RNA Extraction – Basic Practical Knowledge

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OKK-OSSKI
PCR training course
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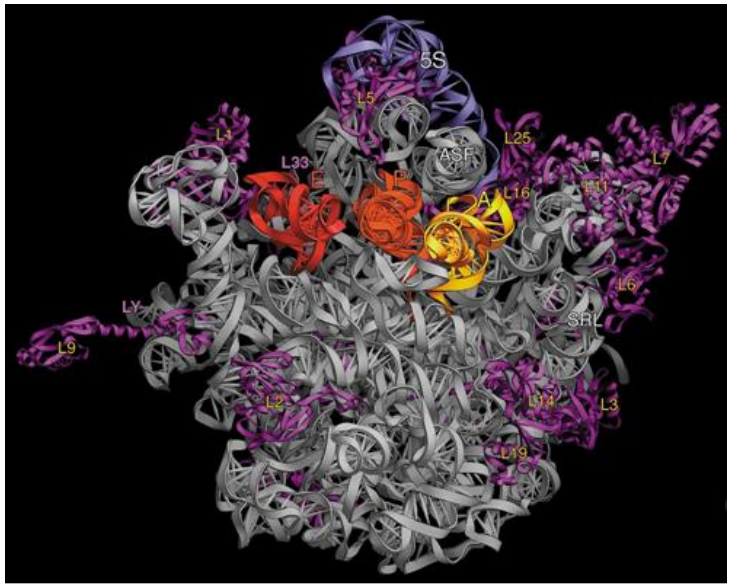
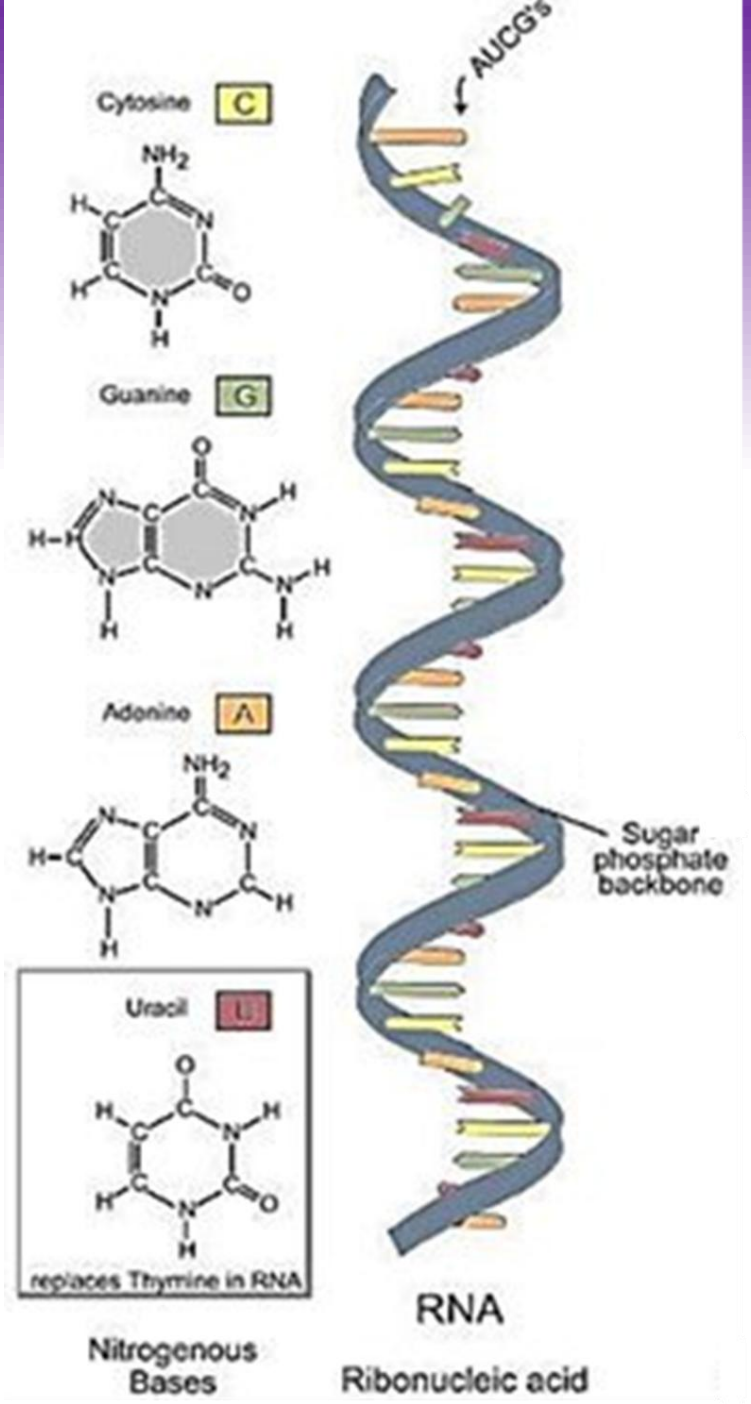


