DNA and RNA Extraction

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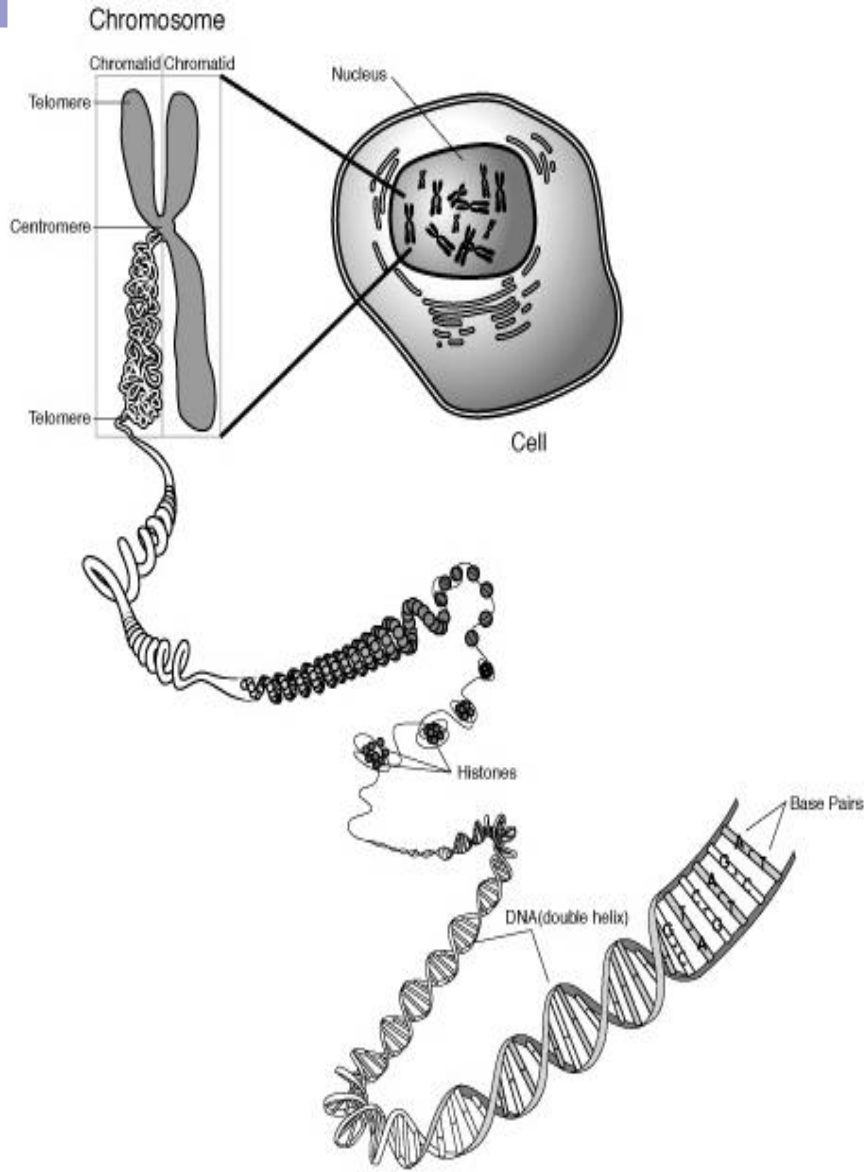


DNA

- Inactive DNA is tightly packed in nucleus
- Resistant to enviromental, chemical and physical damage
- Possible to get extracted relatively easier from a variety of tissue sources

- Fresh and frozen tissues
- Paraffin embedded tissues
- Unstained or stained sections prepared from paraffin embedded and frozen tissues
- Microdissected samples from sections
- Blood
- Body fluids
- Smears...

DNA Extraction

- All fixatives, less or more, give some damage to DNA
- Especially fixatives prepared with acids

(Bouin Solution, Zenker's solution…) and high concentrated acids (decalcification solutions) break DNA in to pieces

- Ideal fixation is with NBF for 12-36 hours

High-throughput, automated DNA and RNA purification systems

- They can process 12-96 samples in 30 minutes-1hour
- Minimize contamination risk
- Reduce inconsistencies in sample yield
- Allow various handling volumes ranging from 1uL to 1000uL.
- These instruments are quite expensive,
- Size, capacity, usage, price, and versatility should be taken into consideration.

