Phenol-Chloroform Isoamyl alcohol (PCI) DNA extraction and Ethanol Precipitation. protocol

Principle of PCI method:

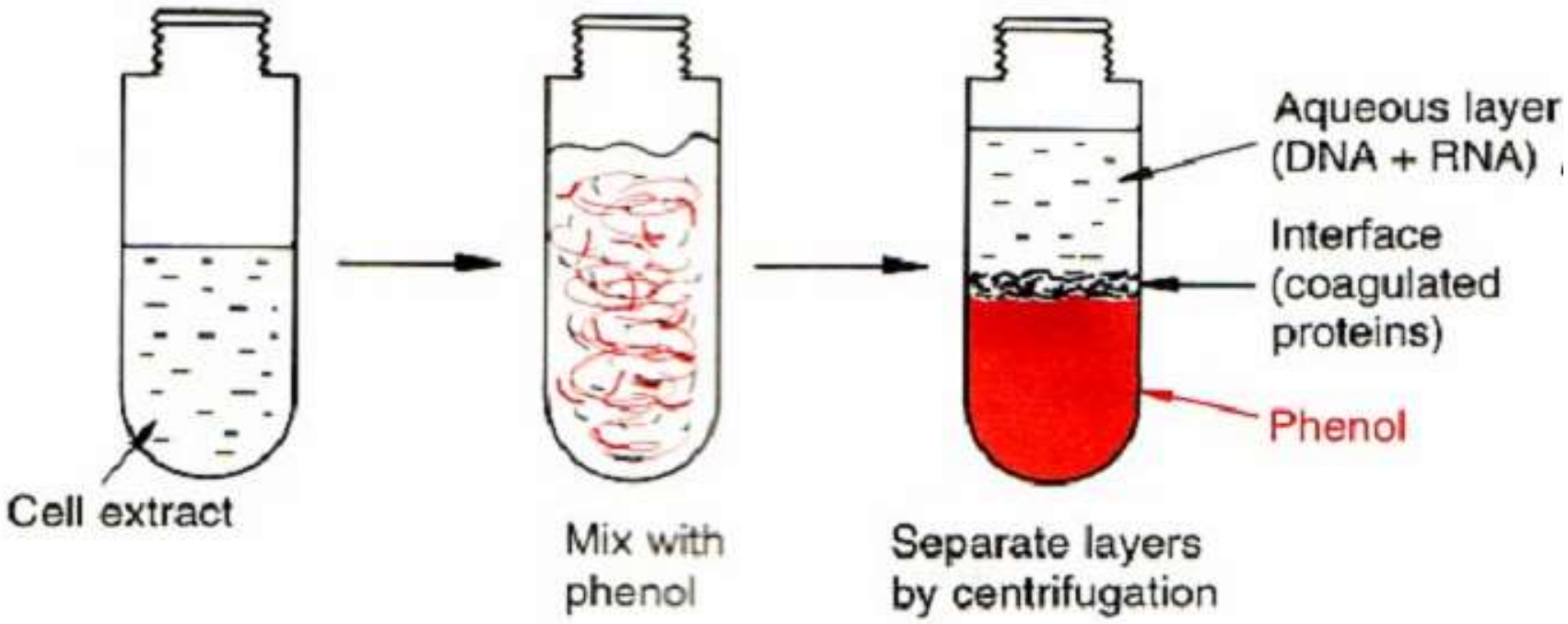
The basic principle of phenol-chloroform DNA extraction method is based on the liquid-liquid extraction of biomolecules.

The protein portions of the cell are denatured and removed by separating DNA into the soluble phase. The entire mechanism of separation is based on the solubility of the biomolecules.

Water is a polar solvent and phenol is a non-polar solvent. Also, phenol is denser than water.

On the other hand, DNA is a polar molecule with a net negative charge on its backbone and protein is non-polar. As we all know that the polar molecule can dissolve in polar solutions thus DNA dissolves in water but not in phenol.

Additionally, water can not mix with the phenol so phenol remains at the bottom due to its higher density. Meanwhile, DNA dissolves in water/chloroform and remains on the top of the phenol as a watery layer(aqueous phase).
When we mix phenol with the cell suspension the protein portion of the cell get digested or denatured and when we centrifuge it, the denatured protein settled into the bottom of the tube along with the phenol.
During the process of mixing, when sample and phenol are mixing together, it forms the foam-like emulsion (emulsification).


Aqueous layer
(DNA + RNA)
Interface
(coagulated
proteins)
Phenol
Cell extract
Mix with
phenol
Separate layers
by centrifugation

Role of each chemical:

Phenol: Phenol (also called **carbolic acid**) is an aromatic organic compound with the molecular formula C_6H_5OH.

DNA is insoluble in phenol because phenol is a non-polar solution.

On the other side, protein has both polar and non-polar group present in it because of the long chain of different amino acids. Different amino acids have different groups present on their side chain.

