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Obtaining RNA from purified Mouse Sperm

Protocol Developed by Kelly Seltzer at Rutger's Snyder Lab

Sperm Collection

1. Combine 2 Cauda into 1 ml PBS
2. Cut up tissue to release tubules (50-80 cuts), incubate at 37°C, 30 minutes; inverting at 15 minutes
3. Centrifuge on the OHAUS Frontier Centrifuge FC5515R for 5 minutes at 1000g to pellet somatic cell debris. Collect supernatant
4. Centrifuge collected supernatant on the OHAUS Frontier Centrifuge FC5515R for 5 minutes at 1000g to obtain pelleted sperm, discard supernatant
5. Wash the sperm pellet with 1ml PBS
6. Re-Pellet Sperm through centrifugation if needed
7. Wash pellet with 1mL somatic cell lysis buffer (SCLB=0.05% SDS and 0.25% Triton-X in PBS) for 10 min on ice
8. Re-Pellet Sperm if needed
9. Wash pellet with 1ml PBS
10. Re-Pellet Sperm if needed
11. Snap freeze the pellet and store at -80°C

RNA Extraction from Sperm pellet

1. Re-suspend the sperm pellet in 700ul QIAzol Lysis Reagent and transfer the entire sample to a flat 2ml tube
2. Add 100ul of 2um glass beads
3. Heat sample for 5 minutes at 65°C with 300 rpm on the OHAUS Incubating Cooling Thermal Shaker
4. Homogenize sample on the OHAUS HT Lysing Homogenizer for 5 minutes at 800 rpm
5. Repeat step 3
6. Repeat Step 4
7. Add 140ul of chloroform to the sample and use the OHAUS Mini-Vortex Mixer to vortex for 15 seconds
8. Incubate at room temperature for 2 minutes
9. Centrifuge on OHAUS Frontier Centrifuge FC5515R for 15 minutes, 4°C, 12,000g
10. Transfer upper aqueous phase to new tube and add 1.5x the solution volume of 100% Ethanol, mix by pipetting
11. Transfer sample to column and centrifuge on the OHAUS Frontier Centrifuge FC5515R at 8,000g for 30 seconds

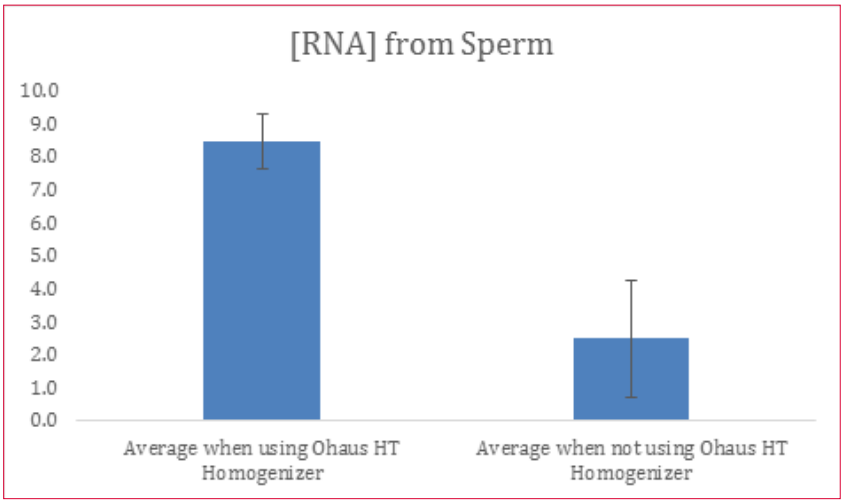
12. ***DNase Digestion***
    a. Add 350ul RWT
    b. Add 10ul DNase I (Qiagen) to 70ul of Buffer RDD and invert to mix
    c. Pipet the Dnase incubation mix (80ul) on to the membrane and incubate at room temperature for 15 minutes
    d. Add 350ul RWT
13. Add 500ul RPE, centrifuge on the OHAUS Frontier Centrifuge FC5515R at 8,000g for 2 minutes
14. Place column into new collection tube and centrifuge at max speed for 1 minute to dry membrane
15. Place column into 1.5ml tube and add 30ul RNase Free H2O, centrifuge on OHAUS Frontier Centrifuge FC5515R at 8,000g for 1 minute
16. Reapply flow through to column and centrifuge again
17. Measure RNA content with a Nanodrop and record resulting data

Results

SAMPLE	SPIN SPEED	Homogenizer	RNA EXT/CLEAN	[RNA]
1	5,000g	No	RNeasy	4.5
2	5,000g	No	Omega	4.9
3	5,000g	No	Omega	2.9
4	5,000g	No	Omega	1.8
5	5,000g	No	Omega	2
6	5,000g	No	Omega	2.8
7	5,000g	No	Omega	2
8	5,000g	No	Omega	2.7
9	5,000g	No	miRNeasy	4.9
10	5,000g	No	miRNeasy	2.7
11	5,000g	No	miRNeasy	1.4
12	5,000g	No	miRNeasy	1
13	700g	No	miRNeasy	5.6
14	1000g	Yes	miRNeasy	9.6
15	1000g	Yes	miRNeasy	8.6
16	1000g	Yes	miRNeasy	7.7
17	1000g	Yes	miRNeasy	8
18	1000g	Yes	Omega	12.1

	Average when using OHAUS HT Homogenizer	Average when not using OHAUS HT Homogenizer
RNA (ug)	8.5	2.5

Results



OHAUS Products Used Within This Procedure

Frontier Centrifuge FC5515R

Incubating Cooling Thermal Shaker

HT Lysing Homogenizer

Mini-Vortex Mixer