DNA Extraction

- There are many different protocoles and commercial kits for DNA extraction from tissues.
- There are three main steps of DNA extraction:
  - Lysis of cell membranes
  - Removing of proteins -Purification
  - Precipitation of DNA

Cell Lysis

- Cutting tissue in pieces:
  - Blender
  - Scalpel
  - Mortar
  - Smashing with liquid nitrogene

Cell Lysis

- Homogenization
  - Pellet pestle
  - Rotor-stator homogenizer
  - Teflon glass homogenizer
  - Sonicator

Lysis Buffer Example: (Hirt's Buffer)

SDS 0.6%,
0.01 M EDTA
0.01 M Tris-HCl, pH 7.4

Hirt B. J Mol Biol. 1967 Jun 14;26(2):365-9

Lysis buffer

- Detergent:
  SDS (sodium dodecyl sulfate)→ membrane lysis
  protein denaturation
- Buffer
  Tris →(trishydroxymethylaminomethane) → to keep DNA soluble and stable
- Chelation (binding divalan ve trivalan metal ions)
  EDTA (ethylenediamine-tetraacetic acid) →inhibition of DNA'ases
- **Proteinase K:** interruption of peptid binds, inhibition of DNA'ases.

Cell Lysis

- Incubation of fragmented tissue+lysis buffer+proteinase K @55ºC water bath overnight
- Amount of lysis buffer depends on material type and amount. 10 mg tissue/500 µl lysis buffer
- Proteinase K 100-200 µg/ml,
  - proteinase K is a serine alkaline protease and active at 37ºC-60ºC

Removing cellular and histone proteins and cell debris.

