Transcriptomics

GENOMICS

BIGNAUD AMAURY
04/10/2023

What’s transcriptomics ?

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

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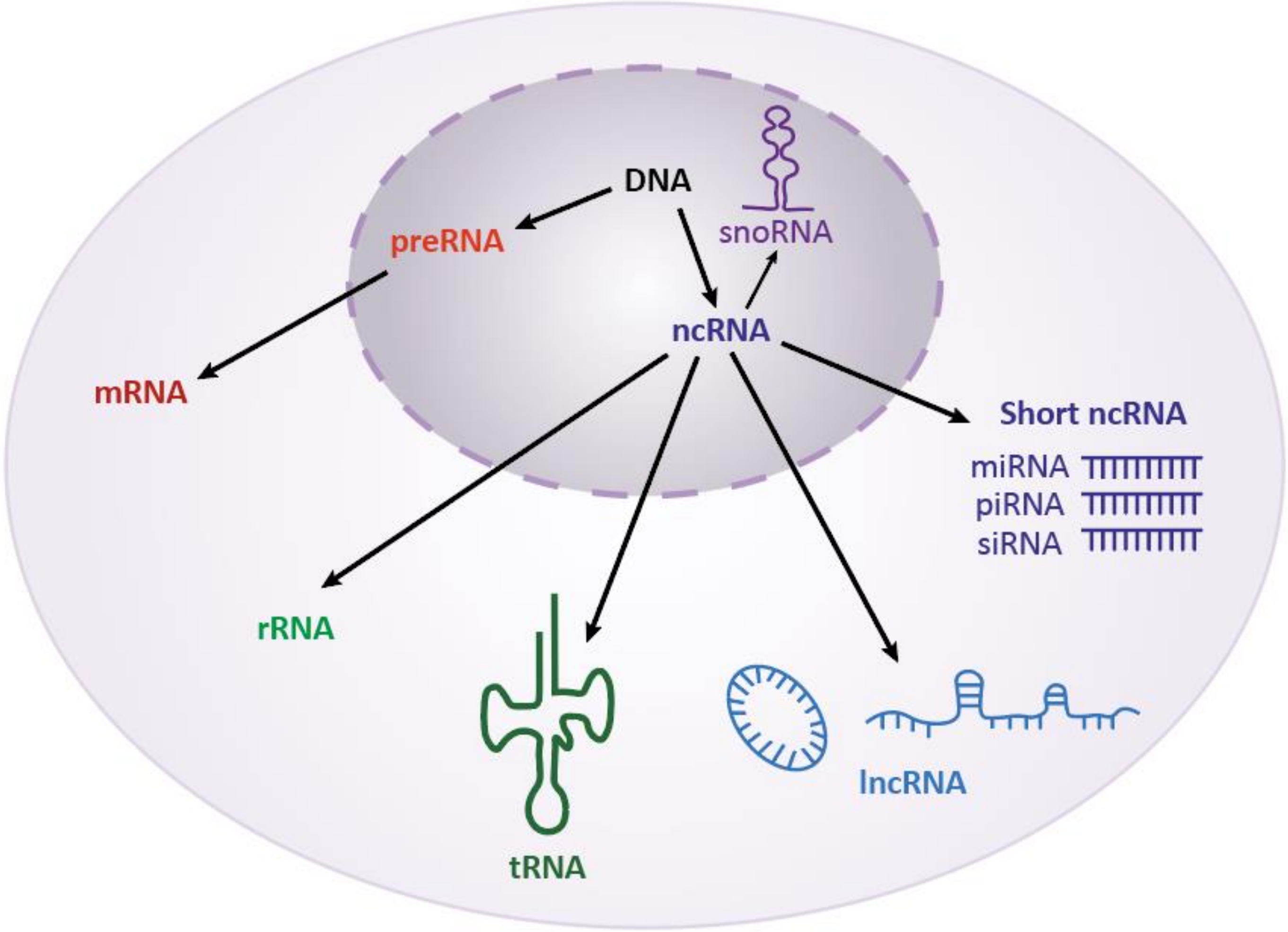

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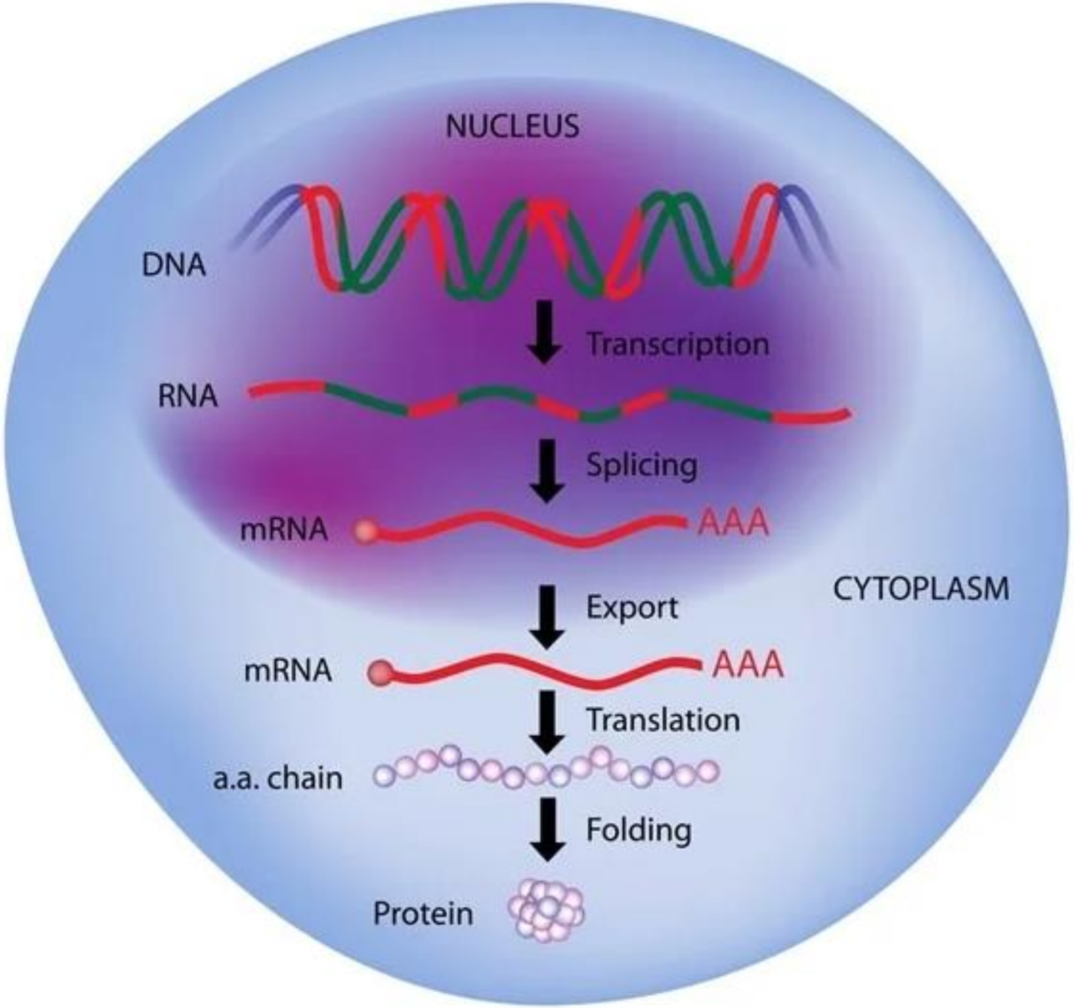
- Generally, the goal is to study **gene expression**.



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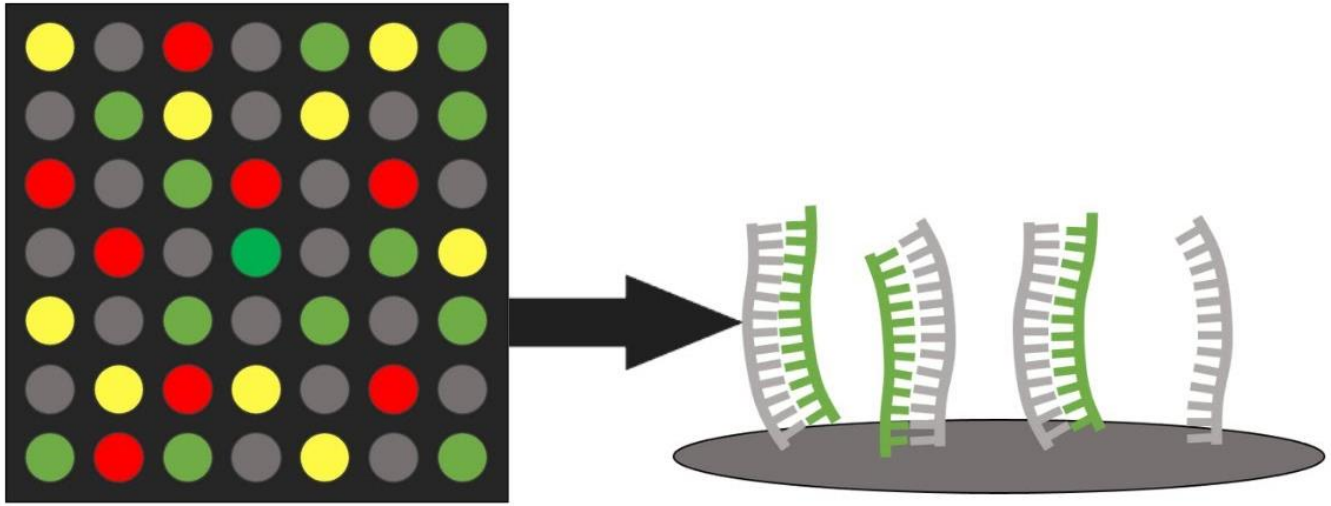
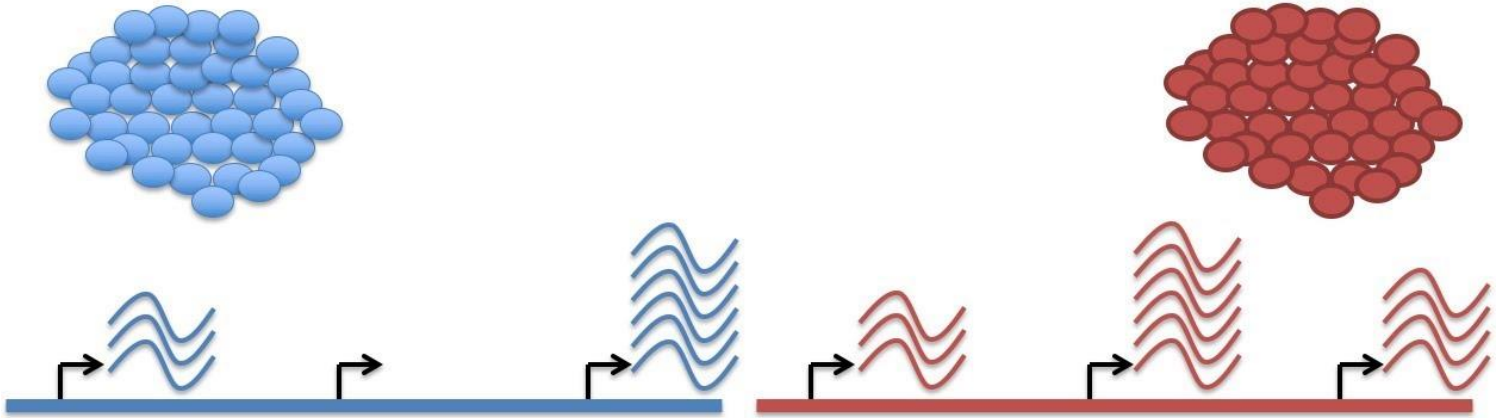
- Based on two main techniques:
  - Microarrays
  - **RNA-seq**





