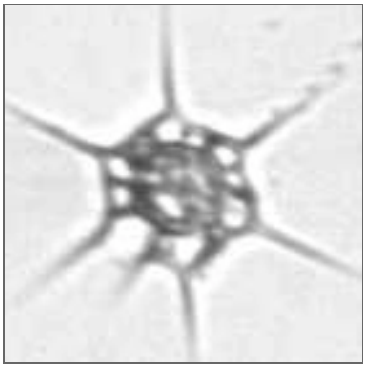

Modified RNeasy Mini Kit protocol for filter extractions - USF edition V.3

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ABSTRACT

RNA extraction protocol for filters - modification of RNeasy mini kit protocol

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https://www.qiagen.com/us/resources/resourcedetail?id=14e7cf6e-521a-4cf7-8cbc-bf9f6fa33e24&lang=en

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Protocol status: Working
We use this protocol and it's working

Created: Feb 12, 2024

Last Modified: Feb 12, 2024

PROTOCOL integer ID: 95111

Prep

1 Wipe all surfaces **inside** the RNA hood with: 20m
1. RNAase ZAP (this is a detergent so do not use much as it can leave a soapy residue)
2. RNAse AWAY (this is a weak base, it can be used liberally but does not evaporate quickly)
3. 70% ethanol (etOH), this can be used liberally and evaporates quickly

use large kimwipes for cleaning surfaces.

2 Turn on the UV light (15 minutes, self-timed) in the RNAhood 15m

(don't open the hood during this time, but just in case the UV light will automatically turn off if you do)

3 Wipe surfaces **surrounding** the RNA hood with:
1. RNAase ZAP (this is a detergent so do not use much as it can leave a soapy residue)
2. RNAse AWAY (this is a weak base, it can be used liberally but does not evaporate quickly)
3. 70% ethanol (etOH), this can be used liberally and evaporates quickly

Pay attention to surfaces you may rest reagents on, lean against, or touch.

4 Preheat the dry bath thermal block to 65ºC.

5 Make sure there are enough DNase aliquots for the day's extractions. Move the aliquots you'll need from the freezer to the fridge so they are thawed when you need them.

!! Each aliquot has 100 µl of DNase. This is exactly enough for 10 samples. !!

6 Set up the tubes you will need for the day by placing them into tube racks in the RNA hood and closing the caps. 10m

Each sample needs:
1x bead-bashing tube (from big red qiagen box, in ziplock bag)
2x RNeasy capped collection tube (from big red qiagen box, in ziplock bag)
1x RNeasy filter column (from big red qiagen box, pink filter columns in individual blister packs)
1x Lo-bind 1.5 mL Eppendorf tubes (in ziplock bag inside RNA hood, extras in light-blue Eppendorf box above hood)

7 Make RLT+BME lysis buffer for the number of samples you have +1 in a fresh 15ml falcon tube: 10m

Per sample / extra:
690 µl of RLT buffer (RLT buffer is in the big red Qiagen box)
10 µl of BME (BME stays in the chemical hood)

for 10 samples + 1 extra:
7590 µl RLT buffer (add this to tube first inside the RNA hood)
110 µl BME (add this to the tube inside the chem hood)

vortex well to mix

8 Take out 1 petri dish for every two samples (10 samples = 5 petri dishes). Put petri dishes in the RNAhood.

9 add 600 µl of RLT+BME lysis buffer to each bead-bashing tube and close the bead tubes tightly.

Close the falcon tube containing the remaining RLT+BME tightly. Keep this tube in the RNA hood waste container until all BME garbage is ready to be disposed of.

