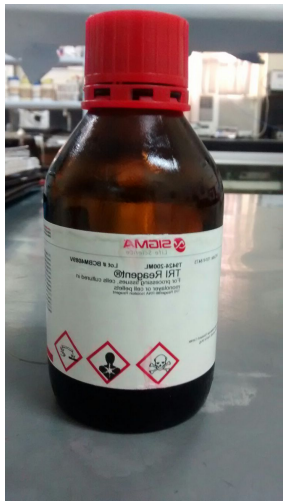
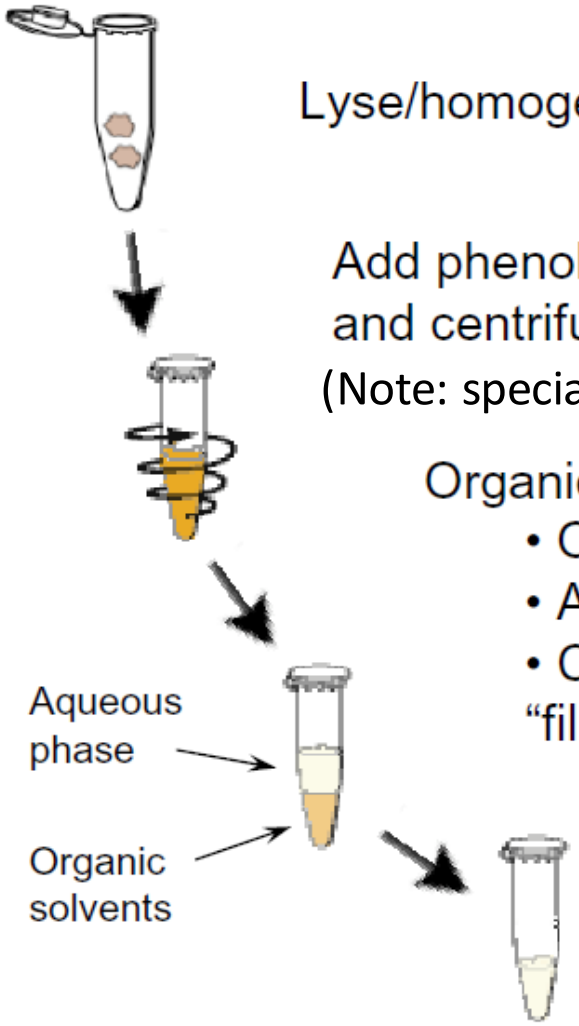
RNA purification techniques

- **Total RNA from biological samples**
  - Organic extraction
  - Affinity purification

- **mRNA from total RNA**
  - Oligo(dT) resins

Organic extraction of total RNA





Lyse/homogenize cells

Add phenol:chloroform:isoamyl alcohol to lysed sample, and centrifuge

(Note: specialized reagetns exist and are communly used: Trizol – Trireagent)

Organic phase separates from aqueous phase

- Organic solvents on bottom
- Aqueous phase on top (contains total RNA)
- Cellular debris and genomic DNA appears as a "film" of debris at the interface of the two solutions

Remove RNA solution to a clean tube; repeat PCI extraction precipitate RNA and wash with ethanol, then resuspend RNA in water

Conditions for precipitations: the same like for genomic DNA

Note: NO Proteinase K digest; chromatin precipitates at interphase

Organic extraction of total RNA

Advantages

- Versatile - compatible with a variety of sample types
- Scalable - can process small and large samples
- Established and proven technology
- Inexpensive

Disadvantages

- Organic solvents
- Not high-throughput
- RNA may contain contaminating genomic DNA

DNase treatment – Phenol extraction – precipitation
DNase treatment – purification via resin

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Affinity purification of total RNA

Lyse cells, and spin to remove large particulates/cell debris

Apply lysate (containing nucleic acids and cellular contaminants) to column with glass membrane

Wash with alcohol to remove contaminants; nucleic acids stick to glass membrane while contaminants wash through. Treat with DNase enzyme to remove contaminating DNA.

