RNA Extraction – Basic Practical Knowledge

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http://rna.ucsc.edu/rnacenter/images/figs/50s_30s_labels.jpg
RNA

- Only one strand – more vulnerable than two-stranded DNA.
- Ribose-phosphate backbone
- Uracil in place of thymine
- Different RNA pool in the nucleolus, the nucleus or in the cytoplasm.
- Complicated secondary and tertiary structures for free energy minimalization.
Nature of information one can obtain through cell RNA content investigation:

- Gene expression-regulation information
- Alternative spliced transcripts investigation
- Gene fusion
- Epigenetic regulation
- Transpozone regulation
Types of RNA in the Cell

- **Micro-RNA - miRNA**: “identification code” denotes mRNAs which are to be decoyed for the ribonuclease protein complex (22-24b)

- **Small interfering RNA – siRNA**: post-transcriptional silencing and methylation of DNA target sites; heterochromatin formation (22-24b)

- **PIWI-interacting RNA – piRNA**: in the cytoplasm they are RNA decomposers, while in the nucleus they draw DNA or histone methylation; they have a role in transposon mRNA breakdown (22-24b)

- **Transfer RNA – tRNA**: amino acid transport to the ribosomes during protein biosynthesis (76-90b)

- **Small nucleolar RNA – snoRNA**: rRNA maturation (around 100b)

- **Small nuclear ribonucleic acid – snRNA**: mRNA maturation (around 150b)

- **Long non-coding RNA - IncRNA**: transcription regulation (around 200b)

- **Messenger RNA – mRNA**: template for protein biosynthesis (1900-2200b)

- **Ribosomal RNA – rRNA**: translation/protein synthesis (t.l. 7216b)
mRNA: facts that must be taken into consideration:

- **Prokaryotes**: polycistronic – more proteins from the same mRNA: at the same time more ribosomal complexes work on the same, just maturated mRNA – which on the other end might simultaneously be decayed by a protein complex

- **Eukaryotes**: the majority is monocistronic mRNA – one protein – one mRNA

- mRNA presence in cells – gene expression analysis
  - Transcription is time-dependent and abundance of mRNA in a cell depends on protein necessity and stimulus.
  - Very variable between different cells in nature and quantity from fewer than 10 copies to several hundreds of copies – good quality RNA isolate is needed for further investigations.
Choosing a well working method...
Major Steps of RNA Isolation Using Membrane-based Assays

- Rnase removal
- Homogenization of sample/Cell lysis
- Lysate filtration
- RNA precipitation
- Bind RNA to a membrane
- DNA digestion
- Cleaning RNA
- Dry membrane
- RNA elution
- Quality and quantity controle
- RNA storage
The Dreadful RNases

- Endoribonucleases or exoribonucleases.
- They are everywhere. RNase7 is secreted by human skin as antipathogene defense.
- Some tissues and cells contain them in abundant measures (e.g. pancreas, some fruits)
- Reactivate after boiling or autoclaving.
How to protect one’s RNA from them?
Protection Against RNases

- We usually never isolate DNA and RNA in the same areas/labs.
- Equipment used for RNA extraction is cleaned thoroughly and kept separate from common lab equipment.
- We treat tables, racks and platforms with various harsh chemicals (eg. DEP, formamide, chloroform, NaOH and SDS combined solution) that destroy RNases.
- Always use and often change gloves. Even for preparation of labware and chemicals.
- At the beginning of the extraction RNA is exposed to RNases released from the sample. Use of different Rnase inhibitor reagents like β-mercapto-ethanol, guanidine isothiocyanate, phenol or SDS in our solutions insures intrinsic protection of RNA.
- At the end of the procedure special care is required because in pure water RNA is very vulnerable. RNA is immediately placed on ice and frozen to -20°C or /especially in case of important samples/ -70°C.
Determining the Correct Amount of Starting Material

Depends:

- RNA content of sample
- Lysis buffer capacity
- The capacity of the method (e.g., phenol-chloroform method vs. membrane-based method) – kits usually emphasize this information
- Usually around $1 \times 10^7$ cells or 10-30mg tissue sample would work.
**Homogenization Methods**

Total disruption of the cell walls and plasma membranes is required;

Different samples require different methods.

