LF aVLMB031 Imaging and Analytical Methods (Autumn 2023): Methods for nucleic Acid Analysis

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PLAN OF THE LECTURE

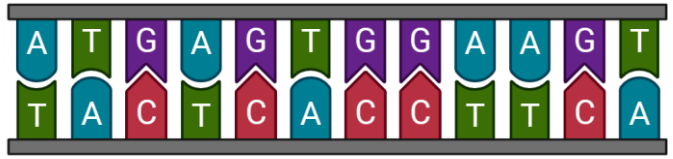
- Introduction
- Isolation of nucleic acids
  - Isolation with high salt concentrations
  - Isolation with phenol – chloroform
- Techniques for nucleic acid analysis
  - DNA analysis
  - RNA analysis
- “Omics” technologies and nucleic acids

- ✓ Understand why it is useful to be able to analyze nucleic acids
- ✓ How nucleic acid analysis can be used in research and clinical practice
- ✓ What the most common methods for nucleic acid analysis are
- ✓ How the “omics” technologies contribute to nucleic acid analysis

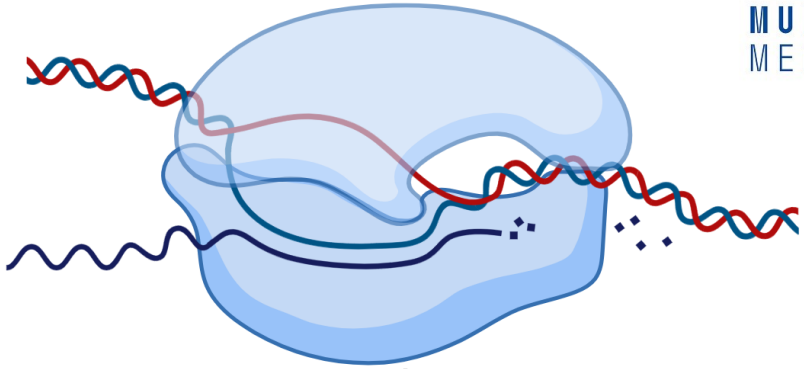
INTRODUCTION

The central dogma of molecular biology

DNA

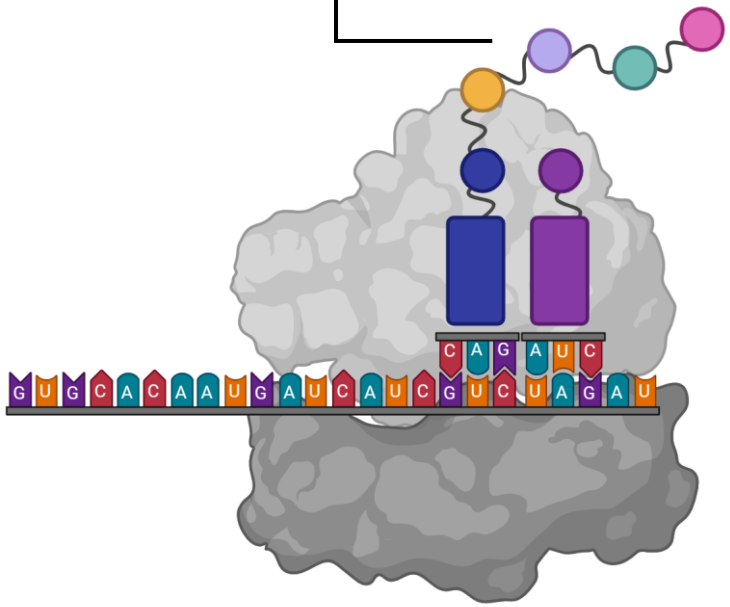


transcription



RNA

translation



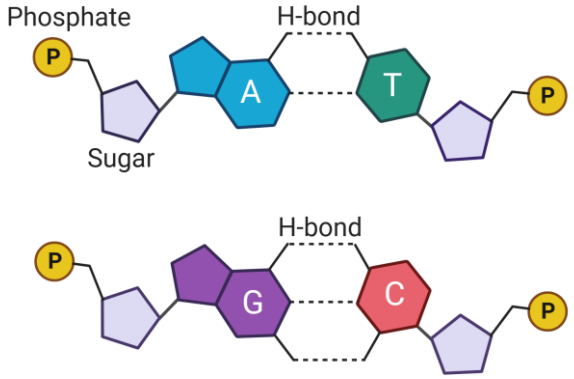
protein

functional role

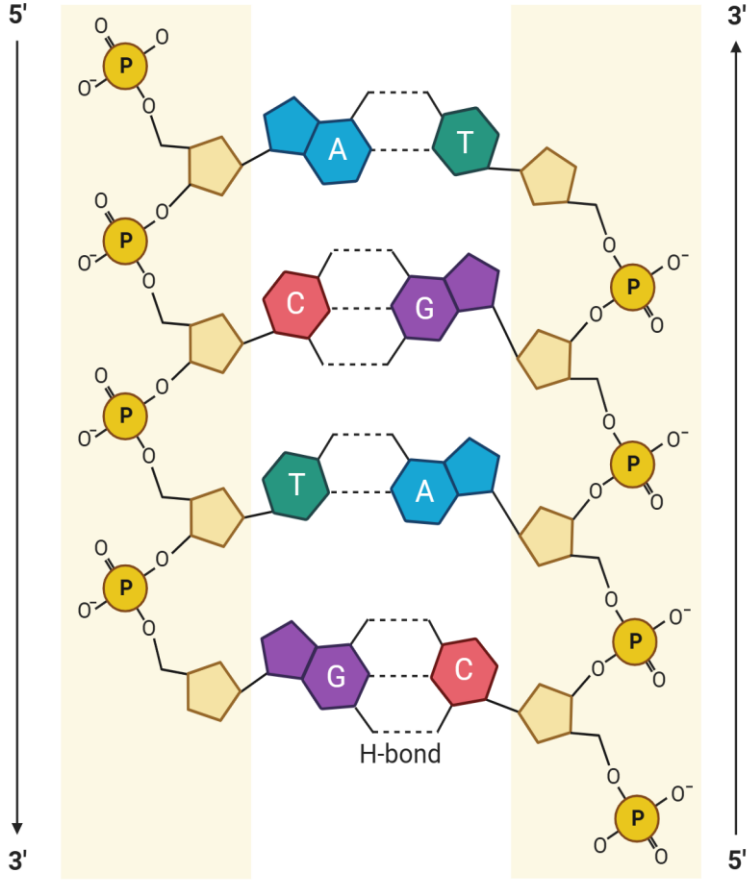
INTRODUCTION

What are the nucleic acids? Let's revise!

