# Isolation of genomic DNA

**The isolation method of choice is dependent upon:**

**The source of the DNA:**
cells, tissue, bacteria, virus etc.;

**The final application:**
Plasmide prep, PCR, restriction, sequencing, fingerprinting, library construction etc.;

**The type of DNA:**
genomic vs plasmid (size)

# Why to prepare DNA

- Many applications require purified DNA.
- Purity and amount of DNA required (and process used) depends on intended application.
- Example applications:

- ➢ Tissue typing for organ transplant
- ➢ Detection of pathogens
- ➢ Human identity testing
- ➢ Genetic research
- ➢ Amplification of region of research interest

# Isolation of Genomic DNA

## Solid tissue (i.e. mouse organ)

Homogenise, chemically or mechanically
**a)** Ultrasould vibration (Vibrazioni ultrasoniche)
**b)** Homogenization (omogeneizzatore)
**c)** Freeze/thaw (congelamento/scongelamento)
(note difference: Alkaline lysis for bacterial plasmids)

} Lysate

OMOGENIZZATORE

SONICATORE

## Single cell suspension (bacteria, cells in cell culture or blood)

**a) Cell wall rupture**
Bacteria (Gram-) – lysozyme or SDS, NaOH (pH alto)
Yeast/fungi - zymolase

} Lysate

**b) Cell membrane rupture**
- Detergents: SDS, sarcosine, triton X-100, CTAB Proteinases; Proteinase K, Pronase E
- Chelators - EDTA
- Guanidine thiocyanate/chloride, urea

} Lysate

# General steps in DNA Isolation from lysates

## 1. Genomic DNA from cell lysates

- SDS/Proteinase K (Organic method)
- Silica columns
- Alkaline method
- Automated method

## 2. Plasmid DNA

- Alkaline lysis method with or without silica columns

## 3. Bacteriophage DNA

- PEG/Salt precipitation method

# General steps in DNA Isolation

1. **Genomic DNA**

   **- SDS/Proteinase K (Organic method)**
   **- Silica columns**
   **- Alkaline method**
   **- Automated method**

2. **Plasmid DNA**

   **- Alkaline/SDS method**
   **- Silica column method**

3. **Bacteriophage DNA**

   **- PEG/Salt precipitation method**

# DNA Preparation from cell lysates
# "The organic method"

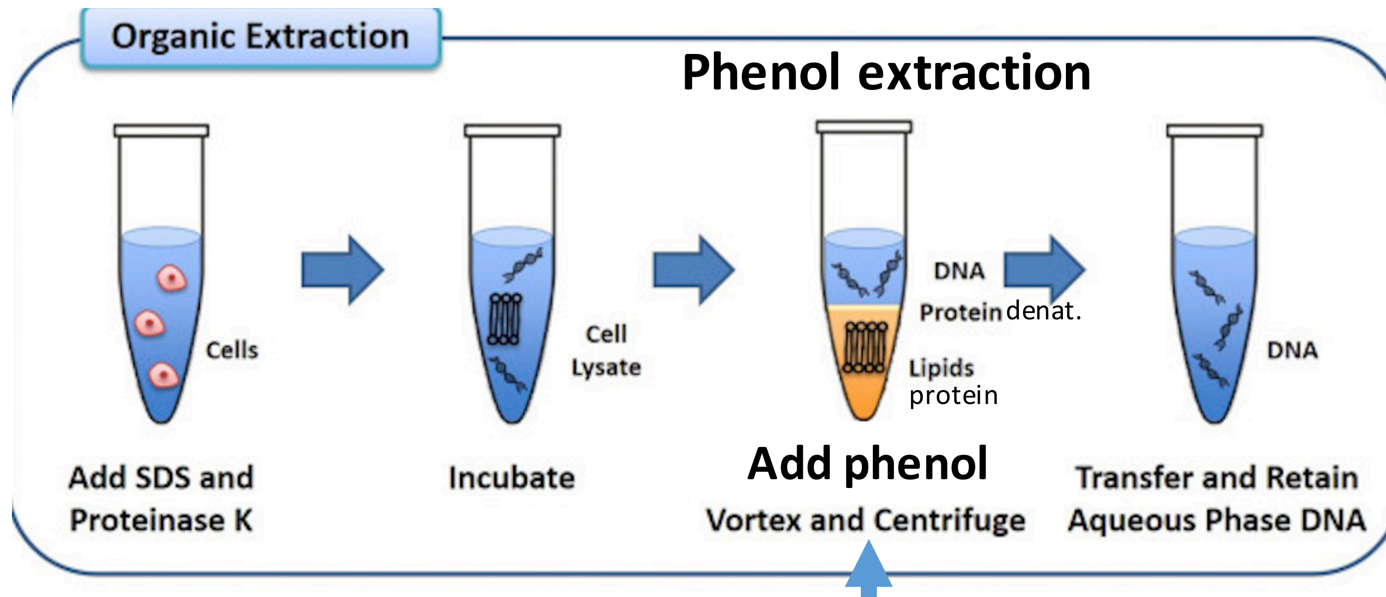
**Step 1: DNA preparation: Open cells, digest proteins, extraction of genomic DNA**
**Step 2: precipitation of DNA → concentrate DNA**

- **Lyse cells (or tissues) in buffer with SDS/PK /(DTT) (500 ul)**
    - SDS = detergent (solubilizes cell membrane)
    - PK = proteinase K (degrades proteins)
    - DTT = reducing agent - breaks disulfide bonds in folded proteins
    - 65°C, agitation, 1 hour - over-night (depending on amount of DNA)
    - At 65°C over-night most RNA is degraded

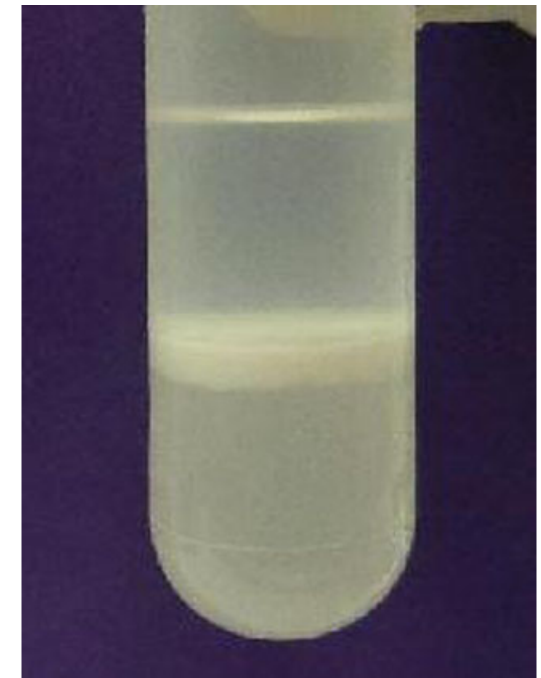
- **Separation of hudrophobic and hydorphilic components: add equal volume of phenol (500 ul); mix**
    - Protein fragments and lipids attracted to hydrophobic phenol
    - Nucleic acids attracted to water
    - → separates aqueous phase (DNA, RNA) from organic phase (lipids, proteins)

**SDS: Sodium dodecyl sulfate**

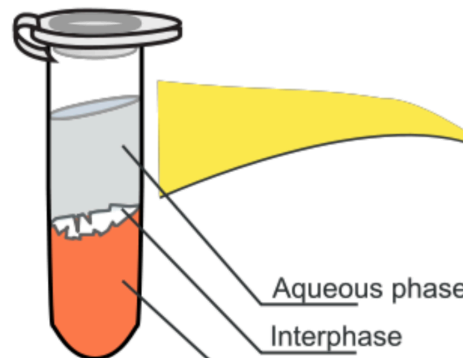
# DNA Preparation from cell lysates
# "The organic method"




Aqueous phase contains DNA (+RNA) and rests of phenol and rests from interphase



Phase separation




Fase acquosa (RNA, DNA)

Interfaccia (Proteine denaturate)

Fase fenolica (Proteine solubile, lipidi)

**Phenol**:
is a strong denaturant of the protein; Denatured proteins, with hydrophobic groups exposed, become soluble in the phenolic phase or precipitate at the phenol-water interphase. Lipids from membranse dissolve in phenol.

