

RNA Extraction

SegoliP Unit

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What are the Key Expectations from an RNA Extraction Procedure?

- ① Maximize RNA recovery
 - Extraction process
- ② Remove inhibitors
- ③ degrade nucleases
- ④ Maximize the quality of RNA
- ⑤ Store RNA/ Use RNA in downstream processes



Types of RNA

RNAs involved in protein synthesis	<p>Messenger RNA or mRNA (1-5%)</p> <p>Transfer RNA or tRNA (10-15%):</p> <p>Ribosomal RNA or rRNA (>80%):</p> <p>Signal recognition particle RNA (SRP RNA)</p>
RNAs involved in post-transcriptional modification or DNA replication	<p>Small nuclear RNA (snRNA)</p> <p>Small nucleolar RNA (snoRNA)</p> <p>Guide RNA (gRNA)</p> <p>Ribonuclease MRP (RNase MRP)</p> <p>Telomerase RNA Component (TERC)</p> <p>Spliced Leader RNA (SL RNA)</p>
Regulatory RNAs	<p>Antisense RNA (aRNA, asRNA)</p> <p>CRISPR RNA (crRNA)</p> <p>MicroRNA (miRNA)</p> <p>Long noncoding RNA (lncRNA)</p> <p>Small interfering RNA (siRNA)</p> <p>Enhancer RNA (eRNA)</p>

RNA Extraction Method

Organic extraction



Spin column



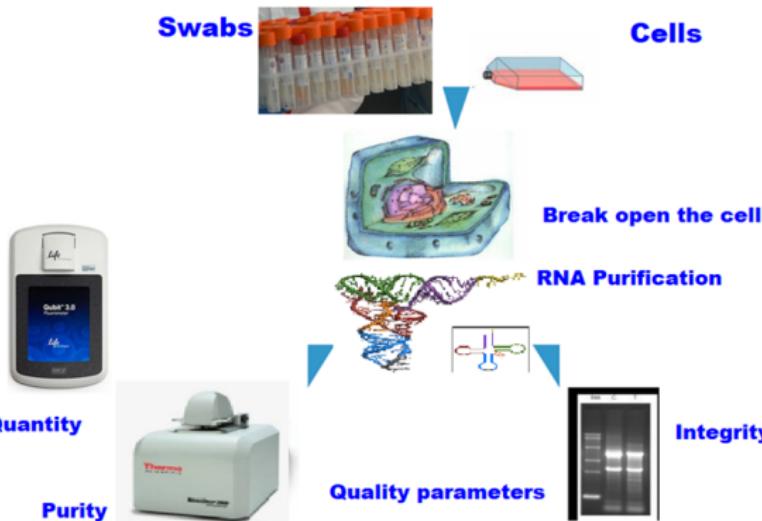
Magnetic beads



Benefits	<ul style="list-style-type: none">Efficient lysis of cells and tissueRapid denaturation of nucleasesStabilization of nucleic acids	<ul style="list-style-type: none">Ease of useHigh yield and purityThroughput flexibility	<ul style="list-style-type: none">No risk of cloggingIncreased target capture efficiencyRapid collection and concentration of sampleAbility to automateScalability
Examples	<ul style="list-style-type: none">Invitrogen <u>RNAzol</u> reagent (<u>Trizol</u>)CTAB	<ul style="list-style-type: none"><u>QiAmp</u> Viral RNA Kit<u>Rneasy</u>Promega	<ul style="list-style-type: none">Applied Biosystems MagMAX kitsTANBead

Purification of RNA

- Cells or tissue must be rapidly and efficiently disrupted. Different tissues/sources raises different issues
- Inactivate RNases
- Denature nucleic acid-protein complexes
- RNA selectively partitioned from DNA and protein
- Store samples in RNA stabilization reagent / freezer



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The whys of extraction reagents

Guanidine thiocyanate / Guanidine isothiocyanate

A chaotropic reagent that is used as a general protein denaturant. Lyse cells and virus particles in RNA and DNA extractions. Prevent activity of RNase enzymes and DNase enzymes by denaturing them. 2M guanidine isothiocyanate can mediate the adsorption of RNA on glass fiber filter membrane or silica magnetic beads.

