Isolation of DNA from Onions

An educational resource from www.edvotek.com

Extracting DNA from Onions (and other plant tissue)

RELEASING THE DNA

1. Carefully slice a small section (about 3-5 grams) of onion tissue from the main body of an onion (not the root tip), and place it in a test tube or small beaker.

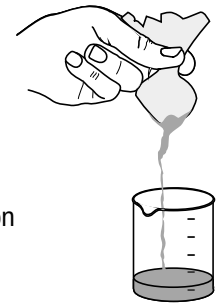
2. Using a pipet, add 5-6 ml of DNA Extraction Buffer into the tube or beaker.

3. Mince and grind the tissue with the eraser end of a pencil or other appropriate instrument.

   The large end of a wooden chopstick works great. A mortar and pestle can also be used to grind the tissue. This releases the cellular contents, including the DNA, from the onion cells.

4. Place a square of cheesecloth (or coffee filter) into a funnel and filter the contents into a clean tube or beaker (squeeze out the excess juice). Measure approximately 5 ml of liquid.

   Alternatively, use a transfer pipet to remove the liquid portion - try to minimize carry over of onion tissue.



SPOOLING THE DNA

5. Carefully overlay the liquid with 5 ml of **very cold** 95-100% Isopropanol.

   Alternatively, use 10 ml of **very cold** 70% clear isopropyl rubbing alcohol.

continued...

Isolation of DNA from Onions

An educational resource from www.edvotek.com

Extracting DNA from Onions (and other plant tissue)

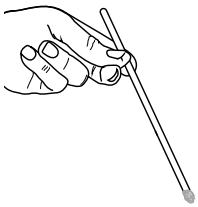
Spooling the DNA, continued

6. Place a glass rod into the test tube or beaker and twirl it at the interface of the two liquids. The DNA will begin to spool (wrap) around the glass rod.

   A pasteur pipet which has been heated to melt the end and form a hook also works well for spooling.

7. Gently lift the glass rod out of the solution from time to time and observe the DNA substance attached to it.

8. After spooling for a minute or two, remove the rod from the test tube or beaker to observe the DNA. The DNA will appear as a viscous, gelatinous-like substance adhering to the glass rod.



   As the DNA adheres to the rod, its initial gelatinous texture will become more compact and fibrous in appearance.

9. Rinse the DNA on the glass rod with 95-100% Isopropanol and allow it to dry for several minutes.

10. Two suggestions for what you can do next with the spooled DNA:

    - To facilitate visualization, the DNA on the glass rod can be stained with a methylene blue-based DNA stain. You should also save the test tube or beaker from which you spooled the DNA to stain the residual DNA in solution.

    - Alternatively, the DNA can be rehydrated with 1x TE buffer and analyzed on a 0.8% agarose gel along with a standard DNA marker.

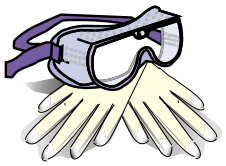
Isolation of DNA from Onions

An educational resource from www.edvotek.com

Staining the Extracted DNA with InstaStain® Methylene Blue

*If the DNA is stained, it can not be used for further analysis or studies, such as electrophoresis.

1. To facilitate visualization, the spooled DNA can be stained.* Make sure you do this on a stack of paper towels:
   - Using a transfer pipet, place approximately 10 drops of distilled water onto an InstaStain® Methylene Blue card to liquify the stain.
   - Transfer 2-3 drops of the blue liquified stain onto the DNA adhering to the spooling rod.
   - Observe the stained DNA. Write a short paragraph describing your observations.



Reminder: Be sure to wear gloves and a lab coat. Be extra careful when handling InstaStain® Methylene Blue. Although it is not toxic, it can permanently stain clothing.

2. Now add 2-3 drops of the liquified blue stain to the tube or beaker from which you spooled the DNA. Observe how the stain reacts with residual DNA in the solution that did not spool onto the rod.
3. Now add 2-3 drops of the liquified stain to a tube or beaker containing only water.
4. Observe and describe the dye in the tube or beaker containing water compared to the solution containing DNA.

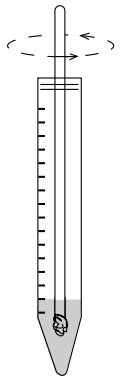
Isolation of DNA from Onions

An educational resource from www.edvotek.com

Preparing the Extracted DNA for Electrophoresis

REDISSOLVING THE SPOOLED DNA

1. Add 1-2 ml of 1x TE Buffer for redissolving DNA to a clean plastic tub and submerge the coated end of the rod into the buffer.
2. Twirl the rod several times to dislodge some of the DNA.
3. Cover the test tube, with the rod still inside, with plastic wrap or foil to prevent evaporation.
4. Allow the DNA to rehydrate at room temperature. High molecular weight DNA can take several days to completely rehydrate and dissolve.



PREPARE THE DNA FOR ELECTROPHORESIS

5. Transfer 0.3 ml of the dissolved DNA to a fresh test tube.
6. Add 30 µl of 10x Gel Loading Solution. (If you are using a transfer pipet, add 1-2 drops.)
7. Mix by tapping.
8. Load 40 µl of the prepared sample on an agarose gel for electrophoresis.