

**INVESTIGATION OF DNA EXTRACTION
AT THE UNIVERSITY OF NORTH CAROLINA
AT PEMBROKE**

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by

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Introduction: DNA extraction is a method which enables scientists to acquire samples of DNA from different sources. This experiment examined a few of these DNA extraction methods and their efficiency. Each method was conducted in the laboratory to test the procedure. Some procedures worked, while others did not. The extraction methods that did not work were then manipulated and tried again. The protocols that worked were then analyzed with a spectrophotometer to obtain the concentration of DNA within each sample. This also detected whether RNA, or other cellular proteins, were in the extracted material. This data was used to determine how well the method isolates pure DNA.

Background: Deoxyribonucleic Acid (DNA) was discovered by Johann Friedrich Miescher in 1869 in the nuclei of human white blood cells. DNA is a large molecule composed of a five-carbon sugar, a phosphate,

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and four nitrogenous bases. These nitrogenous bases are adenine, guanine, thymine and cytosine. These bases are paired to another base by hydrogen bonding. Adenine forms two hydrogen bonds with thymine, while guanine forms three hydrogen bonds with cytosine.

Once bonded, DNA forms a double-stranded structure that is then twisted up clockwise. This double-stranded structure was discovered by James Watson and Francis Crick in 1953; they called this structure the double helix or the duplex molecule. This DNA molecule encodes for the genetic instructions for cells. The DNA is located in the nucleus where the DNA molecule is tightly wound up with proteins into chromosomes. DNA is in every organism dead or alive and is the basis for living organisms. DNA extraction methods give scientists a means of obtaining this invisible DNA molecule by extracting many DNA molecules.

Procedure: This project involved the utilization of five protocols for DNA extraction. The first protocol will be the DNA extraction kit method currently in use here in the Biology department. This kit uses freeze dried E. coli which is placed in a test tube with sodium dodecyl

sulfate (SDS). The SDS detergent breaks down the cell walls, thus causing the cells to lyse (burst), which releases all the cell's genetic material. The test tube is then placed in a water bath at 60-65°C to inactivate the proteins and remove them from the DNA. After thirty minutes in the hot water bath, the contents of the test tube must cool. Once cooled, a 95% ethanol solution is added to allow the DNA to spool. A glass rod is lowered into the solution and rotated clockwise allowing the DNA to wrap around the glass rod. (Biotechnology Kit: DNA Extraction Demonstration)

The second protocol is "DNA Extraction from Cheek Cells" by Roxane Bonner at the University of Arizona Laboratory of Molecular Systematics and Evolution. This method used a saline solution as a swishing solution to obtain the cheek cells. The cells were centrifuged, the swishing repeated and centrifuged once again. Once the second centrifugation is complete, Lysis buffer was added to allow the cells to release their contents. This was incubated at 65-70°C for both one hour and overnight. Once the incubation is complete, sodium chloride was added, mixed well, and the supernatant was transferred to another tube. Cold 95% ethanol was added and the tube gently

rocked until DNA became visible. When DNA was visible, spooling was tried and the sample was allowed to air dry.

The third protocol is “Extraction of DNA from Bovine Spleen” which will require obtaining a bovine spleen from a local slaughter house. A 15g sample of bovine spleen was weighed out and placed into a blender with cold citrate-saline buffer (SSC). The tissue was blended until macerated. This solution was poured into a centrifuge tube and centrifuged for 15 minutes. The supernatant was poured off, which contains the RNA, proteins, and carbohydrates that surround the DNA. The saline/citrate buffer is placed in the tube and mixed. The supernatant was re-centrifuged and discarded once again. Cold sodium chloride was added, mixed, and the liquid was poured off and saved. This was done four times and then all the saved liquid was combined and centrifuged for twenty minutes. The supernatant was poured into a beaker and 95% ethanol was added to the beaker. The solution was gently mixed and spooled with a glass rod. Once the DNA is spooled it was allowed to air dry.

The fourth protocol is the “Salting Out Procedure for Human DNA Extraction”. This protocol utilizes bovine blood which was substituted

for human blood, and was donated by White House Packing Company of Fairmont, North Carolina. This protocol took two days to complete. Day one, nuclei were isolated from blood and Buffer A (0.32 M sucrose, Tris HCl, MgCl₂, 1% Triton X) was added. This solution was mixed and centrifuged for 15 minutes. Buffer B (EDTA, NaCl) was then added to the tube and mixed well. This solution is then transferred to a centrifuge tube, and SDS and proteinase K were then added. This was incubated overnight at 37°C on a rocker. On the second day, the tube was removed from incubation, sodium chloride was added, and the tube was shaken vigorously, then centrifuged for fifteen minutes. The supernatant was poured into a test tube, 100% ethanol was added, and the tube was shaken until a DNA precipitate was evident. The DNA was removed by the spooling method and allowed to air dry.

