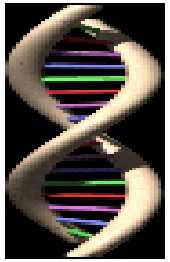
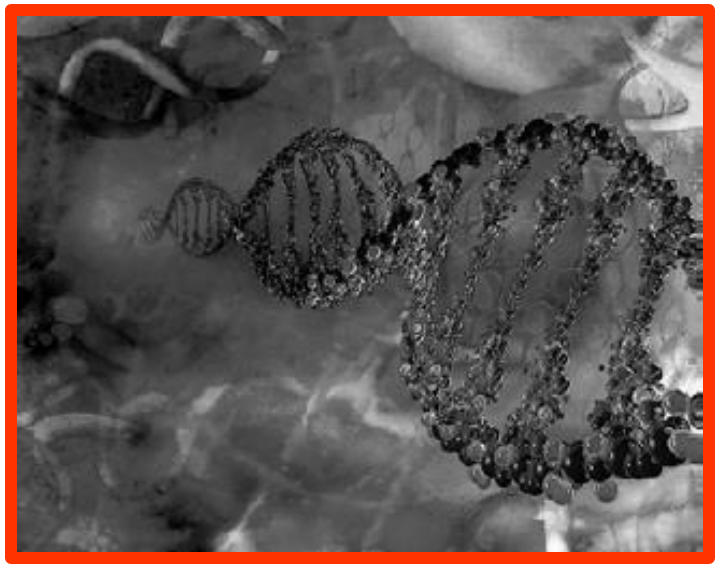
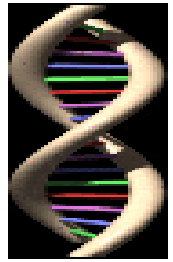
Week 3. DNA Isolation

Preparation of samples to Polymerase Chain Recation (PCR): Principles and Methods of DNA Isolation

