Assessment of Transcriptomic Variability and Tissue Effects in the Normal Mouse

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We approach the normal mouse data of Pritchard et al. [3] using a linear mixed model, emphasizing the following advantages:

- direct modeling of the log2 intensity measurements, not ratios, and accommodation of all known sources of variability across all of the microarrays;
- the flexibility to specify both fixed and random effects, the latter enabling inferences on the former to be extended to general populations of interest;
- a comprehensive analysis framework for complex experimental designs and unbalanced data;
- extensive output statistics suitable for dynamic visual display, including those useful for quality control, inference, and classification.

The experimental design for these data is a popular one, in which half of the experimental intensities arise from a common reference sample. While this design has been shown to be quite inefficient compared with more classical incomplete block or split plot designs Kerr and Churchill [2], we are analyzing the data after the fact and so will attempt make the best of it.
A typical analysis proceeds by forming ratios within each spot and then inputting these ratios to various methods. The ANOVA-based results from Pritchard et al. [3] follow this line, and we greatly welcome more widespread use of classical ANOVA as well as more emphasis on variability assessment.

For this paper, we are able to gain some more efficiency by modeling the log2 intensity measurements directly; that is, by not forming ratios at all. The intuition here is that one sacrifices some valuable information in forming ratios. In particular, the fact that the same reference sample is used on each array can be exploited ipso facto to obtain a highly precise estimate of the reference sample expression before any relative comparisons are made. We accomplish this in our modeling context by assigning a unique treatment identifier to the reference sample, in addition to the three other identifiers for kidney, liver, and testis.