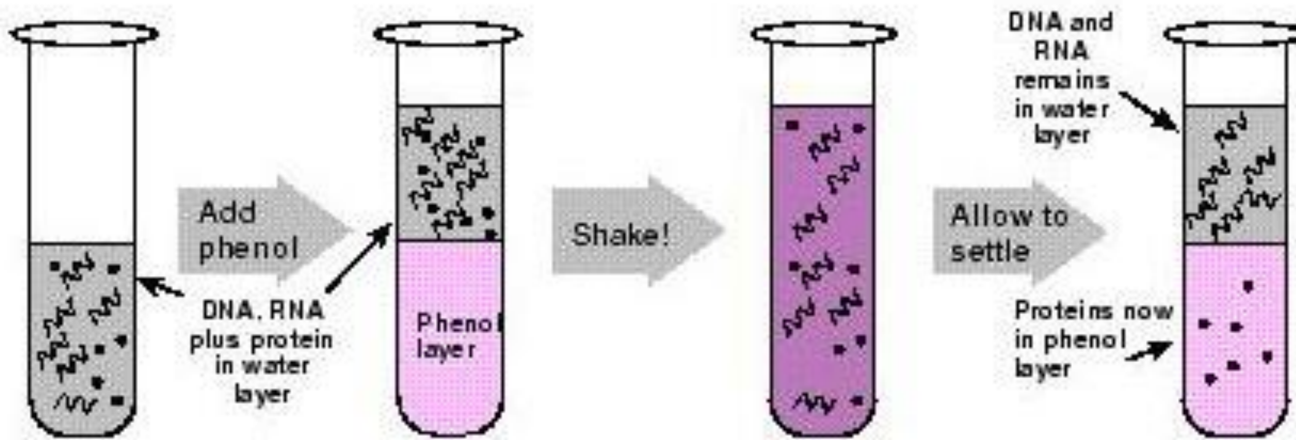
Phenol-Chloroform Extraction

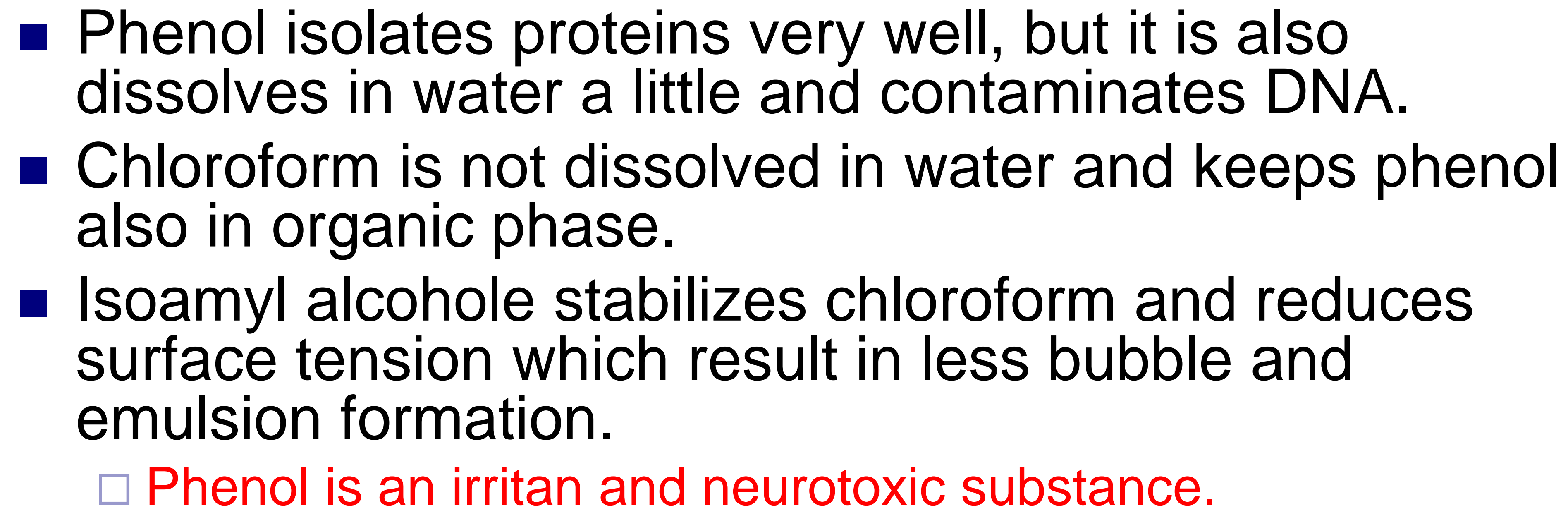
- Phenol and chloroform are organic solvents. Hydrophobic cell components (membrane lipids, hydrophobic polypeptides, polysaccarydes…) are detained in these solvents. …..
- They both are also powerful denaturating agents and also act on protein denaturation.

Phenol-Chloroform Extraction

- 25/24/1 PCI (Phenol/Chloroform/Isoamylalcohole) mixture added to cell lysate with the same amount and mixed well for 10 min/5h.
- Phenol for DNA extraction should be buffered to pH 8
- In pH 8 phenol DNA, in pH 4.5 phenol RNA stays in aqueous phase.

