



# Data Sheet

## ■ Affymetrix Solutions for Cancer Analysis

Over the past decade, microarray technology has revolutionized cancer research by providing a global view of molecular changes involved in disease progression. Affymetrix offers whole-genome solutions for SNP, chromosome copy number, and expression analysis. Researchers can now obtain a better understanding of the complex pathways involved in cancer progression using a comprehensive suite of high-quality, proven assays on a single, industry-standard microarray platform.

Affymetrix provides a broad portfolio of solutions for cancer research. More than 760 scientific publications demonstrate the power of GeneChip® brand microarrays in applications ranging from target discovery to clinical studies. Most comprehensive cancer research programs include one or all of the following fundamental objectives:

- Identify the biochemical cause of the disease.
- Develop better diagnostic methods.
- Improve therapeutic outcomes and patient care.

This data sheet will review some of the key publications that demonstrate the value of GeneChip brand products in achieving these objectives.

### Therapeutic Target/ Biomarker Discovery

#### CHROMOSOME COPY NUMBER ANALYSIS

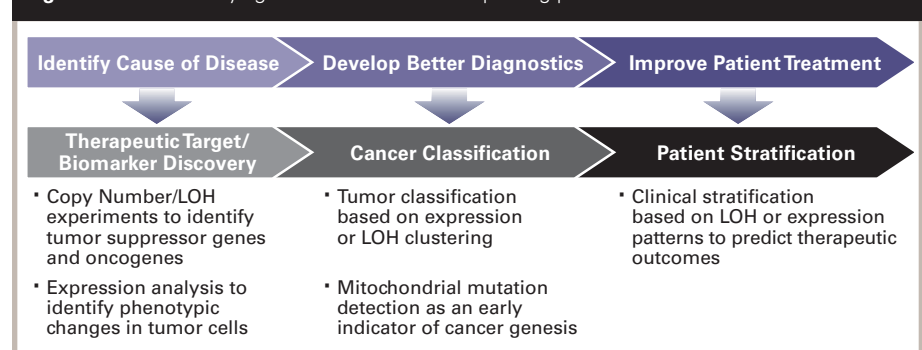
Whole-genome copy number analysis is a powerful approach to identifying critical genes involved in the development and progression of cancer. Many recent publications show the advantages of combining the GeneChip mapping arrays and Copy

Number Analysis Tool (CNAT) to determine regions of chromosomal deletion, amplification, or loss of heterozygosity (LOH). In comparison to existing technologies, GeneChip mapping arrays provide higher resolution, increased throughput, and higher reproducibility. CNAT is a powerful tool for identifying target genes and discovering biomarkers, as well as for clinical classification.

#### GENECHIP® MAPPING ASSAY

The GeneChip Mapping 100K Set offers the highest genomic coverage, providing more power to detect changes in chromosomal copy number. With a mean marker distance of 26kb, the GeneChip Mapping 100K Set enables the identification of micro-deletions and amplifications that cannot be detected by other methods. The GeneChip Mapping 10K Array 2.0 and Mapping 100K Set use the same proven one-primer assay that was developed for the original GeneChip Mapping 10K Array, and has been validated in over 25 publications. Requiring only 250 ng of DNA per array, any of these products can be used for whole-genome SNP analysis at the benchtop.

