

## PROBLEM &gt;

Microarray data is coming under tighter scrutiny from publication standards and reviewers. It is becoming increasingly important and recognized for researchers in the microarray community to monitor data quality and global expression changes by adding spike-in controls to microarray experiments.

# Spike-In Controls Essential to All Microarray Experiments

## SOLUTION &gt;

The SpotReport® Alien® Array Validation System\* is a group of controls that collectively validates microarray data. They can be used for any genome microarray and play a pivotal role in normalization, monitoring hybridization specificity, and checking overall data quality from each microarray.

## Universal Microarray Controls

The SpotReport® Alien® Array Validation System is a carefully selected set of 10 artificial sequences that have been designed to have no sequence homology with any genome. These controls provide you with negative controls that you spot onto any microarray. These are available as oligos, PCR products or amine-modified oligos (for covalent attachment to slides). We offer corresponding mRNA spikes to these sequences for spike-in experiments (Figure 1). Using these control sequences with the corresponding spikes allows you to accurately normalize your data and establish experimental quality and signal linearity of your experiment.

## Determining Microarray Hybridization Consistency

The most common source of variation within microarray systems is local, intensity-dependent systematic variation caused by factors such as poor temperature control and inadequate mixing and distribution of the hybridization solution. Control for hybridization consistency across your microarray by spotting several Alien control genes in sufficient numbers on each microarray. Then, spike a range of concentrations of corresponding Alien mRNA into your labeling reactions (Figure 2) which will monitor variability in fluorescence intensities of the Alien gene replicates and indicate the hybridization consistency across your microarray.

## Data Normalization

To allow normalization using endogenous transcript levels, current microarray analyses assume that relatively few transcripts vary, or that any changes that occur are balanced. When normalization using endogenous genes is carried out, changes in expression levels are calculated relative to the behavior of most of the transcripts. Therefore, using endogenous genes to normalize means that absolute changes in individual genes can't be measured if global shifts in mRNA populations occur.<sup>1</sup> To demonstrate this, the following study used both

external RNA controls and endogenous genes for normalization. Several yeast microarrays were used to monitor global changes under heat shock. The levels of most mRNAs were found to change during human heat shock when external controls were included (Figure 3). Using exogenous genes as controls, even small global changes had a significant effect on the number of genes reported as being differentially expressed. This data would be lost if normalization was carried out on endogenous genes. Use the SpotReport Alien Array validation system to detect global mRNA changes which occur more frequently than is assumed at present. Monitoring these effects is fundamental for accurate determination of changes in gene expression.

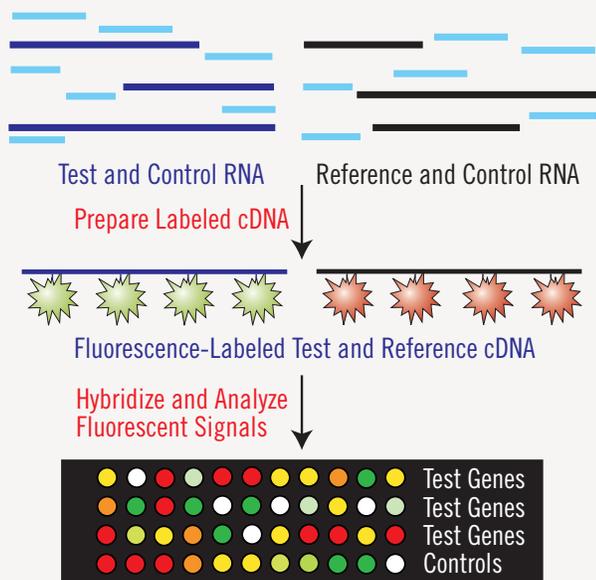
For more information about microarray products, visit our website at [www.stratagene.com/microarrays](http://www.stratagene.com/microarrays)

