Quantification and Visualization of Isolated plant genomic DNA

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Introduction

- Quantification of isolated DNA is done by spectrophotometric method

- The DNA isolated from plant sample have characteristics OD of absorbance maximum at 260nm which is linearly related with the concentration of the DNA in the solution up to the OD value of 2 .

- For a 1-cm pathlength, the optical density at 260 nm (OD_{260}) equals 1.0 for the following solutions:

  - **50 µg/mL solution of dsDNA**
  - **33 µg/mL solution of ssDNA**
  - **20-30 µg/mL solution of oligonucleotide**
  - **40 µg/mL solution of RNA**

- Contamination of nucleic acid solutions makes spectrophotometric quantitation inaccurate.

- Calculate the OD_{260}/OD_{280} ratio for an indication of nucleic acid purity.

- Pure DNA has an OD_{260}/OD_{280} ratio of ~1.8;
- pure RNA has an OD_{260}/OD_{280} ratio of ~2.0.

- Low ratios could be caused by protein or phenol contamination.
- Value less than 1.8 signifies the presence of proteins as impurities.

- **DNA can also be quantified by measuring the UV-induced emission of fluorescence from intercalated ethidium bromide. This method is useful if there is not enough DNA to quantify with a spectrophotometer, or if the DNA solution is contaminated**.

Equipment and Chemical

- UV spectrophotometer /nanodrop
- Quartz cuvette
- Pipette and autoclaved tips
- Tissue paper
- Conical flask
- Beaker
- Distilled water or 1X TE buffer

Protocol

- Use 1X TE as a solvent to suspend the DNA
- According to different dilution amount of DNA and TE buffer can be changed. Final make up volume is taken 3 ml for UV- Vis spectrophotometer
- Measure absorbance at 260 and 280nm

Sample No.	Dilution (X)	ds DNA Amount (µl)	1X TE Buffer (µl)	OD at 260	OD at 280	OD_{260}/OD_{280} ratio	ds DNA Concentration (mg/mL)
1	50	60	2940				
2	100	30	2970				
3	250	12	2988				
4	500	6	2994				
5	1000	3	2997				

Calculate the DNA concentration using following formula (OD 1 for ds DNA at 260 nm = 50 µg/mL)

DNA concentration (µg/µl)= 50 X OD X Dilution factor/1000

A sample of dsDNA was diluted 50X (50 is dilution factor).
The diluted sample gave a reading of 0.65 on a spectrophotometer at OD_{260}. To determine the concentration of DNA in the original sample, perform the following calculation:

dsDNA concentration = 50 µg/mL × OD_{260} × dilution factor
dsDNA concentration = 50 µg/mL × 0.65 × 50
dsDNA concentration = 1.63 mg/mL or 1.63 µg/µl

Visualization of Isolated DNA

- The term gel electrophoresis refers to a technique used for separation and analysis of DNA, RNA , and proteins based on their size and charge.
- DNA fragments are negatively charged, so they move towards the positive electrode.
- Agarose gel electrophoresis is the standard lab procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification.
- Here DNA molecules are separated on the basis of charge by applying an electric field to the electrophoretic apparatus.
- Shorter molecules migrate more easily and move faster than longer molecules through the pores of the gel and this process is called sieving.
- The gel might be used to look at the DNA in order to quantify it or to isolate a particular band.
- The DNA can be visualized in the gel by the addition of ethidium bromide.

