

High-Resolution Quantitative Methylation Profiling with EpiTYPER® and the MassARRAY® System



A complete system for discriminating methylated vs. non-methylated DNA; includes software, reagents, and MALDI-TOF mass spectrometry for detection and quantification.



Interrogate 10s-100s of samples and CpG sites in amplicons from 200 - 600 bp and detect down to 5% differences in methylation.



Validate methylation array, next gen sequencing, or gene promoter study results.



Referenced in over 400 publications in diverse fields, including cancer, differentiation and development, diagnostics research, metabolism, and epigenetics.

Overview

Agena Bioscience's DNA methylation analysis technology (EpiTYPER) is one of the most reliable quantitative methods available today for analyzing DNA methylation changes^{1,2}. The technology, which has been referenced in more than 400 peer-reviewed journal articles, includes the following components:

- EpiDesigner - software for genomic target selection and PCR primer design.
- EpiTYPER Reagent Sets- reagents and consumables for all downstream processes, following bisulfite treatment of DNA.
- MassARRAY Analyzer 4- MALDI-TOF mass spectrometer for robust and precise signal detection and quantification.
- EpiTYPER Reporting Software- for data analysis and graphical presentation of the level of methylation at each CpG site in each sample.

Benefits of the EpiTYPER DNA Methylation Analysis Technology

EFFICIENCY

- Go from bisulfite-treated DNA to data in 8 hours.
- Covers multiple CpGs in amplicons of up to 600 bp.
- Compatible with many sample types, including formalin-fixed paraffin-embedded tissue.

PRECISE & ACCURATE

- High precision (5% CV).
- High inter-laboratory reproducibility.

SENSITIVE

- Detects down to 5% change in methylation levels.

COST EFFECTIVE

- 24-, 96-, and 384-well formats available.
- Multiple CpGs analyzed in one simple reaction, from amplicons as long as 200-600 bp.

SIMPLE WORKFLOW

- No need to design CpG-specific primers.
- No PCR product purification needed.
- Ideal for investigating a few or several hundred target regions.
- Convenient software solutions for comparison between samples.

How it Works

EpiTYPER biochemistry starts with bisulfite treatment of genomic DNA, followed by PCR amplification of target regions. The reverse primers contain a T7 promoter tag. Next, *in vitro* RNA transcription is performed, followed by base-specific RNA cleavage. Finally, the cleavage products are analyzed using MALDI-TOF mass spectrometry (MassARRAY Analyzer). The methylated and non-methylated cytosine residues in the original genomic DNA are easily distinguished using EpiTYPER Software.

Workflow - From Assay Design to Results

STEP 1: ASSAY DESIGN

EpiDesigner is an online automated design tool for DNA methylation experiments on the MassARRAY System. Just enter your target sequences and the software determines primer designs for the most complete DNA coverage. In addition to optimized primer sequences, EpiDesigner delivers an easy-to-read graphical interpretation of the amplicons designed over your target regions, as well as annotating distinct CpG sites covered by the assays.

STEP 2: BISULFITE TREATMENT

Genomic DNA from all samples is extracted and treated with bisulfite. This treatment converts any non-methylated cytosine residues into uracil (shown below in red), while methylated cytosine residues (blue) are unaffected. This step results in the generation of methylation-dependent sequence changes in the DNA template.

