

MassARRAY[®] For Cancer Analysis

Facilitating New Visions for Cancer
Research and Disease Management



MassARRAY Platform - Highly Accurate, Sensitive Cancer Analysis

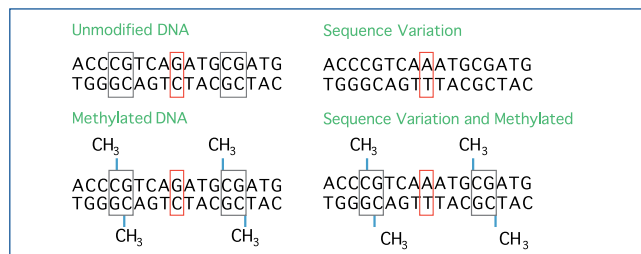
The utility of SNPs as biomarkers in cancer research is well accepted and widely documented. SNPs can be employed to study a variety of different genomic phenomena including disease association, causative mutations, or structural variations. They play a key role as biomarkers for risk, progression, and prognosis. Sequenom's MassARRAY platform and iPLEX[®] Gold provide a flexible and versatile solution for fine mapping of SNPs identified from whole genome studies and other computational methods. iPLEX Gold offers a straight forward workflow for superior assay design efficiency, data processing, and analysis to enable follow up and validation.

iPLEX Gold is the most efficient solution to generate highly multiplexed genotyping assays for efficient and rapid screening of large sample populations. One powerful example is a recently published study that discovered a transforming MET mutation in non-small cell lung cancer³. The same chemistry

can also be used to quantify absolute levels of alleles, genomic copy number, and gene expression.

In addition to SNP genotyping, Sequenom offers a broad portfolio of solutions for cancer research from methylation and gene expression, to low frequency mutation and copy number analysis. At the heart is the MassARRAY platform, based on MALDI-TOF mass spectrometry. Trusted and utilized by leading genetics institutions worldwide, it offers highly accurate, sensitive, and high-throughput methods pivotal to cancer research.

Figure 1: Molecular Modifications associated with Cancer Biology



“We conclude that MassEXTEND LOH analysis (using Sequenom's MassARRAY RT software) is a sensitive, high-throughput, and cost-effective method to screen SNP loci for LOH in formalin-fixed paraffin-embedded tissue.”¹

“This approach is more sensitive than conventional Sanger sequencing, having the potential to detect mutations that are present in only a subset of tumor cells or tumors with high levels of normal cell contamination, which is commonly the case in breast cancer”²

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CCGATGATCGACCAGTATGCGCATGATGATCGAAGTATGCGCATTATGCGCAT
GCGCATTATGCGCATGATGATCGAAGCCGATGATCGACCAGTATGCGCATGA
GCGCATTATGCGCATGATGATCGAAGTATCATGATGATCGAAGCCGATGA



Sequenom for Oncology

Oncogene Mutation Profiling

The MassARRAY system is a highly effective method for profiling hundreds of oncogene mutations in parallel. It offers a higher level of sensitivity compared to conventional sequencing.

In a recent study, R.K. Thomas et al. described a genotyping panel for rapid screening of somatic mutations in cancer samples. They analyzed 238 oncogene mutations in 17 different tumor types with 1,000 human tumor samples. Twelve EGFR mutations in primary lung tumor samples, including three mutant alleles with as little as 9% of the total DNA, were detected with the MassARRAY system. In comparison, Sanger sequencing detected nine EGFR mutations, missing the three low-frequency alleles⁴.

OncoCarta™ Panel

Sequenom now offers its own oncogene panel for somatic mutation profiling. The OncoCarta™ Panel v1.0 is the most comprehensive multi-gene panel available for profiling rare somatic mutations, enabling you to rapidly profile genetic changes associated with tumor initiation and progression.

- Comprehensive—the only panel for somatic mutation analysis with 19 oncogenes and 238 mutations in 24 multiplexes
- Ready-to-go—Pre-designed assays you can run in your lab today
- High sensitivity—detect as low as 10% mutation frequency
- Small input DNA—less than 500 nanograms per sample for 238 mutations, important when using FFPE tissue

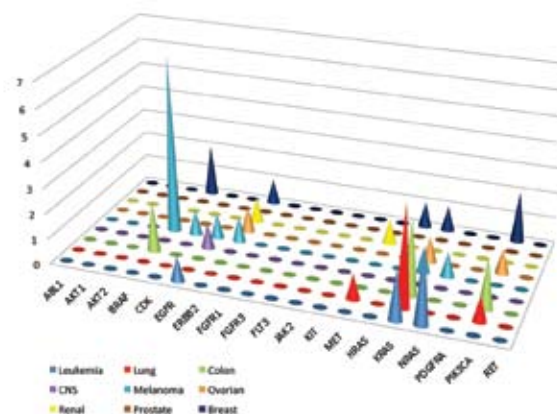
Key oncogenes include EGFR, KRAS, BRAF, and KIT, to name a few of the 19 genes available for investigation (**Table 1**).

