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Nucleic Acid Isolation Methods

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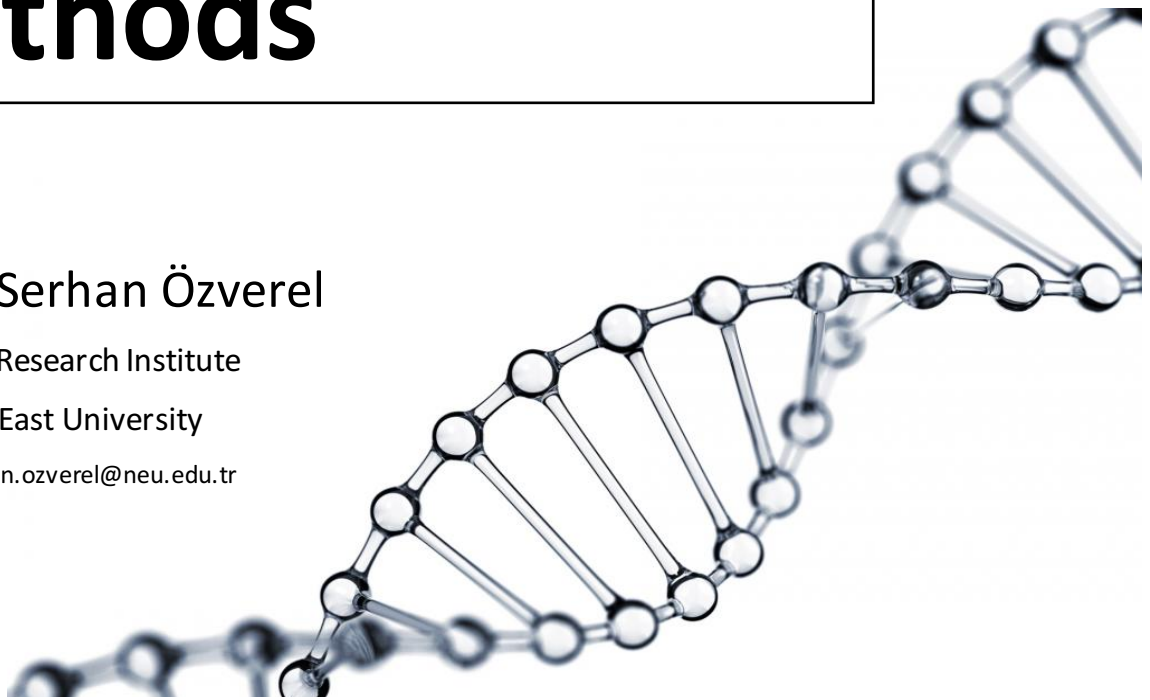
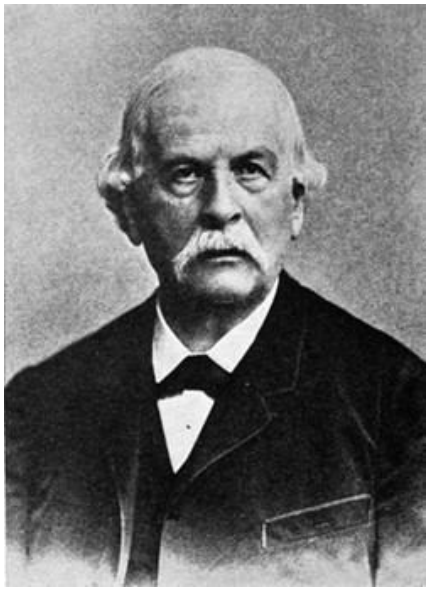


History

- The very **first DNA isolation** was done by a Swiss physician**, Friedrich Miescher** in 1869



His student, Richard Altman

STARTING POINT

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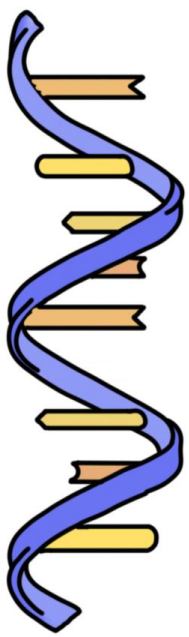
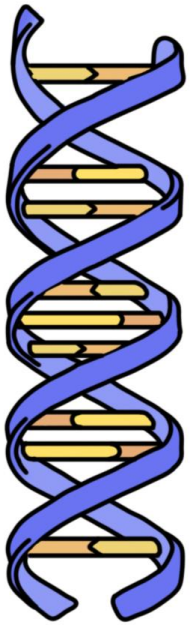
RNA extractions





DNA extractions

- Most molecular applications.

+Diagnostic kits

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DNA isolation

- Effective disruption of cells or tissue; denaturation of nucleoprotein complexes; inactivation of nucleases, for example, RNase for RNA extraction and DNase for DNA extraction; away from contamination

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NUCLEIC ACID isolation

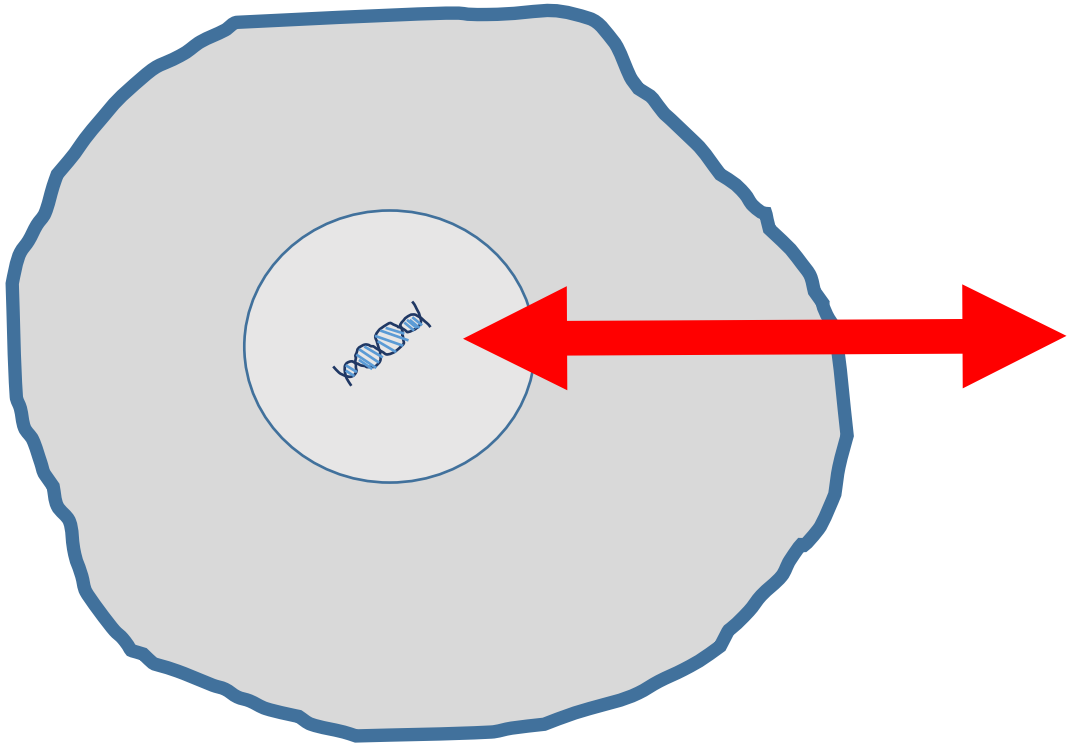
- 3 important steps;

