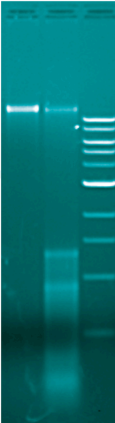


Unravel the Chloroplast Genome



Hereditas

Photosynthetic

Harvest 10 g of spinach washings deep in dark for 48 hrs to remove starch levels. Wash under tap water. Cut into 1 cm strips (directly into ice cold buffer).
 Centrifuge to void excess with 20 ml of cold centrifuge buffer for 2 minutes. The final supernatant (homogenate) should be "thick" with some incompletely homogenized material.
 Filter homogenate through 50 µm nylon mesh into a 10 ml centrifuge tube. A filter funnel will avoid messes. The material in the nylon mesh can be gently squeezed to release more chloroplasts into the supernatant.
 Centrifuge for 20 minutes at 1,000 rpm at 4°C. This will pellet nuclei and cell debris, leaving mostly chloroplasts (intact and broken) in the supernatant.
 Gently pour supernatant into a new centrifuge centrifuge tube and spin at 4,000 rpm for 20 minutes at 4°C. This will pellet the chloroplasts.
 Discard supernatant and resuspend pellet (approx 100 µl) in TE buffer.
 Transfer sample to microcentrifuge tube and add equal volume of equilibrated phenol. Shake intensively for 5 minutes, centrifuge 1 minute at 12,000 rpm. There will be 2 phases, green on bottom and clear on top.
 Transfer the clear upper layer to a new microcentrifuge tube and final volume of 100 µl phenol:chloroform. Shake 5 minutes and centrifuge 5 minutes at 12,000 rpm.
 Transfer upper layer to new microcentrifuge tube and add equal volume of 2:1 chloroform:isoamyl alcohol. Shake 5 minutes and centrifuge 5 minutes at 12,000 rpm.
 Transfer upper layer to new microcentrifuge tube and add 1:1 (v/v) the volume of 5 M ammonium acetate and an equal volume of isopropanol. Store at -20°C for at least 2 hours (I use next lab session).
 Centrifuge 5 minutes at 12,000 rpm. Discard supernatant. You may see a small white pellet (this is the rDNA).
 Resuspend pellet in 200 µl of TE ethanol.
 Centrifuge 5 minutes at 12,000 rpm.
 Discard supernatant and again the pellet, ensuring no ethanol remains in the tube.
 Resuspend pellet in 100 µl TE buffer. This is your DNA sample!
 Mix 5 µl of DNA sample with 1 µl of 6X loading buffer.
 Run sample on 1% agarose gel at 100 volts for ~45 minutes.

Photosynthesis

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