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RNA Extraction with RNA SPK Beads:

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I. Pull out and place in a tu be the amount of RNA beads you need (90uL/samDle; n+l amount) to allow

them to acclimate to room temp. n Hoy, c

2. Place each sample in a 2mL tube with 1 clean BB.

Add IQQuL lysis buffer to each sample. Shake the tubes in the Tissue Lyzer for 1-3min at 30hz.

1. Add 60uL 100% isopropanol to each tube. Mix by pipetting and incubate for I minute.
2. Spin samples down in the centrifuge for 1 min at 1461
3. In a 96-well PCR plate (many samples) OR in autoclaved 1.5mL tubes (few samples), add 90ut- RNA magnetic beads to each well/tube and IOuL Lysis/Binding Enhancer (LBE). Mix well.
4. Move the samples from centrifuge tubes to either the plate or tube. Pipette up and down 15-20 times to mix the sample and beads together well. incubate at room temp for 5-10 minutes. YO
5. Place the plate/tubes on a magnetic rack and allow the beads to separate (2-3min). Remove the

\QVO-OO'Cke( gentle supernatant. when removing NOTE: The supernatant buffers in this to not step remove cause the beads.beads to come off the magnet mtaueeasily, so be

1. Pull plate/tubes off the magnetic rask. Add 150uL RNA Bead} Wash Buffe'r (WB) 1 'pipette up and down 10-15 times to mix the buffer and beads well. Incubate at room temp for 2 min. —no
2. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove the

O supernatant being careful not to remove beads. 9 us-e\_ see @

1. Pull plate/tubes off the magnetic rack. Add 150uL WB2, pipette up and down 15-20 times to mix the buffer and beads well. Incubate at room temp for 2 min. -3 r-CS€nJO t 50 •
2. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove the supernatant being careful not to remove beads. During the separation of beads and WB2, make a mixture of DNase and DNase buffer for your samples. For each sample add 24uL WB2, 3uL DNase and DNase Buffer. Make n+l amount.
3. After removing WB2 supernatant, remove the plate/tubes from the magnet. Add 30uL of the DNase mixture to each sample. Pipette up and down 10-15 times to mix beads and DNase together. Incubate at room temperature for 15-30min.

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| L | the bindin buffer |

1. After incubating, add 100 to each of the samples. Pipette up and down to mix

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|  | well. Incubate for 3-5min.   1. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove the supernatant being careful not to remove beads. 2. Pull plate/tubes off the magnetic rack. Add up and down 15-20 times to mix the |  |
| Tuve | buffer and beads well. Incubate at room temp for 2 min. Place the plate/tubes on the magnetic rack |  |
| ptc | and allow the beads to separate (2-3min). Remove the supernatant being careful not to remove beads. |  |
| h)VeS | Repeat this for a total of 2 times. IS (G ) |  |
|  | 17. On the second removal of WB2, leave the plate/tube on the magnetic rack. Let the beads air dry for 3- a 5min, but being careful to not over dry them (over drying causes lower yield). | er- |
| 60 | 18. Remove the plate/tubes from the magnetic rack after drying. Add 25-50uL autoclave ddH20 to each |  |
| RVIOCD | sample to elute the RNA. Pipette up and down 10-15 times to mix the beads and water well. Incubate at room temp for 5 min.  19. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove all but |  |
| 3(xmpte | 2uL of the water from the plate/tubes being careful not to remove beads. Place the RNA in new tubes.  20. Qubit the RNA for concentration and store at -800C for downstream experiments. |  |

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