cDNA Synthesis and Amplification for Microarrays

This procedure was adapted from protocols for TRIzol (Invitrogen) and the WTA2 TransPlex Whole Transcriptome Amplification kit (Sigma-Aldrich).

Materials

Sigma WTA2 kit, 100 reactions
QIAquick PCR cleanup kit
Agilent DNA 1000 kit for bioanalyzer
Linear acrylamide (5 mg/mL), Ambion
RNase-free chloroform
RNase-free 75% ethanol
RNase-free isopropanol
RNase-free microcentrifuge tubes and pipette tips
Siliconized 1.5 mL microcentrifuge tubes

RNA isolation

1) To 1 mL of thawed RNA-containing TRIzol solution, add 5 µL linear acrylamide and mix tube.
2) Add 200 µL of chloroform and shake vigorously for 20 seconds. Let sit 2-3 min. room temperature
3) Centrifuge at 12,000 x g for 15 min in at 4 °C.
4) Transfer aqueous phase to a new siliconized 1.5 mL microcentrifuge tube with a P200 and add 0.5 mL of isopropanol. Mix by inverting the tube a few times, and incubate the solution for 10 min at room temperature. Invert the tube several times and centrifuge at 12,000 x g for 15 min at 4 °C.
5) Using a P1000, carefully remove supernatant. Watch for the pellet, which will be slightly yellow in color at this step.
6) Add 1 mL of 75% ethanol to the pellet, which should make it appear white. Invert the tube several times, and then centrifuge at 7,500 x g for 5 min at 4 °C.
7) Carefully remove as much supernatant as possible with a P1000. Briefly centrifuge the tube and remove the remaining supernatant with a P10. Air dry briefly but do not allow the pellet to dry completely. Resuspend the pellet in 2.8 µL of RNase-free H_2O.
8) Transfer this solution to an RNase-free PCR tube.

Reverse Transcriptase reaction

1) Thaw the following WTA2 kit components:
    a) Library synthesis buffer
    b) Library synthesis solution
    c) Library synthesis enzyme
    d) Nuclease-free water.

   Mix library synthesis buffer and library synthesis solutions and dissolve any precipitates by briefly heating to 37 °C with thorough mixing.

2) Add 0.5 µL of library synthesis solution to the 2.8 µL solution of RNA.
3) Prime the RNA in a thermocycler: 70 °C for 5 min then hold 18 °C. While priming, make library synthesis mix (volumes/reaction):
    a) 0.5 µL library synthesis buffer.
    b) 0.78 µL water.
    c) 0.5 µL library synthesis enzyme.
4) Add 1.78 µL of the library synthesis mix to the primed RNA at 18 °C. There should be 5.08 µL of total solution per tube.
5) Incubate this mixture in the thermocycler:
    a) 18 °C for 10 min
    b) 25 °C for 10 min
    c) 37 °C for 30 min
    d) 42 °C for 10 min
    e) 70 °C for 20 min
    f) Hold 4 °C

Amplification reaction

1) Thaw the amplification mix and 10 mM dNTP mix.
    a) Prepare the following master mix (given volumes are for one amplification reaction): 63.5 µL RNase free water
    b) 8 µL amplifcation mix
    c) 1.6 µL 10 mM dNTP mix
    d) 0.8 µL1:1000 SYBR green (optional for qPCR, if omitted use equal volume of water)
    e) 0.8 µL amplification enzyme
3) Add 70 µL of master mix to each cDNA sample (will have 75.1 µL total volume)
4) Incubate in thermal cycler using the following parameters:
    a) 94 °C for 2 min
    b) 22 cycles of 94 °C for 30 sec, 70 °C for 5 min
    c) If monitoring by qPCR, proceed 2-3 cycles after plateau.

Purify using QIAquick PCR cleanup kit

1) Elute in 30 µL EB according to the manufacturer's instructions.
2) Quantify DNA concentrations on a Nanodrop spectrophotometer
   Should be >200 ng/µL (> 10 µg)
3) Run sample on Agilent Bioanalyzer using DNA 1000 kit (1.0 µL)
   Should have an average cDNA length of approximately 400 bp