### Microarrays



MICROARRAYS



## The Microarray Process

Microarray analysis is an extremely powerful and widely used tool for gene-expression profiling. It can provide a wealth of information about biological processes. However, with the generation of large amounts of microarray data, it has become increasingly important to address the challenges of data quality and standardization related to this technology.

We have addressed these challenges by developing a comprehensive portfolio of microarray tools designed to control for errors, standardize platforms, and simplify microarray experimentation.



Table 1 THE MICROARRAY PROCESS AND SELECTION GUIDE									
APPLICATION	PRODUCT	ADVANTAGES							
Microarray Preparation									
Array Controls	SpotReport <sup>®</sup> Array Validation Systems	<ul> <li>Sets of exogenous A. thaliana or artificial Alien<sup>®</sup> sequences with corresponding RNA spikes</li> <li>Determine quality of array fabrication process</li> <li>Normalize signals for differences in dye incorporation</li> <li>Assess the sensitivity of your hybridization experiment</li> <li>Validate labeling reactions and test RNA quality</li> <li>Available for oligo or cDNA microarrays</li> </ul>							
Sample Preparation									
RNA Reference	Universal Reference RNAs	<ul> <li>High-quality pool of total RNA for microarray gene-expression profiling</li> <li>Available for human, mouse or rat microarrays</li> <li>Standard for accurate and consistent data comparison</li> <li>Large lot sizes ensure consistency between experiments</li> </ul>							
RNA Purification	Absolutely RNA® Purification Kits	<ul> <li>+ High yields of pure, intact total RNA from tissue or cultured cells</li> <li>+ Three versions: miniprep, microprep and nanoprep for various sample sizes and elution volumes</li> <li>+ Easy and effective on-column DNA removal step</li> </ul>							
RNA Purification	Absolutely RNA® 96 Microprep Kit	<ul> <li>* 96-well plate format for high yields of pure, intact total RNA from cultured cells</li> <li>* Easy and effective on-column DNA removal step</li> </ul>							
Labeling	FairPlay® II Labeling Microarray Kit	<ul> <li>+ Uniform incorporation of fluorescent dyes produces more reliable signals</li> <li>+ High sensitivity to detect low-copy signal</li> <li>+ Includes StrataScript® RT for efficient extension and higher quantities of labeled cDNA</li> </ul>							
Analysis and Validation									
Desktop-Based Analysis Software	ArrayAssist <sup>™</sup> Software	<ul> <li>* Easy to use with multiple microarray platforms</li> <li>* Seamless Affymetrix integration (GCOS, NetAffx<sup>™</sup>)</li> <li>* State-of-the-art Affymetrix analysis including PLIER<sup>™</sup>, GC-RMA and RMA</li> <li>* Fully Integrated with the Interaction Explorer<sup>™</sup> Software (PathwayAssist<sup>™</sup>)</li> </ul>							
Server-based Analysis Software	GeneTraffic <sup>®</sup> Software	<ul> <li>Centralized data management for all microarray platforms</li> <li>Powerful, easy-to-use microarray data analysis tools</li> <li>Seamless Affymetrix integration and analysis (PLIER<sup>™</sup>, GC-RMA, RMA)</li> <li>Rich MIAME-compliant experiment annotation</li> </ul>							
Microarray Validation by Real-T	ime PCR								
Single color or up to 4-color multiplex with powerful data analysis capabilities and fast, accurate results	Mx3000P® QPCR System	<ul> <li>Four optical channels with user selected filters for greater flexibility</li> <li>Broad wavelength range excitation supports most fluorescent dyes</li> <li>Open platform design supports all fluorescent chemistries</li> <li>Precision thermal system for superior uniformity and reproducibility</li> </ul>							
Single color or up to 5-color multiplex with powerful data analysis capabilities and fast, accurate results	Mx3005P <sup>™</sup> QPCR System	<ul> <li>Five optical channels with user selected filters for greater flexibility</li> <li>Defined excitation and emission detection wavelengths are ideal for superior multiplex results, even for five (5) targets simultaneously</li> <li>Custom filter path selection to support FRET dye detection</li> <li>Includes Beacon Designer<sup>™</sup> software for primer/probe design</li> </ul>							
Real-time QPCR with significantly shorter run time (DNA, cDNA and RNA targets)	FullVelocity <sup>™</sup> SYBR <sup>®</sup> Green QPCR and QRT-PCR Master Mixes	<ul> <li>* Sensitive 1-step QRT-PCR in less time</li> <li>* High speed enzyme supports rapid cycling conditions</li> <li>* Quantitative PCR on DNA (cDNA) targets complete 30% faster than conventional methods</li> </ul>							
Pre-optimized 2X mixes for sensitive and reproducible real-time quantification of DNA, cDNA and RNA targets (1 or 2-step)	Brilliant <sup>®</sup> QPCR and QRT-PCR Master Mixes	<ul> <li>+ Made with optimized buffers and performance tested for reproducible results up to 24 months</li> <li>+ Master mix format reduces pipetting steps and minimizes user-error</li> <li>+ dUTP in nucleotide mixes so that UNG can be added for carry over contamination control</li> </ul>							

