MOHAWK COLLEGE OF APPLIED ARTS AND TECHNOLOGY
CHEMICAL AND ENVIRONMENTAL TECHNOLOGY DEPARTMENT

Lab Report

ROOM NO: FE E309

EXPERIMENT NO : **3**

TITLE : **DNA Extraction From Onion**

Submitted by : Lyndsay Grover

Class : **BIOL 10000 lab.**

Partners : Awatif Hagelamin

Instructor : Farag Soliman

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FENNELL CAMPUS
HAMILTON, ONTARIO

Purpose:

The purpose of this experiment was to extract DNA from an onion in a sufficient quantity to be seen, spooled and weighed. This introduces us to the idea of DNA as a tangible element that we can weigh and see how it factors into the weight of the whole organism. Also it is a prelude to the various techniques of DNA testing we will encounter, as extraction is the first step before analysis is able to be completed.

Apparatus:

- Graduated cylinder
- 250mL beaker, 600mL beaker
- glass stirring rod
- Buchner filtration system
- test tubes
- Pasteur pipette
- steam bath, ice bath
- Reagents: dishwashing detergent, sodium chloride, ethanol, TBE buffer

Safety Guidelines:

No harmful reagents are used in this experiment. Use caution when chopping the onion to avoid injury.

Procedure:

1. Set up a hot water bath at 55-60°C and an ice water bath.
2. Prepare solution consisting of 10mL of liquid dishwashing detergent, 1.5g of sodium chloride, adding distilled water to make up to a volume of 100mL in a 250mL beaker.
3. Weigh one large onion and record the weigh in a prepared table.
4. Coarsely chop the onion and place in 1000mL beaker.
5. Cover the chopper onion with the 100mL of solution from step 2. Place the beaker on the steam bath for 10-12 minutes. During this time, press the onion pieces against the side of the beaker with a rubber policeman. Do not keep the mixture in the hot water bath for more than 15 minutes.
6. Cool the mixture in an ice water bath for 5 minutes. During this time, press the chopped onion mixture against the side of the beaker, using the rubber policeman.
7. Filter the mixture using a vacuum into the side arm Erlenmeyer flask using a #2 Buchner funnel and a #3 filter paper. When filtering the mixture try to keep the foam from getting into the filtrate.
8. Add cold ethanol to the beaker mixture by pouring approximately 2.5mL volumes of the cold ethanol down the side of the flask.
9. Weigh a 1.5mL microtube and record the tare weight. Transfer spooled DNA into the microtube.
10. Microcentrifuge for 5 minutes, the remove the supernatant. Extract the spooled DNA in the 1.5mL microtubes using the Savant DNA Speed Vacuum system on low for 5 minutes.
11. Weight the extracted DNA in the 1.5 microtube and record the weight in a prepared table. Calculate % yield of DNA from the onion.
12. Label and save the DNA in a 1.5mL microtube with 0.5mL of 1X TBE buffer solution and refrigerate at (-20°C) for further use.

Amendments to Procedure:
1. Wash and dry 4 test tubes.
2. Transfer solution from Buchner flask into test tubes after filtration.
3. Add ethanol to individual test tubes, no precise amount added.
4. Spool DNA from test tubes into micro tube. Proceed with rest of experiment procedure in the manual.

Calculations:

$$\text{Percent Yield of DNA} = \frac{0.0883\text{g}}{100.900\text{g}} \times 100$$
$$= 0.0875\%$$

Data:

Table 1: Weighing of Onion

Weight of Weighboat (g)	Weighboat + Onion (g)	Onion (g)
0	100.900g	100.900g

Table 2: Weighing of DNA

Microtube (g)	Microtube + DNA (g)	DNA (g)
1.2277	1.2812	0.0535
1.2277	1.2618	0.0348
	Total DNA:	0.0883

Observations:

-detergent type: Compliments Sensitive Skin
- solution description: clear, soapy with a layer of bubbles.
- steam bath not operational, implemented hot plate instead
- solution green after heating and pressing onion to the side of the beaker
- slow to filter
- creates foam layer in flask when filtering due to the soapiness of the solution
- ethanol and solution need to be as cold as possible to achieve best DNA yield
- DNA floats on top of ethanol after the addition
- DNA description: white and stringy
- amendments to procedure were implemented to ensure the maximum amount of DNA was extracted. DNA being easier to spool out of test tubes rather than a large flask.

