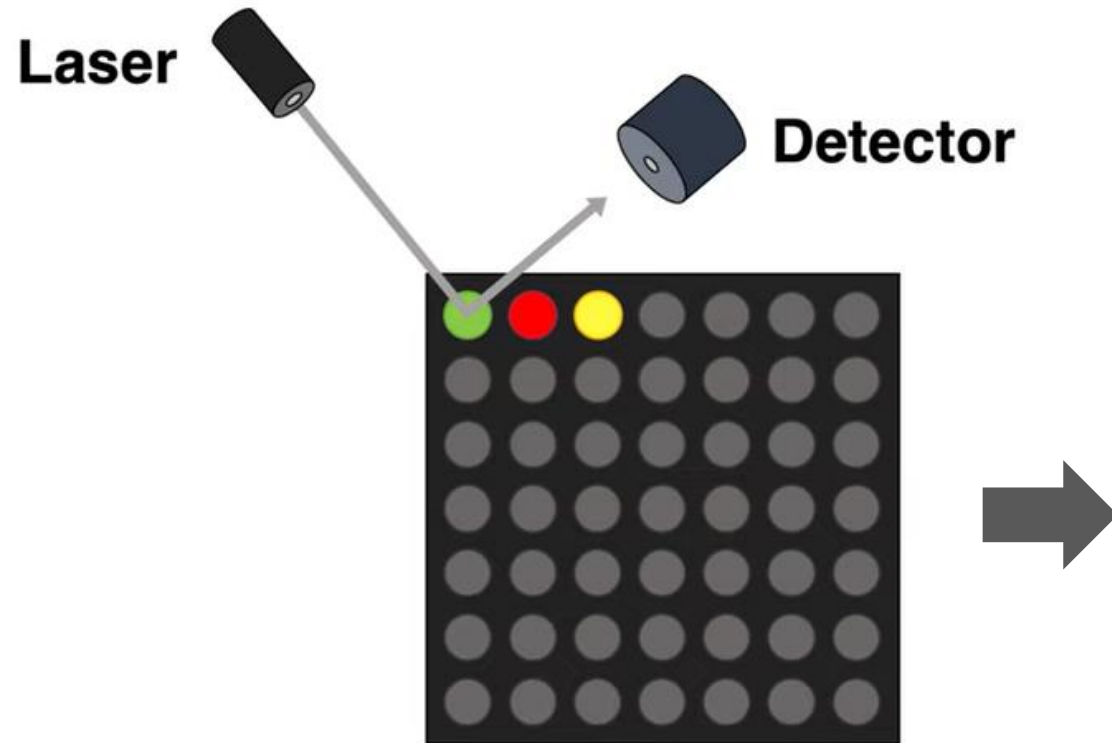
Microarray



- Example of raw output.

	Position	Intensities		Size	Homogeneity	Statistics	Annotations																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
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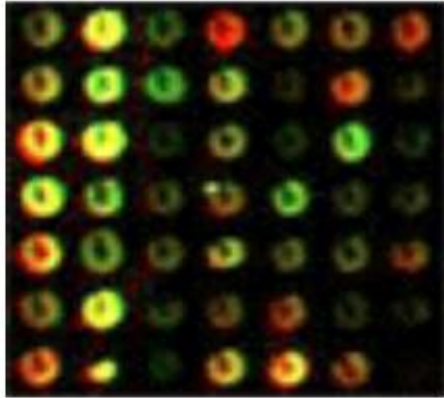
Microarray



- Example of analyzed output.

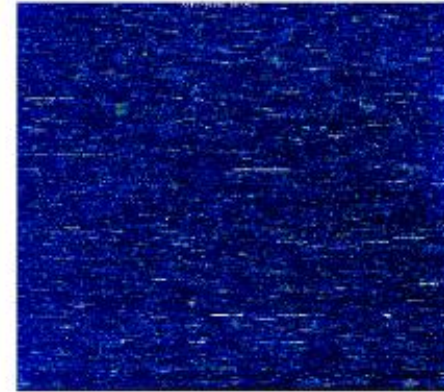
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3	11717559_a_at	ABLIM1	-2.94611096	6.235842407	1.67E-39	4.81E-37
4	11747452_a_at	ABLIM1	-2.146802277	3.36859566	9.47E-53	2.85E-49
5	11749709_a_at	ABLIM1	-2.008194865	3.361132409	9.03E-52	2.12E-48
6	11749710_s_at	ABLIM1	-1.658572984	5.485671844	5.10E-38	1.15E-35
7	11724859_s_at	ACER3	1.684093215	4.849239205	1.51E-19	2.73E-18
8	11746650_a_at	ACER3	1.981104906	6.1517831	6.91E-21	1.45E-19
9	11750700_a_at	ACSL1	1.830780762	9.2329024	1.42E-22	3.55E-21
10	11758337_s_at	ADA	-1.754985051	5.528323031	3.28E-22	7.85E-21
11	11755796_a_at	ADAM9	1.844051869	5.861768073	1.08E-19	1.98E-18
12	11755797_x_at	ADAM9	1.864739912	5.8389587	1.55E-19	2.80E-18
13	11732135_a_at	ADCK3	-1.536367954	4.731076102	2.61E-25	8.75E-24
14	11716710_a_at	ADM	1.532511447	8.763865057	2.81E-20	5.50E-19
15	11753618_a_at	ADM	1.742038275	5.077395157	1.96E-22	4.78E-21
16	11756406_x_at	ADM	1.589010274	8.653189419	1.60E-20	3.21E-19
17	11745774_x_at	AES	-1.511524068	7.445269814	1.25E-30	8.27E-29
18	11739696_s_at	AFF3	-1.656908984	2.904329655	2.64E-27	1.12E-25
19	11731943_x_at	AGAP4	-1.698765651	5.026083247	7.57E-38	1.65E-35
20	11723606_a_at	AGFG1	2.098148491	7.372889389	1.38E-35	1.94E-33
21	11723608_a_at	AGFG1	1.912553661	6.868258961	1.78E-23	4.95E-22
22	11755694_a_at	AGFG1	2.137970819	6.371678765	2.11E-37	4.28E-35