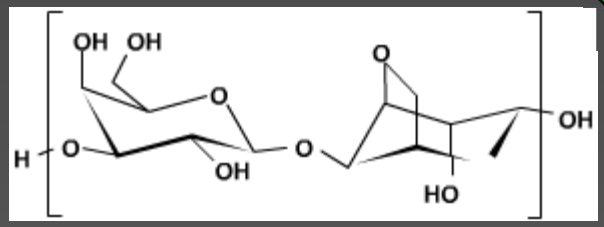
Equipment and accessories

- Weighing machine
- Microwave
- Horizontal gel electrophoresis unit (Casting tray and Gel box)
- Comb
- Power Supply
- UV-transilluminator or gel documentation system
- Pipette and their Different size autoclave tips
- Ice box and ice
- Measuring cylinder
- Beaker

Chemicals and buffers

- Electrophoresis (50x TAE/TBE) buffer
- Agarose
- Autoclave DDW
- Ethidium bromide
- Bromophenol blue
- Xylene Cyano
- DNA samples
- DNA Ladders

Agarose



- Agarose is a polysaccharide obtained from the red algae *Porphyra umbilicalis*. Its systematic name is (1 4)-3, 6-anhydro-a-L-galactopyranosyl-(1 3)-β-D-galactopyranan.
- Agarose makes an inert matrix. Most agarose gels are made between 0.7% and 2% of agarose.
- A 0.7% gel will show good separation for large DNA fragments (5-10kb) and a 2% gel will show good resolution for small fragments with size range of 0.2-1kb.
- Low percentage gels are very weak (Note:- it may break when you lift them) but high percentage gels are usually brittle and do not set evenly.
- The volume of agarose required for a mini gel preparation is around 30-50ml and for a larger gel, it is around
  250ml.

Electrophoresis (TAE/TBE) buffer

- Tris-acetate-EDTA (TAE) running buffer and tris-borate-EDTA (TBE) are commonly used buffers for DNA agarose gel electrophoresis
- TBE buffer has better buffering capacity than TAE, for **extended long runs or repeated runs** in the same buffer
- TAE buffer has better conductivity than TBE, so DNA fragments will migrate **faster** in TAE buffer than TBE.
- TBE buffer supports better agarose cross-linkage, **better resolution** of large DNA fragments in TBE buffer and better resolution of smaller DNA fragments in TAE buffer can be obtained.
- The borate in TBE buffer **inhibits many common enzymes** used in molecular biology, if DNA is being used for any downstream applications involving an enzyme, like PCR or clonal ligation,TAE buffer is better choice.
- And finally, TBE buffer **costs more** than TAE buffer.

Ethidium bromide

- EtBr binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light.
- Stock concentration of 5mg/mL, for it 5 mg EtBr dissolve in 1 ml desionized autoclaved water.
- Ethidium bromide should be stored in a cool, dark place away from strong oxidizing agents. Always keep the container tightly closed when not in use.

loading dye

- *Loading dye serves two purposes:*

1) *it provides a visible dye that helps with gel loading and allows you to gauge how far the DNA has migrated.*
2) *it contains a high percentage of glycerol that increases the density of your DNA sample causing it settle to the bottom of the gel well, instead of diffusing in the buffer.*

- Bromophenol blue migrates almost equal to the migration of ~300bp, whereas Xylene Cyanol migrates arount 3Kb. Similarly Orange G migrates around 50bp and cresol red 1.5Kb.
- Depending on your desired DNA length you can choose your dye fronts. commonly used dyes are BPB and XC-FF, since the range is wide…

6X DNA Loading Dye - 10 ml

- **Reagents needed:**
- 25 mg bromophenol blue
- 25 mg xylene cyanol FF
- 3.3 ml glycerol
- 6.7 ml ddH_2O
- **Directions:**

