

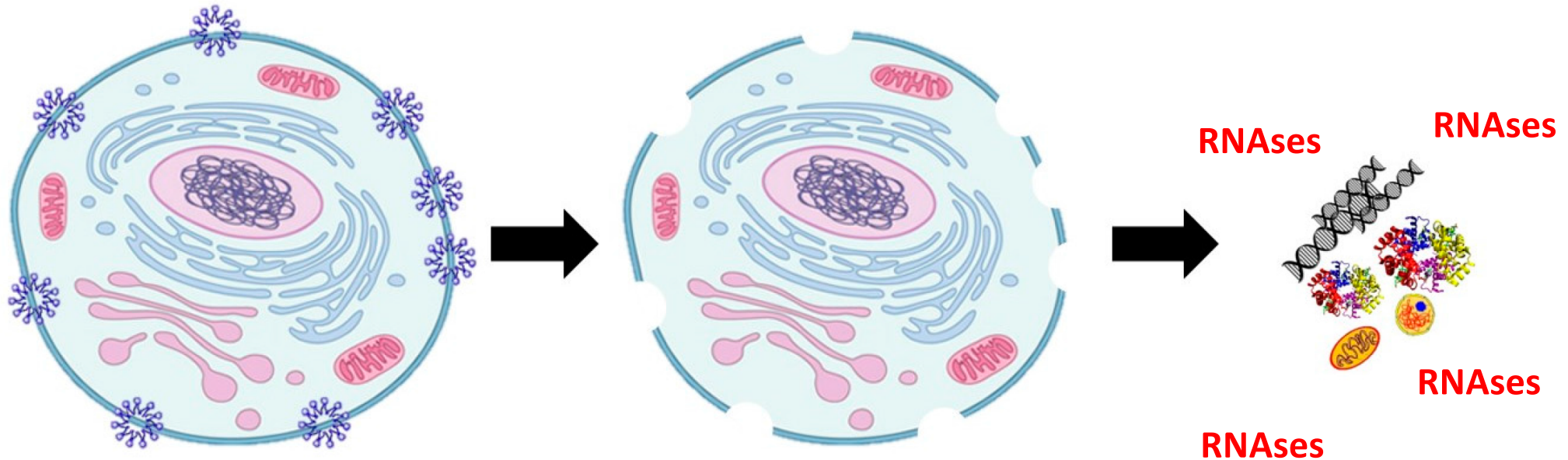
BIO 405L. Cellular and molecular biology laboratory

RNA extraction



Hugo Castillo, Ph.D.

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

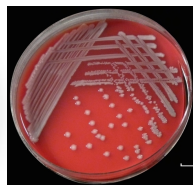


RNases and DNases are highly specific to their substrates, with RNase acting on ribonucleic acids and DNase on deoxyribonucleic acids. RNases are highly stable and resistant to environmental conditions (e.g. temperature, low pH, oxidizing agents), requiring stringent laboratory precautions to prevent 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)

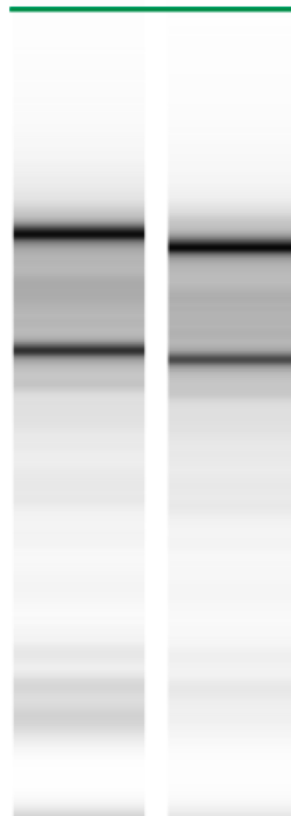
Add 300-400 uL
to pellet

Submerge

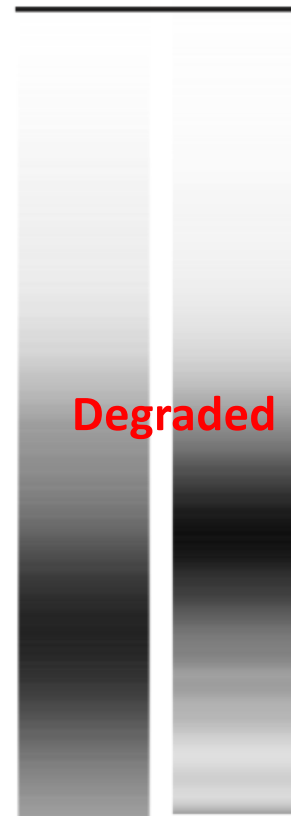


Transport at ambient temperature
(No cold-chain or dry-ice needed)