The Affymetrix GeneChip



Custom spotted arrays

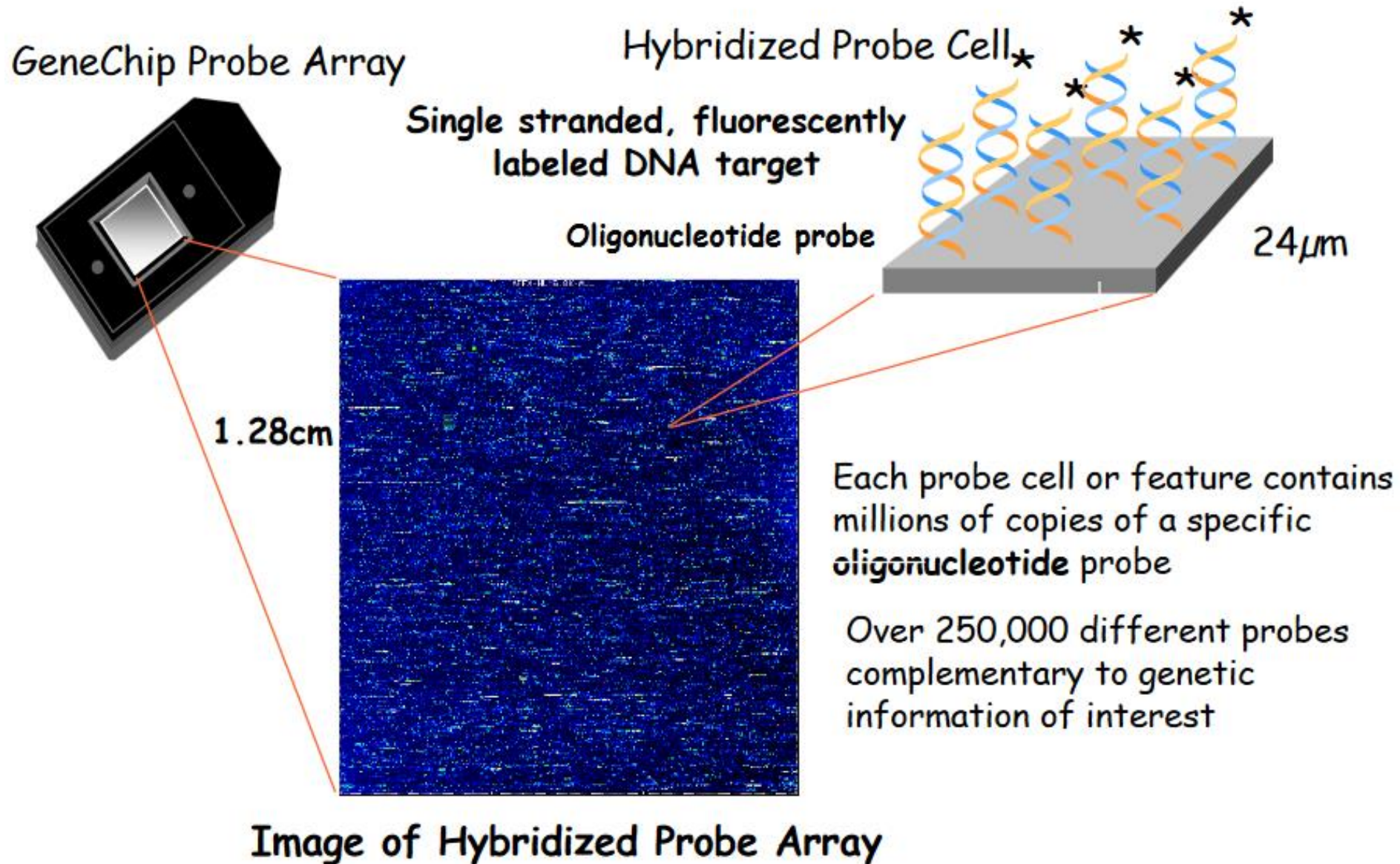
2 channels; yields a ratio between conditions.



Affymetrix GeneChip

1 channel; yields a raw count for one condition.

The Affymetrix GeneChip



Limitations

- High measurement errors

Limitations

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- Issues of isoforms (same DNA molecule)

Crude model where bases are randomly distributed A, T, C, G:

Probability to have 25 same bases: 10^{-15} on a 3×10^9 human genome it means 0.000003% chance to have it.

