DNA Isolation from plant materials

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Protocol

- Collecting plant materials
- Phase Separation
- DNA Precipitation
- DNA Wash
- Redissolving the DNA

Materials

- Plant material.
- CTAB buffer.
- Microfuge tubes.
- Mortar and Pestle.
- Microfuge.
- Absolute Ethanol (ice cold).
- 70 % Ethanol (ice cold) .
- 7.5 M Ammonium Acetate.
- 55 C° water bath.
- Chloroform: Iso Amyl Alcohol (24:1).
- Water (sterile).

Collecting plant materials

- Write your plant binomial nomenclature for example:
  - *Epipremnum aureum*
- The plant most be clean and free of soil, insect and microscopic fungi.
- For extracted DAN you will need a small piece of plant.

Phase Separation

1. Grind 0.05 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer.
2. Transfer CTAB/plant extract mixture to a microfuge tube.
3. Incubate the CTAB/plant extract mixture for about 30 minutes at 55 C° in a water bath

DNA Precipitation

1. After incubation, spin the CTAB/plant extract mixture at 12000 rpm for 5 min to spin down cell debris.
2. Transfer the supernatant to clean microfuge tube.
3. Add 250 µl of Chloroform : Iso Amyl Alcohol (24:1) and mix the solution by inversion.
4. After mixing, spin the tubes at 13000 rpm for 1 min.
5. Transfer the upper aqueous phase only (contains the DNA) to a clean microfuge tube.
6. Add 50 µl of 7.5 M Ammonium Acetate followed by 500 µl of ice cold absolute ethanol.
7. Place the microfuge tube for 1 hr at -20 C° after the addition of ethanol to precipitate the DNA

DNA Wash

1. Following precipitation, the DNA can be pipetted off by slowly rotating/spinning a tip in the cold solution. The precipitated DNA sticks to the pipette and is visible as a clear thick precipitate. To wash the DNA, transfer the precipitate into a centrifuge tube containing 500 µl of ice cold 70 % ethanol and slowly invert the tube. Repeat.
2. (alternatively the precipitate can be isolated by spinning the tube at 13000 rpm for a minute to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol)).
3. After the wash, spin the DNA into a pellet by centrifuging at 13000 rpm fo 1 min. 12.Remove all the supernatant and allow the DNA pellet to dry (approximately 15 min). 13.Do not allow the DNA to over dry or it will be hard to re-dissolve.

Redissolving the DNA

1. 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).
2. 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).
3. After resuspension, the DNA is incubated at 65o C for 20 min to destroy any DNases that may be present and store at 4o C.
4. Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness.

Reference

- http://faculty.ksu.edu.sa/sites/default/files/358_bot.pdf