Apply water to the column; purified RNA washes off the glass and is collected

Affinity purification of total RNA

Advantages

- Eliminates need for organic solvents
- Compatible with a variety of sample types (tissue, tissue culture cells, white blood cells, plant cells, bacteria, yeast, etc.)
- DNase treatment eliminates contaminating genomic DNA
- Excellent RNA purity and integrity

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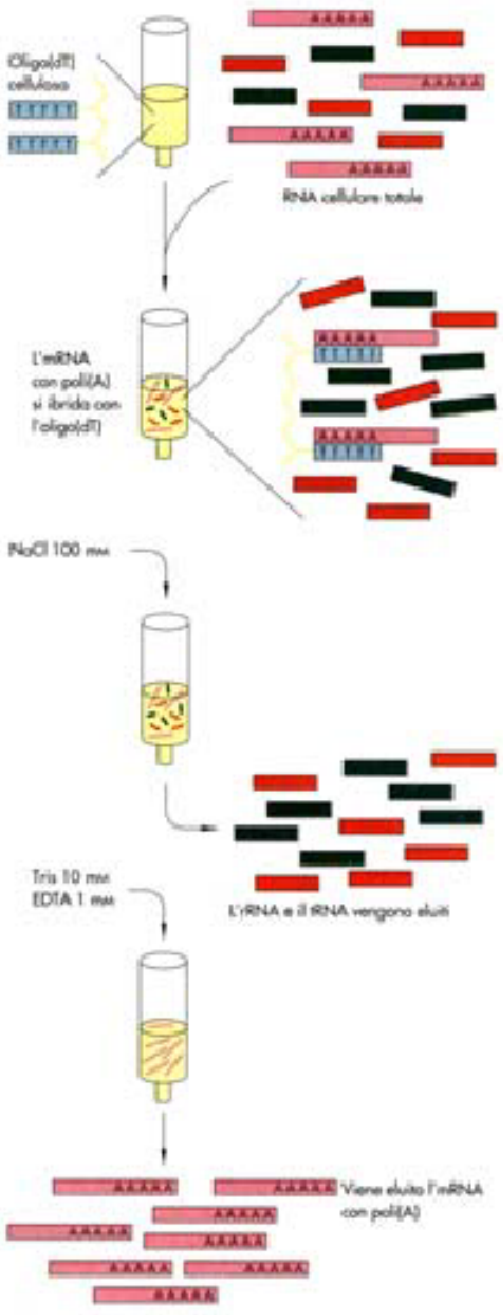
mRNA purification



Solo gli mRNA possiedono "code" di poli(A) lunghe 30-150 residui. Le sequenze di oligo(dT) sono immobilizzate su supporti solidi, in genere di cellulosa.

L'RNA totale viene denaturato e quindi applicato alla colonna in una soluzione salina concentrata (NaCl 500 mM).

Si effettuano molti lavaggi con una soluzione salina meno concentrata (NaCl 100 mM), per rendere più selettivo il legame tra RNA e l'oligo(dT).

Aggiungendo una soluzione di TE (Tris/EDTA) si recupera l'RNA messaggero legato alla colonna.

Eventualmente si ripete il ciclo applicazione-lavaggi-eluizione.

La frazione poli(A)+ RNA contiene sempre una minima parte di RNA non poliadenilato.

Total RNA preparation is passed through a column consisting of a coated polymer with oligo (dT).

Only polyadenylated mRNA will hybridize with oligo (dT), while other species will be eliminated by washing with low concentration pads saline.

The final eluate will be made up of mixture of all mRNA species present in the cell at the time extraction.

Storage of RNA

Buffer

- 1xTE solution (1 mM EDTA, Tris-HCl, pH 6.5): pH should always be <7 (high risk of alkaline hydrolysis)

– no DEPC treatment possible (DEPC destroys Tris)