**Figure 1:** From identifying cause of disease to improving patient treatment.



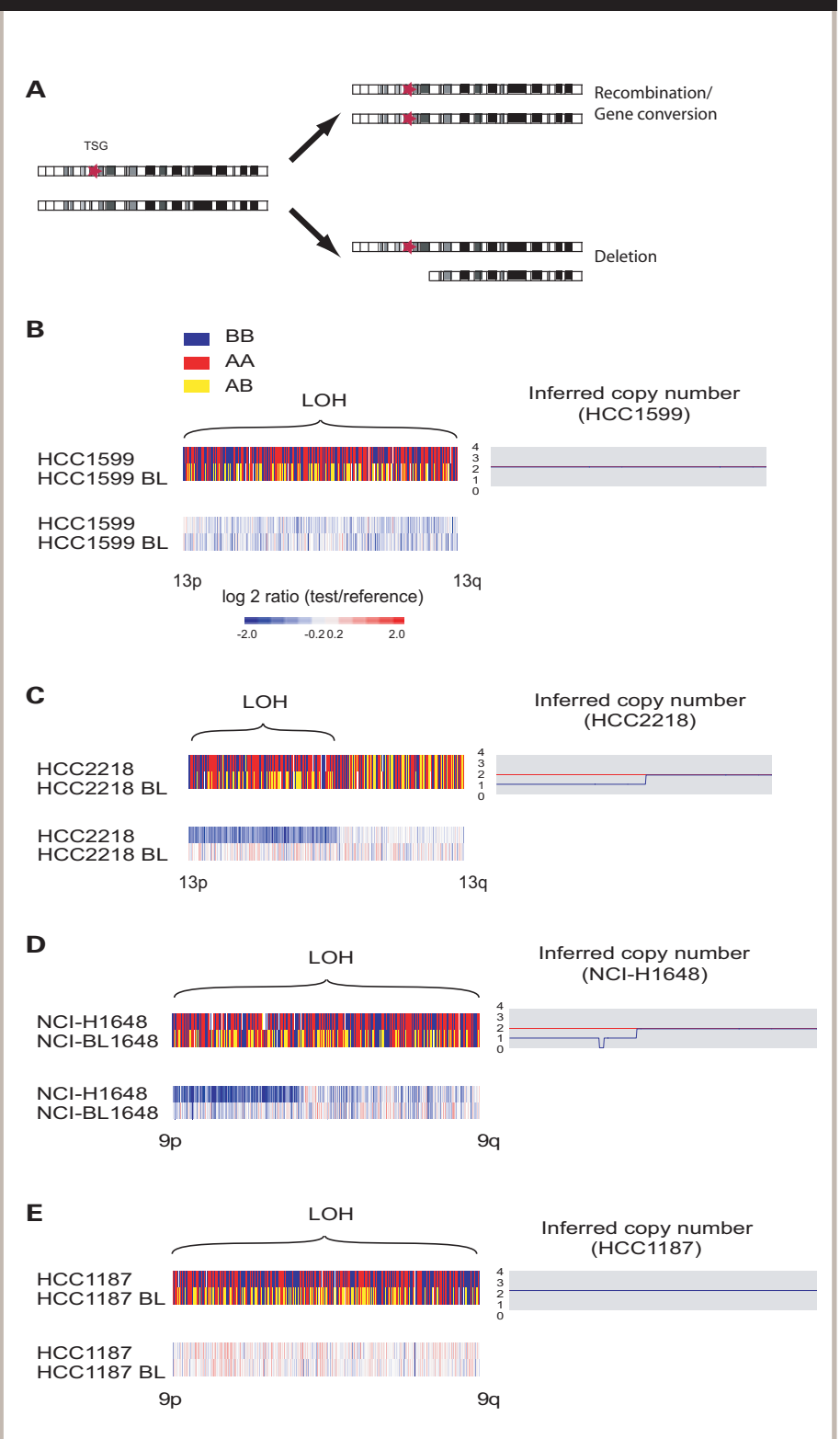
**GENECHIP CHROMOSOME COPY NUMBER ANALYSIS TOOL AND INTEGRATED GENOME BROWSER**

GeneChip CNAT v.2 provides a graphical display of data for rapid identification of clinically relevant amplifications, deletions, and LOH regions by chromosome. CNAT v2 calculates chromosomal copy number based on SNP hybridization signal intensity data from the GeneChip mapping arrays. Genotype calls enable the identification of copy-neutral loss of heterozygosity, as well as allelic imbalance caused by preferential amplification of one allele. Identification of copy-neutral events is not possible using other Comparative Genomic Hybridization (CGH) technologies. Additionally, data can be easily exported from CNAT into Affymetrix' Integrated Genomic Browser to facilitate the rapid identification of disease genes from genomic regions of copy number alteration. Using the GeneChip Chromosome Copy Number Analysis Tool, you can detect regions of chromosomal deletion, amplification, or LOH, and gain a better understanding of underlying molecular events.