+ hydrogen bonds



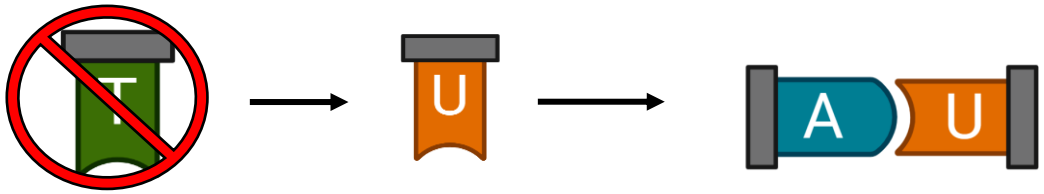
+ phosphate bonds



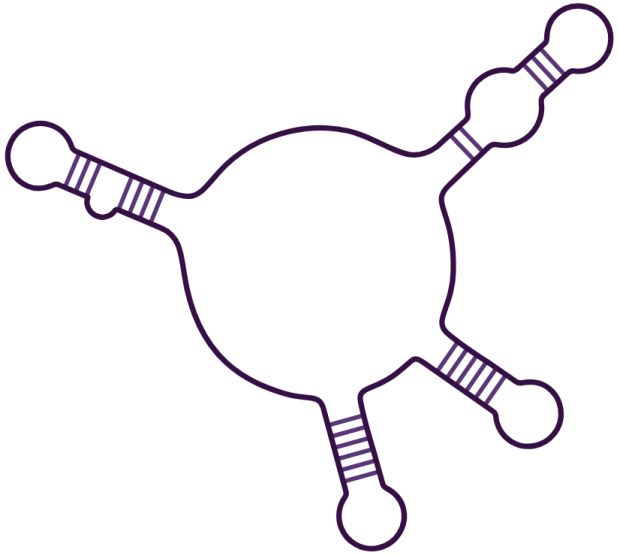
- ✓ *double stranded*
- ✓ *more stable → can be preserved for thousands of years in fossils*
- ✓ *contains all genetic information and regulatory elements*
- ✓ *genes are only a small part of the DNA regions*
- ✓ *coding <u>and</u> non-coding regions can offer valuable information*

INTRODUCTION

What are the nucleic acids? Let's revise!



- ✓ *single stranded*
- ✓ *U instead of T*
- ✓ *less stable*
- ✓ *more "flexible"*
- ✓ *can be transported*
- ✓ *create secondary structures*
- ✓ *can provide information about the coding regions of DNA*
- ✓ *can have regulatory roles itself (!) → rRNA, tRNA, miRs*



secondary structure with loops

INTRODUCTION

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Why is it useful to be able to analyze nucleic acids?

- *Biomedical research*: investigation of molecular mechanisms (that can lead to novel therapies)
- *Translational research*: determination of off-target effects of medicine
- *Basic research*: production of new knowledge → deeper understanding of how the world works
- *Forensics*: DNA fingerprinting
- *Agriculture*: species barcoding → detection of adulterated products

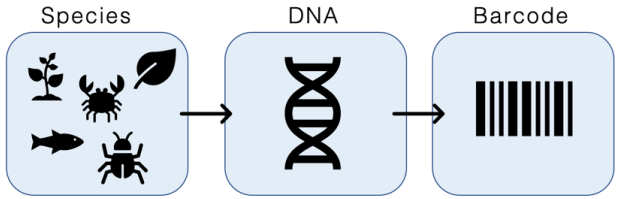
…and what about the clinical practice?

- Identification of foreign DNA/RNA (e.g. virus DNA) or mutated genes (e.g. oncogenes, hereditary diseases)
- Paternity tests
- Karyotypes and prenatal testing
- *Diagnostics*: determination of biomarker levels / risk assessment

INTRODUCTION

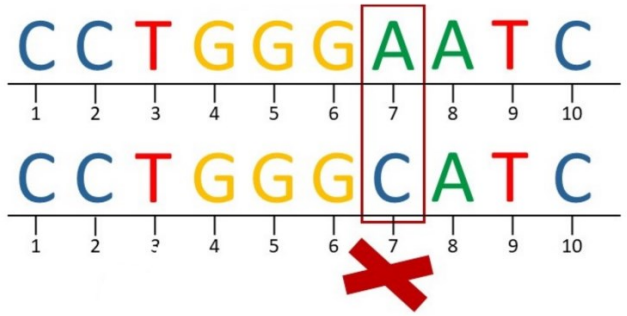
Usage of nucleic acid analysis in research and clinic

Genotyping → telling apart wild type (WT – “healthy”) animals from animals that are genetically modified



Barcoding → determining the origin of products and the presence of foreign / dangerous elements in them

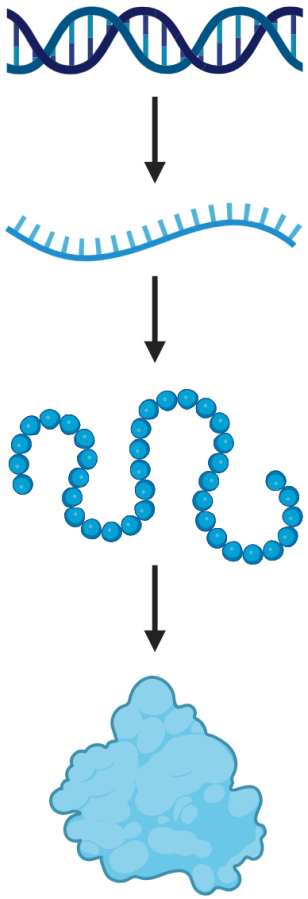
Determination of the expression levels of biomarkers in patients → prediction of risk / severity of disease (prognostics / diagnostics)



Determination of the presence / absence of specific gene or SNP → diagnostics

INTRODUCTION

What is the workflow we need to follow in order to study the nucleic acids?