The final DNA extraction method is “Onion DNA Extraction.” An inch square was cut out of a medium onion, and placed in a blender with Palmolive detergent. The contents was blended for one minute, then the mixture was strained by using a strainer and a coffee filter. Added to the mixture was meat tenderizer and the mixture was mixed well. Six milliliters of the slurry was placed in a test tube that

contained cold ethanol, and allowed to sit for two to three minutes.

The DNA was obtained by taking a glass rod and spooling the DNA.

The DNA was allowed to air dry.

For each protocol, the DNA samples were air dried and reconstituted with a buffer solution. From each solution an ultraviolet absorption was recorded with the use of a spectrophotometer. From these absorption readings, the amount of DNA obtained per mass of tissue used initially was calculated, thus showing which method delivered the highest yield. Also, gel electrophoresis was used to show how large the DNA strands are when compared to each other.

The cost of materials required for each protocol will be taken into account to determine which method is the most cost-effective for an average lab at the University of North Carolina at Pembroke.

Results and Discussion:

DNA EXTRACTION KIT: This kit provides everything needed to conduct the experiment and costs \$32.00 each. The kit provides materials for an entire laboratory of approximately eight groups. This experiment was conducted many times and the results varied widely

each time. The DNA would spool really well one time, but on the next attempt, the DNA would be visible yet unspoolable. This DNA method is the one currently used in Dr. Sue Bowden's Biology 100 laboratories. Their samples were collected at random in two laboratories and analyzed to see the amount of DNA obtained by each student team.

"DNA Extraction from Cheek Cells": This protocol was successful in obtaining DNA. Although visual, not a lot of DNA was able to be spooled. This protocol is fairly inexpensive because it uses the experimenter as the source of DNA. The only problem with this DNA extraction method for the Biology 100 laboratories is that it uses centrifuges, which are not available to the students and also it has long periods of waiting. Though a successful protocol, it is not very applicable in the Biology 100 laboratories; without clinical centrifuges.

"Salting Out Procedure for Human DNA Extraction" : This protocol was changed a little to use bovine blood rather than human blood. This protocol was cheap due to the donation of the bovine blood from White House Packing Company of Fairmont, North Carolina and the equipment already being available. This protocol was followed and the DNA was visible, but unspoolable. This posed a problem because the

DNA was needed to be spooled in order to analyze with the other samples. The protocol was manipulated and changed, however, the changes either would not work or they would yield the same results of the original protocol. The bovine blood was also put through another protocol from the Smithsonian. This protocol was very similar to the salting out protocol and yielded the same results. The idea of charge was also used to try to obtain the small particles of DNA. A glass rod was made positively charged by rubbing it with wool and inserted into the solution of unspoolable DNA. This was in hopes that the negative charge of DNA would be attracted to the positively charged glass rod, but it failed. This procedure was examined intensively, but was determined that it could not be used in the comparison since the DNA was unspoolable.

“Extraction of DNA from Bovine Spleen”: This protocol was rather cheap if all the equipment is already available; such as the centrifuge. This protocol called for spleen from a bovine which was obtained from White House Packing Company in Fairmont, North Carolina. The protocol was not very difficult , but took some time due to the long waits during centrifugation. This protocol would be a good one in

upper level classes, but not in Biology 100 laboratories. This procedure worked each time it was conducted and yielded a great amount of DNA visually. Though when allowed to sit the DNA became yellow looking suggesting that it could have contamination.

“Onion DNA Extraction”: This protocol was the most interesting of all because it was the greatest challenge. The original protocol did not seem to work because it yielded unspoolable DNA. This sparked the idea of change once again and once again it failed. The next solution was thought to be the replacement of an onion with another plant. The potato was chosen and the experiment was conducted using the potato. It also yielded the unspoolable DNA within the test tube. This was confusing due to the fact of hearing great things from high school teachers about this protocol. Further Internet research was conducted, and another protocol was found that was similar to the first, except it added a heating step. This new protocol was conducted, and the onion DNA was now obtainable. The first protocol was once again repeated, but adding in the heating step causing it to yield spoolable DNA. This procedure is very cheap because it uses household items to conduct the experiment. The onion extraction

could be done in any level of lab, high school through Biology 100 laboratories.