http://rna.ucsc.edu/rnacenter/images/figs/50s_30s_labels.jpg

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 terciary structures for free energy minimalization.

http://smart-therapeutics.com/Technology/What-is-RNA

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 interferring 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 - lncRNA: transcription regulation (around200b)
- 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:

- **Procaryotes**: 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
- **Eucharyotes**: 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.

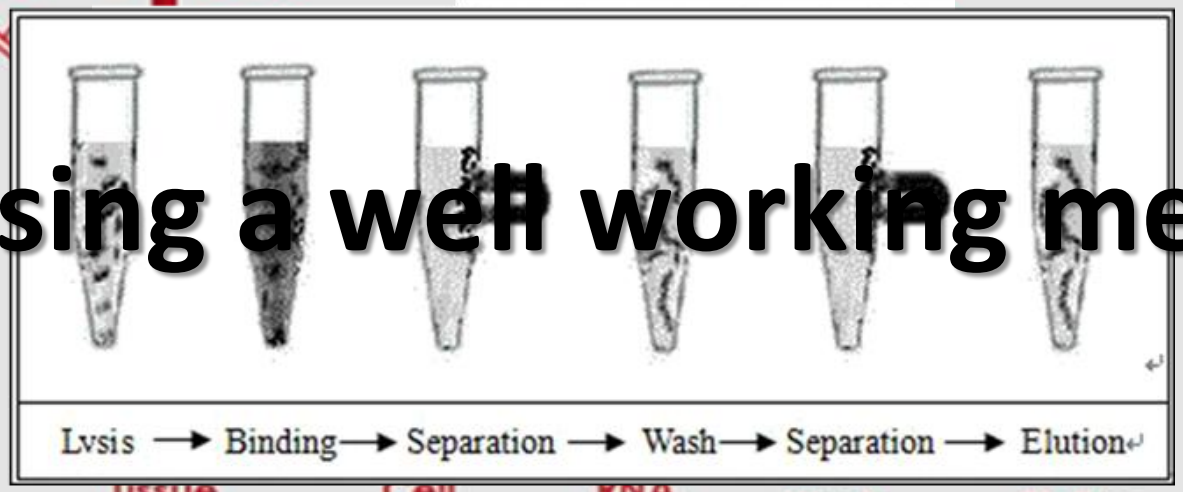
Choosing a well working method…

Phenol chloroform based RNA isolation

Membrane based on- column purification