- RNase free water (DEPC treated); check pH of water: <7.0

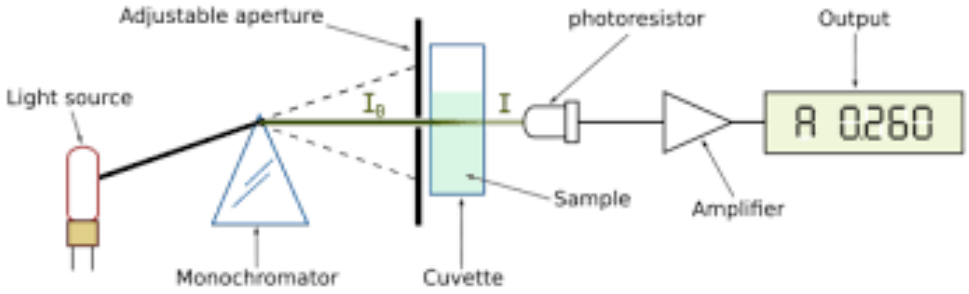
Temperature:

- Always -80°C when aqueous solution
- As precipitate in 70% ethanol, DNA/RNA can be stored at -20°C almost indefinitely, without loosing integity

Quantification of DNA and RNA concentration and purity

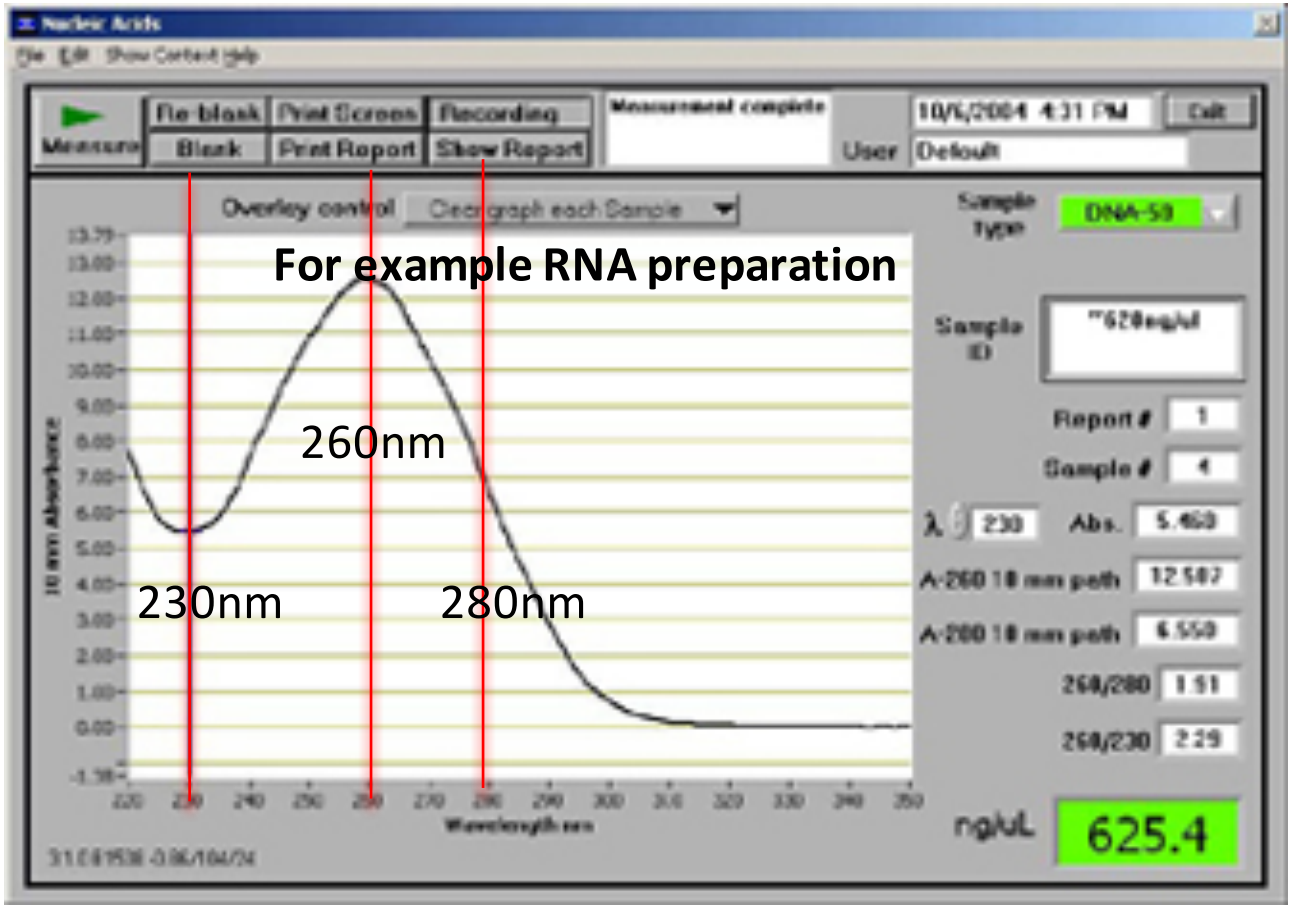
It operates on Beer' s law:

