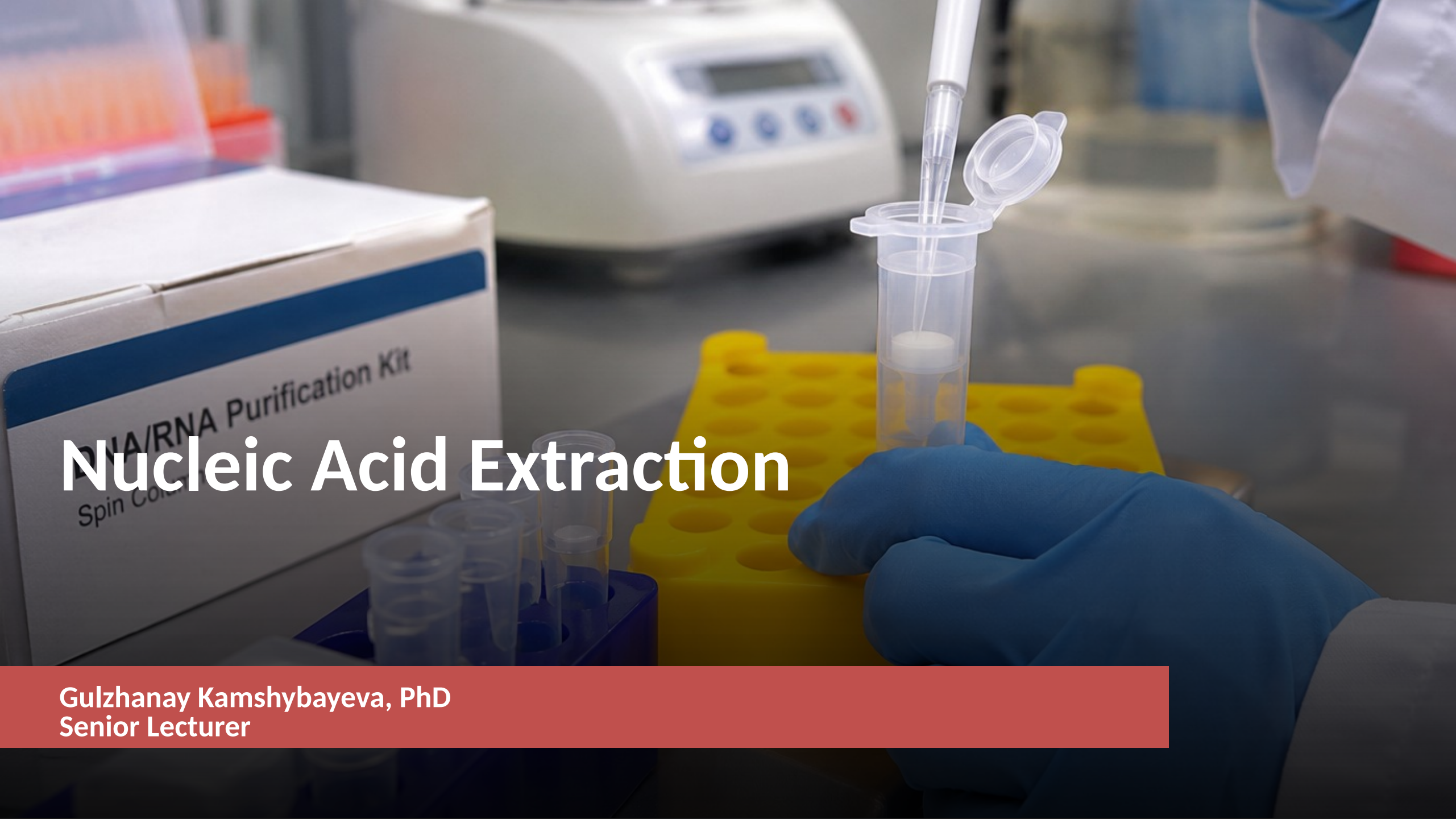