Methods based on magnetic beads

Trizol based RNA isolation



http://www.genmagbio.com.cn/site/a0.php

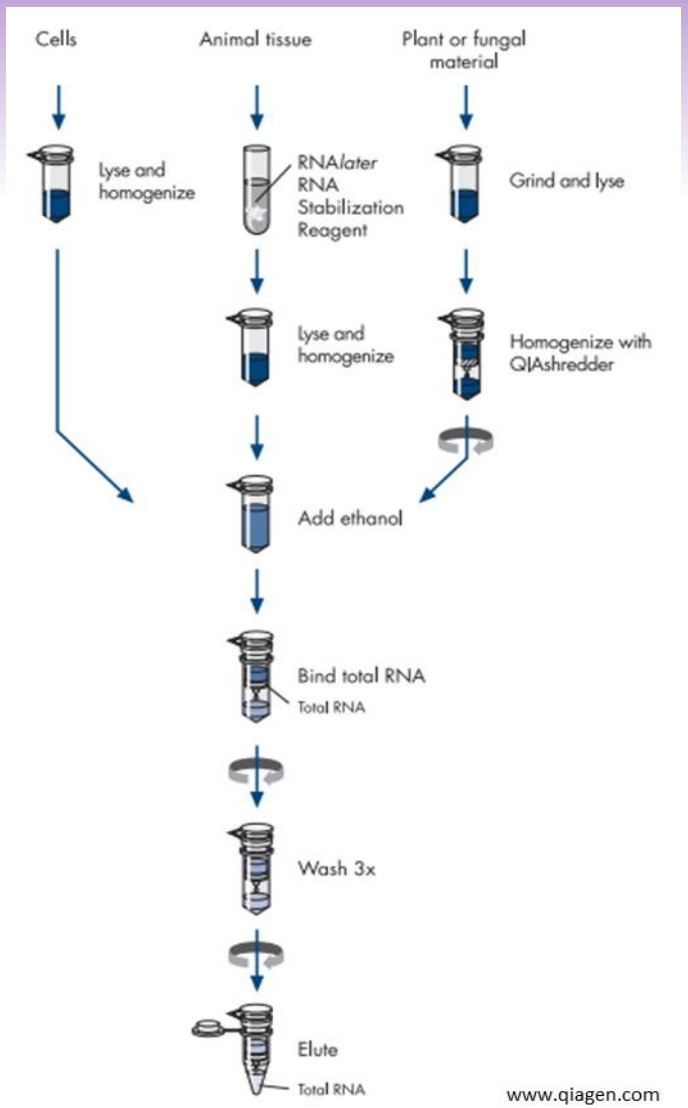
http://www.yeastern.com/Products.php?pid=147&pkid=9&pttype=56

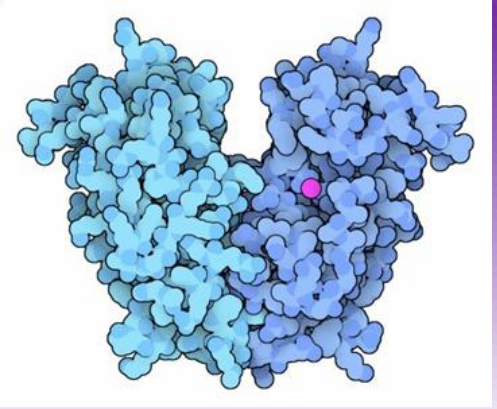
RNA

http://www.rrnursingschool.biz/restriction-enzyme/dna-purification.html

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



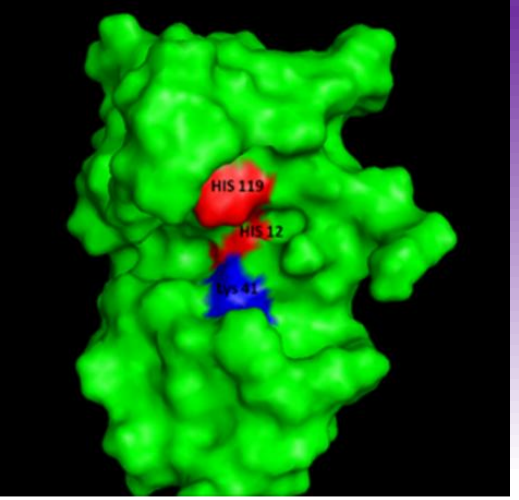


http://sbkb.org/fs/rnase-t

The Dreadful RNases

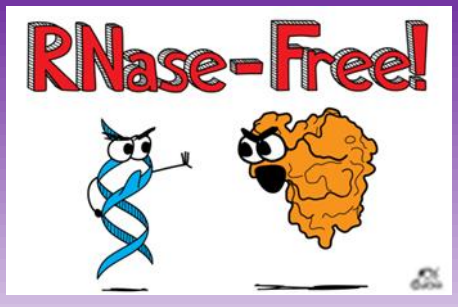
- Endoribonucleases or exoribonucleases.
- They are everywhere. RNase7 is secreted by human skin as antipathogene defense.
- Some tissues and cells contain them in abundant measures (eg. pancreas, some fruits)
- Reactivate after boiling or autoclaving.

