The MIAME Checklist for Datasets A and B*

[* The experimental details described in the MIAME checklist are the same for Dataset A and B; the two datasets differ only in the way the data files were managed. All Supplementary Information, including materials, methods and primary data, can be accessed at http://www.niehs.nih.gov/research/atniehs/core/microarrays/trc/.]

Experiment Design:

- **Type of experiment**: The experiment was designed to determine the reproducibility of gene transcript level measurements between multiple laboratories (n=7) for a single microarray platform called the Standard Spotted Array. Each laboratory was provided with large aliquots from two different mouse standard RNA samples: a liver RNA sample and a five-tissue pooled sample (liver, kidney, lung, brain and spleen in equal amounts) that were prepared in one of the consortium laboratories (see URL for Supplementary Methods, Standard Protocols, Tissue Extraction and Standard RNA Sample Preparation). Each laboratory was provided *Standard Spotted Arrays* that were made in Laboratory 1 by spotting 70-mer oligos (Operon) representing 18,000 mouse genes onto poly-l-lysine coated glass slides.
- **Experimental factors**: Using the standard RNA samples allowed us to focus on variation in the technical and analytical approaches to microarray experimentation, such labeling and hybridization protocols and image analysis, rather than the biological variation.
- **The number of hybridizations performed in the experiment.** Each of the 7 laboratories carried out 8 hybridizations; four that co-hybridized liver RNA versus liver RNA (LvsL) and four that co-hybridized liver RNA versus pooled RNA (LvsP); each set of four hybridizations had two dye-swapped samples (Cy3 versus Cy5).
- **The type of reference used for the hybridizations**: See answer to "Type of experiment" (above).
- **Hybridization design**: See answer to "Type of experiment" and "The number of hybridizations" (above).
- **Quality control steps taken**: See answer to "The number of hybridizations" (above).
- **URL of any supplemental websites or database accession numbers**: Supplemental information, including materials, methods and primary data can be accessed at http://www.niehs.nih.gov/research/atniehs/core/microarrays/trc/.

Samples used, extract preparation and labeling:

- **The origin of the biological sample:** For tissue extraction, twenty-five C57 black male mice (10-11 weeks) were purchased from Taconic Farms and housed five in a cage in pathogen-free rooms determined by sentinel animal survey. They acclimated for 7-10 days until they reached 12 weeks of age. They were housed in shoebox cages on sawdust and fed NTP 2000 chow
- **;** RNA isolation from tissue was performed by one laboratory (see URL for Supplementary Methods, Standard Protocols, Tissue Extraction and Standard RNA Sample Preparation).
- **Manipulation of biological samples and protocols used**: See URL for Supplementary Methods, Standard Protocols, Tissue Extraction and Standard RNA Sample Preparation.
- **Protocol for preparing the hybridization extract**: RNA isolation from tissue was performed by one laboratory and distributed to the other six participating

laboratories (see URL for Supplementary Methods, Standard Protocols, Tissue Extraction and Standard RNA Sample Preparation).

- **Labeling protocol(s)**. The individual laboratories were provided the standard RNA samples and used a variety of resident (in-house) protocols for RNA labeling (see URL for Supplementary Methods, Supplementary Table 4 and Resident Protocols for Laboratories 1-7).
- **External controls (spikes)**. cRNA control *in vitro* Arabidopsis transcripts (70mers) provided by The Institute for Genomics Research (http:pga.tigr.org) were added to each of the standard RNA samples as a means of assessing experimental variability for individual RNA analyses (labeling and hybridizations). A total of 10 Arabidopsis genes were chosen for this spike in design, with fixed concentrations of each transcript in two distinct sets, A3 and A5 (see URL for Supplementary Methods, Standard Protocols, Quality Control Genes).

Hybridization procedures and parameters:

- **The protocol and conditions used during hybridization, blocking and washing**. The individual laboratories were provided the standard RNA samples and used a variety of resident (in-house) protocols for hybridizations to the Standard Spotted Array (see URL for Supplementary Methods, Supplementary Table 4 and Resident Protocols for Laboratories 1-7).