10 Make fresh 70% ethanol with molecular grade ethanol (in RNA hood) and nuclease-free water (also in RNA hood) in a fresh 15mL falcon tube. (need up to 600 µl per sample) 5m

for 10 samples:
7000 µl ethanol
3000 µl water

Vortex well, then keep in the RNAhood until ready to use later in the protocol

Cell Lysis 52m

11 Transfer filter tubes for that day's extraction from the –80 freezer into the liquid nitrogen dewar or a cooler with dry ice 5m
(if using dry ice, make sure all samples are in contact with the dry ice)

12 Using clean forceps (RNAse-away before first use, etOH between samples) remove filter from cryovial. 25m

Keeping the filter over a fresh petri-dish half, cut small pieces of filter with clean dissecting scissors (RNase-away before first use, etOH between samples).

Transfer filter pieces to a bead tube with RLT+BME buffer.

Push the filter pieces down into the beads and buffer so that all filter surfaces remain submerged in the buffer.

Label the bead tube and put the remaining sample back on dry ice or directly back into the –80 freezer.

continue for all filters extracting from that day (max 24, recommend 10 or 12 at the beginning -- 10 is good so all samples can go on one bioanalyzer chip)

13 Transfer all bead tubes to the thermal block dry bath (use the 2 mL side to make sure tubes are deep enou 10m
into the thermal block) and incubate at 65ºC for 10 mins 65 °C 00:10:00

14 During the 10-minute heat incubation, label all other tubes and filter columns to match the sample names on the bead tubes.

15 Attach the bead tube adapter to the vortex-genie2 (bead tube adapter is in a small navy-blue qiagen box on the top shelf above the bench) 5m

remove the vortex pad by firmly lifting it upward. Line up the notch in the tube adapter with the notch in the vortex connector and press down firmly until you hear/feel a click.

move all bead tubes to the vortex adapter (caps facing in) so that the adapter is balanced.

Vortex at the highest speed setting for 5 min 00:05:00

16 Transfer bead tubes to the centrifuge rotor and centrifuge bead tubes for 1 min at 4,000 rcf 1m
4000 rcf, 00:01:00

17 Move centrifuge rotor to RNA hood. Using p1000 (BLUE) pipettor set to 1000 µl, remove ALL supernatant from the bead tube and transfer to a matching labeled RNeasy capped collection tube. 5m

Start by removing liquid above the filter and beads, then "wring-out" the filter by pressing it against the tube wall and collecting additional supernatant. Finally, push the pipette tip down into the beads to collect as much remaining supernatant as possible.

It is OK to collect some beads and other detritus. But be careful because too much bead material will get stuck in the pipette tip.

18 Centrifuge RNeasy capped collection tubes with supernatant from bead tubes for 1 min at 4,000 rcf 1m
4000 rcf, 00:01:00

19 Move centifuge rotor back into the RNAhood. Transfer **450 µl** of supernatant to a clean RNeasy capped collection tube with matching label. 5m

It is OK to have different volumes from different samples, but it makes it much easier to pick the minimum volume that you are able to recover and transfer the same volume for all the samples.

20 Add an equal volume (**450 µl**) of freshly-prepared 70% EtOH to each sample. Gently and carefully mix by pipette until the density gradient is no longer visible. 5m

Tranfser 450 of the sample mixed with 70% EtOH to the top of a labeled matching pink spin column.

(Try to do this with one pipette tip for each sample, add 450 ul of ethanol, mix, transfer 450 ul of sample/ethanol to spin column)

RNA purification

21m 40s

21 Transfer spin columns to centrifuge and spin at 10,000 rcf for 20s 10000 rcf, 00:00:20 20s

22 Discard flowthrough:

Transfer the centrifuge rotor back to RNA hood. Pick up each spin column, separate the collection tube, pour the contents into the waste container, and snap the column back together. Transfer the emptied spin columns to the tube rack.

23 Transfer the remaining 450 µl of sample/ethanol to the top of the matching spin column and centrifuge again at 10,000 rcf for 20 s 10000 rcf, 00:00:20 20s

24 Discard flow-through by emptying the bottom collection tube into the waste and then transferring spin columns to the tube rack.

