

Experiment 1: DNA Isolation from Plant and Animal Tissue and Gel Electrophoresis

Amber Broadbooks, Adrienne Martinez, Quenton Sayles

Submitted: February 19, 2009 to Dr. Bidlack

Revised by Dr. Bidlack on February 21, 2009

DNA Isolation from Plant and Animal Tissue and Gel Electrophoresis

Objective:

The purpose of the experiment is to extract DNA from a sample from both a plant and animal tissue in order to qualitatively evaluate DNA on an agarose gel and quantitatively determine the percent yield of DNA obtained. This can be accomplished by Gel Electrophoresis and Spectrophotometric Methods.

Part 1: DNA Isolation

Equipment:

Microcentrifuge
Microcentrifuge Tubes
DNeasy Mini Spin Columns
QIA Shredder Mini Spin Columns Collection Tubes
Vortex
Mortar and Pestle
Micropipettes with Tips (preferably P-100 and P-200)

Reagents:

Buffers: API, AP2, AP3/E*, AE, AW*
RNase A
N₂ (l)

**Note that Buffers AP3/E and AW are supplied as concentrates. Ethanol must be added to these reagents as indicated on the bottle before using.*

Procedure

1. Initial preparations consist of chilling a mortar and pestle, preparing an ice bath, and pre heating the water bath to 65° C. Once heated, place the Buffer AE into the warm water bath.
2. Obtain a frozen sample of onion, by freezing with N₂ (l) and shatter by pounding the sample with a chilled mortar and pestle until a powder consistency is obtained. Immediately weigh 0.250 g on an analytical balance (it is recommended that no more that 0.100 g be used, but sometimes this is difficult to weigh and completely transfer all of it to the centrifuge tube – just be sure to record the exact amount of sample you use for future calculations).
3. Transfer the sample directly into a 2.00 mL centrifuge tube.
4. Micropipette 400 µL of Buffer AP1 and 4.00 µL of RNase A into the sample and vortex.
5. Incubate the sample for 10 minutes in the 65° C water bath. During incubation mix the sample at least 2-3 times by inversion.
6. Place 130 µL of Buffer AP2 to the centrifuge tube. Mix with a pipette, place in the ice bath, and allow to incubate for 5 minutes.
7. Place the sample in the centrifuge. Spin at 10,000 rpm for 5 minutes. If a pellet has formed, it will not be used in the next step and can be discarded.
8. Transfer the lysate (liquid portion) to a QIAshredder Mini Spin Column and centrifuge for 2 minutes at 10,000 rpm.
9. Transfer the flow through collection from the QIAshredder Mini Spin Column into a new centrifuge tube.

10. Add 750 μL of Buffer AP3/E to the lysate sample and mix thoroughly. If a pellet forms, be sure to suspend this evenly into the mixture in preparation for the next step.
11. Transfer 650 μL of the lysate sample into the DNeasy Mini Spin Column. Do not discard the remaining solution. Centrifuge the mixture for 1.5 minutes at 6,000 rpm. Discard the flow through solution but keep Mini Spin Column for the next step.
12. Using any remaining lysate sample (from the previous step), transfer into the same Mini Spin Column, and repeat centrifugation as in the previous step.
13. Place the DNeasy Mini Spin Column in a new 2.00 mL microcentrifuge tube and add 500 μL Buffer AW to the Column and centrifuge for 2 minutes at 10,000 rpm. Discard the flow through collected in this step, but keep the DNeasy Mini Spin Column, which now contains DNA.
14. Transfer the DNeasy Mini Spin Column to a new 2.00 mL microcentrifuge tube and pipet 100 μL of the pre-heated Buffer AE directly onto the DNeasy membrane. Allow 5 minutes incubation time at room temperature before centrifuging for 2 minutes at 8,000 rpm.
15. Collect the flow-through from the previous step, which contains DNA, and use this sample for subsequent parts of the experiment.

Part 2: Electrophoresis

Equipment:

Microwave
Micropipette with Tips (preferably P-10 and P-100)
Microcentrifuge Tubes
100 mL Graduated Cylinder
Analytical Balance
Heat-Resistant Gloves
250 mL Erlenmeyer Flask
Electrophoresis Chamber with Power Supply
Gel Mold and Combs
UV Light Box

Reagents

Agarose Gel Powder
"Sample" Loading Dye
10X TAE Buffer
DNA Ladder from BstEII digest of lambda
Ethidium Bromide (10 mg /mL) NOTE: strong mutagen, handle with gloves!

Procedure

1. Prepare 400 mL 1X TAE Buffer by adding 40 mL 10X TAE stock solution to 360 mL DI H₂O.
2. Use the 1X TAE Buffer to make a 1.0% agarose gel by adding 1.0 gram of agarose powder to 50 mL 1X TAE Buffer in a 250 mL Erlenmeyer Flask. Then add another 50 mL of the 1X TAE Buffer. Swirl to suspend the agarose in the TAE buffer.
3. Place the 1.0% agarose mixture into microwave and heat for 2 minutes. Remove using heat-resistant gloves. Check for floating particles or wisp strands, if they are present heat for 30 seconds and repeat until the solution is clear. Do not boil solution or heat for more than 4 minutes.

