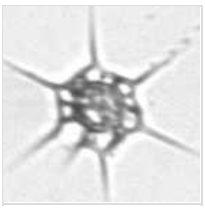


Jul 12, 2022

Modified RNeasy Mini Kit protocol for filter extractions

Margaret M. Brisbin[1]

[1]Okinawa Institute of Science and Technology

dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1

MetaT

Margaret Brisbin
Okinawa Institute of Science and Technology

ABSTRACT

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

DOI

dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1

EXTERNAL LINK

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

PROTOCOL CITATION

Margaret M. Brisbin 2022. Modified RNeasy Mini Kit protocol for filter extractions. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1

CREATED

Jul 12, 2022

LAST MODIFIED

Jul 12, 2022

PROTOCOL INTEGER ID

66553

Prep

1 UV sterilize hood and clean all surfaces with RNAse-away 20m

2 Preheat thermal block to 65ºC

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

4 Take out and label tubes: 10m

Each sample needs:
- 1x bead-bashing tube
- 2x Lo-bind Eppendorf tubes
- 1x RNeasy filter column
- 1x RNeasy capped collection tube

number tubes consecutively from last extraction (if last extraction was #126, start numbering at 127)

5 Make fresh 70% ethanol with molecular grade ethanol and nuclease-free water (need up to 600 µl per sample) 5m

can reuse the 50m falcon tube in the RNA hood

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

Per sample / extra:
- 590 µl of RLT buffer
- 10 µl of BME

vortex to mix

add 600 µl of RLT+BME to each bead-bashing tube

Cell Lysis 52m

7 Transfer filter tubes for that day's extraction into the liquid nitrogen dewar or into a cooler with dry ice 5m

Citation: Margaret M. Brisbin Modified RNeasy Mini Kit protocol for filter extractions
https://dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1

8 Using ethanol-cleaned forceps, remove filter half from cryovial and transfer to a bead tube with RLT+BME buffer. 25m

Get as much of the filter as possible, but do not worry if some small fragments get away.

Push the filter pieces down into the beads and buffer so that the filter breaks apart a bit and all surfaces are covered in buffer.

Write down all information on the cryovial in lab notebook with the corresponding extraction number

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 one bioanalyzer chip)

9 Transfer all bead tubes to the thermal block mixer and incubate at 65ºC for 10 mins **65 °C** **00:10:00** 10m

10 attach the bead tube adapter to the vortex-genie 5m

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

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

11 Centrifuge bead tubes for 1 min at 4,000 rcf **4000 rcf, 00:01:00** 1m

12 transfer bead tubes to RNA hood. Using p1000 pipettor set to 1000 µl remove ALL supernatant from the bead tube and transfer to an eppendorf lo-bind tube. Start by removing liquid above the filter and beads, then "wring-out" thefilter 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. 5m

It is OK to collect beads and other detritus.

13 Centrifuge eppendorf tubes with supernatant from bead tubes for 1 min at 4,000 rcf **4000 rcf, 00:01:00** 1m

Citation: Margaret M. Brisbin Modified RNeasy Mini Kit protocol for filter extractions
https://dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1

14 Move tubes back into the RNAhood. Transfer 500-600 µl of supernatant to clean eppendorf lo-bind tubes. It is OK to have different volumes from different samples, but it makes it much easier to pick the minimum volume you are able to recover and transfer the same volume for all the samples. 5m

15 Add an equal volume (500-600 µl) of freshly-prepared 70% EtOH to each sample. 5m

RNA purification 21m 40s

16 transfer up to 700 µl of cell lysate + 70% EtOH to top of spin column.

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

18 discard flow through. Transfer remaining sample to top of spin column and spin again for 10,000 rcf for 20 s **10000 rcf, 00:00:20** 20s

19 If looking at Qiagen protocol cards, switch over to Part 2 for on-column DNase digestion.

20 Add 350 µl of RW1 to the top of each column. Spin at 10000 rcf for 20 s. Discard flow-through. **10000 rcf, 00:00:20** 20s

21 Prepare DNase mastermix for each sample + 1 extra:

10 µl of DNase stock
70 µl of RDD Buffer (in DNase kits on door of older fridge)

22 Add 80 µl of DNase master mix directly to the center of each spin column. Incubate for 15 minutes at room temperature. **00:15:00** 15m

23 Add 350 µl of RW1 to the top of each spin column. Centrifuge at 10000 rcf for 20s and discard flow-through. **10000 rcf, 00:00:20** 20s

Citation: Margaret M. Brisbin Modified RNeasy Mini Kit protocol for filter extractions
https://dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1

24 If looking at Qiagen Protocol, go back to part one and start at step 5. 20s

Add 500 µl of RPE to spin column. Centrifuge at 10000 rcf for 20s and discard flow-through.
10000 rcf, 00:00:20

25 Add 500 µl of RPE to spin column. Centrifuge at 10000 rcf for **2 minutes** and discard flow-through. 2m
10000 rcf, 00:02:00

26 Transfer spin columns to fresh collection tubes (without cap) and centrifuge at max speed (21300 rcf) for 1 min. 1m
21300 rcf, 00:01:00

27 Transfer spin column to labeled collection tube (with cap). Add 30 µl of RNase-free water directly to the center of the spin column. Centrifuge at 10000 rcf for 1 min. 1m
10000 rcf, 00:01:00

28 Pipette the 30 µl flow-through / eluate from each tube back onto the center of its spin column. Centrifuge at 10000 rcf for 1 minute. **10000 rcf, 00:01:00** 1m

29 Discard the spin column.

Aliquot 2.1 µl of each sample into a PCR tube. Put the rest of the eluted RNA into the -80ºC freezer.