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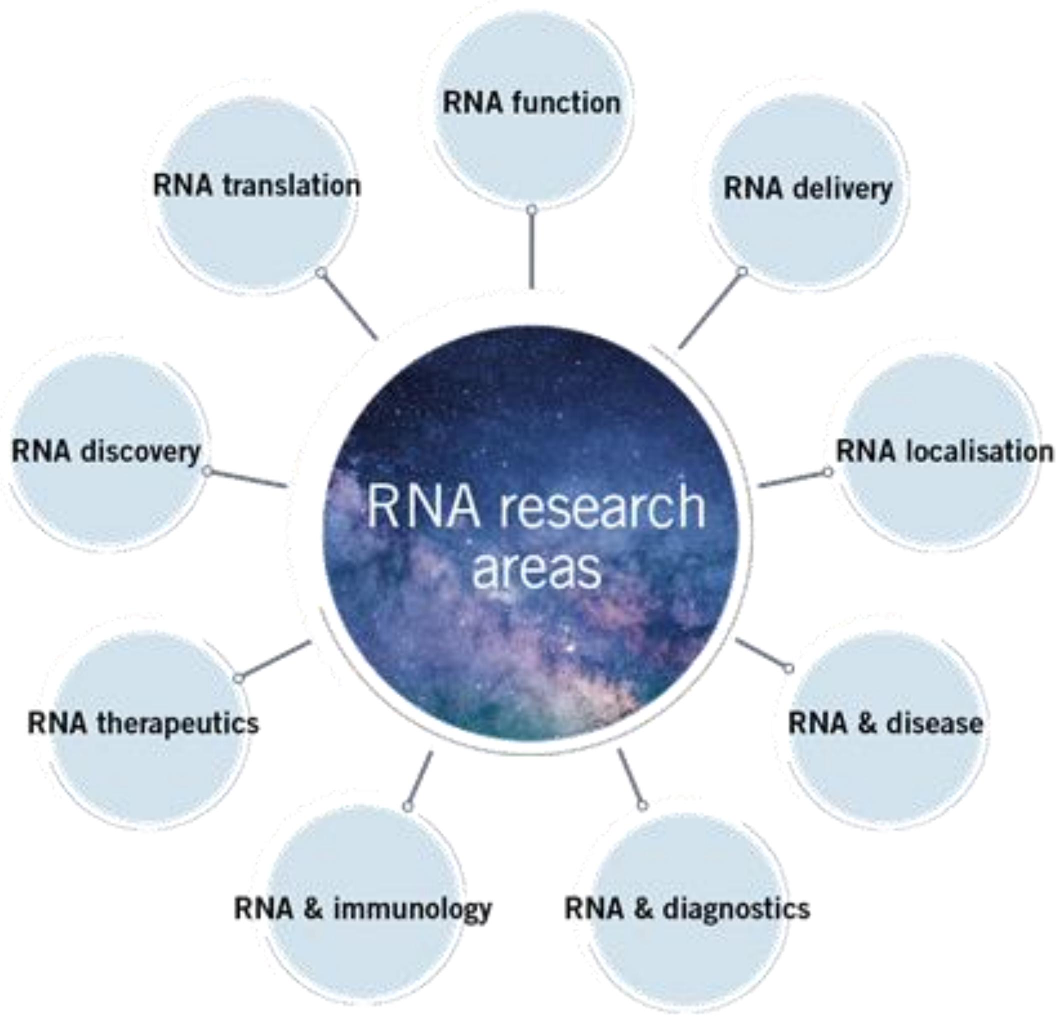
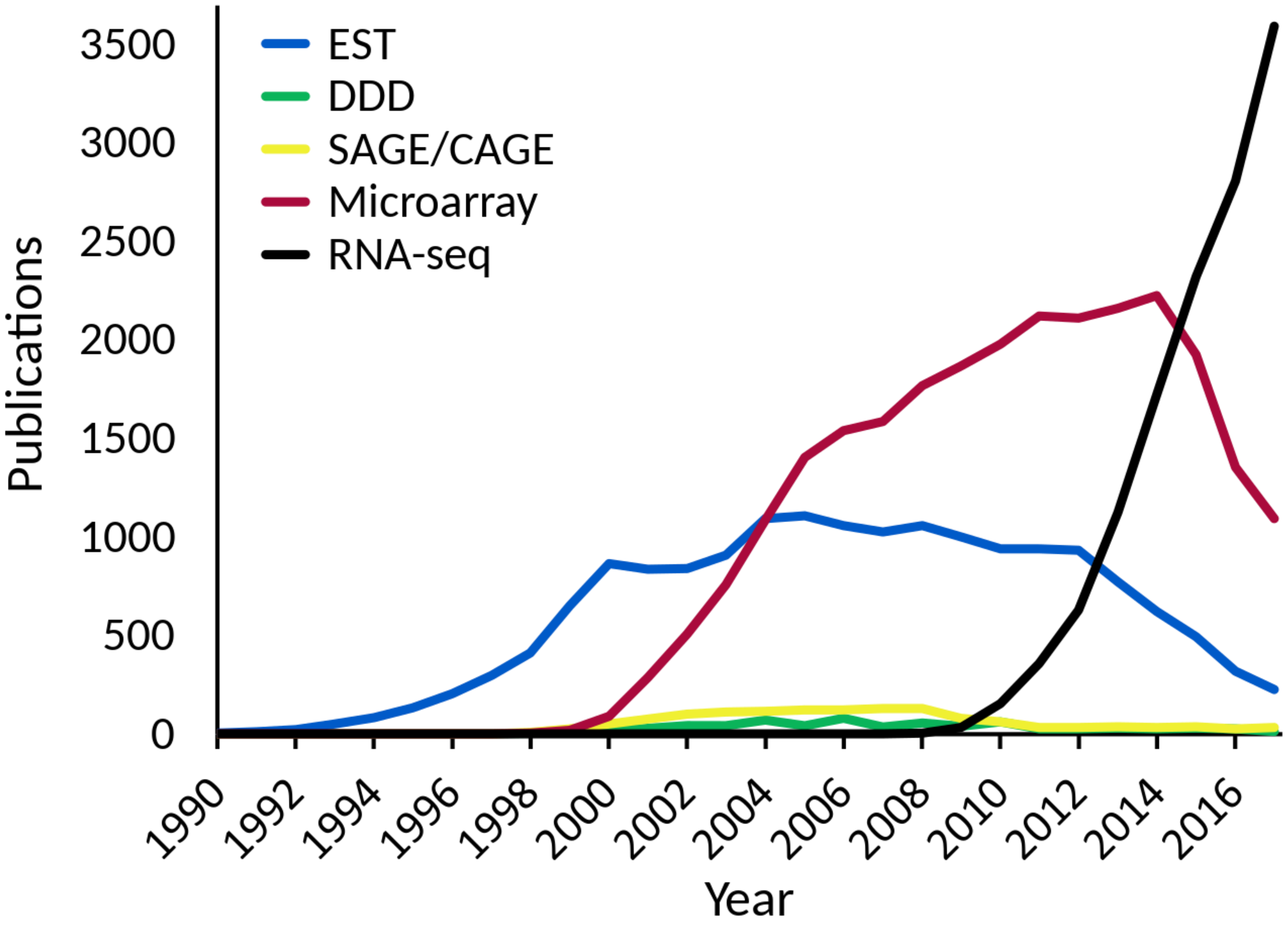
- Based on two main techniques:
  - Microarrays
  - **RNA-seq**
- Goals:
  - Measure the gene expression in various conditions (development stade, healthy vs diseased tissues…).
  - Measure the difference of expression between conditions.
  - Understand functions of genes based on detected variations.
  - Understand diseases.



Transcriptomics methods



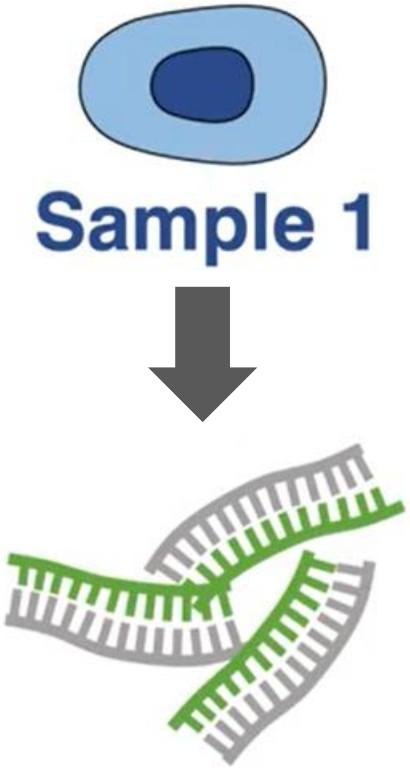
- First method to do transcriptomics was EST (Expressed sequence tag) invented in 1979.
- The DDD (Differential Display cDNA), SAGE/CAGE and microarrays arrived later.
- Now RNA-seq is the most used method with the apparition of the NGS (Next Generation Sequencing) technologies.