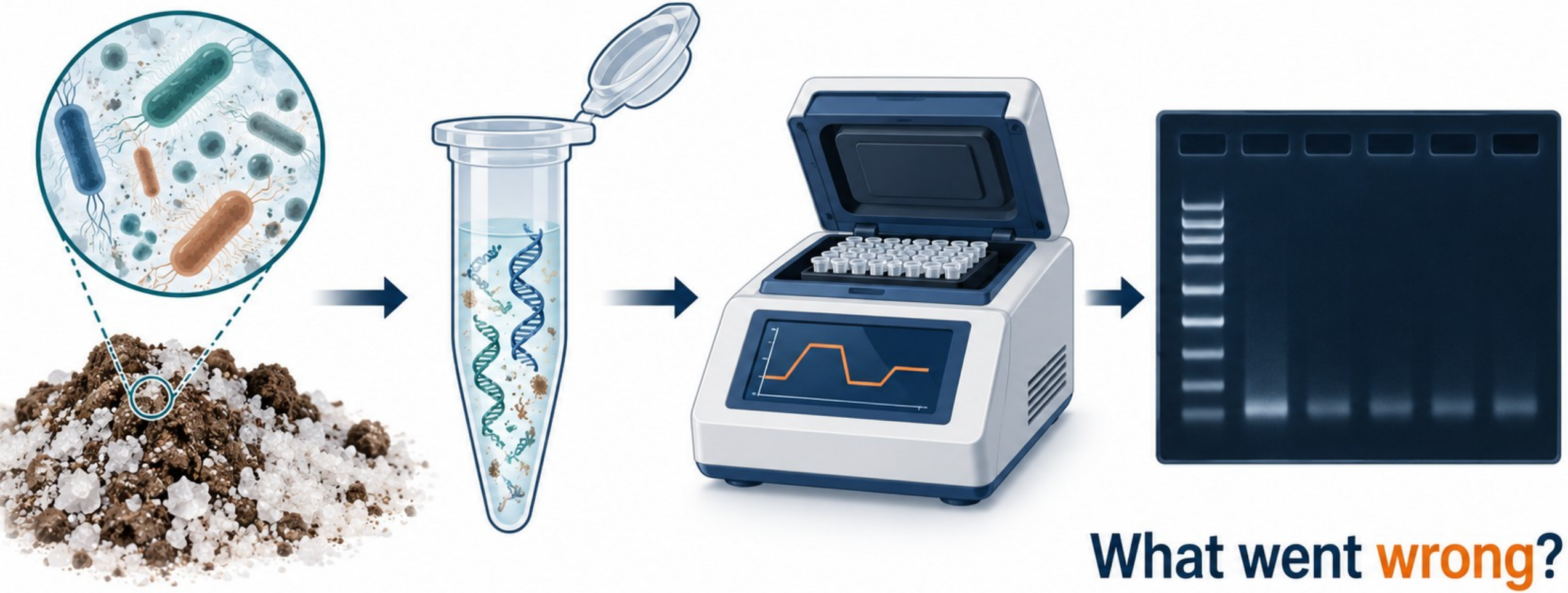