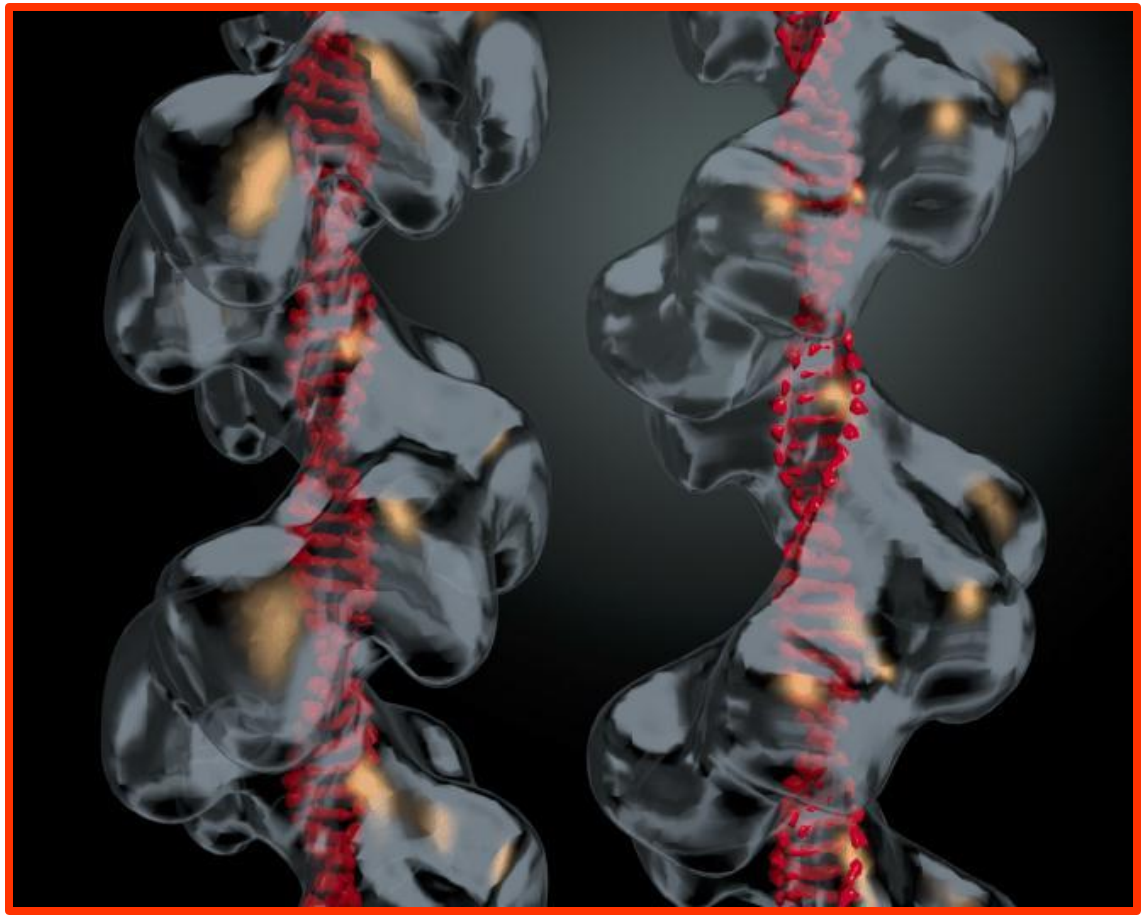






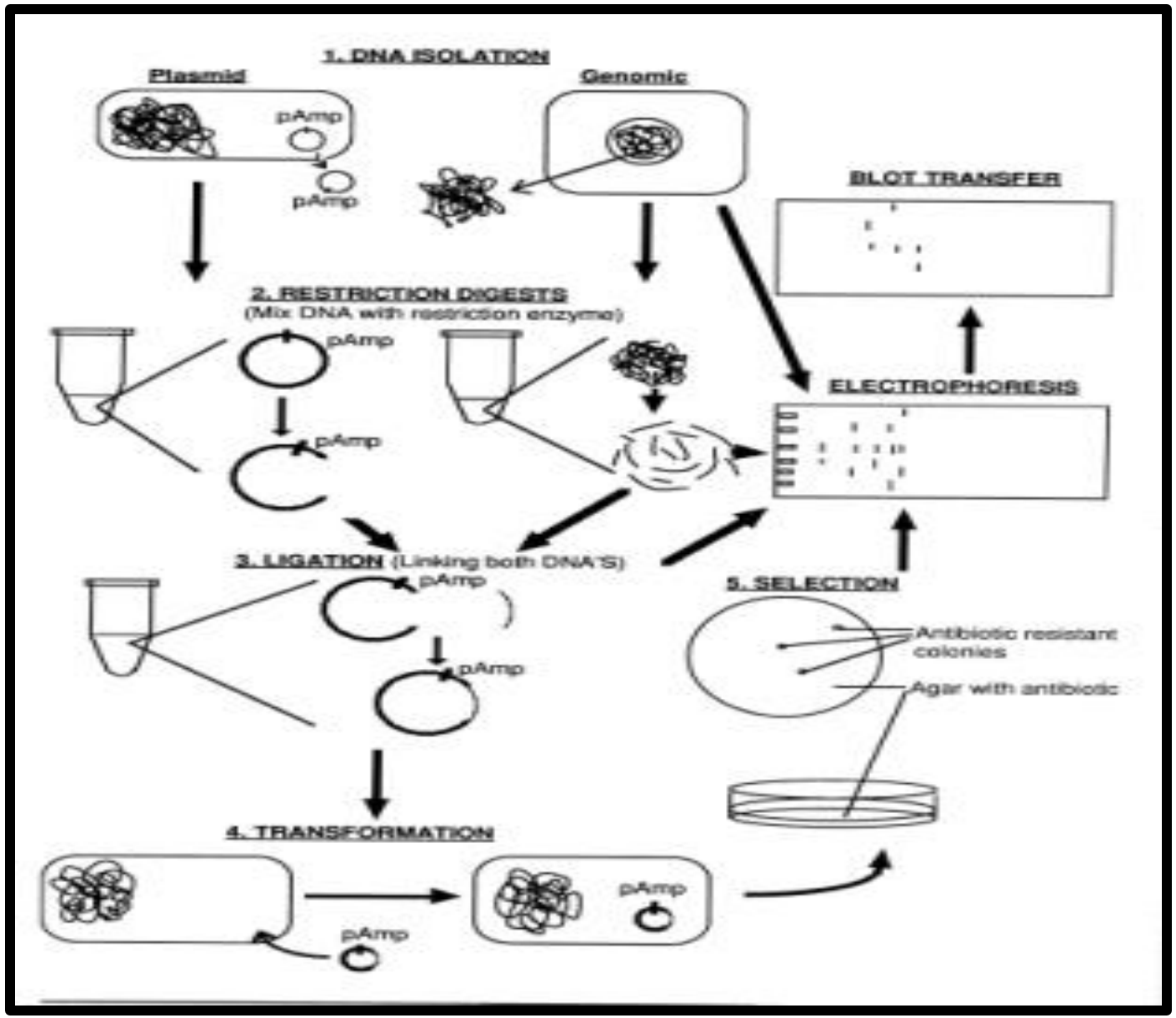
Department of Microbiology

Why do we isolate DNA?



