**BIO 405L. Cellular and molecular biology laboratory**

# RNA extraction

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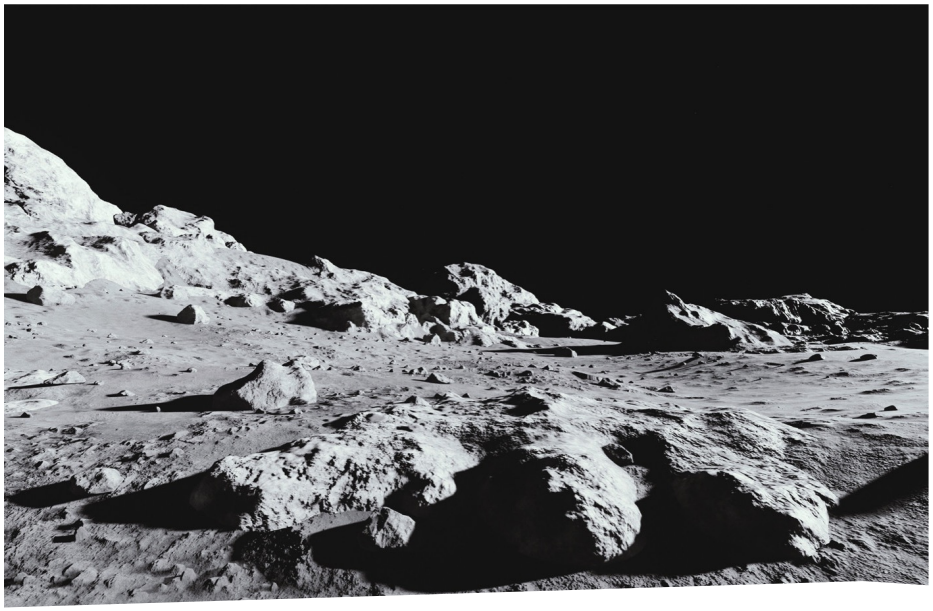
| | RNA Extraction | DNA Extraction |
|---|---|---|
| **Molecules extracted** | mRNA, tRNA, rRNA, and non-coding RNAs | Nuclear and mitochondrial DNA |
| **Stability** | Highly unstable and easily degraded by RNAses activity | More stable and resistant to environmental degradation than RNA |
| **Enzymatic Protection** | Requires strict precautions to prevent RNases contamination, including RNase-free reagents and tools (DEPC-treated) | Relatively resistant to DNases activity, particularly in the presence of $Mg^{+2}$ ions chelators such as EDTA |
| **Lysis Buffer** | Often includes reducing agents such as guanidine isothiocyanate or beta-mercaptoethanol to inactivate RNases | Typically contains EDTA, detergents and salts to lyse cells and stabilize DNA |
| **Purification** | Usually involves phenol-chloroform extraction or silica column purification specific for RNA. | It uses methods similar to those for RNA, but the buffers and binding conditions are optimized for DNA |
| **Precipitation** | Requires isopropanol or ethanol for RNA precipitation, often with co-precipitants like glycogen | Precipitation protocols are similar, typically using ethanol or isopropanol |
| **Quality** | Spectrophotometry (A260/A280 and A260/A230 ratios) and agarose or capillary (Bioanalyzer or Tapestation) electrophoresis | Similar methods, but DNA integrity is assessed based on high-molecular-weight bands |
| **Storage** | Stored at -80°C in RNase-free water or buffer; avoid freeze-thaw cycles to prevent degradation | Stored at -20°C or -80°C in TE buffer or water for long-term stability |
| **Applications** | Studying gene expression (e.g., qPCR, RNA-seq); Functional studies of non-coding RNAs; Viral RNA detection | Genotyping, sequencing, and cloning; Genome studies and mutation analysis; DNA barcoding |

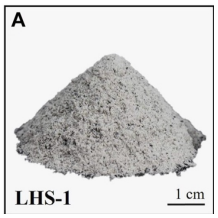


**Will *Escherichia coli* regulate the expression of stress-related genes when it is grown in the presence of Lunar or Martian regolith?**

