Principle of DNA Extraction

Extraction of DNA basically consists of four major steps.

1. Preparation of a cell extract:

To extract DNA from tissue/cells of interest:

- The cells have to be separated and the cell membranes have to be disrupted by using "Extraction buffer". Included in the buffer: EDTA and SDS.
- EDTA (Ethylene diamine tetra acetate) removes Mg^{2+} ions that are essential for preserving the overall structure of the cell membrane
- SDS (Sodium Dodecyl Sulphate) aids in disrupting the cell membranes by removing the lipids of the cell membranes.

Having lysed the cells, the final step in the preparation of a cell extract is removal of insoluble cell debris and partially digested organelles by centrifugation, leaving the cell extract as a reasonably clear supernatant.

2. Purification of DNA from cell extract

In addition to DNA the cell extract will contain significant quantities of protein and RNA. A variety of procedures can be used to remove these contaminants, leaving the DNA in a pure form.

The standard way to deproteinize a cell extract is to add phenol or a 1:1 mixture of phenol:chloroform. These organic solvents precipitate proteins but leave the nucleic acids in aqueous solutions. The aqueous solution containing nucleic acid is removed carefully with a pipette.

For RNA, however, the effective way to remove is by using ribonuclease enzyme, which will rapidly degrade these molecules into ribonucleotide subunits.

3. Collecting DNA

The most frequently used method of concentration is ethanol precipitation. In the presence of salt and at a temperature of -20 °C or less, absolute ethanol will efficiently precipitate polymeric nucleic acids. With a concentrated solution of DNA one can use a glass rod to spool the adhering DNA strands.

For dilution purposes the precipitated DNA can be collected by centrifugation and re-dissolving in an appropriate volume of water.

4. Measurement of purity and DNA concentration

UV absorbance can also be used to check the purity of a DNA preparation. For a pure sample of DNA the ratio of absorbencies at *260 nm and 280 nm (A_{260}/A_{280}) is 1.8*. This is because proteins absorb maximum UV light at A_{280}. Ratio of less than 1.8 is indicative of protein contamination.

If the solution is reasonably pure, DNA concentrations can accurately be measured using UV absorbance spectrometry. This is because the base pairs in DNA absorb UV light, therefore amount of bp is directly proportional to DNA concentration.

For DNA, absorbance at A_{260} (also called optical density, OD) is converted into DNA concentration by following method:

A_{260}/OD of 1.0 = a concentration of 50 μg/ml of double-stranded DNA *(dsDNA)*

A_{260}/OD of 1.0 = a concentration of 33 μg/ml of single-stranded DNA (ssDNA or RNA)

QUANTIFICATION OF EXTRACTED DNA SAMPLE BY SPECTROPHOTEMETRY

50 μg/ml of DNA = 1 OD (optical density)

Therefore the concentration is calculated by using following equation:

OD_{260} of sample X dilution factor X 50 μg/ml (1 OD) = μg/ml DNA

Example;

If 5 μl of extracted DNA in 1000μl (1ml) gives an OD_{260} = 0.14

Dilution factor = 1000 / 5 = 200

0.14 X 200 X 50 = 1400 μg/ml or 1.4 mg/ml

Concentration and purity via OD measurement

Concentration of DNA	➢ 1 A_{260} Unit of dsDNA = 50 μg/ml H_2O ➢ 1 A_{260} Unit of ssDNA = 33 μg/ml H_2O
Notes	➢ OD value should lie between 0.1 and 1.0 to ensure an optimal measurement. ➢ The above mentioned values are based on extinction coefficients of nucleic acids in H_2O, please note that these coefficients – and hence the above mentioned values – differ in other buffers and/or solutions. ➢ Example of calculation: ● volume of dsDNA sample: 100 μl ● dilution: 25 μl of this sample + 475 μl H_2O (1/20 dilution) ● A_{260} of this dilution: 0.44 ● concentration of dsDNA in sample: 0.44 x 50 μg/ml x 20 (=dilution factor) = 440 μg/ml ● amount of dsDNA in sample: 440 μg/ml x 0.1 ml (= sample volume) = 44 μg
Purity of DNA	➢ Pure DNA: $A_{260}/A_{280} \geq 1.8$
Notes	➢ An $A_{260}/A_{280} < 1.8$ indicates that the preparation is contaminated with proteins and aromatic substances (e.g. phenol). ➢ An $A_{260}/A_{280} > 2$ indicates a possible contamination with RNA. ➢ The OD gives no information about the size of the DNA.

Today's Experiment:

DNA extraction using QIAGEN™ mini columns

A ***costly*** however, an ***effective*** method of extracting high quality amplifiable genomic DNA from whole blood, urine, dried blood spot, buffy coat and tissue biopsy samples. Refrigerated samples and reagents from the kit are brought to room temperature before starting the procedure. Check the following equipments and reagents are ready:

- *Water bath at 56°C.*
- *Buffer AE or dd.H_2O for elution.*
- *Buffer AWl, Buffer AW2, and QIAGEN Protease.*
- *If a precipitate has formed in Buffer AL, dissolve by incubating at 70^oC*
- *All centrifugation steps should be carried out at room temperature*

1. Pipette 20µl of QIAGEN Protease into the bottom of a 1.5ml microcentrifuge tube.

2. Add 200 µl sample to the microcentrifuge tube.

3. Add 200µl buffer AL to the sample. Mix by pulse-vortexing for 15sec.

4. Incubate at 56°C for 10 min.

DNA yield reaches a maximum after lysis for 10 min at 56°C, but longer incubation times will not have negative effect on DNA extraction.

5. Briefly centrifuge the 1.5ml microfuge tube to remove drops from the inside of the lid.

6. Add 200µl of ethanol (96-100%) to the sample and mix again by pulse-vortexing. After mixing, briefly centrifuge the 1.5ml microfuge tube to remove drops from the inside of the lid

7. Carefully transfer the mixture from step-6 to the QIAamp spin column (in a 2ml collection tube) without wetting the rim, close the cap, and centrifuge at 6000xg (8000rpm) for 1 min.

Place the QIAamp spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.

Do not over tighten caps. If caps are tightened until they snap they may loosen during centrifugation and subsequently damage the centrifuge.

8. Carefully open the QIAamp spin column and add 500µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000xg (8000rpm) for 1min. Place the QIAamp spin column in a clean 2ml collection tube (provided), and discard the collection tube containing the filtrate.

9. Carefully open the QIAamp spin column and add 500µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000xg; 14000rpm) for 3 min. Continue directly with step-10, or to eliminate any chance of possible buffer AW2 carryover, perform step 9a, and then continue with step 10.

9a. (optional): **Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the collection tube containing the filtrate. Centrifuge at full speed for 1min.**

10. Place the QIAamp spin column in a clean 1.5ml microfuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp spin column and add 200µl Buffer AE or distilled water. Incubate at room temperature for 5min, and then centrifuge at 6000xg (8000rpm) for 1min.

**A second elution step with a further 200µl Buffer AE will increase yields by up to 15%.*

<u>For calculating DNA concentration;</u>

Pipette 2µls in a clean 1ml tube and add to it 198µls of water to give 1/100 dilution factor

Take the OD at A_{260} x 100 x 50 = µg/ml of DNA