Isolation and Restriction Endonuclease Digestion of Onion DNA in the Junior College-High School Biology Laboratory

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Abstract: Chromosomal DNA isolation is a routine procedure in many larger college science laboratories, but is attempted in relatively few junior college or high school science settings. Reasons for not doing so are varied, but the primary concerns include lack of needed equipment, hazardous reagents, and oftentimes the formal education of the science instructor occurred before the molecular biology revolution. With the numerous advances in modern day biology, it is critical that today's students gain experience in the technologies involved in molecular biology through experiments such as the isolation of chromosomal DNA. Techniques of DNA isolation are documented that are not dependent on expensive equipment, but the DNA obtained is often contaminated with significant amounts of protein and is unusable for subsequent molecular biology manipulations. Thus, it was the goal of this project to develop a simple chromosomal DNA isolation procedure that yielded a product of sufficient quality that it could be used in subsequent molecular biology manipulations. A procedure for the isolation of chromosomal DNA from an onion is presented utilizing routine household items such as liquid dishwashing detergent, salt, and coffee filters. The DNA obtained is of sufficient quality so that it can be used in subsequent procedures that further extend the learning experience.

***Keywords*:** onion, DNA isolation, restriction enzymes, agarose gel electrophoresis

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Introduction

The field of molecular biology is one of the fastest growing areas of science. As a result, educators at all levels of the educational system need to adapt to meet the needs of students as they move into this highly technical world. Unfortunately, many educators at the junior college-secondary level are hesitant and often reluctant to implement a molecular biology curriculum primarily due to the lack of proper equipment and formal training in the area of molecular biology. As a result, industrial and university professors have devoted considerable effort to make the field of molecular biology more "user friendly". However, the dependency still exists on expensive, sophisticated equipment that is considered routine in this area. Simplified and abbreviated protocols, such as DNA extraction from an onion or banana, have been developed to facilitate hands on experimentation that can be performed with minimal equipment and cost (1-4). These procedures, which allow visualization of DNA right before the student's eyes, are crude at best and unacceptable for further molecular biology manipulations. The use of hazardous chemicals and instrumentation not routinely available to junior college-high school science laboratories, or the use of expensive kits which are required to clean the preparations for use in subsequent analyses are deterrents to their use. It was our objective to develop an isolation procedure that could be performed using materials common to most science laboratories and would provide DNA of sufficient quality to be used in subsequent molecular biology applications (e.g. restriction enzyme digestion). We report a procedure to isolate DNA from an onion that is non hazardous and can be performed easily using materials common to most laboratories.

Methods and Materials

1. Chop one medium onion into small pieces (5 mm cubes) and place in a household blender

containing 72 ml of water. Blend on high speed for 1 minute.

2. Pour the onion slurry into a 400 ml beaker. Add one tablespoon of salt (approx. 8 grams) and two tablespoons of liquid dishwashing detergent (approx. 8 ml). Mix by stirring. Incubate at 60° C for 15 minutes followed by cooling in an ice water bath for 5 minutes.

3. Place three coffee filters over the top of a 600 ml beaker and pour the onion mixture through it, allowing it to filter out particulate matter. Save the filtrate and discard the filters.

4. Gently add 2 ml chloroform down the side of the beaker containing the filtered solution and swirl gently, being careful not to mix the phases. Carefully pour off the top aqueous phase into a clean 250 ml flask, taking care not to get any of the white chloroform mixture. Repeat this procedure four more times.

5. Determine the volume of the extract by pouring it into a 100 ml graduated cylinder. Add two volumes of ice-cold 95 % ethanol to the mixture and let it incubate on ice for 10 minutes.

6. Using a glass rod, place one end into the solution and spool the DNA onto the rod by gently moving it in a circular motion. Remove the spooled DNA from the glass rod by scraping it into a microcentrifuge tube.

7. Remove as much of the ethanol as possible and allow the DNA contained in the tube to air dry.

8. Resuspend the DNA in 200 µl of water.

Set up a restriction endonuclease reaction as follows:

1. In a small tube with a cap, add the following reagents in the order listed:
   5 µl DNA solution
   11 µl water
   2 µl 10X buffer (supplied with *Rsa*I)
   1 µl *Rsa*I (Promega, Madison, WI)
   1 µl RNase (Promega, Madison, WI)

2. Incubate at 37° C for 1 hour.

To determine if the restriction endonuclease digestion was successful, the reaction mixture and DNA not subjected to endonuclease digestion should be subjected to agarose gel electrophoresis and the results compared. Practical applications and theory of agarose gel electrophoresis can be found in references 5-8.

