Pine Tree Method

This method was originally described by Chang S., Puryear J., Cairney J. (1993) A Simple and Efficient Method for Isolating RNA from Pine Trees. Plant Molecular Biology Reporter 11: 113-116.

◎*Materials and Reagents**

Extraction buffer (For RNA extraction Pine Tree Method)

2% CTAB (hexadecyltrimethylammonium bromide)

2% PVP (polyvinylpyrrolidone K 30)

100 mM Tris-HCl pH 8.0

25 mM EDTA

2.0 M NaCl

0.5 g/L spermidine

Mix and autoclave.

2% beta-mercaptoethanol **(add just before use)**

Chloroform:isoamyl alcohol (24:1)

10 M Lithium chloride

1. Warm 5 ml extraction buffer to 65℃ in a water bath, quickly add 1g ground tissue and mix by inverting the tube and vortexing.

2. Extract two times with an equal volume of chloroform:isoamyl alcohol, separating phases at room temperature by centrifugation for 10 min at 12,000 x g. Centrifuge longer if phases are not well separated.

3. Add 1/4 volume 10 M LiCl to the supernatant and mix. The RNA is precipitated overnight at 4℃ and harvested by centrifugation at 12,000 x g for 20 min. Shorter precipitations time may also be used with lower yield.

4. Optional: wash pellet with 20 ml of 75% ethanol. Vortex briefly. Centrifuge at 10,000 x g at 4℃ for 10 min. Discard supernatant; briefly dry pellet on kimwipe.

5. Dissolve pellet into 100-250 μl DEPC-H_2O and proceed with polyA^+ RNA selection directly.