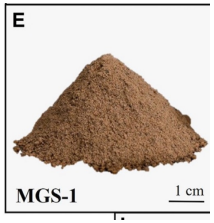
## *E. coli* cells suspended in 1X PBS

Control

Lunar simulant

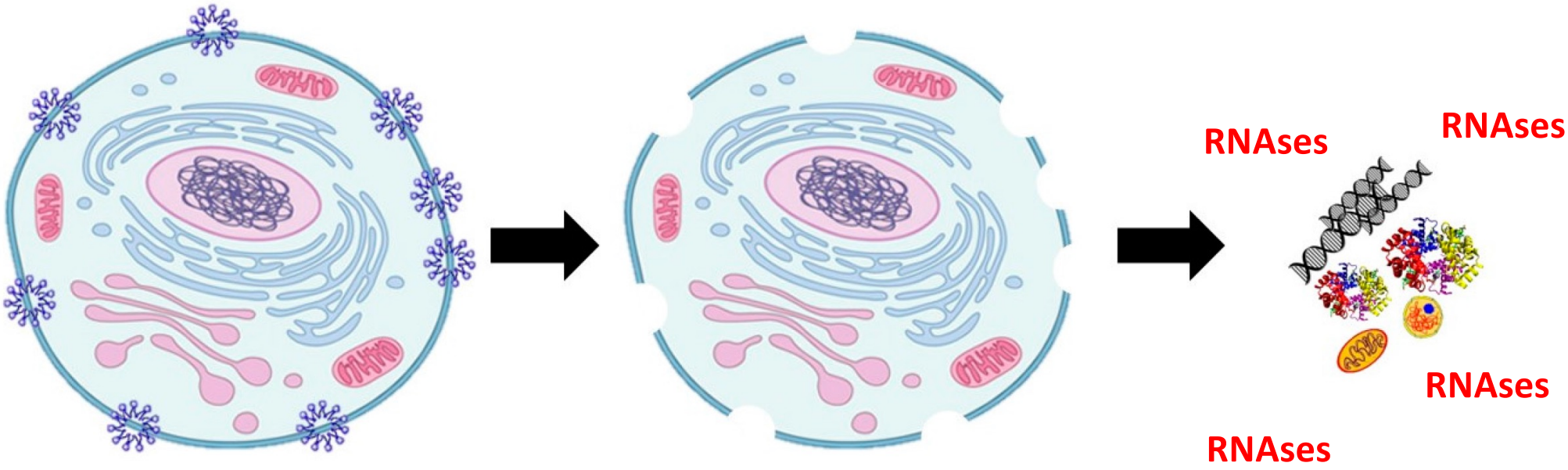



Martian simulant



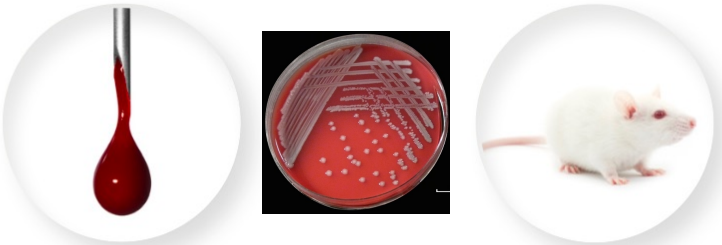

## Incubated at 30 C for 7 days with daily mixing

# RNA extraction and purification from a biological sample uses a combination of physical, mechanical and chemical methods, under reducing conditions to prevent RNA degradation




RNases and DNases are highly specific to their substrates, with RNase acting on ribonucleic acids and DNase on deoxyribonucleic acids. RNases are highly stable and resistant to environmental conditions (e.g. temperature, low pH, oxidizing agents), requiring stringent laboratory precautions to prevent RNA degradation (e.g. RNA protect, DNA/RNA Shield, Trizol). DNase, although effective, is more susceptible to inactivation by chelating agents like EDTA.

