Sequenom Applications Overview

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SEQUENOM®
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About the Company

Sequenom is an established genomic analysis company with two principal divisions: genetic analysis systems and reagents, and noninvasive diagnostics. As an industry innovator in DNA detection technology, Sequenom developed the bench-top MassARRAY® Compact Analyzer, a high-performance nucleic analysis platform that utilizes matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to provide a direct physical measurement of the genetic target material and variations therein. The system delivers precise, quantitative data from complex biological samples and from genetic target material that is only available in trace amounts. The MassARRAY System is used by respected genetics institutions worldwide to perform SNP genotyping/fine mapping, to validate whole-genome studies, and for routine applications that employ fixed SNP panels. In addition to genotyping, the MassARRAY is used for quantitative methylation and gene expression analysis. Sequenom continues to refine these core applications and has developed several new applications for copy number variance (CNV) analysis, oncogene mutation profiling, and comparative sequence analysis for bacteria and viruses. On the diagnostics front, Sequenom is developing a comprehensive portfolio of prenatal test methods by combining the versatility of the MassARRAY® system with the Company's SEQureDx™ technology, which enables the detection of circulating cell-free fetal nucleic acids in a maternal blood sample.

Summary:

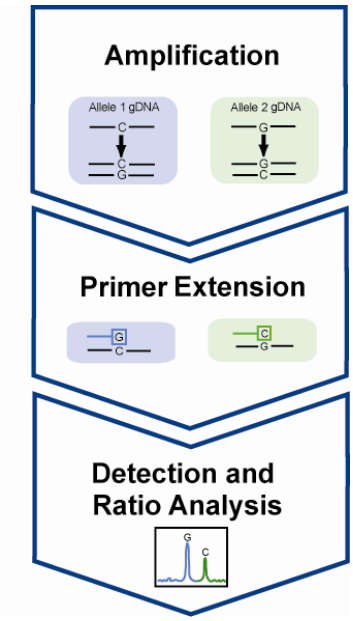
- Established Genomic Analysis Company- over 10 years in business, worldwide distribution
- Two principal divisions- genetic analysis and diagnostics
- High performance nucleic acid analysis platform, MassARRAY Compact Analyzer is a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) instrument
- MassARRAY technology enables the following research use only applications:
    - SNP genotyping/validation
    - Quantitative methylation analysis
    - Quantitative gene expression analysis
    - Copy number variance analysis
    - Oncogene mutation profiling
    - Comparative sequence analysis
- Sequenom is pioneering noninvasive diagnostic tests

Genotyping

Description

Sequenom's iPLEX® Gold assay is a leading technology for SNP genotyping and fine mapping for sub-whole genome study applications. The iPLEX Gold assay combines the benefits of simple and robust single-base primer extension biochemistry with the detection sensitivity of the MassARRAY® System (1).

Figure 1: iPLEX® Gold Genotyping Assay



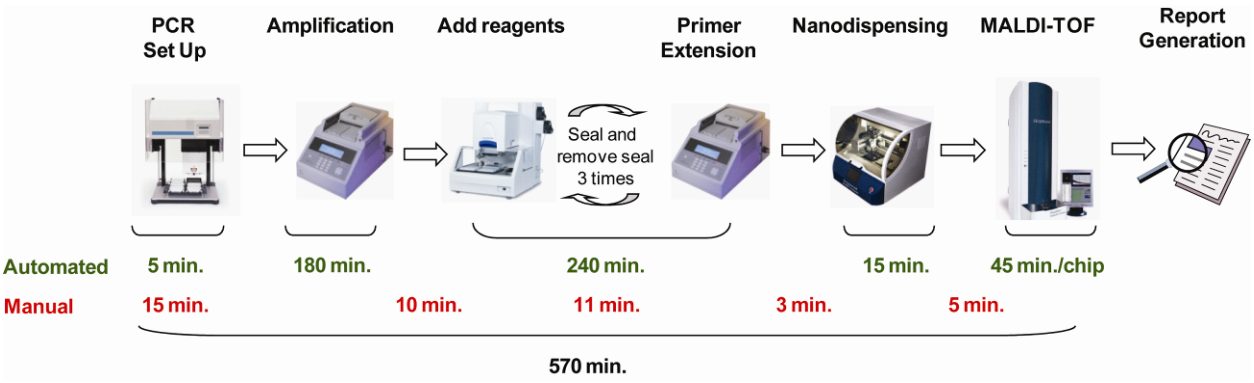
Assay Design – The MassARRAY® System Designer software can automatically design both PCR and MassEXTEND® primers for multiplexed assays. The software has a proven design efficiency of over 95% with a throughput of more than 10,000 assays per day.

MassEXTEND Reaction – MassEXTEND® is a primer extension process designed to detect sequence differences at the single nucleotide level. The iPLEX® Gold assay uses a single termination mix and universal reaction conditions for all SNPs. The primer is extended, dependent upon the template sequence, resulting in an allele-specific difference in mass between extension products. This mass difference allows the data analysis software to differentiate between SNP alleles.

Genotype Calling and Results – After the extension products are spotted on to SpectroCHIP® arrays, the arrays are placed into the MALDI-TOF mass spectrometer. A 384 position SpectroCHIP® array is typically processed in 45-60 minutes. The results are automatically loaded into a database that allows convenient data analysis.

The iPLEX Gold assay is ideal for analyzing several hundred SNPs in 100s to 1000s of samples. The assay is scalable and cost effective, with a simple workflow and convenient design and analysis tools.

Figure 2: MassARRAY Workflow



Advantages

One of the key strengths of the iPLEX Gold assay lies in its multiplexing capabilities. Genotyping reactions can be multiplexed with up to 40 SNPs in each individual reaction, allowing for throughput levels of up to 150,000 genotypes per instrument, per day. The assay design is automated and straightforward, and all primer oligonucleotides are unmodified standard quality, meaning that reagent ordering takes significantly less time than competing technologies. The multiplexing capabilities, convenient assay design tools, and the use of simple reagents allow an extremely rapid turn-around time from experimental design to results using iPLEX Gold, compared to sequencing or bead-based technologies.

Another significant advantage of using the iPLEX Gold assay for SNP genotyping is the ability to obtain highly precise, quantitative results. Most competitive technologies do not rely on direct detection methods such as mass spectrometry, but instead use indirect measurements such as hybridization and/or labeling. Compared to existing sequencing methods, the iPLEX Gold assay provides a significant advantage for the sensitive and quantitative detection of low frequency mutations.

