Ocean Ridge Biosciences
The Brightest Spots in the Business

Part C. DNase Digestion and clean-up [microRNA Profiling]

Adjust Dnase digestion volume to 50 ul if you have less then 15 ug of LMW RNA.

1 Dilute 30 ug of LMW RNA in a volume of 84 ul of Qiagen RNase-free water.
2 Add 10ul of Invitrogen 10X Dnase Digestion Buffer
3 Add 2ul of Rnase Block (Rnase Inhibitor) -mix by gentle vortexing
4 Add 4ul of Ambion Rnase-free Dnase I
5 Mix by inversion or flicking and spin briefly (do not vortex)
6 Incubate at 37C for 30 min
7 Add 4.0ul of 0.5 M EDTA pH 8 , vortex and spin down
8 Adjust volume of Dnased-RNA to 100 ul with Qiagen water if necessary
9 Combine DNased digest RNA with 350 ul of buffer RLT and mix by gentle vortexing (don't centrifuge)
10 Combine RNA/RLT mixture with 1500 ul of 100% ethanol
11 Mix by gentle vortexing (don't centrifuge)
12 Apply the sample to an Rneasy MinElute spin column in a 2ml collection tube.
13 Close the tube gently and centrifuge for 1 min at >10,000 g.
(Repeat till all the sample is loaded on to the column.)
14 Transfer the spin column into a new 2ml collection tube.
15 Pipet 500 ul buffer RPE onto the spin column
(Apply around rim of column to make sure the area is washed)
16 Close the tube gently, and centrifuge for 1 min at >10,000 g to wash the column.
17 Discard the flow-through and reuse collection tube
18 Pipette 500 ul of 80% ethanol to the Rneasy minelute spin column.
19 Close the tube gently, and centrifuge for 1 min at >10,000 g
20 Discard flow-through and collection tube.
21 Transfer the Rneasy minelute spin column into a new 2 ml collection tube.
22 Close the cap of the spin column, and centrifuge in a microcentrifuge at 16,000 g or max speed for 3 min.
23 Discard flow-through and collection tube.
24 Repeat steps 21-23 one more times (important to remove any trace of ethanol)
25 To elute, transfer the spin column to a new 2 ml capless collection tube.
26 Pipette 14 ul Rnase-free water directly onto the center of the silica-gel membrane.
27 Close the tube gently, incubate 1 minute on bench and centrifuge for 1 min at max speed to elute
28 Measure the sample concentration using Ribogreen based fluorescence assay. (optional)
29 Freeze at -70C or ship to ORB on dry ice.