β -mercaptoethanol

An antioxidant and prevents oxidation of polyphenols (polyphenols bind covalently to RNA giving it a brown color and making it useless for most research applications)

Phenol

Its properties allow for effective nucleic acid extraction, particularly as it strongly denatures proteins, it is a nucleic acid preservative, and it is immiscible in water.

The whys of extraction reagents

chloroform:isoamylalcohol(24:1)

Chloroform is, an organic compound. All the cellular compounds which are soluble in chloroform (lipids, proteins) will be **dissolved** into the chloroform. RNA is not soluble in chloroform and will remain dissolved in the aqueous (water) layer.

Isoamylalcohol reduces **foaming** and facilitates the **separation of phases**.

Absolute Ethanol

Used to concentrate and de-salt RNA

Isopropanol

Causes the solution to become more hydrophobic. Polar molecules (like RNA) precipitate out of solution

The whys of extraction reagents

70% ethanol

The salt solution contributes positively charged atoms that are attracted to the negative charge of DNA, effectively neutralizing the DNA's electric charge. This neutralization allows the RNA molecules to aggregate with one another. When ethanol is added, the **RNA clumps together** and **precipitates** at the water/ethanol interface because the RNA is not soluble in ethanol.

EDTA

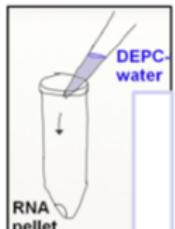
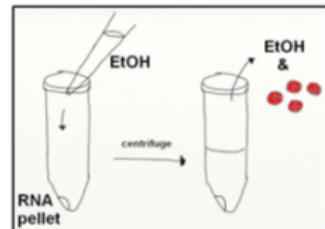
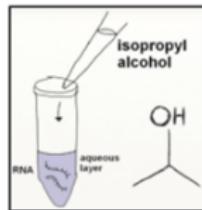
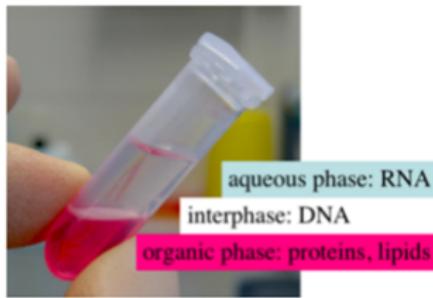
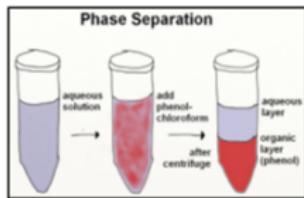
chelates the cations Mg^{2+} and Ca^{2+} , and thereby prevents the degradation and random nicking of RNA by RNases, which are dependent on these cations for activity.

Tris

Maintains a constant pH (between pH 6-8) to prevent hydrolysis at lower pH and denaturation of RNA at higher pH.

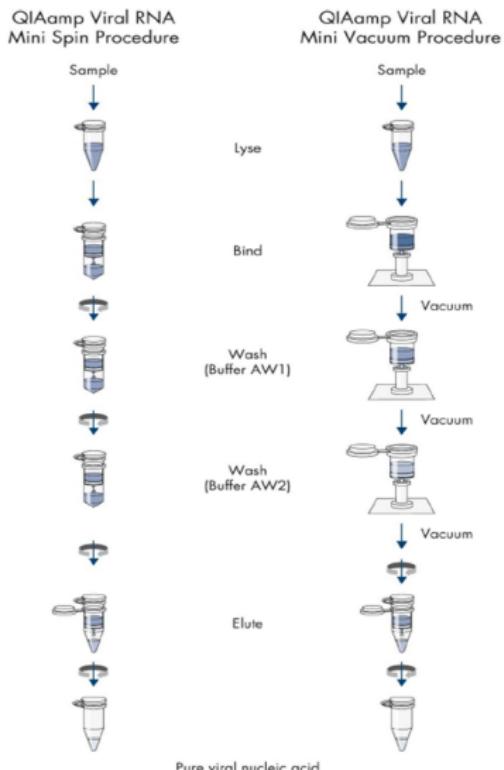
RNA Extraction using Trizol

- Lyse cells/tissue in the lysis buffer and centrifuge to remove particles
- Transfer the supernatant in a fresh tube then proceed to phase separation by adding and mixing with phenol-CHCl3
- Centrifuge to separate phases