**TARGET DISCOVERY: SPECIFIC EXAMPLES FROM PUBLISHED DATA**

A major advantage of the GeneChip DNA mapping arrays is the use of SNPs, allowing simultaneous analysis of copy number alterations and genotype on the same array. Complex patterns of chromosomal gains and losses are difficult to decipher using either CGH or microsatellite analysis alone. Collecting the genotype information in addition to the copy number leads to the understanding of different LOH mechanisms, such as LOH without copy number reduction (Bignell *et al.* 2004, Herr *et al.* 2005, Zhao *et al.* 2004). LOH regions that do not show copy number changes could be the result of mitotic non-disjunction followed by duplication of one parental chromosome, as observed in two breast cancer cell lines (HCC1599 and HCC1187) on chromosomes 13 and 9, respectively (Zhao *et al.* 2004).

**Figure 2:** Single nucleotide polymorphism array analysis and different genetic mechanisms that lead to loss of heterozygosity (LOH).



## Cancer Classification

An early and accurate diagnosis of cancer is critical in determining the most appropriate therapeutic option. This task is difficult or impossible by traditional methods because of atypical clinical presentation or histopathology. By stratifying tumors on a molecular basis, microarrays enable scientists to better classify tumors. Better classification leads to the development of clinically predictive systems for diagnosis, prognosis, and treatment. GeneChip arrays are the first technology powerful enough to scan through tens of thousands of genes in a cancer cell and pinpoint the handful that are significant.

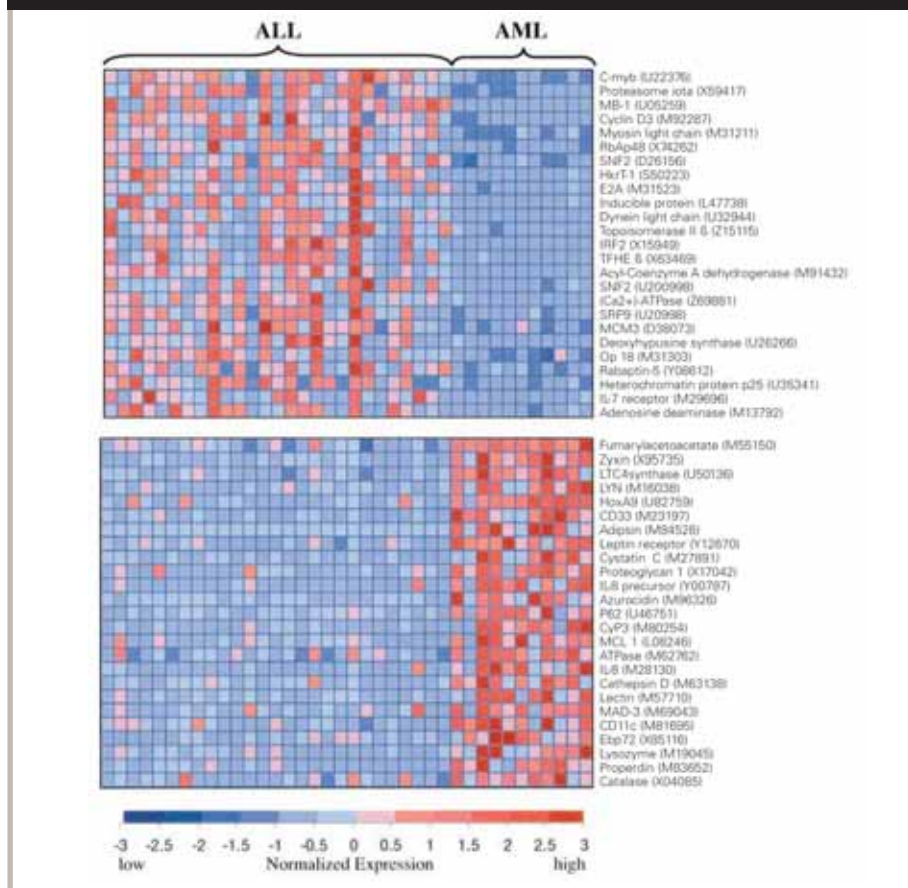
### GENECHIP HUMAN GENOME U133 PLUS 2.0 ARRAY

Cancer research has made significant advances in the understanding of gene expression with powerful tools from Affymetrix. The GeneChip Human Genome U133 Plus 2.0 Array is the first single array representing the whole human genome. The array contains over 54,000 probe sets, representing over 38,500 well-characterized human genes that can be used to explore cancer biology. Increasingly, scientists access the entire human genome for a global view of the changes in gene expression that occur during cancer progression.