25 If following along with Qiagen protocol cards, switch over to Part 2 for on-column DNase digestion.

26 Add 350 µl of Qiagen buffer **RW1** to the top of each column. Spin at 10,000 rcf for 20 s. 20s
10000 rcf, 00:00:20

27 Discard flow-through by emptying the bottom collection tube into the waste and transferring spin columns to the tube rack.

28 Prepare DNase master mix for each sample:

Each sample needs:
10 µl of DNase stock
70 µl of RDD Buffer (in blue Qiagen DNase kit box in the door of fridge)

to make master mix:
Add 700 µl of RDD buffer to 100 µl thawed DNase aliquot.
Gently pipette up and down to mix.
Quick spin.

29 Add 80 µl of DNase master mix directly to the center of each spin column by pipetting onto the middle of t 15m
spin column (not against walls) just above the spin column membrane. !! Do not touch the spin column membrane !!

Incubate for 15 minutes at room temperature. 00:15:00

30 Add 350 µl of Qiagen buffer **RW1** to the top of each spin column. Centrifuge at 10,000 rcf for 20s 20s
10000 rcf, 00:00:20

31 Discard flow-through by emptying the bottom collection tube into the waste and transferring spin columns to the tube rack.

32 If following along with Qiagen Protocol cards, go back to part one and restart at step 5. 20s

Add 500 µl of Qiagen buffer **RPE** to the top of the spin column. Centrifuge at 100,00 rcf for 20s
10000 rcf, 00:00:20

33 Discard flow-through by emptying the bottom collection tube into the waste and transferring spin columns to the tube rack.

34 Add 500 µl of Qiagen buffer **RPE** to the top of the spin column. Centrifuge at 10000 rcf for **2 minutes.** 2m

10000 rcf, 00:02:00

!! Don't forget to change the time on the centrifuge !!

35 Discard entire collection tube and transfer spin columns to the fresh capless collection tubes (in zip-lock bag in the big red Qiagen box).

36 Centrifuge again at **max speed** (21,300 rcf) for **1 min**. 1m

21300 rcf, 00:01:00

!! Don't forget to change the **time and speed** on the centrifuge !!

37 Discard the capless collection tubes and transfer the spin column to the matching labeled 1.5 mL lo-bind eppendorf tube. Add 30 µl of RNase-free water directly to the center of the spin column membrane. Do not touch the filter membrane. 1m

Centrifuge at **10,000 rcf** for 1 min.

10000 rcf, 00:01:00

!! Don't forget to change the **speed** on the centrifuge !!

38 Pipette the 30 µl flow-through / eluate from each tube back onto the center of its spin column filter membrane. Do not touch the filter membrane. 1m

Centrifuge at 10,000 rcf for 1 minute. 10000 rcf, 00:01:00

39 Discard the spin column.

Put RNA samples in the fridge while preparing the Qubit Reagents.

RNA quantification

40 Follow the protocols for the RNA HS Qubit assay.

Use 1 µl of each sample (and 199 µl of qubit reagent) to quantify.

Quantities can range from 20 ng/µl to 200 ng/µl depending on the time of year that the samples were collected.

Record sample concentrations with metadata and extraction #.

Enter data into the spreadsheet to determine the dilution factor for the bioanalyzer.

Storage + cleanup

41 If samples were successful, aliquot exactly one µl to a PCR tube. Put the PCR tube in a small tube rack box, label it with tape inside the box (the –80 causes the tape glue to fail), and put the box in the –80 freezer.

Put the remaining sample tubes in the tube box for the project that is already in the –80.

Empty all tube/tip waste into the garbage and tie up the garbage bag (this is important to contain the BME fumes).

Wipe down RNA hood and surrounding surfaces with 70% etOH.

Start the UV light in the hood (double-check that no biological samples or reagents (e.g. enzymes, primers) are in the hood!)

Turn off the centrifuge and heating block. Put away all boxes and reagents.