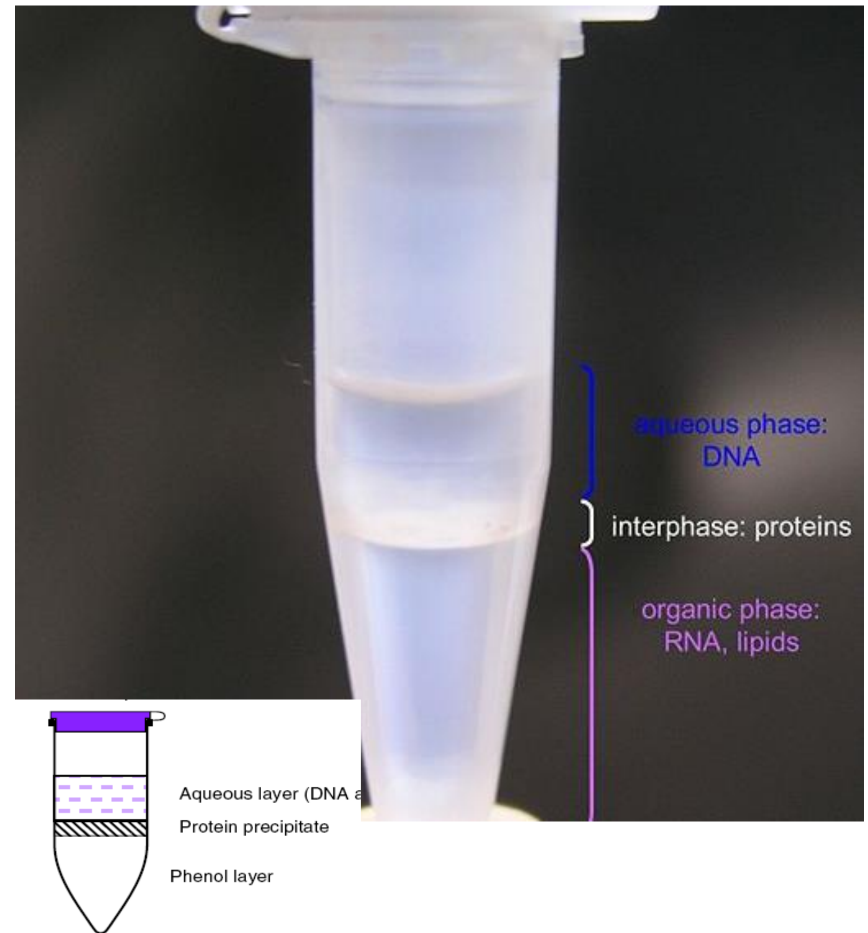
Phenol-Kloroform Extraction





- Phenol isolates proteins very well, but it is also dissolves in water a little and contaminates DNA.
- Chloroform is not dissolved in water and keeps phenol also in organic phase.
- Isoamyl alcohole stabilizes chloroform and reduces surface tension which result in less bubble and emulsion formation.
  - Phenol is an irritan and neurotoxic substance.

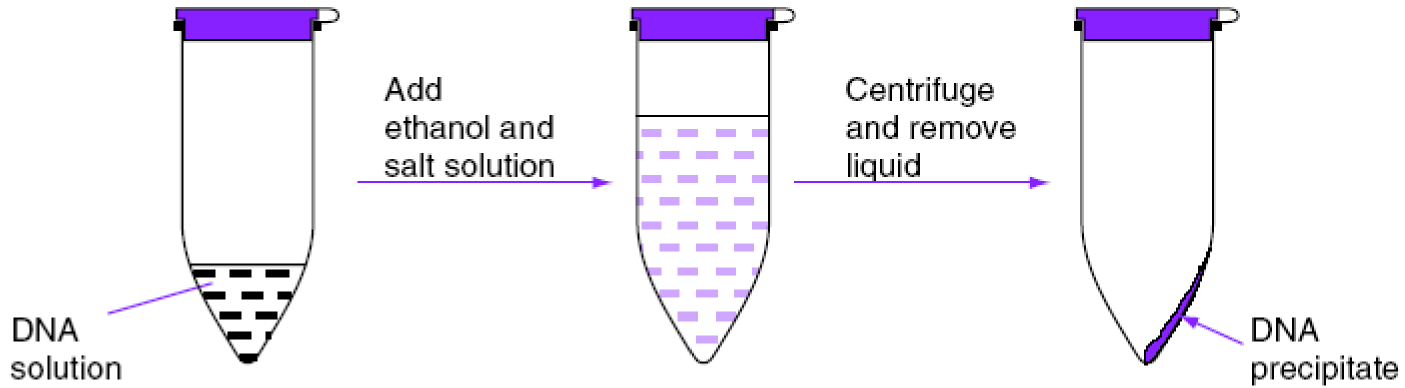
- After PCI, samples separated by centrifuge, and aqueous phase is taken to another tube and CI is repeated (1-3 times) until it becomes clear.
- CI: 24/1 Chloroform/Isoamyl alcohole



DNA Precipitation

- Add aquous phase
  - Absolute ethanol x2.5, cold or
  - Isopronole x1 RT
  - Salt (amonium acetate, sodium acetate, lityum chloride)

  When we add absolute ethanol to aqueous phase the solution generally includes more than 80% ethanol, because DNA can stay soluble in up to 65% of ethanol, it starts to precipitate in small particles.



After ethanol @ -20ºC O/N or @-80ºC 1-2hrs, after isopropanole @ RT 10-15m)..

DNA Precipitation

- Centrifuge @ +4 ºC
- Wash pellat with 70% ethanol(after isopropanol several times)
- Dry @ RT
- Dissolve pellat in TE(Tris-EDTA) buffer or in ultra pure water @37ºC for several hours.