Solid Phase RNA extraction (Spin Columns)

- Lysis of cells
- ETOH Precipitation
- Spin column: RNA adsorption to silica gel membrane
- Two-different wash steps: removal of contaminants
- Elution - pure and concentrated RNA



Solid Phase RNA extraction (Spin Columns)



Figure: RNA extraction from wastewater

Automated RNA extraction

Uses beads technology to purify RNA. The silicon dioxide layer coating on the magnetic beads adsorbs and purifies nucleic acid from the samples.

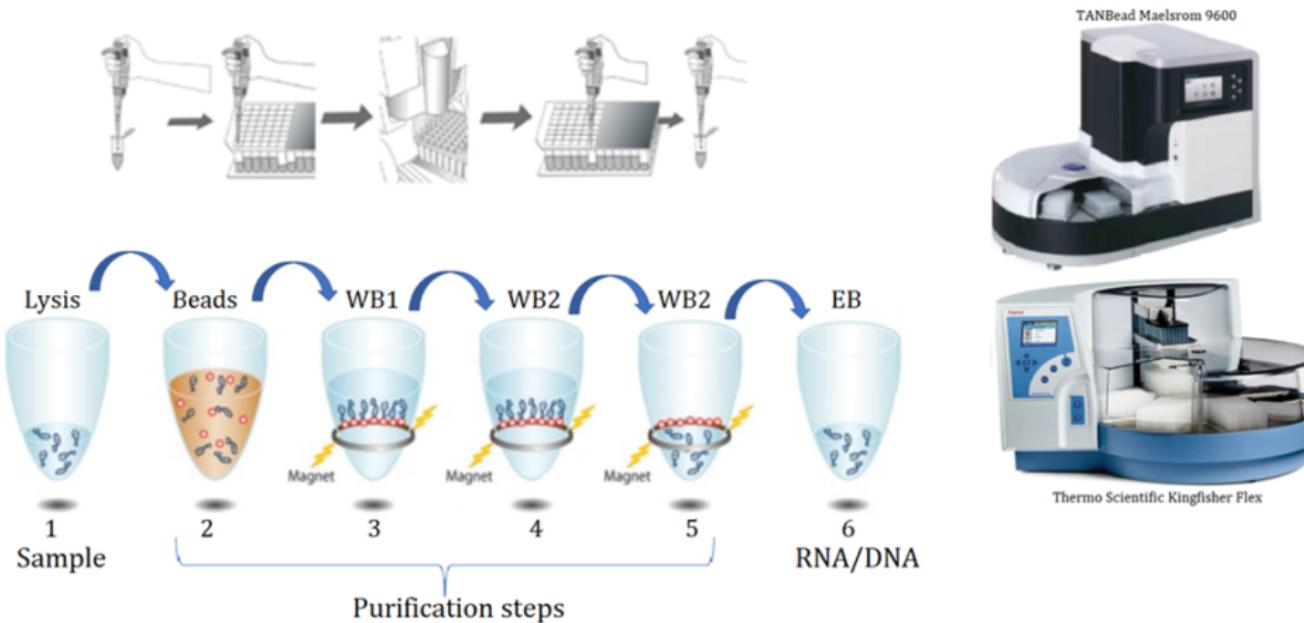
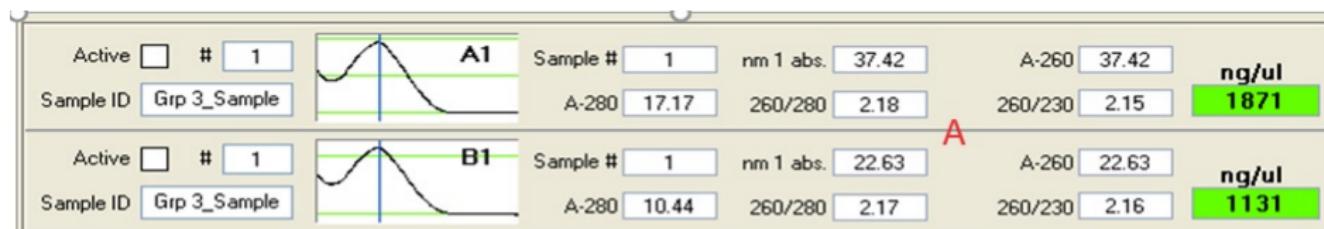