# **Microarray Preparation**

Microarrays are constructed by carefully dispensing precise and uniform amounts of probe on the surface of a glass slide by a process referred to as *printing*. An imperative part of building a quality microarray is the incorporation of external control genes on your microarray that will measure variables such as print quality, dye bias, hybridization efficiency, signal linearity, and array orientation.

### SpotReport<sup>®</sup> and SpotReport<sup>®</sup> Alien<sup>®</sup> Array Validation Systems

It is imperative to control for variability in microarray experiments. SpotReport<sup>®</sup> and SpotReport<sup>®</sup> Alien<sup>®</sup> Array Validation Systems<sup>®</sup> collectively validate experimental microarray parameters such as print quality, hybridization efficiency and specificity, assay sensitivity and dynamic range of detection, and dye bias for data normalization (Table 2). Use our standard SpotReport system for mammalian arrays or the SpotReport Alien system for plant, mammalian, or microbial arrays. Simply resuspend in the buffer of your choice and spot onto your microarray. Figure 1 shows how to spike the mRNAs into the labeling reactions for hybridization onto your microarray. SpotReport systems are available for either cDNA or oligo arrays, and are also available as amine-modified cDNA or oligos for all slide attachment chemistries.

- Hybridization efficiency across the slide
- Conduct normalization on control spots
- Monitor experimental quality
- Assess dye bias
- ✓ Verify print quality
- Measure assay sensitivity
- Assess dynamic range of the experiment
- Ø Determine microarray orientation



#### Table 2

PARAMETERS CONTROLLED BY SPOTREPORT® ARRAY VALIDATION SYSTEMS

### Figure 1

### HOW SPOTREPORT® ARRAY VALIDATION SYSTEM WORKS

Varying amounts of the control RNA spikes are added to the test and reference RNA. The RNA are converted to fluorescence-labeled cDNA and hybridized to test and control genes spotted on an array. The control genes are either PCR products or oligos (70-mers). After hybridization, the fluorescent signals are analyzed.

# Sample Preparation

The next step in the microarray process is to isolate and label sample target material that will be hybridized to the array. It is critical to assess RNA quality because traces of phenol, DNA contamination, and RNA degradation can contribute to poor labeling and signal quality in microarray experiments. Use of microarray standards such as our Universal Reference RNA allow for data to be compared between experiments and labs.

### **RNA Isolation and Purification Kits**

Absolutely RNA<sup>®</sup> Purification Kits allow you to efficiently isolate pure RNA for labeling. The kit offers a fast, streamlined protocol using spin columns, which eliminate phenol contamination. The kit also has an on-column DNase treatment that ensures removal of genomic DNA, and is stored at room temperature. Absolutely RNA<sup>®</sup> Miniprep Kit isolates RNA from 10<sup>5</sup> to  $10^7$  cultured cells or 5 to 40 mg of tissue while Absolutely RNA<sup>®</sup> Microprep Kit isolates total RNA from up to 5 x 10<sup>5</sup> cells (Figure 2).

### FairPlay® II Microarray Labeling Kit

The FairPlay<sup>®</sup> II Microarray Labeling Kit<sup>b</sup> solves the problems associated with preparation of fluorescent cDNA for microarrays and provides a reliable and reproducible way to convert total RNA or poly A RNA to fluorescencelabeled cDNA for hybridization to microarrays. Our newly improved FairPlay II kit doubles the yield of full-length fluorescence-labeled cDNA for microarray experiments and gives you improved signal-to-noise ratios and increased hybridization signals with higher correlations (Figure 3). Made with our high-quality StrataScript<sup>®</sup> Reverse Transcriptase<sup>c</sup>, these improvements provide the highest yield, while maintaining an even representation of full-length cDNA compared to other commercial labeling systems.



