Standard Operating Procedures for the CTAB Method

Operation Steps

1. **Sample Preparation**: Pipette 1000 μL of CTAB lysis buffer into a 2.0 mL EP tube. Add lysozyme and an appropriate amount of the sample. Incubate the mixture in a 65 ℃ water bath, inverting and mixing periodically to ensure complete lysis of the sample.
2. **Centrifugation and Phase Separation**: Centrifuge the lysed sample to collect the supernatant. Add a mixture of phenol (pH 8.0): chloroform: isopentanol (25:24:1) to the supernatant, invert and mix thoroughly, then centrifuge at 12,000 RPM for 10 minutes.
3. **Organic Phase Removal**: Transfer the supernatant to a fresh tube. Add a mixture of chloroform: isopentanol (24:1), invert and mix well, then centrifuge at 12,000 RPM for 10 minutes.
4. **Precipitation**: Transfer the supernatant to a 1.5 mL centrifuge tube. Add isopropyl alcohol, mix by inverting, and precipitate the nucleic acids at -20 ℃.
5. **Washing and Drying**: Centrifuge at 12,000 RPM for 10 minutes. Carefully remove the supernatant without disturbing the pellet. Wash the pellet with 1 mL of 75% ethanol, repeating this step twice. Collect any residual liquid by centrifugation and remove it using a pipette tip.
6. **Drying**: Air-dry the pellet on a laminar flow hood or at room temperature. Note that DNA samples should not be overdried, as this may hinder subsequent dissolution.
7. **Resuspension**: Resuspend the DNA pellet in ddH_2O. If necessary, incubate at 55-60 ℃ for 10 minutes to facilitate dissolution.
8. **RNA Digestion**: Add 1 μL of RNase A to the dissolved DNA sample to digest any residual RNA. Incubate at 37 ℃ for 15 minutes.