**PHENOL IS HIGHLY TOXIC – WORK IN FUME HOOD WITH PROTECTION GLASSES**

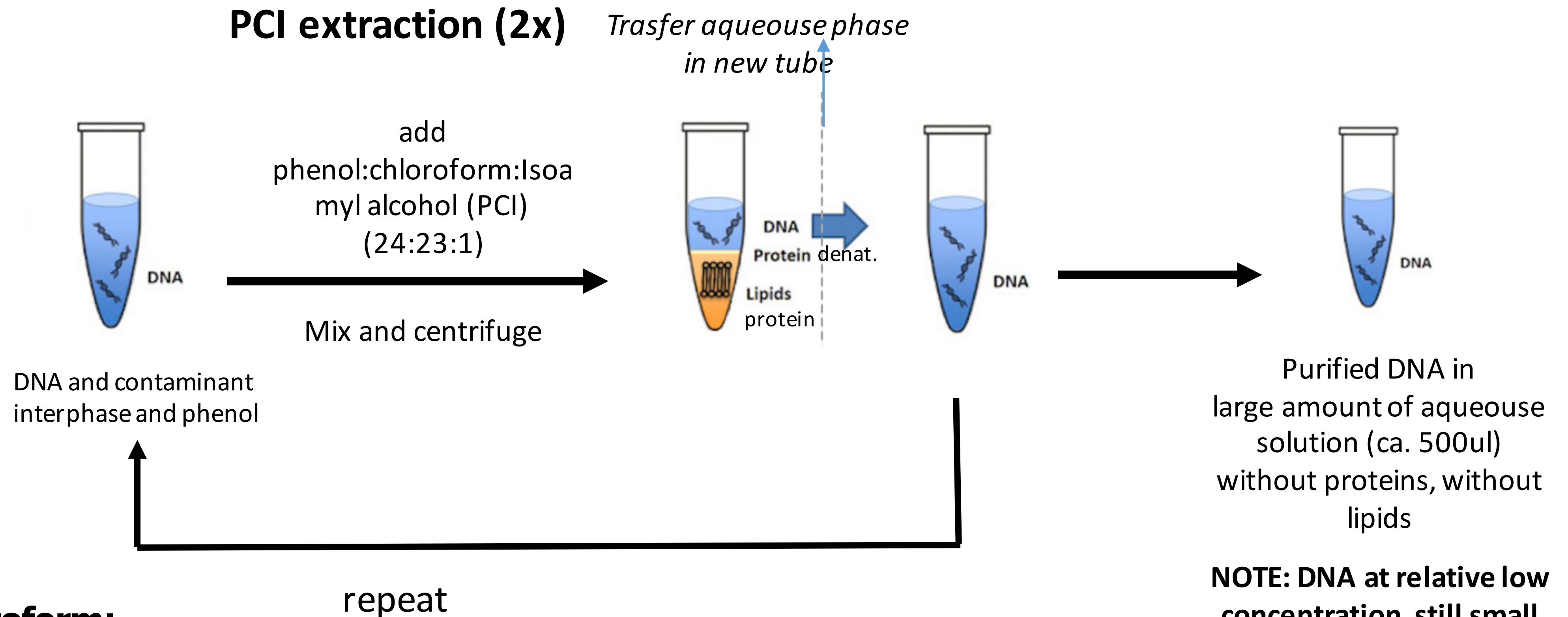
# DNA Preparation from cell lysates
# "The organic method"

**Step 1: DNA preparation: Open cells, digest proteins, extraction of genomic DNA**
**Step 2: Precipitation of DNA → to concentrate DNA**

- **Elimination of rests of proteins, lipids, phenol from aqueous phase: add equal volume (500 ul) of PCI; mix**
**→ PCI: mix of Phenole, Chloroform, Isoamylacohol**

**SDS: Sodium dodecyl sulfate**

# DNA Preparation from Cells – The organic method




Purified DNA in large amount of aqueouse solution (ca. 500ul) without proteins, without lipids

**NOTE: DNA at relative low concentration, still small contamination with organic solvents can be present (PCI)**

**Cloroform:**

- completes protein denaturation
- removes lipids
- its high density it facilitates the separation of the aqueous phase (containing the deproteinized DNA) from the organic one (phenolic) stabilizing the interface between the two phases.

**Isoamyl alcohol:**

Reduces the foam that forms during the course extraction.

**PHENOL, CHLOROFORM ARE HIGHLY TOXIC – WORK IN FUME HOOD WITH PROTECTION GLASSES**

# DNA Preparation from cell lysates
# "The organic method"

**Step 1: DNA preparation: Open cells, digest proteins, extraction of genomic DNA,**
**Step 2: Precipitation of DNA → to concentrate DNA**

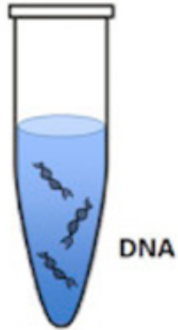
- **Rendering DNA insoluable**
- **Generate a DNA pellet by centrifugation**
- **Wash pellet to remove contaminants (Salts and contaminants from extraction)**
- **Dissolve in acqueous buffer**

**SDS: Sodium dodecyl sulfate**

# DNA Precipitation

## 1. Precipitation

**DNA is rendered insolable → precipitates and can be isolated → Precipitate is dissolved in storage buffer**




***3 options to set set up of DNA precipitation using high salt conditions in alcohol***

1. Na-Acetate: 0,3M final conc
+ 1x volume Isopropanol

2. Na-Acetate: 0,3M final conc
+ 3x volume Ethanol

3. NaCl: 0,5M final conc
+1x volume Isopropanol

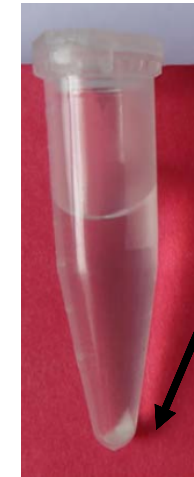
4. Nacl: 0,5M final conc
+3x volume Ethanol

**Practical exmple:**
500 ul DNA solution
1. Add + 55,5 ul 3M Na-Acetate
( final conc. 0,3M)
2. Add + 550 ul Isopropanol
3. Place in freezer at -20°C for 1 hour (DNA precipitates)
4. Centrifuge 13.000rpm 30 minutes at 4°C

+ Salt
+ Isopropanol or EtOH
→ DNA precipitate forms

DNA-precipitate in suspension
(cloudy appearance)

*centrifugation*
*at min. 8000g at 4°C*

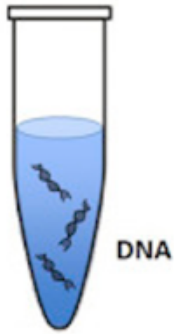


***DNA pellet***

1. Precipitation

# DNA Precipitation

**DNA is rendered insolable → precipitates and can be isolated → Precipitate is dissolved in storage buffer**

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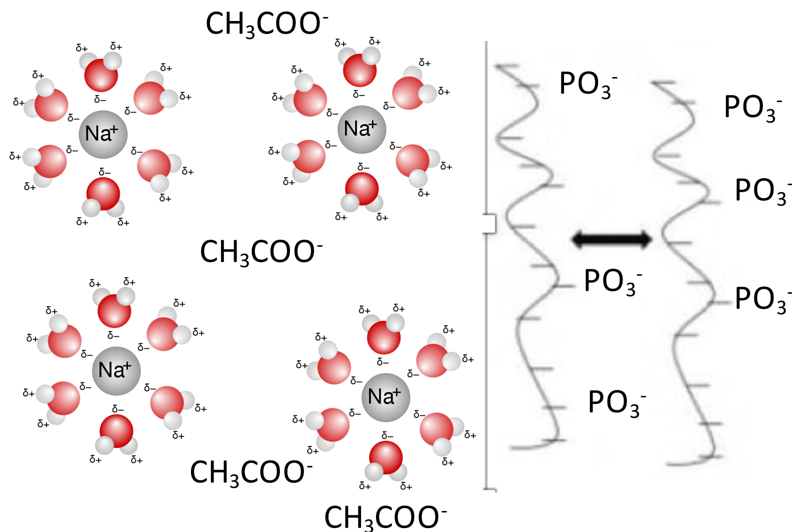
+ 3x volume Ethanol

3. NaCl: 0,5M final conc
+1x volume Isopropanol

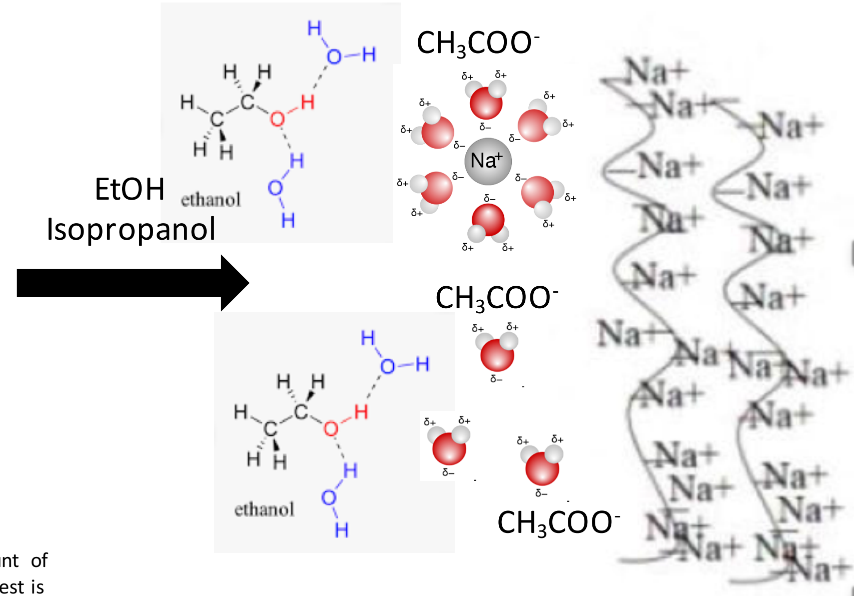
4. Nacl: 0,5M final conc
+3x volume Ethanol

