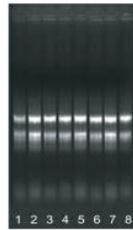
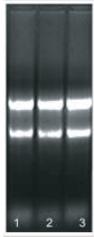


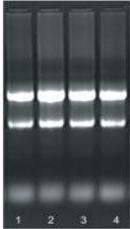
Choose primer sets that amplify the processed mRNA across the splice junctions, making the unprocessed genomic DNA an unamplifiable target;
 Choose primer sets that amplify cDNA made from processed mRNA and not made from an unprocessed genomic DNA template, which would be too large to PCR-amplify effectively;
 Treat the RNA sample with RNase-free Dnase 2.

3. Pictures of Experiment

Picture 1: Total RNA extracted from animal tissues Picture 2: Total RNA extracted from rice leaves



Picture 3: Total RNA extracted from culture cells



Total RNA pictures above are strictly operated according to Total RNA Extraction Reagent

TROUBLE SHOOTING

Q=Question; R=Reason; S=Solution

Q1. Why the yield of extraction is low?

- R1: Incomplete homogenization. (Such as low room temp, inadequate volume);
 S1: prolong lysis time or maximize volume.
 R2: The RNA pellet is not dissolved completely;
 S2: Please prolong the time of dissolve RNA; and do not let the RNA dry completely before dissolving.
 R3: The yield of RNA varies greatly from tissue to tissue. Normally new tissues or cells contain a plenty of RNA.
 S3: Choose fresh, plenty cell-contained tissue.
 R4: The speed and centrifuge gravity can not meet the requested rate, please increase the speed or prolong the centrifugation time appropriately.
 S4: Suggest maximize speed of centrifugation or prolong the centrifugation time

Q2 Why RNA degradation?

- R1: Tissues were not immediately processed or frozen after removal from the animal;
 S1: During grind samples into powder, please add the Liquid N2 make sure tissue samples biologic active
 R2: Queous solutions or tubes were not RNase-free;
 S2: All plastic wares and tips should be RNase-free.
 R3: Cells were dissolved by longtime trypsinase digestion; Forget to ware groves or not to change groves frequently during the procedure;
 S3: Beginner had better wear glove and respirator
 R4: The RNA do not be stored at the appropriately temperature.
 S4: Please store the RNA at -70?

Q3. Why there is DNA contamination when extraction total RNA?

- R1: Too much Sample with inadequate Total RNA Extraction Reagent;
 S1: Please use more Total RNA Extraction Reagent or decrease amount of tissue on the base of suggested volume
 R2: Inter phase contamination when moving upper aqueous phase;
 S2: Please transfer the aqueous phase careful.
 R3: Samples were not stored at room temperature more than 15min, Insoluble particle remain because of uncompleted homogenization before chloroform extraction;
 S3: Process tissue according to manual, be sure to completely homogenize sample under lower room temp, delay incubate time.
 R4: After adding chloroform, shake too vigorously;
 S4: Gently mix
 R5: Too many particulate matter remains after homogenization.
 S5: Please watch Option I?
 R6: Contamination has strongly relationship with the origin of sample.
 (For example: the sample rich in protein, fat etc.);
 S6: Please watch Option I

Q4 Why OD260/OD280 ratio is too low? (<1.65)

- R1: RNA sample was diluted in water instead of TE prior to spectrophotometric analysis.
 Low ionic strength and low pH solutions increase absorbance at 280 nm;
 S1: Please dissolve sample in TE or Tris-HCl
 R2: Homogenizing sample with inadequate Total RNA Extraction Reagent volume;
 S2: Please use more Total RNA Extraction Reagent or decrease amount of tissue.
 R3: Samples were not stored at room temperature more than 15 minutes for complete lyses after homogenization;
 S3: Please delay the lysis time, for the sample lysis completely.
 R4: The aqueous phase was contaminate by the phenol phase;
 S4: Please carefully transefer the aqueous phase.
 R5: Incomplete dissolution of the final RNA pellet; S5: Please delay the time of dissolve RNA