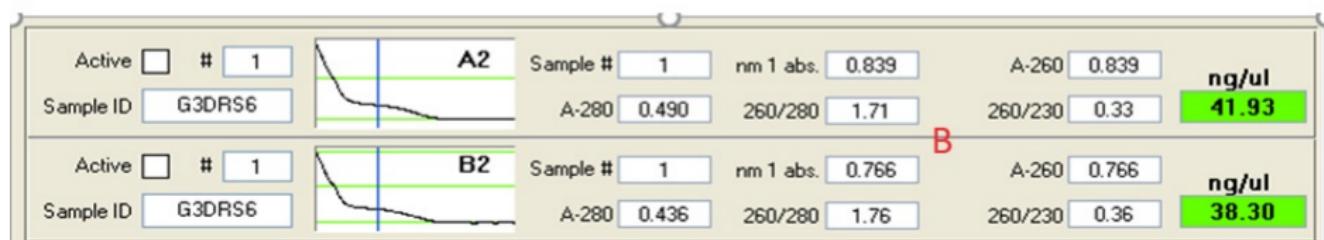
Figure: Automated Extraction

RNA Quantification: Spectrophotometer

- The peak of absorption for RNA is 260 nm and a solution whose absorbance at 260 (A_{260}) = 1 OD contains $\infty 40\mu\text{g RNA/ml}$. \Rightarrow Conc=40xOD260
- For a good RNA solution, the ratios of A_{260}/A_{230} and A_{260} / A_{280} must be about 2.2 and 2.0, respectively.



A



B

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Figure: Nano drop RNA readings

RNA Quantification: Flurometer

- The Qubit is a fluorometer that uses **dyes** that only emit a signal when **bound** to specific target molecules.
- The assay is highly selective for RNA over dsDNA and thus gives a better quantification compared to other methods.

Assay Name	Sample Name	Original Sample Conc.	Conc. units	Sample Volume (uL)	Dilution Factor	Extended Low Range	Core Range	Extended High Range	Excitation	Std 1 RFU	Std 2
RNA HS	S1		18.1	ng/uL		2000	0.1-0.2	0.2-100	100-120	BLUE	2.882129
RNA HS	S2		18.4	ng/uL		2000	0.1-0.2	0.2-100	100-120	BLUE	3.227984
RNA HS	S3		20.4	ng/uL		2000	0.1-0.2	0.2-100	100-120	BLUE	3.372091
RNA HS	S4		68.2	ng/uL		2000	0.1-0.2	0.2-100	100-120	BLUE	2.795665
RNA HS	S5		131	ng/uL		2000	0.1-0.2	0.2-100	100-120	BLUE	3.026235



RNA Quality Check: Gel Electrophoresis

- The speed of migration of the RNA depends on its size, form and net charge.
- The net charge dictates the direction of migration.