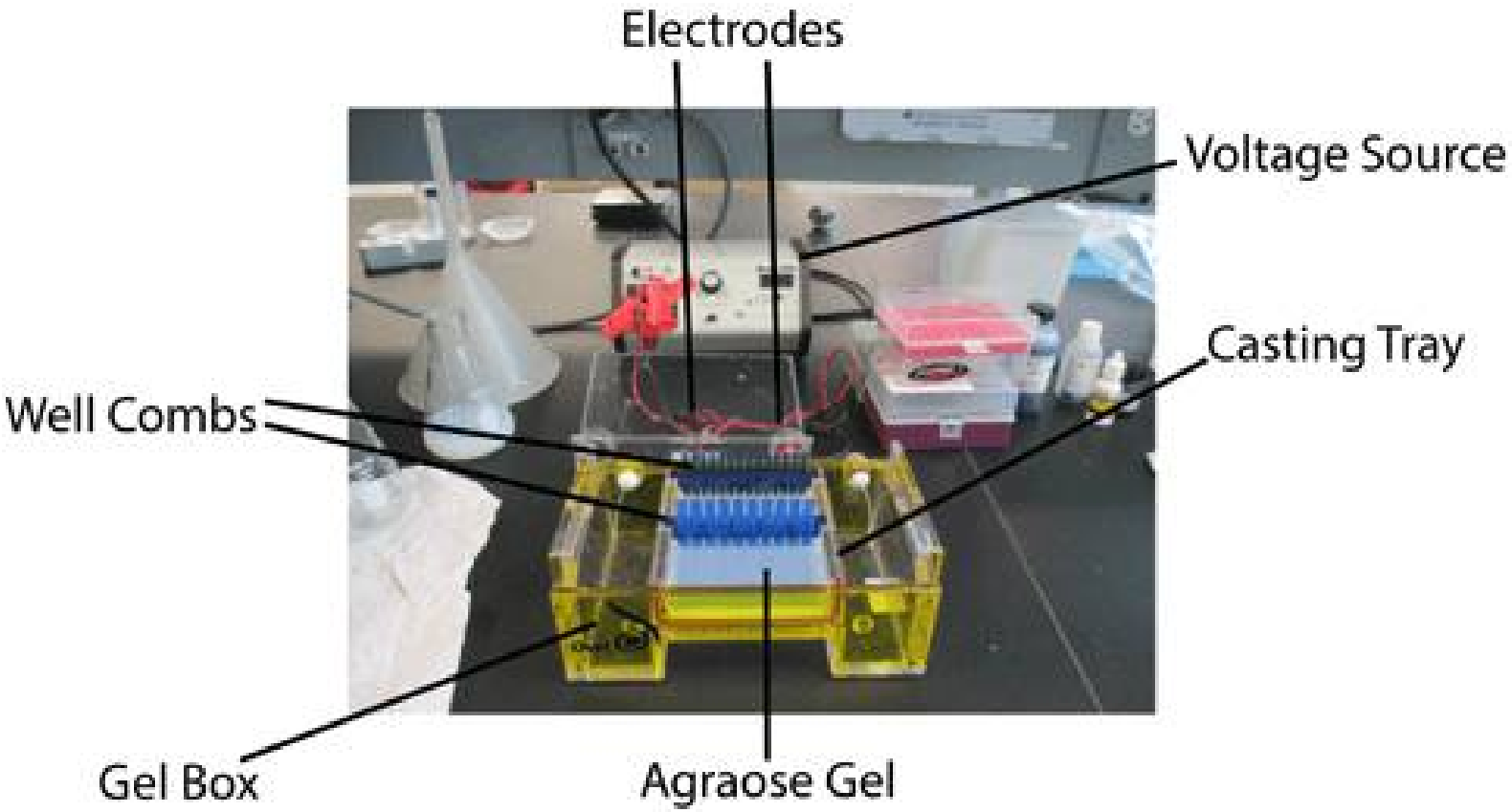
➢ Add 25 mg of bromophenol blue to 6.7 ml of ddH_2O and mix.

➢ Add 25 mg of xylene cyanol FF and mix.

➢ Add 3.3 ml of glycerol and mix.

➢ Aliquot and freeze at -20 °C for long-term storage.

Agarose gel electrophoresis Unit



Before Running the gel…..