Summary

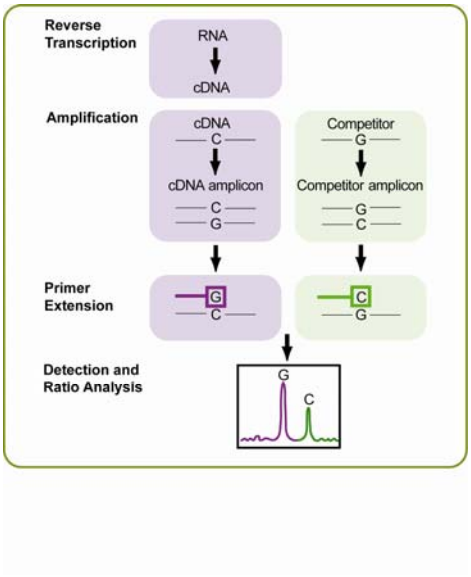
- The MassARRAY platform with the iPLEX Gold genotyping assay constitutes a complete fine mapping solution for genotyping
- High multiplexing (up to 40 assays in one reaction) allow for throughput levels of up to 150,000 genotypes per instrument, per day
- Semi automated workflow, convenient assay design tools and unmodified oligos provide ease of use and rapid assay turnaround
- Obtain highly precise and quantitative data
- Direct detection methods allow detection of low frequency mutations

Quantitative Gene Expression

Description

MassARRAY QGE is the ideal solution for multiplexed gene expression analysis and post-array validation. It improves upon the sensitivity and accuracy of gene expression quantification expected from real-time PCR, and couples the benefit of higher throughput made available by medium-density arrays as depicted in the diagram below. MassARRAY QGE enables sensitivity to single copy number detection, high-level multiplexing, with minimal assay optimization. Independent studies have also shown a high rate of concordance between MassARRAY QGE, microarray data, and real-time PCR (2). MassARRAY QGE provides orders of magnitude greater sensitivity than real-time quantitative PCR, and permits very closely related genes to be assayed reliably and quantitatively (3).

Figure 3: Overview of Sequenom Quantitative Gene Expression



Total RNA or mRNA is reverse-transcribed to cDNA. The resultant cDNA and a synthesized competitor that differs in only one nucleotide undergo competitive PCR. Following amplification, remaining nucleotide triphosphates are deactivated by phosphatase treatment (not shown). A single base primer extension step is performed, and the primer extension products are quantitatively analyzed using MALDI-TOF MS.

MassARRAY QGE, cDNA and a synthetic competitor designed with a single nucleotide mismatch undergo real-competitive PCR (rcPCR). The same primers anneal to and co-amplify both templates with equal stoichiometry and kinetics, preserving the initial cDNA: competitor ratio. Co-amplification of the cDNA and competitor in the same PCR reaction ensures that quantification is not affected by the PCR reaction conditions. Since the cDNA concentration of any one transcript is unknown, a competitor titration is set up to determine the competitor concentration at which amplification of cDNA and competitor is equal, termed EC_{50}.

A standard curve, dubbed the competitor titration, is initially performed to determine the relative amount of cDNA within the sample. The log of the competitor concentration is plotted against the cDNA frequency to measure absolute copy number. A sample is run against a synthetic competitor during each assay. This feature helps to control for inter-assay variability since each reaction contains an internal standard (competitor) that uses the identical primer set and reaction conditions as the gene being evaluated. Once the approximate range of target concentration is determined, the number of titration points can be minimized to reduce set-up and reagent costs.

Advantages

The benefits of MassARRAY QGE include:

- Higher sensitivity from end-point measurement.
- Greater multiplexing capability due to non-fluorescent detection.
- Less PCR optimization made possible by universal reaction conditions, end-point measurement, and computational primer design.

In addition, a wide range of expression levels from 1 to >2.83 x 10^8 copies can be quantified within the same multiplex if desired. See specifications below for more information.

Table 1: QGE Specifications

Property	Specification
Limit of detection	1 copy per reaction
Precision	~10% CV
Specificity of detection	Single nucleotide
Dynamic range of detection	15 logs with titration; 2-3 logs without titration
Assay format	96, or 384 spots per chip
Multiplex capability	1-25
Input total RNA	5 pg-1 µg
Chips per shift	6 (Compact)
Samples per shift (1-plex)	2K (Compact)
List price per reaction	$1.20*

MassARRAY QGE Applications

MassARRAY® QGE can be used for the following applications:

- Allelotyping (see below)
- Splice Variant Analysis (see below)
- Viral load determination (see below)
- Copy number variance (see page 12)
- Biomarker characterization
- Loss of heterozygosity

Allelotyping

The process for analyzing allele-specific expression is similar to that used when conducting disease-association SNP/gene screens with pooled DNA populations, with the exception that cDNA is used as a template for PCR as opposed to genomic DNA. These methodologies work by comparing the ratio of alleles from one group to another to determine if any statistically significant difference in allele frequency exists between them. When assaying for allele-specific expression differences, it is also important to establish that no significant assay bias exists at the genomic DNA level. This is achieved by assaying both cDNA and genomic DNA samples from the same individual for the same cSNP. Since genomic DNA alleles exist at a 1:1 ratio in heterozygous individuals, the frequency of each allele should be equal. If any bias is observed, then it should be accounted for when comparing allele ratios in the cDNA. In addition to comparing the ratio of alleles to determine differences in transcript levels, the number of each cDNA molecule present in the reaction can also be determined using competitive PCR coupled with MALDI-TOF MS and the MassEXTEND procedure. In the case of allele-specific expression, a third allele representing the competitive template is introduced and its concentration is titrated to determine the transcript levels of each of the wild-type alleles. With this process, the difference in transcript levels correlated with each allele can be precisely determined (4).

Alternative Splicing

Researchers at the University of Pennsylvania School of Medicine used MassARRAY® Quantitative Gene Expression to help discover that introns associated with mRNA are an important molecular guide to forming nerve cell electrical channels (5, see also 6). The MassARRAY QGE method combines real competitive polymerase chain reaction (rcPCR) in conjunction with MALDI-TOF mass spectrometry to

quantify nucleic acid levels. MassARRAY QGE was the technology of choice to determine the levels and endogenous populations of intron-containing transcripts because it used fewer exon-spanning extension primers to identify multiple, alternatively-spliced transcripts and could reliably detect and quantify low abundance transcripts. These experimental results are the first evidence that an intron-containing RNA outside of the nucleus serves a critical cellular function.

