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Dockets Management Branch  
Division of Management Systems and Policy  
Office of Human Resources and Management Services  
Food and Drug Administration  
5630 Fishers Lane, Room 1061, (HFA-305)  
Rockville, MD 20852

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Dear Sir/Madam:

Professor Jane Chang and I would like to submit the attached comments on *Multiplex Tests for Heritable DNA Markers, Mutations and Expression Patterns; Draft Guidance for Industry and FDA Reviewers*.

Sincerely,

Jason C. Hsu  
Professor

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**Comments on  
Multiplex Tests for Heritable DNA Markers, Mutations and Expression Patterns;  
Draft Guidance for Industry and FDA Reviewers**

by

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and

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To provide as much as of a safeguard to the public as possible in the use of microarrays for monitoring gene expressions, including their potential off-label use, we believe the design and analysis of experiments executed to validate the performance of microarrays should follow the statistical principles of Randomization, Replication, and Blocking. Our rationale is as follows.

### **Randomization**

Randomization is the basis for valid statistical inference. It removes systematic bias due to unintended factors. Randomization helps to ensure unbiased estimation of quantities of interest. Unbiased estimation is an important safeguard for the public.

Variations observed in gene expressions may be attributable to differences in the groups being compared, in the samples, or differences in nuisance factors such as spot, array, and dye (if more than one dye is used). Random assignment of replicate samples to levels of factors (e.g., array) prevents systematic bias from extraneous factors. Randomization of the spotting of genes on a microarray may help to avoid systematic spot bias in the statistical analysis of gene expressions.

### **Replication**

Replication allows the estimation of experimental error, necessary in the calculation of measurement precision. We agree that measures of precision are best presented as confidence intervals. Control of precision of estimates, in terms of widths of confidence intervals, can be achieved by increasing the number of replications.

Replication can be accomplished by spotting each gene more than once on a microarray, and by having replicate samples across multiple arrays.

### **Blocking**

Blocking on known nuisance factors in sampling is a statistical technique that may improve the precision of estimation. Blocking is effective if within block variation is small, compared to between block variations. Array may be considered a blocking factor in microarray experiments, for example.