RNA extraction from maize endosperm using SDS-TRIZOL protocol

One of the most common problems to extract RNA from tissues such as maize endosperm is the high levels of starch. Guanidine based protocols dissolve the starch, making a viscous matrix which limits RNA yields. This combo protocol (SDS-TRIZOL) takes advantage of the SDS extraction method, which separates the starch from the nucleic acids and uses TRIZOL extraction procedures to enrich the RNA fraction. We also take advantage of PHASE LOCK tubes (by Eppendorf) to minimize pipetting and facilitate the removal of organics during the extraction procedure. Several Phenol-chloroform can be done in a single tube without the need for pipetting out the aqueous phase.

Sample preparation

- Dissect the endosperm from frozen kernels quickly, without letting it thaw completely.
- Place it in a tube and in liquid nitrogen immediately.
- 100 mg of endosperm will yield roughly 100 ug of total RNA.

SDS extraction (Based on Prescott and Martin, 1987)

- Start from 100mg of endosperm (roughly one 14 DAP endosperm or half 20 DAP endosperm) individually placed in a tube.
- Pick them up from liquid nitrogen and add 200 ul of RNA extraction buffer (50mM TRIS pH 8.0, 150mM LiCl, 5mM EDTA pH 8.0, 1% SDS – all stocks made on DEPC treated water).
- Quickly grind it using a RNAse Free plastic pestle (a system with a power drill works great).
- After grinding add 200 ul of 1:1 Phenol (equilibrated in TRIS pH 8.0) -Chloroform, shake it well and place them on ice for 5'. Mix occasionally.
- Transfer the solution to a PHASE LOCK tube (Eppendorf)
- Centrifuge the samples for 10’ @ 10000g, 4oC;
- Add 200 ul Phenol-Chloroform, shake it well;
- Centrifuge the samples for 10’ @ 10000g, 4oC;
- Add 200 ul of Chloroform, shake it well and place them on ice for 5’. Mix occasionally.
- Centrifuge the samples for 10’ @ 10000g, 4oC;

**TRIZOL extraction**

- Pour the aqueous phase into a new PHASE LOCK tube (the organic phase will be locked in the bottom of the tube).
- Add 1 ml of TRIZOL.
- Shake it well for 15 sec. Incubate for 5’ @ room temperature.
- Add 200 ul of Chloroform, shake it well and incubate for 2-3’ @ room temperature;
- Centrifuge for 10’ @ 10000g, 4oC;

**RNA precipitation**

- Transfer the aqueous phase to a fresh microfuge tube;
- Add 500 ul of Isopropanol, mix well and incubate for 10’ on ice;
- Centrifuge for 10’ @ 10000g, 4oC.
- At this point the RNA precipitate can be seen;

**RNA washing and dissolving**

- Pour out the supernatant;
- Add 1 ml of 70% ETOH (in DEPC treated water);
- Centrifuge for 5’ @ 10000g, 4oC;
- Pour out the supernatant (be careful, pellet might be loose);
- Air dry the RNA pellet;
- Add 50ul of DEPC treated water;
- Dissolve the pellet
**DNAse treatment (protocol suggested by Invitrogen)**

- For each 1-2 ug of total RNA add:
  - 1ul of 10x reaction buffer
  - 1 ul of DNAse (amplification gradient)
  - fill up to 10 ul with DEPC treated water
- Incubate @ RT for 15’;
- Add 1
- 1ul of 25mM EDTA pH 8.0;
- Incubate @ 65oC for 15’ to heat inactivate DNAse;
- Place on ice for 1’
- Dilute the RNA to the desired concentration.