AZENTA INFO ABOUT RNA YIELDS OK FOR SEQ:
Total RNA → <100ng not ideal… so aim for 100ng total RNA and up
Concentration → prefer 50ng/ul, but 20ng/ul is good. Main thing is total RNA >/= 100ng.
10-15ng/ul ok, but likely won't be able to assign a RIN, and they'll sequence with our permission
Final vol → not important, main thing is total RNA >100ng and concentration ideally 20ng/ul+

Sending samples:
Can send eluted RNA in a samples box on dry ice
Need to be at SAFS desk by 12noon
Ideal to send on monday or tuesday as it needs to be sent priority overnight to Azenta

Sample and collection information:
Sea star coelomic fluid was collected using a 1ml syringe and 25G needle. The sea water was drained, and the needle was inserted in the "armpit" to access coelomic fluid in the main body cavity. The amount of fluid collected varied greatly from star to star, depending on disease state and just generally.

The coelomic fluid was immediately placed into a 1.7ml nuclease-free microfuge tube and placed on wet ice.

The samples were taken to the dry lab at USGS Marrowstone and spun down to pellet the cells. The supernatant of the fluid was removed, and 500ul was added to the pellet. The samples were stored at -80C.

The samples were then transferred to UBC… driven. Dry shippers? Can't remember.

Then I needed them at UW, so Alyssa drove them to me on dry ice.

Supplies for extraction and quantification:
Zymo Research Quick DNA/RNA Microprep Kit (D7005)
Qubit RNA HS Kit
Pipets (P1000, P200, P100, P20, P10)
Pipet tips (P1000, P200, P100, P20, P10)
100% EtOH
1.7ml nuclease-free microfuge tubes
M gloves

LABEL ALL TUBES

SAMPLE PREPARATION
Get samples from -80C and place on wet ice
Let thaw
Spin 5,000g 20 mins to pellet
Remove RNAlater

Treat pellet like blood:

1. Add 300ul **1x DNA/RNA Shield** and resuspend pellet
2. Add 15ul reconstituted **Proteinase K** and 30ul **PK Digestion Buffer**
3. Mix well and let incubate at RT for 30 mins
4. Vortex samples, centrifuge at max speed 2mins. Transfer 300ul of supernatant to new nuclease-free tube.
5. Add 300ul **DNA/RNA Lysis Buffer** and pipet to mix well.

Sample Purification

1. Transfer the samples to yellow spin column and spin 16,000g for 1min
2. DNA/RNA
    a. DNA → put yellow column in fridge
    b. RNA:
        i. Add 600ul **100% EtOH** and mix well. Transfer to clear spin column and centrifuge 16,000g for 30s

DNAse Treatment

1. 400ul **DNA/RNA Wash Buffer**
2. 40ul of DNAse Treatment
    a. (n+1) x 5ul DNAse I = 25ul DNase I
    b. (n+1) x 25ul DNA Digestion Buffer = 175ul DNA Digestion Buffer
3. Let sit for 15mins

Purification:

3. 400ul **DNA/RNA Prep Buffer**. Centrifuge 16,000g 30s. Discard flow-through
4. 700ul **DNA/RNA Wash Buffer.** Centrifuge 16,000g 30s. Discard flow-through.
5. 400ul **DNA/RNA Wash Buffer**. Centrifuge 16,000g 2MINS. Discard flow-through.
6. 15ul **DNase/RNase-free Water**. Centrifuge 16,000g 1 min.

QUBIT:
4 samples, 2 standards, 1 extra prep.
Working solution:
7 x 1ul RNA HS Dye = 7 ul RNA HS dye
7 x 199ul RNA HS Buffer = 1393 ul RNA HS Buffer

Standards:
10ul S1 + 190ul working solution
10ul S2 + 190ul working solution

Samples:
1ul of sample + 199ul working solution.

Vortex well 5 sec.

Incubate RT 2 mins.

Run on Qubit with RNA HS, record data