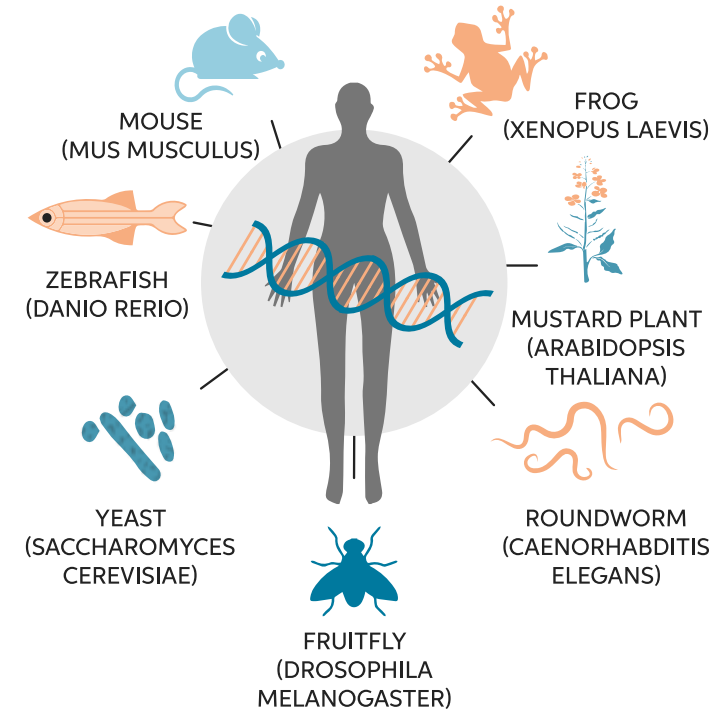
Reality a lot of sequences are more frequent, repeated sequences, gene homologues so a probe can have homologs.

Limitations

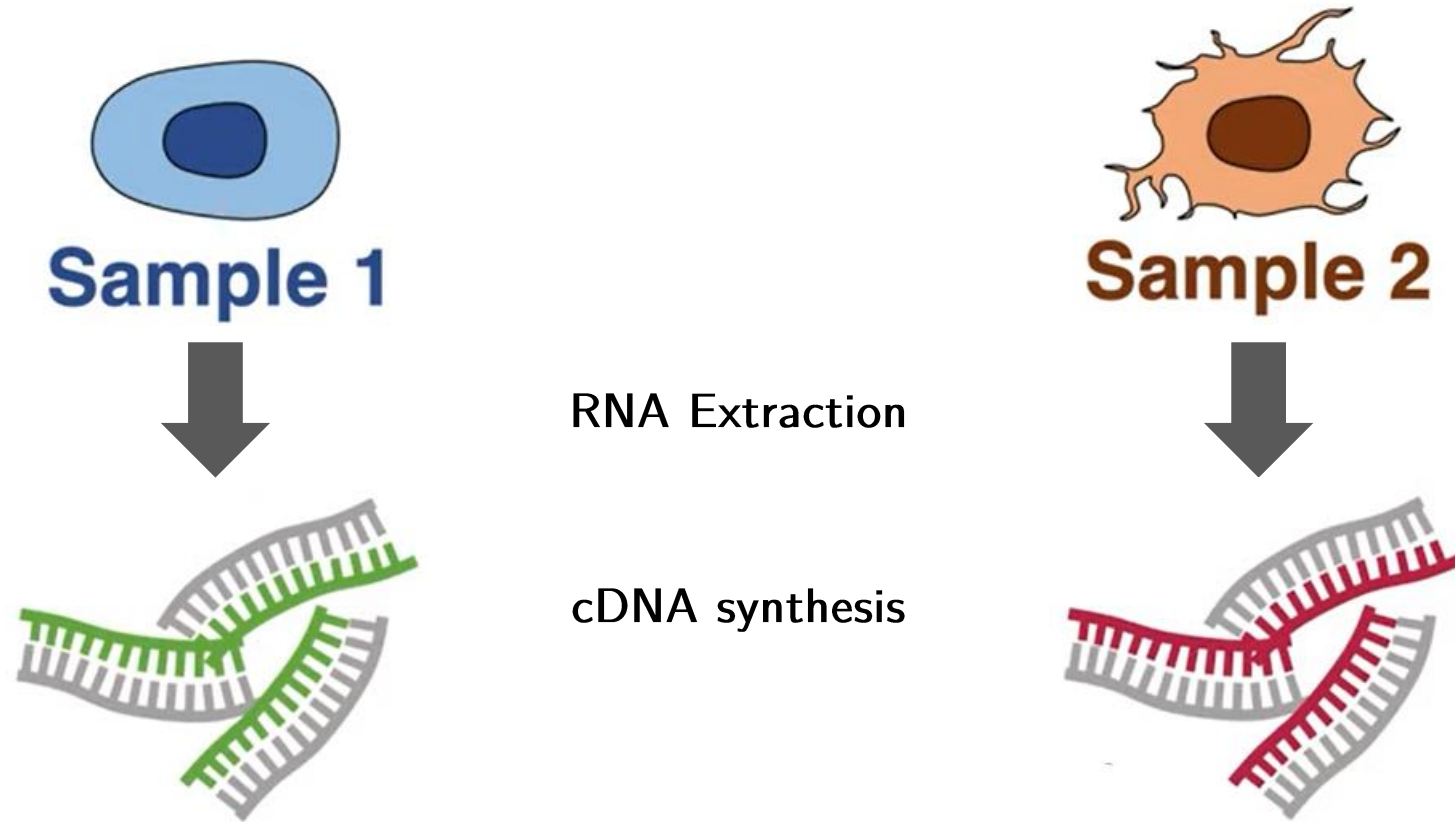
- High measurement errors
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- Higher cost of experiment than a RNAseq as the price of sequencing plummeted.

