

Microarray Resource

Illumina® TotalPrep™ cDNA Cleanup Protocol

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A. Required reagents:

DEPC-treated water

Ethanol (ACS grade or equivalent proof)

*cDNA Binding Buffer

*Wash Buffer

B. Equipment and supplies:

Microcentrifuge with 1.5 ml rotor

Micropipettors

Aerosol-barrier tips

Vortex mixer

Powder-free gloves

Air incubator at 55°C

1.5 ml RNase-free microcentrifuge tubes

*cDNA Filter Cartridges

*1.5 ml Collection tubes

*1.5 ml cDNA Elution Tubes

*Reagents supplied with Illumina® TotalPrep™ RNA Amplification Kit (Ambion)

I. Illumina TotalPrep cDNA Cleanup

* *All centrifugations in this procedure should be done at 10,000 x g (~10,000 rpm) at room temperature.*

* Before beginning the cDNA purification, preheat Nuclease-free Water to 50-55°C for at least 10 min.

*Check the cDNA Binder Buffer for precipitation before use. If a precipitate is visible,

redissolve it by warming the solution for 37°C for up to 10 min and vortexing vigorously. Cool to room temperature before use.

*Before using the Wash Buffer for first time, add 24 ml of 100% Ethanol.

1. Transfer entire sample from the 0.2 microcentrifuge tube to a 1.5 ml RNase-free microcentrifuge tube.

2. Add 250 ul of cDNA Binding Buffer to each sample, and mix thoroughly by pipetting up and down 2-3 times, then flicking the tube 3-4 times. Spin briefly.

*Check that the cDNA Filter Cartridge is firmly seated in its wash tube.

3. Pipet the entire cDNA sample/cDNA Binding Buffer onto the center of the cDNA Filter Cartridge.

4. Centrifuge for 1 min, or until the mixture is through the filter.

5. Discard flow-through and replace cDNA Filter Cartridge in the wash tube.

*Make sure that the ethanol has been added to the bottle of Wash Buffer before using.

6. Apply 500 ul Wash Buffer to each cDNA Filter Cartridge.

7. Centrifuge for 1 min, or until all the Wash Buffer is through the filter.

8. Discard flow-through and spin the cDNA Filter Cartridge for an additional minute to remove trace amounts of Wash Buffer.

9. Transfer cDNA Filter Cartridge to a cDNA Elution Tube.

*It is important that the Nuclease-free water is at 50-55°C for the cDNA Elution. Colder water will be less efficient at eluting the cDNA, and using hotter water ($\geq 58^\circ\text{C}$) may result in reduced cRNA yield.

10. Apply 10 ul of the preheated Nuclease-free water to the center of the filter in the cDNA Filter Cartridge.

11. Incubate at room temperature for 2 min and then centrifuge for ~1.5 min, or until all the Nuclease-free Water is through the filter.

12. Apply a second aliquot of 9 ul of preheated Nuclease-free water and centrifuge for 2 min. The double stranded cDNA will now be in the elute (~17.5 ul).

*Store purified cDNA at -20°C or proceed to Illumina[®] TotalPrep[™] IVT to synthesize cRNA.