### CANCER CLASSIFICATION SPECIFIC EXAMPLES FROM PUBLISHED DATA

In their seminal study published in *Science*, Golub *et al.* made advances identifying new cancer classes and assigning tumors to known classes using Affymetrix® microarrays. Figure 3 shows a clustering of the 50 genes most highly correlated with the ALL-AML class distinction, with each row corresponding to a gene and each column corresponding to the expression level in each of the 38 leukemia samples. Ultimately, the researchers' work showed that cancer classification can be achieved with gene expression patterns even in the absence of any *a priori* understanding of the underlying biology, discovering and predicting cancer classes for other types of cancer is feasible.

**Figure 3:** Golub, T.R. *et al.* Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. The genes shown are the top 50 genes that most highly correlate with the two class distinctions, ALL and AML. Red represents high expression, whereas blue represents lower expression.



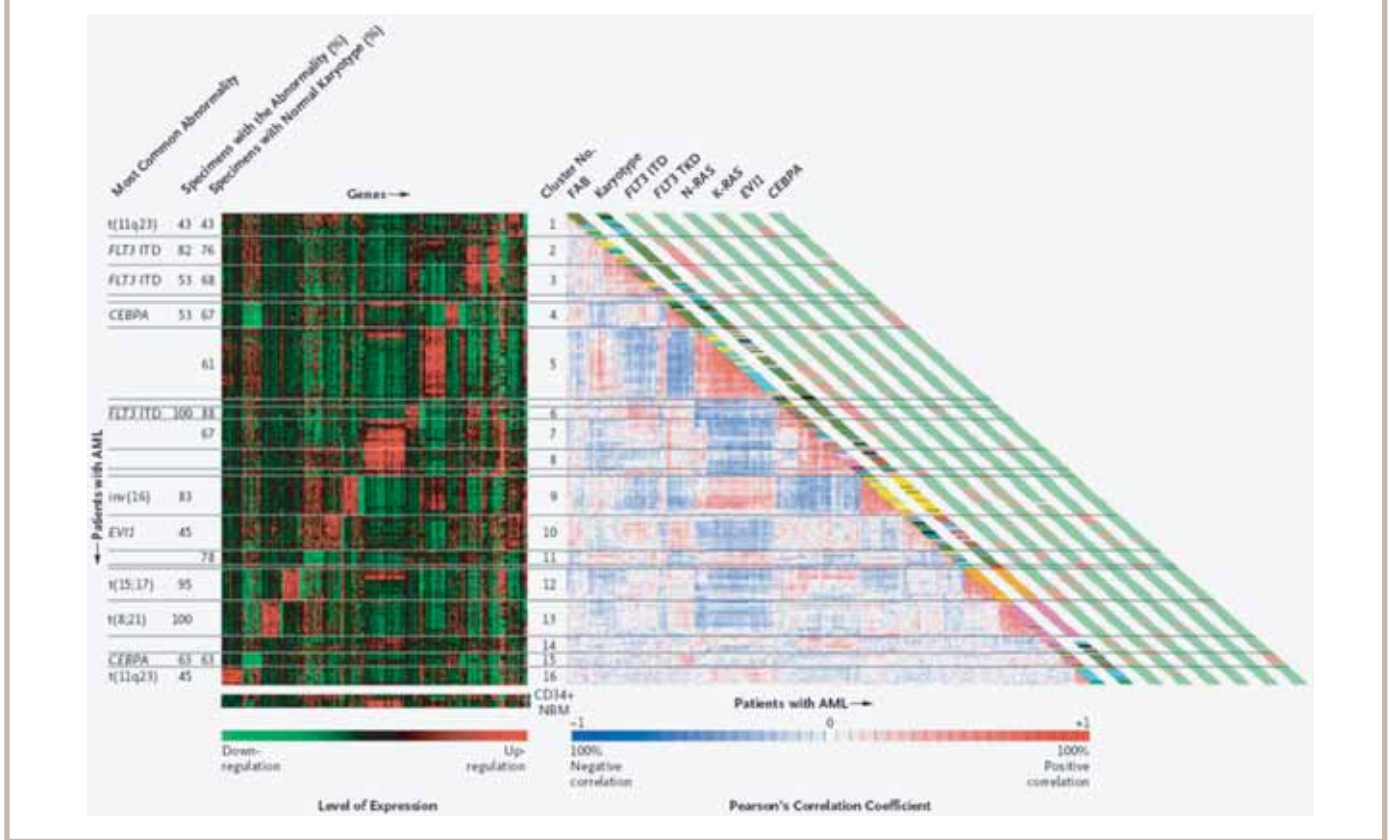
In a more recent report, Valk *et al.* used Affymetrix GeneChip® Human Genome U133A Arrays to document one of the first large-scale efforts to catalog the diversity of gene expression profiles in 285 AML patients. On the basis of unsupervised clustering analyses, the researchers were able to classify AML patients into a total of 16 true subgroups (see Figure 4) according to unique molecular signatures.