Limitations

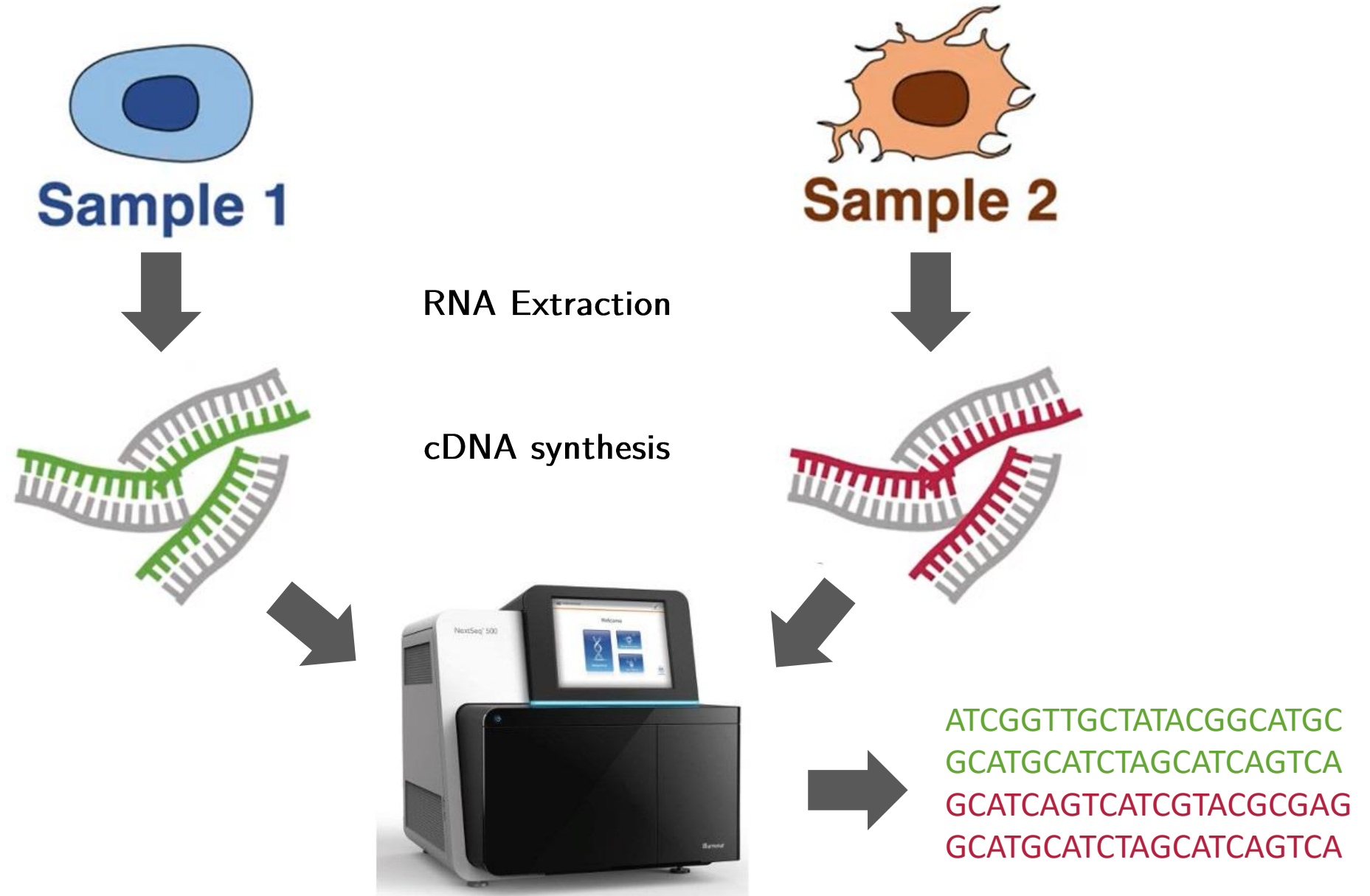
- High measurement errors
- Issues of isoforms (same DNA molecule)
- Higher cost of experiment than a RNAseq as the price of sequencing plummeted.
- Probes limitations, works for model organisms, very expensive if you want to design personalized probes.