**Just add DNA/RNA Shield™**



Biological **Liquid**

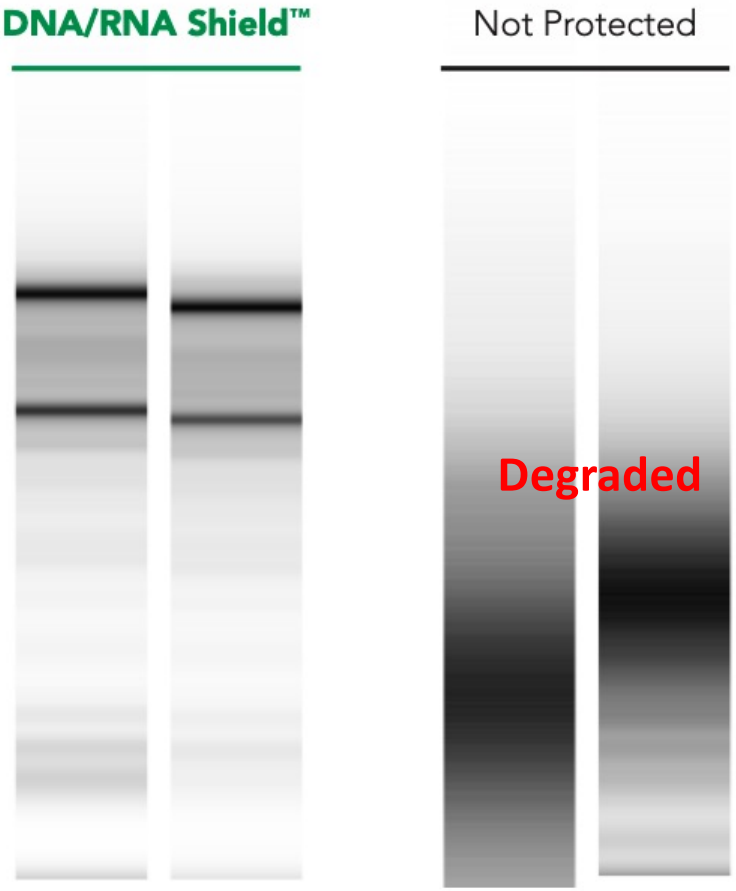
**Solid** Tissue/Biopsy

Add 3 volumes
(Mix well)

