RNA isolation (Total RNA Extraction from High Polysaccharide Plants)

Fatemeh Mehrpooyan*

MSc., Genetic and Molecular Biology, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

*sahar_mehr8261@yahoo.com

- ***What is RNA Extraction?***

RNA extraction is the purification of RNA from biological samples. This procedure is complicated by the ubiquitous presence of ribonuclease enzymes in cells and tissues, which can rapidly degrade RNA. Several methods are used in molecular biology to isolate RNA from difficult samples. Here CTAB modified method is described.

- ***Which method is more efficient?***

Phenol–chloroform extraction (abbreviated PC or PCI) is a liquid–liquid extraction technique in biochemistry. It is widely used in molecular biology for isolating DNA, RNA and protein. Equal volumes of a phenol:chloroform mixture and an aqueous sample are mixed, forming a biphasic mixture. This method may take longer than a column-based system such as the silica-based purification, but has higher purity and the advantage of high recovery of RNA: an RNA column is typically unsuitable for purification of short (<200 nucleotides) RNA species, such as siRNA and miRNA.

- ***How does it work?***

Total RNA will be extracted from samples using a modified method based on Kiefer et al., 2000. One hundred to one hundred and fifty mg of plant tissue will be grinded using cold mortar and pestles under liquid nitrogen. The powdered tissues then will be placed in a 2 ml microcentrifuge tube and 1 ml of pre-warmed (65 °C) extraction buffer (100 mM Tris-HCl, 25mM EDTA, 2M NaCl, 2% CTAB w/v, 2% PVP w/v, 2% mercaptoethanol) will be added to the tube. After that, an equal volume of chloroform/isoamylcohol (24:1 v/v) will be added and the tube will be vortexed at 1,800 rpm at room temperature for 5 minutes. The tube will be then centrifuged for 15 minutes at 4 °C, 10,600 x g and the supernatant will be transferred to a new 2 ml microcentrifuge tube. Five hundred µl of chloroform/isoamylcohol (24:1; v/v) then will be added to the supernatant. The tube will be vortexed at room temperature followed by centrifugation for 15 minutes at 4 °C, 10,600 x g. The supernatant will be transferred to a 2 ml microcentrifuge tube and five hundred µl of Phenol: Chloroform: Isoamylalcohol (PCI) (24:1:25; v/v/v) will be added to it. The tube will be again vortexed at room temperature followed by centrifugation for 15 minutes at 4 °C, 10,600 x g. Another time the supernatant will be transferred to a new 2 ml microcentrifuge tube. After that, five hundred µl of chloroform/isoamylcohol (24:1; v/v) will be added to the supernatant. The tube will again be vortexed at room temperature followed by centrifugation for 15 minutes at 4 °C, 10,600 x g. Later, the supernatant will be transferred to a 1.5 ml microcentrifuge tube and 3 volumes of cold absolute

ethanol (4 °C) and 0.1 volume of NaOAc (Sodium Acetate) will be added to it. The tube will be incubated at -80 °C overnight and followed by centrifugation for 30 minutes, 4 °C at 17,900 x g. The pellet then will be washed with cold 70% ethanol and centrifuged for 5 min, 4 °C at 17,900 x g. Eventually, the supernatant will be discarded and the pellet will be re-suspended in 50 µl of DEPC treated dH2O.

- ***What modifications?***

Two steps of chloroform:Isoamylalchohol (CI) extraction followed by one step of Phenol:Chloroform:Isoamylalcohol (PCI) extraction are used respectively to eliminate the proteins and polysaccharide contamination from the extract; moreover, one extra step of CI extraction is included to remove any phenol residues to avoid interference during PCR. This is compared to the original CTAB method where only two steps of CI extraction are suggested for washing steps. These extra extraction steps helped increase the purity of samples with high polysaccharide contamination in plant samples.

- ***Contact us***

For more information or troubleshooting on your Transformation, please do not hesitate to contact us at ijpp@iau-saveh.ac.ir. You can simply mention your problem by attaching your results. We look forward to hear from you soon.

- ***Read more on***

http://www.protocol-online.org/prot/Plant_Biology/RNA/