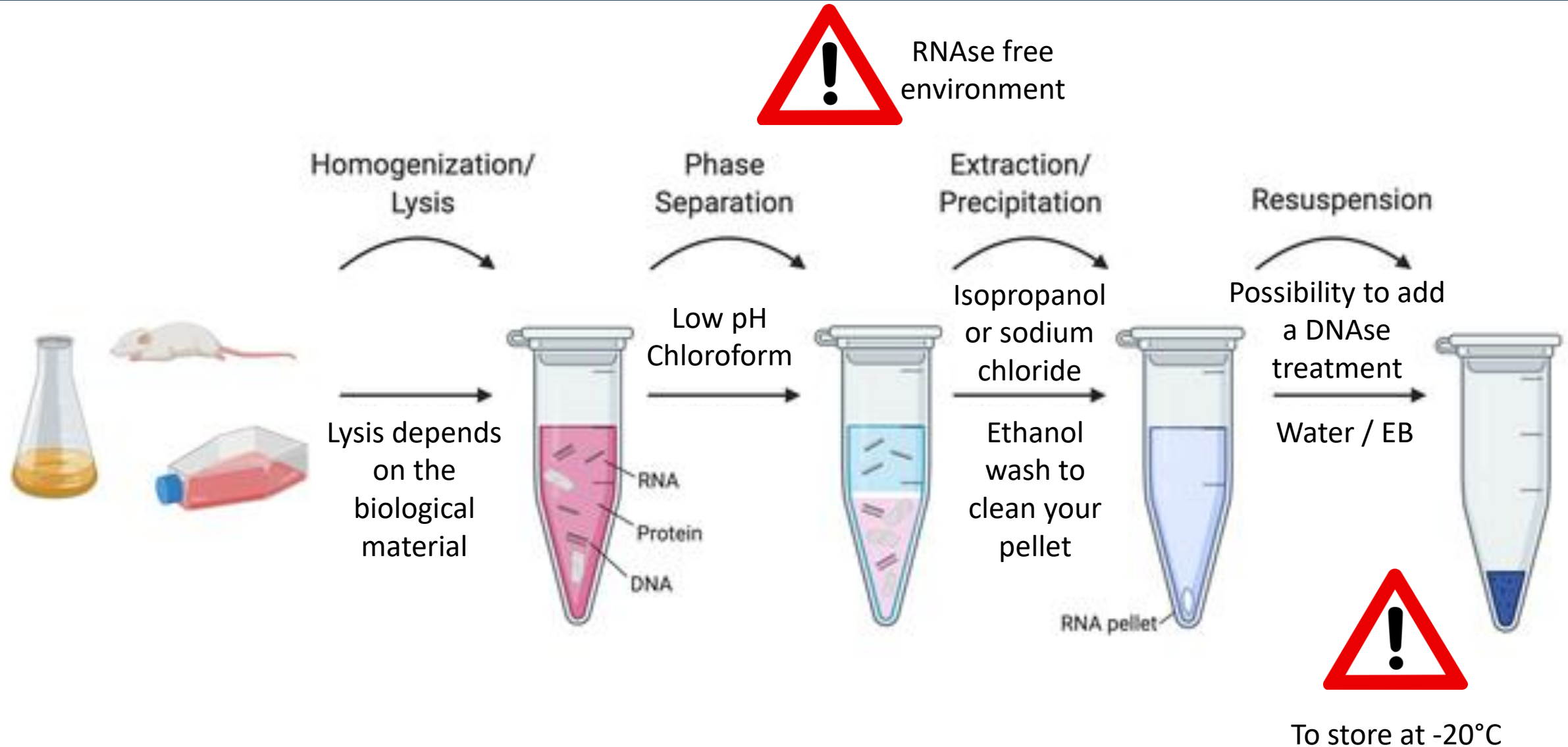
RNA sequencing



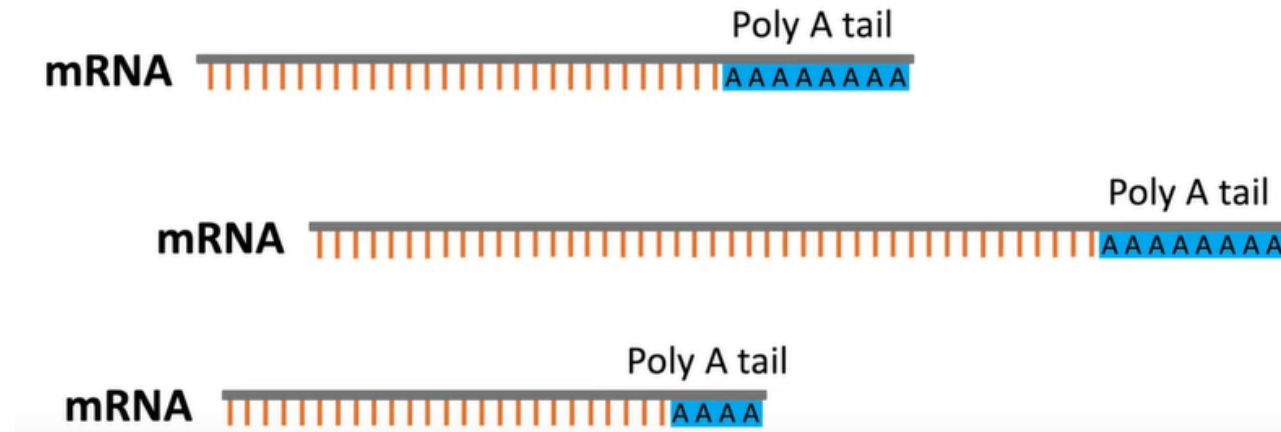
RNA sequencing



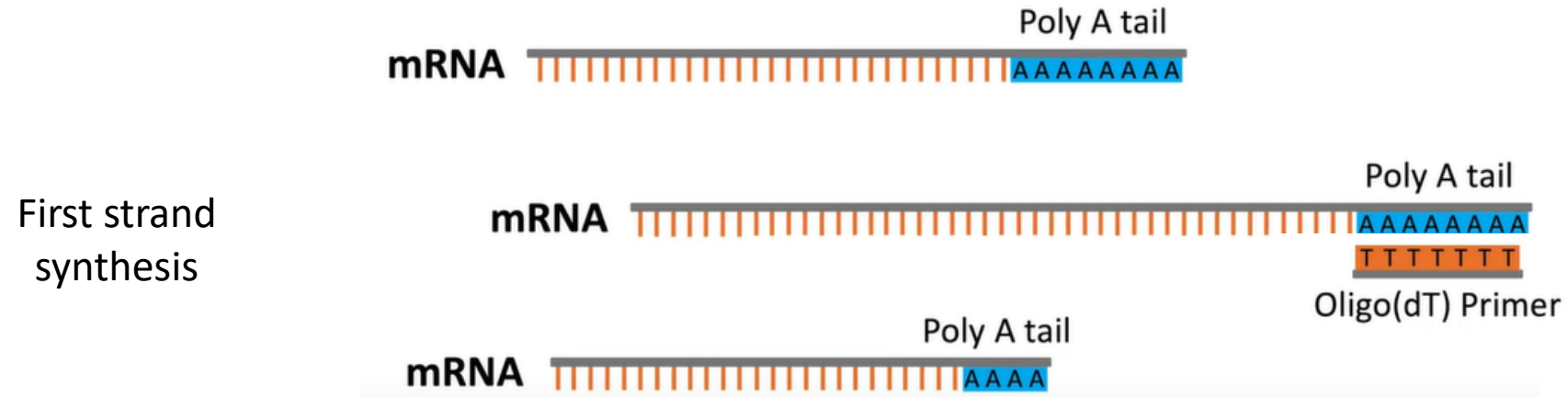
The RNA extraction



cDNA synthesis