LYSIS

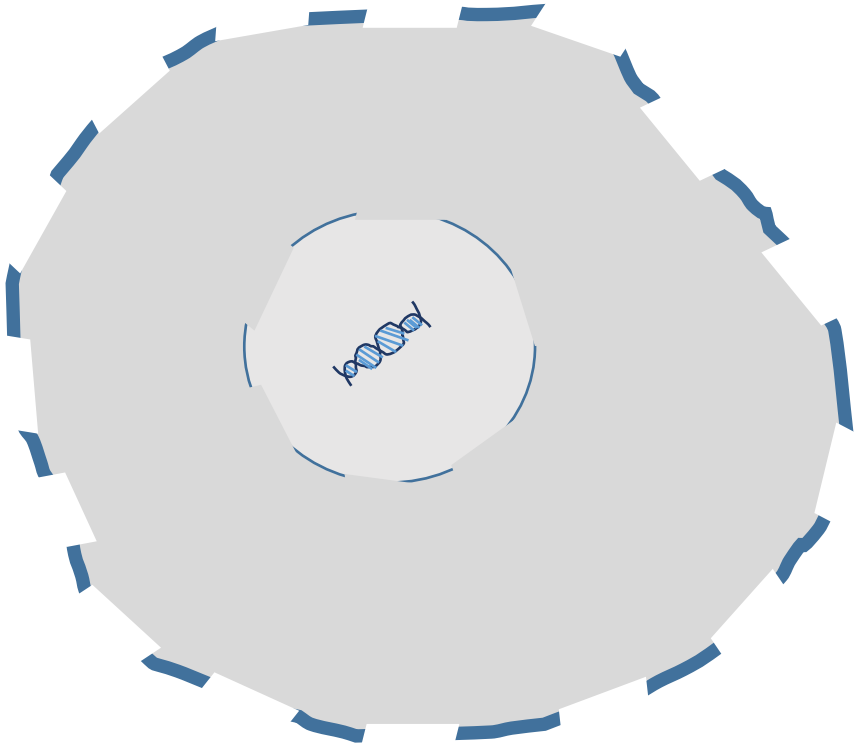
PRECIPITATION

PURIFICATION

LYSIS



LYSIS



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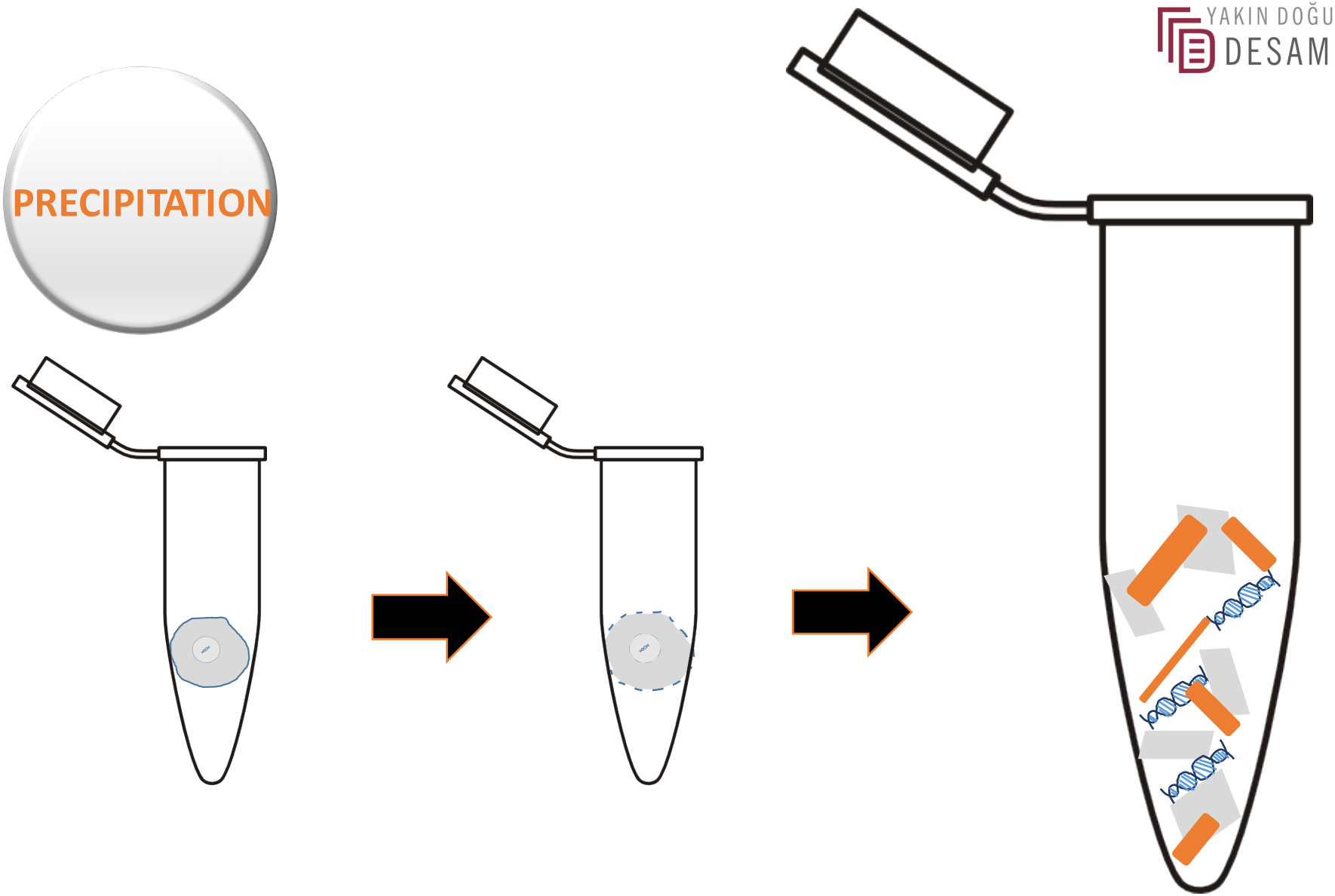
PRECIPITATION



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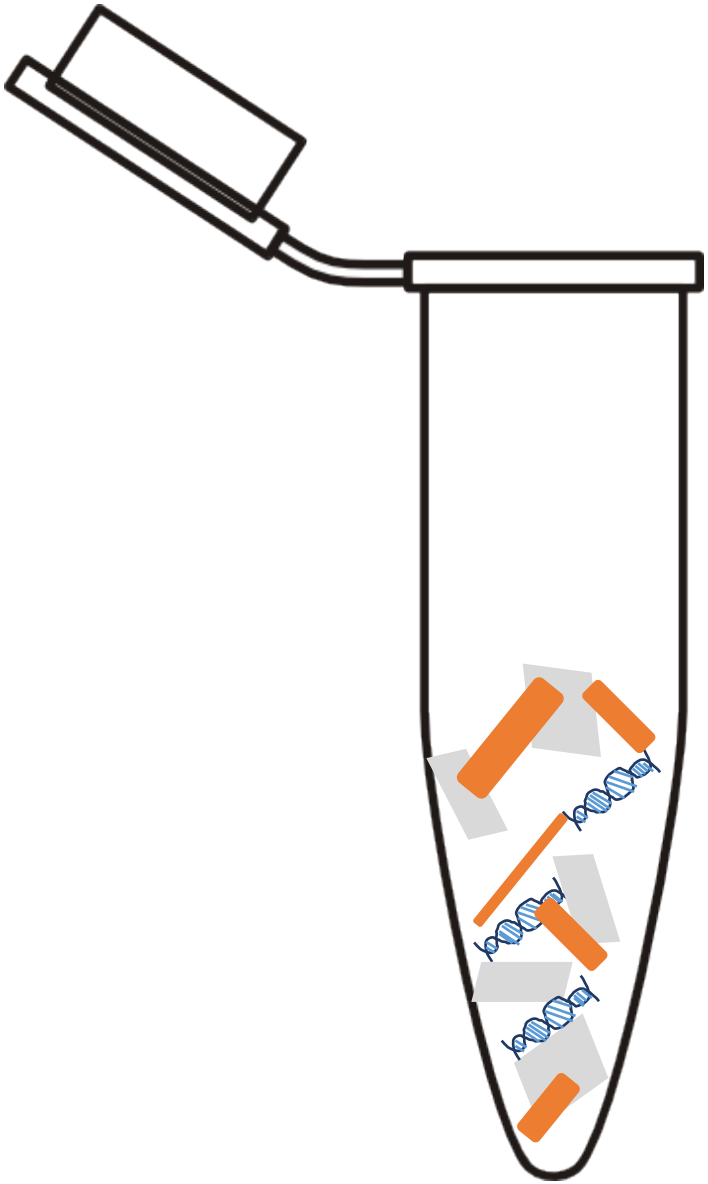
PRECIPITATION



Important!