- **Manual**:
  - Dounce (Alexander Dounce 1909–1997) – glass mortar with ceramic pestle; cells are wrecked but not organelles. (e.g., plants, filamentous fungi)

- **Mechanical**:
  - Scalpel or syringe and needle (e.g., animal cell monolayers)

- **Automatic homogenizator** (e.g., animal tissues)

- **Bead homogenizer** (plants, cells or tissues)

- **Enzimatic** (e.g., yeast)

- **Cryopulverization**

Incomplete homogenisation:
- Lower amounts of RNA
- Incomplete binding to the membrane

http://www.wenk-labtec.com/

http://shop.midsci.com/scategory/M50/316

http://cellcrusher.com/
Homogenization of Sample and Cell Lysis - Considerations

Cultured cells:
- Easier to lyse – no/little homogenization required
- Needle and syringe
- Homogeneous RNA pool

Tissue sections
- Different lysis difficulties due to ECM composition
- Homogenizers: manual, mechanical or automated
- Directly homogenise frozen samples
- Smaller sample requisite
- Heterogeneous RNA pool
- Hard to reproduce
Cell Lysis

Strong denaturating agents:

• Total RNA extraction
• Releases all nucleic acid content
• Proteins are also denatured and inactivated. Rnases, too.
• Dnase treatment required
• Tissue lysis also feasible.

Mild lysis buffer:

• Keeping cell compartments intact
• Separated isolation of different RNA pools of different compartments
• Attention to RNases! Intrinsic inactivators are inevitable to use.
Purifying RNA

- Sample filtration might be needed in case of insufficient homogenization.
- RNA precipitation is usually performed by ethanol or isopropanol.
- Binding RNA to a membrane facilitates purification/otherwise it is imperial not to loose the precipitate.
- RNA binds strongly to the silica membrane in presence of high salt concentration, and strong denaturing agents (e.g., guanidium salts).
- DNA digestion by DNase I – very sensible to physical denaturation and functionates at room temperature.
- After centrifugation and washing steps, the nucleic acid can be washed off with low salt aqueous solution!
RNA Concentration and Quality Control

- **Quantity might be determined by:**
  - spectrophotometric quantification: 260nm: 1U corresponds to 40µg/ml
  - Multi-Mode Microplate Reader: at 260/280nm using the Gene5. program / Nucleic Acid Quantification

- **RNA quality control:**
  - 260/280nm: <1.8 protein contamination. Optimal: 2.00 (1.8-2.3)
  - 260/230nm: <1.8 other organic contaminants. Optimal: 2.0-2.4
  - 260/240nm: <1.4 ionic contamination. Optimal: 1.4

- **Gel electrophoresis:**
  - agarose gel at a 1.2% concentration. For mammalian rRNA, a 28S:18S rRNA ratio of 2:1 is generally representative of good-quality RNA.
  - Special RNase free and denaturating formaldehyde agarose gel electrophoresis.

- **Bioanalyser:** software-based electrophoresis analysis
RNA Quality Control 2.

Phenol-chloroform method

28S RNA ~5kb long
18S RNA ~2kb long
5SRNA
trNA

Membrane-based on-column isolation

28S RNA - ~5kb long
18S RNA - ~2kb long

Storage

• Short term (<1 week) dissolved in nuclease-free water or alcoholic precipitate at -20°C.

• Long-term storage (2-6 months): dissolved in nuclease-free water at -80°C.

• In pure formamide (100%), FORMAzol® or RNALater® up to two years at -20°C, BUT: it must be cleaned prior to reverse transcription!

• Aliquoting samples is very useful in case of multiple downstream use – to avoid thaw-freeze cycles
Thank You for Your attention!

http://evolution.berkeley.edu/evolibrary/article/ellington_03