- DNA extraction from FFPE tissues:

After deparaffinization, the same protocole is performed

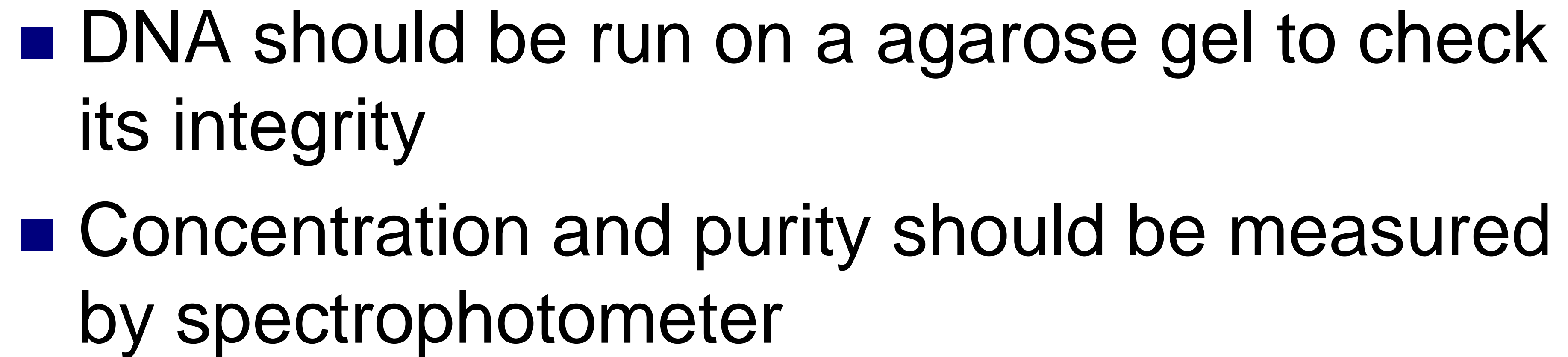
  - Put 50µicron section in tube
  - With 1 ml Xylene @45ºC, 30 min., 2 times
  - 100%, 90% and 75% ethanol, 30 min
  - Wash with PBS

NaSCN(sodium thiocyanate): protein denaturation

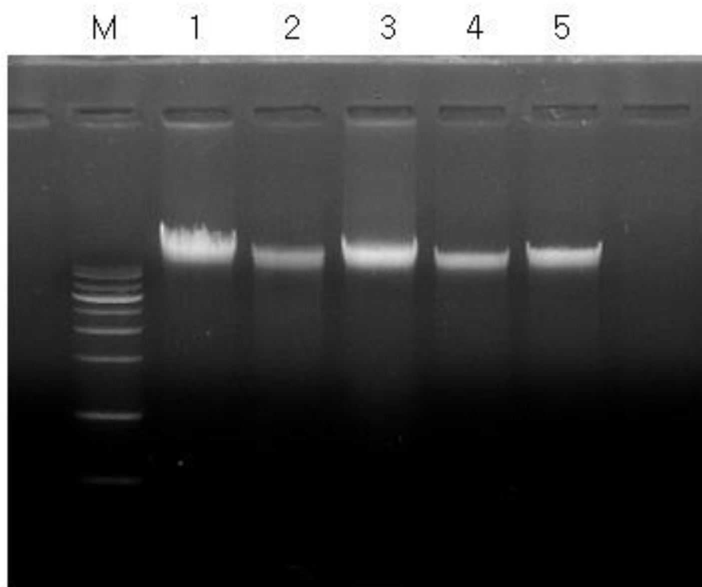
Incubation with 1M NaSCN @37º O/N

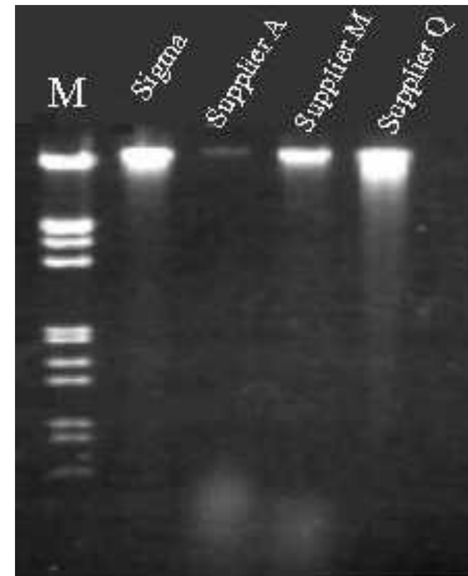
DNA isolation from blood

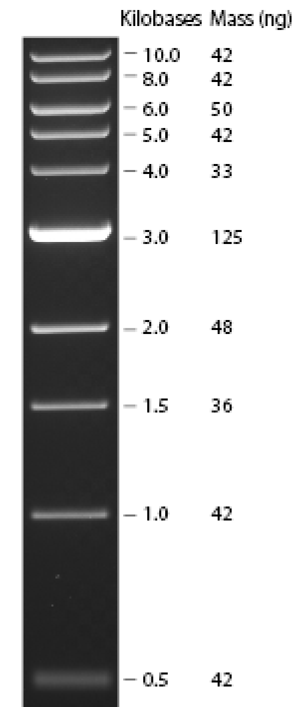
- Blood should be in EDTA or citrate tube and be stored @-80ºC
- Red blood cell lysis (incubation with citrate buffer 2-3 times, remove supernatant)
- Lysis
- Phenol-Chloroform Extraction