Viral Load Determination

Researchers at the University of Michigan Medical School use Sequenom Quantitative Gene Expression technology to detect HPV DNA in the serum or peripheral blood fraction (PBF) of patients with cervical carcinoma, head/neck cancer, or bladder cancer caused by schistosomiasis infection (7). The authors compared two methods of detection: real-time fluorescence-based PCR assays, and real competitive PCR and mass spectrometry using the MassARRAY platform. The results clearly show that the MassARRAY detection sensitivity was superior to that of fluorescence-based PCR assays, establishing it as the most sensitive, specific, and quantitative detection method for monitoring occurrence, recurrence, and potential treatment efficacy of malignancies and dysplasias associated with HPV. HPV-16 DNA was detected in all of the tested schistosomiasis-associated bladder cancer samples, whereas the fluorescence-based PCR technique was not reproducible or accurate. Both head/neck and cervical tumors result in liberation of HPV DNA into the serum and/or PBF, providing the means to monitor tumor occurrence and treatment. MassARRAY for QGE can detect HPV-16 DNA with the highest sensitivity, which is superior to fluorescence-based PCR assays, *in situ* hybridization, and other ELISA-based DNA tests, while maintaining critical levels of specificity.

Copy Number Variation

Description

Human genomic variation ranges from large, microscopically visible chromosomal rearrangements to single nucleotide polymorphisms (SNPs). Although copy number variations (CNVs) occurring in large chromosomal segments have been described for many years, the ubiquitous presence of submicroscopic variations (> 1 Kb to several Mb) are a focus of investigation. Many studies are being undertaken to examine the roles these smaller CNVs play in population diversity and in disease response. Sequenom has developed three methods for CNV analysis using the MassARRAY® platform, combining design flexibility, high resolution, and absolute or relative quantification of CNV regions.

Sequenom has developed two methods for analysis of putative CNV regions that contain characterized SNPs. Using the SNP allele ratio (SAR) approach, you can determine whether a heterozygote sample deviates from the core heterozygote cluster, which could indicate CNV. Using the allele-specific copy number (ASCN) approach, a competitor amplicon is designed with a single nucleotide mismatch that serves as the internal standard for absolute, allele-specific quantification. ASCN is performed using previously-described real-competitive PCR (rcPCR) followed by iPLEX primer extension and detection on the MassARRAY® platform for absolute quantification of your CNV region of interest.

Absolute quantification for CNV regions combines real-competitive PCR (rcPCR) with the iPLEX primer extension reaction, followed by detection and ratio analysis. Examining CNV regions via the ACN method takes advantage of a competitor amplicon designed with a single nucleotide mismatch that serves as the internal standard for absolute quantification. The assay design is flexible, and does not rely on existing SNPs. In addition, ACN assays can be designed with up to 24 targets in the same plex.

Advantages

The SAR and ASCN methods are useful for validating whole-genome array experiments or investigating suspected CNV regions as a follow-up to targeted SNP genotyping studies:

- Using the SNP allele ratio (SAR) approach, you can determine whether a heterozygote sample deviates from the core heterozygote cluster, which could indicate CNV.
- ASCN is performed for absolute quantification of your CNV region of interest.

The ACN method provides highly quantitative, informative data for absolute copy number, and can be used for:

- Validating genome-wide data following array CGH
- Creating a high resolution quantification for a select panel of CNVs

Summary

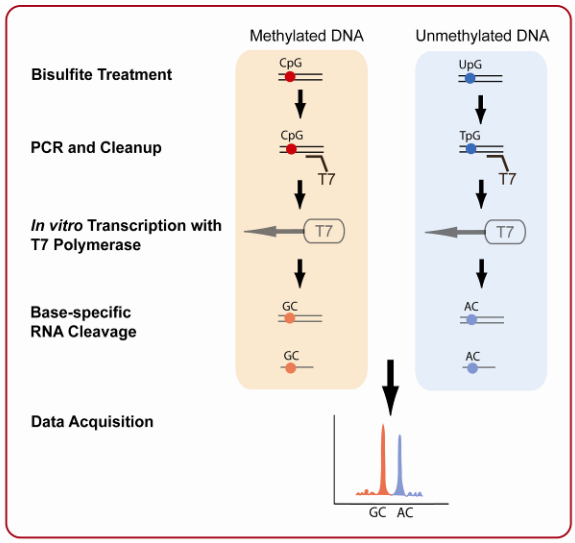
- You can perform absolute or relative quantification of CNV using MassARRAY, starting with existing SNPs or without prior knowledge of SNPs
- Methods are based closely on iPLEX and QGE assays, with simple software script modifications
- Sequenom's CNV analysis allows you to validate genome wide data following array CGH methods, create your own panel of CNVs, or quantify your CNVs of interest in many samples

Methylation

Description

DNA methylation and related chromatin changes play essential roles in regulation of gene expression. Hypermethylation of CpG dinucleotides within regions known as CpG islands in the 5' regulatory regions of genes is a well-characterized means of transcriptional silencing (8, 9). Sequenom has developed a comprehensive suite of products and tools for methylation analysis. Sequenom's EpiTYPER® technology allows precise and quantitative DNA methylation detection based on bisulfite conversion, MassCLEAVE™ chemistry and MALDI-TOF-MS.

Figure 4: Overview of EpiTYPER DNA Methylation Analysis



In bisulfite-treated methylated DNA, CpGs are unchanged (left, red) while in unmethylated DNA, CpGs are converted to UpG (right, blue). DNA is PCR-amplified with reverse primers that add a T7 polymerase binding site. During PCR in unmethylated DNA, uracil is converted to thymidine (TpG). *In vitro* transcription with T7 polymerase produces RNA, and base-specific cleavage results in RNA fragments. MALDI-TOF mass spectrometric (MS) analysis differentiates fragments corresponding to methylated CpGs (GC) from those with unmethylated CpGs (AC).