Nucleic Acid Extraction

Gulzhanay Kamshybayeva, PhD
Senior Lecturer

A high DNA concentration — but PCR fails

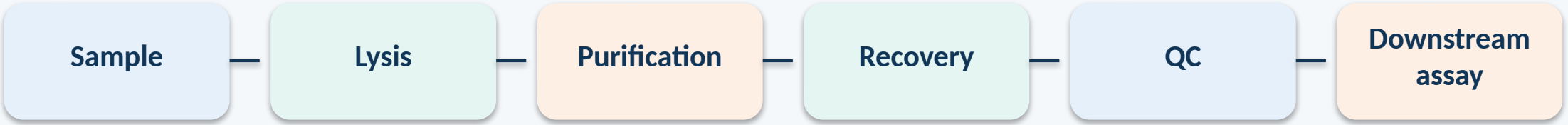
What went wrong during extraction or quality control?



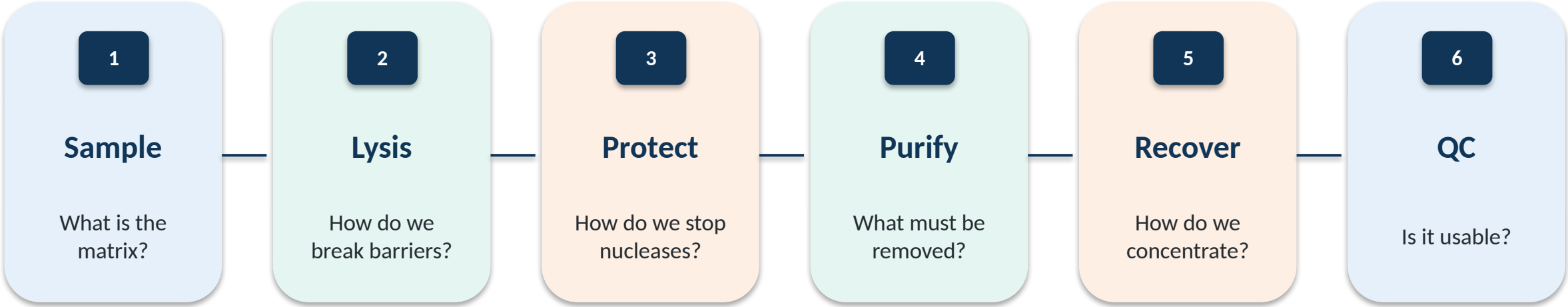
What went wrong?

Nucleic Acid Extraction and Quality Control

From biological sample to fit-for-purpose DNA and RNA



Extraction is a chain of decisions



The downstream application defines what “good DNA/RNA” means.

The sample matrix determines the extraction challenge

Gram-negative bacteria	Gram-positive bacteria	Plant tissue	Yeast / fungi	Soil / rhizosphere
Outer membrane + thin peptidoglycan	Thick peptidoglycan	Cellulose wall; polysaccharides; polyphenols	Rigid wall with glucans/chitin	Mixed cells + minerals + humic substances
Often easier to disrupt chemically	Often needs lysozyme + stronger disruption	Grinding + chemistry to control contaminants	Mechanical and/or zymolyase-based lysis	Bias + inhibition are major risks

The pre-analytical phase starts before lysis

1

Sampling

Representative material

Avoid cross-contamination

2

Preservation

Control temperature

Limit nuclease activity

3

Transport

Minimize delay

Avoid freeze–thaw cycles

4

RNA-specific

Stabilize transcript profile

Work rapidly / RNase-aware

A perfect extraction protocol cannot fully rescue a badly collected or degraded sample.