- DNA should be run on a agarose gel to check its integrity
- Concentration and purity should be measured by spectrophotometer



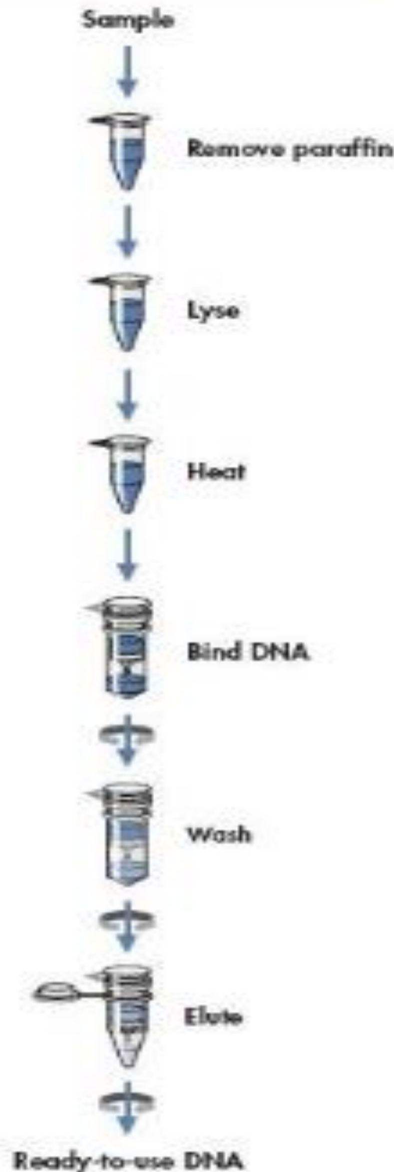




- An easily seen band contains roughly 20 ng of DNA

Simplified methods of DNA Isolation

- **Salting out of proteins:** Proteins are precipitated by saturated salt solutions (6M NaCl) after lysis.
- **Boiling:** Boiling tissue with DW for 20 min.
- **Boiling with Chelex 100:** A chelating agent. Polar resin particles bind polar cellular components.
- **Simplified proteinase K extraction:**
  - Lysis buffer (**50mM KCL, 1.5mM MCl2, 10mM TRIS-HCL, 0.5%Tween 20, pH9) and 200** µg/ml proteinase K @ 55ºC, 24hrs incubation
  - Proteinase K inactivation by boiling 10 min @97ºC and centrifuge 14000 rpm for 5min. Keep supernatant @4ºC or -20ºC