The study demonstrated that the well-recognized cytogenetic abnormalities seen in AML are associated with distinct gene expression signatures, suggesting that detection of these events in the future might be accomplished using gene expression-based techniques. Moreover, the study

identified several novel clusters with some specimens showing normal karyotypes. In addition, it also demonstrated that one unique cluster corresponded to very poor patient prognosis. Such research highlights the benefits of using GeneChip microarrays as high-capacity surveying tools that help elucidate the molecular pathology of disease, and discover markers that predict disease progression, responses to treatments, and patient prognosis.

Just as hierarchical clustering approaches have been used to classify cancers using gene-expression profiles, several researchers have shown that hierarchical clustering of LOH regions can also be used. In 2004, Janne *et al.* showed that non-small-cell and

**Figure 4:** Valk *et al.* Prognostically useful gene-expression profiles in acute myeloid leukemia. Adapted correlation view of specimens from 285 patients with AML involving 2856 probe sets (right), and the levels of expression of the top 40 genes that characterized each of the 16 individual clusters (left).



small-cell lung cancer samples could be distinguished based on LOH clustering. Oral squamous cell carcinoma has also been evaluated by hierarchical clustering using the GeneChip® Mapping 10K Array (Zhou *et al.* 2004a; Zhou *et al.* 2004b).

#### SOMATIC MUTATION ANALYSIS OF MITOCHONDRIAL GENOME

The accumulation of somatic mutations in mitochondrial genomes is associated with several types of cancer. Complete sequence analysis of the mitochondrial genome is useful in characterizing the pattern of mutation rates and may potentially serve as an early detection method. The GeneChip Mitochondrial Resequencing Array provides a high-throughput method for identifying somatic mutations in the entire mitochondrial coding region.