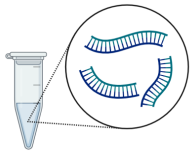


obtain the sample

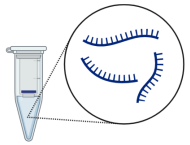
isolation of DNA/RNA

quantity/quality control

downstream analysis

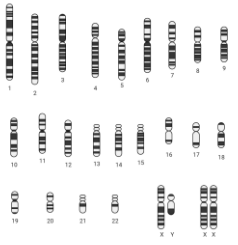


pure DNA

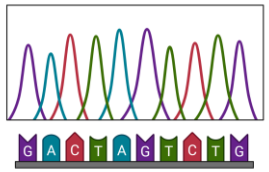


pure RNA

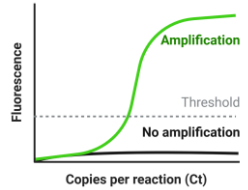
karyotypes



sequencing

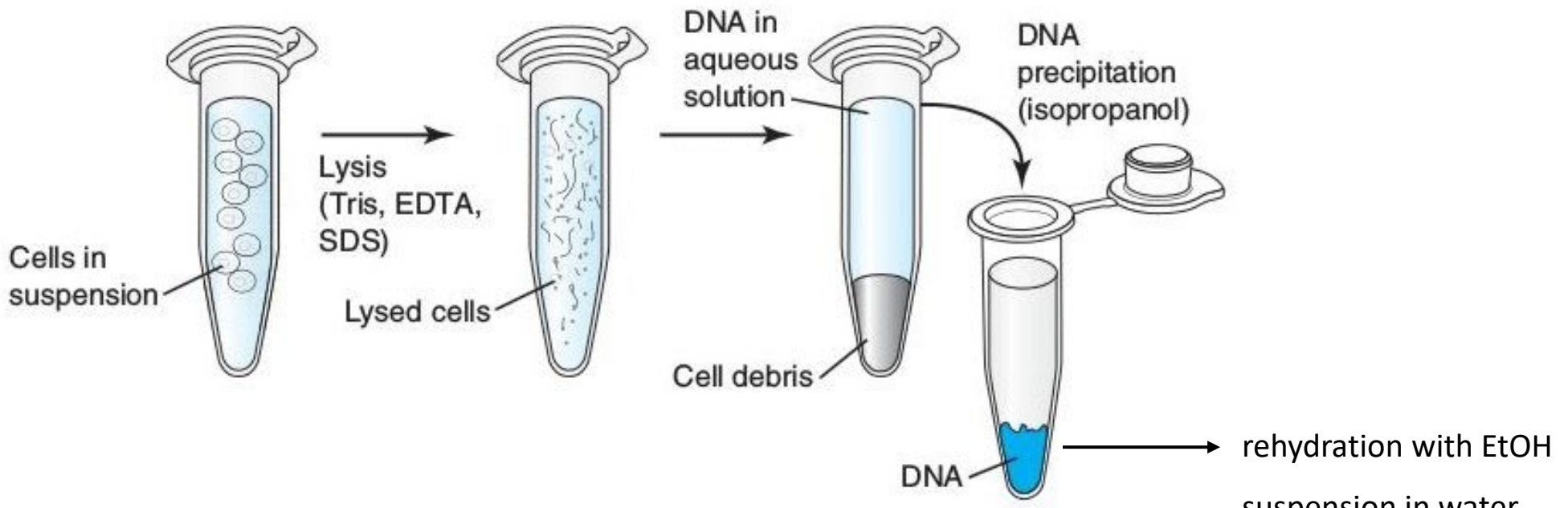


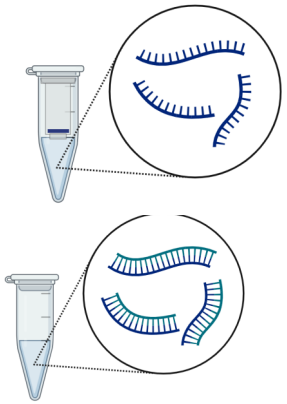
qPCR



ISOLATION OF NUCLEIC ACIDS (I)

High salt concentration





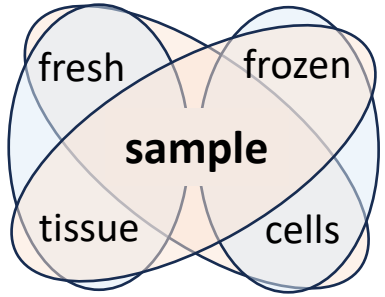
High salt concentration → makes the proteins and debris precipitate → nucleic acids stay in the supernatant

SDS, EDTA → denature proteins and destroy membranes → help precipitation of proteins / separation of nucleic acids

Isopropanol → organic solvent (nucleic acids can't be diluted in it) → precipitation of nucleic acids

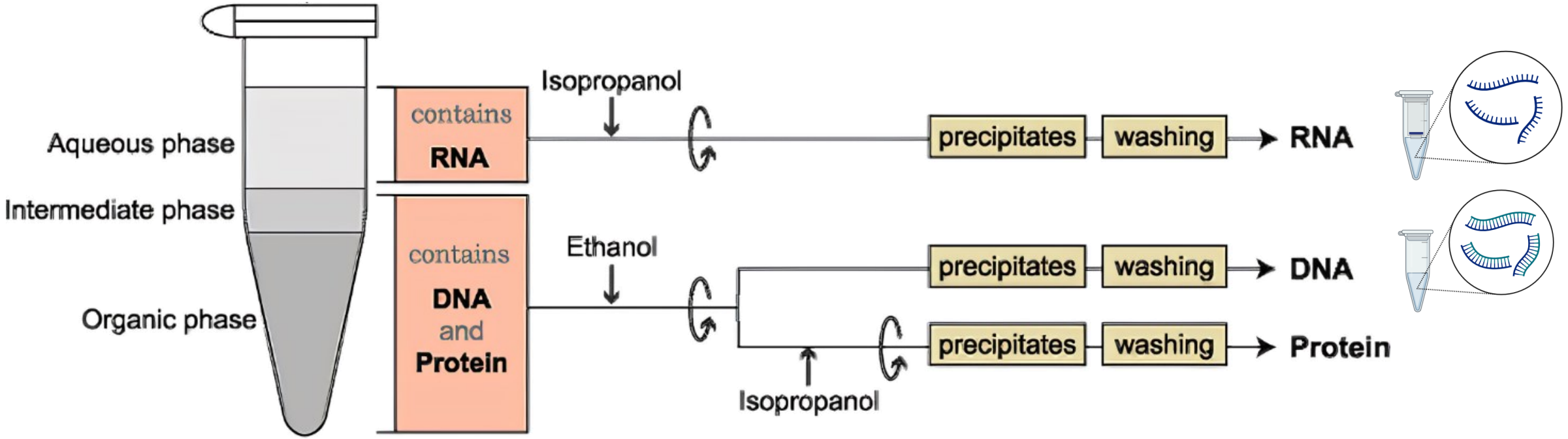
ISOLATION OF NUCLEIC ACIDS (II)