DNA extraction with commercial kits

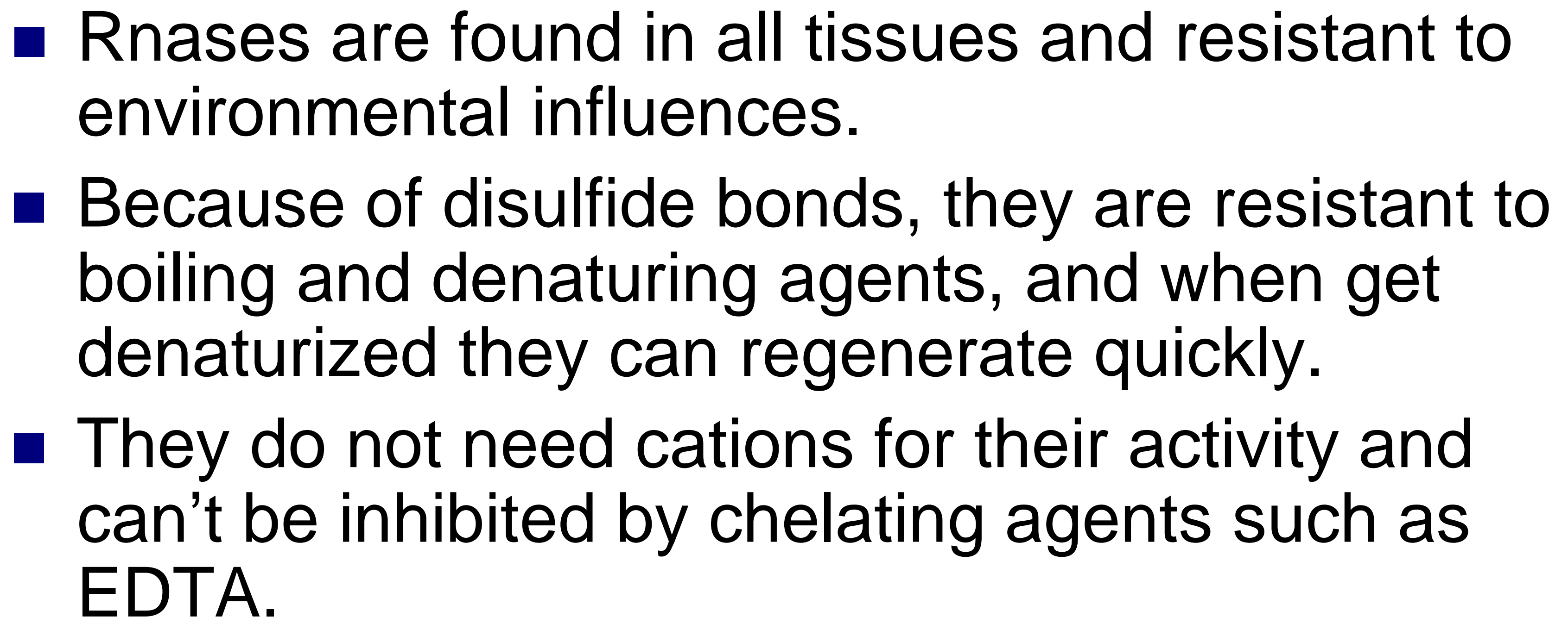
- They have different methods for DNA purification. Some kits use silica-gel based columns for binding DNA, other components removed by washing steps. DNA elution is performed by buffers with low level of salt.
- Some other kits include protein denaturation with high concentrated salt and alcohole precipitation.
- Another group of kits depend on binding of DNA with monodispersed magnetic particles.

Total RNA in cells

- 6% of total weight of bacteria
- 1.1% of more developed organisms.
- A mammalian cells contains about 10-15µg of total RNA.
  - 80-85% rRNA (28S, 18S and 5sRNA)
  - 15-20% low molecular weight RNA types (tRNA, small nuclear RNAs..)

- mRNA is 1-5% of total RNA

- **RNA**
  - Transfers transient information for dynamic protein translation of gene expression
  - It is critical to stop signal when the mission completes!
  - As it has hydroxyl group in its ribose residues, it is more reactive than DNA chemically and can be broken easily by RNase .


Rnases are found in all tissues and resistant to environmental influences.
Because of disulfide bonds, they are resistant to boiling and denaturing agents, and when get denaturized they can regenerate quickly.
They do not need cations for their activity and can’t be inhibited by chelating agents such as EDTA.

- Exogenous RNAases can be eliminated with prophylactic measures.
- DPEC(diethylpyrocarbonate) which is a chemical agent inhibites RNAase activity

Preventive Measures

Body fluids (such as perspiration):Use powder free gloves all the time. Try not to touch contaminated surfaces during extraction process.

Pipettes: Use a separate automatic pipette set and certified RNA-ase free tips and tubes.

Chemicals:Use separate chemicals, baked spatulas and untouched weigh boats or weighing papers

Preventive Measures

Solutions:

- DEPC 0.05% should be added
- Incubate O&N @RT
- Autoclave for 30 minutes to remove any trace of DEPC.

DEPC inactivated Tris, so Tris-based buffers cannot be used with DEPC; Purchase RNase-free Tris and use DEPC-treated or nuclease-free water for Tris-buffered solutions.

Preventive Measures

- Non-disposable glassware and plasticware:
- Bake glassware at 250° C overnight. Rinse plasticware with 0.1N NaOH/1mM EDTA and then (DEPC)-treated water.
- Water: Check RNase activity of your deionized water source simply by incubating an RNA sample with that water and run the RNA then on a agarose gel to check degradation.