### Figure 2 EXCEPTIONAL TOTAL RNA PURITY

We assessed the purity of a total RNA sample isolated from a brain cell line using the Absolutely RNA® Kit method with an Agilent® Bioanalyzer. An RNA ratio (28S/18S) greater than 1.5 indicates very pure RNA. The data shown here yielded an RNA ratio 1.72, demonstrating the recovery of very pure RNA using the Absolutely RNA kit method.



### Figure 3

#### PERFORMANCE OF FAIRPLAY® II MICROARRAY LABELING KIT

The number of spots with Cy<sup>™</sup>3 and Cy<sup>™</sup>5 hybridization intensities >500 was determined following normalization using the Lowess sub-grid using our GeneTraffic<sup>®</sup> 2.8-9 DUO Two Color Microarray Analysis Software.



### Sample Preparation (continued)

#### **Universal Reference RNA**

With the wide use of microarrays, the key concern for researchers is how to standardize data sets to make them useful for future experiments and other researchers through cross platform comparisons. Furthermore, with the generation of a substantial amount of data from microarray experiments, it is essential to monitor data quality. To increase the chances of detecting biological variability, technical variability needs to be minimized. Using our Universal Reference RNA provides standardization and monitors technical variability in microarray experiments (Figure 4).

Pooled total RNAs from various tissues can act as a compilation of expressed genes in the genome and can be used as a common reference for microarray ratio analysis. We co-developed the Universal Human Reference RNA with the Brown and Botstein labs at Stanford University. Our Universal Reference RNAs are widely accepted with many supporting publications (Table 3, ref. 1-4), especially where researchers are conducting cross platform comparisions (Table 3, ref. 5) and investigating human clinical samples (Table 3, ref. 6-11). Our human, mouse (Table 3, ref. 12), and rat Universal Reference RNAs are comprised of high quality pooled total RNA from multiple cell lines providing broad gene coverage for data set comparison over long periods of time (Figure 5). These Universal Reference sets are produced at industrial-scale lot sizes and are extensively quality controlled.

#### Benefits of Using a Reference RNA

The use of reference RNA in microarray experiments allows you to simplify microarray data comparisons within time course experiments and perform experiments across multiple microarray platforms. It is also critical for clinical studies that occur over long time periods, and deconvolves chip-to-chip variability and technical errors. Our Reference RNAs also provide a good quality control tool for any microarray.

PARWISE DESIGN Reprimental Design: Sample A and sample B are Sample A Are Carlo Area Sample A Area Sample Area Area Area Area Area Area Area A





### Figure 4

### TWO EXPERIMENTAL DESIGNS TO CONSIDER FOR TWO-COLOR MICROARRAY EXPERIMENTS

Comparison of the pairwise design to the reference design of a two-color microarray experiment. The reference design is simple, requiring fewer hybridizations and allows you to compare all your data to a single reference.

### Figure 5 UNIVERSAL MOUSE REFERENCE RNA

Compugen 7,500-spot oligo mouse array provided by Chuck Perou (University of North Carolina). Averages of 96% and 80% gene coverage were obtained at single-and double-signal intensities above background.

### Universal Reference RNA Monitors Data Quality in Affymetrix GeneChip® Experiments

The Affymetrix microarray system has stringent controls in place. However, over time, equipment changes such as scanner settings and software upgrades must be made to ensure optimal data collection. In these situations, our Universal Reference RNAs are practical and reliable tools to monitor how ongoing hardware or software changes affect your data. Coverage data on the Affymetrix GeneChip<sup>®</sup> U133A 2.0 Plus is available on our website at www.stratagene.com/microarrays. With the next generation GeneChip<sup>®</sup> Human Exon 1.0 ST Arrays available from Affymetrix, there is a requirement for RNA samples with a high representation of genes to validate the authenticity of these new splice isoform predictions. The Universal Reference RNAs are a perfect solution for this application since the cell lines composed in these pooled RNAs have been individually and carefully selected to give the broadest possible gene coverage across each genome.