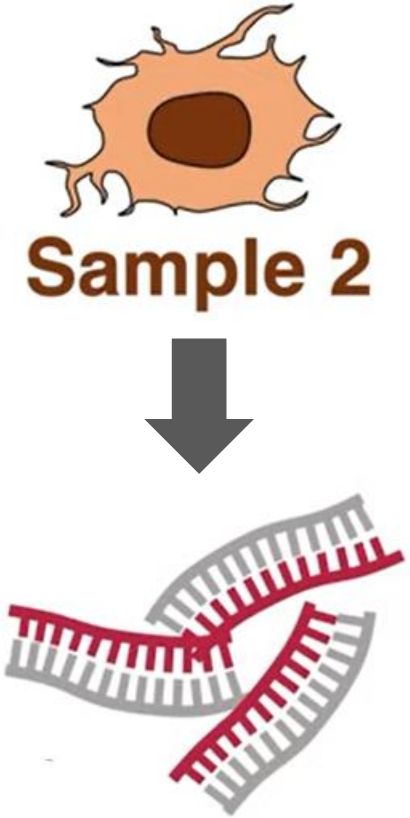
Microarray

Microarray

RNA Extraction

Microarray

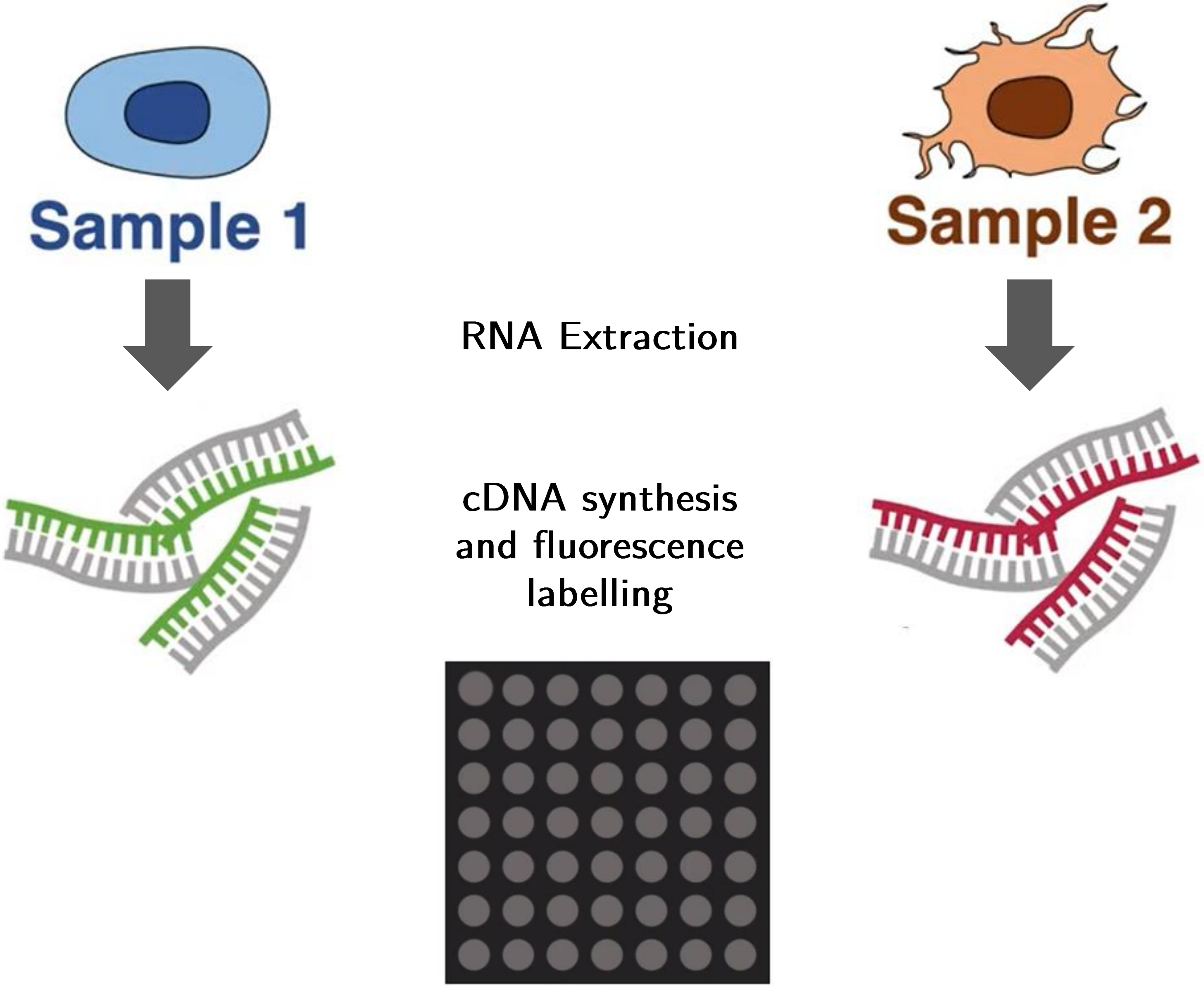




RNA Extraction

cDNA synthesis
and fluorescence
labelling

Microarray



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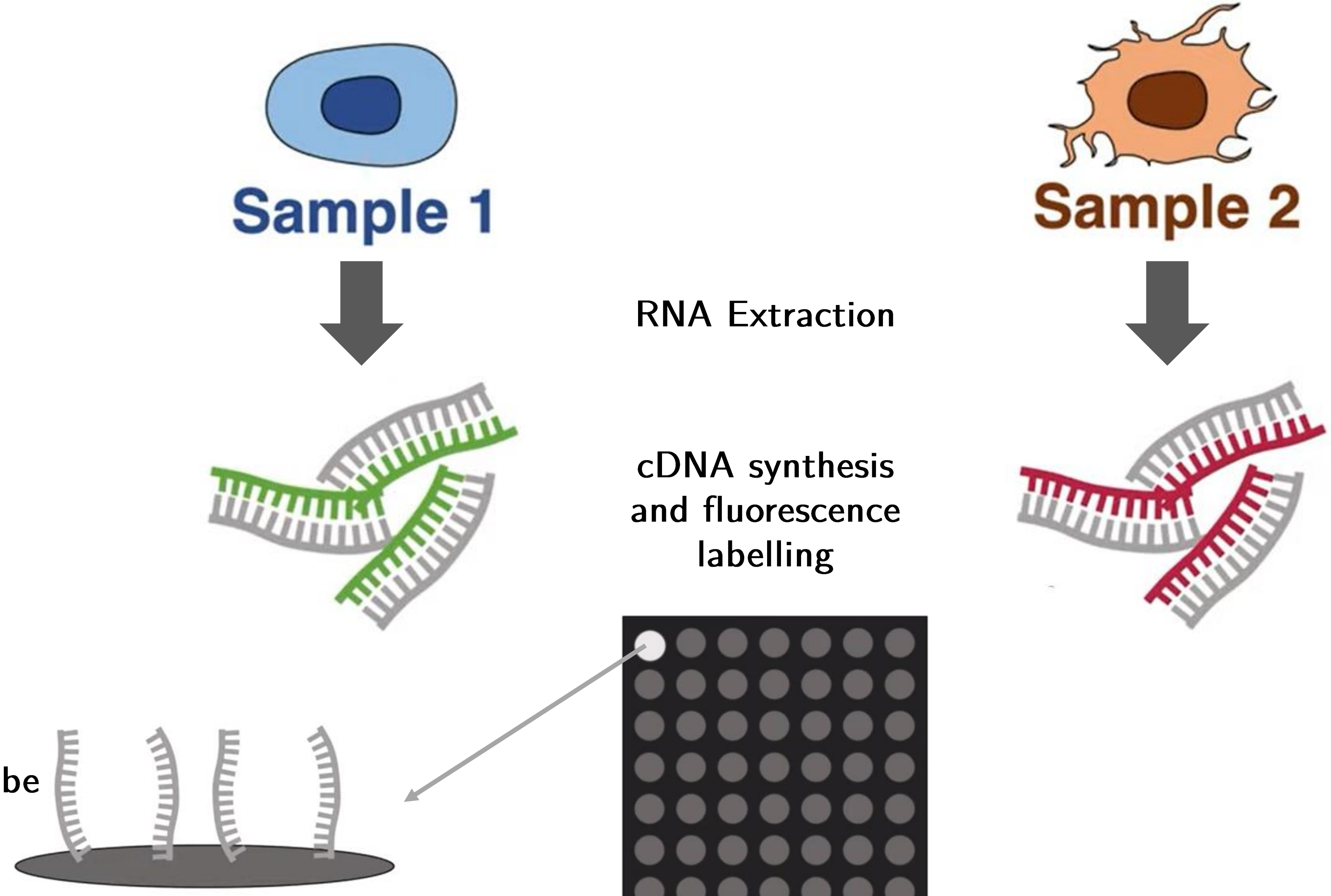

RNA Extraction

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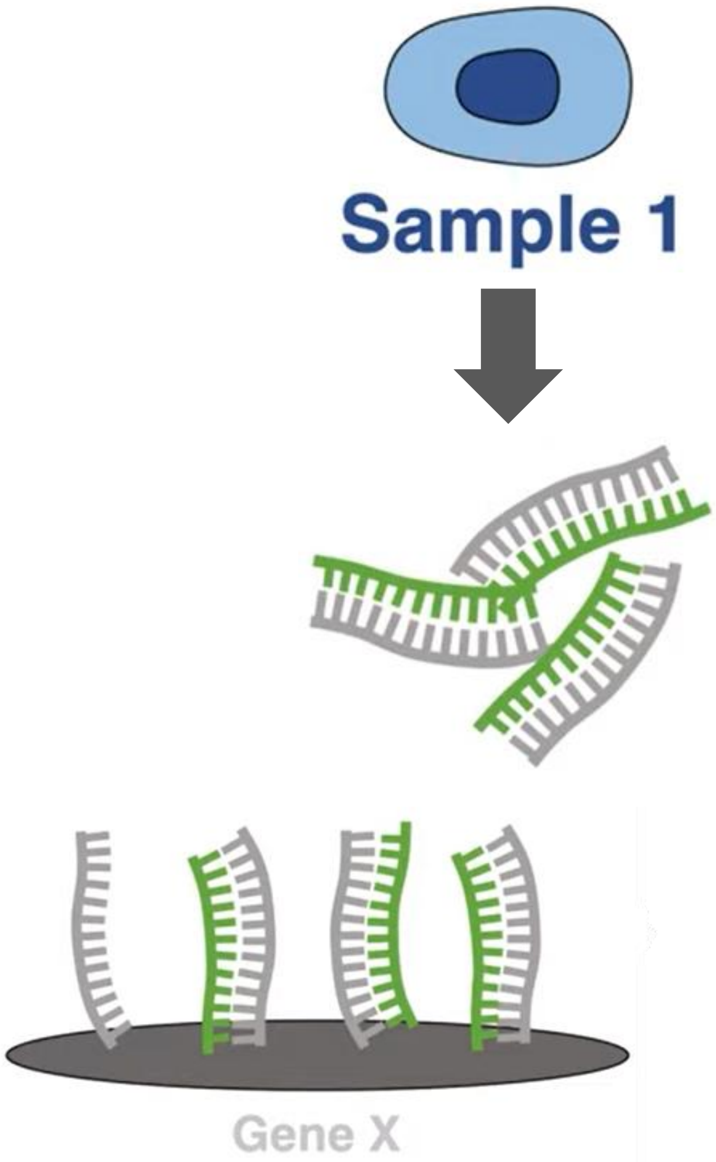
DNA probe