Discussion:

This lab is a good introduction for Biotechnology students to the extraction procedures required before DNA analysis can be completed. Using an onion was a good choice for introduction experiment as it because of its low starch content which allows the DNA to be seen clearly, so that we are easily able to distinguish the DNA from the rest of the solution. This also introduces DNA to us on a tangible level where we can see it and touch rather than just the theory surrounding it that we have come to know.

This experiment is a prelude to experiment 4 where we use the DNA that we have extracted to analyze it using the spectrophotometer. This allows us to follow through completely from extraction to analysis

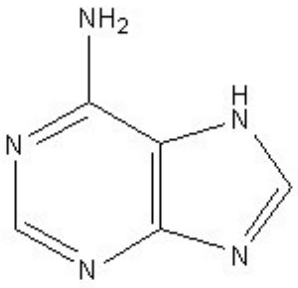
to better gain an understanding of the process followed in industry. This follow through without a disconnect really help us understand the importance of our performance in the lab as well as our results because they affect how we will perform the next experiment.

Sources of Error:

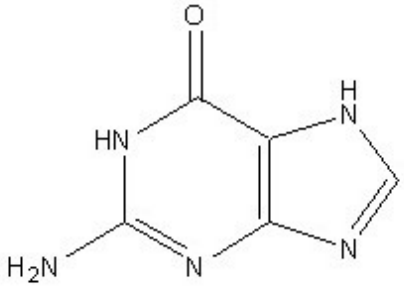
Possible Sources of Human Error: Improper measurements of detergent. Improper measurements of sodium chloride. Chopping the onion too finely. Heating the onion for too long or at too high of a temperature. Not pressing the onion against the walls of the beaker. Loss of DNA during spooling and transfer.
Possible Sources of Equipment Error: Balance reading incorrect. Steam bath unstable temperature. Vacuum filtration not working to maximum capacity.
Possible Sources of Experiment Error: Improper ethanol concentration.

Post Laboratory Questions:

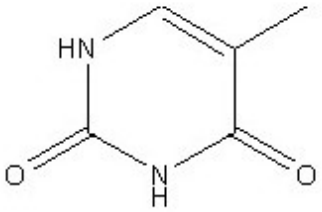
1. The parent molecules of DNA are the nitrogenous bases: Adenine, Guanine, Thymine and Cytosine. They are called bases due to their similarity in function to chemical bases.



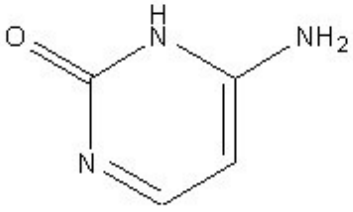
adenine



guanine



thymine



cytosine

2. Scientist would want to extract DNA from plant cells in order to analysis the gene sequences that are coded in the DNA. By looking at the gene sequences they can determine what proteins the DNA codes for and see if they can alter the codons for those proteins to create a protein needed for another purpose. They could also use the understanding of what proteins the plant creates to see if it is possible to synthesize something with the plant, i.e. bio-pharmaceuticals synthesizing vaccines in tobacco plants.
3. I would expect the DNA yield to be higher. This is due to the fact that the cold would not denature the DNA but it would denature the enzymes that break down the DNA in to smaller pieces preventing it from being extracted. So more DNA would be able to be extracted since less of it would be broken down.
4. The amount of ethanol used during the precipitation step would not affect how much DNA is extracted. The ethanol does not cause DNA to be extracted it simply causes it to separate from the solution. The amount of DNA extracted is determined in the previous steps.

Conclusion:

After reviewing the results of the percent yield of DNA I believe we extracted a decent amount of DNA from our onion. Although 0.0875% seems like an extremely small number when you take into consideration the size of DNA in comparison to an onion it is a considerable amount to be extracted. As well taking human error into consideration and the fact that something of the DNA may have been lost in the spooling and transferring procedure. Taking that loss into consideration it means that more DNA could have possibly been extracted. DNA was successfully extracted from the onion and a considerable amount was recovered. When observing the amount of DNA we were able to extract I believe that we extracted a sufficient amount of DNA in order for us to carry out Experiment 4 and perform a spectrophotometric analysis of the DNA we extracted.

References:

Biotechnology, An Introduction
S.R. Barnum, 2nd Edition, 2005.

Biotechnology 1, Laboratory Manual.
Mohawk College, Custom Courseware, 2010.