The role of salt in the protcol is to neutralize the charges on the sugar-phosphate backbone. This reders DNA/RNA insolubile and leads to precipitation

1. Addition of sodium acetate or NaCl: In acqous solution,ionic bonds of salt breaks up into $Na^+$ and $[CH_3COO]^-$ or $Cl^-$.
2. $H_2O$ has a high dielectric constant, and shields Na+ ions. $Na^+$ is not captured by $PO_3^-$ in the DNA backbone
3. Ethanol/Isopropanol are polar, small sized alcohols that are solubile in water. They have a much lower dielectric constant. Mixing of water with EtOH or Isopropanol reduces the dielectric constant in the environment.
4. Reduced dielectric constant enables $Na^+$ to interact with the $PO_3^-$. This shields its charge and makes the nucleic acid less hydrophilic, thus causing it to drop out of the solution




Coulomb's law, or Coulomb's inverse-square law, is an experimental law of physics that quantifies the amount of force between two stationary, electrically charged particles. The electric force between charged bodies at rest is conventionally called electrostatic force or Coulomb force.



# DNA Precipitation

## 2. Washing of with 70% Ethanol

- Remove supernatant from top of pellet
- Carefully add 70% Ethanol (in $H_2O$) on top of pellet

(now residual salt from precipitation will be dissolved in $H_2O$)

- Pellet does not dissolve; remains visible
- Centrifuge for 10 seconds to force pellet to bottom of tube
- Remove carefully supernatant
- Let pellet air dry at room temperature for 5-10 minutes to allow the evaporation of EtOH
- Dissolve pellet instorage buffer

**Why: 70% Ethanol disolves salt (NaCl or Na-Acetate) that can co-precipitate during centrifugation.**

**Contaminating salt in the DNA preparation can have negative effect on enzymatic reactions uisng the prepared DNA!!!!**

**PRECIPITATION METHOD IS APPLICABLE TO ALL TYPES OF NUCLEIC ACIDS (RNA, genomic DNA, plasmid DNA)**

# DNA storage

## Buffer

• DNA, RNA and oligonucleotide are storage in 1xTE solution (1 mM EDTA, Tris-HCl, pH 7.2): pH should always be <7,5 (otherwise risk of alkaline hydrolysis)

## Temperature:

•Everyday use: +4°C (generic samples)

•Storage for long time: -20°C or -80°C; (long term storage, valuable samples)

•A precipitate in 70% ethanol can be stored at +4°C almost indefinitely, without loosing DNA/RNA integrity

## Note: Organic method is not limited to de-novo DNA preparation; also for DNA purifucaton purposes after enzymatic reactions

- *To remove proteins from DNA*

  For example restriction digest plasmid:

  - digest with EcoRI

  Purify DNA

  - Phenol, PCI extraction (to remove restriction enzyme, stays in hydrophobic phase)
  - DNA precipitation (Buffer component remains in acqueous supernatant)
  - 70% EtOH wash
  - dissolve DNA in strorage buffer

- *To change buffer of DNA solution*

  You have used DNA for an enzymatic reaction using a defined buffer; for another enzymatic reaction you need a different buffer

  Purify DNA:

  - Phenol, PCI extraction (to remove enzyme; stays in hydrophobic phase)
  - DNA precipitation (buffer component remains in acqueous supernatant)
  - 70% EtOH wash
  - dissolve DNA in new buffer
  - Start second enzymatic reaction

# General steps in DNA Isolation

## 1. Genomic DNA

- SDS/Proteinase K (Organic method)
- Affinity method using **Silica columns**
- Alkaline method
- Automated method

## 2. Plasmid DNA

- Alkaline/SDS method
- Silica column method

## 3. Bacteriophage DNA

- PEG/Salt precipitation method

# DNA preparation using silica columns

New DNA purification methods are based on purification of DNA from crude cell lysates (see earlier) by selective binding to a support material.

**Support Materials**

- Silica
- Anion-exchange resin

**Advantages**

- Speed and convenience
- No organic solvents
- Amenable to automation/miniaturization

**Disadvantage**

- DNA fragmentation
- Low yield

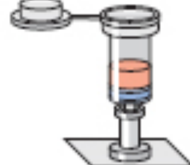
For example: https://www.qiagen.com/it/resources/resourcedetail?id=62a200d6-faf4-469b-b50f-2b59cf738962&lang=en

# DNA preparation using silica columns

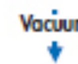
QIAamp Spin Procedure | QIAamp Vacuum Procedure

Sample | Sample

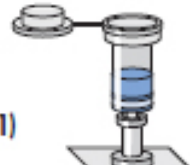
Lyse



Bind



Vacuum



Wash
(Buffer AW1)

centrifuge | vacuum

## Main steps in genomic DNA preparation:

**1. Cell lysis** in buffer containing high concentration of SDS and high concentration of EDTA (binds bivalent ions such as $Mg^{2+}$)

**2. Passage on an insoluble resin** (silica resins) that specifically binds DNA in the presence of salts (see column concept in mini prep)

**3. Wash resin** using specialized washing buffers to remove contaminants (proteins, etc…)

**4. Elute bound DNA with elution buffer** (that also serves as storage buffer)

Note: same principal like in mini-prep for plasmids, however genomic DNA is eliminated in mini prep…
(why: in mini prep alkaline lysis causes the precipitation of genomic DNA, but not plasmid DNA)

# Isolation of RNA

***Total RNA contains:***

- Messenger RNA (mRNA): 1-5%
  *Serves as a template for protein synthesis*

- Ribosomal RNA (rRNA): >80%
  *Structural component of ribosomes*

- Transfer RNA (tRNA): 10-15%
  *Translates mRNA information into the appropriate amino acid*

- other small RNAs: miRNAs, siRNAs, snoRNAs, snRNAs, etc…

## *RNA is unstable:*

RNA is a molecule that is easily degraded by high pH or ribonucleases (RNases). RNases are highly abundant in the environment – also in the laboratory!

RNases do not require enzymatic cofactor, resitant to high temperatures, abundantly present

**ALWAYS WHEN WORKING WITH RNA:**
…. reduce risk of contamination with RNases:

- Be careful not to introduce exogenous RNases (wear gloves, work on cleaned surface or fume hood).
- Use only solutions and materials that are sterile or treated with DEPC (diethylpyrocarbonate; TOXIC!!)) and autoclaved– binds covalently to histidine, lysine, cystein and tyrosin - protein inactivation. Alternatively, Rnase free solutions can be bought from lab-reagent suppliers.
- Store the RNA samples on ice during their handling.
- When not used, store RNA preparations at -80°C or as precipitate (4°C or -20°C)
- Use dedicated materials (test tubes, tips, filters, etc.) and solutions for RNA prep
- Clean working surface

**RNase Inhibitors:**
- **add proteins that act RNAse inhbitors to reactions involving RNA**
- **Treat solutions when possible with DEPC+autoclave**

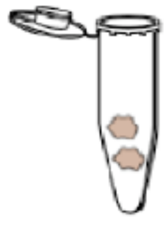
# RNA purification techniques

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

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

- **mRNA from biological samples**
  - Oligo(dT) resins

# Organic extraction of total RNA

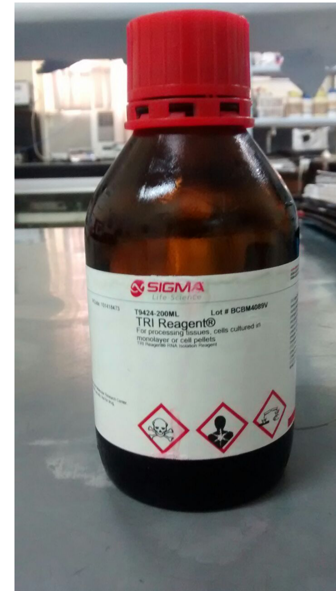


**Option 1: Collect cells as pellet (for example Hela cells cultivated in plates)**

- Apply specialized, commercial available reagents for RNA preparation: Trizol – Trireagent)
- Reagent lyses cells

**Option 2: Collect tissue pieces (for example mouse liver)**

- Apply reagent for RNA preparation: Trizol – Trireagent) and mechanically dissociate tissue
- Reagent lyses cells




Organic reagent for RNA preparation

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 (interfaccia)

Aqueous phase

Organic solvents

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

Same procedure 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

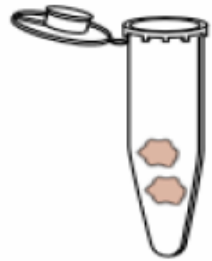
DNase treatment – Phenol extraction – precipitation