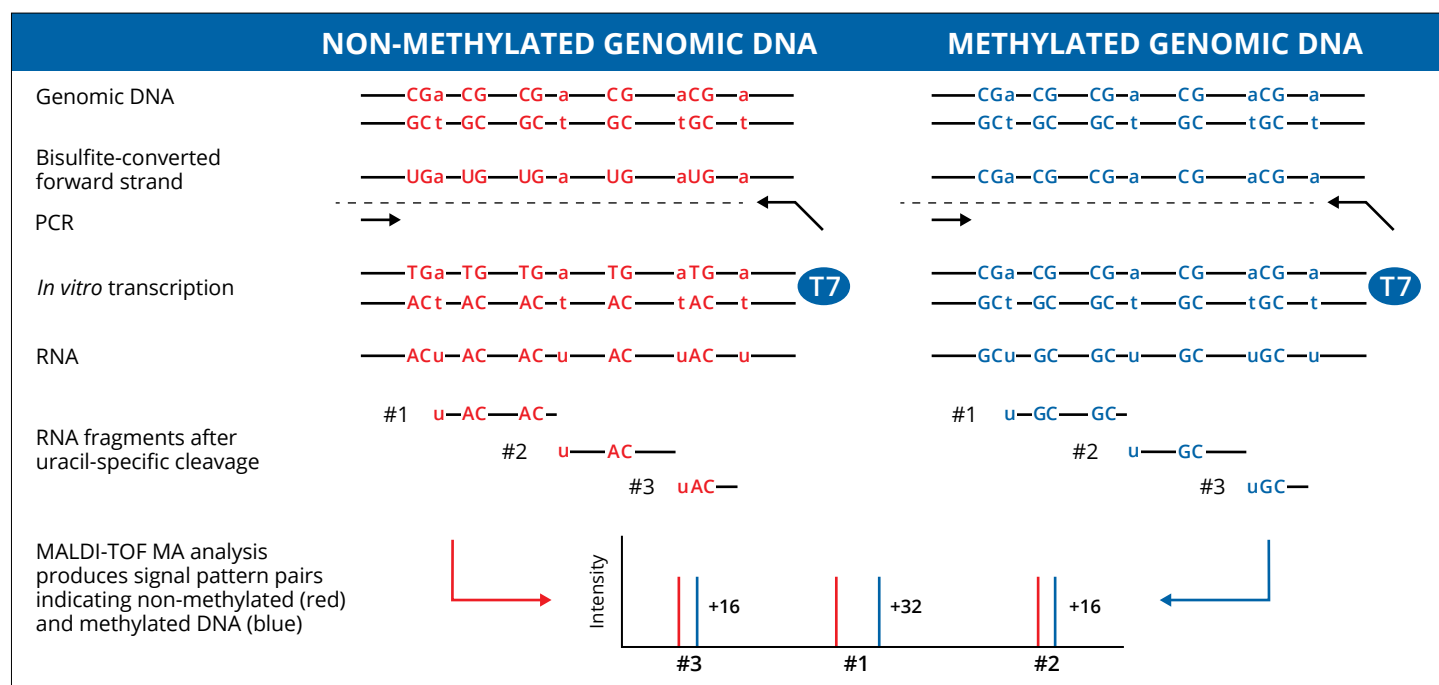
STEP 3: PCR, IN VITRO TRANSCRIPTION, AND RNA CLEAVAGE DESIGN

The EpiTYPER Assay starts with PCR using T7-promoter-tagged reverse primers to amplify the target regions while preserving the bisulfite-induced sequence changes. After SAP treatment, *in vitro* transcription is performed and the resulting RNA transcripts are specifically cleaved at uracil residues. The resulting fragments differ in size and mass, depending on the sequence changes generated through bisulfite treatment. This difference allows the data analysis software to generate quantitative information for each analyzed target fragment.

STEP 4: DATA ACQUISITION AND ANALYSIS

The EpiTYPER reaction products are dispensed onto a SpectroCHIP® Array (Chip). The Chip is then placed in the MALDI-TOF mass spectrometer for data acquisition, which typically requires 15-60 minutes. The results are automatically loaded into a database for data analysis with EpiTYPER software.

▼ **Figure 1. Overview of EpiTYPER Assay.** Nucleotides that determine U-specific cleavage are shown in lower-case for illustration purposes.



EpiTYPER Software

The EpiTYPER software provides an advanced and convenient solution for the quantitative analysis of CpG methylation. Numerical and graphic interpretation tools are available and the data are automatically matched to the provided sequence. Basic statistical analysis and confidence ratings are available for built-in quality control.

DATA ANALYZER MODE

Data Set Selection

- Clicking on one or both of these buttons will determine which data set is included in tab panes and EpiGram.

Spectrum Pane

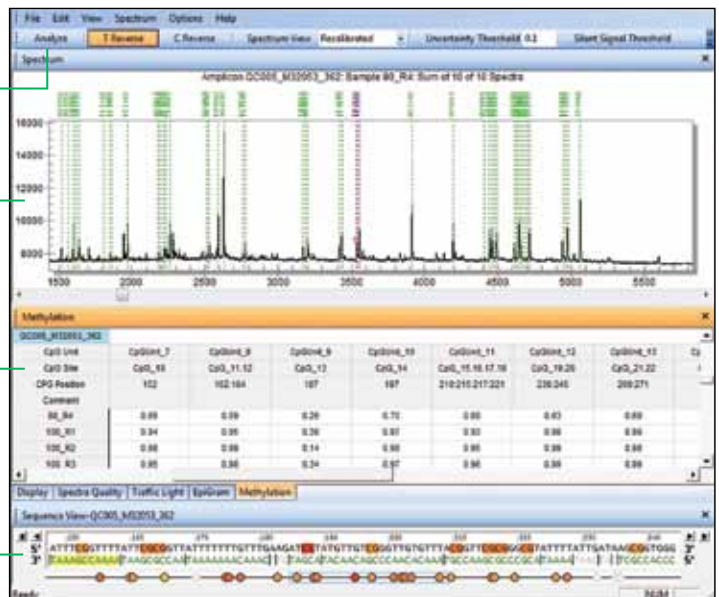
- Automatically displays the mass spectrum for all analyzed amplicons and identifies the selected CpG site (red arrow in spectrum).

