

### *Sperm Collection*

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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*

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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

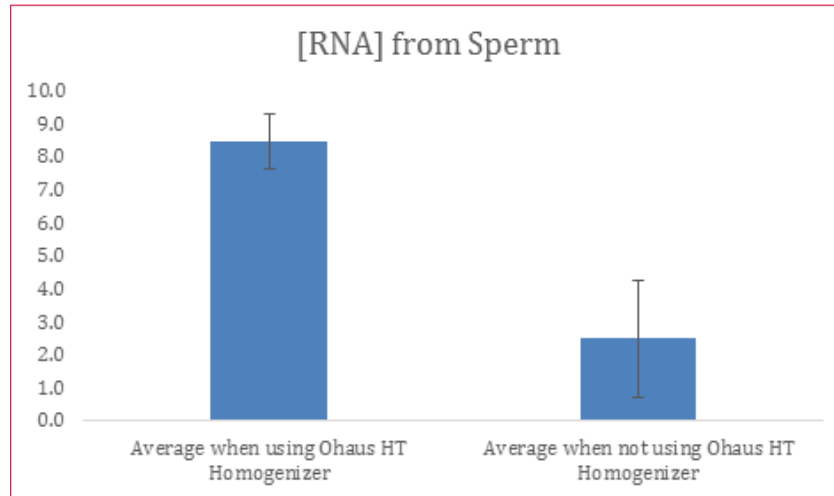
## Results

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

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### OHAUS Products Used Within This Procedure

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Frontier Centrifuge FC5515R



Incubating Cooling Thermal Shaker



HT Lysing Homogenizer



Mini-Vortex Mixer