To improve quality of DNA

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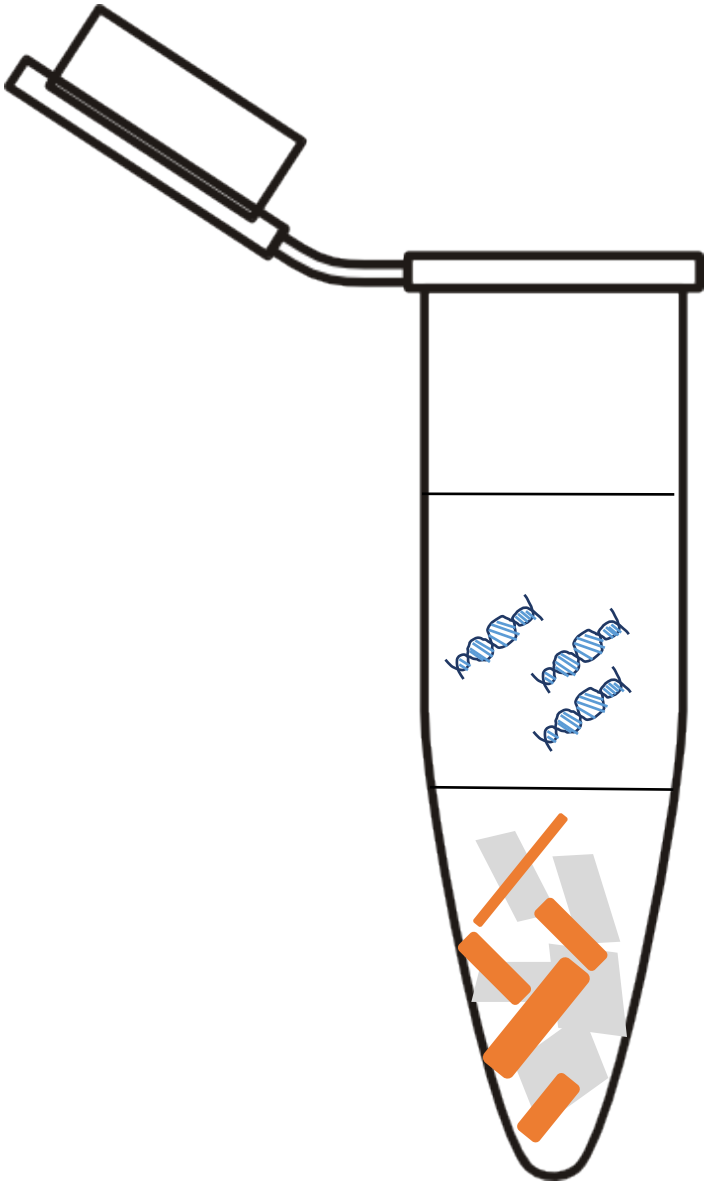
PURIFICATION



After precipitation

DNA is rinsed with Ethanol or isopropanol

Removal of cell debris and unwanted materials

Storage and handling


HOTEL
DILLAC
CRITERION
TDK
2013
THE LION KING
SONY
ONE WAY
NO STOPPING ANYTIME
from the Museo Nazion
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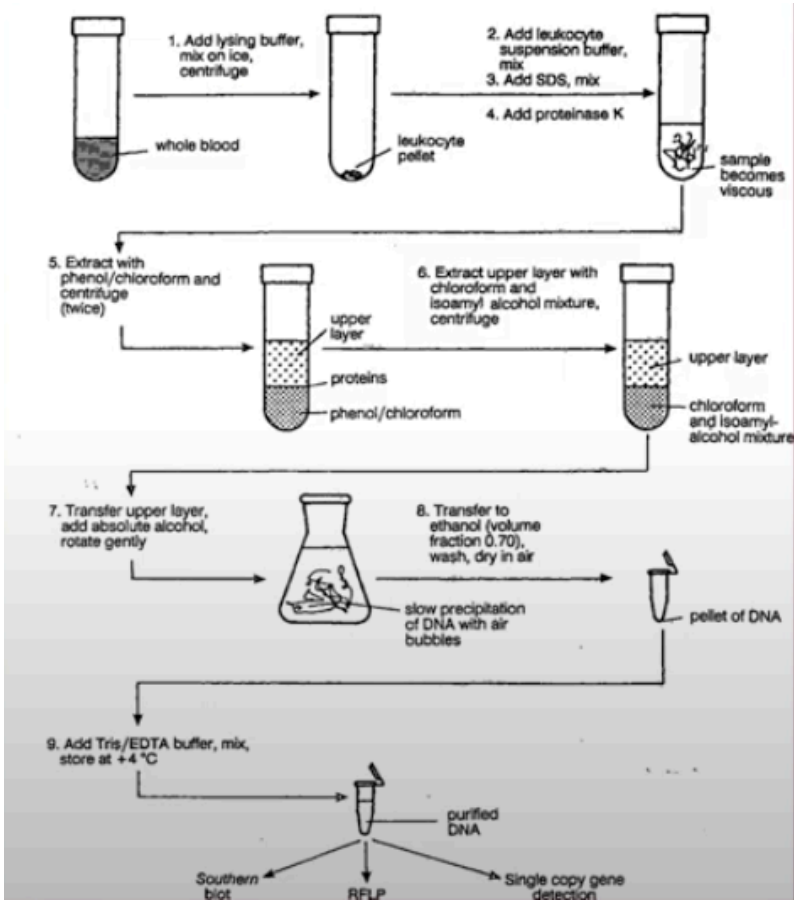
In the Past, the process of extraction and purification of nucleic acids