Also, the folding of the protein into the secondary, tertiary and quaternary structure depends on the polarity of the amino acids. The bonds between amino acids are broken by the addition of phenol and protein get denatured.

Ultimately, we can say the protein become unfolded by addition of phenol.

Chloroform:

Chloroform increases the efficiency of phenol for denaturation of the protein. Here, chloroform allows proper separation of the organic phase and aqueous phase which keeps DNA protected into the aqueous phase.

Chloroform denatures the lipid as well.

Isoamyl alcohol:

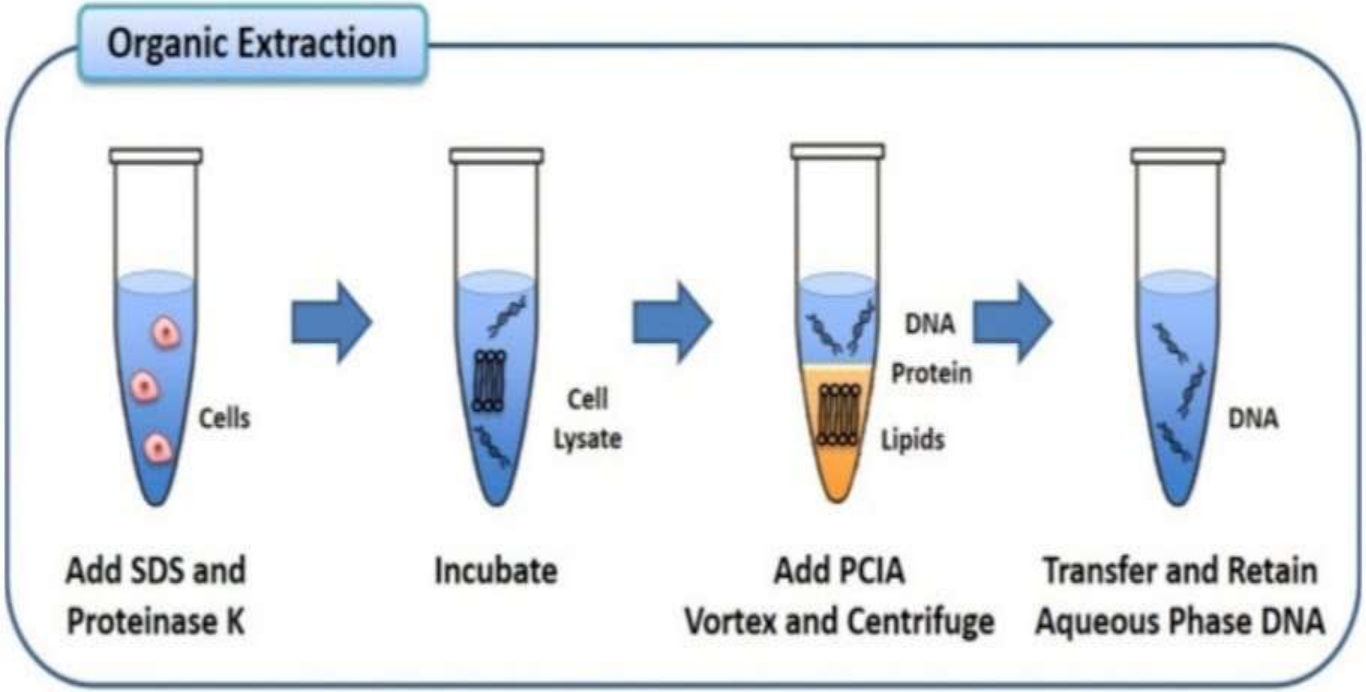
In the phenol-chloroform DNA extraction method, Isoamyl alcohol helps in reducing foaming between interphase. It prevents the emulsification of a solution.

The liquid phase contains DNA and the organic phase contains lipid, proteins and other impurities. The precipitated protein denatured and coagulated between both these phases. This will create the cloudy, whitish- foam between interphase.

The anti-foaming agent, isoamyl alcohol stabilized the interphase by removing the foaming. This will increase the purity of DNA.

Chemical	Role in DNA extraction
Tris	It maintains the pH of the solution and also permeabilizes the cell membrane.
EDTA	It is a chelating agent and blocks the activity of DNase enzyme.
SDS	It is an anionic detergent which helps in denaturation of cell membrane protein.
NaCl	Prevents the denaturation of DNA
MgCl2	Protects DNA from mixing with other cell organelles
TE buffer	It dissolves DNA

DNA extraction phenol chloroform method



organic method

DNA extraction from whole blood protocol

1- 0.5-2.0 ml of blood sample is transferred to a centrifuge tube with 12-15 ml size.

2. Centrifuge the samples at 4000 rpm for 15 minutes at room temperature, to obtain a cellular pellet, and discard the supernatant.