Spot

Microarray



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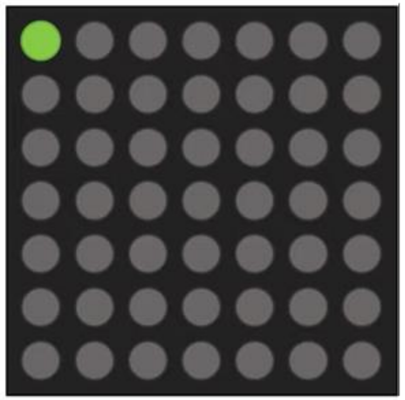
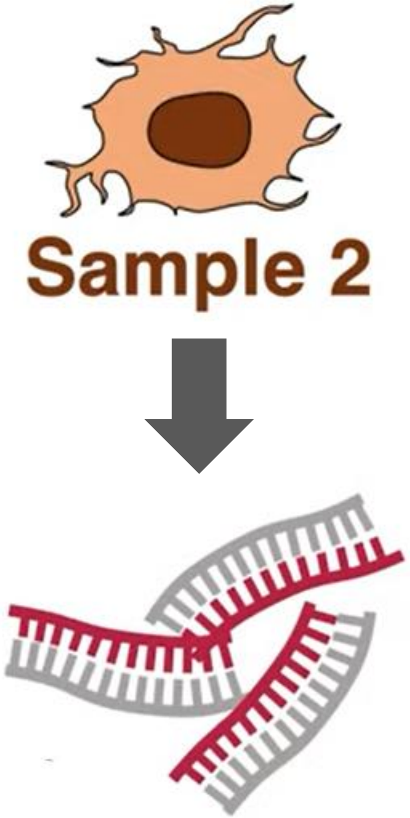
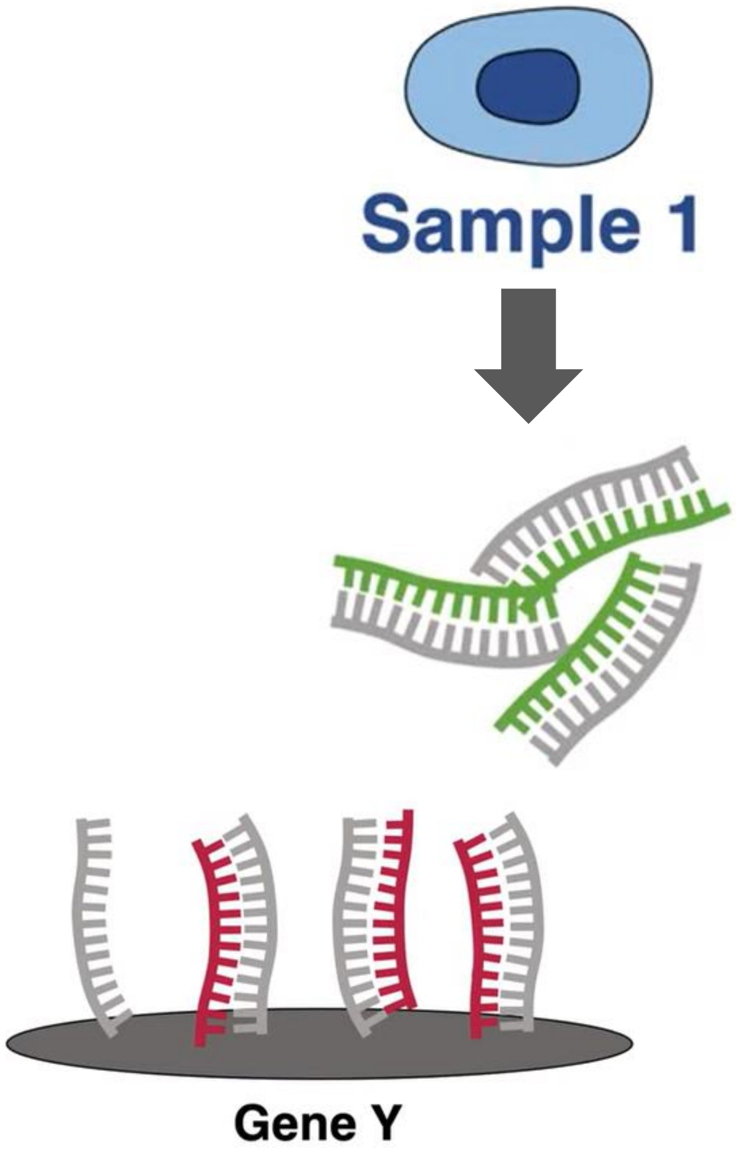
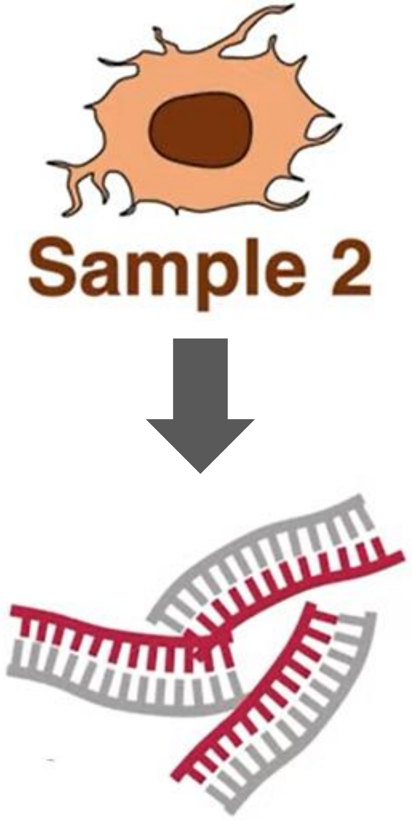




- Gene X expressed only in sample 1.

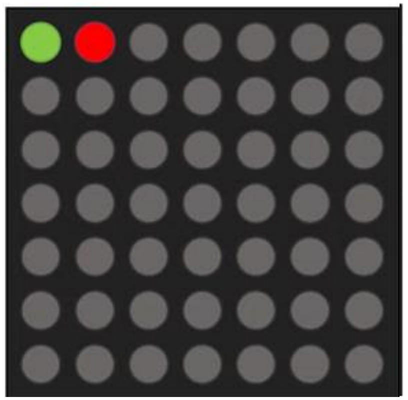
Microarray





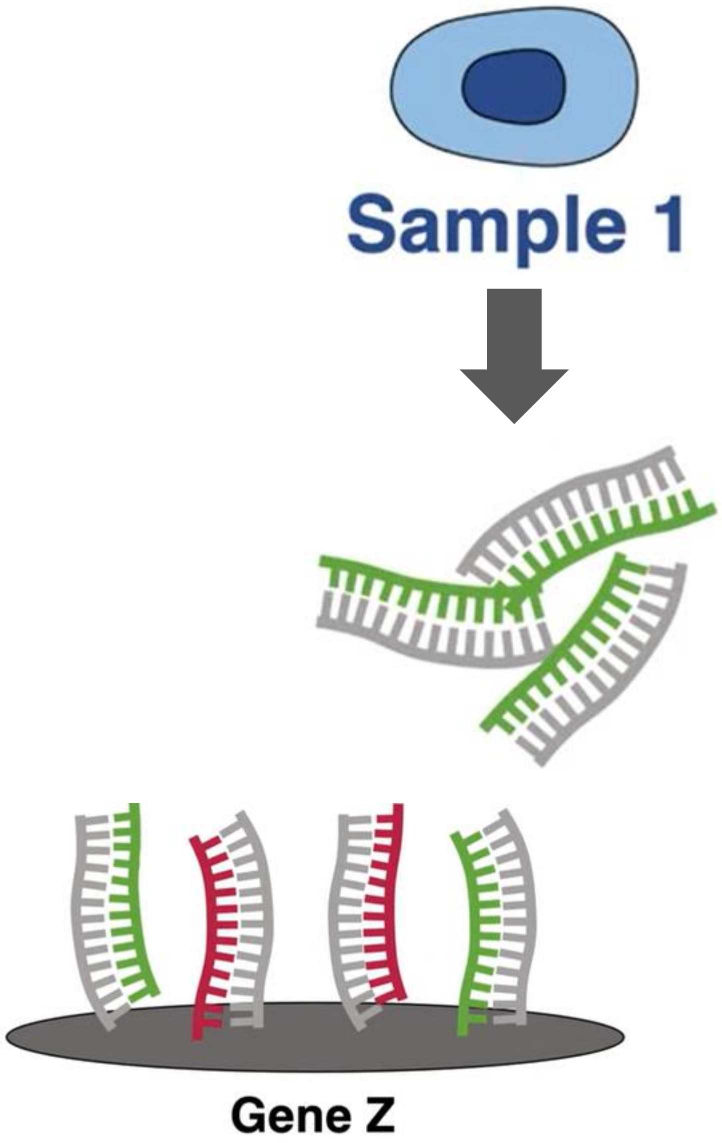
RNA Extraction

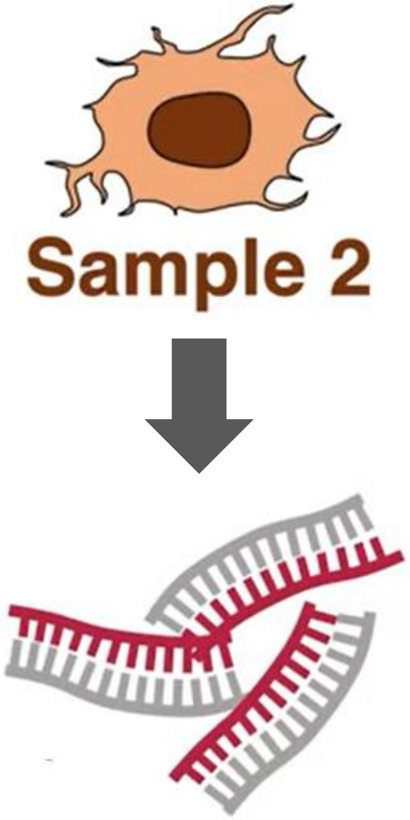
cDNA synthesis and fluorescence labelling



- Gene X expressed only in sample 1.
- Gene Y expressed only in sample 2.