When monochromatic light (light of a specific wavelength) passes through a solution there is usually a quantitative relationship (Beer's law) between the solute concentration and the intensity of the transmitted light, that is, the more concentrated the specimen is, the less light is transmitted through it.



Measurement with a SPECTROPHOTOMETER

→ **Measure absorbance of DNA/RNApreparation = concentration**



Maximum absorbance:

RNA, DNA: 260 nm

Protein: 280 nm

Solvents/carbohydrates: 230 nm

CRITICAL PARAMETERS OF DNA AND RNA PREPARATIONS

- **Quantity**
- **Purity**
- **Integrity**

- **Purity** (contamination with proteins/carbohydrates/solvent)

- **ratio A_{260}/A_{280}**
- **ratio A_{260}/A_{230}**

Nucleic acids absorb at A=260nm

Proteins absorb at A=280 nm

Carobohydrates and phenol (solvents) absorb at A=230nm

Ratio A260/A280 = quantification of protein contamination

For DNA preparations: ratio: 1.6-1.8 (A_{260}=0.234; A_{280}=0,137: ratio =1,7 → OK)

For RNA preparations: ratio: 1.8-2.0

Il ratio is out of range : **contamination with proteins** (DNA A_{260}=0.234; A_{280}=0,199: ratio =1,1 → NO)

Ratio A260/A230 = quantification of contamination with carbohydrates and phenol (solvents)

