Lecture 28: Methods of preparing genomic DNA

Why DNA as genetic material:

DNA is deoxyribonucleic acid made of two anti-parallel chains of four different nucleotides (A, T, G, C) connected to each other by phosphodiester bonds. Nature selected the DNA as genetic material (except retro viruses), due to its high structural stability over RNA and proteins. It carries all the genetic information in form of genes which get transcribed into RNA and then translated in the form of functional units that is protein.

Methods of DNA isolation:

There are several techniques for isolation of genomic DNA, based on the type of sample, from which DNA has to be isolated, required DNA quality and quantity, molecular weight of DNA and its downstream applications. All the procedures involves some common steps like, disruption of sample, lysis of cells, removal of proteins and other contaminants and lastly recovery of DNA. The disruption of samples and lysis of cells can be achieved by different methods depending on starting materials. The most common method for cell disruption and lysis are lysozyme treatment or treatment with detergent, which leads to rupturing of cell wall and release of cell components. In order to prevent the DNA from degradation the lysis method should be very gentle and if mechanical disruption is needed, it should be mild to prevent the DNA from fragmentation. There should be any chelating agent to chelate the Mg^{++} ions required for DNase action to prevent the enzymatic degradation of DNA. The RNA can be removed by RNase treatment and proteins can be generally removed by proteinase K treatment followed by extraction by salts, organic solvents, or interaction of DNA with solid matrix (anion exchange or

silica based technology). The DNA can be recovered by either ethanol precipitation or precipitation with isopropanol (Fig. 1)

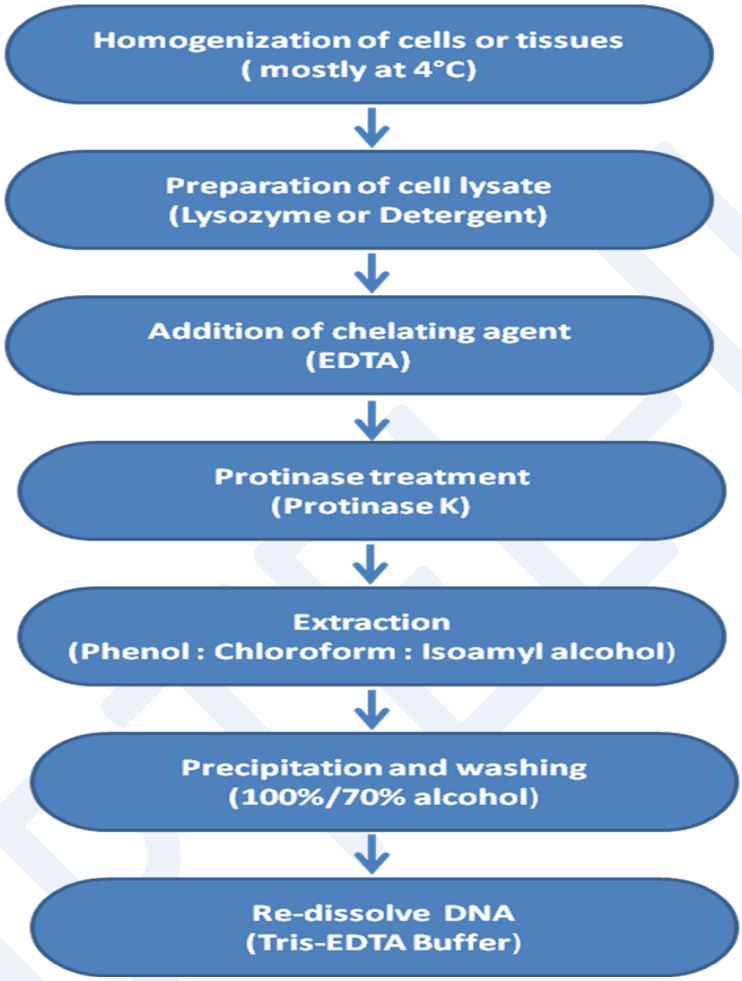

Figure 1: Different steps of genomic DNA prepration.