RNA quantification

30 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 #.

RNA Quality Check

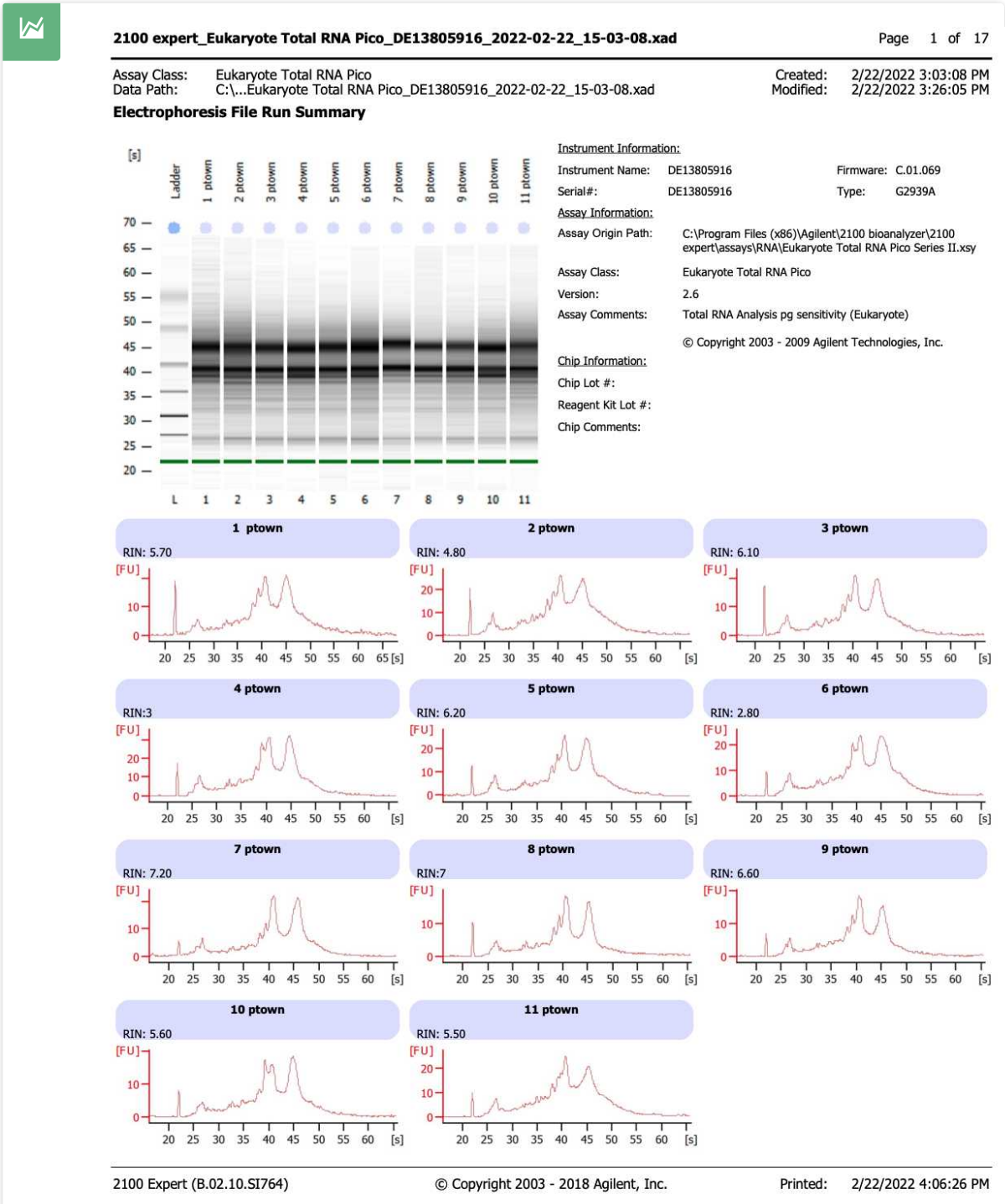
31 Follow protocols for the Pico RNA 6000 Bioanalyzer assay

Citation: Margaret M. Brisbin Modified RNeasy Mini Kit protocol for filter extractions
https://dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1

(reagents are in the "old fridge" and chips are in the drawer in the Apprill Lab (Fye) where the Bioanalyzer lives.

Dilute all samples to 5 ng / µl before running.

32



Expected bioanalyzer results

Citation: Margaret M. Brisbin Modified RNeasy Mini Kit protocol for filter extractions
https://dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1

Citation: Margaret M. Brisbin Modified RNeasy Mini Kit protocol for filter extractions
https://dx.doi.org/10.17504/protocols.io.8epv59qd6g1b/v1