DNase treatment – purification via resin

# RNA purification techniques

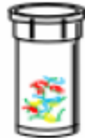
- **Total RNA from biological samples**
  - Organic extraction
  - Affinity purification
- **mRNA from total RNA**
  - Oligo(dT) resins
- **mRNA from biological samples**
  - Oligo(dT) resins

# Affinity purification of total RNA

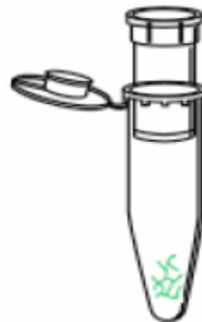


Lyse cells, and spin to remove large particulates/cell debris

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



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



Apply water to the column; purified RNA washes off the glass and is collected (alternatively, as specialized elution buffer can be used)

RNA can be stored at -80°C (or alternatively precipitated and stored in 70% EtOH at 4°C or -20°C)

# Affinity purification of total RNA

**Advantages**

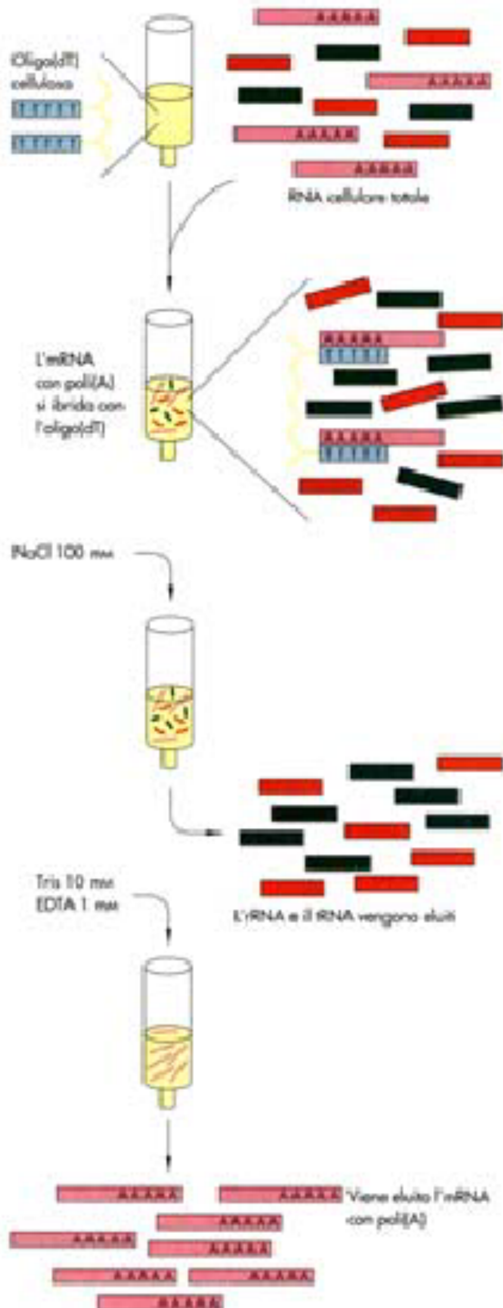
- Eliminates need for organic solvents
- Compatible with a variety of sample types (tissue, tissue culture cells, white blood cells, plant cells, bacteria, yeast, etc.)
- DNase treatment eliminates contaminating genomic DNA
- Excellent RNA purity and integrity

# RNA purification techniques

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

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

# 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.

# *RNA storage:*

## 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 or DEPC treated water; check pH of water: 6.5 - 7.0

## Temperature:

- Always -80°C when aqueous solution
- As precipitate in 70% ethanol, DNA/RNA can be stored at 4°C or -20°C almost indefinitely, without loosing integity

# CRITICAL PARAMETERS OF DNA AND RNA PREPARATIONS

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

# CRITICAL PARAMETERS OF DNA AND RNA PREPARATIONS

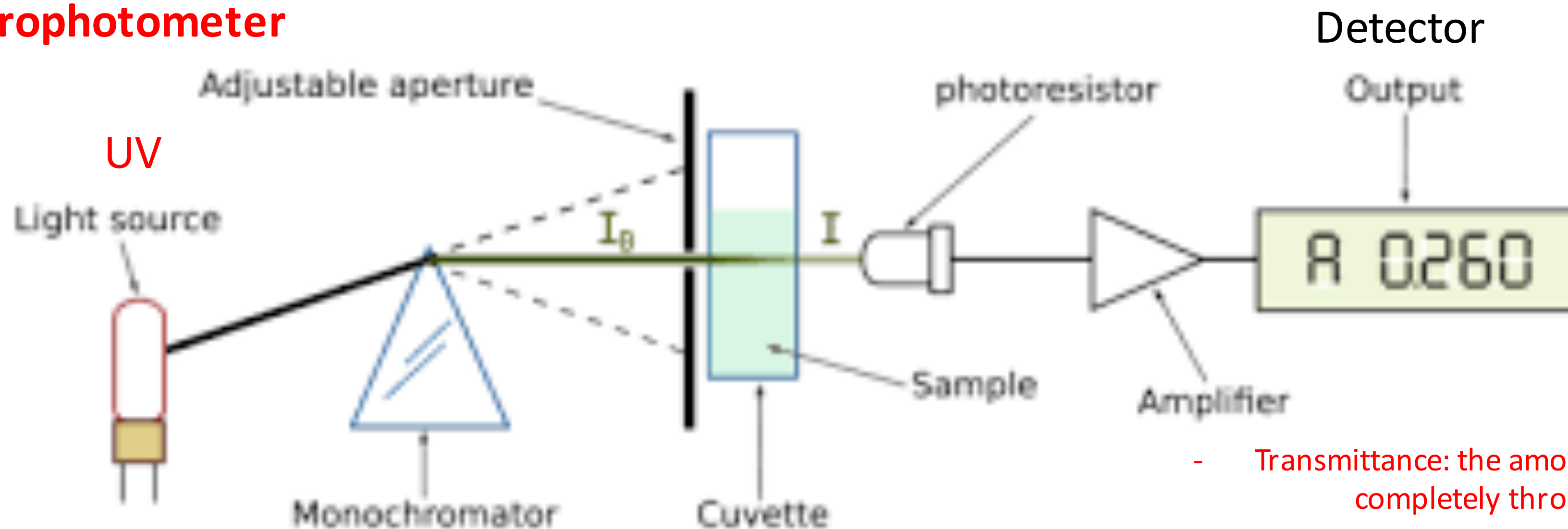
- Quantity
- Purity
- Integrity

# 1. Quantification of DNA and RNA concentration

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.

**Spectrophotometer**




- Transmittance: the amount of light that passes completely through the sample

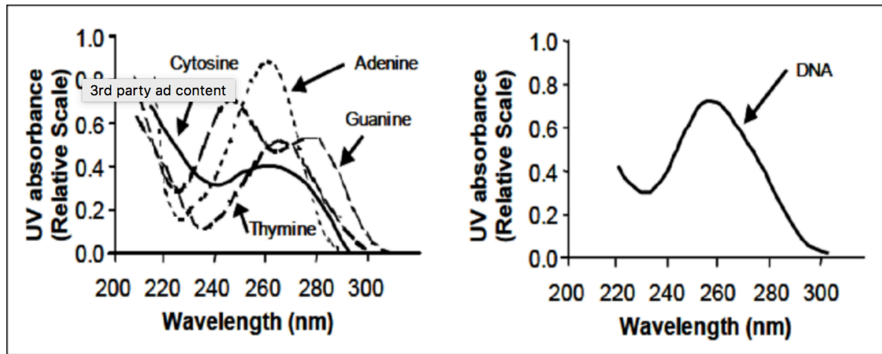
Absorbance: measurement of light that is absorbed by the sample

Monochromator:
Selects precise wavelenght

**Optical density (OD) = Log (Intensity of incident light / Intensity of Transmitted light)**

**OD depends on type of molecule, wavelength and concentration**

# Concentration measurments using Spectrophotometers



5: UV absorbance of Nucleotides (left) and Nucleic Acid (right) at pH7

Heterocyclic rings of nucleic acid show maximum absorbtion at 260 nm

- Spectophotometer measures absorbance (A): measurement of light that is absorbed by the sample

- Molecules have <u>specific</u> exctinction coefficients (**ε**). For nucleic acid concentration measurments, **ε,** at 260 nm wavelength is used.