- *When agarose dissolve, Be careful stirring, eruptive boiling can occur.*
- *EtBr is a known mutagen b*ecause it can bind with DNA, it is highly toxic.
- *EtBr* may potentially cause carcinogenic or teratogenic effects, although no scientific evidence showing either health effect has been found. Exposure routes of *EtBr* are inhalation, ingestion, and skin absorption.
- *Wear a lab coat, eye protection and gloves when working with this chemical.*
- *When using UV light, protect your skin by wearing safety goggles or a face shield, gloves and a lab coat.*

Procedure

0.8% agarose solution prepration

- Measure 0.8 g of agarose.
- Mix agarose powder with 100 mL 1xTAE in a microwavable flask.
- Microwave for 1-3 min until the agarose is completely dissolved.

Agarose gel formation

- Let agarose solution cool down to about 50 °C.
- Add ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5 µg/mL.
- Pour the agarose into a gel tray with the well comb in place.
- Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

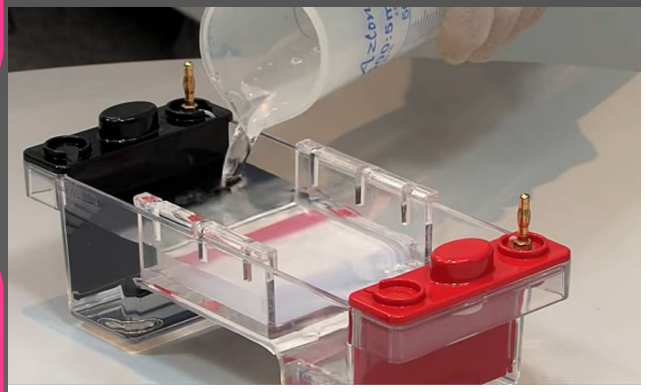
Gel casting tray

Sealing of ends and placing combs

Pouring of cooled 1% Agarose gel containing

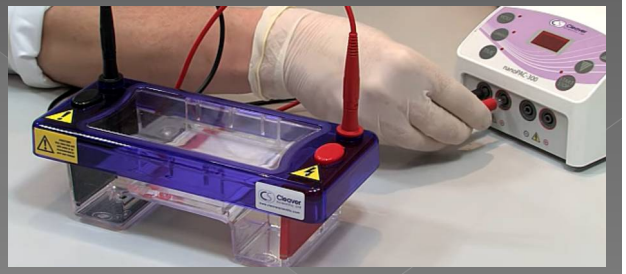
Procedure of loading sample

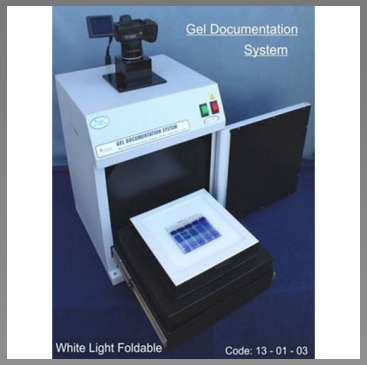
- Add loading dye (5:1) to each of your DNA samples.
- Once solidified, place the agarose gel into the gel box (electrophoresis unit).
- Fill gel box with 1xTAE (or TBE) until the gel is covered.
- Comb will be removed.
- Carefully load a molecular weight ladder into the first lane of the gel.
- Carefully load your samples into the additional wells of the gel.