Phenol - Chloroform

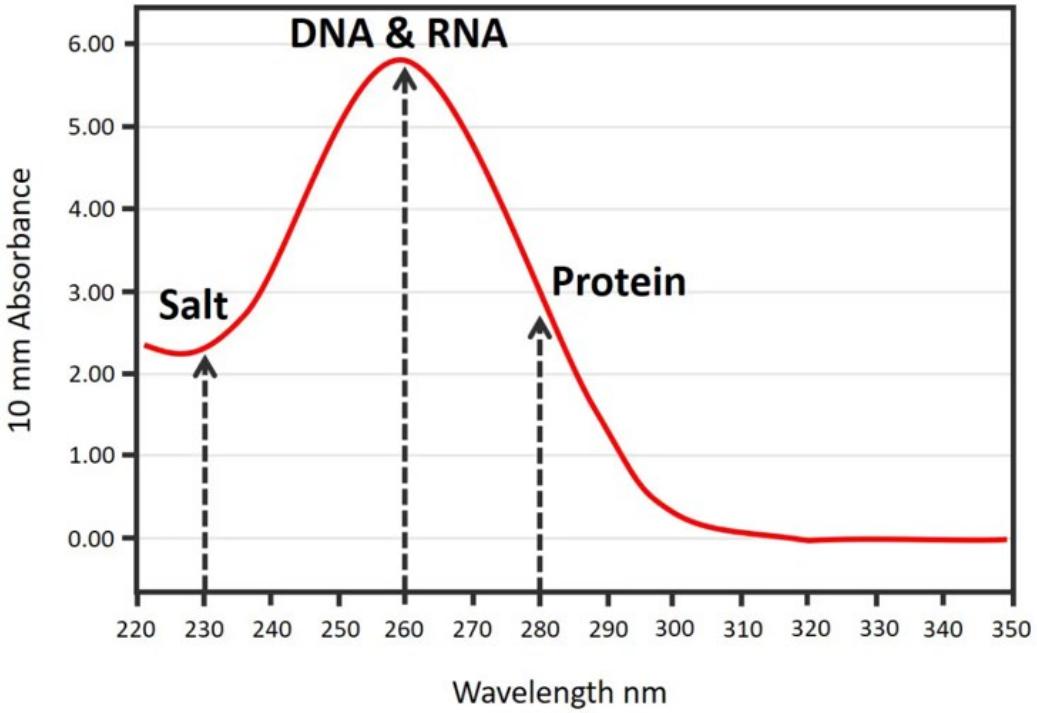


Lysis with phenolic reagent
(e.g. TriZOL, ExtraZOL)

centrifugation



QUALITY AND QUANTITY CONTROL

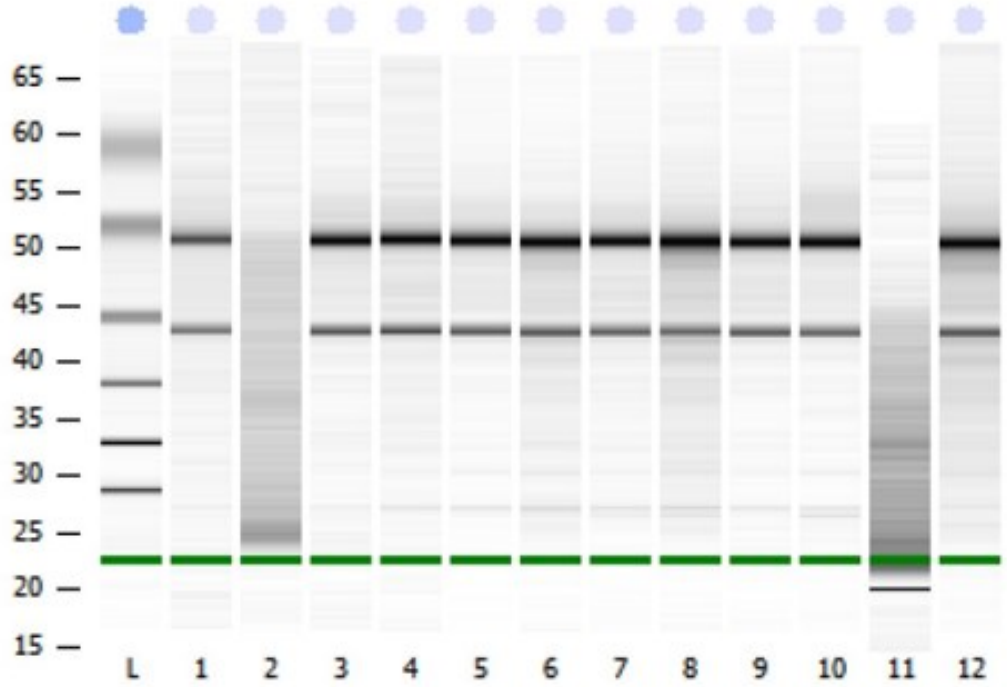


1. Assessment of quantity and purity

- ✓ Done with Nanodrop or Bioanalyzer instruments
- ✓ Produces ratios indicative of purity (260/280 & 260/230)
- ✓ Determines RNA quantity (ng of RNA per µl)