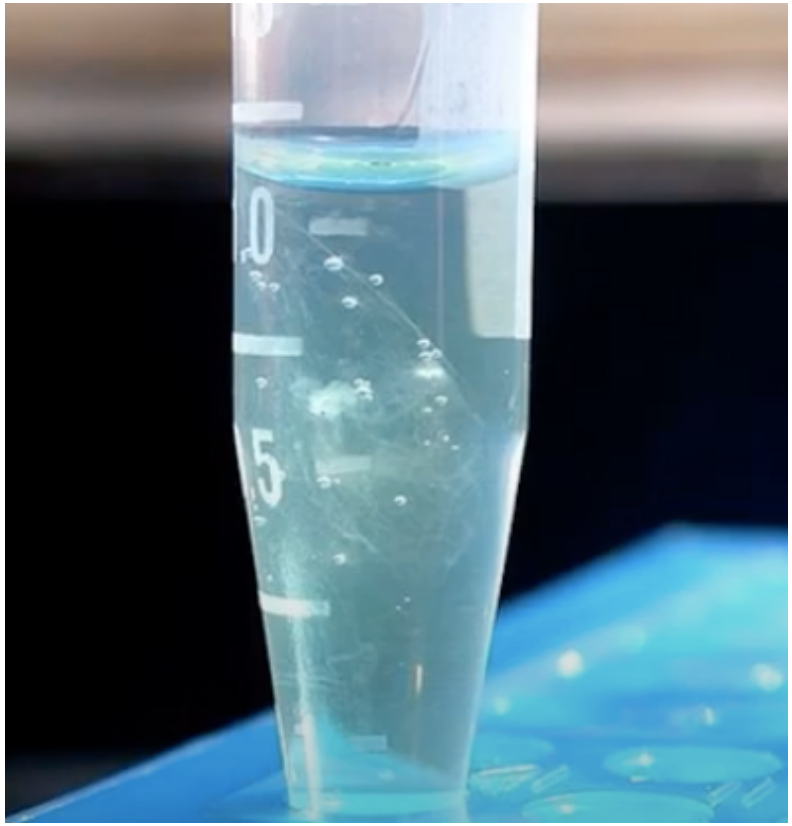
COMPLICATED

LABOR-INTENSIVE

TIME-CONSUMING

Phenol Chlorophorm method





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Protocol

- Centrifugate cells at 1000 x g for 1 minute and discard supernatant.
- Resuspend the cell pellet in 100 µL cold PBS and mix by pipetting up and down repeatedly.
- Add 100 µL of cell **lysis buffer** and 2 µL of 20 mg/mL proteinase K and mix by vortexing.
  - **(Optional):** Add 3 µL of RNase A
- Incubate in a thermal mixer at 56°C for 1 hour with agitation at 1400 rpm. Alternatively if a thermal mixer is not available, use a 56°C water bath or heating block and vortex occasionally.
- Thoroughly extract the samples with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) and mix well by inverting the tube until the phases are completely mixed.
- Spin at max speed for 5 minutes, and carefully transfer the upper aqueous layer to a fresh Eppendorf tube.
- To precipitate DNA add 1 mL of 100% EtOH (room temperature), close tube and gently invert until DNA precipitate forms.
- Incubate the tube at room temperature for 15 – 30 minutes.
- Spin at max speed for 5 minutes and carefully remove and discard supernatant.
- Wash the DNA pellet with 1 mL 70% EtOH (-20°C) and invert several times.
- Spin at max speed for 2 minutes, and carefully remove and discard supernatant.
- Dry the DNA pellet at room temperature overnight or dry using a vacuum concentrator
- Resuspend DNA pellet in an appropriate volume of TE buffer.

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- Incubate in a thermal [illegible] at 56°C for 1 hour with agitation at 1400 rpm. Alternatively if a thermal mixer is not available, use a 56°C water bath or heating block and vortex occasionally.

Cell Lysis Buffer Recipe

Reagent	Final Concentration	per 500 mL
1 M Tris pH 8.0	10 mM	5 mL
5 M NaCl	100 mM	10 mL
0.5 M EDTA pH 8.0	10 mM	10 mL
10% SDS	0.50%	25 mL
dH_2O	[redacted]	to 500 mL

- Add 20 µL of a 20 mg/mL Proteinase K per 1 mL of lysis buffer

- Dry the DNA pellet at room temperature overnight or dry using a vacuum concentrator
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- **Resuspend** DNA pellet in an appropriate volume of TE buffer.

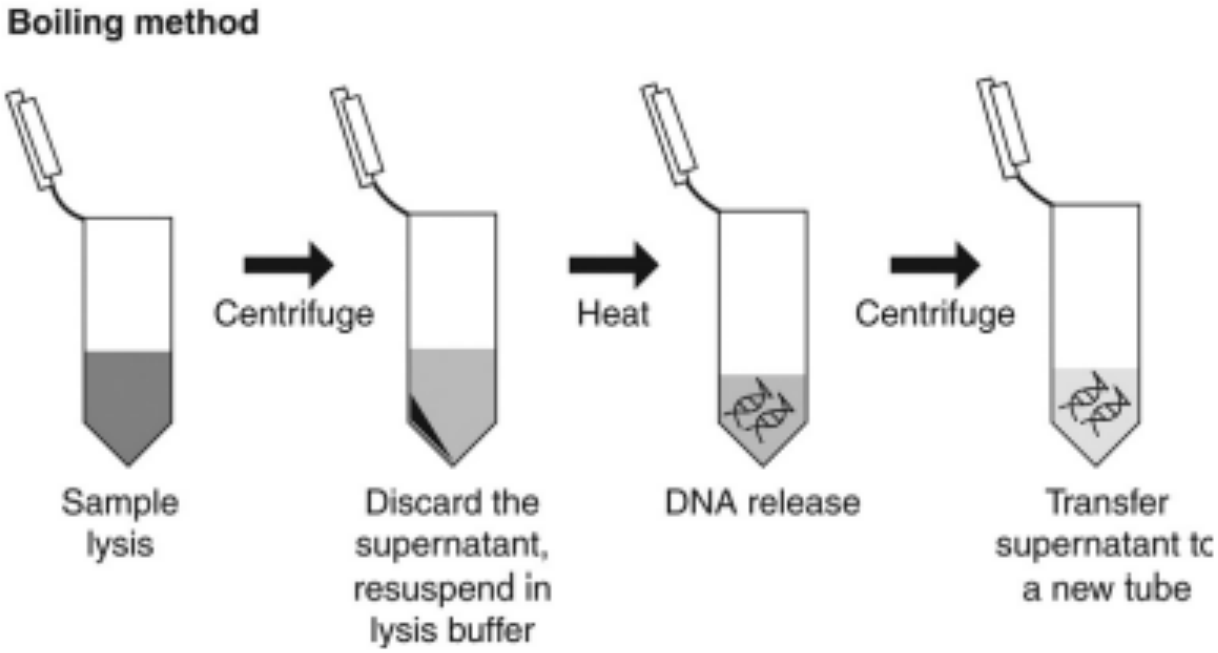
- **Advantages:**

- Cheap
- Good amount of product

- **Disadvantages:**

- Labor-sensitive
- A lot of repeating steps

Boiling Method



- **Advantages:**

- Cheap
- Easy
- Less Laborious

- **Disadvantages:**

- Lower yield
- Lower Purity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In the Past, the process of extraction and purification of nucleic acids

COMPLICATED

LABOR-INTENSIVE

TIME-CONSUMING

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NOW, the process of extraction and purification of nucleic acids

MANUAL - PROTOCOLS

MANUAL - PROTOCOLS have come a long way…

Complete Kits

Commercial Offerings

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NOW, the process of extraction and purification of nucleic acids

Magnetic Bead Method

Silica Spin Column Method

Paper Method

High Purity

Good Yield

FAST

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REPEATED CENTRIFUGATION STEPS