Results

As shown in Figure 1, DNA isolated from an onion using the protocol described was of sufficient quality that it could be digested by the restriction endonuclease *Rsa*I. This is demonstrated by a shift in the molecular weight of the undigested onion DNA from the greater than 10 kb – 0.5 kb region to the 4 kb – 0.3 kb region on the agarose gel.

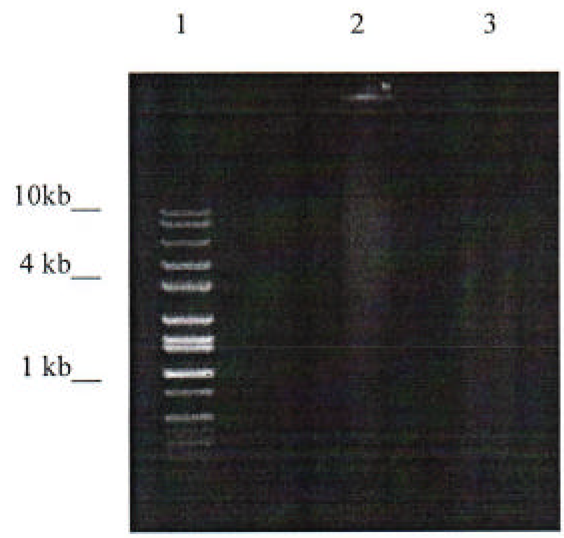

Figure 1. *Agarose gel electrophoresis of* RsaI *digested onion DNA. Lane 1: DNA molecular weight markers; Lane 2, undigested onion DNA; Lane 3,* RsaI *digested onion DNA; kb, kilobases.*

Discussion

Manipulation of DNA through molecular biology experimentation requires DNA free of contaminating materials. Impurities, such as protein, bind through non-covalent interactions and make the DNA unsuitable for subsequent applications. Protocols suitable for the junior college or secondary school science laboratory generally accomplish their goal by just purifying a crude preparation of DNA, but students could be exposed to additional theories through hands on experiences if they could further manipulate the DNA. To do so, however, requires that the DNA be of sufficient quality to be digested by restriction endonucleases (also called restriction enzymes).
We report an onion DNA extraction procedure that is applicable to introductory laboratories with a broad range of abilities that is based on routine equipment. In developing this procedure, we tried to find a substitute for chemicals normally used in these types of procedures. Household dishwashing detergent coupled with table salt at 60° C was used to disrupt the cell membrane. Lysis under these conditions could be accomplished using lower temperatures, but 60° C proved optimal in removing a significant amount of contaminating protein. We tried boiling the lysate for

10 min, but it provided no benefit over the 60° C incubation (data not shown). After filtration through coffee filters, chloroform was added down the side of the flask were it went to the bottom of the filtrate. We were careful not to mix the organic and aqueous phases. A thorough mixing would have facilitated the removal of contaminating protein, but also would have required the use of a centrifuge to separate the layers. Using a gentle swirling action, a white interface develops which is indicative of denatured protein. We determined five of these extractions were sufficient; however, we could have repeated it many more times and continued to remove contaminating protein. After the final chloroform extraction, the DNA was precipitated from the aqueous solution by addition of two volumes of 95% ethanol. The alcohol molecules compete with water molecules surrounding the DNA and cause it to fall out of solution. The DNA is very "sticky" and readily adheres to a glass rod that is swirled in it.

To determine if the isolated DNA could be digested with a restriction endonuclease, a reaction was set up using the enzyme *Rsa*I. Restriction enzymes cleave DNA at specific sequences and have proven invaluable in molecular biology. If DNA in these reactions is "dirty", the specific base pair sequences that need to be recognized by the restriction enzyme will be "covered up" and the enzyme will not cut. Alternatively, the enzyme may be inhibited by contaminants. The enzyme *Rsa*I recognizes the sequence GTAC in the DNA and makes a double stranded cut at that site. As evidenced by the agarose gel in Figure 1, it can be seen that digestion of the DNA into smaller fragments was achieved by comparing it to undigested DNA.

In summary, a routine DNA isolation procedure was modified to make it more appealing to educators not possessing the equipment or background for a more sophisticated procedure. Expensive and hazardous chemicals were replaced with cheaper, less corrosive materials. The real value of this approach is that the isolated DNA is of sufficient quality that it can be further manipulated. Thus, students whose attention and imagination are captured with the routine DNA isolation procedure can now investigate theories behind restriction enzyme digestion as well as other molecular biological applications. This procedure has been successfully performed in a freshman college biology class. Observing the appearance of DNA upon ethanol precipitation combined with its visualization by agarose electrophoresis was well received by the students. We have not performed the experiment at the high school level, but the manuscript was reviewed and the process was observed by a high school science teacher. She was of the opinion that the experiment could be performed and would be enjoyed by her students.

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