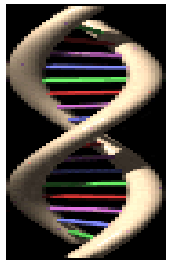
- Molecular cloning (antibacterial peptides, hormones, enzymes, etc. in *E. coli*)
- Molecular diagnosis (PCR)
- Hybridisation methods (Southern blotting)
- Molecular Typing (RFLP)
- Protection (i.e. DNA vaccines)
- Forensic Medicine
- Maternity / Paternity tests

Stages of Molecular (DNA) Cloning



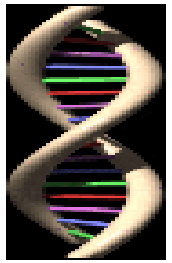
DNA Isolation and Purification Principles

DNA Isolation



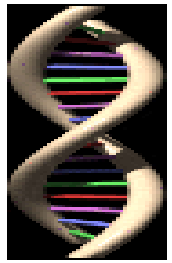
- Physical characteristics of this molecule lets us to separate DNA from other molecules in the environment (matrices)
- DNA – easiest isolation with salts, detergents, and alcohol
  - Soap (detergents) destructs the cellular structure
  - salts (especially Na^{+}) selectively binds DNA
  - Alcohol precipitates DNA

DNA Isolation-2

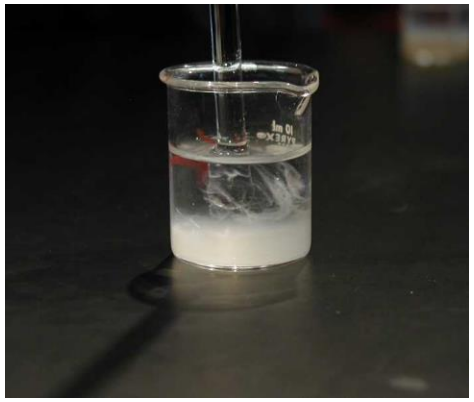


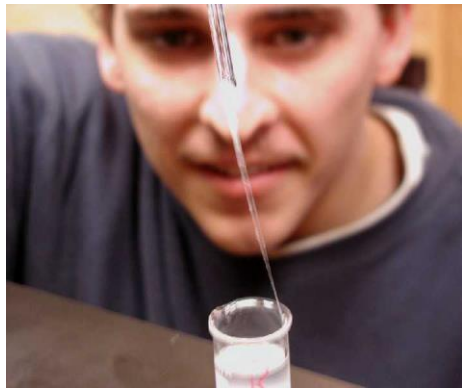
- Cells were grounded and destructed in special solutions.
- Incubation (generally in high temperatures; boiling method)
- Removing of cell residues-santrifuging
- Adding of alcohol (ethanol or isopropanol)
- Cooling and santrifuging
- Collection of DNA in the bottom of eppendorf tube as a pellet

DNA Isolation-3

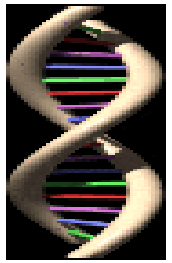


- DNA seals to the glass
- We can use this characteristic in DNA purification
- Ethanol treatment of glass rod seperates DNA from the rod



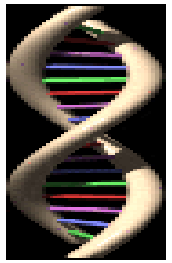


DNA Isolation-4



- DNA and RNA has similar physical characteristics
- Some enzymes (**RNAses**) are used for disruption of RNA in the matrices
- As well, enzymes (**Proteazlar**, **Proteinase-K**) are used for the denaturation of proteins binding to DNA molecules

DNA Isolation-5



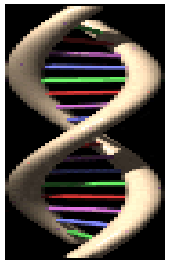
- DNA exists in the cell as a complex with some proteins (histones, non-histone proteins, High mobility group (HMG) proteins) and RNA. In microorganisms like viruses , DNA is found in a protein coat.
- DNA isolation can be performed basically in three steps:

1. Destruction of cell-wall

2. Separation and Disruption of DNA-protein complexes

3. Separation of DNA from other molecules in the matrices

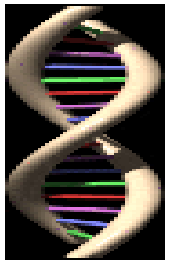
Lysis of Cell Wall:



- 1. First stage is the weakening of cell wall:
  - Physical (freezing-thawing)
  - Chemical (lyzozyme, EDTA)
- 2. Destruction stage:
  - Ionic detergents (sodium dodecyl sulphate, SDS)
  - Non-ionic detergents (Triton X-100)
  - Chemical treatment times differs according to the organism
- Method used for destruction depends to two factors:
  - 1. Length of DNA
- i.e.: DNAs longer than 15 kb arehighly sensitive so time of application should be shorter and should work carefully
  - 2. Organism
- Cell wall content of the organism require use of different chemicals for the purpose :
- In Bacteria---------- lysostaphine (Staphylococci), lyzozyme (Streptococci), proteinase K
- In yeasts ---------- novozyme

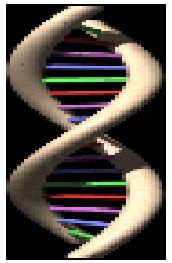
i.e.: *Shizosaccharomyces pombe*

Resolution of DNA-Protein Complexes



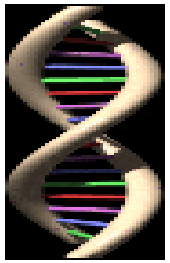
- Denaturation ---------- phenol extraction
- By the help of phenol proteins are denatured and removed from the environment
- pH of the phenol is important; since in alcalic pH (pH 8.0) RNAs are removed, in asidic pH (pH 5.0) DNA are removed.

Seperation of DNA from other molecules in the environment



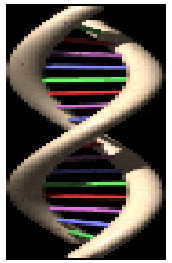
- Treatment of DNA with physical and chemical substances:
- Chemical precipitation of i.e.: ethanol
- Chemicals increase the level of precipitation i.e.: isopropanol
- Physical precipitation of DNA: santrifuges
- Tuning of santrifuge rpm depends on the weight of the molecule.

Nukleic Acid Purification



- The purpose of nukleic acid use defines the level of purity of nucleic acid
- According to the purpose sometimes no purification is needed and other times lots of consecutive steps are needed
- The simplest purification step is treatment of 70% ethanol: removes salts
- Phenol/chloroform treatment removes protein from the environment

Purity of nucleic acids



- Column Chromatography – removement of small DNA fragments and other nucleotides
- Cesium density santrifugation – isolation of high purity DNA
- Electrophoresis and cutting of pure DNA from the agarose gel

Preparation of the equipment and the solutions used in DNA isolation

Equipment and expandatures;

- Nuclease free (nuclease free, DNAase, RNAase-free, PCR-grade) plastic expandatures (ependorf tubes, filtred pippet tips)
- Automatic pippettes used only for the purpose of DNA isolation
- Water bath, heating block
- Refrigerated santrifuge, vortex, sterile laminar flow, tube rocks, ice-buckets, homogenizators

