ISOLATION OF CHLOROPLASTS

https://www.youtube.com/watch?v=6JJBvh-NQZA&t=191s
The chloroplast is an important organelle found in plant cells that conduct photosynthesis. Photosynthesis is the process used by plants, algae and certain bacteria to harness energy from sunlight and turn it into chemical energy.

Chloroplasts are the best starting material for studies of chloroplastic processes such as carbon assimilation, electron flow and phosphorylation, metabolic transport, or protein targeting.

The chloroplast fraction can be further extracted to obtain membrane, stroma, or thylakoid proteins as well as chloroplastic DNA and RNA.
the chloroplast

Thylakoid
Thylakoid space (lumen)
Thylakoid membrane

Chloroplast envelope
Outer membrane
Intermembrane space
Inner membrane

Granum

Stromal thylakoids (lamellae or frets)
Granal thylakoids

Stroma

Nucleoid (DNA rings)

Ribosome
Plastoglobulus
Starch granule

Thylakoid

H₂O
NADP⁺

ADP

ATP

NADPH

O₂
تركيب كلوروبلاست:

1: غشاء خارجي
2: غشاء داخلي (1+2+3: الغلاف) 
3: فراغ بين الغشائين 
4: الحشوة أو السدى أو الستروما (محلول مائي) 
5: تجويف الثايلاكوب (داخل الثايلاكوب) 
6: غشاء الثايلاكوب 
7: جرائج 
8: صفيحة ثايلاكوب 
9: حبة نشاء
10: ريبوسوم
11: كرية بروتين دهني cpDNA
12: ريبوسوم
Materials (per groove)

- Fresh spinach
- clean sharp sand
- 50 mL 0.5 M sucrose
- cheese cloth, 12 x 12 inches
- Ice
- 25 mL graduated cylinder
- mortar and pestle (or blender)
- Centrifuge
- glass filter funnel
- two 16x150 mm test tubes in rack
- three 13x100 mm test tubes in rack
- plastic capped 15 mL centrifuge tube
- glass stirring rods
Procedures

1 - Prepare, weigh and homogenize:

Grind 8 g deveined spinach with ½ tsp clean sharp sand in mortar and pestle to a paste.
2-Suspend in 0.5 M sucrose:

Measure out 16 mL ice-cold 0.5 M sucrose solution in a 25 mL graduated cylinder. Add in 3-4 mL increments, grind to smooth pulp with each addition.
3-Filter

homogenate through about eight layers of clean cheese cloth in a glass funnel into an iced 16x150 mm test tube
4-Pour filtrate back into 25 mL cylinder and **record volume**. Save ~0.5 mL of the filtrate and examine it at 400x under microscope.
5- **Centrifuge at low speed:** prepare a balance tube against the filtrate in a 16x150 tube and spin at 50x g for 10 minutes
6- **Decant** the top 10 mL into a clean cold centrifuge tube, discard sediment. Record volume. Save ~0.5 mL supernatant to examine under microscope.
7- **Centrifuge** the supernatant at 1000x g for 10 minutes to precipitate chloroplasts. Carefully decant all of the supernatant into 16x150 mm tube but save the pellet. Discard supernatant *if* you have a significant pellet.
8-Resuspend pellet from the last step the filtrate in ice-cold 0.5 M sucrose with a clean, ice cold stirring rod. Record final volume. Keep on ice at all times. Examine suspended organelles under microscope.