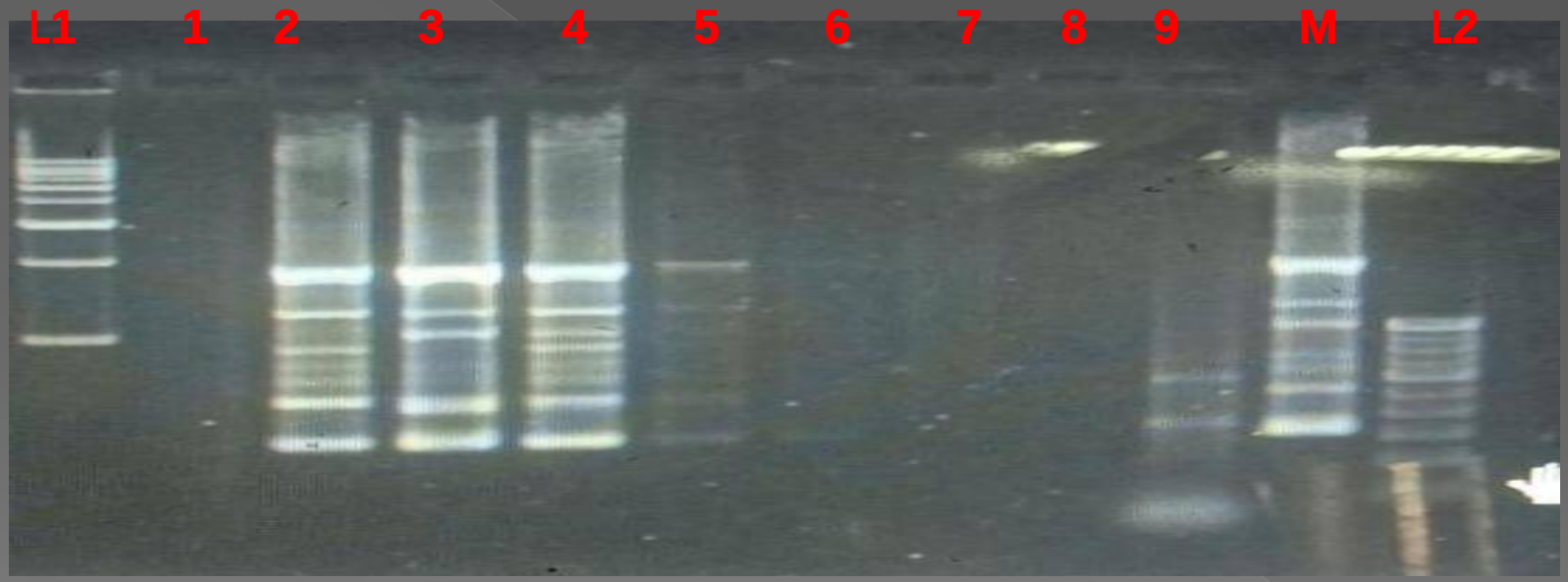


Run the gel and visualize DNA fragments

- Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel.
- Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.
- Using any device that has UV light For visualize DNA fragments.







Lane L1: GeneRuler 100 bp, Lane L2: GeneRuler 1 Kb DNA Ladder (Bangalor Genei),

Tips

- *Make sure to use the same buffer for gel preparation as the one in the gel box (do not mix different buffers and do not use water).*
- It is a good idea to microwave for 30-45 sec, stop and swirl, and then continue towards a boil.
- *If you add EtBr to your gel, you will also want to add it to the running buffer when you run the gel. If you do not add EtBr to the gel and running buffer.*
- Pour slowly to avoid bubbles which will disrupt the gel. Any bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip.
- A few simple ways to increase the resolution (crispness) of your DNA bands include: a) running the gel at a lower voltage for a longer period of time; b) using a wider/thinner gel comb; or c) loading less DNA into the well. Another method for visualizing very short DNA fragments is polyacrylamide gel electrophoresis (PAGE), which is typically used to separate 5 - 500 bp fragments.

- If you have similarly sized bands that are running too close together, you can adjust the agarose percentage of the gel to get better separation. A higher percentage agarose gel will help resolve smaller bands from each other, and a lower percentage gel will help separate larger bands.

- For each sample you want to load on a gel, make 10% more volume than needed because several microliters can be lost in pipetting. For example, if you want to load 1.0 µg in 10 µL, make 1.1 µg in 11 µL.

- *In electrophoresis Unit, Black is negative, red is positive. The DNA is negatively charged and will run towards the positive electrode.* ***Always Run to Red.***

- If you will be purifying the DNA for later use, use long-wavelength UV and expose for as little time as possible to minimize damage to the DNA.

THANKS