DNA isolation from plant materials

Collecting plant materials

DNA isolation using

**Protocol**

1. Collecting plant materials
2. Phase Separation
3. DNA Precipitation
4. DNA Wash
5. Redissolving the DNA

**Materials**

* Microcentrifuge
* Microcentrifuge tubes
* Mortar and Pestle
* 65C and 37C water bath
* Micropipettes several size
* The nuclei lysis solution (ice cold)
* The protein precipitation solution
* The Dnase solution
* Isopropanol
* 70 % Ethanol (ice cold)

**Collecting plant materials**

1. The plant most be clean and free of soil, insect and microscopic fungi.
2. Keep the specimen inside clean container or zip lock bag.
3. Label information should be placed inside the zip lock bag with specimen which include: Taxon name, collection name, Date of collection.
4. If some time must elapse before shipping, refrigerate but do not freeze the plant.
5. Get a 2 sample from every specimen one for molecular work other to keep it in the university herbarium.
6. For extracted DAN you will need a small piece of plant.
7. Plant tissues may be efficiently powdered by first freezing in liquid nitrogen or dry ice/ethanol before DNA extraction.

**Phase Separation**

1. Tack a small amount of plant understudy and put it in the labeled tube then add 100µL of the nuclei lysis solution.
2. put the mixture (the sample/the nuclei lysis solution) in clean pestle and start grinding for 1 minute or until you see some color change (chlorophyll released with plants)
3. Then add the remaining 500 µL of the nuclei lysis solution and mix it agine.
4. Incubate extract mixture for about 15 min at 65⁰C in a recirculating water bath.
5. After incubation, add 3µL of RNase solution which contains RNase is going to break down a lot of RNA that’s present in the solution.
6. Shack the tube and incubate it at water bath 37C for 15 minutes, this process is recommended in order to minimize RNA carry-over into the DNA.

***DNA Precipitation***

1. Add 200 µL of protein precipitation solution to the tube then Put the tube on ice for 4mintes. The protein precipitation will tack the proteins which are soluble and force them to come out so that we can get rid of them when we want to extract the DNA.
2. Pay attention to color change in tube, it may be become milky
3. Put the sample in centrifuge for 4 minutes 13000rpm.
4. After centrifuge, we can notice that all of the cellular detritus has lodged itself on the side of the tube wall.
5. Remove the liquid supernatant and leave the rest of the material behind.
6. Add 600 µL of isopropanol in the sample and invert it several time
7. Spin them for 1 minute at maximum speed.
8. Carefully remove the isopropanol from the sample.

**DNA Wash**

1. Wash the DNA precipitate twice with 600 µL of ethanol to washes away any excess impurities.
2. Tack off the ethanol from tube gently.
3. Make sure that the pellet that was there in the side of the tube.
4. Air dry the DNA by storing in an open tube for 5-15 seconds after removing the ethanol. (If the DNA is exposed to air for more than a few seconds, it will be much more difficult to dissolve.)
5. Add 100 µL DNA rehydration solution to the tube, cap the tube and shake it up.
6. Incubate in water bath at 65C for 45-60 minute.
7. If you are going to use the DNA immediately you can keep it on ice until you go the PCR step or you can put it at -20 until you are ready to use.

**DNA Solubilization**

* Resuspend the DNA in sterile DNase free water (approximately 50-400 μl H2O; the amount of water needed to dissolve the DNA can vary, depending on how much is isolated). RNaseA (10 μg/ml) can be added to the water prior to dissolving the DNA to remove any RNA in the preparation (10 μl RNaseA in 10ml H2O).

Notice: Please check the video below

* [**http://www.dnalc.org/view/16959-DNA-Barcoding-Protocol-Isolating-DNA.html**](http://www.dnalc.org/view/16959-DNA-Barcoding-Protocol-Isolating-DNA.html)

References

* Williams J. **DNA Barcoding Protocol: Isolating DNA,** DNA Learning Center, goes through the steps involved in isolating DNA from an animal or plant sample. **Available online at** [**http://www.dnalc.org/view/16959-DNA-Barcoding-Protocol-Isolating-DNA.html**](http://www.dnalc.org/view/16959-DNA-Barcoding-Protocol-Isolating-DNA.html)
* **Xin**Z. and **Chen J. 2012.**  **A high throughput DNA extraction method with high yield and quality. Plant methods.** Available online at: <http://www.plantmethods.com/content/8/1/26>