Microarray





RNA Extraction

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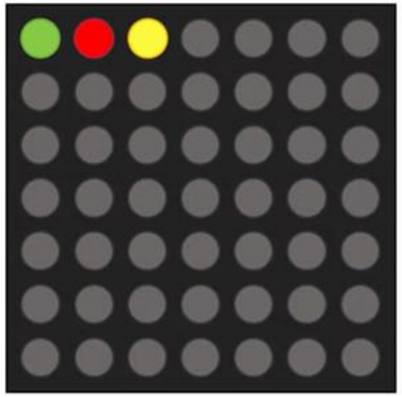
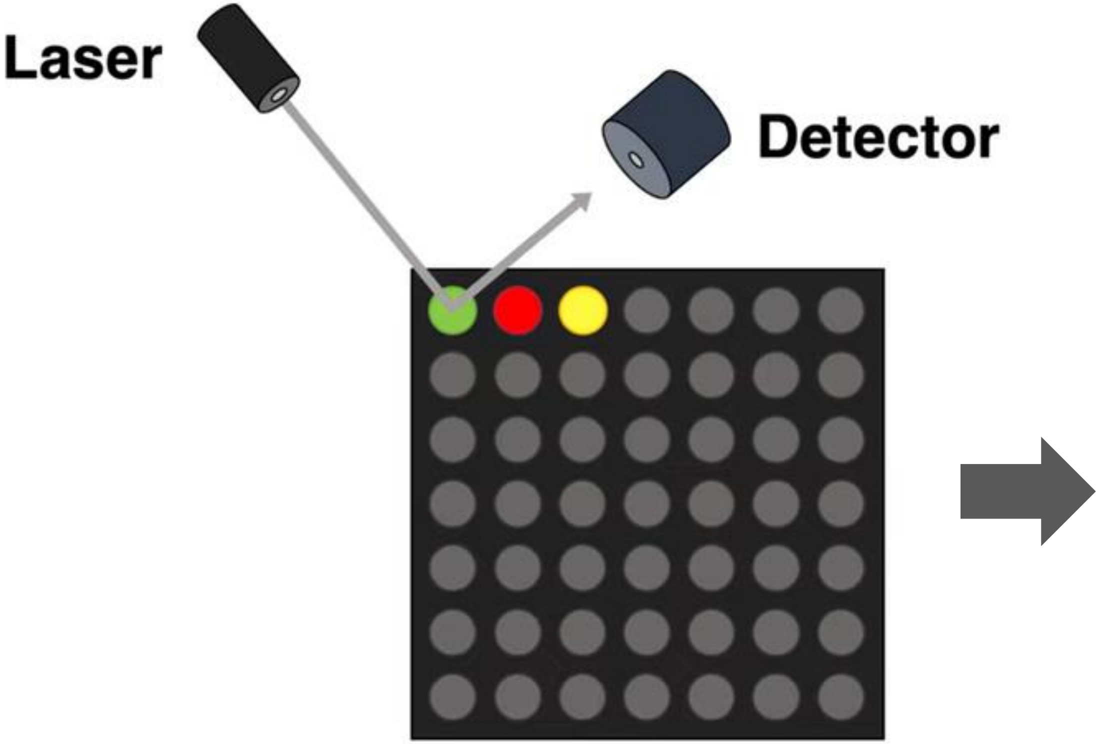
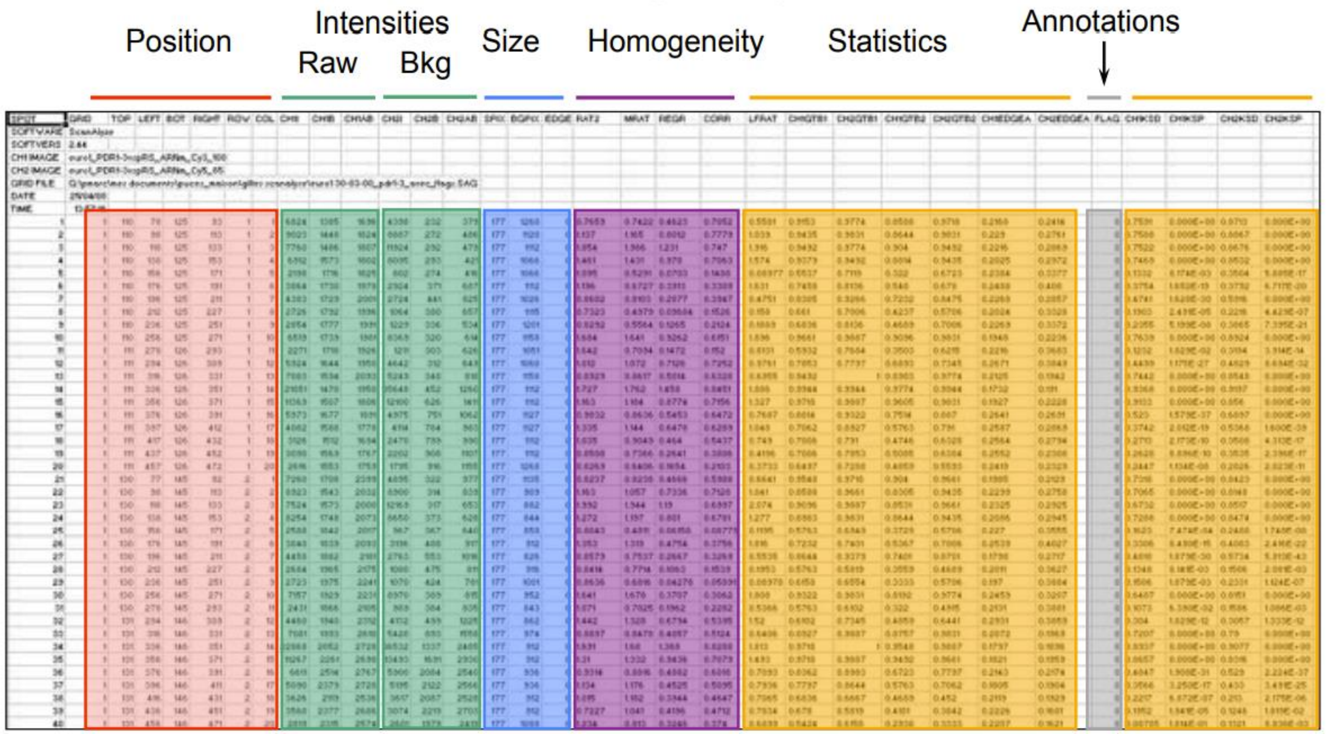


- Gene X expressed only in sample 1.
- Gene Y expressed only in sample 2.
- Gene Z expressed in both samples.

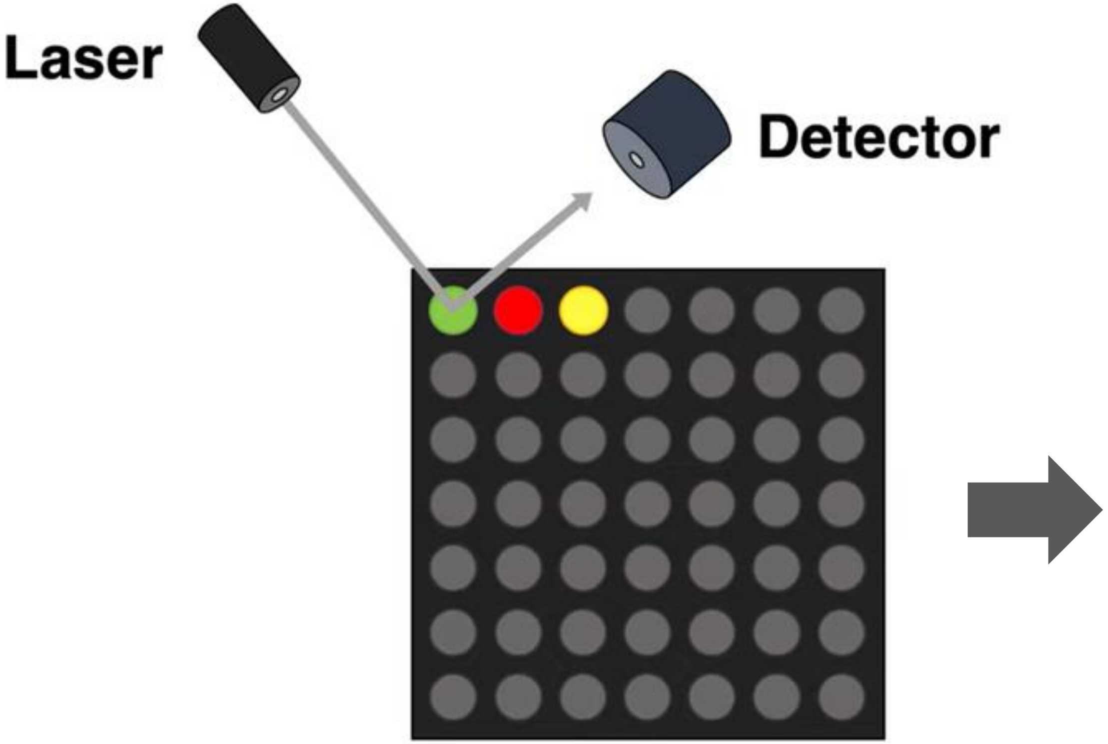
Microarray



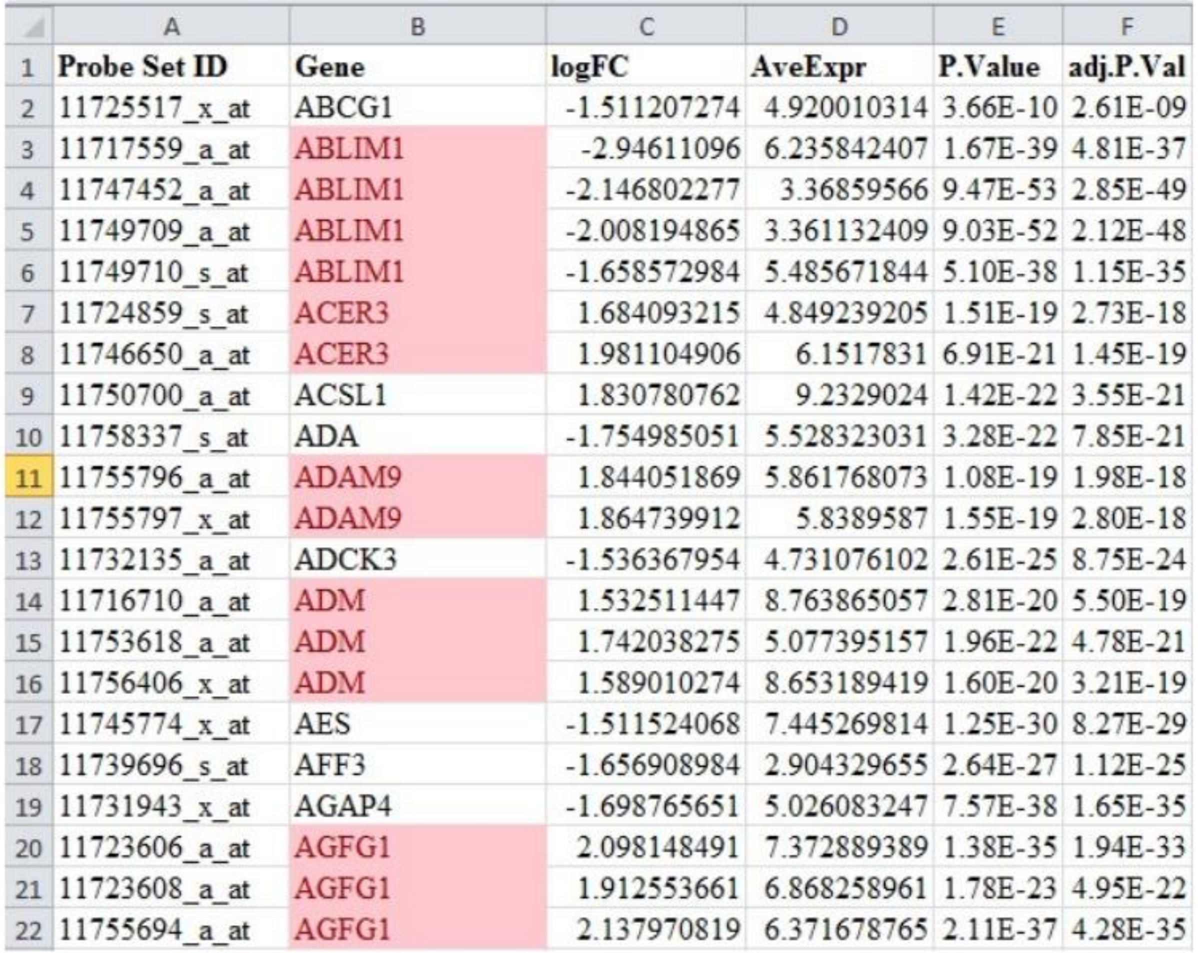
- Example of raw output.