Solutions

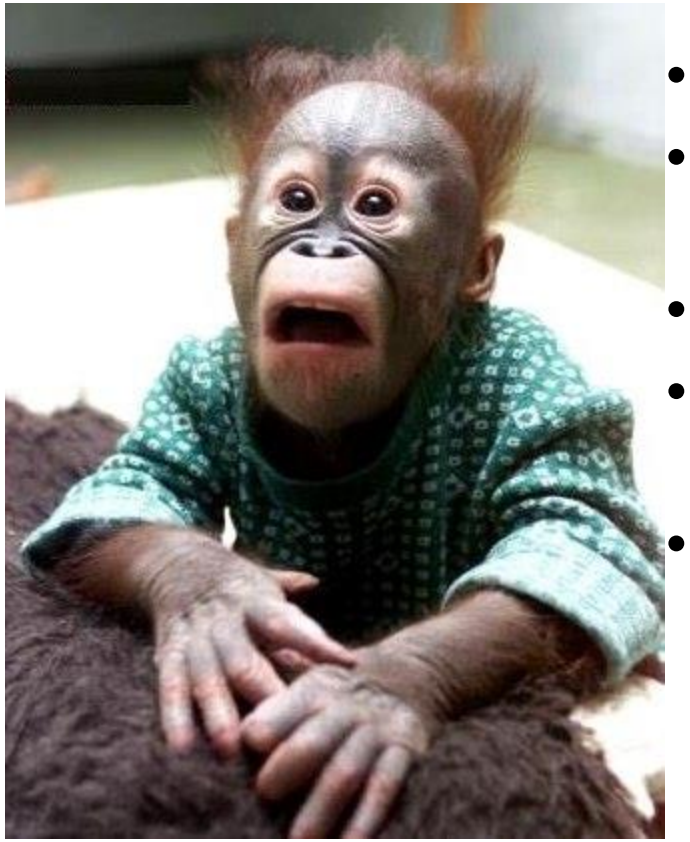
- TE (Tris-EDTA) buffer
- DEPC treated water, distilled water
- PBS, NaCl (%0.9 w/w)
- Proteinase-K (10mg/ml)
- Lysostaphine, lysozime enyme, RNAase
- Lyzis buffer (SDS+TNE, Tris-HCl, NaCl, EDTA)
- Phenol, chloroform, isoamylalcohol (separetly or in a mixture)

DNA Isolation Methods

DNA Isolation Methods

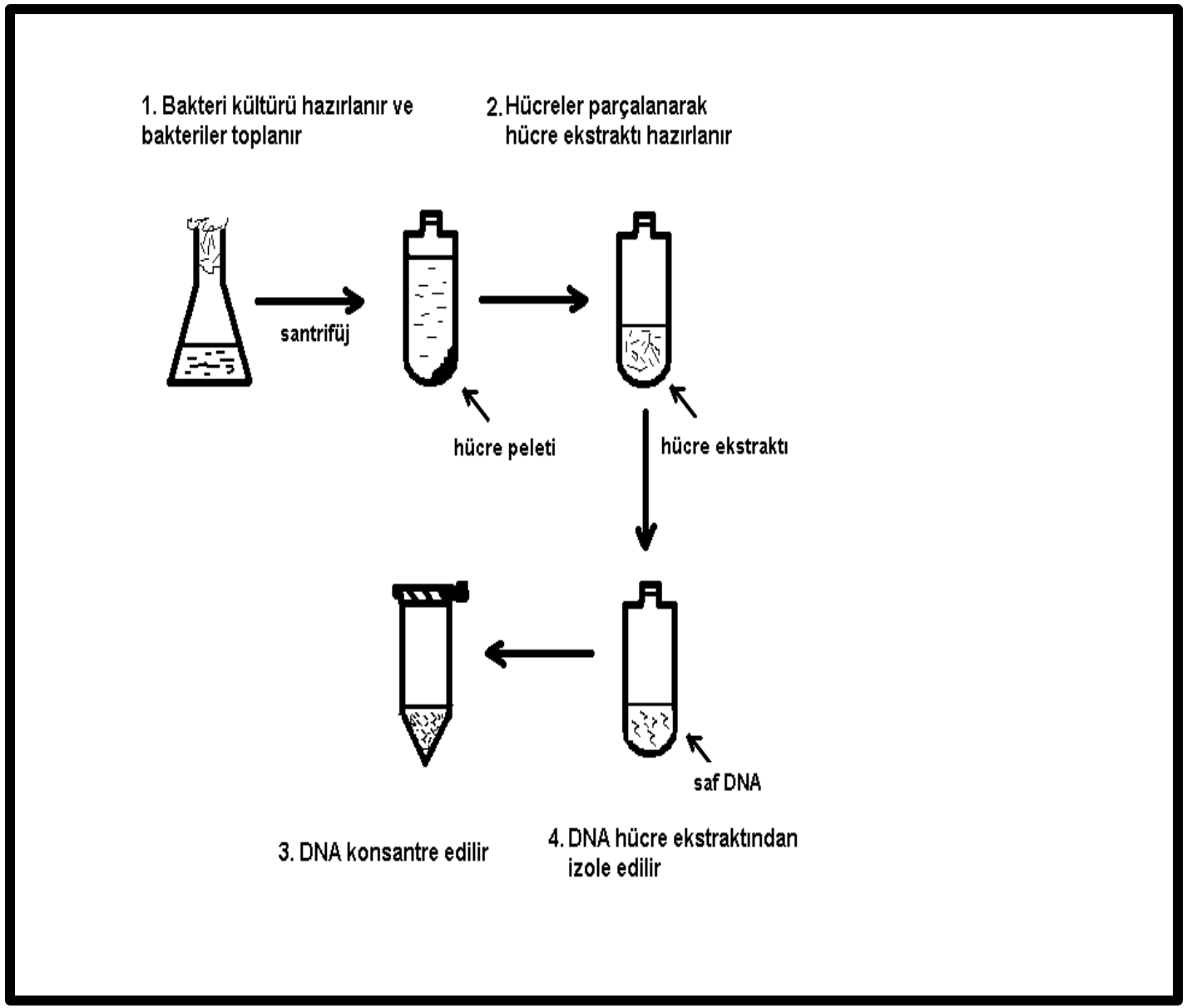
- Easiest: direct use without isolation
- Boiling method
- Phenol/chloroform extraction
- Alcali-lysis method
- Commercial kits according to instructions