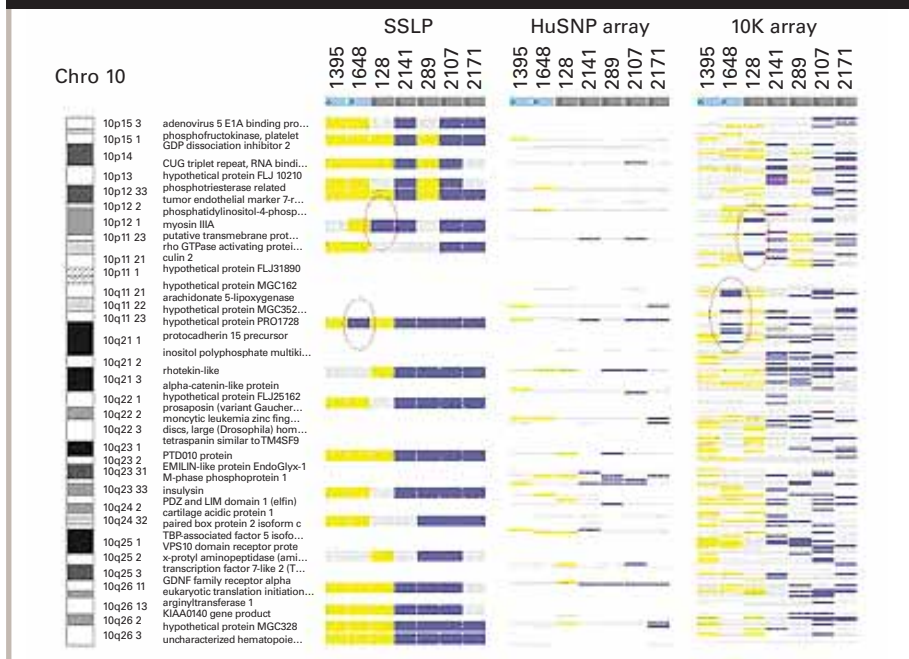
#### GENECHIP MITOCHONDRIAL RESEQUENCING ARRAY

The GeneChip Mitochondrial Resequencing Array is a useful tool for detecting the accumulation of somatic mutations. Each array interrogates more than 15,000 bases of the mitochondrial coding sequence selected from the MitoMap database. Eight unique oligonucleotide probes are tiled at each base position allowing for the detection of both known and novel mutations. The Mitochondrial Resequencing Array enables rapid sequencing of the entire genome in less than 48 hours. Using long-range PCR to amplify the entire genome in only three reactions reduces the cost and labor involved in mitochondrial analysis. Furthermore, the Mitochondrial Resequencing Array is the only system capable of performing highly sensitive detection of heteroplasmy from clinical samples.

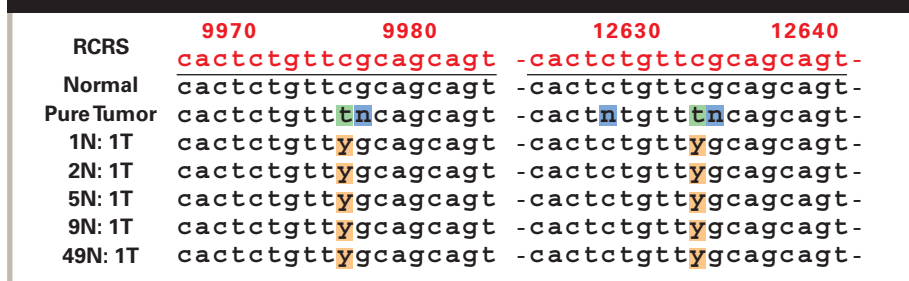
#### MITOCHONDRIAL SOMATIC MUTATIONS: SPECIFIC EXAMPLES FROM PUBLISHED DATA

In a study by Maitra *et al.*, the Mitochondrial Resequencing Array was found to be a highly accurate, high-throughput sequencing technology for the detection of mitochondrial mutations in clinical samples. In a serial dilution experiment, the array was able to detect the presence of mutations at a 50:1 proportion of normal to tumor cells (Figure 6). No other technology has shown this level of sensitivity to detect mutations in mixed-cell populations. Furthermore, this study confirmed the detection of somatic mutations in both tumors and body fluids such as urine and pancreatic juice, supporting the hypothesis that this method could be used as a non-invasive early diagnostic tool.

**Figure 5:** The GeneChip® Mapping 10K Array identifies regions of LOH that are missed by HuSNP. Blue-LOH; yellow-retention; gray-uninformative.



**Figure 6:** Detection of aberrant clonal population in 50-fold-diluted tumor DNA.



## Patient Stratification and Prognosis

### CLINICAL STRATIFICATION/ THERAPEUTIC RESPONSE

Genetic differences between individuals have been shown to have a significant impact on therapeutic outcomes. By helping understand the genetics of disease and patient response to treatment, microarrays are enabling researchers to predict which drugs will work for which patient with which disease. There are over 40 examples of GeneChip array profiling used in clinical trials to classify disease markers enabling predictions of drug efficacy and clinical trial outcomes.

