RNA extraction and sample preparation for Affymetrix Gene 1.0 ST arrays

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1. For many of the cell types, multiple sorts were pooled to collect enough cells. RNA was extracted from over 100,000 cells to as few as 10,000 cells.

2. RNA was prepared using the RNeasy Micro kit (Qiagen #74004) following manufacturer instructions for "Cells," with the following exceptions:

> **a.** The protocol was started at step 2 (disruption with RLT). β-ME was not added. To disrupt the cells, they were pipetted with a P1000, vortexed for 5 minutes, and centrifuged for 2 seconds. When multiple tubes of cells were pooled, the first was disrupted, the RLT from the first tube was moved to the second one, the cells were disrupted in the second tube following the same method, and this was repeated until all the cells were pooled.
> **b.** Step 3 (homogenization) was skipped.
> **c.** In step 10, samples were washed 3x with RPE, rather than one.

3. Samples were prepared for the Affymetrix Gene 1.0 ST arrays using the Nugen WT-Ovation Pico RNA Amplification System (3300), WT-Ovation Exon Module (2000), and the Encore Biotin Module (4200), following manufacturer instructions. For purification following the Pico and Exon kits, the Qiagen QIAquick PCR Purification Kit (28104) was used following the instructions provided by Nugen.

4. Fragmented and labeled product was submitted to the Duke Institute for Genome Sciences and Policy Microarray Facility for hybridization.

Citations:

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