DNA/RNA Shield™



Not Protected



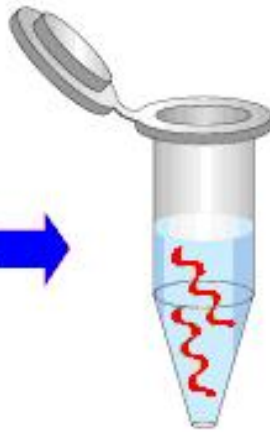
Degraded

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

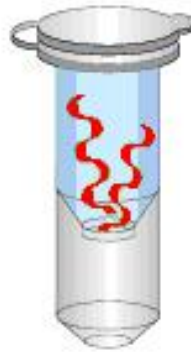
Sample
Preparation



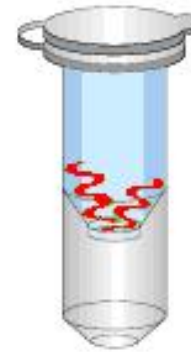
Lysis



RNA Binding

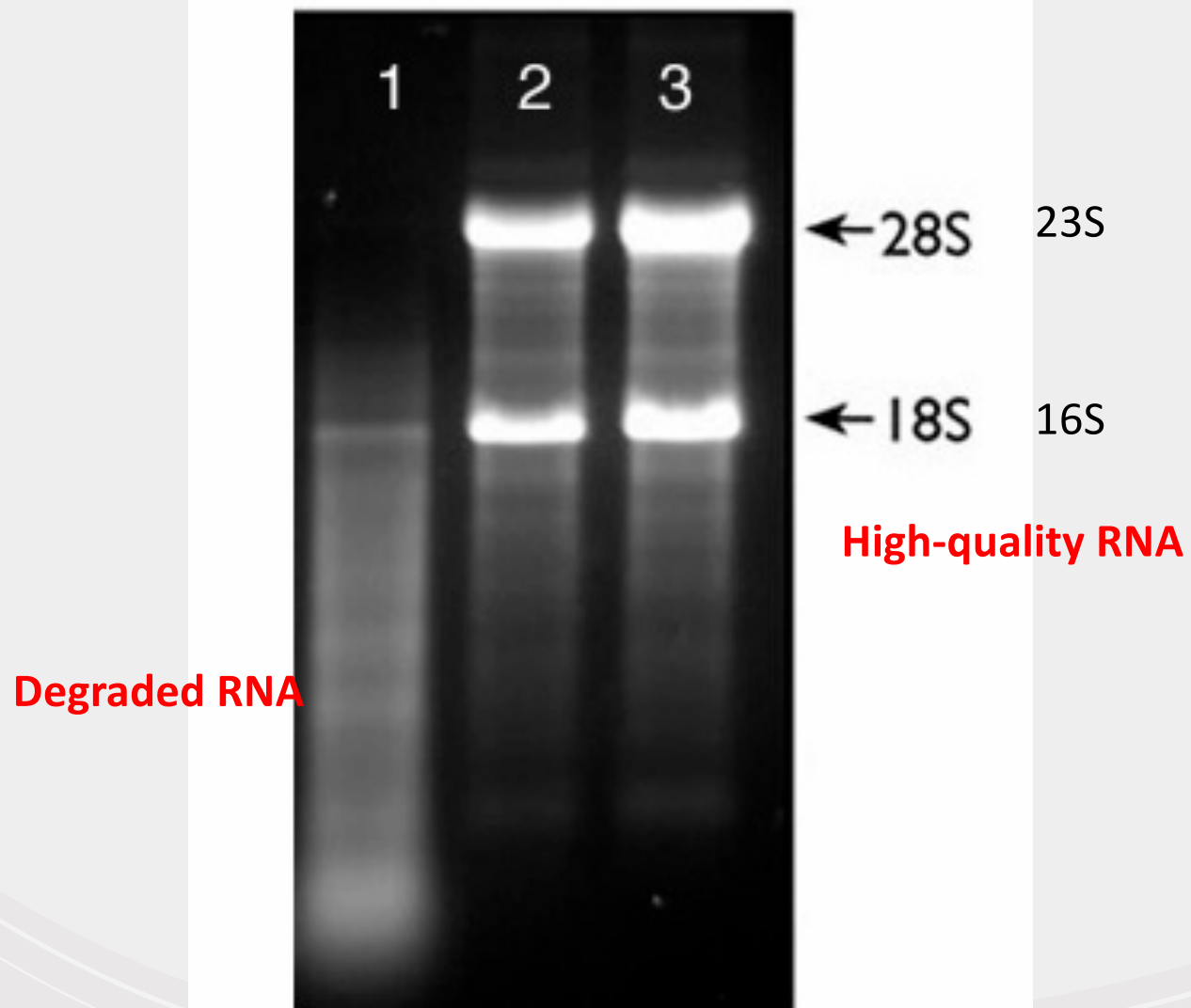


Wash



RNA Elution





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

RNA integrity measurement using capillary electrophoresis with the Agilent TapeStation.