Measurement data and specifications:

- **The quantitations based on the images**: The individual laboratories used a variety of resident (in-house) protocols for scanning and image analysis (see URL for Supplementary Methods, Supplementary Table 5 and Resident Protocols for Laboratories 1-7). Gene expression florescence intensities (for red and green channels) were measured for all spots represented on the Standard Spotted Array.
- **The set of quantitations from several arrays upon which the authors base their conclusions.**
  - **Type of scanning hardware and software used**: The individual laboratories used a variety of resident (in-house) equipment for scanning the Standard Spotted Arrays to generate Dataset A and B (see URL for Supplementary Methods, Supplementary Table 5 and Resident Protocols for Laboratories 1-7).
  - **Type of image analysis software used**: The laboratories used a variety of resident (in-house) image analysis software packages for analyzing the raw images for the Standard Spotted Array to generate Dataset A and B (see URL for Supplementary Methods, Supplementary Table 5 and Resident Protocols for Laboratory 1-7).
  - **A description of the measurements produced by the image-analysis software and a description of which measurements were used in the analysis**. The laboratories used a variety of resident (in-house) protocols for scanning and image analysis of the Standard Spotted Array to generate Dataset A and B. The measurements used in the analyses are summarized below for the various image analysis software packages.

Software	Foreground Intensity	Background Intensity
GenePix	F532 Mean, F635 Mean	B532 Median, B635Median
MolecularWare	Spot Mean Intensity w595, Spot Mean Intensity w685	Background Median Intensity w595, Background Median Intensity w685
ArraySuite	SR_Mean, SG_Mean	SR_bkMean, SG_bkMean
QuantArray	ch1 intensity, ch2 intensity	Ch1 background, ch2 background
SpotOn	Rmean, Gmean	bgRmed, bgGmed

- **The complete output of the image analysis *before* data selection and transformation**. See URL for Supplementary Information, Download the Primary Data, Raw Data for Dataset B.
- **Data selection and transformation procedures**. Median Pearson correlation coefficients were calculated between background corrected log signal intensity values for all nucleotide sequences represented on the Standard Spotted Array for co-hybridizations of standard liver RNA versus standard liver RNA (LvsL) samples and standard liver RNA versus standard pooled RNA (LvsP) samples (see Methods).
- **Final gene expression data table(s) used by the authors to make their conclusions *after* data selection and transformation**. See URL for Supplementary Information, Download the Primary Data, Raw Data for Dataset B.

Array Design:

- **General array design.** The Standard Spotted Array was manufactured by Laboratory 1 using sequence-verified mouse oligonucleotides (70mers) representing 18K unique mouse gene elements purchased from Operon and spotted onto poly-l-lysine coated slides using a GeneMachine OmniGrid Arrayer**.**
- **For each feature (spot) on the array, its location on the array and the ID of its respective reporter**. Microarray slides are printed on a Gene Machine OmniGrid Arrayer and Axon GenePix 4000B scanner, and the format dictated from these two files refers to the location of the spots.
- **For each reporter, its type.** Sequence-verified mouse oligonucleotides (70mers) representing 18K unique mouse gene elements were purchased from Operon. Arabidopsis 70mer probes corresponding to the cRNA Arabidopsis control *in vitro* transcripts (spike ins) were randomly spotted onto the slides. Sequence information can be obtained from The Institute for Genomic Research (http:pga.tigr.org).
- **For commercial arrays**. Not applicable.
- **For non-commercial arrays, the following details should be provided**:
- **The source of the reporter molecules**: Sequence-verified mouse oligonucleotides (70mers) representing 18K unique mouse gene elements were purchased from Operon. Arabidopsis 70mer probes corresponding to the cRNA Arabidopsis control in vitro transcripts were provided by TIGR (http:pga.tigr.org). Stratagene Spot report was used.
- **The method of reporter preparation**. To be provided, if accepted.
- **The spotting protocols used**. The Gene Machine OmniGrid Arrayer was used to spot the slides**.**
- **Any additional treatment performed prior to hybridization**.