- **ε,** at 260 nm wavelength passing through a 1cm cuvette for

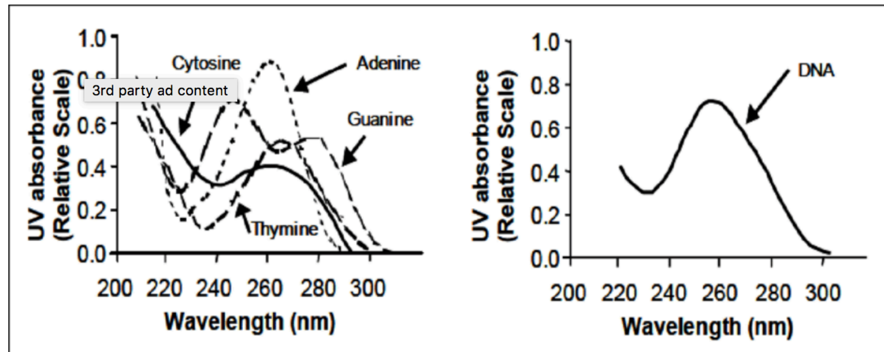
**dsDNA: 0.020 (µg/ml)$^{-1}$ cm$^{-1}$**
**→ at 50µg/ml absorbtion measured: A=1**

**RNA: 0.025 (µg/ml)$^{-1}$ cm$^{-1}$ → 40µg/µl**
**→ at 50µg/ml absorbtion measured: A=1**

**c (µg/ml) = A x dilution factor x ε**

**50 (µg/ml) = 1 x 1 x 50**

# Concentration measurments using Spectrophotometers




5: UV absorbance of Nucleotides (left) and Nucleic Acid (right) at pH7

Heterocyclic rings of nucleic acid show maximum absorbtion at 260 nm

**Typical situation in the laboratory:** Plasmid Mini prep **done; now determine the concentration of DNA**

Take 3 ul of mini prep (total volume 30µl) and dillute **100 fold** (3 µ l mini prep + 297 µ l water)

Transfere dilluted DNA prep into cuvette

Measure OD at 260nm = “A260”

**A260 = 0,200**

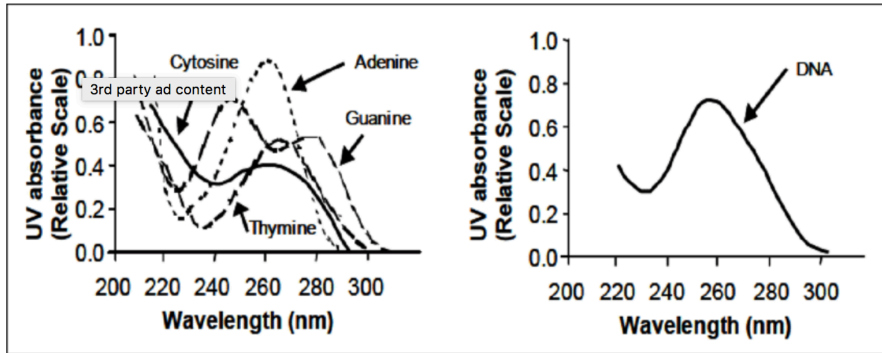
**c (µg/ml) = A x dilution factor x ε**

**1000 (µg/ml) = 0,200 x 100 x** 50

**DNA concentration is: 1000 (µg/ml) = 1 µg/µl**

**Total amount of DNA in mini prep: 30µg**

# Concentration measurments using Spectrophotometers

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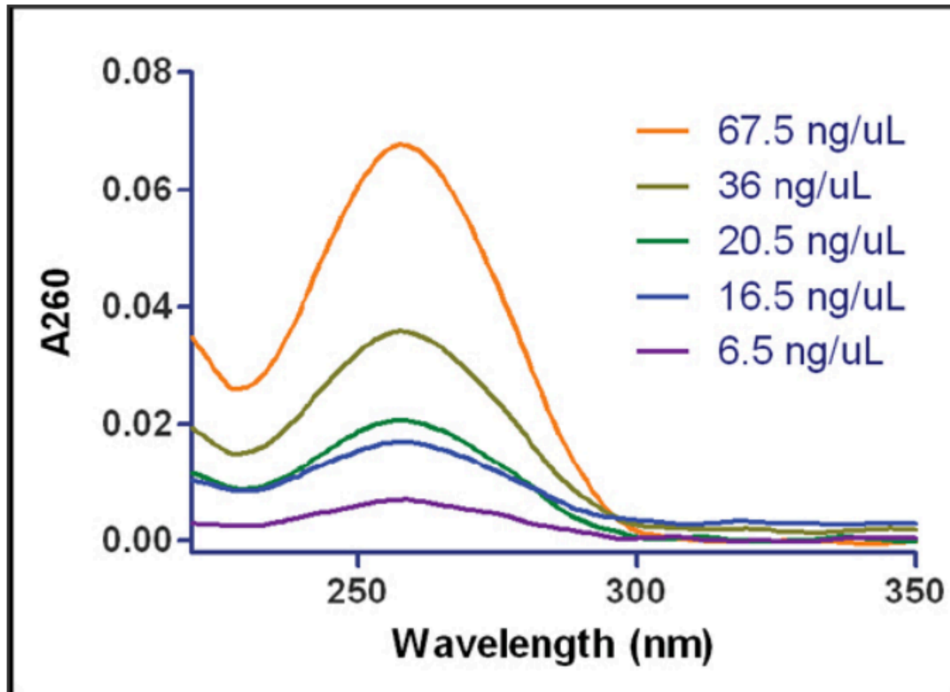
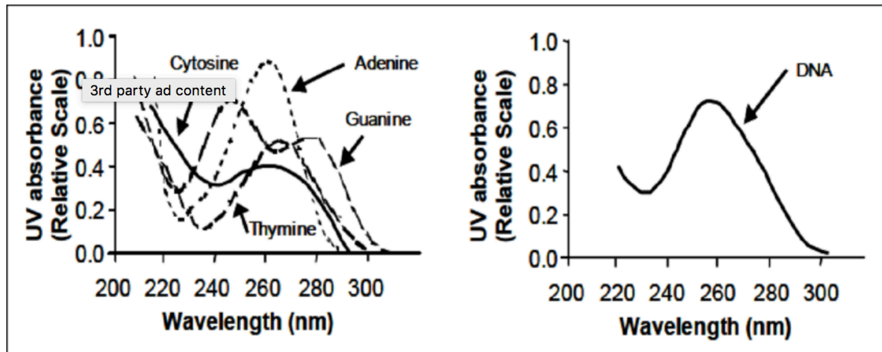



**c (µg/ml) = A x dilution factor x ε**

**Low A value: low concentration**
**High A value: high concentration**

# Concentration measurments using Spectrophotometers



5: UV absorbance of Nucleotides (left) and Nucleic Acid (right) at pH7

Heterocyclic rings of nucleic acid show maximum absorbtion at 260 nm

**Typical situation in the laboratory: RNA prep done; now determine the concentration of RNA**

Take 3 ul of RNA prep (total volume 60 µ l) and dillute **100 fold** (3 µ l RNA prep + 297 µl water)

Transfere dilluted DNA prep into cuvette

Measure OD at 260nm = "A260"