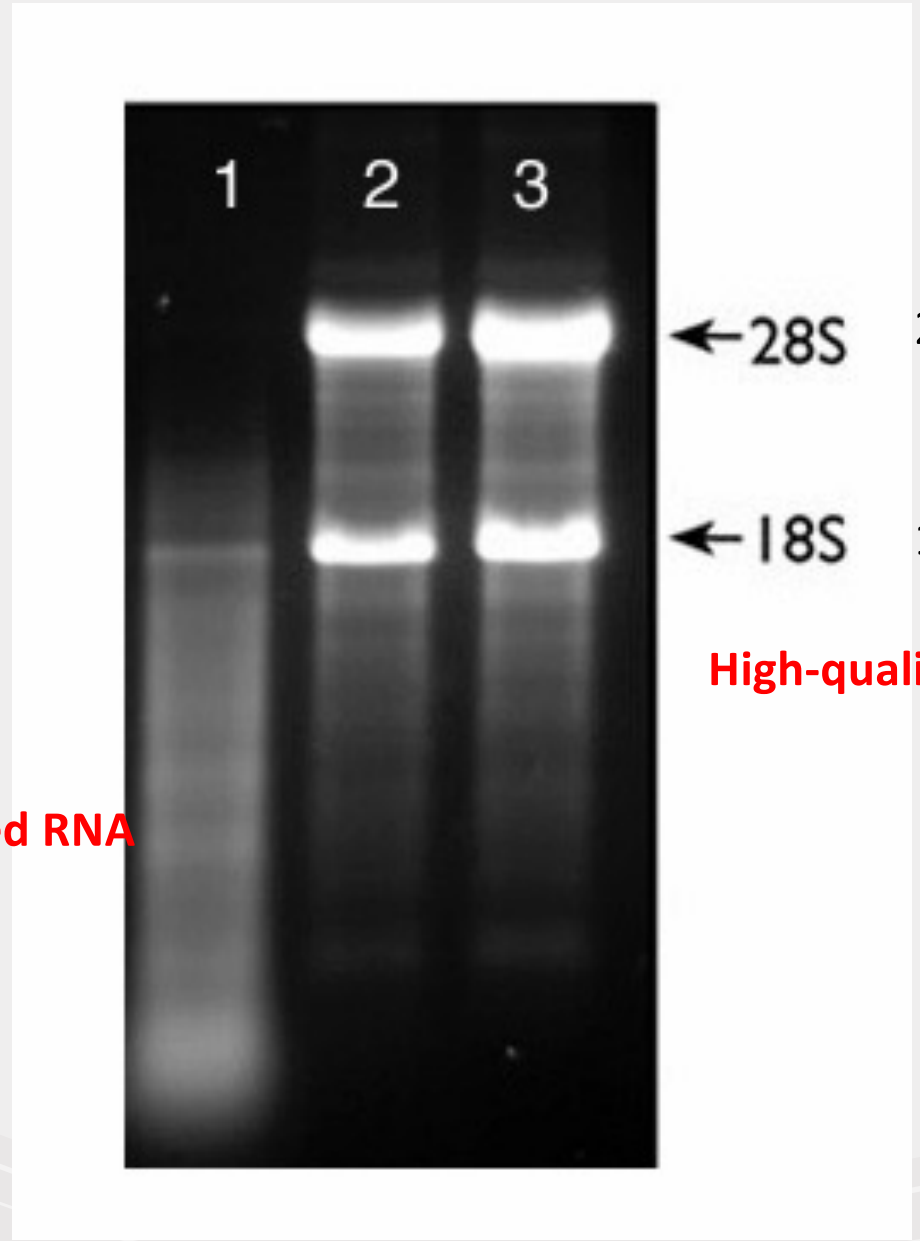
Add 300-400 uL
to pellet

Submerge

**Transport at ambient temperature**
(No cold-chain or dry-ice needed)

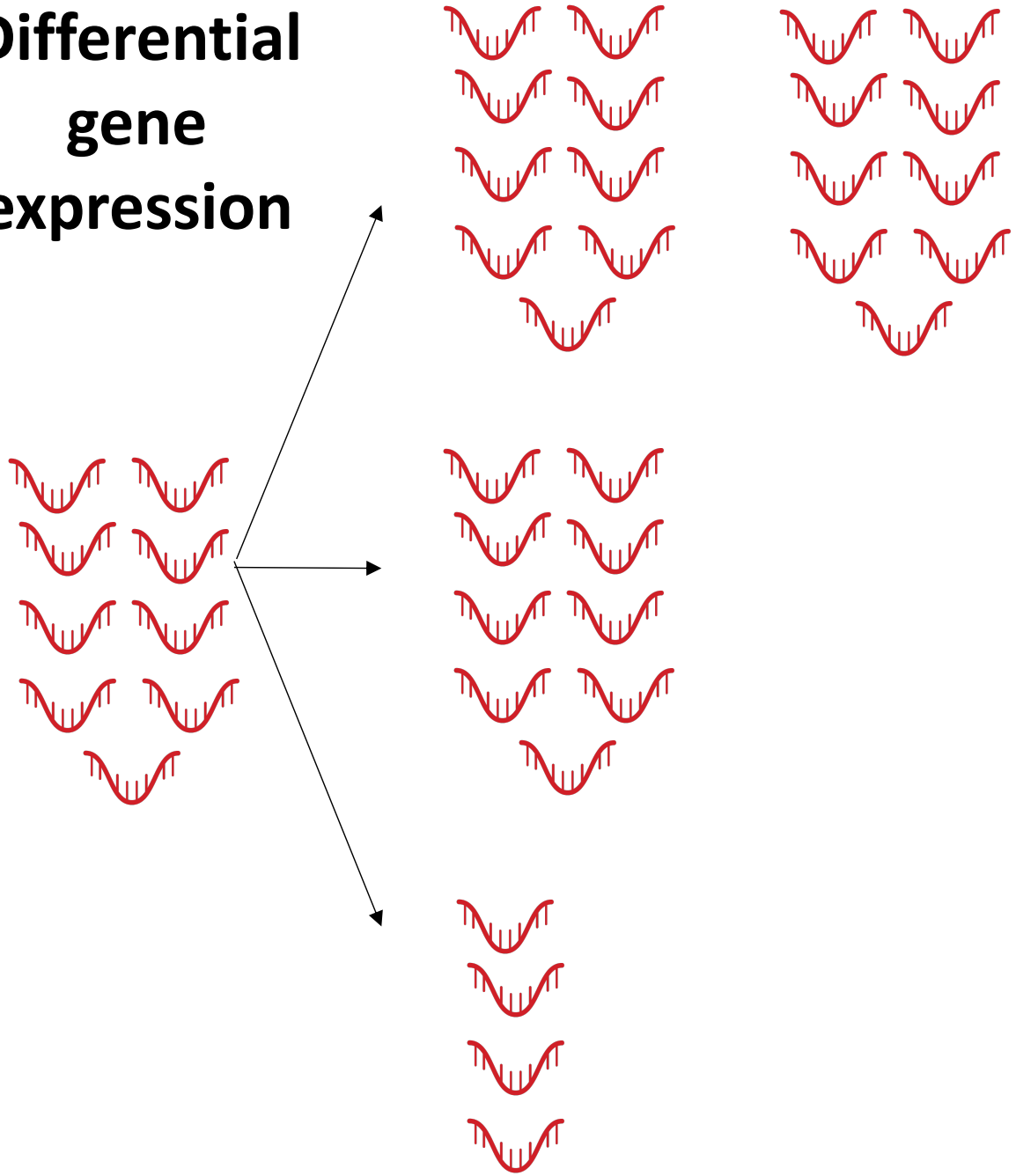



High quality RNA from blood stored in DNA/RNA Shield™
that was freeze-thawed from -80°C to room temperature.




**260/280 > 1.7 ((higher indicates RNA contamination**

# Differential gene expression



2X, upregulation

1X, no regulation

0.5X, downregulation