After each protocol had been completed, samples were analyzed by spectrophotometry and gel electrophoresis. The samples analyzed were: bovine spleen, E. coli kit, cheek cells, and onion cells. The amount of sample was limited, due to evaporation during storage. Each eppendorf tube contained sample and TE buffer that equaled 100 μ l, except for the bovine spleen which contained 200 μ l total. In each spectrophotometer quartz tube, a 1:100 dilution was made (0.014 μ l of sample: 1.386ml of TE buffer). The spectrophotometer was blanked with 3ml of TE buffer before each reading. Two absorptions were obtained from each sample, one at 260nm and the other at 280nm. The following results were obtained:

SAMPLE	260 nm WAVELENGTH	280 nm WAVELENGTH
<i>E. coli</i> KIT	0.038	0.026
<i>E. coli</i> KIT	1.280	0.716
<i>E. coli</i> KIT	0.202	0.119
<i>E. coli</i> KIT	0.537	0.286
<i>E. coli</i> KIT	0.125	0.054
CHEEK CELLS	0.032	0.021
CHEEK CELLS	0.041	0.026
ONION CELLS	0.011	0.022
ONION CELLS	0.023	0.008
BOVINE SPLEEN	0.055	0.070

Using the UV absorptions of each sample, calculations were done to determine the concentration of the DNA.

Estimation of Purity of DNA

This calculation indicates the degree of purity of the sample, thus showing much protein and organic solvent is contaminating the sample. The calculation was performed on each sample as follows:

Absorption at 260nm/Absorption at 280nm

or

A₂₆₀ / A₂₈₀

Solving for Concentration of DNA and Total Mass of DNA

To calculate the concentration of double-stranded DNA, the absorption or the optical density (OD) was multiplied by the double stranded DNA conversion factor (1 A₂₆₀=50 μ g/ml) and then multiplied by the dilution factor . The following equation was used:

$$\text{OD}_{260} \times 50\mu\text{g/ml} \times 100$$

The total mass of the double stranded DNA was calculated by taking the concentration of DNA and multiplying it by the volume. The following equation was used:

$$\text{concentration } \mu\text{g/ml} \times \text{volume of sample}$$

The volume of sample was 0.100 ml for all samples except for the bovine spleen DNA its volume was 0.200ml.

The results of these calculations are in the following table.

SAMPLE	DNA PURITY	DNA CONCENTRATION μg/ml	DNA TOTAL MASS μg
<i>E. coli</i> KIT	1.462	190	19
<i>E. coli</i> KIT	1.788	6400	640
<i>E. coli</i> KIT	1.697	1010	101.0
<i>E. coli</i> KIT	1.878	2685	268.5
<i>E. coli</i> KIT	2.315	625	62.5
CHEEK CELLS	1.524	160	16
CHEEK CELLS	1.577	205	20.5
ONION CELLS	0.500	55	5.5
ONION CELLS	2.875	115	11.5
BOVINE	0.7857	275	55
SPLEEN			

The purity ratios that are greater than 1.8 is highly purified DNA.

The sample that contained the purest DNA was the onion cells, though it was not very consistent. The most consistent in purity was the *E. coli* kit and the cheek cells protocols.

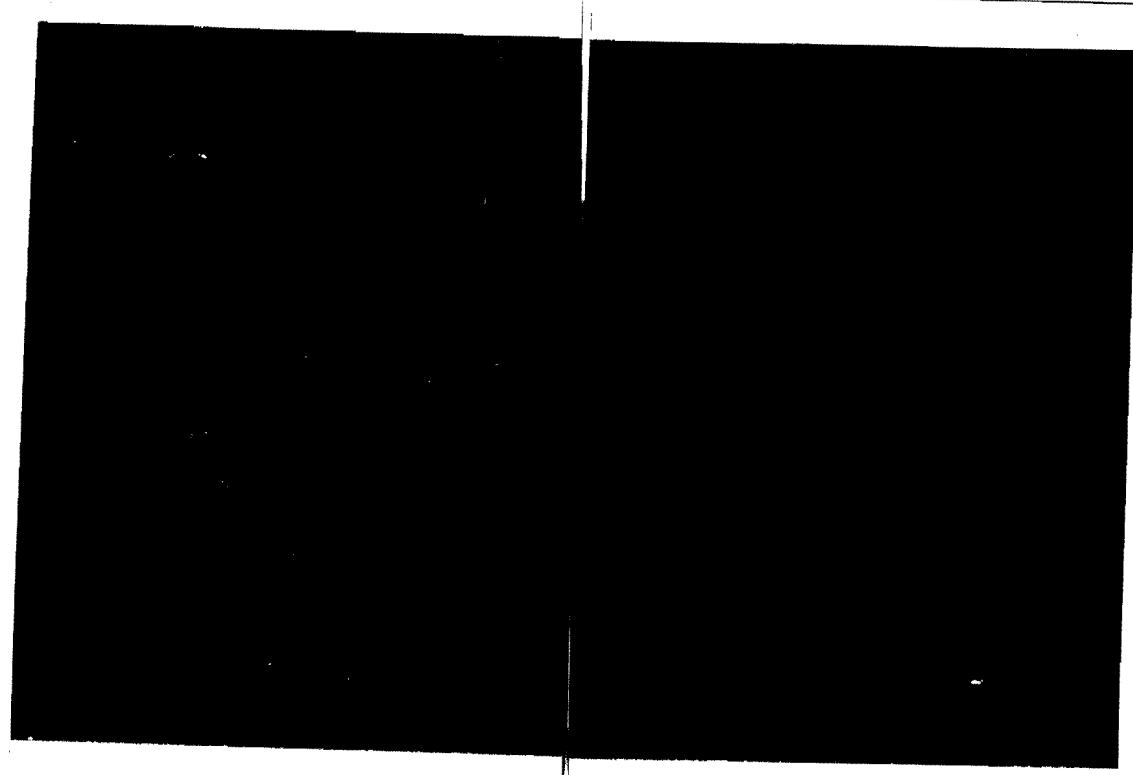
The DNA concentrations were used to calculate the amount of DNA sample to be added to the gel electrophoresis. A 1:7 dilution was used for the set up of the gel electrophoresis. The total amount of