**A260 = 0,200**

**c (µg/ml) = A x dilution factor x ε**

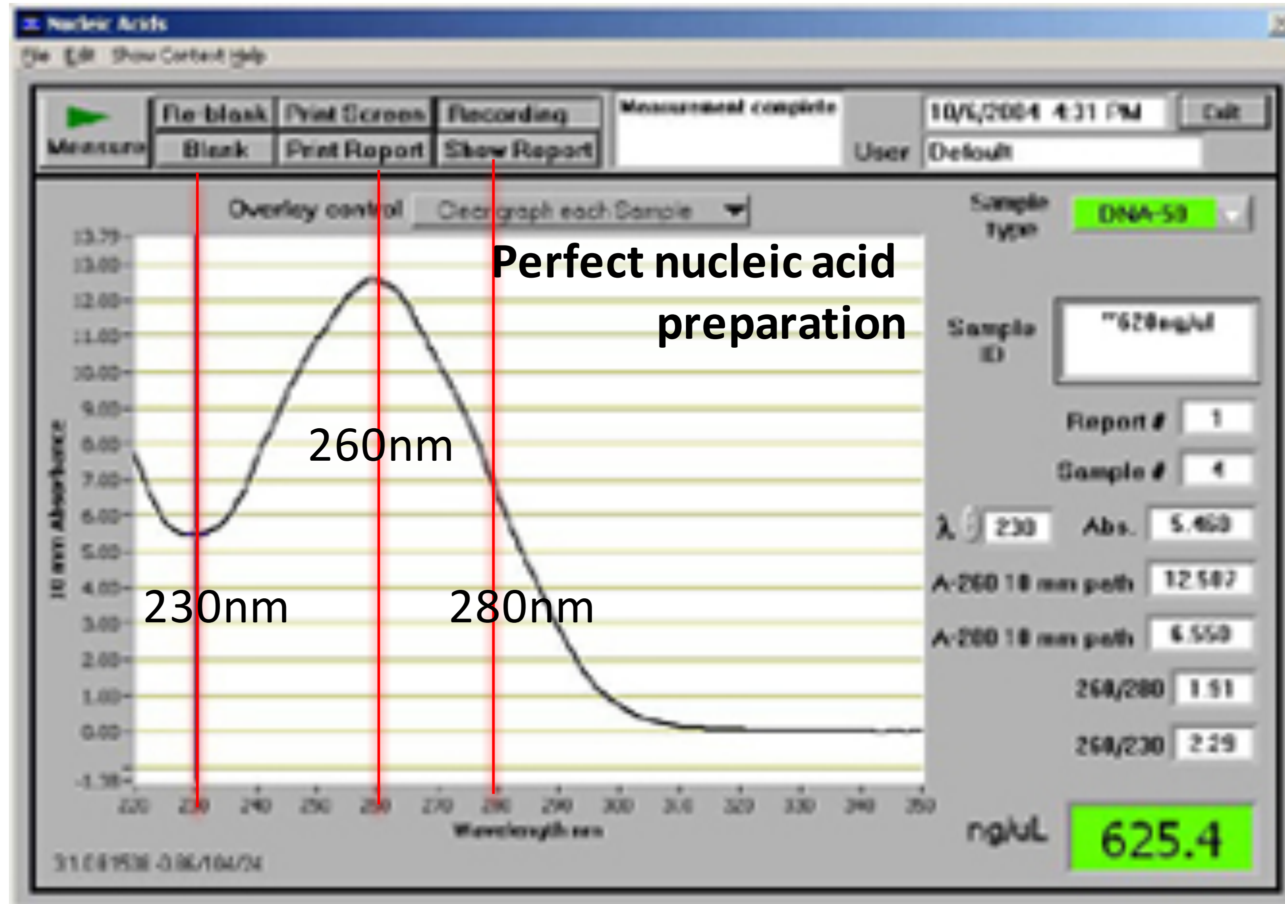
**800 (µg/ml) = 0,200 x 100 x 40**

**DNA concentration is: 800 (µg/ml) = 0,8 µg/µl**

**Total amount of DNA in mini prep: 48µg**

# 2. Determination of DNA and RNA purity

**Measuring absorbance (A) using a SPECTROPHOTOMETER at different wave lengths**
**→ Measure absorbance of DNA/RNApreparation = concentration**




**Maximum absorbance:**

**Molecule of interest:**
**RNA, DNA: 260 nm**
**Contaminats (inprecise purification)**

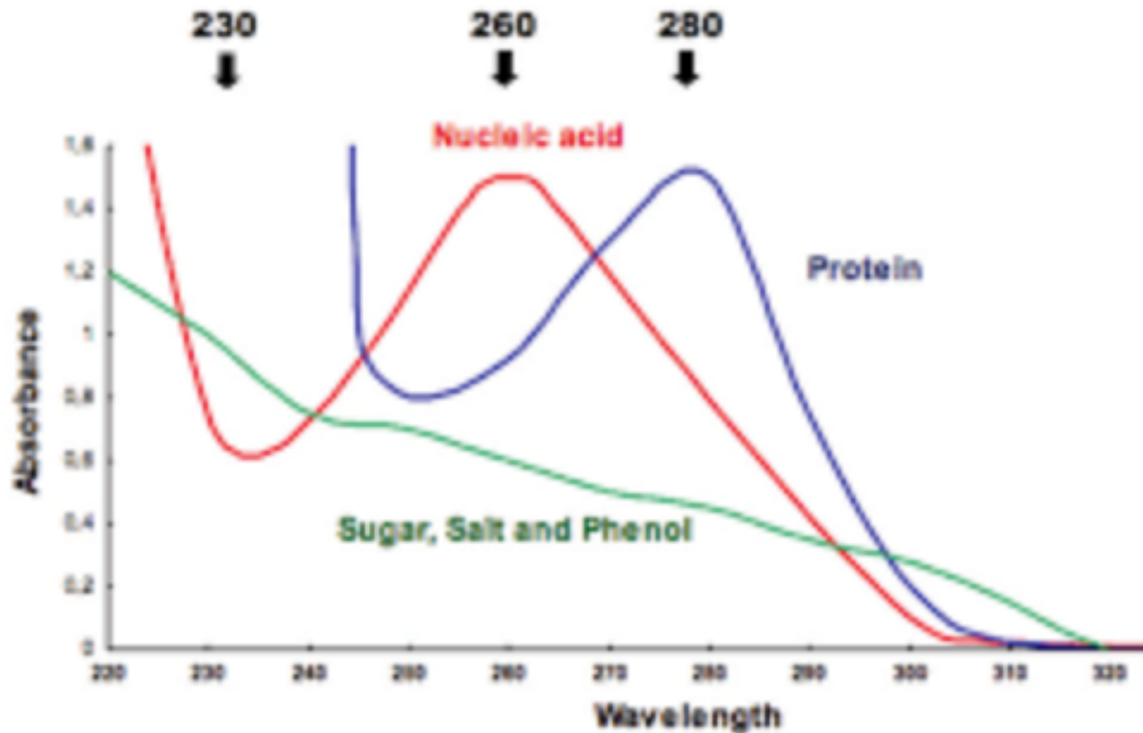
1. **Protein: 280 nm**

**2. Organic compounds /salt/carbohydrates: A230 nm**

# Determination of DNA and RNA purity

**Measuring absorbance (A) using a SPECTROPHOTOMETER at different wave lengths**

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




**Maximum absorbance:**

**Molecule of interest:**
**RNA, DNA: 260 nm**

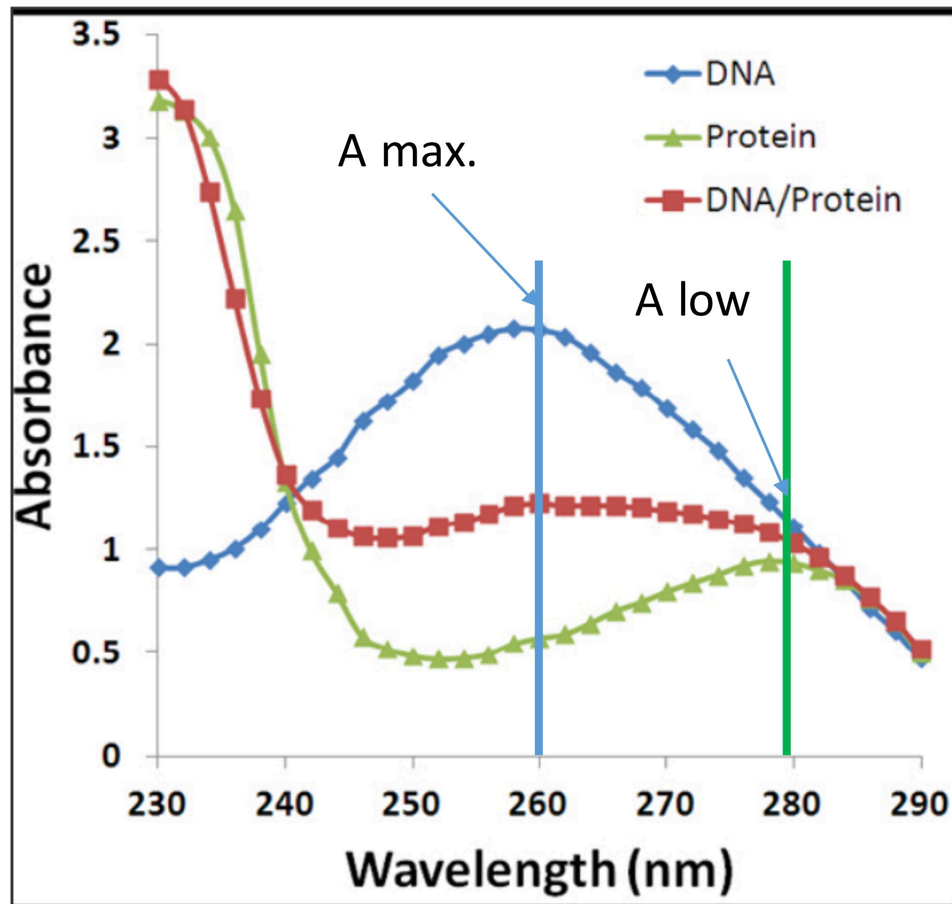
**Contaminats (inprecise purification)**
**1. Protein: A280 nm**

**2. Organic compounds /salt/carbohydrates: A230 nm**

# Determination of DNA and RNA purity

**Measuring absorbance (A) using a SPECTROPHOTOMETER at different wave lengths**
**→ Measure absorbance of DNA/RNApreparation at 230nm, 260nm, 280nm**