Bisulfite treatment of genomic DNA converts non-methylated cytosine into uracil while methylated cytosines remain unchanged. Next, a PCR amplification step is carried out to yield an amplicon with a T7 promoter tag. The advantage of this method is that the PCR primers are independent of the genomic DNA methylation state, meaning they bind to both methylated and non-methylated template, as opposed to methylation-specific primers. Only two primers are needed to screen for methylation changes within a region of several hundred bases in a single experiment whereby the length of the PCR amplicon is only limited by the degradative side effects of the bisulfite treatment to the template. Next, *in vitro* RNA transcription is performed on the reverse strand, followed by RNase A cleavage at specific bases (U or C). Cleavage products are generated for the reverse transcription reactions for both U (T) and C in separate

reactions, and these are spotted on a chip containing a matrix compound to assist in laser deionization. Within the cleavage products, methylation-dependent C to T variation appears as G/A generated from the reverse strand by base-specific cleavage. These G/A variations result in a mass difference of 16 Da per CpG site, which is easily detected by the MassARRAY Compact Analyzer, resulting in a signal pair pattern from the methylated and non-methylated template DNA. The relative amount of methylation is determined by comparing the signal intensity between the mass signals of methylated and non-methylated template. EpiTYPER® generates quantitative results for each cleavage product analyzed. Each cleavage product encloses either one CpG site or an aggregate of multiple CpG sites. An analyzed unit containing one or multiple CpG sites is called a "CpG unit". For both T and C reactions, the resulting cleavage products have the same length and differ only in their nucleotide composition.

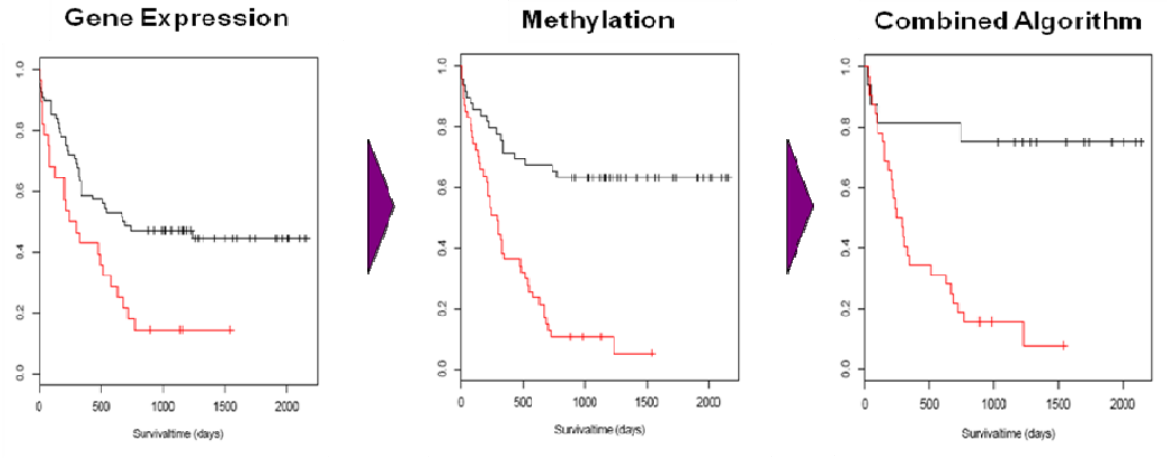
Advantages

MassARRAY® EpiTYPER technology represents a paradigm shift in quantitative DNA methylation analysis. The speed and accuracy of the MassARRAY® system enable cost-effective and quantitative analysis of the methylation status of multiple CpGs in one PCR amplicon. The data quality and reporting functions of EpiTYPER® make it the superior solution for methylation analysis, post-array validation, or targeted gene promoter analysis.

Large scale studies that evaluate quantitative methylation for multiple CpG sites in various gene regions and a large number of samples are difficult to implement using current technologies. The amount of information generated in such large-scale studies requires powerful data analysis solutions. Sequenom offers the EpiTYPER technology to enable high-throughput cytosine methylation profiling of candidate genes.

The relationship between changes in DNA methylation and gene expression is statistically significant. Here we demonstrate an integrated approach where genome-wide analysis using microarray data can be done to identify the genes that are differentially expressed. Once these genes are discovered, quantitative methylation analysis can be applied and a subset of methylation-regulated genes can be identified. EpiTYPER analysis can be used in combination with gene expression profiling to discover a clinically meaningful molecular marker set that is capable of accurately predicting survival. Figure 5 below illustrates Kaplan-Meier survival estimates, where a combined algorithm results in better cluster-defined analysis.

Figure 5: Kaplan-Meier Survival Estimates



Epigenetic Analysis Tools from Sequenom

EpiBrowser

Sequenom's EpiBrowser is a user-friendly tool that enables you to quickly browse the database and display genes/samples of interest quickly and logically in charts, tables, heatmaps and epigrams. The database not only provides information on the methylation status of CpG sites throughout the cancer related panel, but also provides validated primer sequences for amplifying the regions of interest that our team has interrogated. These validated primer sequences are available for download free of charge to facilitate your methylation studies.

EpiPanels

Sequenom has designed and validated assays for thousands of genomic regions. During the course of research and methylation service efforts over the past two years, we observed that a subset of genes is frequently analyzed. The methylation status of the genes in this subset is commonly subject to change, and is relevant to many areas of cancer research. In 2007, the Sequenom® Standard EpiPanel was compiled, representing the first high resolution, fine mapping panel for a subset of putative epigenetic targets. The Sequenom Standard EpiPanel offers the speed and convenience of using "off the shelf" validated amplicon designs. Using EpiTYPER® together with the amplicons found in the EpiPanel enables a first-of-its-kind combination of fast, inexpensive, and quantitative analysis that is not available elsewhere. For each gene, PCR amplicons were designed to cover the majority of CpG-dense areas in close proximity to, or overlapping with the annotated transcription start.

In each EpiPanel figure, a subset of amplicons to cover the entire CpG island is shown. All amplicon designs provide good quality results in our model system, but the preferred amplicon designs are indicated. A detailed table lists the amplicon name, position, length and CpG coverage of each set and a primer index lists the sequences to order for each amplicon.

Additional panels include amplicon designs to design CpG sites in promoter regions of genes known to be involved in neoplastic transformation and imprinting (Cancer EpiPanel), maternal or paternal imprinting (Imprinting EpiPanel), and in mouse studies (Mouse EpiPanel).

EpiDesigner

Assays for any genomic region of interest that are not currently included in the EpiPanels, assays can be easily designed using Sequenom's EpiDesigner software. This program allows simple design of primers for bisulfite treated genomic DNA and recommends primer pairs for individual assays.