For RNA and DNA: ratio must be ca. 2.2

ratio < 2,2: contamination with solvent/carbohydrate

http://cshprotocols.cshlp.org/content/2007/11/pdb.ip47.full

Quantification of DNA and RNA concentration and purity

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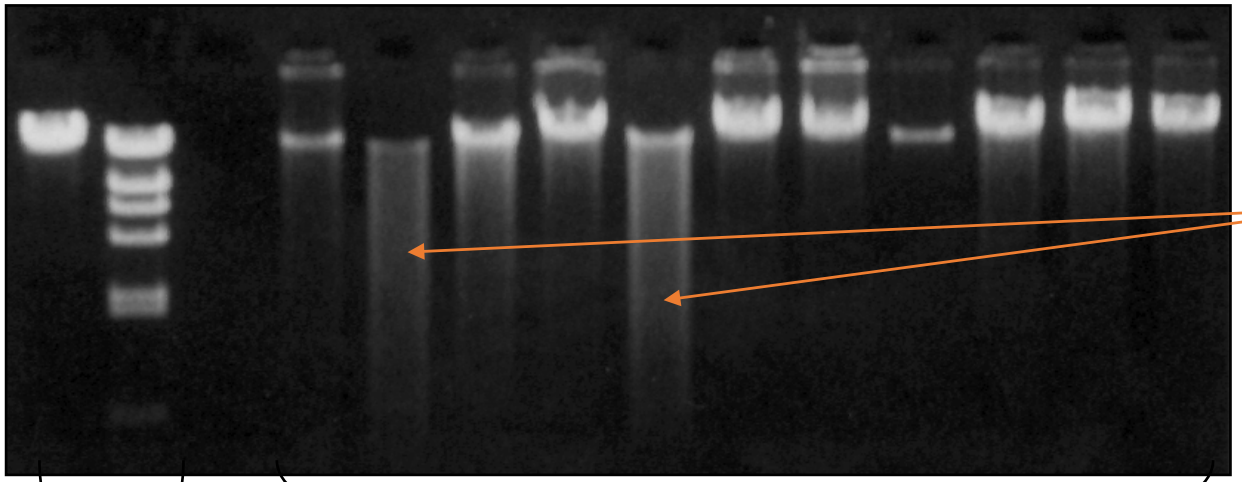
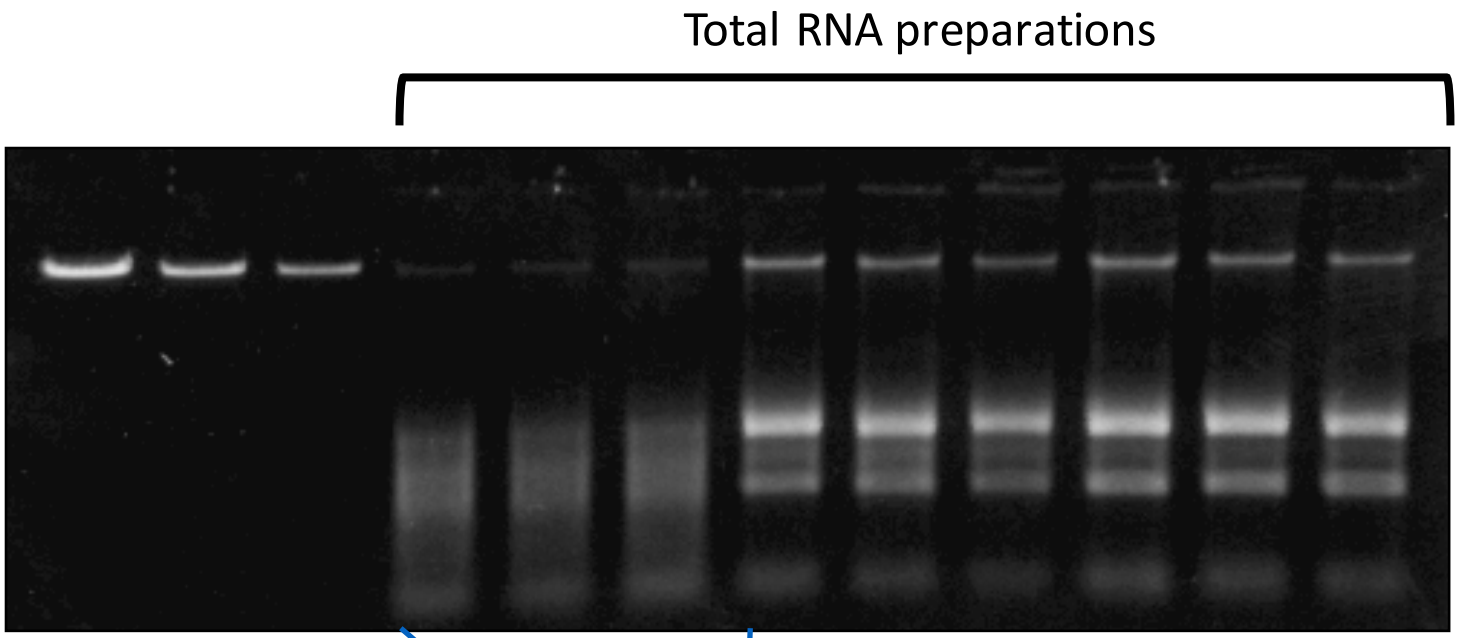
• DNA / RNA Integrity

Easiest method: Gel electrophoresis

Gel di agarosio, le bande di DNA sono state messe in evidenza introducendo nel gel etidio bromuro una sostanza che si inserisce tra le basi del DNA diventando fuorescente.