## REFERENCE

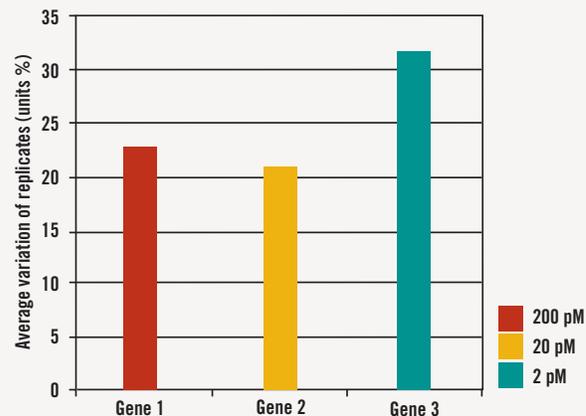
1. van de Peppel, J., et al, (2003) *EMBO Report* 4: 387-393

\* Patent pending. See license reference 6 on page 65.

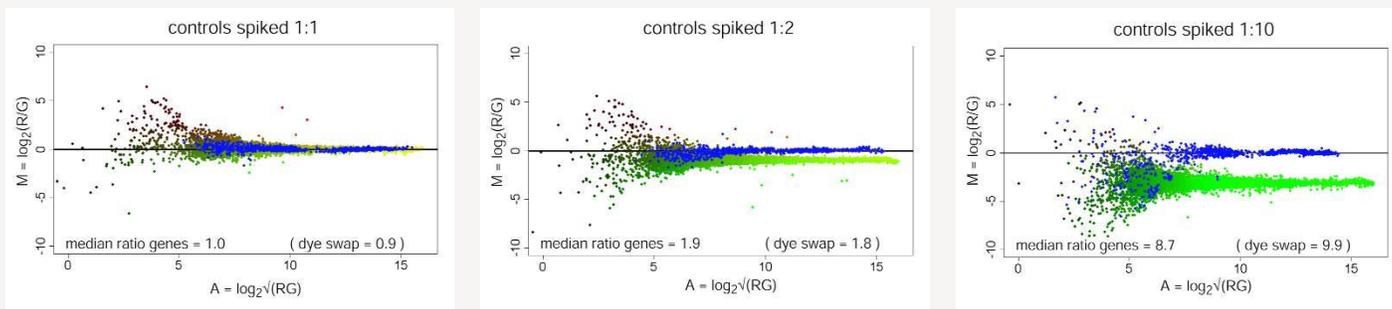
- + Controls for hybridization specificity
- + Assess overall experimental quality
- + Accurately normalize your data



**Figure 1**  
**How to Use SpotReport® Alien® mRNA**  
 The SpotReport® Alien® genes are spotted onto your microarray. Sample RNA are spiked with varying amounts of our high-quality SpotReport Alien mRNA spikes. After the labeling reaction, the SpotReport Alien mRNA spikes will hybridize to the genes spotted onto your microarray.



**Figure 2**  
**Monitoring Hybridization Efficiency Across a Microarray**  
 If the average variation of replicates is >30%, the consistency is low and local normalization is needed



**Figure 3**  
**External Controls Can Accurately Detect Global Transcript Changes<sup>1</sup>**  
 The external control mix (blue) was added at a ratio of (A) 1:1, (B) 1:2 and (C) 1:10 to paired aliquots of a single yeast total RNA preparation (green/red).

SpotReport® Microarray Systems			
	Contents	Catalog	Price
<b>SpotReport® Alien® cDNA Array Validation System</b>			
10 Artificial PCR/RNA standard positive and negative controls	Prints 500-1000 slides	252550	\$1623
<b>SpotReport® Alien® Oligo Array Validation System</b>			
10 Artificial PCR/RNA standard positive and negative controls	Prints 500-1000 slides	252270	\$1623
<b>SpotReport® Amine Oligo Array Validation System</b>			
10 <i>A. Thaliana</i> PCR/RNA standard positive and negative controls	Prints 500-1000 slides	252610	\$1420
<b>SpotReport® Alien® Amine Oligo Array Validation System</b>			
10 Artificial PCR/RNA standard positive and negative controls	Prints 500-1000 slides	252630	\$1623
<b>SpotReport® Alien® mRNA Spike Set</b>			
10 Artificial mRNA spikes	100 ng each	252575	\$528