Reference	Titles	Publications
1 .	Indirect Measurements of Differential Gene Expression with cDNA Microarrays	Belbin, T., et al. 2004 Biotechniques 36: 310-314
2	Short Technical Report: Towards a Universal Standard-Comparing Two Methods for Standardizing Spotted Microarray Data	Ryan Weil, M,. et al. 2002 Biotechniques 32: 1310-1314
3	Universal Reference RNA as a Standard for Microarray Experiments	Novoradovskaya, N., et al. 2004 BMC Genomics 5: 1-13
4 :	Suitability of Stratagene Reference RNA for Analysis of Lymphoid Tissue	Dybkaer, K., et al. 2004 Biotechniques 37: 470-474
5	Interlaboratory Comparability Study of Cancer Gene Expression Analysis Using Oligonucleotide Microarrays	Dobbin, K. K., et al. 2005 Clinical Cancer Research 11: 565–572
6 : :	Different Gene Expression Profiles between Microsatellite Instability-High and Microsatellite Stable Colorectal Carcinomas	Kim, H., et al. 2004 Oncogene 23: 6218-6225
7	Gene Expression Profiling and Subgroup Identification of Oligodendrogliomas	Huang, H., et al. 2004 Oncogene 23: 6012-6022
8 :	The Mouse X Chromosome is Enriched for Sex-Biased Genes not Subject to Selection by Meiotic Sex Chromosome Inactivation	Khil, P., et al. 2004 Nature Genetics 10: 642-646
9 ·	Lung Cancers Detected by Screening with Spiral Computed Tomography have a . Malignant Phenotype when Analyzed by cDNA Microarray .	Bianchi, F., et al. 2004 Clinical Cancer Research 10: 6023–6028
10	Microarray-Based Screening for Molecular Markers in Medulloblastoma Revealed STK15 as Independent Predictor for Survival	Neben, K., et al. 2004 Cancer Research 64: 3103–3111
11	Global Gene Expression Profile of Nasopharyngeal Carcinoma by Laser Capture . Microdissection and Complementary DNA Microarrays	Sriuranpong, V., et al. 2004 Clinical Cancer Research 10: 4944–4958
12	Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease	Reddy, P.H., et al. 2004 Human Molecular Genetics 13: 1225–1240
13	Maximizing Gene Expression Microarray Data Quality with Stratagene's Universal Reference RNA	Taylor, C., 2003 Strategies 16: 10-11

Table 3

ARTICLES AND PUBLICATIONS: APPLICATIONS OF UNIVERSAL REFERENCE RNAS AS A STANDARD

# Microarray Analysis

The final part of microarray analysis is data visualization and interpretation. Raw images are acquired using either a laser or CCD-based imaging system. Data acquired from the raw images is then analyzed and filtered before statistical methods can be applied for interpretation. Extracting differences in gene-expression levels through statistics allows you to examine relative differences between samples. Bioinformatics tools can be employed to explore biological relationships that exist between genes/proteins of interest and existing pathways for further study.

### ArrayAssist<sup>™</sup> Software

ArrayAssist<sup>™</sup> Software is a fully-integrated desktop software package that easily analyzes Affymetrix GeneChip<sup>®</sup> and spotted microarray data. This includes up-to-date probe level analysis (RMA, GC-RMA and PLIER<sup>™</sup>). This desktop software incorporates powerful statistical analyses, clustering and visualization tools, and the latest in probe-level analysis algorithms (RMA and GC-RMA), which together improve the precision and sensitivity of GeneChip expression data (Figure 6). The software is optimized for speed and scalable to process hundreds of microarrays. An integrated, searchable database of current gene annotations for all GeneChip microarrays is kept current with the latest NetAffx<sup>™</sup> annotations. GeneTraffic® Microarray Database and Analysis Software

GeneTraffic\* Software is a client-server based microarray data management and analysis software package intended for core facilities or large-scale microarray users with support for Affymetrix GeneChip or two-color microarray platforms. It extends the capabilities of the ArrayAssist software by storing all data, including raw images, on the server, which can be accessed using your PC. Multiple users can access the data at the same time, and even work on the same project simultaneously. Our GeneTraffic Software has a complete application programming interface (API) to integrate data in the GeneTraffic server with other software applications to complete any microarray workflow. Our GeneTraffic software is an ideal product for any high-throughput microarray facility.



Figure 6 K MEANS CLUSTERING Easy-to-use statistical analysis, clustering, and visualization tools.

# Microarray Validation by Real-Time PCR

Real-time QPCR techniques provide definitive microarray data validation by combining more sensitive two-fold expression change resolution and increased accuracy using sample replicates. We provide quantitative PCR systems that meet performance needs for accurate and reproducible data validation.