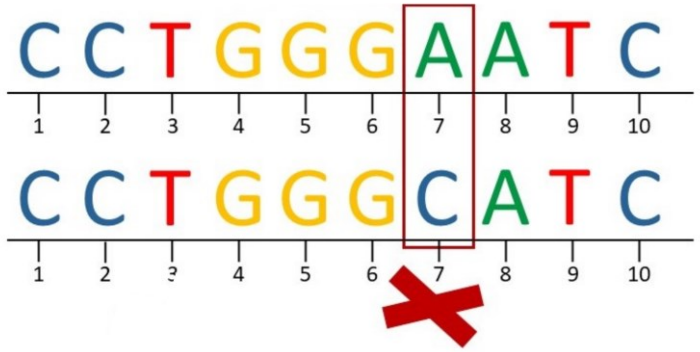
2. Assessment of integrity

- ✓ Done with electrophoresis
- ✓ Determines intact of degraded RNA
- ✓ Intact RNA shows two big bands that correspond to the two ribosomal subunits



TECHNIQUES FOR NUCLEIC ACID ANALYSIS

SNP / chromosomal analysis – FISH (fluorescent in-situ hybridization)

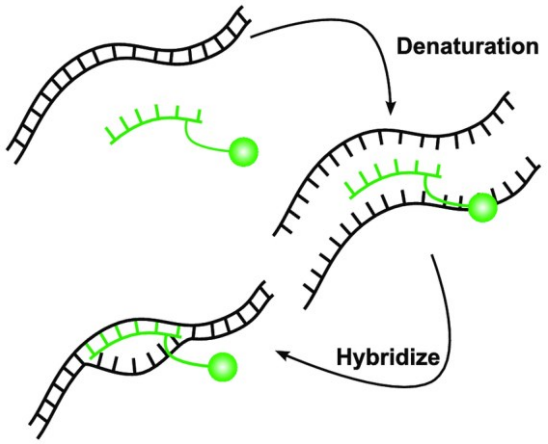


SNPs
single nucleotide polymorphisms

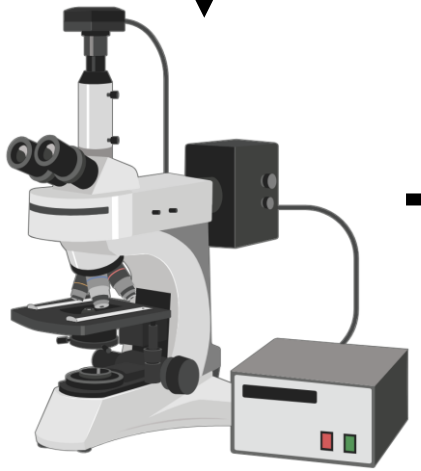
↓ *genes*
introns
non-coding regions
regulatory elements

may correlate with health problems
(e.g. heart disease, metabolic conditions, cancer)

