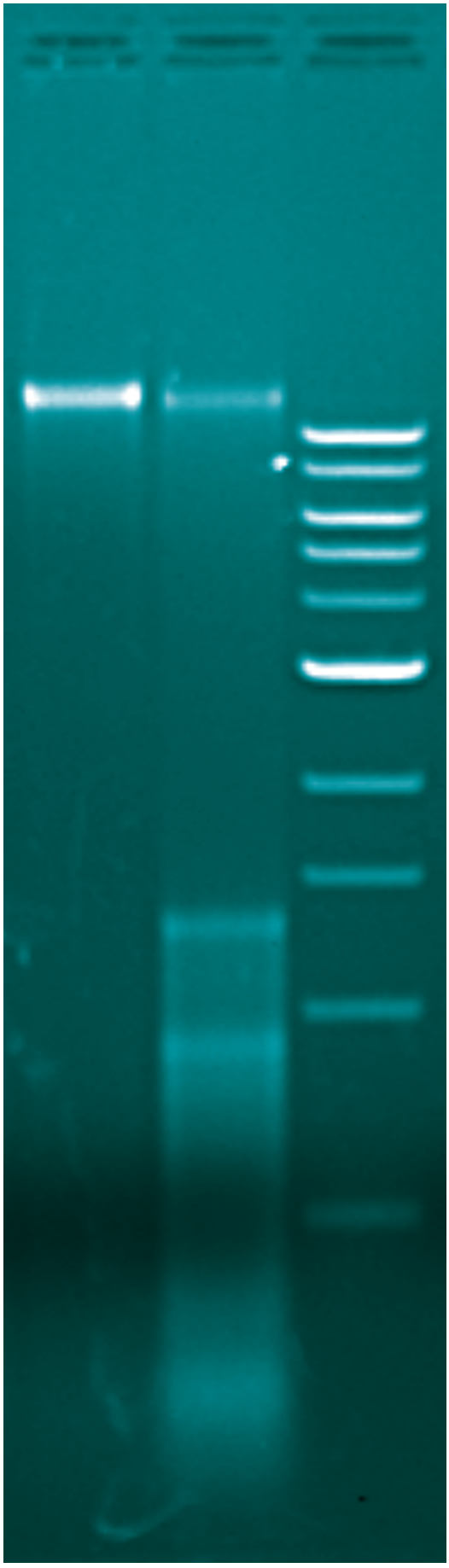
Unravel the Chloroplast Genome



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· Harvest 10 g of spinach seedlings (kept in dark for 48 hrs to reduce starch levels). Wash under tap water. Cut into 1 cm strips (directly into ice-cold buffer).
· Grind in ice-cold mortar with 20 ml of cold complete buffer for 2–4 minutes. Don't grind excessively: Homogenate should be "chunky" (with some incompletely homogenized material).
· Filter homogenate through 50 µm nylon mesh into a 40 ml Oakridge centrifuge tube. A filter funnel will avoid messes. The material in the nylon mesh can be gently squeezed to release more chloroplasts into the tube.
· Centrifuge for 20 minutes at 1 000 rpm at 4ºC. This will pellet nuclei and cell debris, leaving mostly chloroplasts (intact and broken) in the supernatent.
· Gently pour supernatant into a new Oakridge centrifige tube and spin at 4 000 rpm for 20 minutes at 4ºC. This will pellet the chloroplasts.
· Discard supernatant and resuspend pellet (gently!) in 200 µl ST buffer.
· Transfer sample to microfuge tube and add equal volume of equilibrated phenol. Shake intensively for 5 minutes, centrifuge 5 minutes at 12 000 rpm. There will be 2 phases, green on bottom and clear on top.
· Transfer the clear upper layer to a new microfuge tube. Add equal volume of 1:1 phenol:chloroform. Shake 5 minutes and centrifuge 5 minutes at 12 000 rpm.
· Transfer upper layer to new microfuge tube and add equal volume of 24:1 chloroform:isoamyl alcohol. Shake 5 minutes and centrifuge 5 minutes at 12 000 rpm.
· Transfer upper layer to new microfuge tube and add 1/10th the volume of 5 M ammonium acetate and an equal volume of isopropanol. Store at –20ºC for at least 2 hours. (Until next lab session).
· Centrifuge 5 minutes at 12 000 rpm. Discard supernatant. You may see a small white pellet (this is the DNA).
· Resuspend pellet in 200 µl of 70% ethanol.
· Centrifuge 5 minutes at 12 000 rpm.
· Discard supernatant and air-dry the pellet, ensuring no ethanol remains in the tube.
· Resuspend pellet in 10 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.

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