Which DNA method?
How to decide?



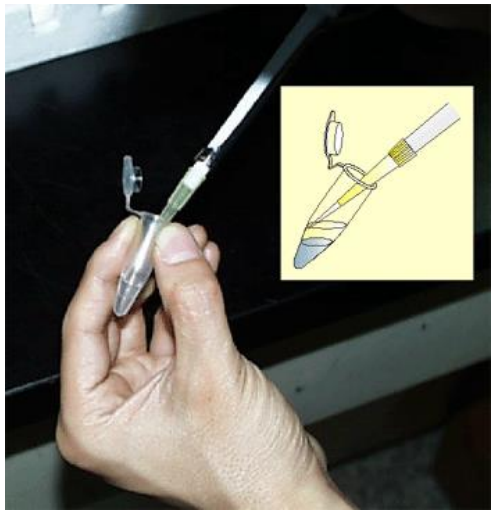
- Total time and labor for the job
- Reliability of isolation method regarding diagnosis
- Contamination risks
- Isolation of sufficient amount and pure DNA for amplification
- As the number of processes increases DNA losses also increses

DNA isolation from bacteria



Homogenisation

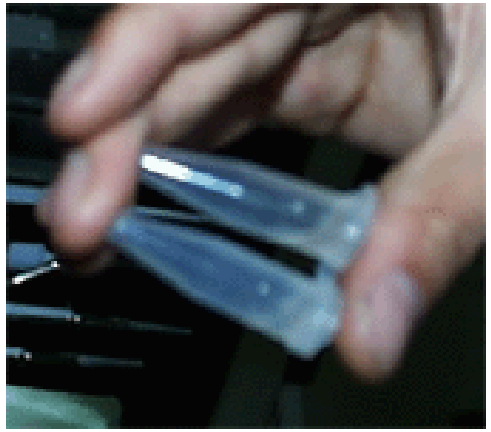
Pipetting of supernatant



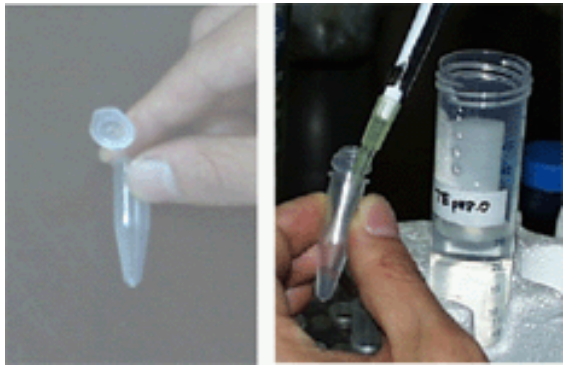
Removing of ethanol

Phenol extraction

Following ethanol and isopropanol tubes are shaken manually



Elution of DNA



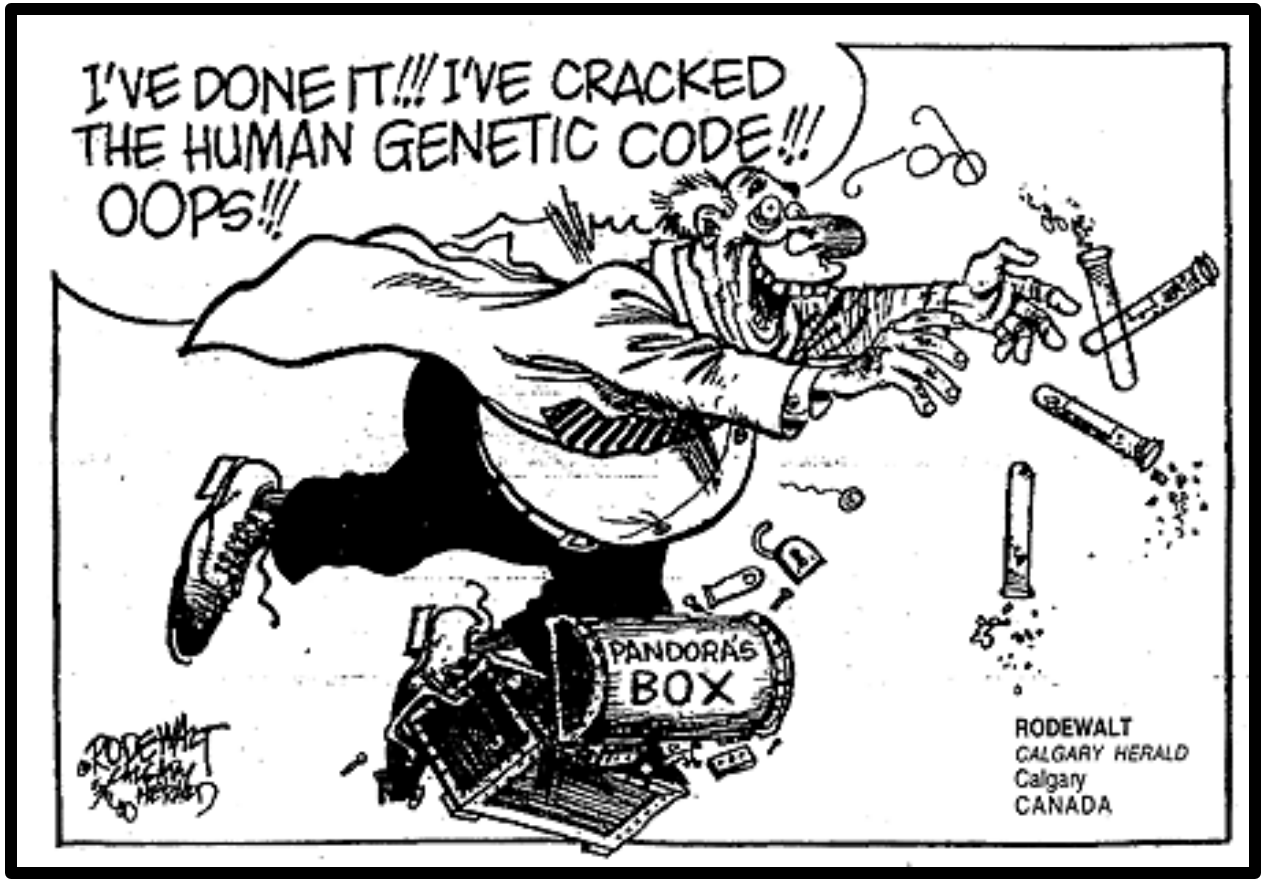
Commercial DNA Isolation Kits

- Genomic DNA isolation kit (Fermentas)
- DNA isolation kit for blood/bone marrow/tissue (Roche)
- High Pure PCR Template Preparation Kit (Roche)
- DNeasy Tissue Kit / QIAamp DNA Mini Kit / QIAquick PCR Purification Kit (Qiagen)
- MasterPure™ Complete DNA and RNA Purification Kit (Epicentre)
- 200-400 € prices

Storage of Isolated DNA

- In DNA TE (Tris-EDTA, pH 7.4-8.3) buffer, DEPC (diethylpyrocarbonate) treated water or just in steril water.
- Although DNA can be stored in room temperature
- For daily storage at 4ºC
- For long-term storage at -20ºC
- For longer storage (years) at -70ºC.
- Do not forget that DNA has a fragile structure. Be careful not to freeze-thaw DNA a lot since it can cause mechanical destruction of DNA

Problems faced with DNA isolation



These are;

- Cross-contaminations
- External (environmental) contaminations
- Loss of DNA due to wrong manupilations
- Insufficient DNA isolation