Table 1: Oncogenes in the OncoCarta™ Panel

Gene	No. Mutations	Gene	No. Mutations
<i>ABL1</i>	14	<i>JAK2</i>	1
<i>AKT1</i>	7	<i>KIT</i>	27
<i>AKT2</i>	2	<i>MET</i>	5
<i>BRAF</i>	24	<i>HRAS</i>	6
<i>CDK</i>	2	<i>KRAS</i>	12
<i>EGFR</i>	43	<i>NRAS</i>	8
<i>ERBB2</i>	7	<i>PDGFR</i>	11
<i>FGFR1</i>	2	<i>PIK3CA</i>	13
<i>FGFR3</i>	5	<i>RET</i>	6
<i>FLT3</i>	2		

The OncoCarta™ Panel was validated against NCI60 cell line DNA and FFPE tumor samples. We observed >90% concordance with known mutations as described in the COSMIC database. Interestingly, we also detected a high percentage of novel mutations, including 18% in the NCI60 samples, and >40% in the FFPE set. We believe this is due to the higher level of sensitivity with the OncoCarta™ Panel, and the mutations are worthy of further investigation for biological relevance. **Figure 2** shows the distribution of mutations across tumor types.

Figure 2: Distribution of Mutations across Tumor Types found with the OncoCarta™ Panel v1.0





High-Throughput Quantitative Methylation Profiling

Epigenetic changes hold great promise as early detection markers as they frequently occur before clinical onset of disease. Large scale studies to identify key epigenetic markers are in demand. Wide CpG coverage, use of large sample numbers, and efficient data analysis are key to large scale studies. In response to these needs, Sequenom offers the EpiTYPER technology to enable high-throughput cytosine methylation profiling of candidate genes.

- Parallel analysis—quantify multiple CpG sites in amplicons up to 600 bp
- Better modeling—correlate methylation and gene expression for better predictive survival models
- Broad range— measure a broad dynamic range of transcript levels for a given sample within the same well

EpiBrowser and Cancer EpiPanel for Rapid Methylation Profiling

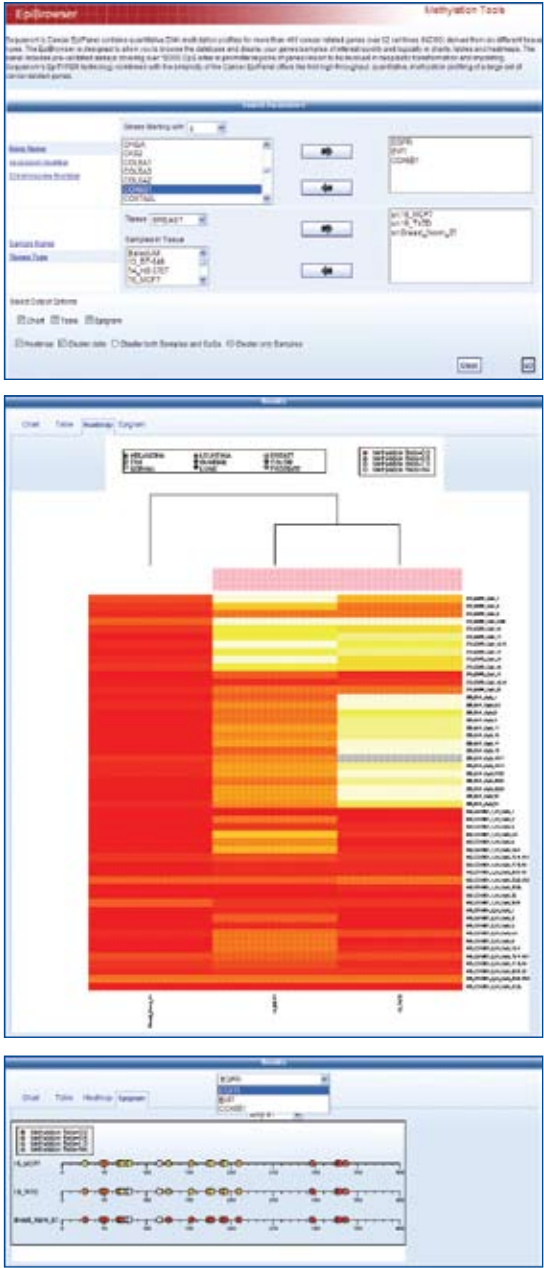
Sequenom’s Cancer EpiPanel contains quantitative DNA methylation profiles for more than 400 cancer related genes over 59 cell lines (NCI60) derived from nine different tissue types.