4. After letting the 1.0% agarose solution cool on bench top, add 10.0 μL of ethidium bromide (10.0 mg/mL) by pipette and swirl to mix (use gloves while doing this because ethidium bromide is a mutagen).
5. Assemble the Gel Mold and pour the 1.0% agarose solution carefully (pouring into one corner of the mold and allowing it to spread evenly). Set assembly aside until it is firm.
6. Place firm 1.0% agarose gel into electrophoresis chamber and pour the remaining 500 mL 1X TAE Buffer into chamber to cover gel completely.
7. In a labeled centrifuge tube mix 25 μL of sample DNA and 5 μL of sample loading dye.
8. In a labeled centrifuge tube mix 15 μL of the supplied DNA ladder and 5 μL sample loading dye.
9. Centrifuge both sample DNA and DNA ladder for 30 seconds at 5000 rpm to remove air bubbles.
10. Load 25 μL of sample DNA and 25 μL DNA ladder into separate lanes in the agarose gel taking note of lanes that each were placed in not overfilling the wells.
11. Place lid on the chamber and connect the wire closest to the wells to the negative current and the wire furthest from wells to the positive current on the power supply. DNA will be attracted to the positive end.
12. On a setting of 100 V on power supply, run the samples through the gel until loading dye is two-thirds of the way through the gel.
13. Turn off electrophoresis power supply and carefully remove gel and identify the DNA bands using the UV light box (Use appropriate eye-protection!).

Part 3: Spectrophotometric Analysis

Equipment

Varian Gary 50 Spectrophotometer

1.0 mL Quartz Cuvette

Micropipette with tips (preferably P-100 and P-200)

Reagents

DI Water

Procedure

1. Prepare the DNA Sample by using all remaining DNA (somewhere around 50 to 75 μL) and adding enough DI water to fill a 1.00 mL cuvette $\frac{3}{4}$ to the top. For example, use 50 μL of DNA and 450 μL of DI water to make 1:10 mixture of DNA plus water.
2. Mix by inversion with the aid of parafilm.
3. Using DI Water as a blank, scan the DNA sample with the Varian Gary 50 Spectrophotometer with a spectrum range of 230 nm - 330 nm. Look for peaks at 260 and 280 nm.
4. Using the Advanced Scan mode obtain a scan at both 260 nm and 280 nm for the prepared DNA Sample.

Data Analysis and Calculations (to be integrated into lab report)

Part 1: Determining relative purity and / or contamination. Use the Absorbance Ratio (A_{260}/A_{280}) and these general trends to determine if results for your sample revealed mostly DNA or if it was contaminated with protein or RNA. If the absorbance Ratio (A_{260}/A_{280}) is

0.0 < 1.8 = Protein Contamination

1.8 to 1.9 = DNA

1.9 to 2.0 = RNA Contamination

For example, if absorbance peak at A_{260} was 0.075 and the absorbance peak at A_{280} was 0.040. The A_{260}/A_{280} ratio would then be 1.875, which would be indicative of a fairly pure DNA sample since the ratio lies between 1.8 and 1.9

Part 2: Determining DNA in your onion sample. As a general rule of thumb, an absorbance reading (also called optical density) of 1.00 indicates that the concentration of double stranded DNA is about 50 $\mu\text{g} / \text{mL}$. Using Beer's Law and an extinction coefficient of $\epsilon = 20 \text{ g}^{-1} \text{ cm}^{-1} \text{ L}$ at A_{260} for double stranded DNA, calculate the amount of DNA in your original solution of DNA (100 μL) eluted from the DNeasy spin column in the last step. Then calculate the amount of DNA in your original onion tissue in units of μg DNA per gram of onion tissue. Note that you may have diluted your DNA before placing it into the spectrophotometer and this, too, should be used in calculation for DNA in the originally eluted DNA sample.

For example, let's start with 0.250 grams of onion tissue from which you extracted DNA and eluted 100 μL of DNA in the last step. If your A_{260} reading at the end of the experiment is 0.075 after elution of DNA, and you diluted your sample in the cuvette by mixing 50 μL of DNA with 450 μL DI water, the amount of DNA in your eluted DNA would be:

$A = \epsilon bc$, and rearranging gives $c = A / \epsilon b$. Assume the path length is 1.0 centimeters.

Such that, $c = 0.075 / (20 \text{ g}^{-1} \text{ DNA cm}^{-1} \text{ L})(1.0 \text{ cm})$ and c (in the cuvette) is $0.00375 \text{ g DNA} / \text{L}$ (or $3.75 \mu\text{g} / \text{mL}$). Taking into account that your DNA was diluted in the cuvette, the actual concentration of DNA in original eluted DNA sample would be $(0.00375 \text{ g DNA L}^{-1})(500 \mu\text{L} / 50 \mu\text{L}) = 0.0375 \text{ g DNA L}^{-1}$

And the amount of DNA in your original sample would be:

$(0.0375 \text{ g DNA L}^{-1})(1 \text{ L} / 1,000,000 \mu\text{L})(1,000,000 \mu\text{g} / \text{g})(100 \mu\text{L DNA} / 0.250 \text{ g onion tissue}) = 15 \mu\text{g DNA per gram of onion tissue.}$