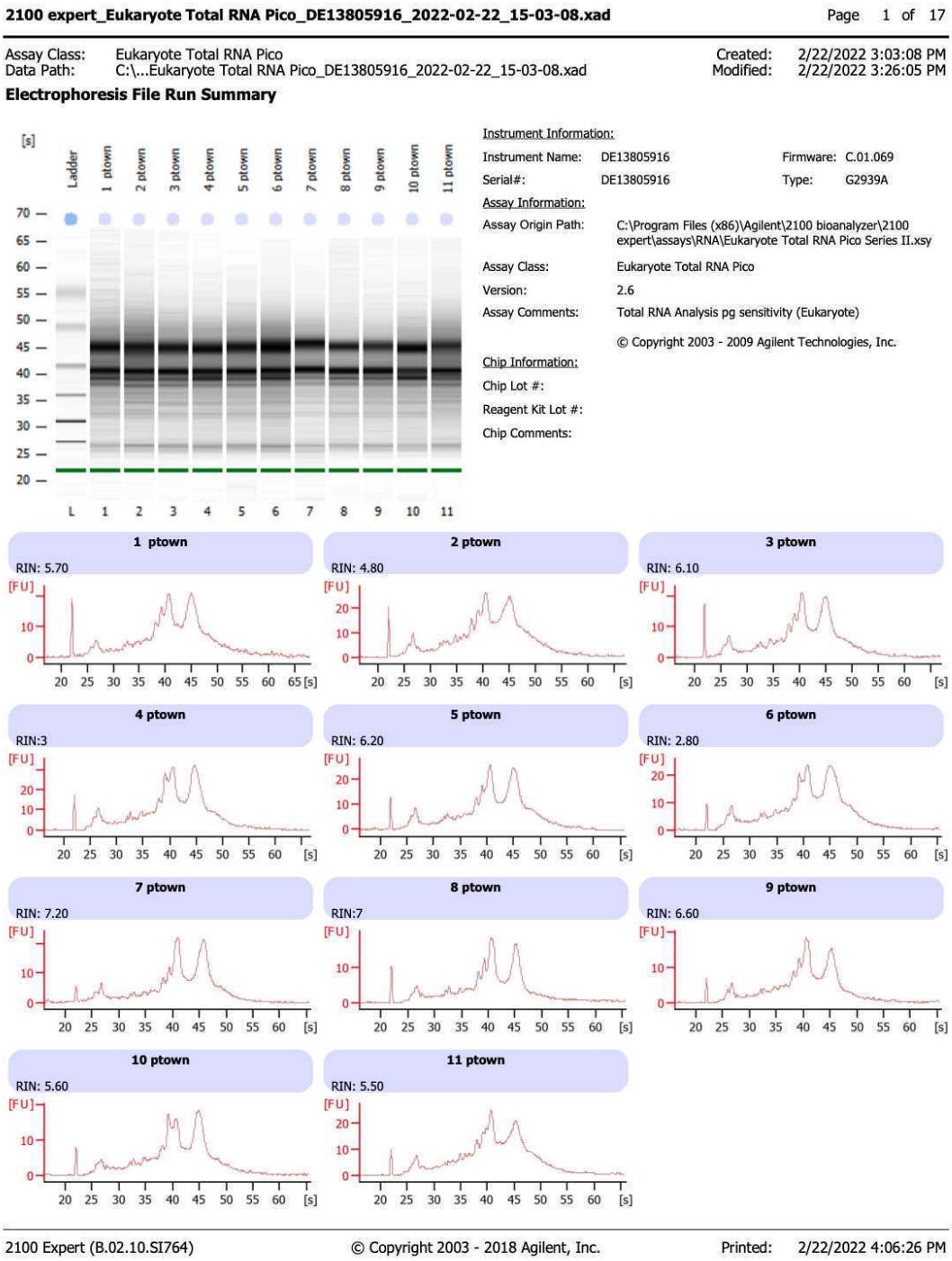
RNA Quality Check

42 Follow protocols for the Pico RNA 6000 Bioanalyzer assay

Dilute all samples to 5 ng / µl before running.

43

Expected result



Expected bioanalyzer results