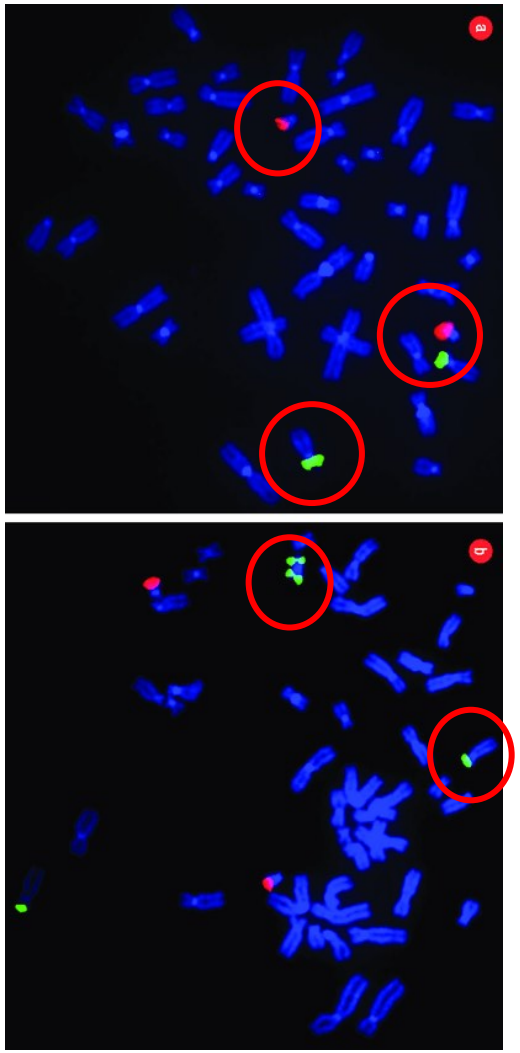
→ *karyotype*



↓ *observation in fluorescent microscope*



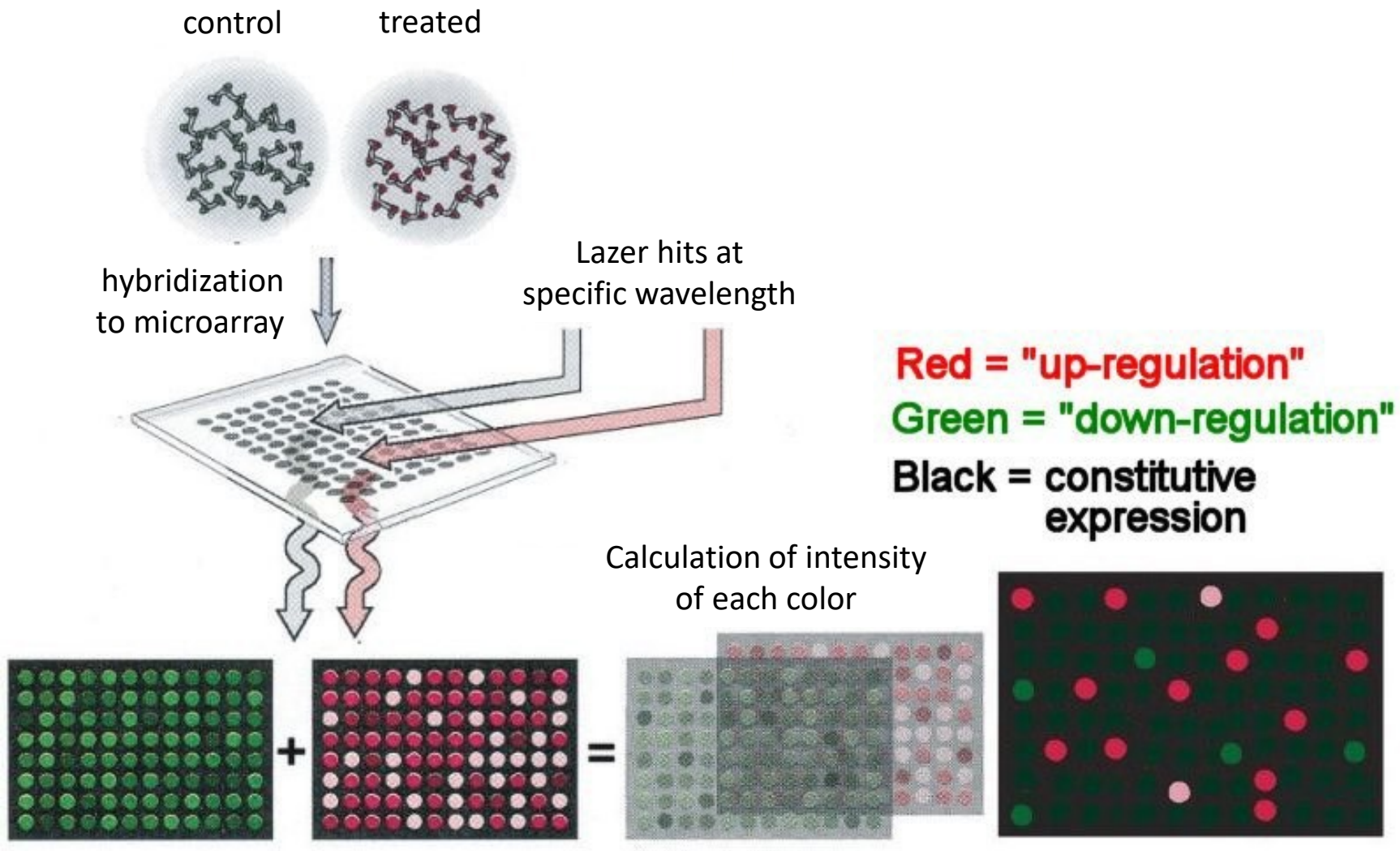
→



TECHNIQUES FOR NUCLEIC ACID ANALYSIS

Microarrays – use for gene expression

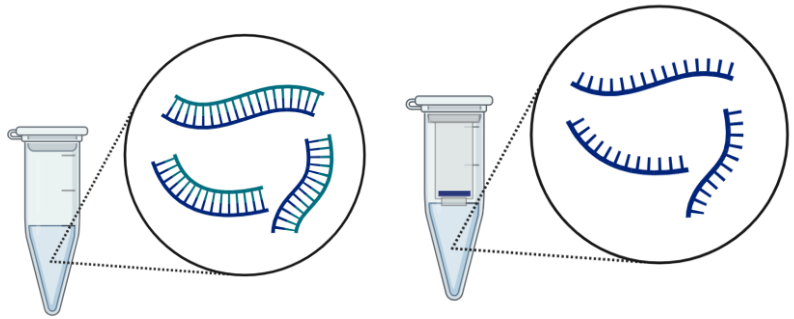
Labeling of samples with fluorescent dyes



- ✓ Detection of SNPs
- ✓ Investigation of both alleles (in case the input material is DNA)
- ✓ Investigation of RNA transcripts or patient samples at once

TECHNIQUES FOR NUCLEIC ACID ANALYSIS

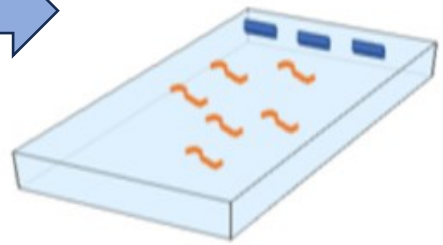
Southern / Northen blot



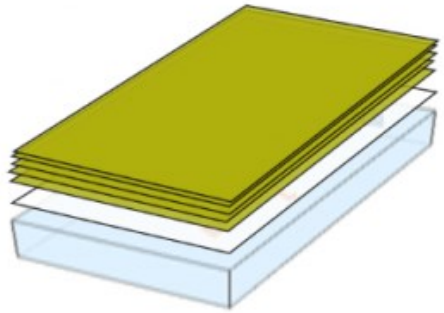
isolated DNA

isolated RNA

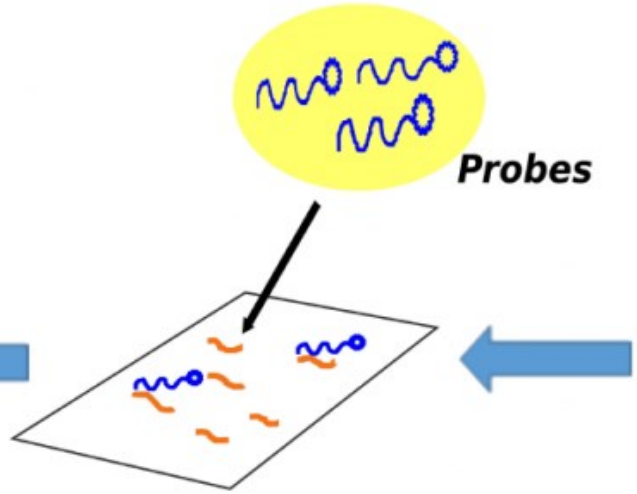
digestion to fragments
(only for DNA)



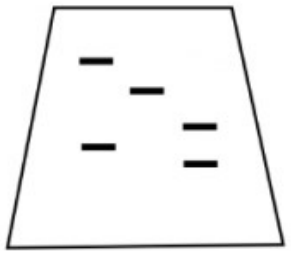
electrophoresis
(separation of fragments)



transfer
(stabilization of fragments to membrane)



hybridization
(probe binding to specific region)