Summary

EpiTYPER

- The most comprehensive and cost effective, high throughput, quantitative technology for DNA methylation analysis
- Target sequenced up to 600 bp in one reaction to quantify differential methylation within large promoter regions
- Delivers individual methylation rations forCpGs within a sequence

EpiBrowser

- Allows you to display methylation profiles of selected genes and samples

EpiPanels

- Pre-Designed, validated assays for quantitative finemapping
- Detailed tables list amplicon name, position, length, and CpG coverage
- Several panels available with commonly-studied genes, as well as genes involved in cancer, imprinting, and in mouse studies

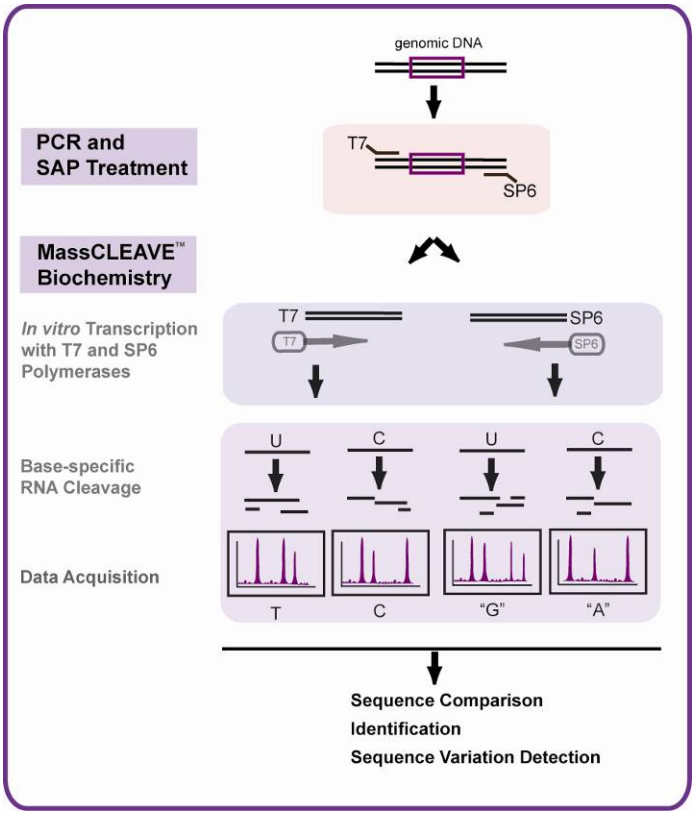
EpiDesigner

- A simple primer design tool for bisulfite treated genomic DNA

Molecular Typing

Sequenom has recently developed a new application for comparative sequence analysis and molecular typing for bacteria and viruses. iSEQ™ comparative sequence analysis enables analysis of one or more target regions in multiple samples in a homogenous assay format. The iSEQ software enables automatic sample identification and mutation detection by comparison with user-defined reference sequence sets.

Figure 6 Overview of MassARRAY® iSEQ Comparative Sequence Analysis



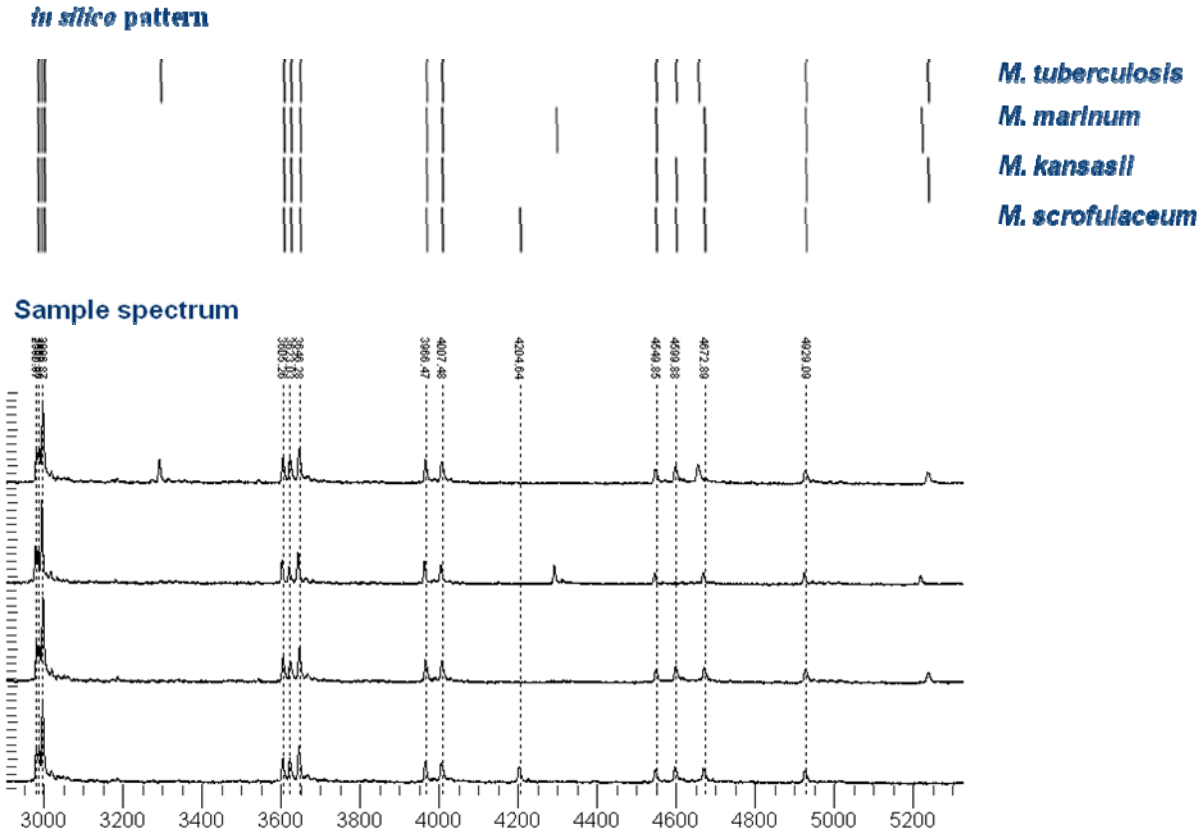
Microbial genomic DNA is PCR-amplified with forward and reverse primers that add T7 polymerase binding sites, followed by a cleanup step (not shown). *In vitro* transcription with T7 polymerase produces RNA, and base-specific cleavage results in fragments that differ by mass. The mass signal patterns obtained from MALDI-TOF MS are compared with a set of reference sequences (not shown) to identify the microbial DNA.