Physical and mechanical disruption

Lyophilization

Drying can make some tissues easier to grind

Useful in some plant/material workflows

Liquid N_2 grinding

Brittle frozen tissue is pulverized

Excellent for plant tissue; limits enzymatic activity

Bead-beating

High-energy collision with beads

Strong for microbes and soil; may shear DNA

Sonication

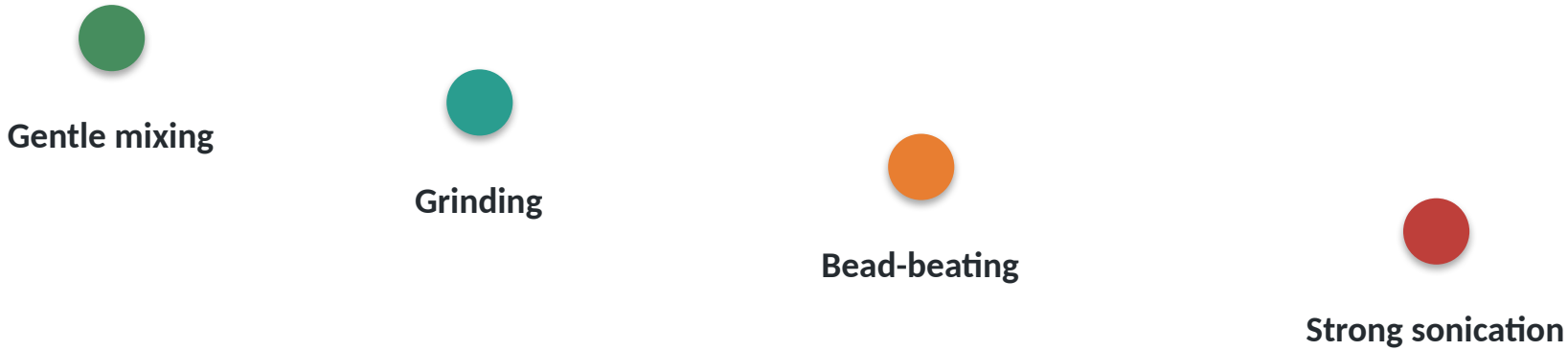
Ultrasonic energy disrupts cells

Fast, but can fragment nucleic acids

Mechanical lysis is a trade-off: recovery vs integrity

More aggressive disruption

Higher cell disruption / yield



Potential DNA shearing increases →

For long-read sequencing, preserve high-molecular-weight DNA. For routine PCR, modest fragmentation may be acceptable.

Chemical lysis: detergents disrupt membranes

SDS

Anionic detergent

- Disrupts lipid membranes
- Strongly denatures proteins
- Helps release nucleic acids
- Useful when robust protein denaturation is acceptable

Triton X-100 / NP-40

Non-ionic detergents

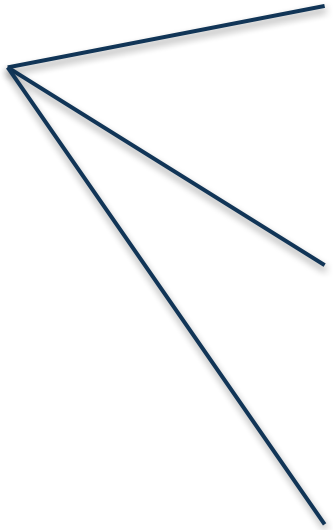
- Disrupt membranes more gently
- Less protein denaturation than SDS
- Useful when preserving some macromolecular structure
- Choice depends on the extraction goal

Chaotropic salts: denature proteins and reshape binding chemistry

Common examples

Guanidinium thiocyanate — GITC

Guanidinium hydrochloride — GuHCl



Protein denaturation

Strong nuclease inhibition

Promote nucleic-acid binding to silica under suitable high-salt conditions

Enzymatic lysis targets specific biological barriers

Lysozyme	**Target: Peptidoglycan**	Bacteria; especially useful for Gram-positive pretreatment
Proteinase K	**Target: Proteins and many nucleases**	Broadly used during DNA/RNA purification
Zymolyase	**Target: β-glucan-rich yeast/fungal cell wall**	Yeast and some fungal workflows

Enzymes are often combined with detergents and mechanical disruption rather than used alone.

Protecting nucleic acids: inhibit DNases and RNases

Chelators: EDTA / EGTA

Bind divalent cations such as Mg^{2+}

Many nucleases require metal ions for activity.

Chelation therefore reduces nuclease activity.

Reducing agents: β-ME / DTT

Reduce disulfide bonds

Can help inactivate RNases during RNA extraction.

Especially useful in RNase-rich tissues and RNA workflows.

Important: these reagents reduce nuclease activity; they are not universal substitutes for clean technique.