Microarray

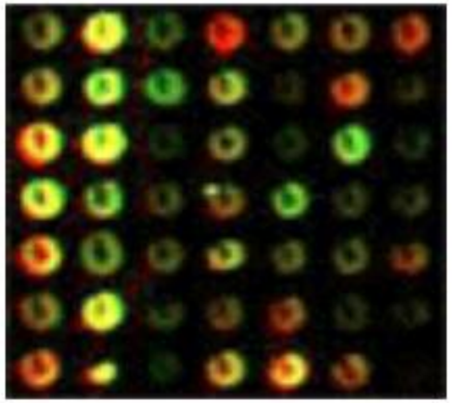


- Example of analyzed output.



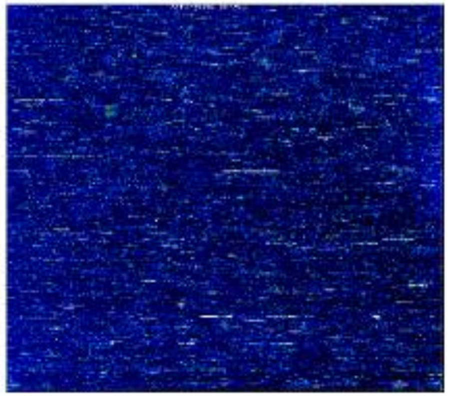
	A	B	C	D	E	F
1	**Probe Set ID**	**Gene**	**logFC**	**AveExpr**	**P.Value**	**adj.P.Val**
2	11725517_x_at	ABCG1	-1.511207274	4.920010314	3.66E-10	2.61E-09
3	11717559_a_at	ABLIM1	-2.94611096	6.235842407	1.67E-39	4.81E-37
4	11747452_a_at	ABLIM1	-2.146802277	3.36859566	9.47E-53	2.85E-49
5	11749709_a_at	ABLIM1	-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

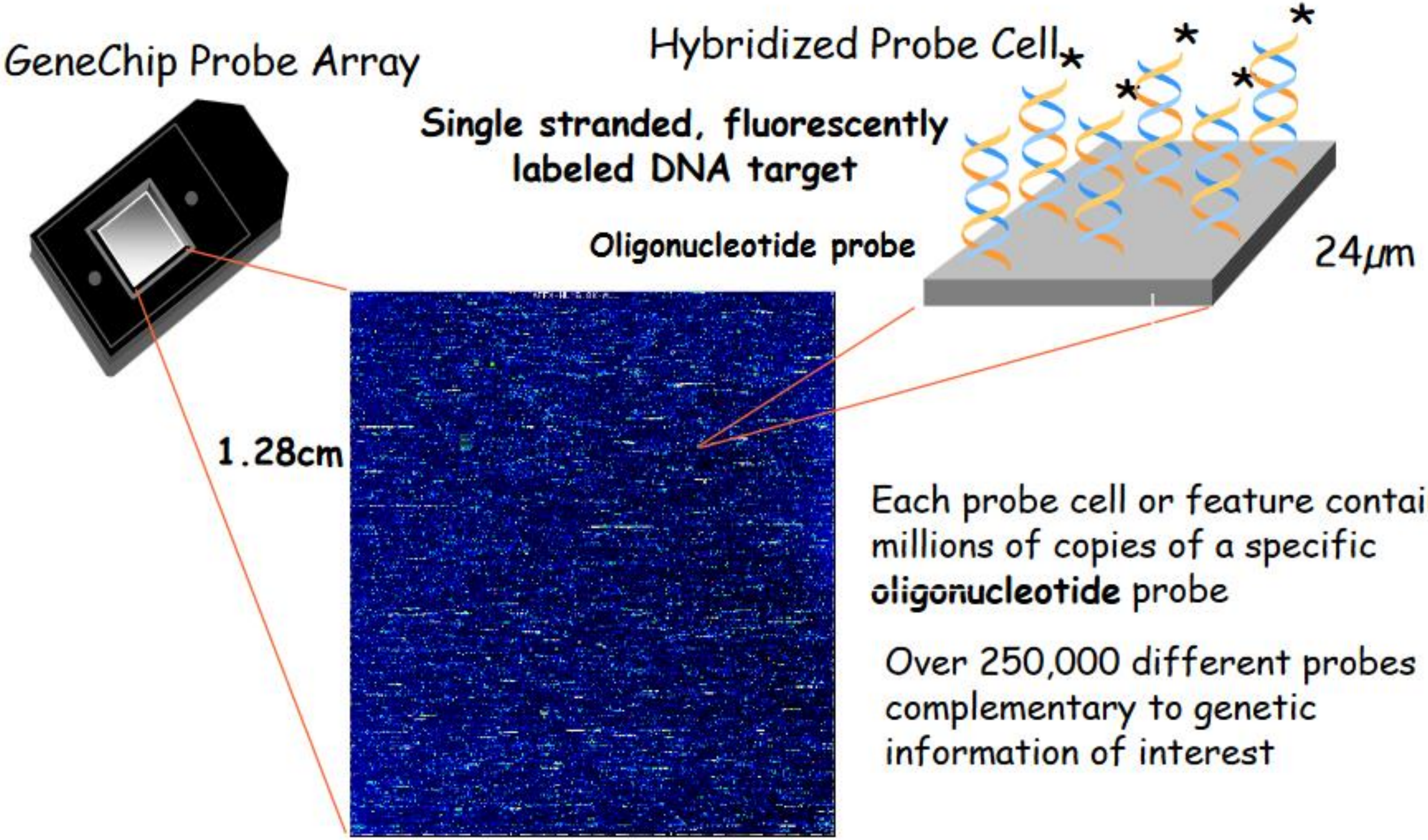
2 channels; yields a ratio
between conditions.



Affymetrix GeneChip

1 channel; yields a raw
count for one condition.

The Affymetrix GeneChip



Each probe cell or feature contains millions of copies of a specific **oligonucleotide** probe

Over 250,000 different probes complementary to genetic information of interest

Image of Hybridized Probe Array

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 $3x10^{9}$ human genome it means 0.000003% chance to have it.

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

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- Higher cost of experiment than a RNAseq as the price of sequencing plummets.

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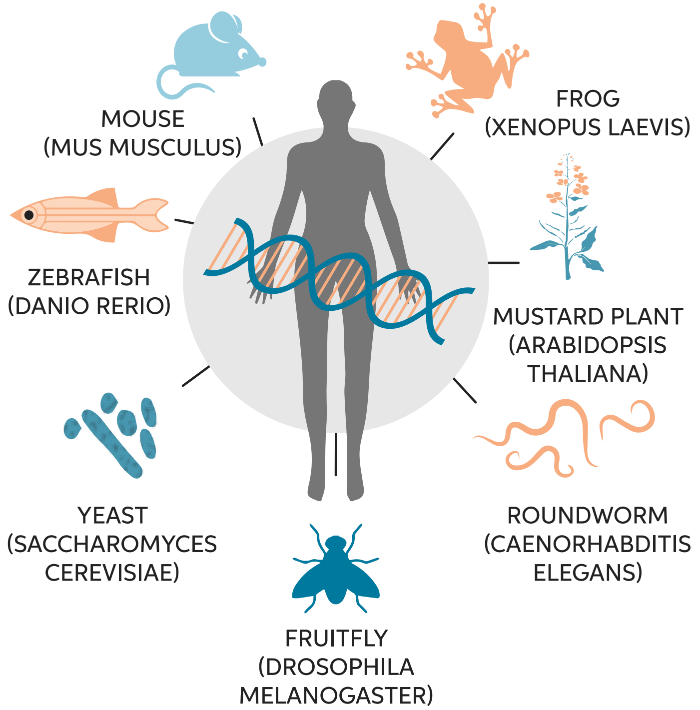
- Issues of isoforms (same DNA molecule)
- Higher cost of experiment than a RNAseq as the price of sequencing plummets.
- Probes limitations, works for model organisms, very expensive if you want to design personalized probes.

