

# SC/BIOL 4160 Photosynthesis



artwork by Michael Hagelberg

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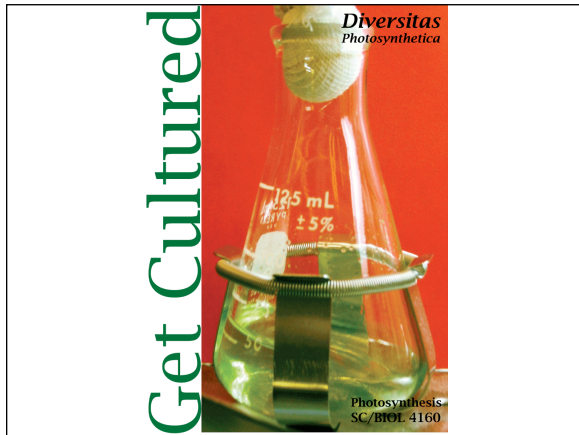
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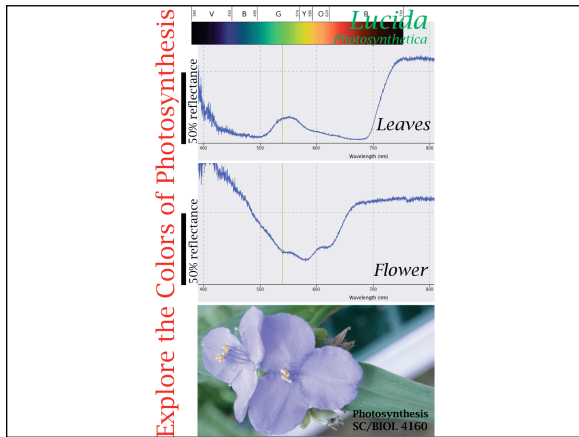
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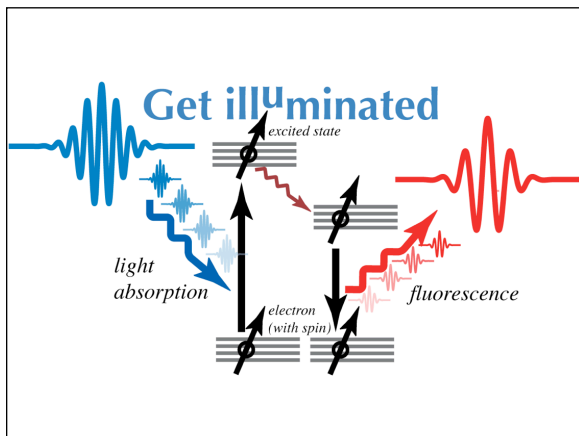
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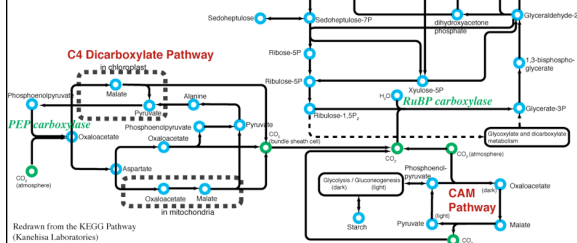








# Discover pathways of carbon fixation



Redrawn from the KEGG Pathway (Kanehisa Laboratories)

**Photosynthesis SC/BIOL 4160**

## Get Glowing

**Fluere Photosynthetica**

Photosynthesis SC/BIOL 4160

## Unravel the Chloroplast Genome

### Hereditas Photosynthetica

Harvest 10 g of spinach seedlings kept in dark for 48 hrs to induce starch levels. Wash under tap water. Cut into 1 cm strips (directly into ice cold buffer).

Grind to a fine powder with 20 ml of cold extraction 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 50 ml centrifuge tube. A filter funnel will avoid messes. The material in the nylon mesh can be easily separated to release more chloroplasts into the filtrate.

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.

Centrifuge supernatant into a new 50 ml centrifuge tube and spin at 4,000 rpm for 20 minutes at 4°C. This will pellet the chloroplasts.

Resuspend supernatant and resuspend pellet (spin at 100 at 4°C buffer).

Transfer sample to microcentrifuge tube and add equal volume of equilibrated phenol. Shake thoroughly 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 microcentrifuge tube and final volume of 1.5 phenol:ethanol:water. 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 1/10th the volume of 5 M ammonium acetate and an equal volume of isopropanol. Store at -20°C for at least 2 hours. (Hard 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 70% ethanol.

Centrifuge 5 minutes at 12,000 rpm.

Discard supernatant and dry the pellet, ensuring no ethanol remains in the tube.

Resuspend pellet in 10 µl TE buffer. This is your DNA sample.

Use 5 µl of DNA sample with 1 µl of dx loading buffer.

Run sample on 1% agarose gel at 100 volts for ~45 minutes.

**Photosynthesis SC/BIOL 4160**

## Create Oxygen

### Aerobica Photosyntheticiam

**Photosynthesis SC/BIOL 4160**

