3. Gentle swirling of the Petri dishes following overnight digestion in protoplasting solution releases the protoplasts from the leaves. Sieving of the protoplasts allows separation of the protoplasts from the remaining leaf tissue.
4. During protoplast isolation, gentle pipeting is essential to ensure that protoplasts are not damaged. This is achieved by cutting off the ends of pipet tips and slowly resuspending or collecting the protoplasts.
5. When the sucrose solution is overlaid with protoplasts, care must be taken not disturb the boundary between the two solutions.
6. When looked at under the light microscope, the overall appearance of individual protoplasts is determined. If there is evidence of burst cells and "grainy" cellular material among the healthy cells, then it is necessary to carry out a second spin in the sucrose solution, which should remove any debris.
7. During PEG transformation of protoplasts with plasmid DNA, the mixture should not be left standing for more than 30 min, because PEG treatment, although less damaging than other such chemicals used, can render protoplasts unrecoverable.
8. As experience is gained in isolating protoplasts, the need for FDA staining becomes less essential. The general appearance of the protoplasts under the light microscope will allow for determination of viability.

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