RNA Isolation Protocol –Damla D. Bilgin-IGB, UIUC.

Materials:

Acid Phenol:Chloroform: Ambion Cat# 9722
Ammonium Thiocyanate: Sigma A7149
Chloroform (HPLC Grade): Fisher Cat# C606SK-1
Glycerol: Fisher G33-500
Guanidine Thiocyanate: Ambion Cat# 9422
Phenol: Sigma P4557
3M Potassium Acetate pH 5.5: Ambion Cat# 9610
3M Sodium Acetate pH 5.5: Ambion Cat# 9740

HMT Preparation Protocol:

Added		Conc.	20mL	40mL	100mL	200mL
	Phenol Solution	*38%*	7.6mL	15.2mL	38.0mL	76.0mL
	Guanidine Thiocyanate	*0.8M*	1.89g	3.78g	9.45g	18.9g
	Ammonium Thiocyanate	*0.4M*	0.638g	1.276g	3.19g	6.38g
	3M Sodium Acetate pH 5.5	*0.1M*	0.668mL	1.336mL	3.34mL	6.68mL
	Glycerol	*5%*	1.0mL	2.0mL	5.0mL	10.0mL

Add nuclease-free water to complete to volume.

Protocol

1. Pre-spin empty 50[1]mL Phase Lock Gel-Heavy tubes (PLG) briefly to collect gel on tube bottoms (1500g for 1 min @ 4˚C is sufficient to collect gel at tube bottoms).
2. Pre-cool mortar with liquid nitrogen. Add excess liquid nitrogen so it does not fully evaporate.
3. Add frozen tissue to mortar containing liquid nitrogen.
4. Grind to a fine powder, carefully adding more nitrogen, if necessary, as it evaporates to ensure tissue does not thaw[2]. *Be careful not to splash tissue out of the mortar*
5. Once ground sufficiently, let nitrogen evaporate off and add 6[1]mL of HMT and keep on grinding.
6. Cut the end off a pipette tip with a clean razor and transfer the sample to 50[1]mL PLG tube.
7. Add 2[1,3] mL HMT to the mortar again and rinse the mortar and transfer the liquid to the same PLG tube. (Total of 8[4]mL)
8. Add 1/5 volume of chloroform (1/5 * Total vol. of HMT added)
9. Mix by rocking[6] and incubate at room temperature for 5 minutes.
10. Centrifuge swing out rotor program 2 (2000g for 10min @ 4˚C)
11. Transfer and measure aqueous phase to a new 50[1]mL PLG tube add cold 3M Potassium Acetate pH 5.5 (1mL Potassium Acetate for every 3mL of aqueous layer)
12. Mix by swirling[6]
13. Add equal volume of acid phenol:chloroform (1mL / 1mL aqueous layer)
14. Mix by swirling[6]
15. Incubate on ice for 1hr
16. Centrifuge swing out rotor, program 2 (2000g for 10min @ 4˚C)
17. Transfer[7] and measure[5] the aqueous phase to 'oak ridge' tube
18. Add 0.25 mL of 0.8 M sodium citrate/1.2 M NaCl per 1 mL of aqueous phase transferred.
19. Mix by swirling[6]
20. Add 0.25mL of absolute EtOH / 1mL aqueous (0.25*aqueous phase transferred)
21. Mix by gentle inversion[6] and incubate at -20 ˚C for at least 30 minutes (overnight incubation produces higher yields).
22. Centrifuge fixed rotor, program 3 (11,000g for 10min @ 4˚C)
23. Decant the supernatant (Being careful not to lose pellet)
24. Add 2mL of 70% EtOH, vortex
25. Centrifuge, program 3 (11,000g for 10min @ 4˚C)
26. Discard supernatant (being careful not to lose pellet)
27. Repeat 70% EtOH wash
28. Centrifuge, program 3 (11,000g for 10min @ 4˚C)
29. Discard supernatant (being careful not to lose pellet)
30. Air dry the pellet
31. Dissolve in RNase free water (200 µL)

Notes:
1 – depends on starting material
2 – dark green = thawed
3 – Add appropriate amount of HMT until glue-like consistency
4 – Based on amount added of HMT added in step 7
5 – Volume should be equal to amount HMT added
6- DO NOT SHAKE!
7 – If Phase Lock broke or there seems to be gel in sample then transfer sample to a new PLG tube and add equal amounts of acid phenol:chloroform and centrifuge again with a water a counter balance.

Name: Date:

Quick Glance Flow Chart: Amount Used:

	Sample	Sample	Sample	Sample
	_____	_____	_____	_____
-Add HMT to mortat to transfer and wash	_____	_____	_____	_____
-Add 1/5 volume **chloroform**	_____	_____	_____	_____
-Incubate 5 min while rocking -Centrifuge Program 2 -Transfer and **measure aqueous** to new PLG	_____	_____	_____	_____
-Add 1/3 volume **KAc** and swirl	_____	_____	_____	_____
-Add 1 vol. of **acid phenol:chloroform** - swirl	_____	_____	_____	_____
-Incubate 1 hour on ice -Centrifuge Program 2 -Transfer and **measure aqueous** to oak ridge	_____	_____	_____	_____
-Add ¼ volume of **NaCitrate/NaCl** and swirl	_____	_____	_____	_____
-Add ¼ volume of **100% EtOH**	_____	_____	_____	_____
-Gently Invert and incubate at -20˚C overnight -Centrifuge Program 3 -Decant supernatant -Add 2mL **70% EtOH**		2mL		
-Centrifuge Program 4 -Discard supernatant -Add 2mL **70% EtOH**		2mL		
-Centrifuge Program 4 -Discard Supernatant -Air Dry pellet -Dissolve in 200µL of **nuclease-free water**		200µL		

Sample #____ → Sample #____ →

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