REMOVAL OF SUPERNATANTS

MECHANICAL TREATMENTS

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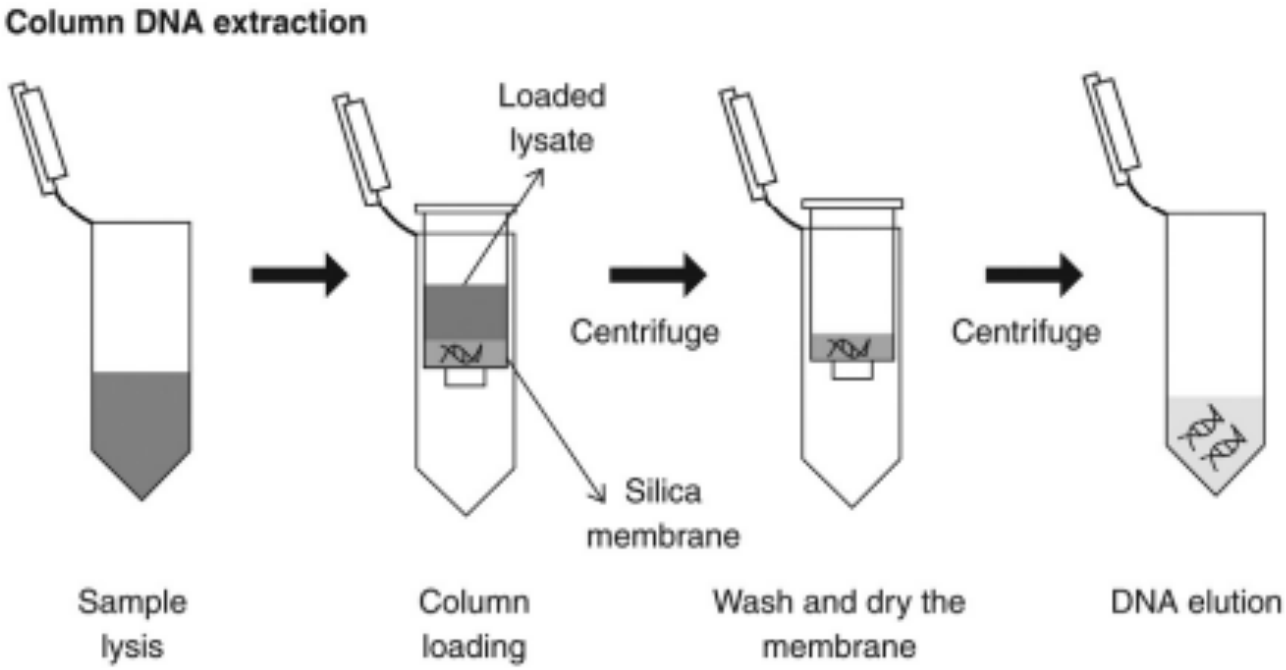
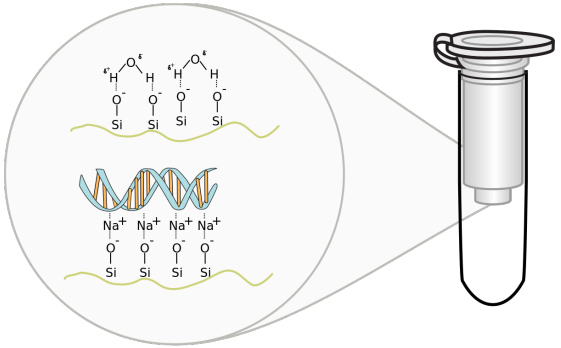
REPEATED CENTRIFUGATION STEPS

REMOVAL OF SUPERNATANTS

MECHANICAL TREATMENTS

Silica Spin Column Protocol





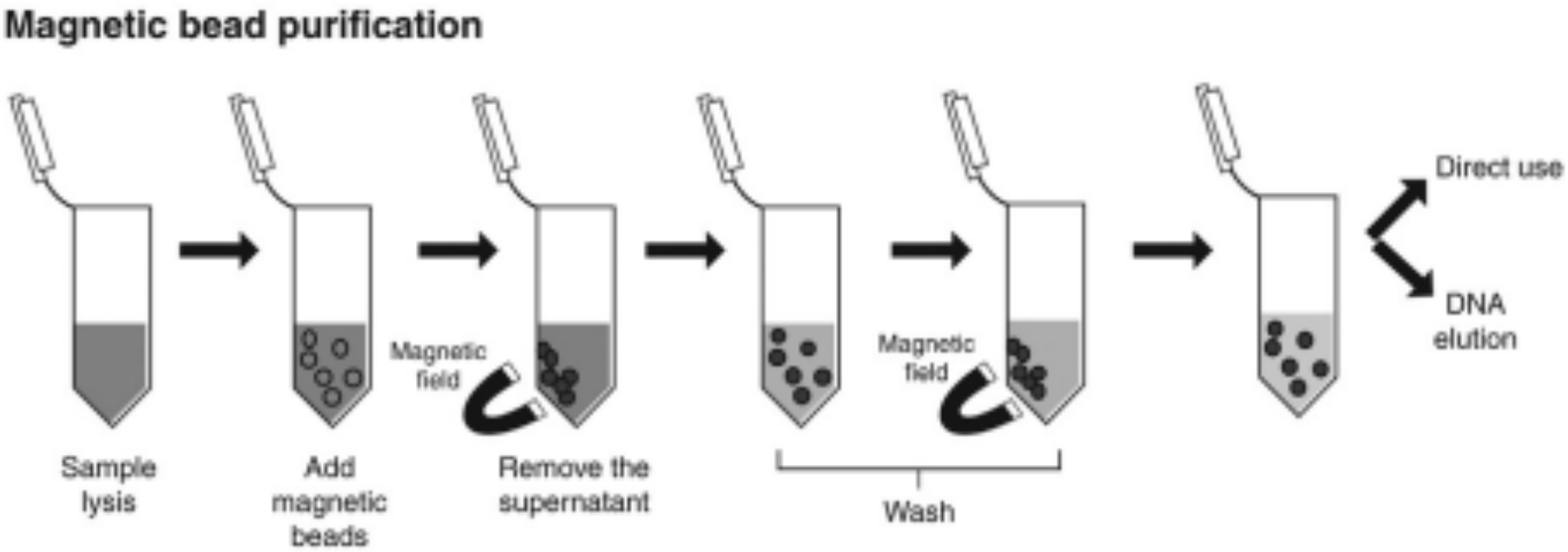
- **Advantages:**

- Simple
- Can be applied to large number of samples
- High yield
- High Purity

- **Disadvantages:**

- Cost
- Can be time consuming and labor-intensive

Magnetic Beads



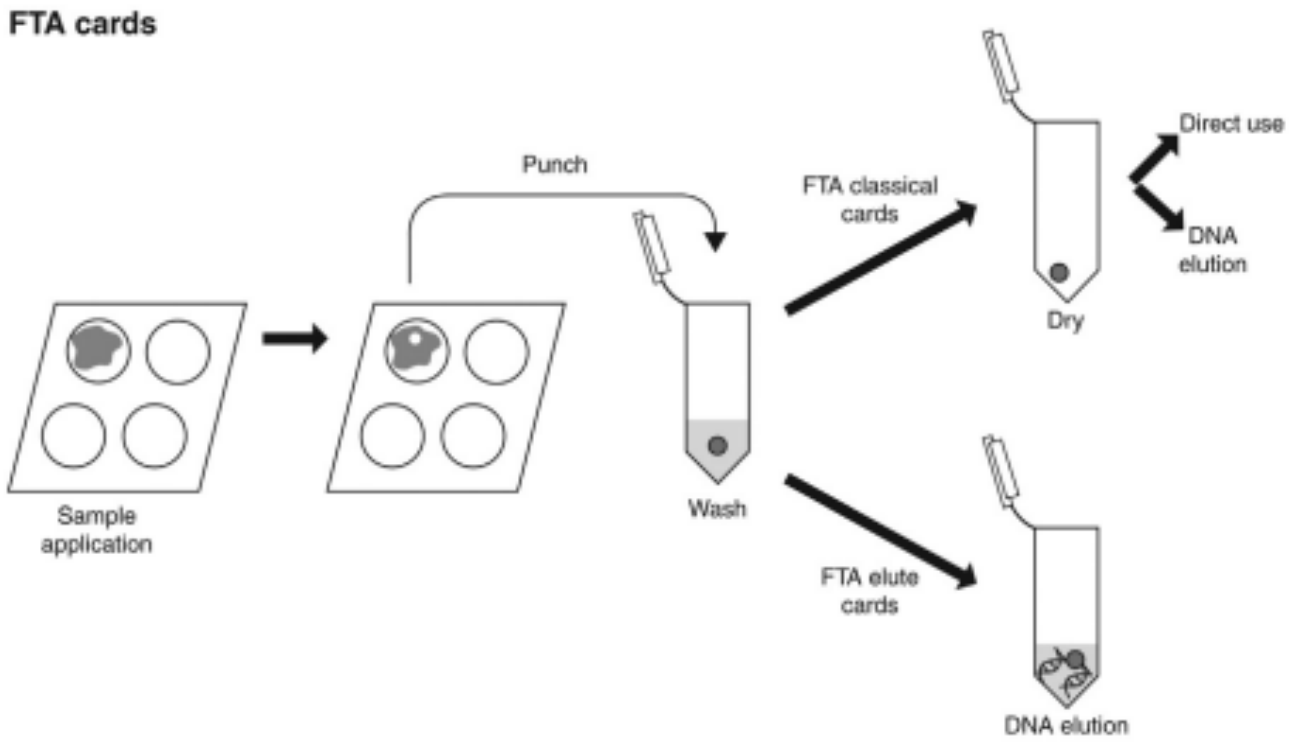
- Advantages:

- Fast
- Simple to handle
- Good yield
- Good quality
- Less labor sensitive

- Disadvantage:

- High Cost

FTA Paper Cards



- Advantages:

- Cost Effective storage
- Fast

- Disadvantages:

- Limitations / max vol applied on card
- Insufficient yield

In general, new methods;

- Advantages;

Fast

Safe

More Pure DNA

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RNA Extraction

- RNAses → Everywhere
- More difficult than DNA extraction
- Easily degraded.
- Should be processed quickly

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MANUAL - PROTOCOLS

Complete Kits

Commercial Offerings

★★★★★

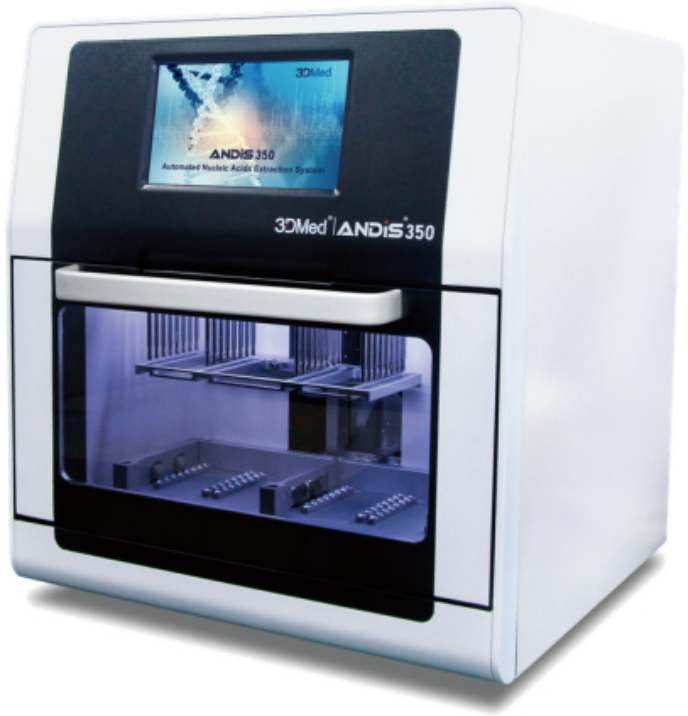
AUTOMATED- PROTOCOLS

AUTOMATED-SYSTEMS

Automated Systems

- Routine clinical laboratories.

- ADVANTAGES:
  - Fast
  - Efficient
  - Load many samples
  - Higher yield
  - Lower risk of contamination



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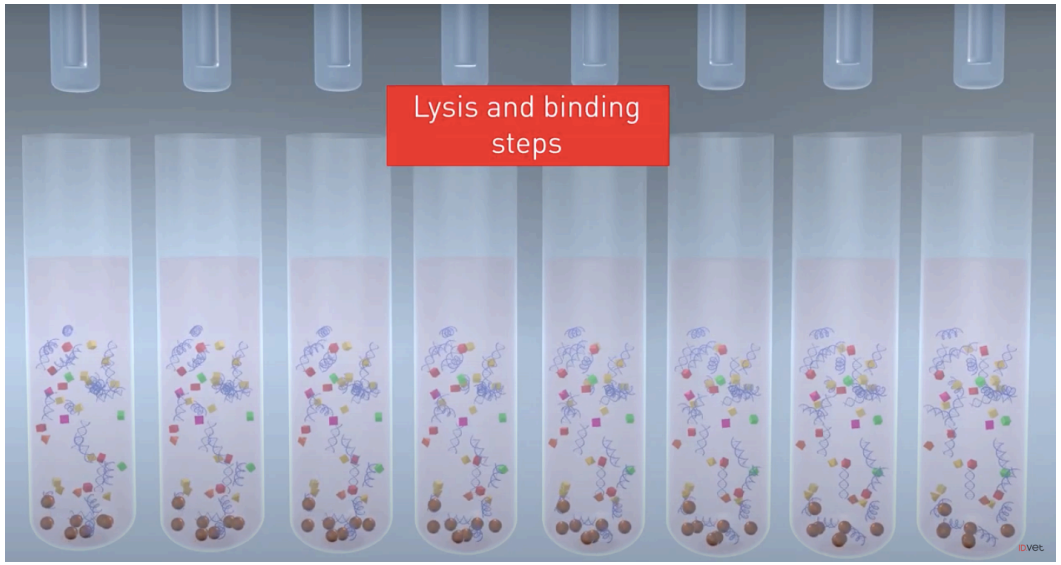

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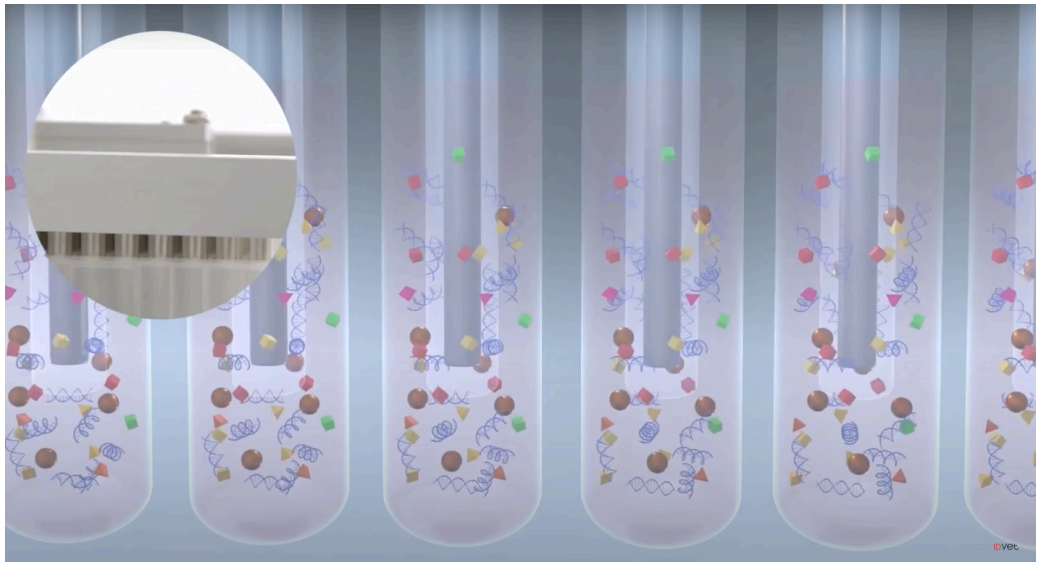