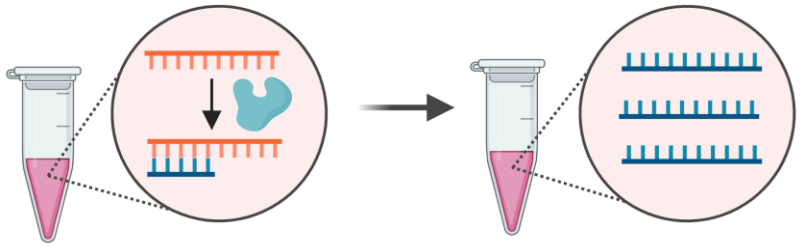


Visualization

TECHNIQUES FOR NUCLEIC ACID ANALYSIS

qPCR

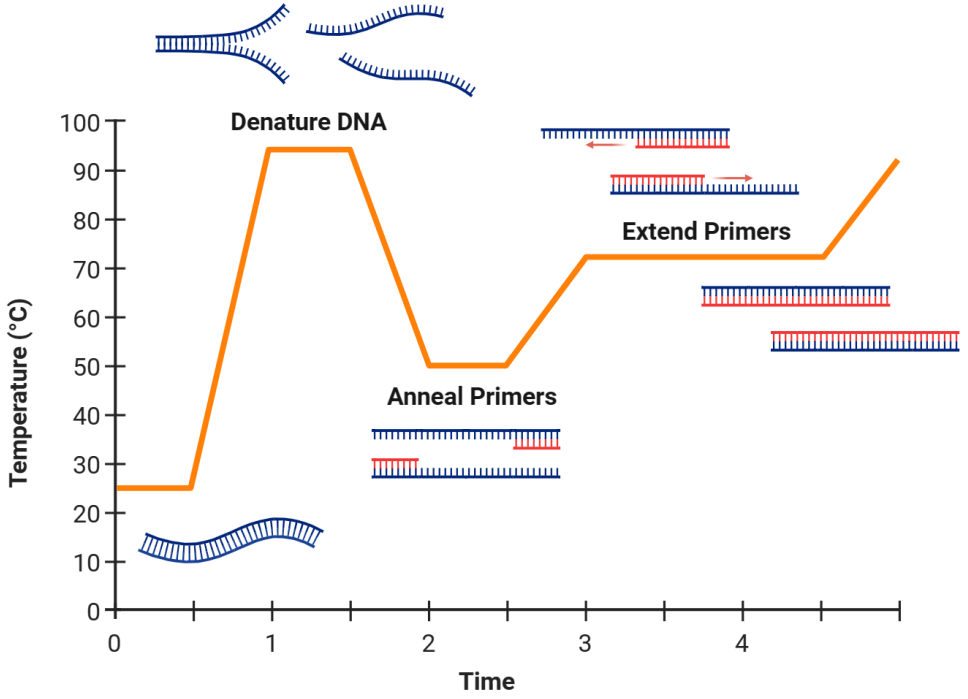
reverse transcription
from RNA to cDNA



- ✓ Proper conditions: pH, co-factors
- ✓ Enzyme
- ✓ Random primers
- ✓ dNTPs

RT-PCR
multiplication of cDNA of interest

- ✓ Proper conditions: pH, co-factors
- ✓ Enzyme
- ✓ Specific primers / probes
- ✓ Fluorescent agent



TECHNIQUES FOR NUCLEIC ACID ANALYSIS

qPCR

+

- ✓ Practical, easy to use and optimize
- ✓ Realtively fast and reproducible results
- ✓ Extremely sensitive and more specific than serological tests
- ✓ Wide applicability

-

<u>From a clinical perspective:</u>

- Speed depends on laboratory so it may miss the relevant time frame
- Resources available in the clinic for urgent cases or (equipment, trained staff)
- Diagnosis of infectious disease – false positives/false negatives

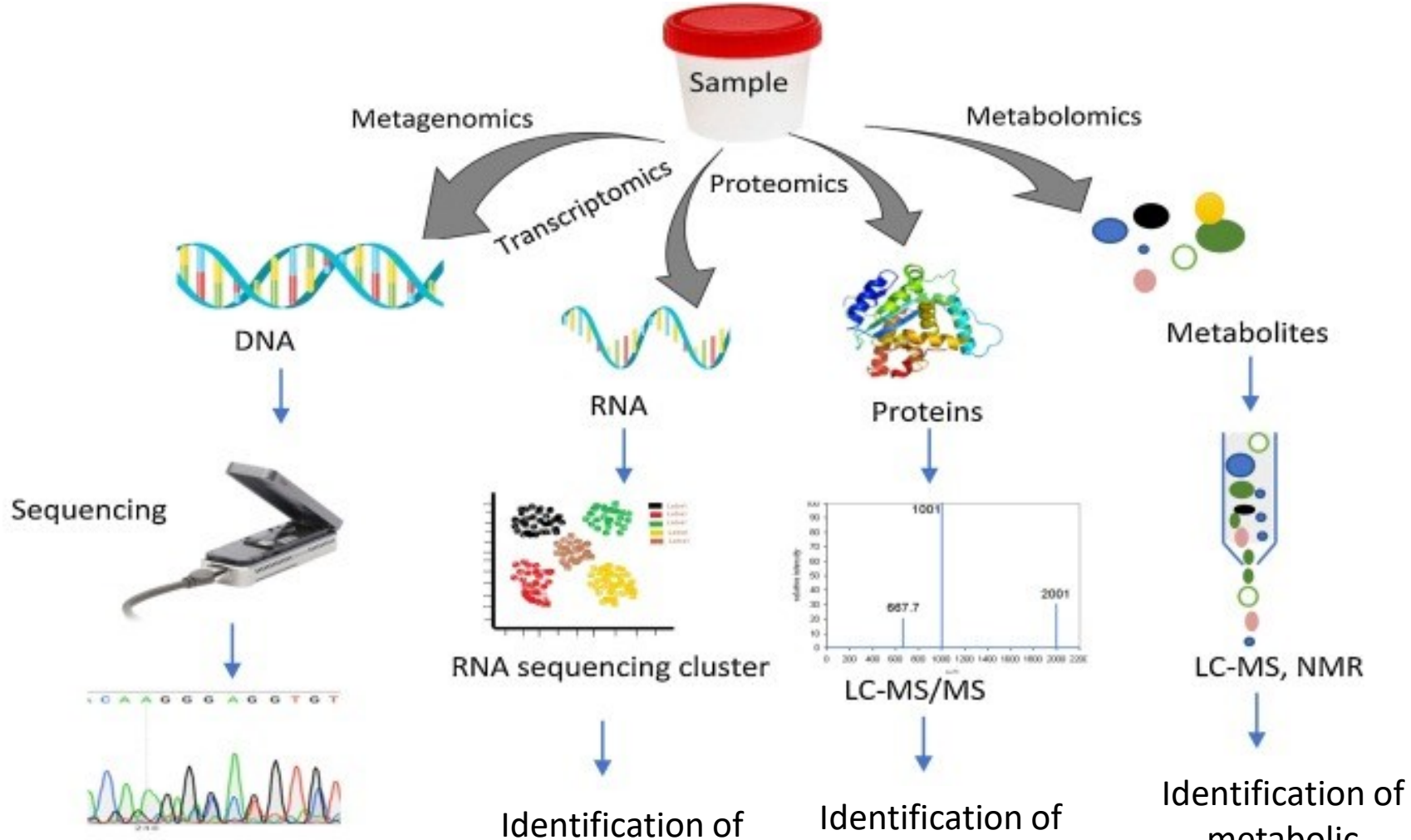
<u>From a biomedical research perspective:</u>