Methylation Pane

- Obtain customized visual presentation of amplicon data in the most useful format for your needs.

Sequence View Pane

- This slender display shows the entire forward and reverse nucleotide sequence for the selected amplicon.



CUSTOM VIEWS OF AMPLICONS

Display Options

- These menus allow fine-tuning of program parameters, panes to display, and methylation color coding of CpG sites.

EpiGram Pane

- This pane provides graphical representations of the CpG sites within the selected amplicon. Each is color coded to represent the degree of methylation, providing a quick, reliable comparison between samples and CpG sites.



MassARRAY System – An Ideal Genetic Analysis System

EpITYPER DNA methylation analysis is performed on Agena Bioscience's MassARRAY System, which uses MALDI-TOF mass spectrometry for robust and precise signal detection and quantification.

The MassARRAY System can also be used for other nucleic acid analysis methods, including SNP and somatic mutation detection, and indel and CNV analysis.

All methods offer flexible assay design, fast time-to-results, and the ability to run from tens to thousands of samples daily. These features make it the ideal genetic analysis system for validation and fine mapping studies in basic and translational research settings.



The Complete Solution for Genomic Analysis

✓ HIGH PERFORMANCE

Accurate MALDI-TOF mass spectrometry detection provides unparalleled specificity and sensitivity for the most reliable results.

✓ MAXIMUM FLEXIBILITY

Analyze any combination of SNPs and samples to meet varying study requirements.

✓ HIGHLY SCALABLE

24-, 96-, and 384-well options for low to high-throughput applications.

✓ EASY TO USE

Data acquisition software streamlines your workflow, and robust assay design software automates primer design and optimization to maximize efficiency and minimize experimental variability.

✓ A VARIETY OF GENOMIC APPLICATIONS

Ready-to-use reagent sets for somatic mutation analysis, genotyping, methylation analysis, and quantitative applications (gene expression and copy number variation) provide a broad and flexible menu with short turnaround time.

✓ CHALLENGING SAMPLE TYPES

Due to the short amplicon lengths needed for the assays, virtually all DNA sample types are amenable to analysis on the MassARRAY System.

- | | |
|---------------------------------|-------------------------|
| • Blood plasma | • Micro-dissected cells |
| • Serum | • Buccal cells |
| • Fresh frozen tissue | • Ear punches |
| • Biopsy samples | • Hair follicles |
| • Formalin-fixed tissue samples | |

Ordering Information

Reagent sets are available in 24-, 96-, and 384-well formats, and are designed for use with the MassARRAY System with EpiTYPER software.

EpiTYPER COMPLETE REAGENT SETS

CAT. NO.	DESCRIPTION	FORMAT
11377	Complete EpiTYPER Methylation Reagent Set	10 x 384 Reagents, SpectroCHiPs, and PCR
11379	Complete EpiTYPER Methylation Reagent Set	8 x 24 Reagents, SpectroCHiPs, and PCR
10252	EpiTYPER Reagent and SpectroCHIP Set	10 x 384 Reagents and SpectroCHiPs
10249	EpiTYPER Reagent and SpectroCHIP Set	2 x 384 Reagents and SpectroCHiPs
10247	EpiTYPER Reagent and SpectroCHIP Set	2 x 96 Reagents and SpectroCHiPs

EpiTYPER ACCESSORY REAGENTS

CAT. NO.	DESCRIPTION	FORMAT
10219A	MassCLEAVE T7 Kit (T Cleavage)	10 x 384 Reagents only
11324	PCR Accessory and Enzyme Set (3,840 reactions)	10 x 384 PCR only
11327	PCR Accessory Set (3,840 reactions)	10 x 384 PCR only
01735	PCR Enzyme (3,840 reactions)	10 x 384 PCR only
10131	EZ 96 DNA Bisulfite Treatment Kit	192 Rxns Bisulfite kit
10132	EZ DNA Bisulfite Treatment Kit	50 Rxns Bisulfite kit

Published Studies Using the MassARRAY System



Visit **www.agenabioscience.com** to search our online database for published studies using the MassARRAY System in your area of interest.

REFERENCE

1. A systematic comparison of quantitative high-resolution DNA methylation analysis and methylation-specific PCR. Claus R, et al. Epigenetics 7(2012) 772-780.
2. Quantitative high-throughput analysis of DNA methylation patterns by base-specific cleavage and mass spectrometry. Ehrich M, et al. PNAS 102(2005) 15785-15790.

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