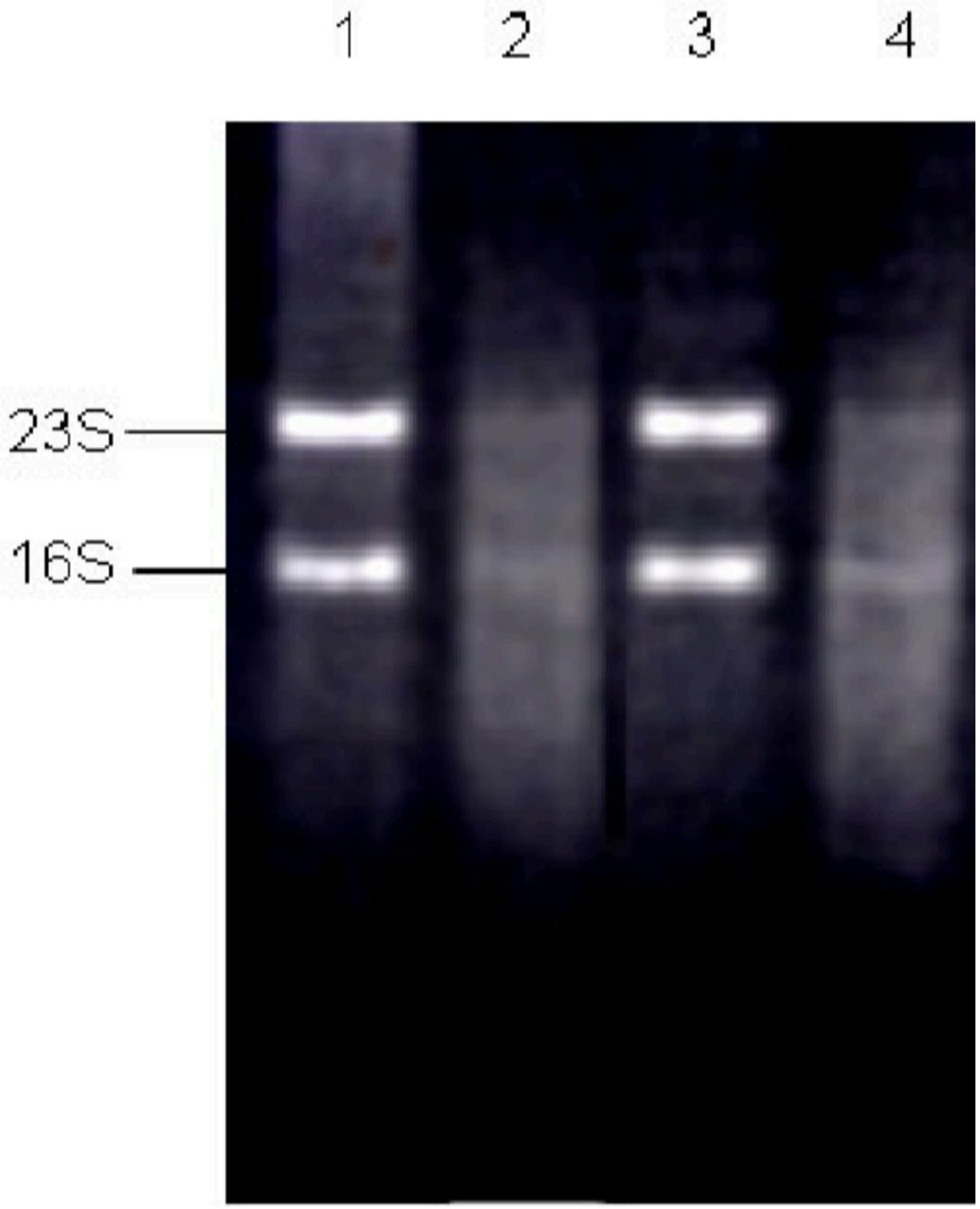


Determination of RNA integrity



Attenzione: Se si è purificato mRNA, esso appare come una scia

Determination of RNA integrity – poly A RNA (mRNA)



polyA RNA (mRNAs)

Lane 1+2 Patient 1 blood cells used to prepare RNA

Lane 1: total RNA

Lane 2: poly A RNA fraction prepared from total RNA

→ smear! (scia)– **normal pattern for poly A** RNA (no degradation!!)

→ **Lane 3+4 Patient 1 blood cells used to prepare RNA**

Lane 3: total RNA

Lane 4: poly A RNA fraction prepared from total RNA

→ smear! (scia)– **normal pattern for poly A** RNA (no degradation!!)