How to protect one’s RNA from them?



http://graylab.jhu.edu/courses/540.414/

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-etanol, 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 (eg: phenol-chloroform method vs. membrane based method) – kits usually emphasize this information
- Usually around $1*10^7$ cells or 10-30mg tissue sample would work.

Total RNA yields obtained with the RNeasy Mini Kit

Source	Starting material	Average yield (µg)
Animal cells		
LMH	1×10^6	12
HeLa	1×10^6	15
COS-7	1×10^6	35
Lymphocytes (unstimulated)	1×10^6	0.5
Mouse tissue		
Liver	10 mg	40
Lung	10 mg	10
Spleen	10 mg	35
Yeast cells		
S. cerevisiae	1×10^7	25

www.qiagen.com

Homogenization Methods

Total disruption of the cell walls and plasma membran[illegible]e required

Different sam[illegible]

Homog[illegible]

compo[illegible]

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

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

- Man[illegible] with[illegible] s. (eg.
- Mec[illegible] al cell mon[illegible]
- Autc[illegible]
- Bead homogenizer (plants, cells or tissues)
- Enzimatic (eg. yeast)
- Cryopulverization

http://cellcrusher.com/

Incomplete homogenisation:

- Lower amounts of RNA
- Incomplete binding to the membrane

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, mechanincal 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 denaturated and inactivated. Rnases, too.
- Dnase treatment required
- Tissue lysis also feasible.

Mild lysis buffer:

- Keeping cell compartements 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 etanol 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 (eg .: 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

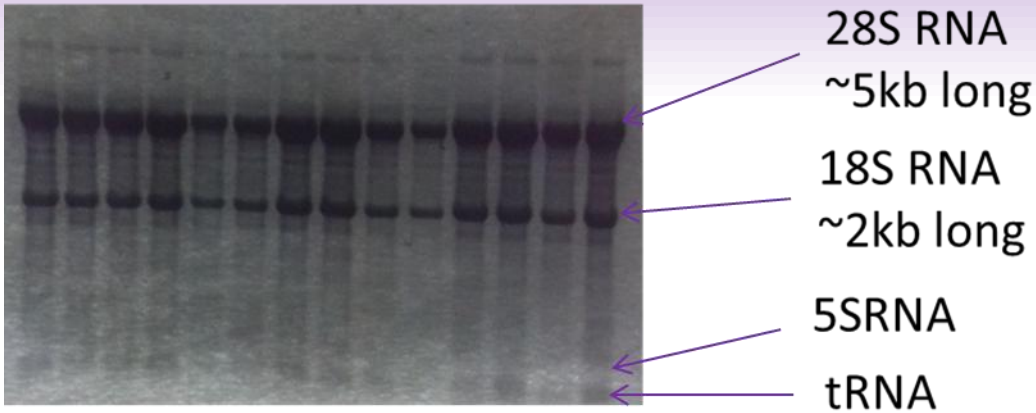
- **Quantity might be determined by**:
  - spectrophotometric quantification: 260nm: 1U corresponds to 40µg/ml
  - Multi-Mode Microplate Reader: at 260/280nm using the Gene5. program / Nucleic Acid Quantification