Sequences are used as the reference for the automated comparative analysis using the simulation tool. Users import a collection of sequences that you have either obtained from public or proprietary databases in FASTA format, and the simulation tool performs a simulated cleavage and then calculates the masses for all fragments. The simulation tool reports the sensitivity of the reference set cleavage patterns, and gives you the discriminatory power, which tells you that you can differentiate each sequence from the others. The biochemistry previously described is performed on samples, followed by automated dispensing and four spectra are acquired on the MassARRAY. The results are automatically generated by the software and the best matches to your reference sequence are called out as sample

identification. Any variations in the sequence compared to the reference are also flagged. These data can also be used for cluster analysis.

MassCLEAVE and MALDI-TOF mass spectrometry has been successfully used to identify environmental *Bordetella* strains (11-12) and *mycobacteria* (13) with 24 isolates unequivocally identified in the study. Figure 7 below exemplifies the identification and differentiation of four mycobacterial species on the basis of their mass signal fingerprints of the 16S rDNA hypervariable region.

Figure 7: 16s rDNA typing of mycobacteria using MassARRAY Comparative Sequence Analysis



Comparative sequence analysis by MassARRAY has also been applied to multi-locus sequence typing (MLST) in *Neisseria meningitidis* (14). MLST is a house-keeping gene based approach, which has been shown to determine population structures. In *N. meningtidis* typing results of a combination of seven marker regions determine the sequence type of the sample.

The iSEQ application for MassARRAY® can be employed in various fields including clinical microbiology, epidemiological and surveillance monitoring, biodefense, agricultural and food science applications,

forensics and basic clinical research. iSEQ offers a versatile, comprehensive solution that delivers superior accuracy, reproducibility, quicker detection time, and low running costs. Together with our genotyping and gene expression applications, a wide range of automated, high throughput and cost effective molecular typing schemes can be performed on Sequenom's MassARRAY System, including those based on SNP analysis, sequence comparison, or on quantification of microbial or viral RNA or DNA.

Summary

- iSEQ is used to rapidly type and identify bacteria, viruses and other haploid organisms
- Flexible, user-defined reference sequence database
- Comparative sequence analysis technology is automated and high throughput
- Mutation profiling and clustering analysis can be performed

Oncogene Profiling

Sequenom has developed an oncogene panel based on 238 oncogene mutations analyzed by Thomas *et al.* (15) using Sequenom genotyping by primer extension and MALDI-TOF mass spectrometry. The OncoCarta™ Panel v1.0 offers rapid, parallel analysis of over 230 simple and complex mutations across 19 common oncogenes.

Table 2: Oncogenes included in the OncoCarta panel

Oncogene	Mutations	Oncogene	Mutations
***ABL*-1**	G250E, Q252H, Y253H, Y253F, E255K, E255V, D276G, F311L, T315I, F317L, M351T, E355G, F359V, H396R	***JAK*-2**	V617F
***AKT*-1**	V461L, P388T, L357T, E319G, V167A, Q43X, E17del	***KIT***	D52N, Y503_F504insAY, W557R/R/G, V559D/A/G, V559I, V560D/G, K550_K558del, K558_V560del, K558_E562del, V559del, V559_V560del, V560del, Y570_L576del, E561K, L576P, P585P, D579del, K642E, D816V, D816H/Y, V825A, E839K, M552L, Y568D, F584S, P551_V555del, Y553_Q556del
***AKT*-2**	S302G, R371H	***MET***	R970C, T992I, Y1230C, Y1235D, M1250T
BRAF	G464R, G464V/E, G466R, F468C, G469S, G469E, G469A, G469V, G469R, G469R, D594V/G, F595L, G596R, L597S, L597R, L597Q, L597V, T599I, V600E, V600K, V600R, V600L, K601N, K601E	***PDGFRa***	V561D, T674I, F808L, D846Y, N870S, D1071N, D842_H845del, I843_D846del, S566_E571>K, I843_S847>T, D842V
***CDK*-4**	R24C, R24H	***PIK3CA***	R88Q, N345K, C420R, P539R, E542K, E545K, Q546K, H701P, H1047R/L, H1047Y, R38H, C901F, M1043I
EGFR	R108K, T263P, A289V, G598V, E709K/H, E709A/G/V, G719S/C, G719A, M766_A767insAI, S768I, V769_D770insASV, V769_D770insCV, D770_N771>AGG/V769_D770insASV/V769_D770insASV, D770_N771insG, N771_P772>SVDNR, P772_H773insV, H773>NPY, H773_V774insNPH/PH/H, V774_C775insHV, T790M, L858R, L861Q, E746_T751del, E746_A750del, E746_T751del, E746_T751del, S752D, L747_E749del, L747_T750del, L747_S752del, L747_T751del, L747_S752del, P753S, A750P, T751A, T751P, T751I, S752I/F, S752_I759del, L747_Q ins, E746_T751del, I ins (combined), E746_A750del, T751A (combined), L747_E749del, A750P (combined), L747_T750del, P ins (combined), L747_S752del, Q ins (combined)	***H-RAS***	G12V/D, G13C/R/S, Q61H/H, Q61L/R/P, Q61K
ERBB2	L755P, G776S/LC, G776VC/VC, A775_G776insYVMA, P780_Y781insGSP, P780_Y781insGSP, S779_P780insVGS	***K-RAS***	G12C, G12R, G12S, G12V, G12D, G12A, G12F, G13V/D, A59T, Q61E/K, Q61L/R/P, Q61H/H
***FGFR*-1**	S125L, P252T	***N-RAS***	G12V/A/D, G12C/R/S, G13V/A/D, G13C/R/S, A18T, Q61L/R/P, Q61H, Q61E/K
***FGFR*-3**	G370C, Y373C, A391E, K650Q/E, K650T/M	***RET***	C634R, C634W, C634Y, E632_L633del, M918T, A664D
***FLT*-3**	I836del, D835H/Y		

The OncoCarta panel consists of a set of pre-designed and pre-validated mutations for sensitive and efficient screening. Tumor samples from fresh, frozen or formalin-fixed paraffin embedded (FFPE) tissue as well as cell lines can be used. The assay consists of 24 wells per sample, and requires no more than 20 ng of DNA per well. The panel is suited for simultaneous identification of both wild type and up to 3 different mutant alleles are included. Only non-synonymous coding mutations previously reported to occur as somatic mutations in human cancers were selected for the OncoCarta™ Panel v1.0 (16).