General Steps for preparation of genomic DNA:

1) Disruption
2) Lysis
3) Removal of protein and other contaminants
4) Recovery of DNA

Note: In some methods step 1 and 2 are combined.

Crude lysate preparation:

DNA can be purified very simply by incubating cell lysate at higher temperature (95°C, 20 min.) or to treate the cell lysate with proteinase K and the product can be directly used for further applications. This method is comparatively very simple and straight forward but, the quality of purified DNA will be poor and can be useful for few applications only. The DNA isolated with this method will not be at optimum pH and get degraded during storage, further more incomplete inactivation of proteinase K results several complications. The DNA will be contaminated with salts and several other compounds which can inhibit the enzyme activity in downstream process.

DNA extraction with salts:

The salting out phenomenon can be used for DNA isolation. The crude extract can be directly treated with high salt (potassium acetate or ammonium acetate) concentration for precipitation of proteins and other contaminants. The precipitates can be removed by centrifugation, and the supernatant can be further treated with alcohol for DNA precipitation. Removal of proteins and other contaminants using this method may be inefficient, and RNase treatment, dialysis, and/or repeated alcohol precipitation are often necessary before the DNA can be used in downstream applications. DNA yield and purity are highly variable using this method.

DNA extraction with organic solvents:

Extraction with organic solvents is a convention method where the cell lysate treated with detergents are mixed with a definite proportion of phenol, chloroform and isoamyl alcohol. Here the protein and other contaminants are separated with organic phase and the DNA remains in aqueous phase, which can be further precipitated with alcohol. The procedure is comparatively

time consuming and use of several toxic chemicals may interfere with downstream applications. Phenol is the most common interfering agent. This method is used for general DNA applications and not suitable for sensitive and high throughput applications.

DNA extraction with Cesium chloride (CsCl) density gradients:

CsCl density gradient method yields good quality of DNA, suitable for several downstream applications but, the use of toxic chemicals, comparatively longer time consumption and extensive labor restrict the usefulness of this method. The crude cell lysate was precipitated with alcohol. Resuspende the DNA and mixed with CsCl + EtBr. Centrifuge the mixture in a ultra centrifuge at high speed for longer time to collect the DNA in a single layer. The DNA layer was extracted with isopropenol to remove the EtBr. The DNA was then precipitated with ethanol.

DNA extraction by anion exchange chromatography:

Anion exchange chromatography method is comparatively faster and yields high quality of DNA which can be used for several purpose. This method doesn't required any toxic chemicals and intense labor, which makes it popular and can be adopted for routine purpose.

As clearly reflected with the name this method involves the exchange of anions. The matrix is positively charged and interacts with negatively charged phosphate group of DNA in low salt condition. The cell lysate was passed through matrix, which binds the DNA. Protein and other contaminants were washed with medium salt buffer and DNA was eluted with high salt concentration. The Eluted DNA was then precipitated with alcohol.

DNA extraction by silica based technology:

Silica gel based technology for extraction of DNA is the most popular, rapid and easy technology which yields ready to use DNA. This method yields highly purified DNA which can be used for any downstream application. It doesn't required extraction and precipitation of DNA. This method is based on selective adsorption of DNA on silica bed. The cell lysate was centrifuged to precipitate the cell debris and the supernatant was passed through silica bed. The buffer used for lysis ensures that only DNA adsorb on the silica bed and other components comes in flowthrouth. There are several kits in market based on this technology (Fig.2).