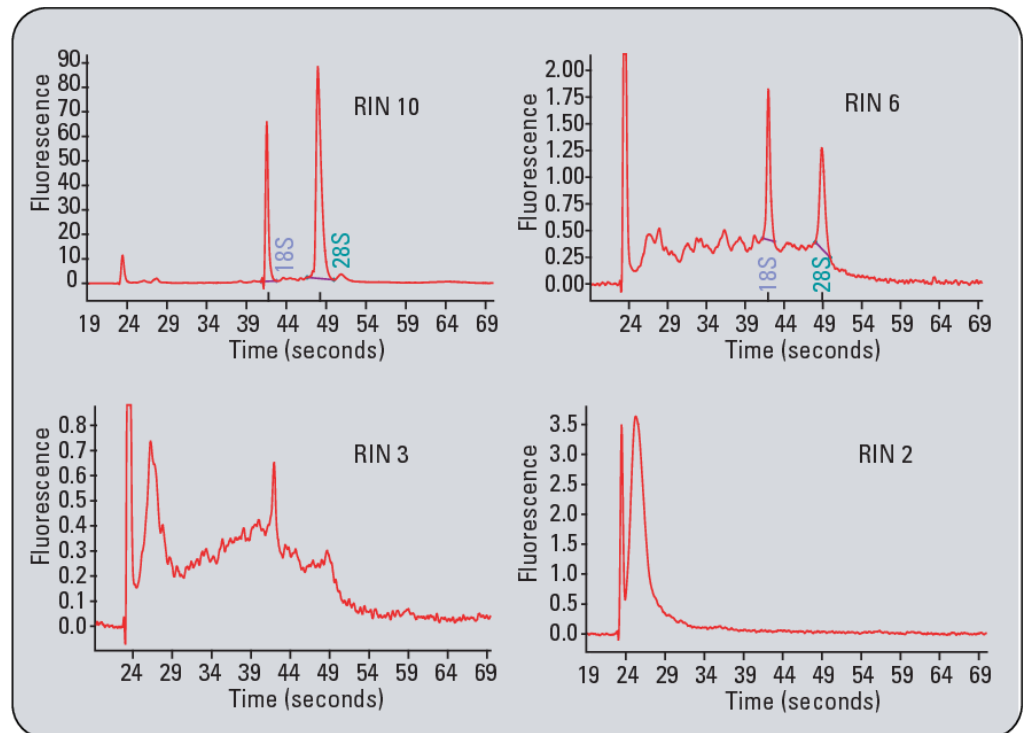
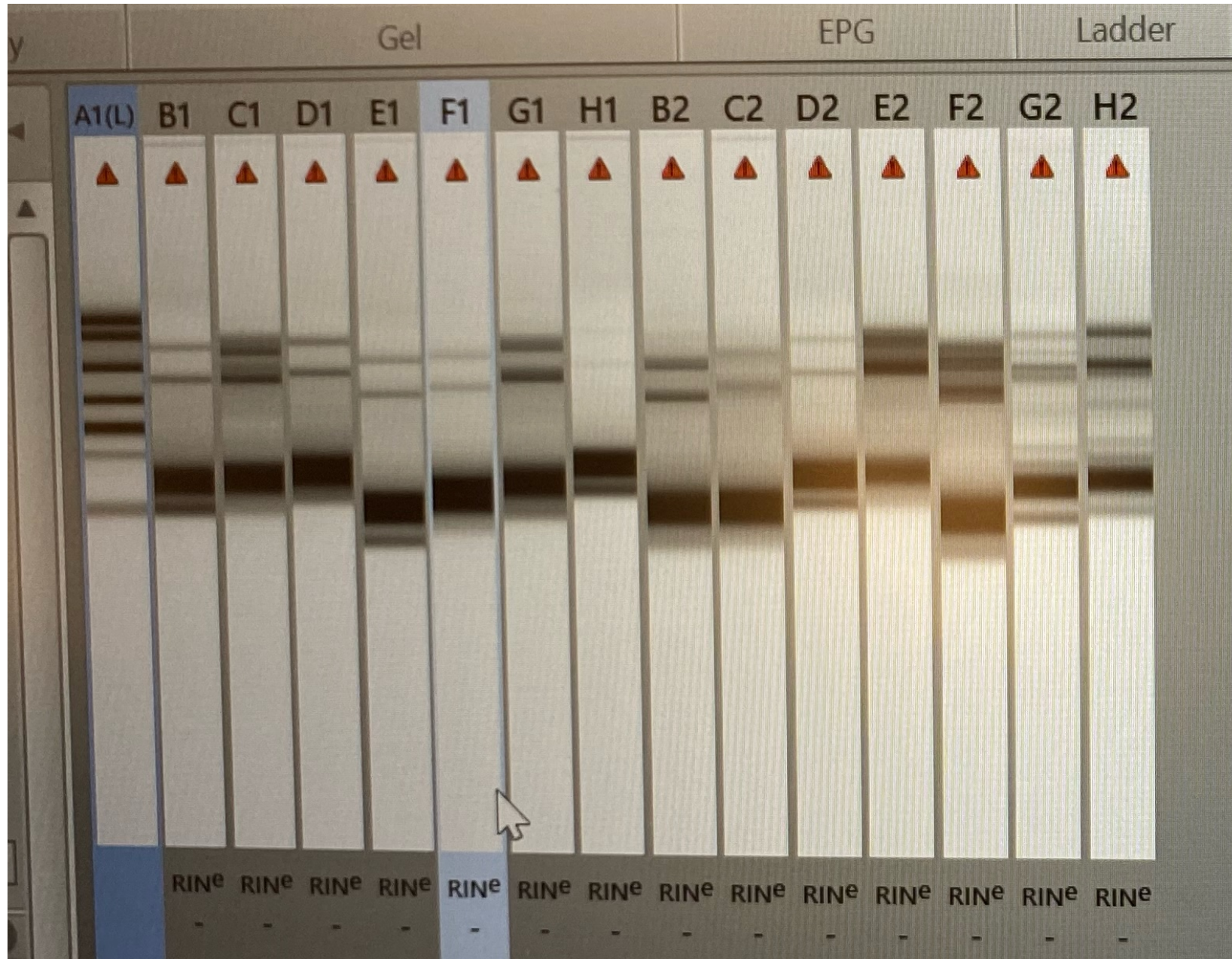
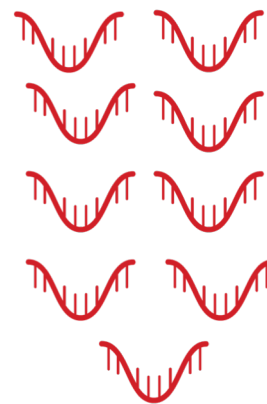
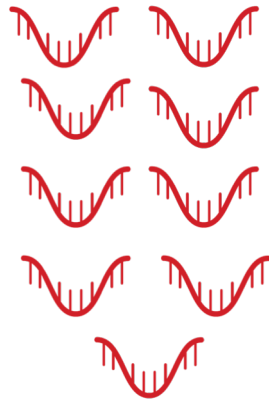
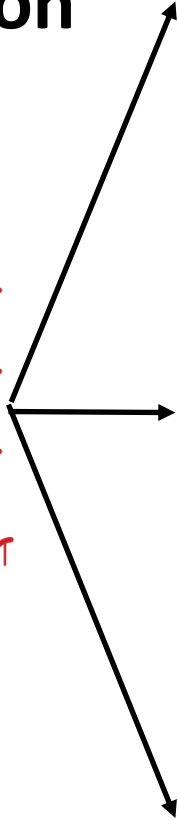


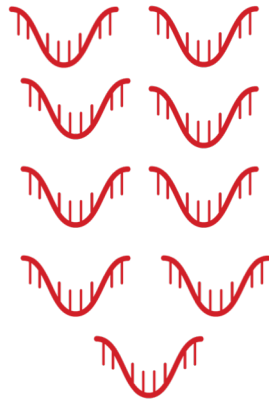
Figure 2



Differential gene expression



2X, upregulation



1X, no regulation



0.5X, downregulation