**PROTEIN CONTAMINATION in nucleic acid preparation**




**Molecule of interest: RNA, DNA: 260 nm**
**Contaminats (inprecise purification)**

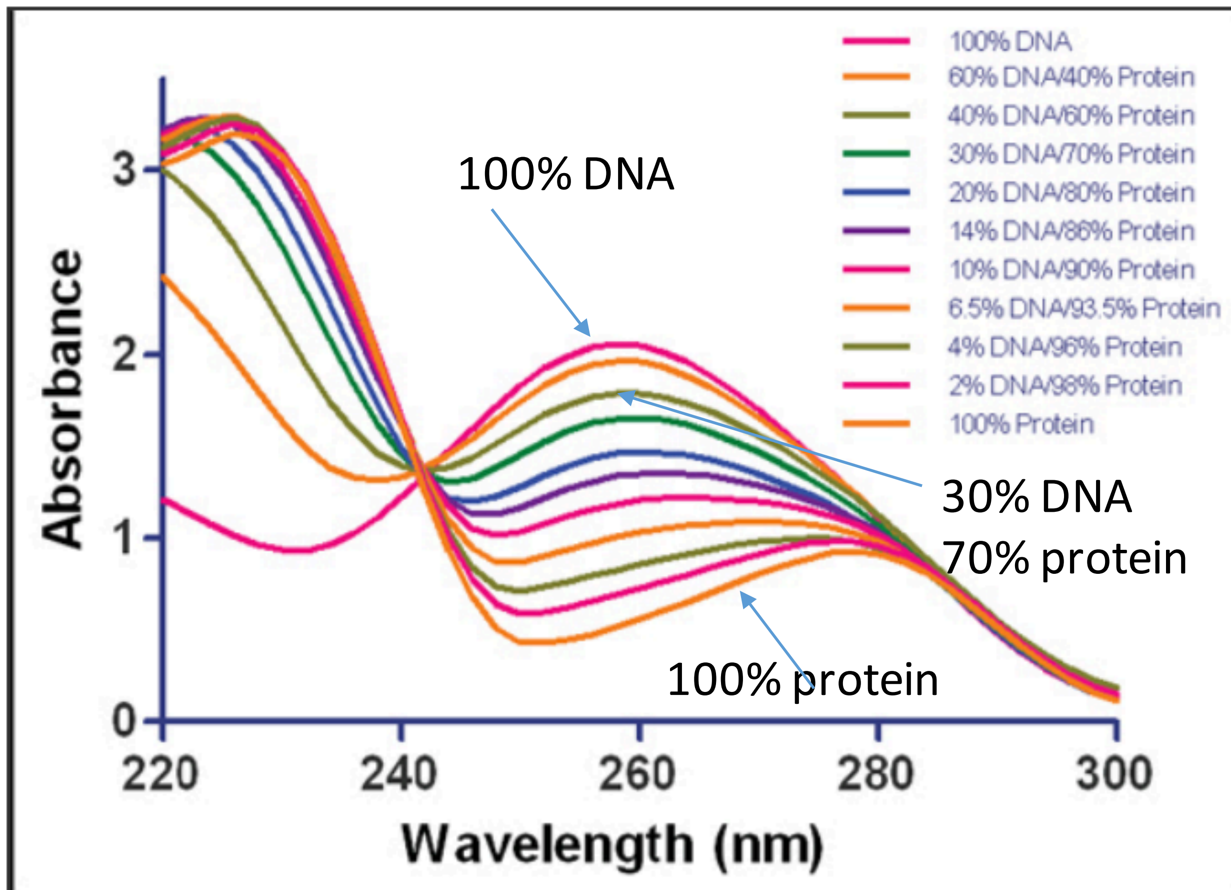
**Protein: 280 nm**

**Use ratio A260/A280 as inidcator for protein contamination**

# Determination of DNA and RNA purity

**Measuring absorbance (A) using a SPECTROPHOTOMETER at different wave lengths**
**→ Measure absorbance of DNA/RNApreparation at 230nm, 260nm, 280nm**

**PROTEIN CONTAMINATION in nucleic acid preparation**




**Maximum absorbance:**

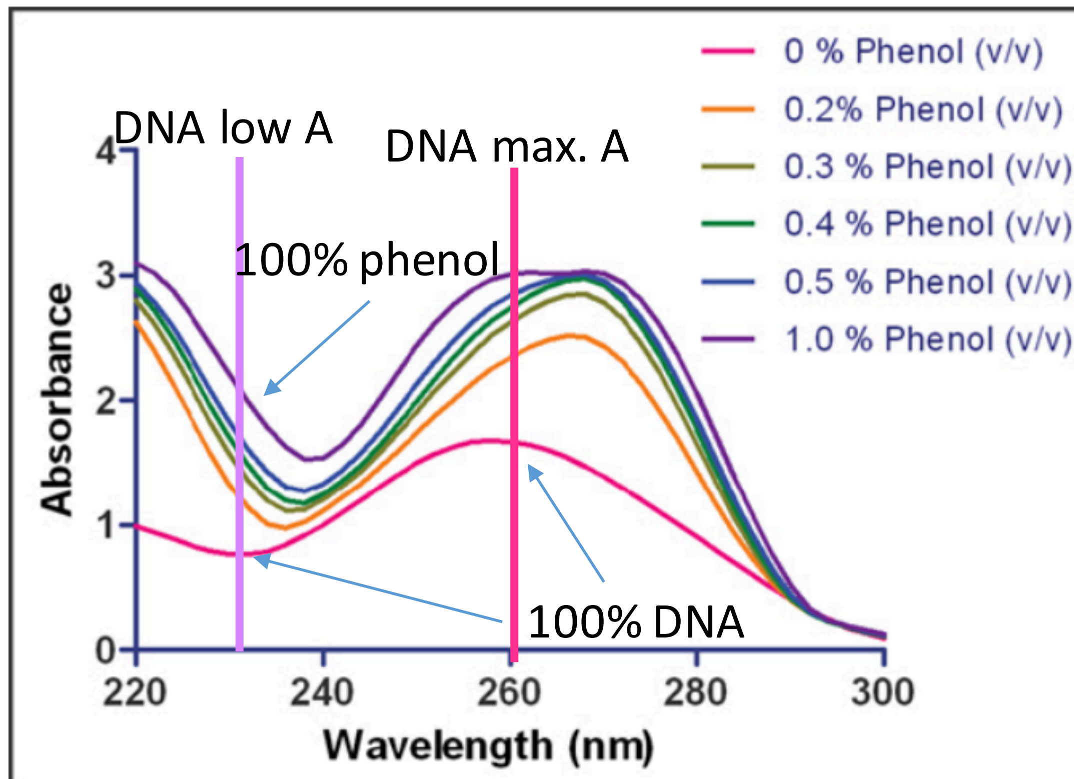
**Molecule of interest: RNA, DNA: 260 nm**
**Contaminats (inprecise purification)**

**Protein: 280 nm**

# Determination of DNA and RNA purity

**Measuring absorbance (A) using a SPECTROPHOTOMETER at different wave lengths**
**→ Measure absorbance of DNA/RNApreparation at 230nm, 260nm, 280nm**

**Organic compounds /salt/carbohydrates contamination in nucleic acid preparation**




**Maximum absorbance:**

**Molecule of interest:**
**RNA, DNA: 260 nm**
**Contaminats (inprecise purification)**

1. **Protein: 280 nm**

**2. Organic compounds /salt/carbohydrates: A230 nm**

# Determination of DNA and RNA purity

- **ratio $A_{260}/A_{280}$ → PROTEIN CONTAMINATION**
- **ratio $A_{260}/A_{230}$ → ORGANIC COMPOUND, SALT, CARBOHYDRATE CONTAMINATION**

**Ratio A260/A280 = quantification of protein contamination**
For DNA preparations: ideal A260/A280 ratio: 1.6-1.8 ($A_{260}$=0.234; $A_{280}$=<u>0,137</u>: ratio =1,7 → OK)
For RNA preparations: ideal A260/A280 ratio: 1.8-2.0
Il ratio is out of range : **contamination with proteins** (DNA $A_{260}$=0.234; $A_{280}$=<u>0,199</u>: 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

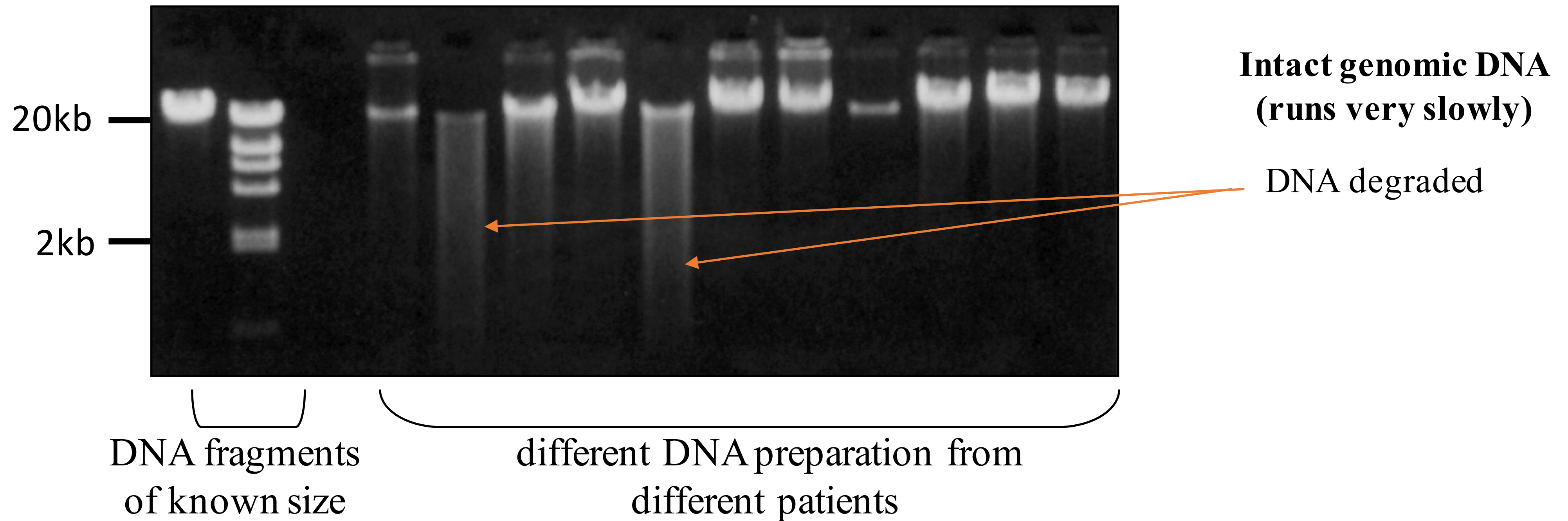
# CRITICAL PARAMETERS OF DNA AND RNA PREPARATIONS