Summary

- Pre-designed and pre-validated SNP panel for efficient and cost effective mutation screening
- Analyze 238 mutation over 19 oncogenes using ~ 500 ng DNA per sample
- Use fresh, frozen or FFPE samples
- Detect and quantify mutation frequencies
- For research use only, not for diagnostic use

Genetic Services

Sequenom offers a variety of applications to meet your research needs. Our dedicated services team offers customized project designs, fast turnaround times, and readily accessible expertise to translate your assays and samples into easy to read data.

Table 3: Sequenom's Genetic Services Department Offerings

Application	Description	Offering
Genotyping	Cost-effective SNP validation and fine mapping	– Assays by Sequenom for design and oligo mixing for MassARRAY® users. – Full-service assay design and production – Multiplexing up to 36 SNPs per well – High throughput capability for rapid sample screening
Methylation	Accurate, quantitative analysis of multiple CpG sites	– Read up to 600 bp per amplicon to interrogate multiple CpGs – Relative methylation ratios between 10-90% with 5% standard deviation – Choose from pre-validated assays from the EpiPanels – Have your own assays custom-designed
Gene Expression	Precisely measure RNA transcript levels from a variety of species and sample types	– Quantify as little as one copy per cell – Comprehensive data normalization for accurate quantification – Multiplexing up to 24 targets per well – High precision over a large dynamic range
OncoCarta™ Panel	Oncogene panel for somatic mutation profiling of human cancer tissue samples	– 238 mutations from 19 oncogenes in 24 wells – Key genes include *EGFR*, *KRAS*, and *BRAF* – Screen DNA from a wide variety of sample types, including FFPE – As low as 10% mutation frequency

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Appendix I: MassARRAY Compact Instrument Specifications

Table 4: Physical Dimensions of MassARRAY Compact Analyzer

Dimension	Imperial	Metric
Width	20.0 in	50.8 cm
Height	42.2 in	107.2 cm
Depth	26.5 in	67.3 cm
Weight	176.0 lb	80.0 kg

Table 5: MassARRAY Compact Analyzer Space Requirements

Direction	Imperial	Metric	Reason
Above	43.0 in	109.2 cm	To allow instrument to fit
Left and Right	24.0 in	60.1 cm	To allow outside doors to open for maintenance
Behind	6.0 in	15.2 cm	To allow access to power switch and space for power cable and ventilation

Sufficient laboratory space is crucial for successful installation and efficient maintenance of the instrument. Make sure the location of the instrument provides the minimum clearances described above. The work surface must not subject the instrument to vibration. The work surface must be able to support the weight of the instrument (176.0 lb/80.0 kg).

Table 6: Instrument operation conditions for MassARRAY Compact Analyzer

Condition	Acceptable Range
Temperature	65-75°F (18-24°C)
Humidity	20-50% Relative Humidity

The MassARRAY® Analyzer Compact can generate up to 2,000 Watts (6,830 BTU) per hour, which must be dissipated. The data system and additional equipment also contribute to the cooling load, although the exact amount of heat depends on the configuration.

Table 7: Electrical and power specifications for MassARRAY Compact Analyzer

Characteristic	Requirement or Description
Site Power	• 100-240 VAC, 47-95 Hz (single phase voltage) • Uninterruptable power supply • Properly grounded • It is recommended the receptacle providing power to the instrument be on a dedicated circuit • Receptacle must be within 4 feet of instrument
Voltage Detection	Instrument automatically detects and selects proper voltage within the range stated above
Power Consumption	3.0A maximum

Correct grounding of the electrical installation must be guaranteed by the customer:

- Do not use the neutral wire as the safety ground. The ground wire should be an isolated ground carrying zero (0) current except in the case of a fault.
- Interruption of the protective conductor may cause a shock hazard for the user and can damage the instrument.

MassARRAY Compact Analyzer Power Supply (North America and Japan)

- We strongly recommend using an uninterruptable power supply such as an ABCE800-11 or ABCE1100-11 or equivalent for each instrument. The specs are listed in the table below.

ABCE800-11	**ABCE1100-11**
Input Voltage: 120VAC 60HZ	Input Voltage: 120VAC 60HZ
Output Voltage: 120 VAC 60 HZ	Output Voltage : 120 VAC 60 HZ
Output Current: 6.7 amps	Output Current: 9.0 amps
Backup Time: 6/20 min (full/half load)	Backup Time: 6/20 minutes (full/half load)
Input Plug: 5-15P	Input Plug: 5-15P
Output Receptacles: (6) 5-15R	Output Receptacles: (6) 5-15R
Dimensions: 8.0”H x 5.8”W x 17.5”D	Dimensions: 9.0”H x 8.3”W x 19.5”D

MassARRAY Compact Analyzer Power Supply (Europe, Asia, and Australia)

- We strongly recommend using an uninterruptable power supply with the following specifications:

Input Voltage 240VAC 50 HZ
Output Voltage: 240 VAC 50 HZ
Min. Acceptable VA Rating: 2000
Input Plug: Local Type
Dimensions: as per manufacturer

An emergency-off stop switch may be installed to interrupt the electric circuits and other systems in an emergency or for maintenance.

MassARRAY Compact Analyzer Network Configuration Requirements

A configured port on your network must be available, and the IT department of your institution should be notified of the installation.

MassARRAY Compact Analyzer Safety Considerations

The standard nitrogen laser emits high intensity radiation at 337 nm, 150 µJ max
(Class IIIb laser classification).

Laser radiation emitted is invisible, but can cause damage.

To safeguard the user, both side doors of the MassARRAY® Analyzer Compact are equipped with locks to prevent users from accessing the laser. During normal operation mode with all covers closed, no laser radiation can be transmitted outside the instrument. The instrument uses high voltages. Access to high voltage cables and high voltage feedthroughs is restricted behind the side doors of the instrument as far as possible. Accessible high voltage connections are marked with danger signs according to regulations. Electromagnetic interference from NMR (nuclear magnetic resonance), cellular phones and radio transmitters may have a negative effect on system performance.