### Mx3000P<sup>®</sup> QPCR System

The Mx3000P<sup>®d</sup> system is the lowest-priced fully-featured real-time PCR instrument. It has four customer-selected filters, for accurate and reproducible data and features an open platform which supports all fluorescent chemistries with no required use of ROX. The Mx3000P software is powerful, intuitive, user-friendly, and can control up to six systems with a single computer. The Mx3000P instrument is optimized for use with our Brilliant<sup>®</sup> and FullVelocity<sup>™e</sup> QPCR reagent kits.

### Mx3005P<sup>™</sup> QPCR System

The Mx3005P<sup>™d</sup> system is our advanced real-time PCR instrument that is also affordably priced. It comes with five customer-selected filters and a custom filter path feature to support FRET chemistry applications. The software for the Mx3005P system is the most powerful available today and controls up to six systems using a single computer. Our Mx3005P system also supports all fluorescent dyes and chemistries including our Brilliant and FullVelocity kits.



### Figure 7 MX3005P™ QPCR SYSTEM

The Mx3005P<sup>"</sup> system provides a 96-well format thermal block optimized for 25-µl volumes and accepts plates, strip tubes, and individual tubes. Dimensions: 14<sup>"</sup> (33 cm) W x 20<sup>"</sup> (46 cm) D x 18<sup>"</sup> (43 cm) H.



### Figure 8

### AMPLIFICATION PLOT SHOWING TEN ORDERS OF DYNAMIC RANGE

Eight replicates of ten-fold dilutions of plasmid DNA for  $\beta$ -actin target. Standard curve efficiency 98.2% and Rsq 0.999.



### Microarray Validation by Real-Time PCR (continued)

### **QPCR Reagent Kits**

Precise quantification of gene expression data is easily performed in real time, using either one- or two-step quantitative reverse transcription PCR (QRT-PCR). We offer two families of QPCR reagents for the quantification of expressed genes, Brilliant and FullVelocity master mixes, each of which uses a different PCR enzyme formulation to provide efficient, reproducible quantification.

### Brilliant® QPCR and QRT-PCR Master Mixes

Brilliant QPCR reagents provide high sensitivity and broad dynamic range required for precise validation of microarray data. Our Brilliant kits are made with our exclusive enzymes and reagents, and meet rigorous standards for stability and reproducibility. Brilliant QPCR master mixes utilize SureStart *Taq*\* DNA Polymerase<sup>e</sup> for high specificity, and our StrataScript reverse transcriptase provides superior real-time sensitivity compared to RNase H(+) reverse transcriptases. While optimized on our Mx3000P and Mx3005P QPCR Systems, Brilliant master mixes can be used on most QPCR instruments for highly sensitive results.

### FullVelocity<sup>™</sup> SYBR<sup>®</sup> Green QPCR and QRT-PCR Reagents

FullVelocity QPCR master mixes provide a fast and economical system for validation of microarray data. FullVelocity real-time results are produced 30 to 50% faster than with traditional *Taq*-based methods. Key to the FullVelocity technology is our unique high-speed archaeal DNA polymerase engineered to excel in rapid, two-step cycling conditions. FullVelocity SYBR Green master mixes are formulated with two specificity enhancers for high specificity during every cycle.

For a complete list of our QPCR reagents, see Table 4.

REAL-TIME QPCR REAGENTS GUIDE									
Format	DNA (cDNA)	<b>RNA Quantification</b>							
ronnat	Quantification	1-Step	2-Step						
SYBR®-based Master Mix	Brilliant® SYBR® Green QPCR Master Mix	Brilliant® SYBR® Green QRT-PCR Master Mix, 1-Step	Brilliant® SYBR® Green QRT-PCR Master Mix, 2-Step						
SYBR®-based Master Mix	FullVelocity™ SYBR® Green QPCR Master Mix	FullVelocity™ SYBR® Green QRT-PCR Master Mix, 1-Step	FullVelocity <sup>™</sup> SYBR® Green QRT-PCR Master Mix, 2-Step						
Probe-based Master Mix	Brilliant® QPCR Master Mix (up to 2 targets) or Brilliant® Multiplex QPCR Master Mix (up to 4 targets)	Brilliant® QRT-PCR Master Mix, 1-Step	Brilliant® QRT-PCR Master Mix, 2-Step						