- Quantity
- Purity
- Integrity

# DNA Integrity

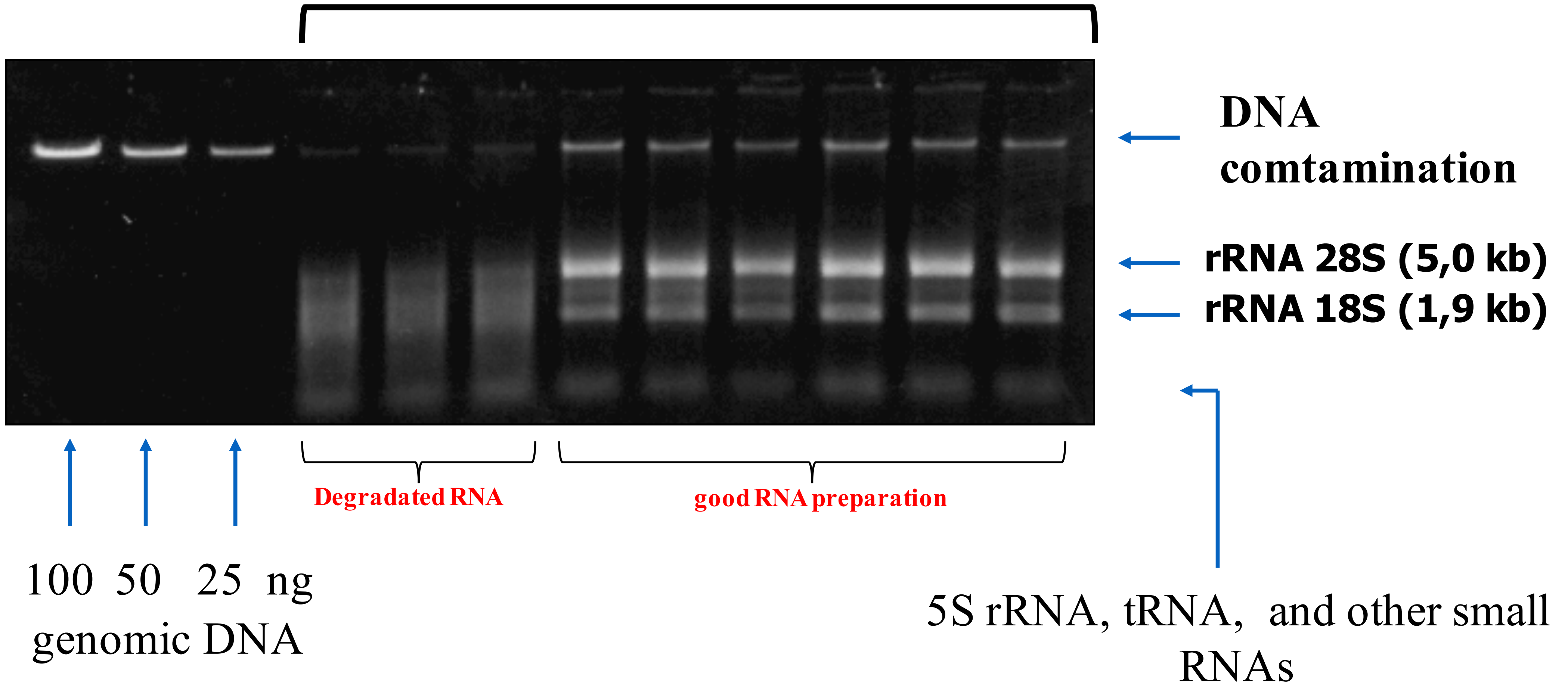
**Easiest method: Gel electrophoresis**

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.



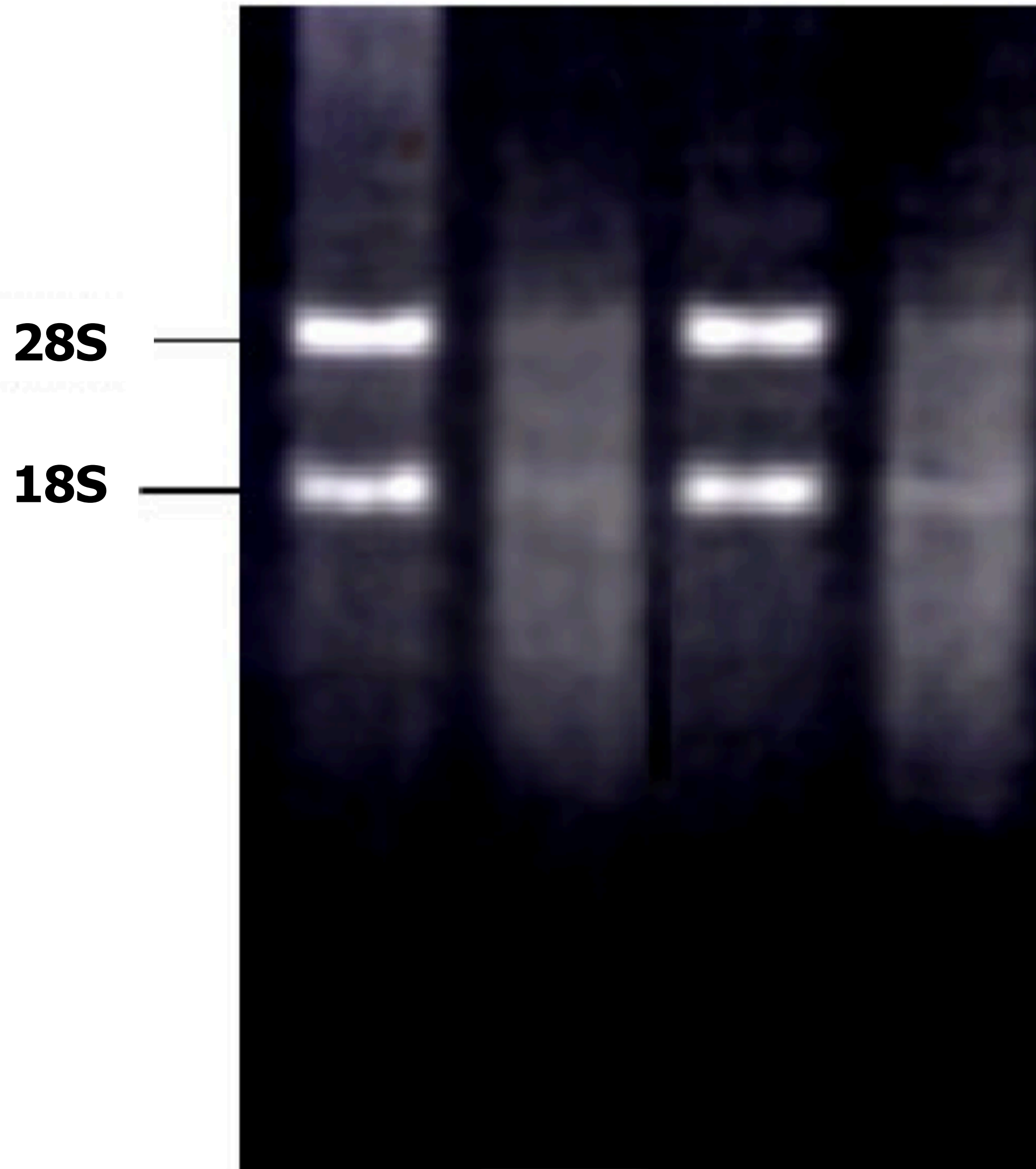
# RNA integrity

Total RNA preparations




**DNA contamination can be removed by DNase treatment and subsequent re-purification of total RNA**

# Determination of RNA integrity – polyA 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!!)

→ If mRNA is degrated, it will appear a smear at low moelcular weight (<2kb)