Purification of chloroplasts using a continuous Percoll gradient centrifugation

Preparation of buffers

Grinding buffer

50 mM HEPES (pH 7.5 with NaOH)
0.33 M sorbitol
0.1 %(w/v) BSA (fatty acid free)
1 mM $MgCl_2$
1 mM $MnCl_2$
2 mM $NaNO_3$
1 mM NaH_2PO_4
2 mM EDTA(2Na)
2 mM Na iso-ascorbate

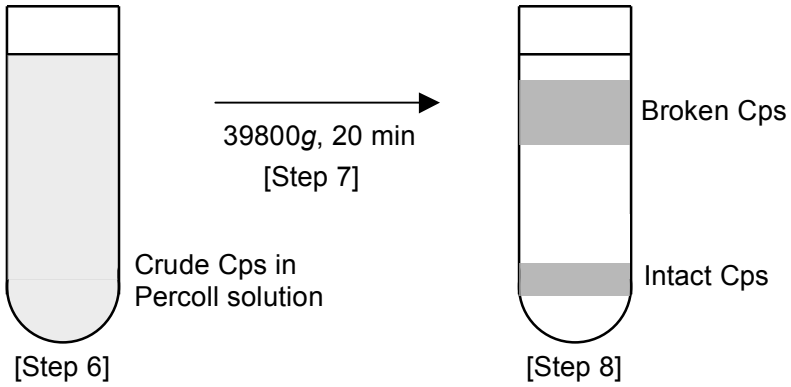
Percoll solution

50 %(v/v) Percoll
50 mM HEPES (pH 7.5 with NaOH)
0.33 M sorbitol
1 %(w/v) BSA
1 mM $MgCl_2$
1 mM $MnCl_2$
2 mM $NaNO_3$
1 mM NaH_2PO_4
2 mM EDTA(2Na)
2 mM Na iso-ascorbate
5 %(w/v) PEG-6000
1 %(w/v) Ficoll 400

Procedure

1. Cut 15 g of leaves into small pieces of 1-2 mm each using a razor blade.
2. Homogenize leaf pierces with 100 ml of semi-frozen grinding buffer using a homogenizer for 5-10 s.
3. Filter the homogenate through 4 layers of cheesechloth.
4. Centrifuge at 2500*g* for 90 seconds (Hitachi RPR 20-2) and wash twice with Grinding buffer.
5. Resuspend the pellet in 1 ml of Grinding buffer using a small paintbrush.
6. Add 35 ml of Percoll solution and mix gently.
7. Centrifuge at 39800*g* for 20 min (Hitachi RPR 20-2).

8. Collect a green layer near the bottom and resuspend in 20 ml of Grinding buffer.
9. Centrifuge at 2500*g* for 90 seconds and wash twice with Grinding buffer.
10. Pool the pellet containing purified chloroplasts.
11. Check if the chloroplasts are intact using the ferricyanide test (Lilley et al. 1975, New Phytol. 75:1).



Notice

1. All procedures should be done at 4°C or on ice.
2. If the presence of BSA or ascorbate causes any problems for your analysis, use Grinding buffer which does not contain these materials after the Percoll centrifugation.
3. This procedure is best suited for wheat. We have also confirmed that this can be applied for peas and spinach.
4. When you use plants other than wheat and find the pellet of starch at the bottom of the tube after step 8, reduce the starch content by placing the seedlings in darkness for 1-2 days to obtain intact chloroplasts at high yield.