cDNA synthesis



cDNA synthesis

First strand
synthesis



cDNA synthesis

First strand
synthesis



cDNA synthesis

First strand
synthesis



cDNA synthesis

First strand
synthesis



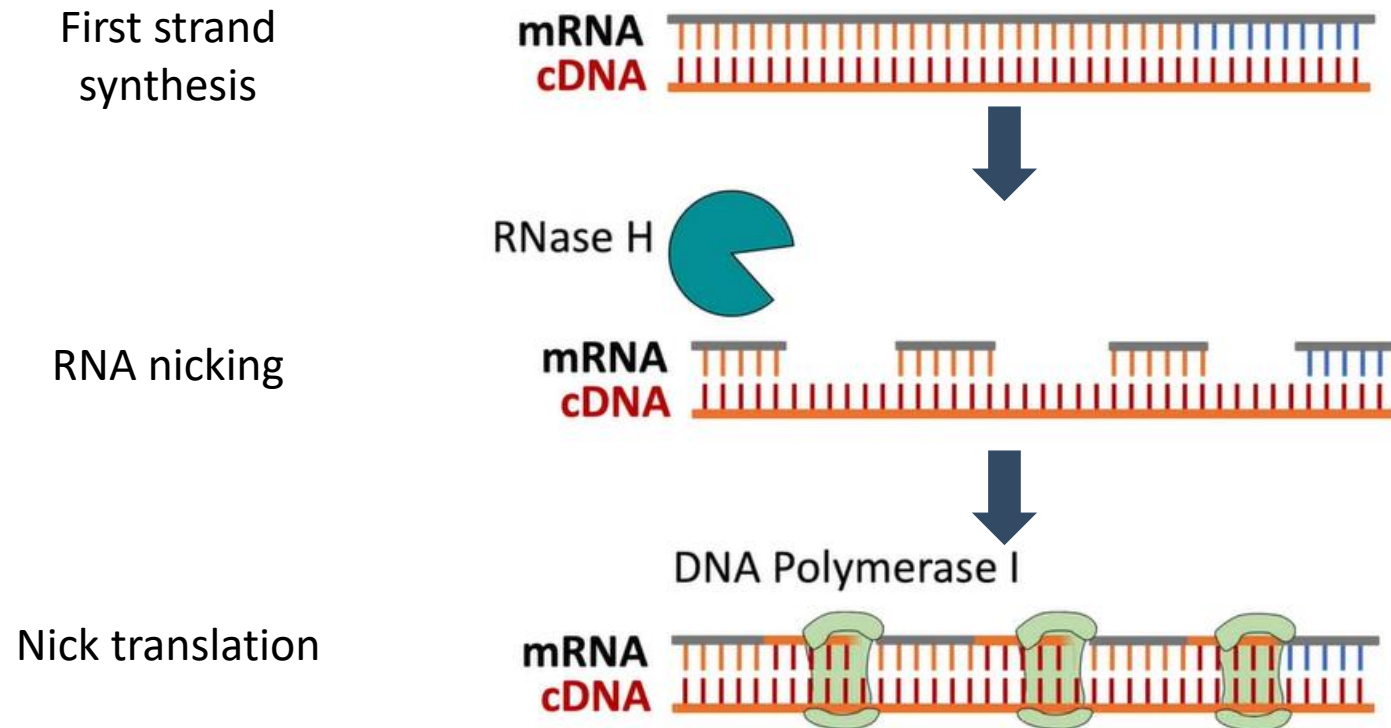