Why RNA extraction is usually more demanding

1

RNA is chemically more reactive

2′-OH group increases susceptibility to hydrolysis

2

RNases are widespread and robust

Skin, dust and laboratory surfaces can introduce RNases

3

Expression profiles can change rapidly

Delay can alter the biological signal before extraction

4

DNase treatment may be required

Residual genomic DNA can confound expression analysis

Purification strategies: same goal, different chemistry

M2

1

Organic extraction

Phenol / chloroform

Phase partitioning

2

Silica columns

Spin-column chromatography

Adsorption to silica

3

Magnetic beads

Silica or SPRI-type beads

Magnetic solid phase

4

Anion exchange

Charged resin

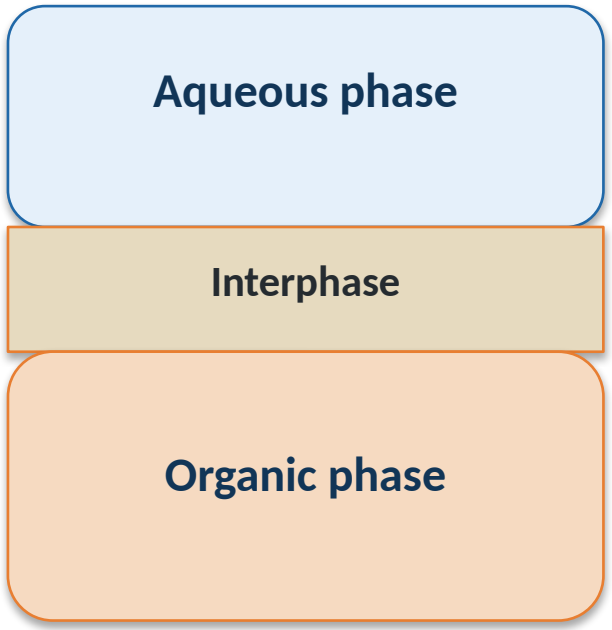
Electrostatic interaction

Choose according to sample type, purity requirement, throughput, safety, cost, and downstream use.

Phenol–chloroform extraction: phase partitioning

M2

After mixing + centrifugation



Nucleic acids preferentially remain in the aqueous phase under neutral/alkaline conditions.

Denatured proteins and lipids accumulate mainly at the interphase and in the organic phase.

Residual phenol can inhibit downstream enzymatic reactions.

Strength: high recovery. Limitation: hazardous, operator-dependent, carryover risk.

pH changes nucleic-acid partitioning: the logic of TRIzol

M2

Neutral / alkaline phenol

~ pH 7.5–8

DNA + RNA

mainly in aqueous phase

Acidic phenol / TRIzol-type

~ pH 4–4.5

RNA

preferentially remains aqueous

DNA → largely interphase / organic-associated

Partitioning depends on pH, ionic conditions and reagent formulation; treat these as operational principles, not rigid universal boundaries.

Silica spin columns: reversible binding under high-salt conditions

M2

1 **Bind** Chaotropic salts + alcohol create conditions that favor nucleic-acid adsorption to silica

2 **Wash** Ethanol-containing buffers remove proteins, salts and lipids while nucleic acid stays bound

3 **Dry** Residual ethanol must be removed; carryover can inhibit PCR and enzymes

4 **Elute** Low-salt buffer or nuclease-free water releases nucleic acid from silica

Spin-column errors: where good protocols fail

M2

Column overload

Too much biomass or nucleic acid

Reduced purity / poor recovery

Incomplete drying

Residual ethanol

PCR / enzyme inhibition

Poor lysis

Cells remain intact

Low yield / representation bias

Small elution volume

Higher concentration, lower total recovery

Trade-off between yield and concentration

Magnetic beads: two related but distinct strategies

M2

Silica-coated magnetic beads

Binding chemistry resembles silica columns

Magnet replaces centrifugation

Useful for extraction automation

SPRI-type carboxylated beads

PEG + salt drive reversible DNA binding

Excellent for cleanup and size selection

Common in NGS library workflows

Do not treat all magnetic-bead methods as a single identical binding mechanism.

Why magnetic beads dominate high-throughput workflows

M2

96/384-well plates	Parallel processing
No spin columns	Fewer manual transfers
Programmable washes	Reproducible timing
Scalable volumes	From microliters to plates
NGS-compatible	Cleanup + size selection