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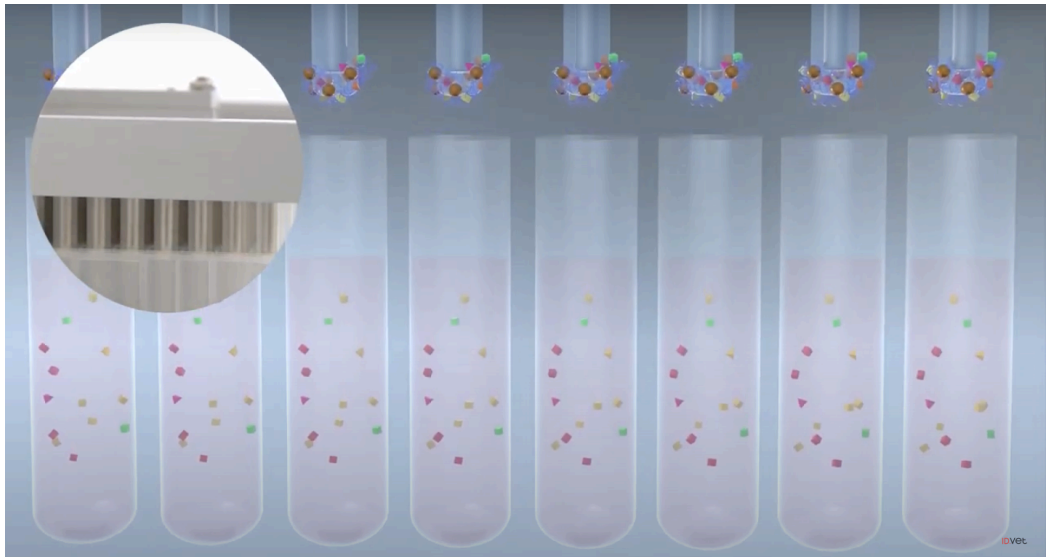

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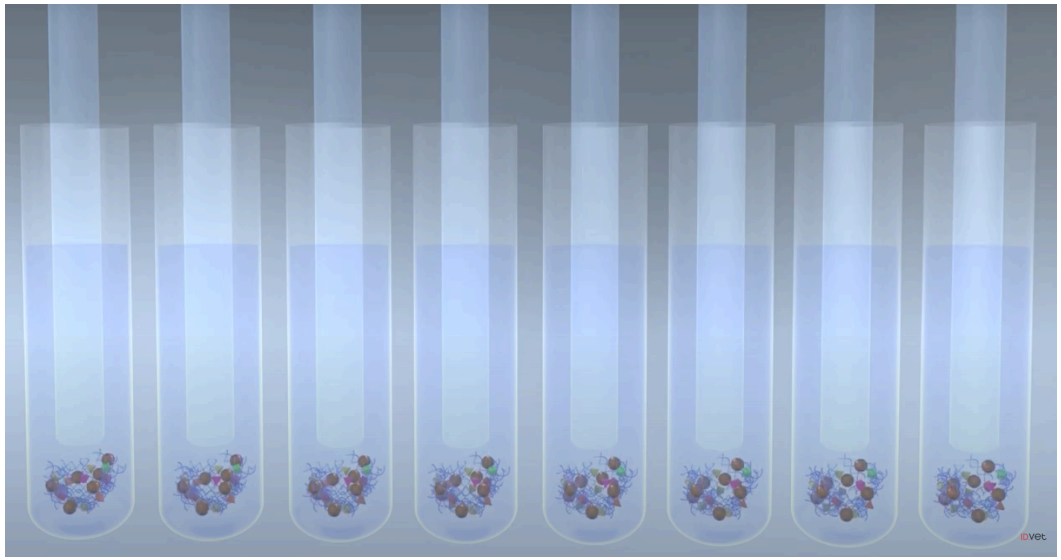

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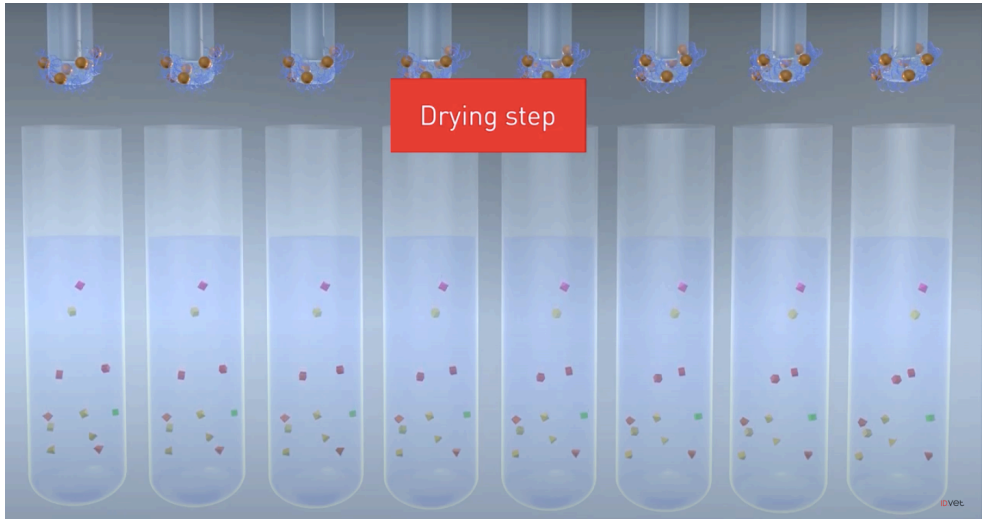

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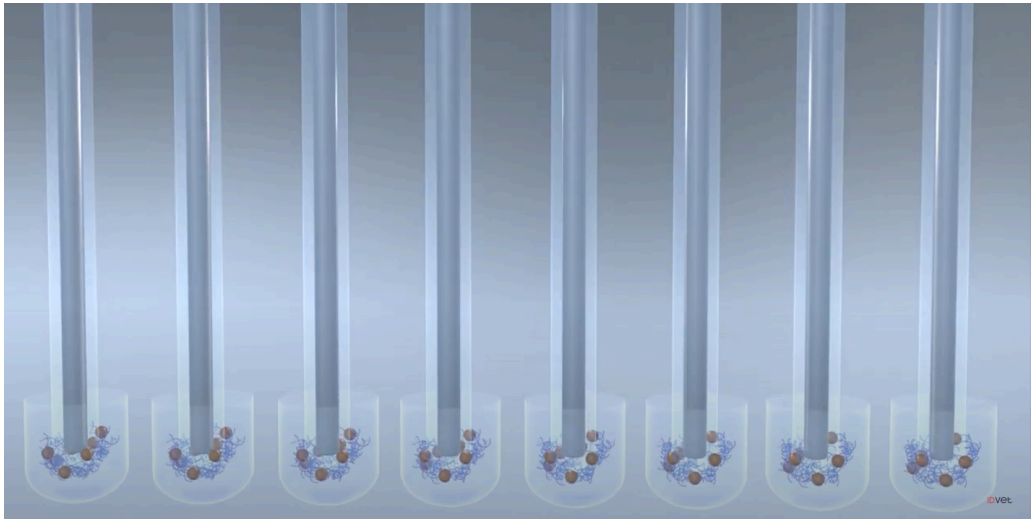