DNA in each well was 300ng. The concentration of DNA ng/ μ l X 300ng gave the amount of sample in μ l that equaled 300ng. Some of the samples were diluted before they were obtained for the gel dilution, because their concentration of DNA was already greater than 300ng.

The following table shows the amounts used for the gel electrophoresis:

SAMPLE	DNA CONCENTRATION ng/ μ l	AMOUNT OF DNA μ l	AMOUNT OF TE BUFFER μ l	AMOUNT OF DYE μ l
<i>E. coli</i> KIT	190	1.58	4.42	1
<i>E. coli</i> KIT	6400 1:21 Dilution 305	1	5	1
<i>E. coli</i> KIT	1010 1:4 Dilution 252.5	1.19	4.81	1
<i>E. coli</i> KIT	2685 1:9 Dilution 298	1	5	1
<i>E. coli</i> KIT	625 1:2 Dilution 312.5	1	5	1
CHEEK CELLS	160	1.88	4.12	1
CHEEK CELLS	205	1.46	4.54	1
ONION CELLS	55	5	1	1
ONION CELLS	115	2.61	3.39	1
BOVINE SPLEEN	275	1.09	4.91	1
DNA MARKER 7μl				

After running the large 0.8% gel electrophoresis for approximately three hours at 100 volts, the gel was removed from the unit and placed in ethidium bromide, which stained the gel overnight.



← Bovine Spleen
← DNA Marker

← Checkcell

electrophoresis:

were taken. The following pictures show the results of the gel

The next day the gel was viewed under a UV light source and pictures

The gel electrophoresis was inconclusive due to the samples sitting for a long period of time. During this time, the DNA was probably sheared by nucleases (enzymes that cut DNA) or by moving the tubes around during the research process. The gel did suggest that the bovine spleen and the cheek cells DNA were more hardy than the other samples, because both appeared on the gel electrophoresis.

CONCLUSION:

For the laboratories at the University of North Carolina at Pembroke the protocols examined could be implemented in the General Biology Lab, Cell Biology, and Genetics. The E. coli kit, onion, and cheek protocols could be used in the General Biology Laboratory because the equipment and procedure are not that difficult to use. The bovine spleen would be more applicable to the higher classes due to the larger equipment being used and the long waiting time.

The expense for each protocol was examined and it was determined that the cheapest protocol would be the cheek protocol. This is because all of the equipment and chemicals are already here at

the University. The only expense in the onion protocol is the onion itself , which cost approximately fifty cents. The bovine spleen had little expenses because the spleen was donated to the University by White House Packing Company in Fairmont, North Carolina. The most expensive protocol was the *E. coli* kit which is ordered and cost approximately thirty-two dollars, but it comes with everything needed.

The protocol which preformed the best visually was the bovine spleen because it yielded so much spoolable material after each extraction. This would be an excellent way to show students what DNA looks like. The overall best protocol which was consistent in giving DNA each time was the cheek cells. The onion protocol is a good one to use to show students how plants have DNA. This protocol is an easy one to do and could even be performed at a high school level.

The comparison of the protocols was not very accurate due to the limited number of samples obtained from each protocol. This is due to the eppendorf tubes leaking and allowing sample to evaporate resulted in the sample being thrown away. The samples were thrown away because when the absorption was read from each tube it did not

**give a proper reading so it was determined that the evaporation
effected the results.**

Personal Reflection:

This research project has taught me many things about the field of biology and science in general. I learned about how difficult it is to conduct a research project. The failures are greater than the successes, which causes disappointment more than rejoice. I think this project has enabled me to grow as a biologist with not only the equipment and chemicals, but the reality is that science does not always turn out the way you want it to turn out. To quote Albert Einstein, the famous research scientist, "If we knew what it was we were doing , it would not be called research , would it?" I think this sums up exactly what scientific research means to me being a future biologist.

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