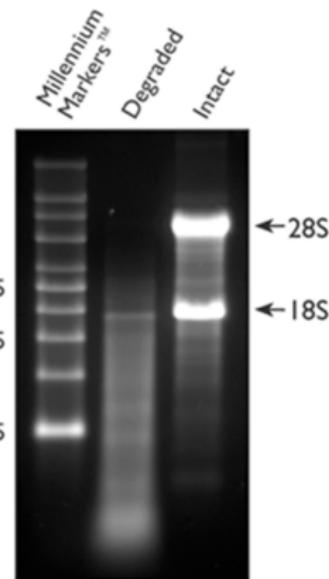
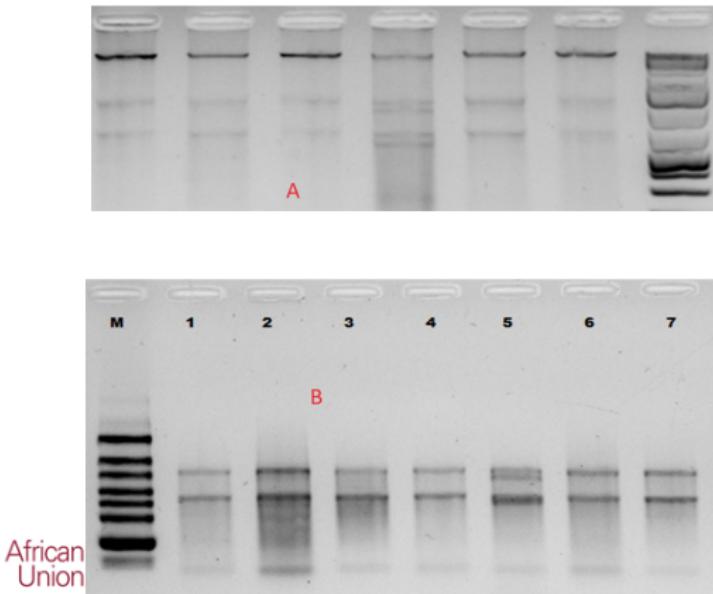


Figure: RNA Gel Image

RNA Quantification: Tape Station / Bioanalyzer

- Total RNA assessed by **capillary electrophoresis** with an Agilent 2100 bioanalyzer.
- Evaluates the proportion of RNA detected before, between and after the rRNA peaks to determine a **relative integrity number (RIN)** for the RNA sample analysed.
- Perfectly **intact RNA** has a **RIN of 10** whereas completely **degraded RNA** has a **RIN of 1**.

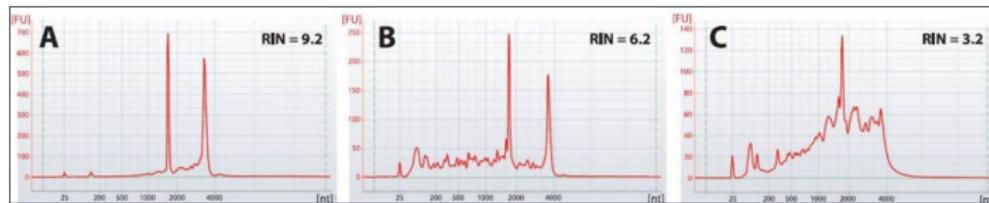


Figure: Bioanalzer RNA readings



Preventing RNases Contamination

- ① Wearing gloves throughout experiments.
- ② Changing gloves after touching skin (e.g., your face), door knobs, and common surfaces.
- ③ Having a dedicated set of pipettes that are used solely for RNA work.
- ④ Using tips and tubes that are tested and guaranteed to be RNase-free.
- ⑤ Using RNase-free chemicals and reagents, glassware and metalware, wash with DEPC-treated water, NaOH or H₂O₂]
- ⑥ Designating a "low-traffic" area of the lab that is away or shielded from air vents or open windows as an "RNase-free Zone".
- ⑦ Decontaminating shared laboratory surfaces and equipments with RNaseZap (or NaOH).
- ⑧ Include a chaotropic agent (guanidine) in the procedure.



RNA Storage: Short-term storage

- For short-term storage, RNA samples can be resuspended in nuclease-free water or buffer and stored at -80°C.
- Using a buffer solution that contains a chelating agent is a better way to store RNA. It prevents heat-induced strand scission. Chelation of divalent cations such as Mg⁺² and Ca⁺² will prevent heat-induced strand scission.



RNA Storage: Long-term storage

- For long-term storage (more than a few weeks), RNA samples are best stored at **-20 to -80°C as a salt/ethanol slurry.**
- To do this, take the RNA through all the steps of a regular precipitation with salt (e.g., 1/10 volume of 3 M NaOAc, pH 4.8) and ethanol (2 volumes of 100% ethanol) and store the mixture at -80°C without pelleting the RNA out of solution
- The combination of low pH, low temperature and high alcohol content will stabilize the RNA and inhibit all enzymatic activity.
- Other alternatives: RNA in frozen aliquots at -20°C or Lower.



Questions

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kea leboha!

Thank You!

Asante Sana!

Matôndo!

Merci!

Obrigado!



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