RNA isolation date :

Modified RNaqueous-Micro Protocol for LCM samples

(Julie Pelletier/HaradaLab January 2007)

Before you begin the procedure: -Warm Wash Solutions 1 and 2/3 to RT.
-Preheat Incubator to 42°C
-Thaw LCM Additive (-20°C) and keep on ice.

Combining the vials for one biological replicate:

-Thaw LCM samples and place on ice. Check to see if any crystallization has occurred. If so, add 20ul of lysis buffer to the samples and note the additional volume added.

-Combine the samples into a 1.5ml tube. Rinse the empty 0.2ml tubes with 20-30ul lysis buffer and add to combined samples. Measure the total volume of lysis buffer.

-Calculate what the final volume should be (buffer added to caps while cutting, buffer added later for dissolving the crystals and rinsing the tubes) and the volume of liquid missing. Add RNase free water to 85% of that volume and 15% of lysis buffer. (For example, if the measured volume is 500ul and should be 600ul: add 85ul RNase-free water and 15ul lysis buffer.) Vortex well and spin down briefly.

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	Sample Name						
1	LCM Collection volume						
2	Rinse Lysis Solution						
3	**Final Volume (1+2)**						
4	Measured Volume						
5	Volume to complete (3-4)						
6	Added Water						
7	Added Lysis Solution						
	Final Volume (4+6+7=3)						

RNA Isolation

-Incubate Samples in Lysis Solution in water bath at 37°C for 1-2min, then in air incubator at 42°C for 1 hour (a minimum of 30 minutes). Vortex and spin down briefly.

-Assemble spin column and collection tubes for each samples and label caps. Prewet the filter with 30ul of Lysis Solution and incubate at room temperature for at least 5 minutes, while the two following steps are performed. Centrifuge 30 sec at max speed to remove solution.

-Add 3% volume of LCM additive (3ul for 100ul lysate). Vortex and spin down briefly.

-Add 1.25V of 100% ethanol. Mix by pipetting up and down ten times.

Sample Name								
LCM Additive (3%)								
100% ETOH (1.25V)								

-Make sure the Lysis solution has been removed by centrifugation before loading the lysate. Apply 400ul of the lysate/ethanol mixture onto column and centrifuge 1min/10,000g to bind RNA. Repeat until all the lysate/ethanol mixture has been passed through the filter.

-Wash filter with 180ul Wash Solution 1. Centrifuge for 1min/10,000g. Discard flow-through.

-Add 20ul of DNase Solution, preheated to 37°C, to filter: Mix gently by pipetting:
-5ul DNase I (Qiagen)
-15ul RDD buffer

-Incubate at 37°C, 20-30 minutes.

-Wash filter: -180ul Wash Solution 1. Centrifuge for 1min/10,000g.
-180ul Wash Solution 2/3. Centrifuge for 30sec/16,000g.
-180ul Wash Solution 2/3. Centrifuge for 30sec/16,000g. Discard flow-through.

-Centrifuge 1min/16,000g to dry filter.

-Transfer column to a labeled, clean 1.5ml Micro Elution tube.

-Add 10ul of Modified Elution Solution (RNAse-free 0.01mM EDTA), preheated to 94°C, to the center of the filter. (Make sure to pipet the Elution Solution up and down every time a new tip is used since accurate pipetting of hot liquids can be difficult.)

-Incubate at room temperature, 5 minutes. Centrifuge 1min/16,000g.

-Repeat Elution step with an additional 10ul of preheated Modified Elution Solution.

-Mix RNA by quick vortexing and spin down briefly. Cool on ice.

-Concentrate sample by SpeedVac (without heat) down to desired volume (10ul), wiping interior surfaces of SpeedVac with RNase Zap before use.

-Measure RNA concentration using Ribogreen (2.5ul RNA + 2.5ul 200X diluted RG).

-Aliquot RNA for Bioanalyzer (if desired) and store at -80°C.