The EpiBrowser allows you to browse the database and display your genes and samples of interest quickly and logically in charts, tables and heatmaps and EpiGrams. The panel includes pre-validated assays covering over 12,000 CpG sites in promoter regions of genes known to be involved in neoplastic transformation and imprinting.

In **Figure 3**, the EpiBrowser queries EVI1, COX6B1, and EGFR against three breast cancer cell lines (MCF7, T47D, and normal).

Sequenom’s EpiTYPER technology combined with the simplicity of the Cancer EpiPanel offers the first high throughput, quantitative, methylation profiling of a large set of cancer-related genes.

Figure 3: EpiBrowser Methylation Tool



Methylation and Gene Expression

EpiTYPER for methylation analysis can be used in combination with gene expression profiling to discover clinically meaningful molecular marker sets that are capable of accurately predicting survival. We present an integrated approach where genome-wide analysis using microarray data identifies differentially-expressed genes.

The MassARRAY system allows for investigation of methylation and gene expression relationships. Typically a high level of promoter methylation correlates with gene silencing. For this study, a scatter plot shows differences of methylation and expression levels between normal and tumor samples (**Figure 4**). The expected negative correlation between methylation and expression changes is exemplified in MGP1 and AQP1.

Methylation and gene expression markers can provide a better prediction of the identification of poor and good survival groups in non-small cell lung cancer (NSCLC)⁵.

Once these genes are discovered, quantitative methylation analysis can be applied and a subset of methylation-regulated genes can be identified. **Figure 5** illustrates Kaplan-Meier survival estimates where a combined algorithm results in better cluster-defined analysis.

Figure 4: Methylation and Gene Expression Scatter Plot

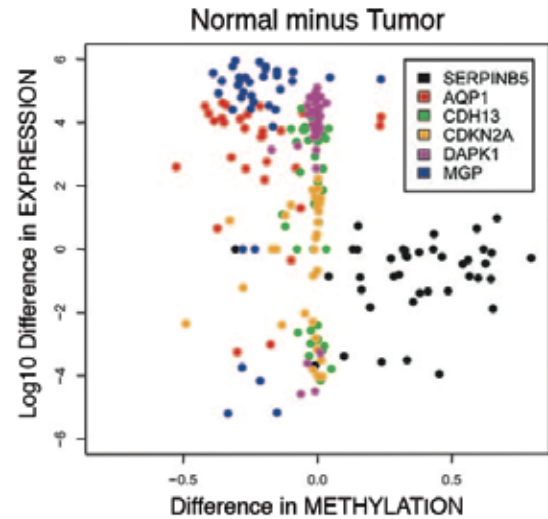
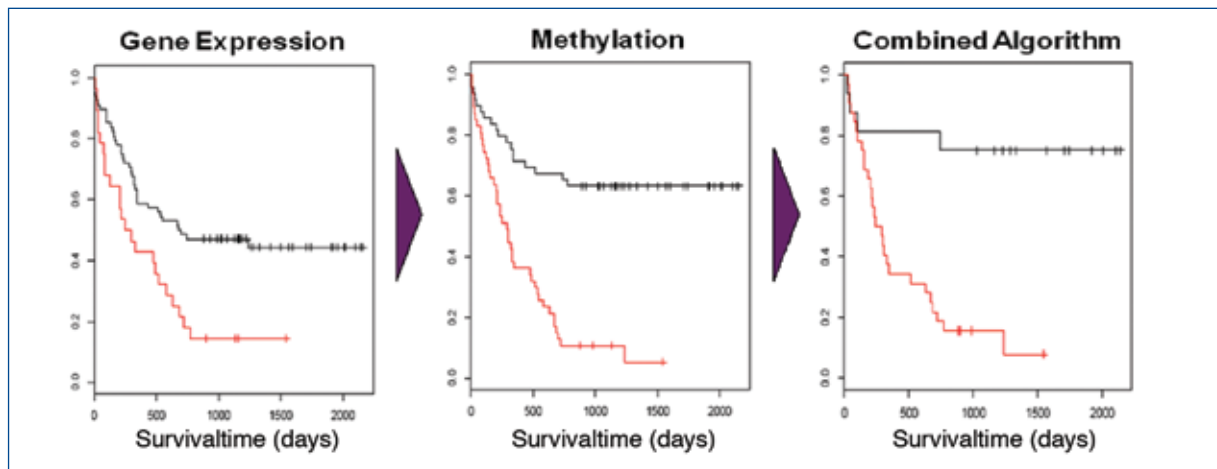