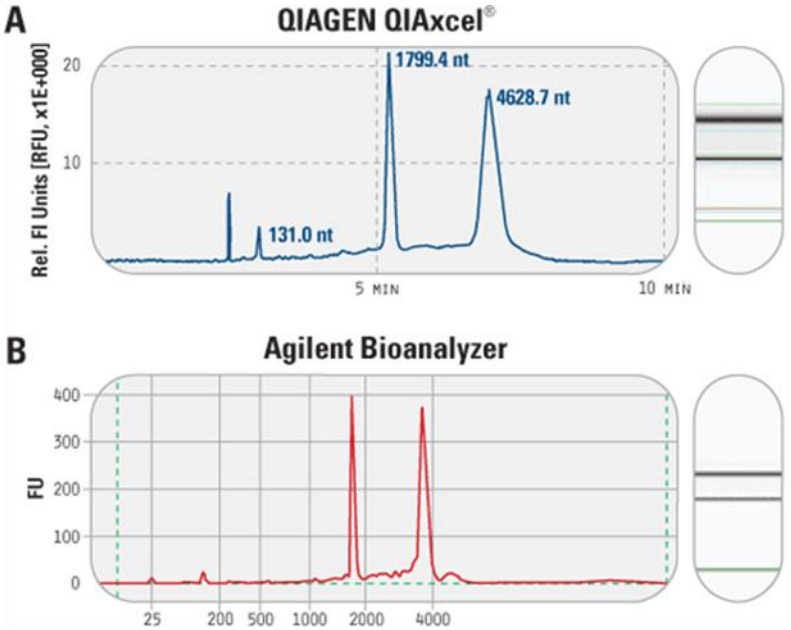
- **RNA quality control**:
  - **260/280nm**: <1.8 protein contamination. Optimal: 2.00 (1.8- 2.3)
  - **260/230nm**: <1.8 other organic contaminants. Optimal: 2.0-2.4
  - **260/240nm**: <1.4 ionic contamination. Optimal: 1.4

  - **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.

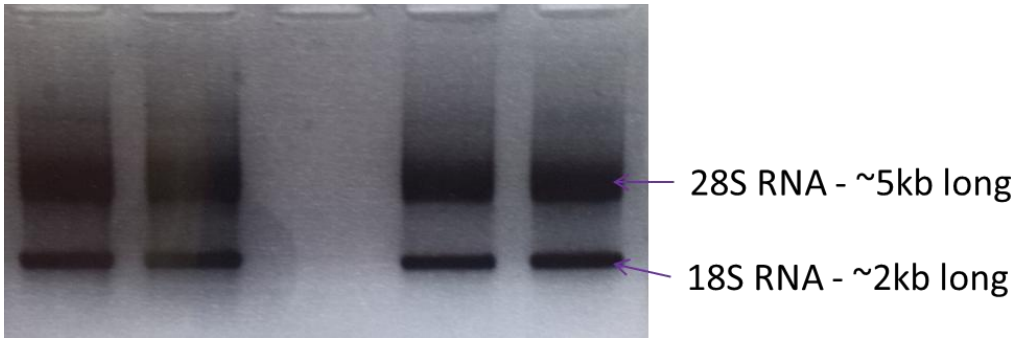
Phenol-chloroform method





http://www.sabiosciences.com/pathwaymagazine/pcrhandbook/qiagen-rneasy-kits.php

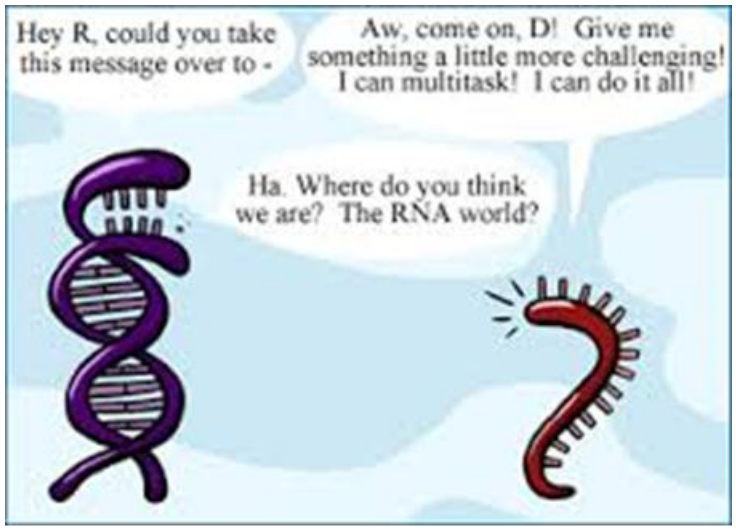
Membrane-based on-column isolation



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-freez cycles

Thank You for Your attention!



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