### Key References: Therapeutic Response

A recent Phase III clinical trial by Novartis Pharmaceuticals used expression profiles to predict the success or failure of Gleevec® treatment on chronic myelogenous leukemia (McLean, L. A., *et al.* 2004). They analyzed gene expression patterns from patients prior to treatment and found a 31 gene “No Response” signature, which predicts a 200-fold higher probability of failed therapy.

Similarly, in a Phase II clinical trial conducted at the Dana Farber Cancer Research Institute for the Millennium Pharmaceutical drug Velcade®, researchers used GeneChip expression arrays to collect pharmacogenomic data from myeloma patients treated

with the drug (Mulligan, G., *et al.* 2002). The scientists discovered a pattern consisting of 30 genes that correlate with response or lack of response to therapy. Clinical utility of biomarkers will be further assessed in a Phase III trial.

## Conclusion

Affymetrix offers a broad portfolio of solutions for cancer research on a single industry-standard microarray platform. By providing a global view of the molecular changes involved in disease progression, GeneChip® arrays enable groundbreaking research in the areas of target discovery, tumor classification, and patient stratification. A growing number of publications illustrate the power of GeneChip arrays to facilitate the discovery and development of predictive systems for diagnosis, prognosis, and treatment—enabling improved clinical outcomes and patient care.

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## Ordering Information

### Expression Products

#### GeneChip® Human Genome U133 Plus 2.0 Array

- 900466** GeneChip® Human Genome U133 Plus 2.0 Array (contains 6 arrays)
- 900467** GeneChip® Human Genome U133 Plus 2.0 Array (contains 30 arrays)

### Software Products

#### GeneChip® DNA Analysis Software Version 3.0

- 690040** GeneChip® DNA Analysis Software 3.0 (initial annual license)

#### GeneChip® Chromosome Copy Number Analysis Tool (CNAT) Version 2

[www.affymetrix.com/support/developer/tools/affytools.affx](http://www.affymetrix.com/support/developer/tools/affytools.affx)

### DNA Analysis Products

#### GeneChip® Mapping Arrays and Assay Kit

- 900540** GeneChip® Human Mapping 10K Array Xba 142 2.0 (contains 30 arrays)
- 900441** GeneChip® Mapping 10K Xba Assay Kit (sufficient for 30 reactions)
- 900518** GeneChip® Human Mapping 50K Array Xba 240 (contains 30 arrays)
- 900523** GeneChip® Human Mapping 50K Array Hind 240 (contains 30 arrays)
- 900520** GeneChip® Mapping 50K Xba Assay Kit (sufficient for 30 reactions)
- 900521** GeneChip® Mapping 50K Hind Assay Kit (sufficient for 30 reactions)

#### GeneChip® Mitochondrial Resequencing Array

- 510987** GeneChip® Mitochondrial Resequencing Array

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##### Japan

+81-(0)3-5730-8200

#### AFFYMETRIX, INC.

3380 Central Expressway  
Santa Clara, CA 95051 USA  
Tel: 1-888-DNA-CHIP (1-888-362-2447)  
Fax: 1-408-731-5441  
[sales@affymetrix.com](mailto:sales@affymetrix.com)  
[support@affymetrix.com](mailto:support@affymetrix.com)

#### AFFYMETRIX UK Ltd

Voyager, Mercury Park,  
Wycombe Lane, Wooburn Green,  
High Wycombe HP10 0HH  
United Kingdom  
UK and Others Tel: +44 (0) 1628 552550  
France Tel: 0800919505  
Germany Tel: 01803001334  
Fax: +44 (0) 1628 552585  
[saleseurope@affymetrix.com](mailto:saleseurope@affymetrix.com)  
[supporteurope@affymetrix.com](mailto:supporteurope@affymetrix.com)


#### AFFYMETRIX JAPAN K.K.

Mita NN Bldg., 16 F  
4-1-23 Shiba, Minato-ku,  
Tokyo 108-0014 Japan  
Tel: +81-(0)3-5730-8200  
Fax: +81-(0)3-5730-8201  
[salesjapan@affymetrix.com](mailto:salesjapan@affymetrix.com)  
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