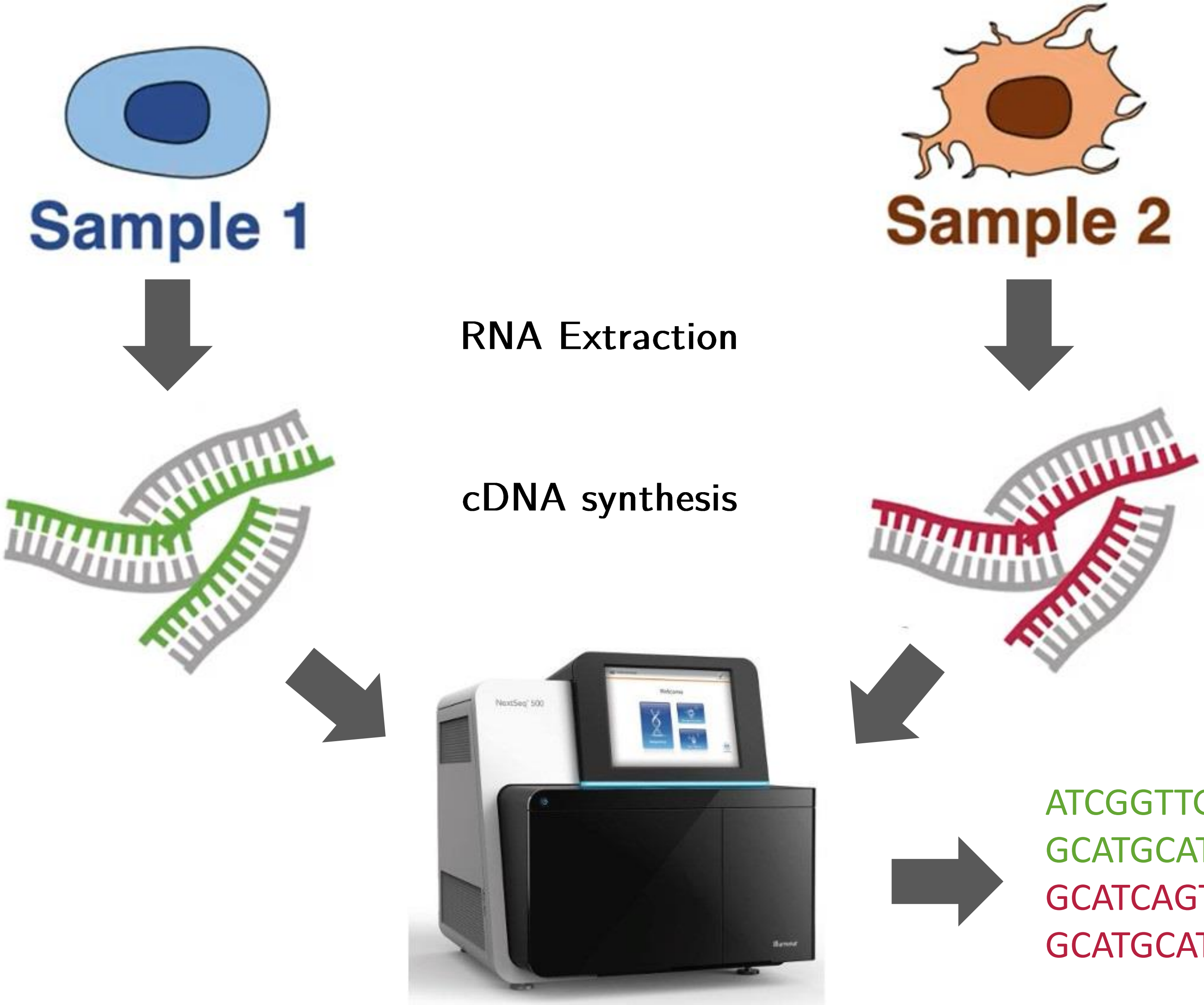


RNA sequencing

RNA Extraction

cDNA synthesis

RNA sequencing



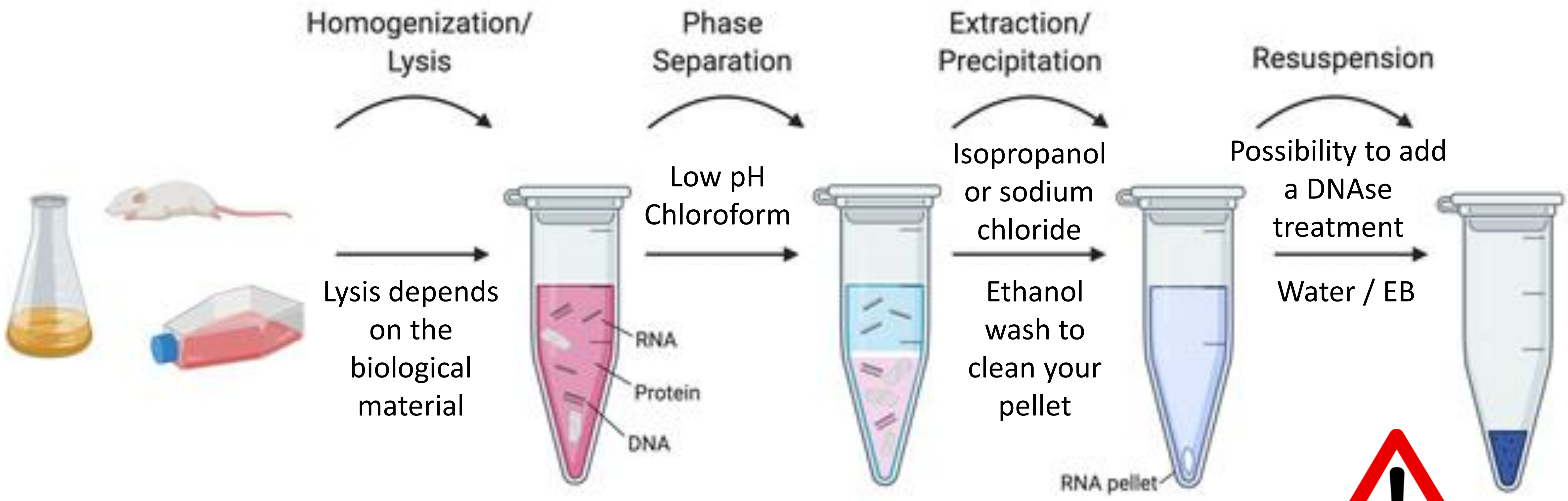
RNA Extraction

cDNA synthesis

ATCGGTTGCTATACGGCATGC
GCATGCATCTAGCATCAGTCA
GCATCAGTCATCGTACGCGAG
GCATGCATCTAGCATCAGTCA

The RNA extraction

RNAse free environment



Lysis depends on the biological material

Low pH Chloroform

Isopropanol or sodium chloride

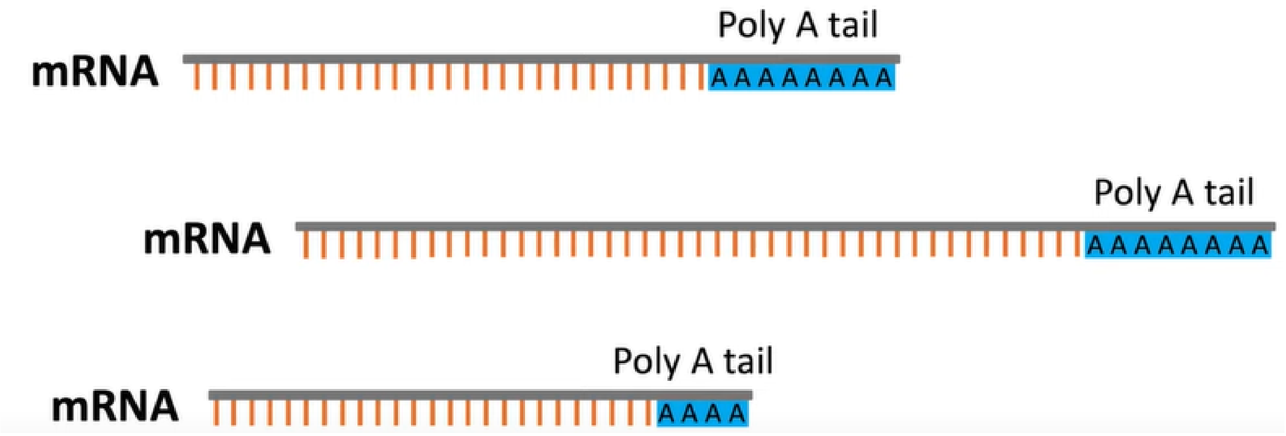
Ethanol wash to clean your pellet

Possibility to add a DNAse treatment

Water / EB

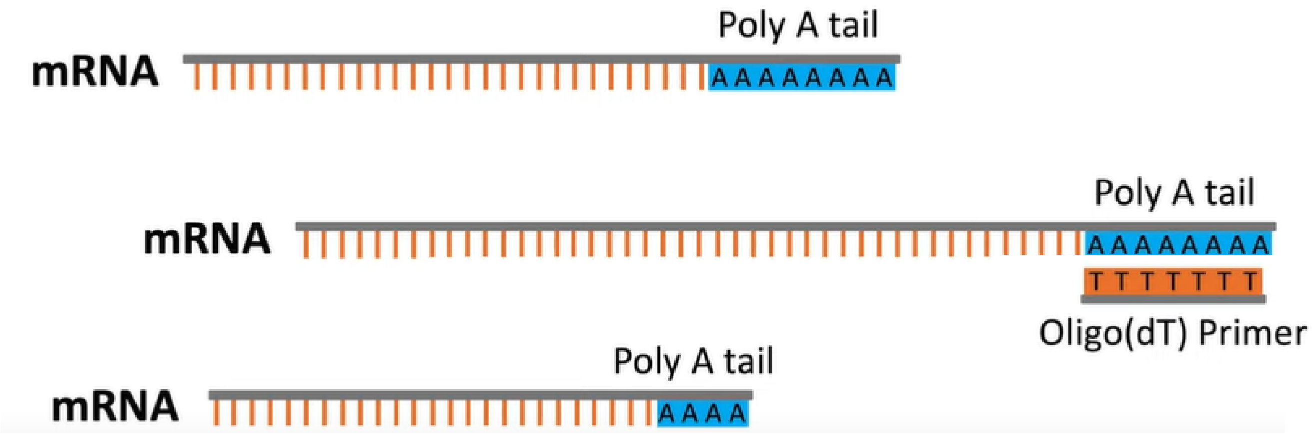
To store at -20°C

cDNA synthesis



cDNA synthesis

First strand synthesis



cDNA synthesis

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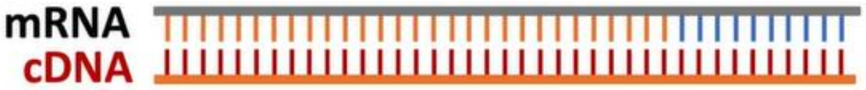
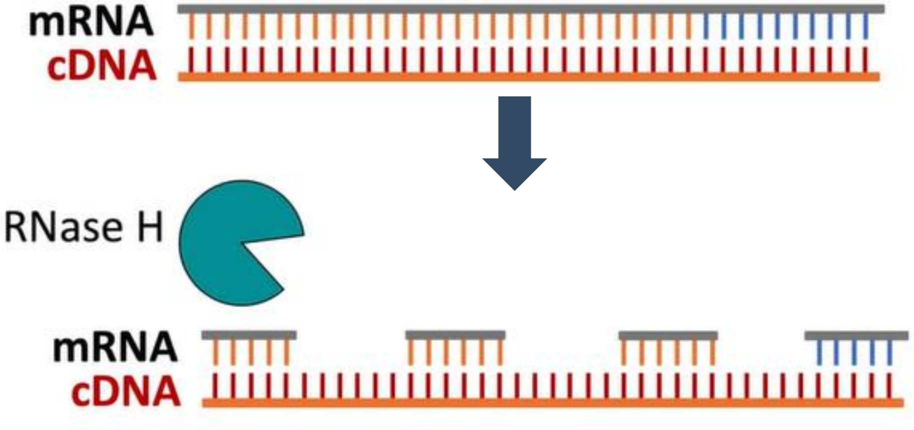