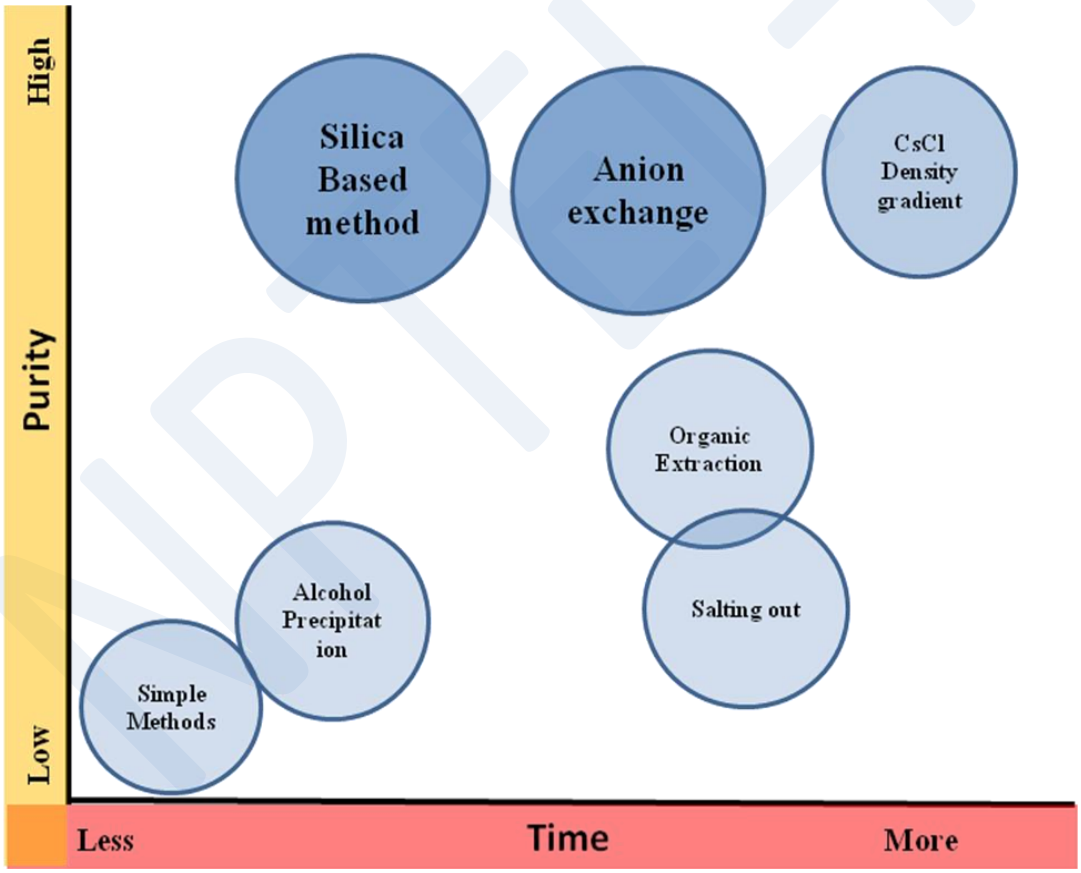

Figure 2: Comparison of different DNA preparation methods.

Determination of concentration of purified DNA:

After purification the integrity of purified sample can be checked on agarose gel. The concentration (µg cm^{-3}) of DNA can be estimated by spectrophotometer.

50 XA_{260} = Concentration of DNA Sample

Type of Nucleic Acid	Extinction Coefficient [$cm^{-1}M^{-1}$]
Double Stranded DNA	50
Single Stranded DNA	33
RNA	40

DNA is the polymer of nucleotides consists of pentose sugar, nitrogenous bases (A, T, G, C) and phosphate. The nitrogenous bases contain aromatic ring structure, which absorbs maximum at 260 nm, this counts the uv-spectrophotometric DNA estimation. The possible contaminants of DNA (protein & lipids) and reagents used during DNA preparation (phenols) also absorbs at 280 nm. The purity of DNA sample can be determined by the ratio of A_{260} to A_{280}. If the ratio is ≥1.8, means DNA is comparatively in pure form.