Figure 5: Kaplan-Meier Survival Estimates

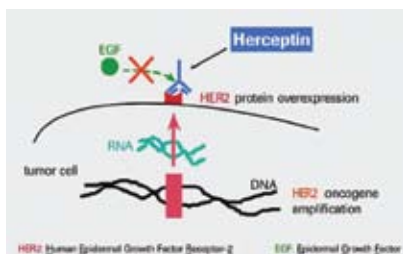


Sequenom for Oncology

Gene Amplification in Cancer

Gene amplification provides a hallmark for tumor initiation, classification, and treatment. The normal process of replication is flawed, and instead of making a single copy, many copies are produced. If an oncogene is included in the amplified region, over-expression can lead to deregulated cell growth. iPLEX Gold was used to measure HER2/neu gene amplification in breast cancer cell lines. The HER2 gene encodes for a receptor of the

Figure 6: Mechanism of Herceptin

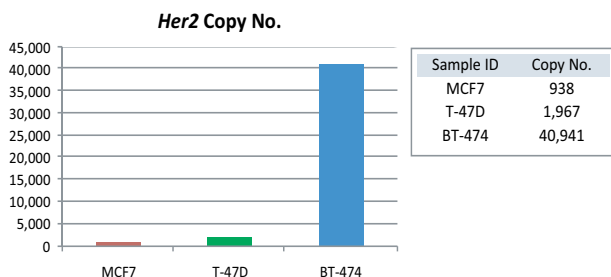


EGF receptor family and is amplified in approximately 30% of invasive breast cancer cases. The drug Herceptin® selectively blocks the receptor on the cell surface effectively reducing tumor growth (**Figure 6**).

In order to classify the tumor and to decide if Herceptin will be an effective treatment, tumor tissue is analyzed for over-expression of the receptor on the cell surface or gene amplification of the HER2 gene.

HER2 gene copies were assessed in three breast cancer cell line (**Figure 7**). The MCF7 line represents normal amounts of HER2 protein expression. T-47D has previously been shown to have a 2-fold increase in copy number compared to normal cell lines. BT-474 has significant gene amplification associated with high HER2 protein expression. Our data confirms previous results, and provides a highly accurate method for measuring copy number changes over a wide dynamic range. Multiple regions of interest can be queried within the same assay for rapid CNV analysis.

Figure 7: HER2 DNA Concentration



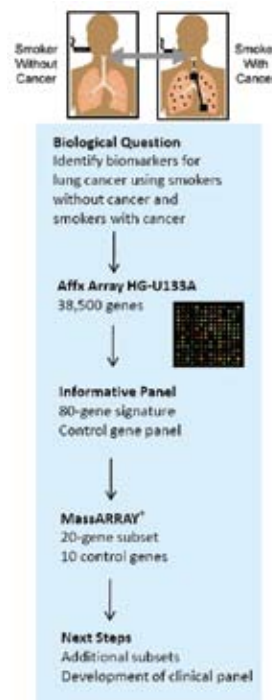
Biomarker Discovery & Validation

The MassARRAY system provides a powerful tool to follow-up on whole-genome expression arrays. Using iPLEX Gold chemistry, assays are routinely designed with up to 24 targets per multiplex. These assays can be used to analyze 100s to 1000s of samples for highly accurate validation studies.

- High multiplexing—up to 24 targets per well to improve cost-effectiveness and data quality
- High-throughput validation—investigate 100s to 1000s of samples to rapidly validate microarray data
- High sensitivity—detect as low as one copy per cell (attomolar concentrations)
- Flexibility—easily design and change assays based on your clinical research needs

In a recent collaborative study between Boston University and Sequenom, a sample population of smokers with and without cancer was profiled with an Affymetrix® HGU133A array to identify candidate lung cancer biomarkers (**Figure 8**). An 80-gene signature was identified from the array. Post-array validation was successfully performed using iPLEX Gold. We were able to design a single multiplex to query 20 of the key genes, and readily validate our initial results against a larger sample number.

Figure 8: MassARRAY for Post-Array Validation



■ Publications

1. Van Puijenbroek, M. et al. (2005)
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2. Stemke-Hale, K. et al. (2008)
"An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer."
Cancer Research 68 (15): 6084-91
3. Teng, T. et al. (2006)
"A transforming MET mutation discovered in non-small cell lung cancer using microarray-based sequencing."
Cancer Letters 239, 227-233.
4. Thomas, R.K. et al. (2007)
"High-throughput oncogene mutation profiling in human cancer."
Nature Genetics, doi: 10.1038/ng1975."
5. Ehrich, M. et al. (2006)
"Cytosine methylation profiles as a molecular marker in non-small cell lung cancer."
Cancer Res 66 (22): 10911-10918.

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