cDNA synthesis

First strand synthesis

RNA nicking

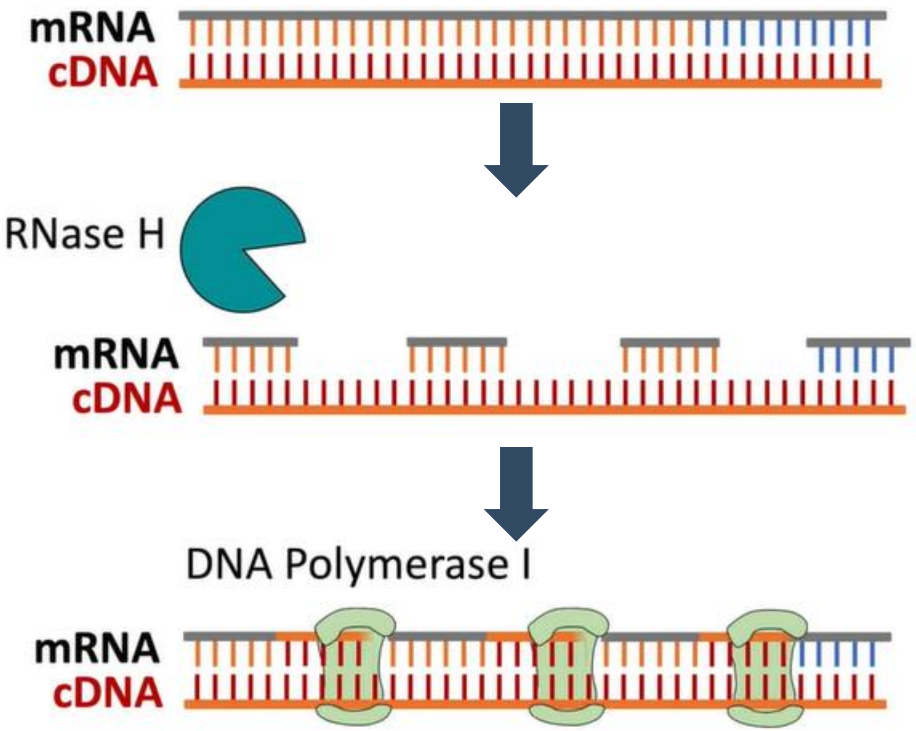


cDNA synthesis

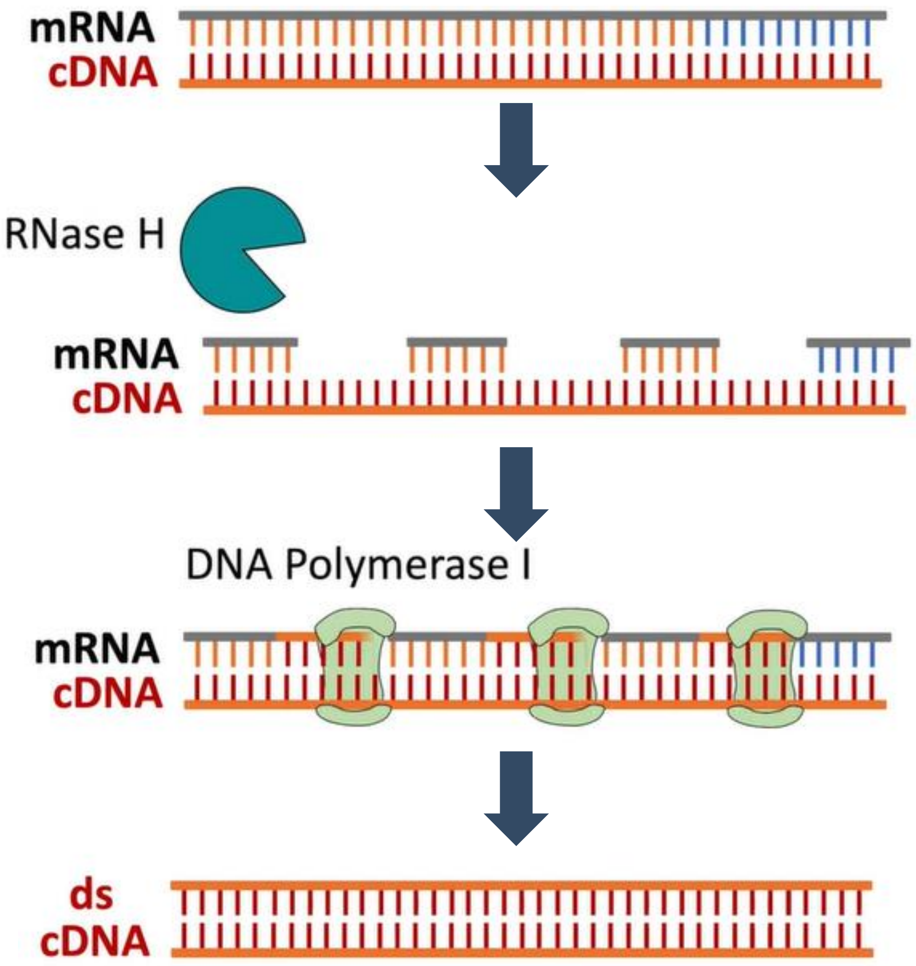
First strand synthesis

RNA nicking

Nick translation



cDNA synthesis



First strand synthesis

RNA nicking

Nick translation

Excision activity of the polymerase and ligase

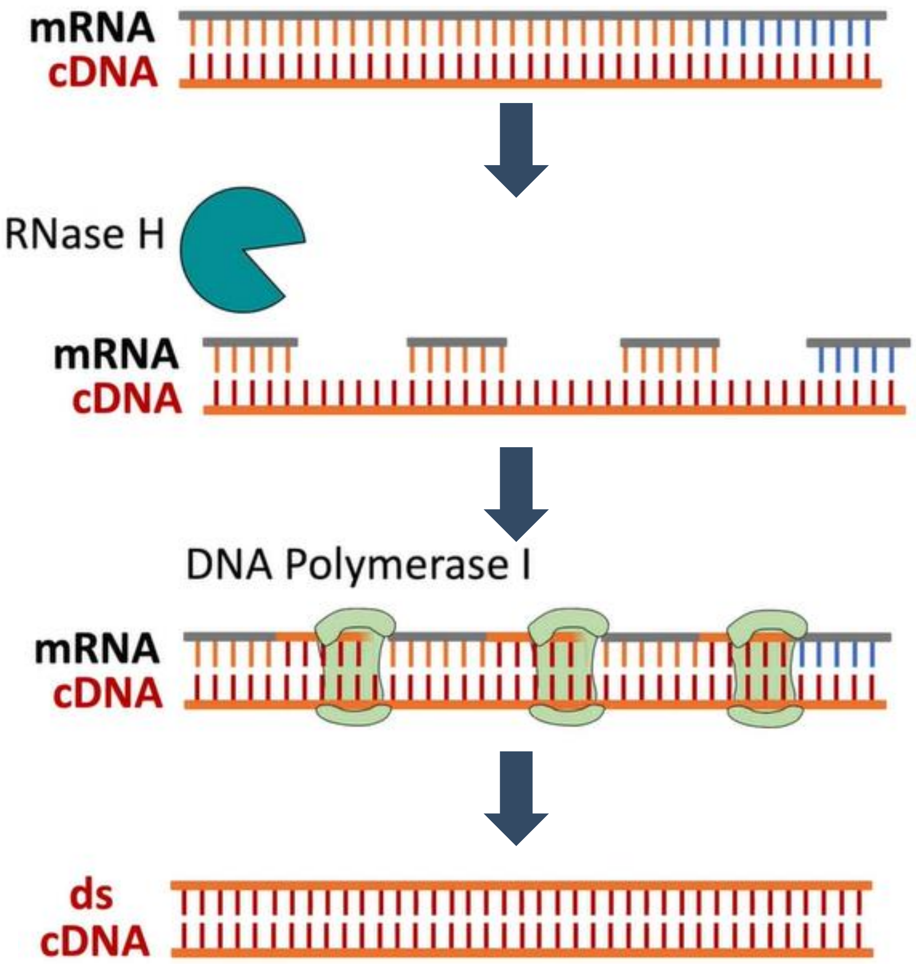
cDNA synthesis

First strand synthesis

RNA nicking

Nick translation

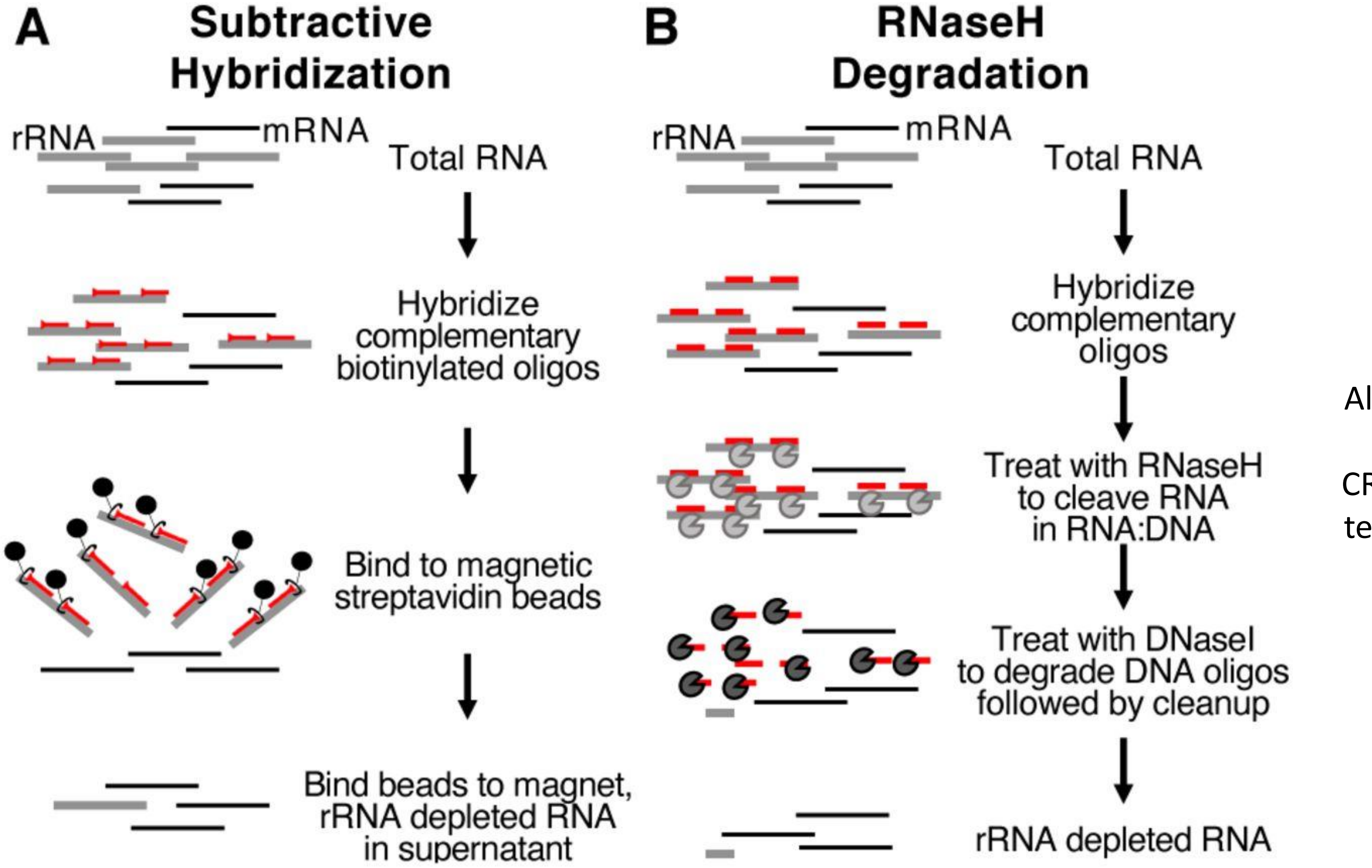
Excision activity of the polymerase and ligase



→ PCR amplification

The rRNA depletion

You can work on cDNA which are more stable



Alternative using CRISPRcas9 technology