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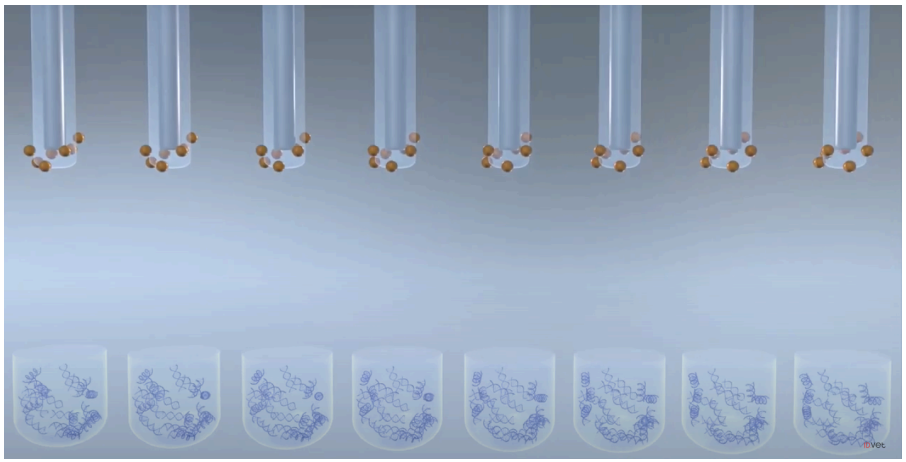
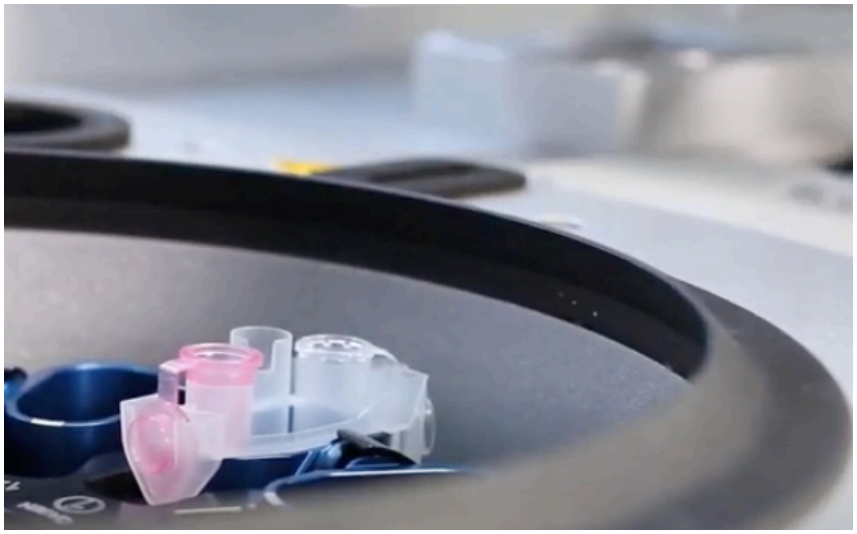
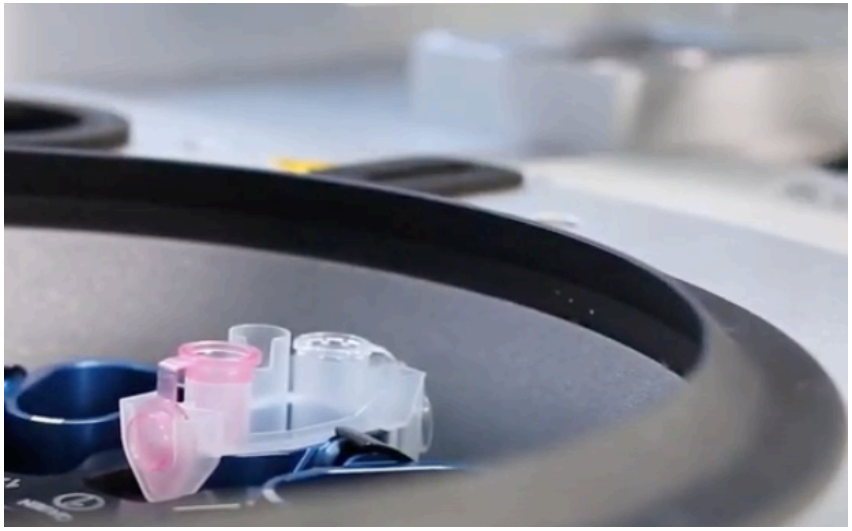
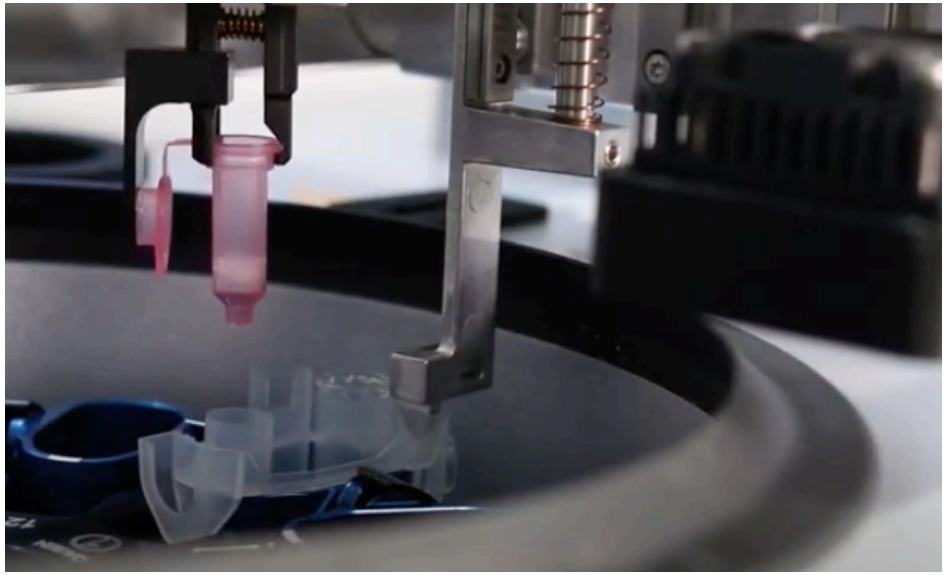
7









Automated Systems

YIELD

PURITY

REPRODUCABILITY

SPEED

ACCURACY

RISK OF CROSS CONTAMINATION

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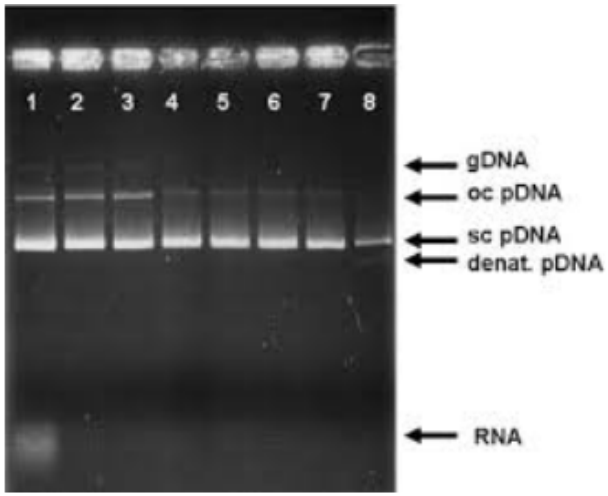
After performing isolation..

- Concentration
- Quality /Purity

Bioanalyzer



Gel electrophoresis

Spectrophotometry


World Health Organization
THE BIG STORY
CORONAVIRUS:
WHO DECLARES PANDEMIC
WHO DIRECTOR-GENERAL

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Nucleic acid isolation – Diagnosis ?

- We need to be faster!
- VTM
- Protocols changed!

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Thank you.