Do not bypass or disable any of the safety features associated with the MassARRAY® Analyzer Compact. Do not operate the instrument with the covers removed.

MassARRAY Compact Analyzer Required Components (Consumables and Equipment)

PCR machine:

- Thermocycler with 384 well blocks

Pipettes:

- One set of single channel pipettes (*e.g.* Eppendorf 2.5 µl, 20 µl, 200 µl and 1000 µl volume)
- Filter tips

Centrifuge:

- Plate centrifuge capable of at least 3,200 x *g*

Appendix II: Nanodispenser RS1000 Instrument Specifications

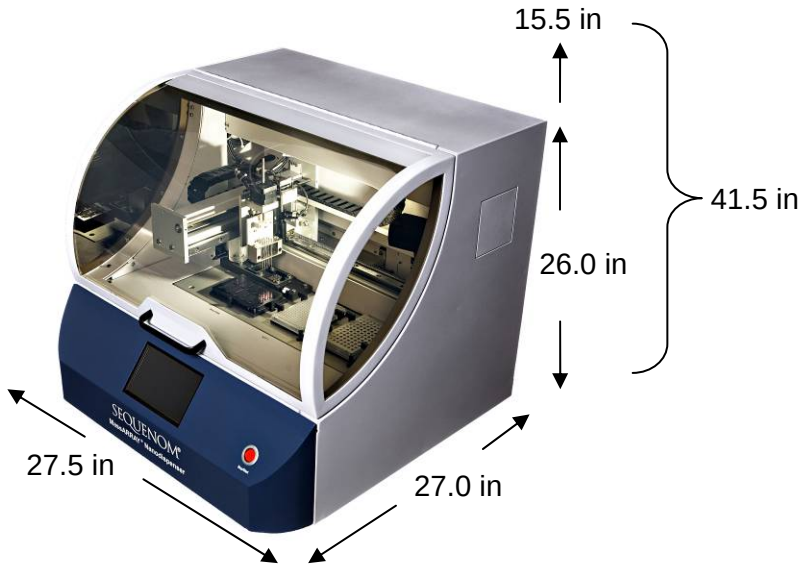

Table 8: Physical Dimensions of the Nanodispenser RS1000

Dimension	Imperial	Metric
Width	27.5 in	69.9 cm
Height	26.0 in	66.0 cm
Depth	27.0 in	68.6 cm
Weight	155.0 lb	70.3 kg

Table 9: Nanodispenser RS1000 Space Requirements

Direction	Imperial	Metric	Reason
Above	16.0 in	40.6 cm	To allow enclosure door to open fully
Left	12.0 in	30.5 cm	To allow access to supply and drain ports
Right	12.0 in	30.5 cm	To allow access to power switch and cable on back of instrument
Behind	6.0 in	15.2 cm	To allow access to power switch and space for power cable and ventilation

Sufficient laboratory space is crucial for successful installation and efficient maintenance of the instrument. Make sure the location of the instrument provides the minimum clearances described above. The work surface must not subject the instrument to vibration. The work surface must be able to support the weight of the instrument (155.0 lb/70.3 kg).

important

The MassARRAY® Nanodispenser RS1000 requires a source of deionized water (>18 MΩ).

Table 10: Environmental Conditions for Nanodispenser RS1000

Condition	Acceptable Range
Temperature	65-75° F (18-24°C)
Humidity	20-50% Relative Humidity

Table 11: Electrical and Power Specifications for Nanodispenser RS1000

Characteristic	Requirement or Description
Site Power	• 100-240 VAC 50-60 Hz 15A • Properly grounded • It is recommended the receptacle providing power to the instrument be on a dedicated circuit • Receptacle must be within 4 feet of instrument
Power Supply Fuse	T4AL 250V, 5X20 mm (for replacement only; two fuses)
Voltage Detection	Instrument automatically detects and selects proper voltage within the range stated above
Power Consumption	3.2A maximum

Hardware and Software Requirements

There are no requirements for hardware or software. The instrument has an integrated computer, touch screen, and control software. Optionally, you may connect an external monitor, keyboard, and pointing device. There is a VGA port to connect an external monitor. The monitor operates as a clone display of the touch screen on the instrument. Two USB 2.0 ports may be used to connect an external keyboard and pointing device.

The instrument has an Ethernet port for connection to a network. This port should be used only for sample tracking and processing data. It should not be used for general networking.

Required Components (Consumables and Equipment)

100% Ethanol

Appendix III: MassARRAY Liquid Handler Instrument Specifications

Table 12: Physical Dimensions of MassARRAY Liquid Handler

Dimension	Imperial	Metric
Width	25.5 in (+ 8.0 either side)	64.7 cm (+ 20.3 either side)
Height	27.0 in	58.0 cm
Depth	25.0 in	63.5 cm
Weight	110.0 lb	50.0 kg

Table 13: MassARRAY Liquid Handler Space Requirements

Direction	Imperial	Metric	Reason
Above	37.0 in	94.0 cm	To allow instrument to operate
Left & Right	24.0 in	60.9 cm	To place 4L Supply and Waste bottles on either side of the instrument
Behind	6.0 in	15.2 cm	To allow space for power cable and ventilation

Sufficient laboratory space is crucial for successful installation and efficient maintenance of the instrument. Make sure the location of the instrument provides the minimum clearances described above. The work surface must not subject the instrument to vibration. The work surface must be able to support the weight of the instrument (110.0 lb/50.0 kg).

The MassARRAY® Matrix Liquid Handler requires a source of deionized water (>18 MΩ).

Table 14: Environmental Conditions for MassARRAY Matrix Liquid Handler

Condition	Acceptable Range
Temperature	65-75°F (18-24°C)
Humidity	20-50% Relative Humidity

Table 15: Electrical and Power Specifications for MassARRAY Matrix Liquid Handler

Characteristic	Requirement or Description
Site Power	• 110-240 VAC 50/60 Hz • Properly grounded • It is recommended the receptacle providing power to the instrument be on a dedicated circuit • Receptacle must be within 4 feet of instrument
Voltage Detection	Instrument automatically detects and selects proper voltage within the range stated above
Power Consumption	<3.0A (with workstation and monitor)

The instrument should be powered off when changing cables or peripherals to avoid damage to electrical circuitry.

Water Supply

The MassARRAY® Matrix Liquid Handler requires access to a deionized water system producing >18 MΩ resistivity.