RNase H



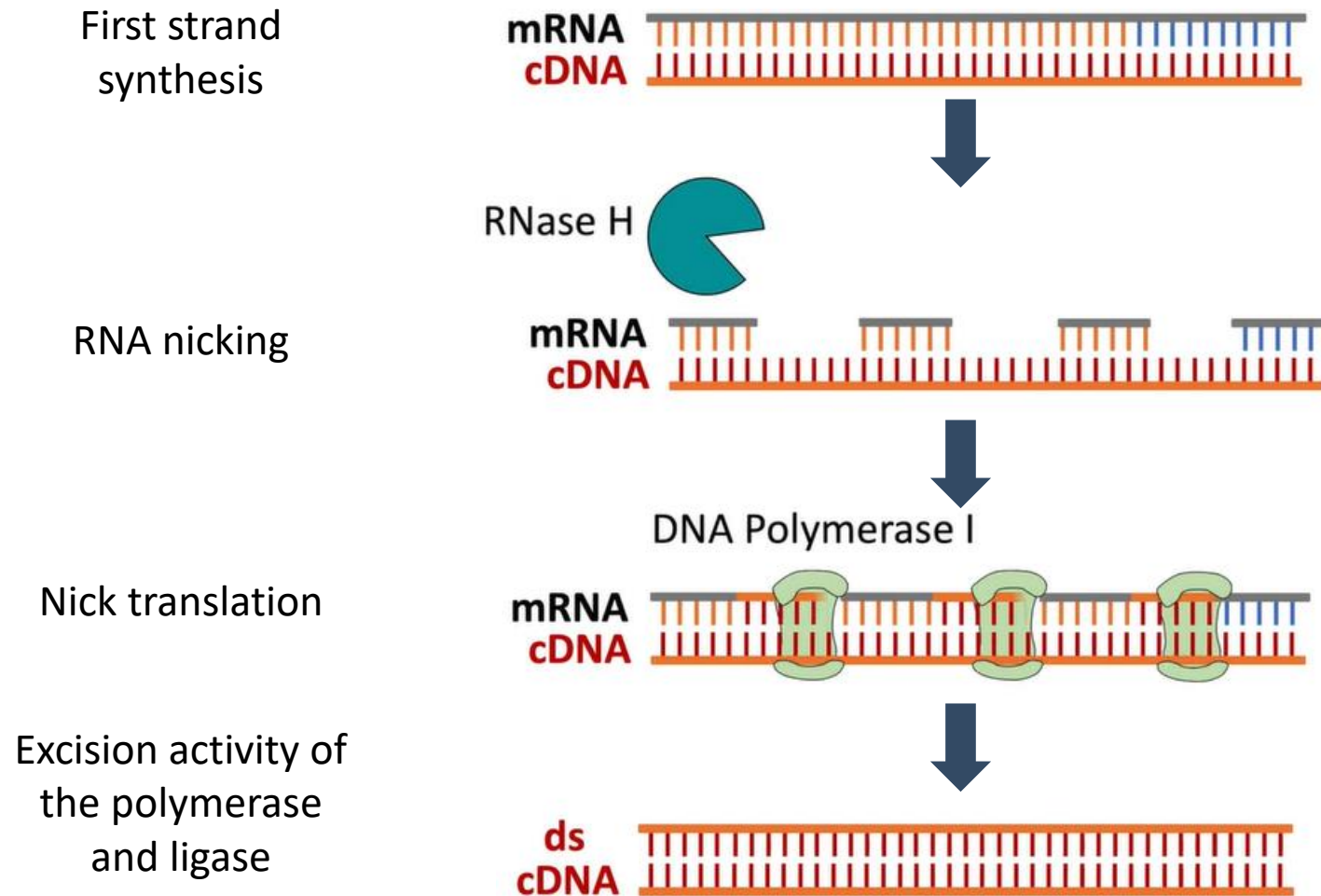
RNA nicking



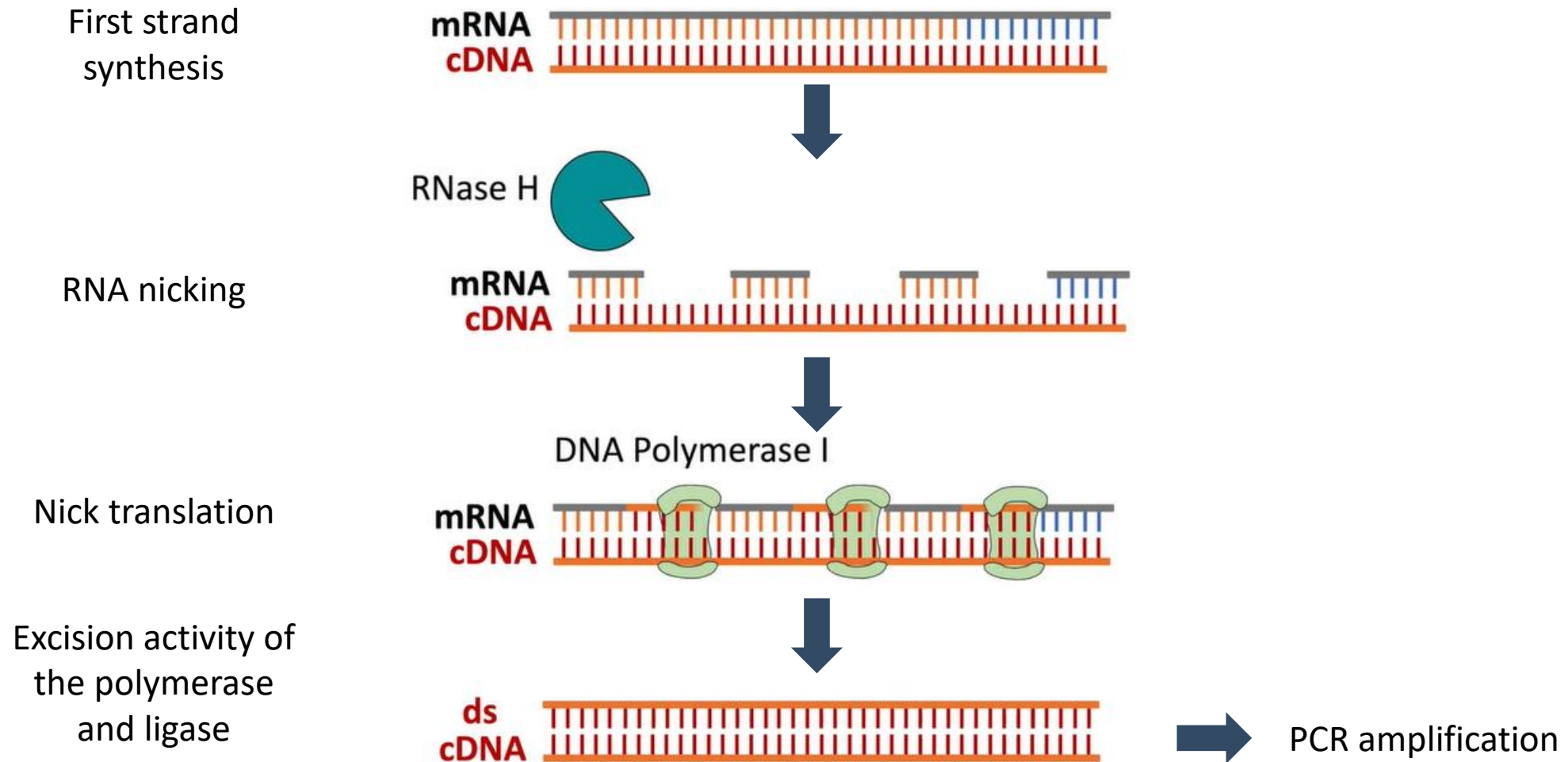
cDNA synthesis



cDNA synthesis



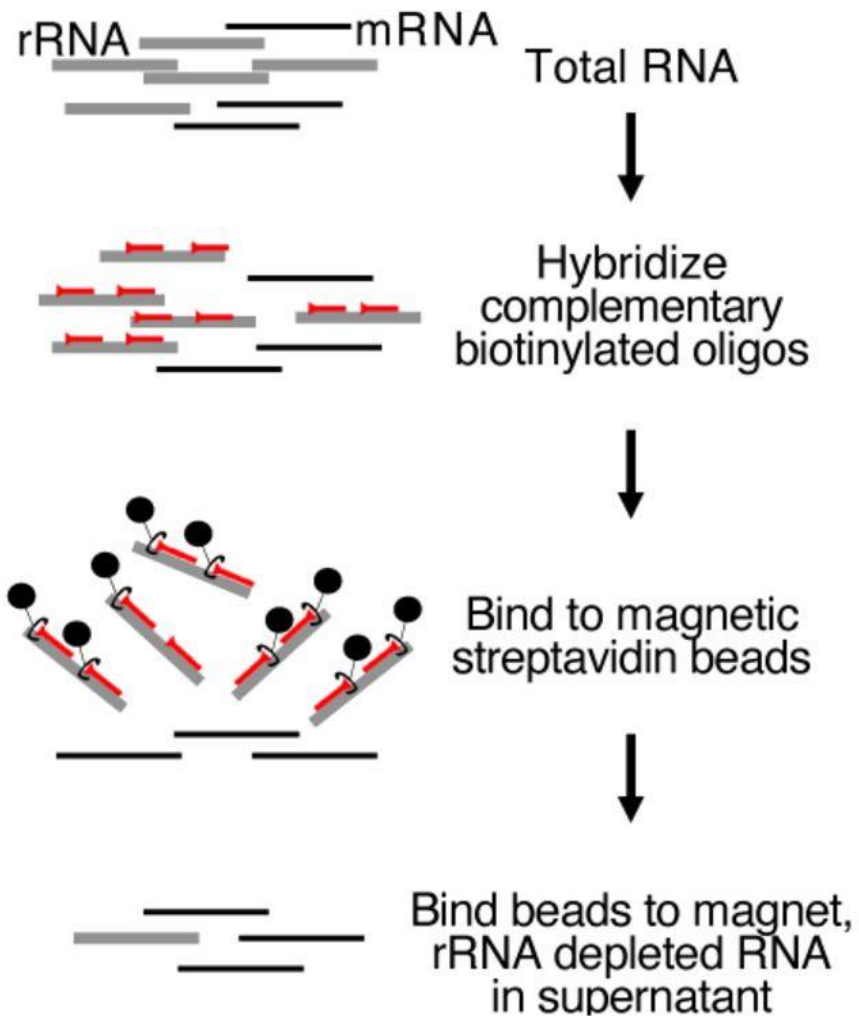
cDNA synthesis