### Table 4

QPCR REAGENT SELECTION GUIDE

# **Ordering Information**

PRODUCT	QUANTITY / FORMAT	CATALOG NO.
Microarray Preparation		
SpotReport®-3 cDNA Array Validation System	3 exogenous controls. Standard positive and negative controls. (Prints between 500-1000 slides)	252005
SpotReport®-10 cDNA Array Validation System	10 A. Thaliana controls. Standard positive and negative controls. (Prints between 500-1000 slides)	252010
SpotReport® Oligo Array Validation System	10 A. Thaliana Oligo controls. Standard positive and negative controls. (Prints between 500-1000 slides)	252170
SpotReport® Alien® cDNA Array Validation System	10 Artificial controls. Standard positive and negative controls. (Prints between 500-1000 slides)	252550
SpotReport® Alien® Oligo Array Validation System	10 Artificial controls. Standard positive and negative controls. (Prints between 500-1000 slides)	252270
SpotReport® Amine Oligo Array Validation System	10 A. Thaliana controls. Standard positive and negative controls. (Prints between 500-1000 slides)	252610
SpotReport® Alien® Amine Oligo Array Validation System	10 Artificial controls. Standard positive and negative controls. (Prints between 500-1000 slides)	252630
All SpotReport $^{\circ}$ cDNAs, oligos, and mRNA spikes are av	ailable separately. Please visit www.stratagene.com/microarrays.	
Sample Preparation		
Absolutely RNA® Miniprep Kit	50 preps	400800
Absolutely RNA® Microprep Kit	50 preps	400805
Universal Human Reference RNA	400 µg	740000
Universal Mouse Reference RNA	400 µg	740100
Universal Rat Reference RNA	400 µg	740200
FairPlay® II Microarray Labeling Kit	10 rxn	252006
	30 rxn	252007
Microarray Analysis		
ArrayAssist™ Software	Academic	999110
ArrayAssist™ Software	Commercial	999111
GeneTraffic® UNO	For Affymetrix GeneChip® expression arrays	GT001
GeneTraffic® DUO	For two-color data	GT002
GeneTraffic® Multi	For one- and two-color microarray data analysis	GT003
Microarray Validation by QPCR		
Brilliant® QPCR Master Mix Kits	SYBR® Green-based detection, 400 rxn x 25 μl Probe-based detection, 400 rxn x 25 μl	600548 600549
Brilliant® QRT-PCR Master Mix Kits, 1-step	SYBR® Green-based detection, 400 rxn x 25 µl Probe-based detection, 400 rxn x 25 µl	600552 600551
FullVelocity™ SYBR® Green OPCR Master Mix Kits	SYBR® Green-based detection 400 rxn x 25 µl	600581
FullVelocity <sup>™</sup> SYBR <sup>®</sup> Green QRT-PCR Master Mix, 1-step	SYBR® Green-based detection, 400 rxn x 25 µl	600582
cDNA Synthesis for 2-Step QRT-PCR		
StrataScript® First Strand cDNA Synthesis Kit	50 rxn	200420
QPCR Systems		
Mx3000P® QPCR System	4-Color system (110y) with notebook computer	401403
	4-Color system (110v) with desktop computer	401405
	4-Color system (230v) with notebook computer	401406
	4-Color system (230v) with desktop computer	401407
Mx3005P™ QPCR System	5-Color system (110v) with desktop computer	401434
	5-Color system (110v) with notebook computer	401435
	5-Color system (230v) with desktop computer	401436

5-Color system (230y) with notebook computer

#### LEGAL LANGUAGE

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Agilent Bioanalyzer is a registered trademark of Agilent

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- b. Some uses of the FairPlay® II microarray labeling kit may require licenses from third parties in certain countries.
- c. Purchase of this PCR-related product does not convey any rights under the *foreign counterparts* of the PCR patents owned by Roche Molecular Systems. A license to use the PCR process, *where such process is covered by patents*, accompanies the purchase of certain reagents from Stratagene when used in conjunction with an Authorized Thermal Cycler.
- d. This instrument is an Authorized Thermal Cycler. Its purchase price includes the up-front fee component of a license under the non-U.S. counterparts of United States Patent Nos. 4,683,195, 4,683,202 and 4,965,188 owned by

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- r. Use of labeling reagents may require licenses from entities other than Stratagene. For example, use of fluorogenic probes in 5' nuclease assays may require licenses under U.S. Patent Nos. 6,214,979, 5,804,375, 5,210,015 and 5,487,972 owned by Roche Molecular Systems, Inc. and under U.S. Patent No. 5,538,848 owned by Applied Biosystems. TadMan\* is a registered trademark of Roche Molecular Systems. Inc.

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