

## AMC 3' IVT EXPRESSION ASSAY METHODS

June 2004-September 2007

Array feature size\* = 11  $\mu\text{m}$   
AMC Standard Labeling Assay

Microarray assays were performed in the Affymetrix Microarray Core, a unit of the OHSU Gene Microarray Shared Resource using procedures adapted from the Affymetrix GeneChip Expression Analysis Technical Manual, rev.5

**Target labeling.** Messenger RNA is amplified and labeled from 2  $\mu\text{g}$  of total RNA in two steps. In the first step, mRNA is converted to double-stranded cDNA using Superscript Reverse Transcriptase (Invitrogen) and an oligo-dT primer linked to a T7 RNA polymerase binding site sequence (Affymetrix). In the second step, amplified and labeled cRNA (the target) is produced in an in vitro transcription reaction using T7 RNA polymerase and biotin-CTP (Affymetrix.). Following removal of free nucleotides, target yield is measured by UV<sub>260</sub> absorbance.

**Target quality assessment.** Approximately 200 ng of each sample cRNA target along with a control cRNA target is analyzed on the RNA 6000 LabChip using the 2100 Bioanalyzer (Agilent Technologies). The target quality is determined based on cRNA yield and size distribution produced from the in vitro synthesis reaction. Samples that fail quality control are discarded or relabeled.

**Array hybridization and processing.** Labeled target is fragmented at 95° C in the presence of high magnesium concentration. The fragmented material is combined with biotinylated hybridization control oligomer and biotinylated control cRNAs for BioB, BioC, BioD and CreX (Affymetrix) in hybridization buffer. Ten  $\mu\text{g}$  of target is hybridized with the GeneChip (*insert array name*) array (Affymetrix) overnight, followed by washing, staining with streptavidin-phycoerythrin (Molecular Probes), signal amplification with biotinylated anti-streptavidin antibody (Vector Labs), and a final staining step on the Fluidics Station 450 (Affymetrix). The distribution of fluorescent material on the processed array is determined using the Affymetrix 3000 GeneArray laser scanner with the 7G upgrade. Image inspection is performed manually immediately following each scan. All array scanning and data processing on the Affymetrix system is performed with GeneChip Operating System (GCOS) software.

**Data analysis.** The probe-level Cel file is processed with Affymetrix Microarray Suite, version 5.0 (MAS 5.0) software. The GeneChip expression arrays contain control probe sets for both spiked and endogenous RNA transcripts (e.g., BioB, BioC, BioD, CreX and species-specific actin and GAPDH). Following image processing and absolute analysis of the array pattern with MAS, six values are examined to assess overall assay performance: background, noise, average Signal, % Present, ratio of Signal values for probe sets representing the 5' and 3' ends of actin and GAPDH transcripts, and total Signal for probe sets for BioB, BioC, BioD and CreX. Assays demonstrating poor or marginal performance are flagged. Using MAS 5.0, array data is globally scaled to a uniform, average target intensity for all assays prior to further analysis.

\* For protocol modification for the Human Genome Focus Array and other GeneChip arrays with feature sizes greater than 11  $\mu\text{m}$ , please contact the AMC.