3. Add 2ml of lyses buffer 2X, then the sample inverting several times and left the mixture at 4°C for 10-30 minutes.

4. Centrifuge the mixture at 3000rpm for 10 minutes to obtain a nuclear pellet, and then discard the supernatant.

5. Re-suspended the nuclear pellet in 1ml of salt/EDTA buffer and mixing by using vortexes briefly.

6. To re-suspending the pellet, add 100µl of 10% SDS, and 10µl of 20mg/ml Protinase K

7. The latter mixture was then incubated at 37°C overnight.
Or **The optimal temperature for Protinase K activity ranges between 50-65 ˚C for 10-30 minutes. The higher temperatures help with protein unfolding, easing the ability for proteinase K to breakdown those proteins.**
8. Following incubation, 1ml of phenol (saturated with 0.1M Tris HCl pH 8.0) was added, and then mixed on a rotary mixer, for 10 minutes, followed by centrifugation at 2000rpm for 5 minutes (using a bench centrifuge).
9.The supernatant was then transferred to another tube, then re-extracted with 1ml chloroform: Isoamyl alcohol (24:1), and then mixed on a rotary mixer, for 10 minutes, followed by centrifugation at 2000rpm for 5 minutes.
10. The supernatant from the last step was mixed gently with 0.5ml of 7.5M Ammonium acetate and 3ml of absolute ethanol, to precipitate DNA, and the mixture was cooling at 4°C for 2-4 hours.

12. If the precipitate DNA visualize then removed with a Pasteur pipette whose end has been sealed and shaped into a U and left to dissolve in 200µl of sterile Tris/EDTA buffer on a rotary mixer overnight.
13. If the DNA precipitated becomes fragmented and not visualized, collect it by centrifugation at 4000rpm.
14. Discard the supernatant and add 200 µl of sterile Tris/EDTA buffer and on a rotary mixer overnight to dissolve the DNA.

https://www.youtube.com/watch?v=JNl1kjw9ZDQ

Salting out DNA isolation
https://youtu.be/um-ys5VKUJk

Homework: explain the DNA replication process

Preparation of materials used in phenol chloroform isoamyl DNA extraction method

Lyses buffer 2X: It was prepared by dissolving 0.829 g of Ammonium Chloride, and 0.092 g of Potassium Hydrogen Carbonate in 0.4 ml of 0.5M EDTA (pH 8.0). The volume made up to 100ml by distillated water, autoclaved and store at 4°C.

Salt/EDTA Buffer: It was prepared by dissolving 0.44 g of Sodium Chloride in 4.8ml of 0.5 M EDTA (pH 8.0). The volume was made up to 100ml by distilled water, autoclaved and stored at 4°C.

TE Buffer: was prepared by adding 2.5 ml of 1M Tris buffer (pH 8.0), to 0.5 ml of 0.5 M EDTA (pH 8.0). The volume was made up to 250 ml by distilled water, autoclaved and stored at 4°C.

Tris HCl 1M pH 8.0: was prepared by dissolving 60.55 g of Tris base in 400ml of distilled water. Concentrated HCl was added to adjust the pH to 8.0.The volume was made up to 500ml by the distilled water and sterilized by autoclaving.

Tris HCl buffer 0.1M pH 8.0: was prepared by dissolving 12.11 g of Tris base in 800 ml of distilled water, pH was adjusted to 8.0 by concentrated HCl. The volume was made up to 100 ml by adding distilled water.

EDTA 0.5M pH 8.0: was prepared by dissolving 93.05 g of EDTA in 400 ml of distilled water and stirred vigorously on a magnetic stirrer. pH was adjusted to 8.0 using 10N NaOH. The volume was made up to 500ml by distilled water, and sterilized by autoclaving.

Chloroform: Isoamyl alcohol (24:1): To prepare 100ml of this mixture, 96ml of chloroform was added to 4ml of Isoamyl alcohol and stored in a dark bottle at 4°C until use.

NaOH 10M: was prepared by dissolving 40 g of NaOH in 80 ml of distilled water. The volume was made up to 100 ml by distilled water.

Phenol: was prepared by melting Crystalline phenol at 68°C water bath, and then overlaid with an equal volume of 1M Tris pH 8.0, and allowed to stand overnight at 4°C.The 1M Tris was then discarded to be replaced by 0.1M Tris pH (8.0), stored at 4°C for up to 4 weeks.

SDS Solution 10%: was prepared by dissolving 100g of SDS in 900ml of distilled water with heating at 68°C to assist dissolution. The volume adjusted to one liter using distilled water.

Ammonium acetate 7.5M: was prepared by dissolving 5.782 g ammonium acetate in 10ml distilled water, sterilized by filtration through 0.22µm micropore filter.

Ethanol 70%: was prepared by a mixing 70 ml of absolute ethanol and 30ml of distilled water.