LiCl / Urea extraction of RNA

This protocol is based on Auffray and Rougeon (1980) Eur. J. Biochem. 107: 303-314 and has been modified in the Benoist/Mathis lab over the years. It works for all mouse organs we tried and is especially useful for RNA preparation from organs rich in RNAses, like the pancreas.

Homogenizer: We use a Polytron (Kinematika AG, Littau, Switzerland), Fisher's PowerGen Model 700 should work fine as well.

Solutions (LiCl/Urea and PK buffer are to be prepared fresh):

LiCl/Urea:

	conc.	for 20ml:	for 50 ml:
LiCl	3M (M=42.3)	*2.54g*	*6.35g*
Urea	6M (M=60.06)	*7.2g*	*18g*
NaOAc, 3M stock (pH5.2)	10mM	*66.7µl*	*166.7µl*

SDS 20%

PK buffer *(prepare it at RT, or SDS/EDTA will precipitate):*

	conc.	for 10ml:
Tris pH8.0	10mM	*50µl Tris 2M*
EDTA, 0.5M stock	2mM	*40µl*
NaCl, 5M stock	200mM	*400µl*
SDS, 20 % stock	0.5 %	*250µl*
Proteinase K, 20mg/ml stock	200µg/ml	*100µl*

Procedure:

1 The organ has to be freshly dissected out or should be frozen in liquid nitrogen right after dissection and kept at -80°C in a 14ml Falcon polypropylene tube.

2 Homogenize the organ in 2ml LiCl/Urea + 50µl SDS in a Polytron homogenizer (2 times 15 seconds with the Polytron at maximum speed; move the tube around the probe to detach any piece of tissue stuck to the side).

3 Incubate overnight at 4°C (on ice).

4 Centrifuge 10 min at 9000 rpm and 4°C in the Sorvall SM-24 rotor. Remove the liquid.

5 Resuspend the pellets in 2 ml LiCl solution, without adding SDS. Incubate for at least 30 minutes on ice.

6 Centrifuge 10 min at 9000 rpm and 4°C, as above. Remove the liquid.

7 Resuspend the pellets in 400-500µl PK buffer. Transfer into 1.5ml Eppendorf tubes. Incubate for >20 min at 37°C.

8 Extract by adding 250µl phenol, vortex thoroughly; add 250µl chloroform, vortex; and centrifuge 5 minutes at 13000 rpm. Transfer the aqueous phase into a fresh tube.

9 Add 500µl chloroform, vortex, and centrifuge 5 minutes at 13000 rpm for. Transfer the aqueous phase into a fresh tube.

10 Precipitate RNA with 2.5 volumes of 100% ethanol. Gently mix. Leave 30 min at -80°C or 2 hours at -20°C. Centrifuge at 13000rpm for 10 minutes, 4°C. Remove the supernatant carefully and discard it.

11 Wash with 1ml of 75%ethanol. Centrifuge 5 minutes at 13000 rpm, 4°C.

Dissolve the pellet in H_2O or formamide (20 to 200µl, depending on expected yield and future applications).

Notes: Yields from an adult mouse: thymus or spleen: 200µg, liver: >1 mg, bone marrow (2 thighbones): 30µg, lymph nodes (inguinal+axilary+mesenteric): 35µg, islets: 1µg (need several mice), cell suspension: $1\mu g/10^6$cells (from pellets frozen in liquid nitrogen).

For a small quantity of tissue, you might reduce the volume of LiCl for homogenization (minimum 1-1.5ml), increase the time of centrifugation after precipitation in ethanol (20-30 min).

For pancreas RNA, the organ must be disrupted or frozen as fast as possible after the mouse is sacrificed. To dissect the pancreas, don't pin the mouse down, lay it on its right side, and cut the skin. The spleen should be visible through the peritoneum. Open the peritoneum, hold the pancreas with forceps right under the spleen and pull it out cutting what's holding it along the spleen.