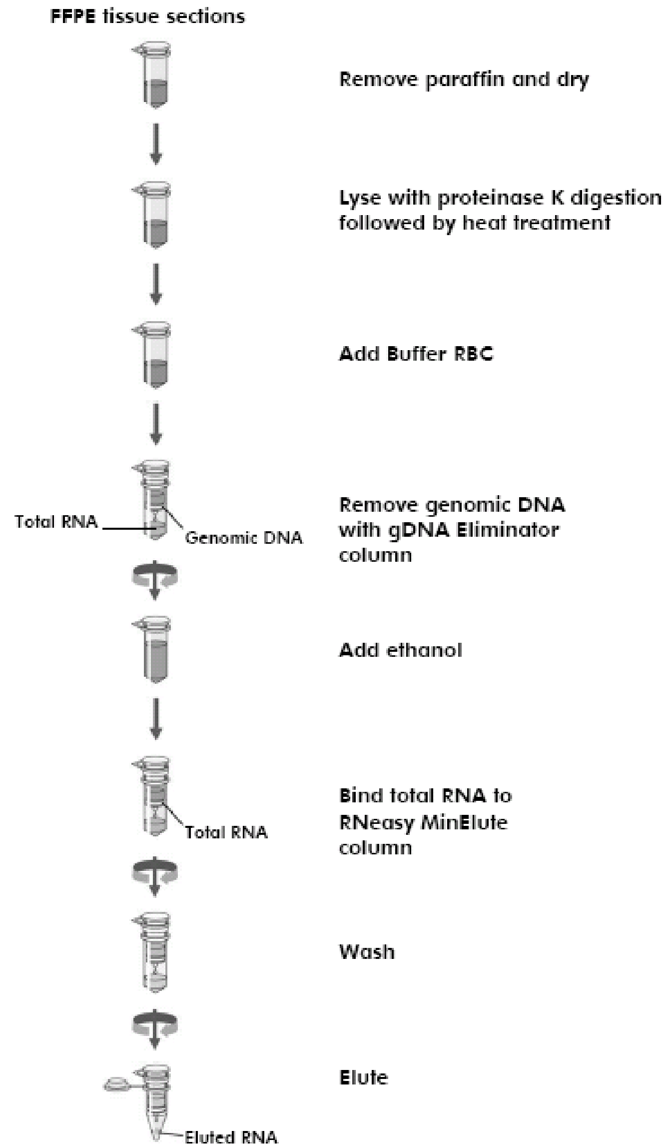
RNA Extraction

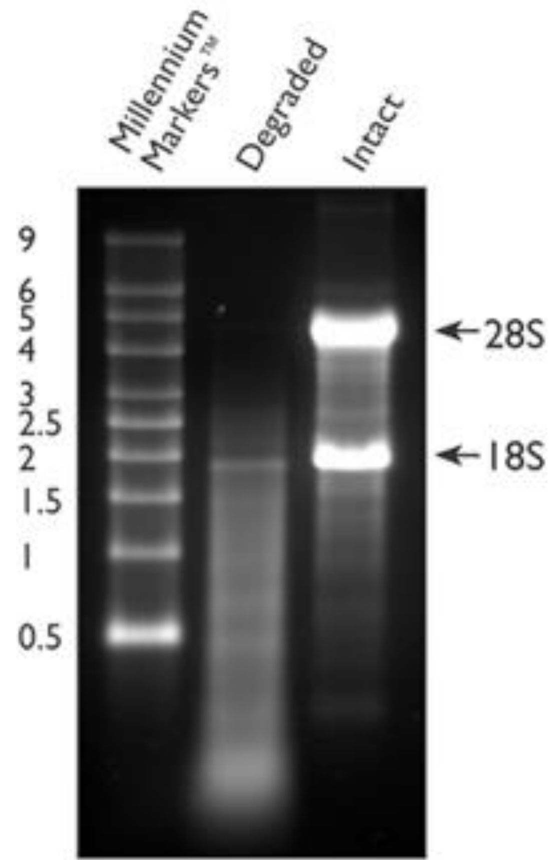
- Lysis of cell membranes
- Guanidium thyhiocyanate-phenol-chloroform extraction
- Guanidinium thiocyanate is a powerful denaturating agent. It denaturates proteins (including Rnases) , also separates rRNA from ribosomal proteins.
- Guanidium thiocyanate is commonly used in RNA extraction

RNA Extraction

- Trizol
  - Mono-phasic solution of phenol and guanidine isothiocyanate
  - It is a commercially available, ready to use reagent
- Commercial kits
  - Silica-column based
  - Magnetic bead technologies

RNeasy FFPE Procedure





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