Development of Standards and Methods for Measuring Gene Expression Assay Performance

NIST scientists are developing standards that can be used to assess microarray performance quantitatively, with a focus on "spike-in," or externally added controls. NIST is also developing methods to use them to assess performance of DNA microarrays in quantitation of mRNA for gene expression determination. More reliable DNA microarray-based measurements will enable better gene expression determinations to be performed and new innovations in medical diagnostics.

M. Salit, J. McDaniel, M. Satterfield (Div. 831)

DNA Microarrays are being used to measure gene activity – Gene Expression – across whole genomes, inexpensively and rapidly. The genes being expressed in a cell contribute strongly to the nature of the cell, and their measurement can be used to indicate cell status. Microarray results have been shown to be significantly variable across different platforms, across different laboratories, and even within laboratories in single studies. While this reported variability has presented a major barrier to more broad adoption of the technology, the inability to routinely quantitatively assess performance impedes refinement of the technology, its method of application, and limits confidence in results.

NIST is developing the capabilities for routine quantitative assessment of DNA microarray performance. This is expected to remove impediments to the refinement of this important technology, thus enabling more confidence in results.

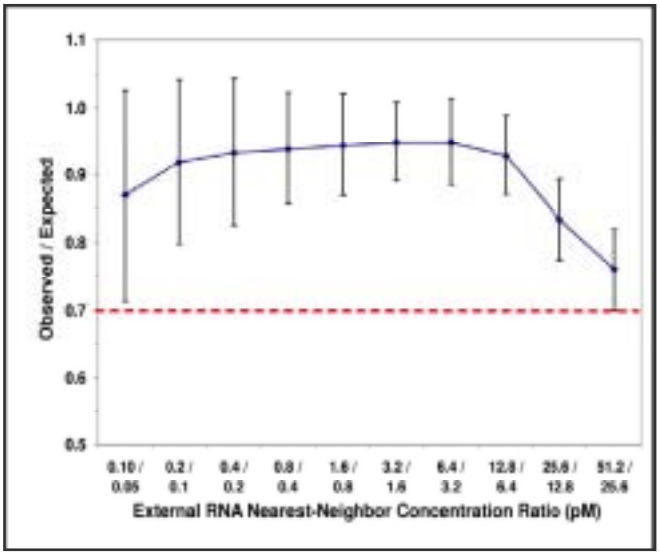
External RNA Controls Consortium Status

NIST has been hosting an industry-led consortium, the External RNA Control Consortium (ERCC), in an effort to develop standard approaches to assess gene expression assay performance. This group, now with more than 90 self-declared member organizations, with participation by more than 150 individuals from those organizations, is working together to develop "spike-in," external controls that can be added to samples to assess assay performance.

Along with these spike-in controls, working under the auspices of the Clinical Laboratory Standards Institute (CLSI), the ERCC has developed and published a guidance document, "*Use of External RNA Controls in Gene Expression Assays; Approved Guideline.*" This document provides protocols for preparation and use of the spike-ins, along with discussion of the metrics that should be determined to assess performance.

A DNA sequence library has been developed to make these controls from, with 176 different sequences. These sequences are unique with respect to known genomes, and to each other. Most of these sequences were deposited in the library by ERCC members, with NIST developing 48 synthetic sequences for the library.

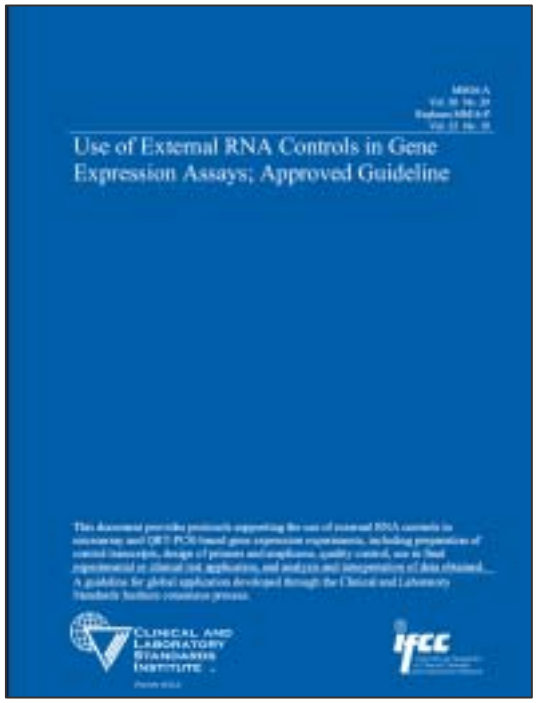
RNA is being made from these sequences, and will be distributed to 12 microarray and quantitative PCR laboratories for evaluation in a collaborative study. The design of the collaborative study is described in a paper, "*Proposed methods for testing and selecting the ERCC external RNA controls*" (possible figure for the external RNA testing program shown below.



How well do spike-in controls predict performance of genes under study?

To understand further the degree to which observed spike-in performance can be used to establish performance of the genes under study, the NIST team, including researchers from both the Biochemical Science and Analytical Chemistry Divisions, are collaborating with researchers from Imperial College (IC) in London. Together, we are evaluating a large database of experiments from IC that used a common set of spike-in controls, and establishing that the response from these controls varied in a manner similar to

that observed of the non-control genes. Along with the spike-in controls, the variability of so-called endogenous controls is being evaluated, laying the groundwork for further standards and method development.



Publications:

- ***Proposed methods for testing and selecting the ERCC external RNA controls***
  BMC Genomics 2005, **6:**150

- ***"Use of External RNA Controls in Gene Expression Assays; Approved Guidelines"***
  http://www.clsi.org/source/orders/index.cfm?section=Shop&task=3&CATEGORY=MM&PRODUCT_TYPE=SALES&SKU=MM16AE

The figure below shows the variability as a function of signal for the bulk of the genes in an array experiment as a density cloud, the endogenous controls as 'o' or 'c', and the spike-ins as 'P' or 'H.