High-throughput extraction

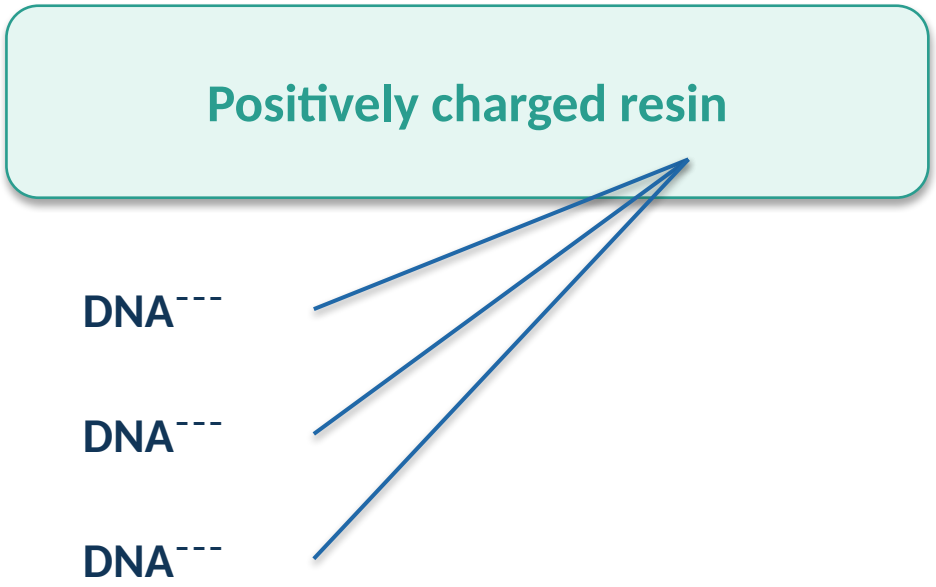
Sample → beads → magnet → wash → elute

Automation reduces handling variability and enables many samples to be processed in parallel.

But poor chemistry cannot be rescued by automation.

Anion-exchange chromatography: separation by charge

Nucleic acids carry a strong negative charge
because of the phosphate backbone.



Increase ionic strength

→ competing ions weaken electrostatic interactions
→ DNA elutes from the resin

Strength: high purity and scalable purification.
Limitation: more instrument / workflow complexity than simple spin columns.

How do the purification methods compare?

M2

Method	Mechanism	Strength	Limitation
Phenol/chloroform	Phase partitioning	High recovery	Hazardous; carryover
Silica spin column	Adsorption to silica	Simple, robust	Capacity; ethanol carryover
Magnetic beads	Solid phase + magnet	Automatable	Cost; chemistry-specific
Anion exchange	Electrostatic binding	High purity / scale	More complex workflow

Method selection = sample matrix + desired purity + throughput + safety + downstream assay.

Alcohol precipitation: why salt + alcohol make DNA/RNA fall out of solution

1

Add monovalent cations

Na^+, NH_4^+ or Li^+ help shield negative phosphate charges

2

Add ethanol or isopropanol

Lower solvent polarity reduces nucleic-acid solubility

3

Collect precipitate

Centrifuge → pellet → wash → resuspend

Precipitation concentrates nucleic acids, but it can also co-precipitate salts and contaminants.

Ethanol vs isopropanol precipitation

Ethanol

- Typically needs a larger volume
- Often gives cleaner salt removal
- 70% ethanol commonly used for pellet washing
- Good general-purpose precipitation

Isopropanol

- Requires a smaller volume
- Efficient precipitation at lower alcohol volume
- Can co-precipitate salts more readily
- Useful when sample volume must be minimized

Co-precipitants help recover very small amounts of nucleic acid

1 **Glycogen** Provides a carrier matrix that improves pellet formation and visibility

2 **Linear polyacrylamide (LPA)** Inert carrier useful for low-concentration DNA/RNA

Use carriers when the expected nucleic-acid mass is too low to form a reliable visible pellet.

Resuspension and storage: protecting the sample after extraction

1	**TE buffer**	Tris maintains pH; EDTA suppresses metal-dependent nucleases
2	**Nuclease-free water**	Simple and compatible with many assays; less buffering/protection
3	**Temperature**	Short term: cold; long term: appropriate freezing according to sample and protocol
4	**Freeze–thaw cycles**	Repeated cycles can damage RNA and high-molecular-weight DNA
5	**HMW genomic DNA**	Avoid vigorous vortexing and repeated passage through narrow tips