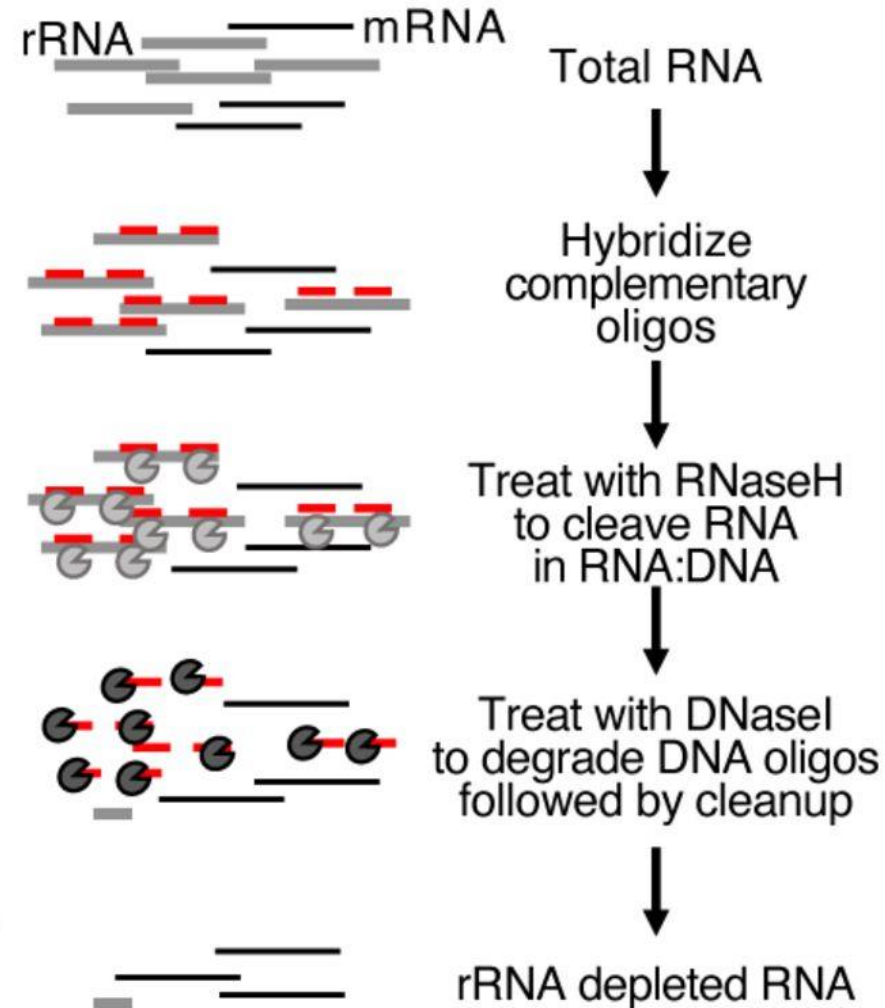
The rRNA depletion

You can work on cDNA which are more stable

A Subtractive Hybridization



B RNaseH Degradation



Alternative using CRISPRcas9 technology