-Primers: sequence must be known, primers must be well designed

-Sensitivity/Contamination

“OMICS” TECHNOLOGIES AND NUCLEIC ACIDS (I)

What are the “omics” technologies?



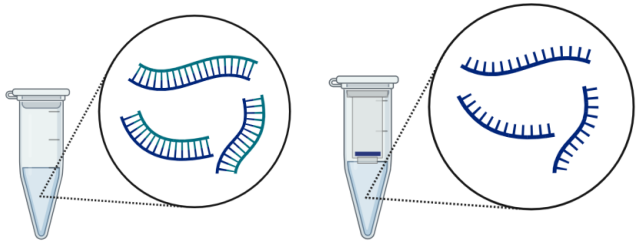
Investigation of the “totality”

Collective characterization of the DNA, RNA, proteins or metabolites of samples / patients

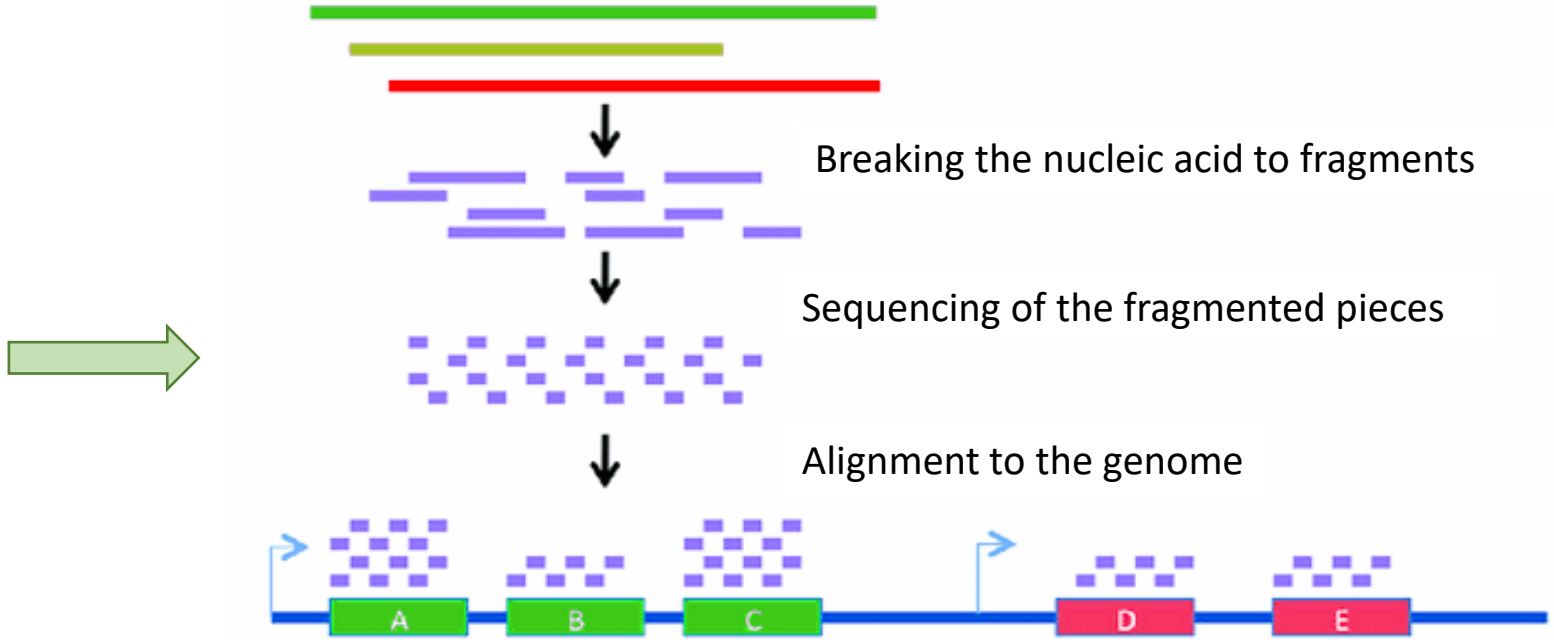
“OMICS” TECHNOLOGIES AND NUCLEIC ACIDS (II)

What is DNA / RNA sequencing?

- ✓ Determination of the nucleotide sequence of the whole genome / transcriptome of a patient
- ✓ Detection of mutations in the genomic DNA or of alteration in the expression of all genes
- ✓ Production of the “genomic profile” or “transcriptomic profile” of the patient



isolated DNA isolated RNA



…IN CONCLUSION…

- ✓ **Nucleic acids can offer valuable information regarding:**
  - The expression of various genes
  - The presence / absence of polymorphisms connected to diseases
  - The origin of products
- ✓ **Research and clinical practice can benefit from nucleic acid analysis via:**
  - ✓ Determination of the expression profile of genes
  - ✓ Construction of karyotypes
  - ✓ Hybridization of fragments in microarrays
- ✓ **Some common methods for nucleic acid analysis are**
  - ✓ DNA: SNP determination through FISH, Genotyping, Genetic barcoding, qPCR
  - ✓ RNA: RT-PCR, microarrays, Northen blot
- ✓ **The "omics" technologies allow**
  - ✓ Scaling-up of the analyses
  - ✓ Production of the information much quicker
  - ✓ Multiple analysis of many DNA/RNA regions

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Thank you for your attention!

See you at 27th of October in the lab ☺