The MIAME Checklist for Dataset D*

[*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 Commercial 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, 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 Commercial Arrays purchased from Agilent (ToxArray).
- **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 material, 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 male C57 black 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 used a standardized protocol for producing labeled cRNA from the standard RNA samples (see URL for Supplementary Methods, Standard Protocols, Production of Labeled cRNA from Total RNA).
- **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 with a standard protocol for hybridization of labeled RNA to the Standard Commercial Array (see URL for Supplementary Methods, Standard Protocols, Hybridization and Washing of Standard Commercial Arrays).

Measurement data and specifications:

- **The quantitations based on the images**: The individual laboratories used a standardized scanner (Agilent G2565AA Scanner) for producing images from the Standard Commercial Arrays. The laboratories used a standardized image analysis software package (Agilent Feature Extraction and Image Analysis software v A.6.1.1) and set of feature extraction parameters for analyzing the raw images to generate Dataset D. Gene expression florescence intensities (of red and green channels) were measured for all spots represented on the Standard Commercial Array (see URL for Supplementary Methods, Standard Protocols, Scanning with the Agilent G2565AA Scanner and Image Analysis for Standard Commercial Arrays).
- **The set of quantitations from several arrays upon which the authors base their conclusions.**
    - **Type of scanning hardware and software used**. All laboratories used the Agilent G2565AA Scanner (see URL for Supplementary Methods, Standard Protocols, Scanning with the Agilent G2565AA Scanner).
    - **Type of image analysis software used**. The laboratories used the Agilent Feature Extraction and Image Analysis software (v A.6.1.1) and standardized set of feature extraction parameters for analyzing the raw images from the Standard Commercial Arrays to generate Dataset D. (see URL for Supplementary Methods, Standard Protocols, Image Analysis for Standard Commercial Arrays).
    - **A description of the measurements produced by the image-analysis software and a description of which measurements were used in the analysis**. The measurements produced using the Agilent Feature Extraction and Image Analysis software (v A.6.1.1) and used in the analysis include foreground mean, foreground median, background mean and background median (for both red and green channels).
    - **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 D.
    - **Data selection and transformation procedures**. Gene expression ratios were calculated for the quadruplicate arrays (LvsP) in each laboratory using Lowess normalization without background subtraction.

Pearson correlation coefficients comparing the average expression ratios across laboratories were calculated for each gene on the platform.

- **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 D.

Array Design:

- **General array design.** The Standard Commercial Array (Agilent ToxArray) was developed through collaboration between the Toxicogenomics Research Consortium and Agilent. The array includes 60mer probes for over 20,000 mouse genes and transcripts. The array was manufactured with Agilent's SurePrint fabrication platform. Please refer to the Agilent website (www.agilent.com) for additional information about the microarray platform.
- **For each feature (spot) on the array, its location on the array and the ID of its respective reporter**.
- **For each reporter, its type.**
- **For commercial arrays**
- **For non-commercial arrays, the following details should be provided**: Not Applicable
  - **The source of the reporter molecules**:
  - **The method of reporter preparation**.
  - **The spotting protocols used**.
  - **Any additional treatment performed prior to hybridization**.