METHYLATION

# Methylation Profiling





## High-Throughput 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.



Agena® BIOSCIENCE

# Technology 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 1,000 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 SYSTEM** – 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

### Efficient

- 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

- 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.

# ASSAY WORKFLOW

## STEP 1 ASSAY DESIGN

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

Bisulfite treatment converts any non-methylated cytosine residues into uracil, while methylated cytosine residues 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

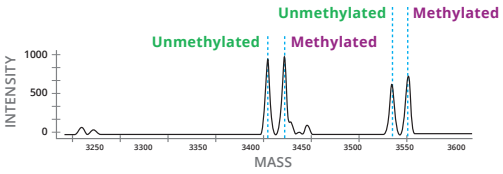

PCR with T7 Tag


RNA transcription and uracil-specific cleavage using RNAse A.

The EpiTYPER Assay starts with PCR using T7-promotertagged 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. The results are automatically loaded into a database for data analysis with EpiTYPER software.



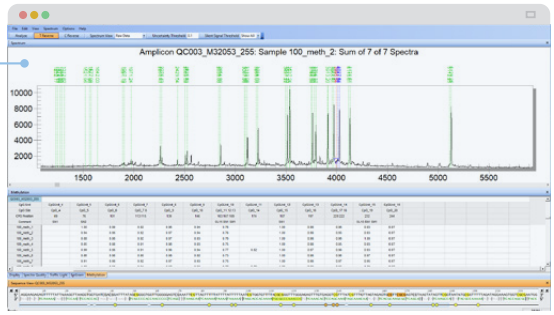

Easily determine percent methylation

# 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.

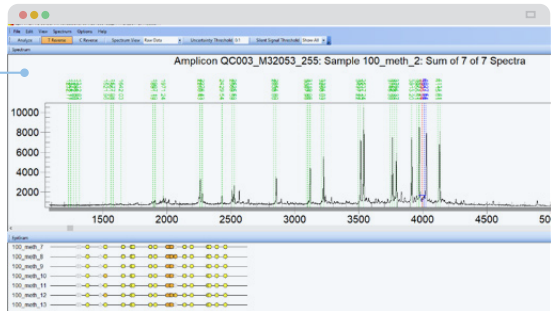
## DATA ANALYZER MODE

Customized visual presentation of amplicon data in the most useful format for your needs. Sequence view shows the entire forward and reverse nucleotide sequence for the selected amplicon.




## CUSTOM VIEWS OF AMPLICONS

Graphical representations of the CpG sites as epigrams within the selected amplicon enable easy comparison of samples and CpG sites.




## ORDERING INFORMATION

| Catalog No. | Item | # Wells/Pads | Chip Format |
|---|---|---|---|
| **MassCLEAVE Reagent Kits** | | | |
| 13165F | MassCLEAVE, PCR Reagent And SpectroCHIP Kit | 960 | CPM 96 |
| 11377D | MassCLEAVE, PCR Reagent And SpectroCHIP Kit | 3840 | CPM 384 |
| 13165 | MassCLEAVE, PCR Reagent And SpectroCHIP Kit | 960 | 96 |
| 11377 | MassCLEAVE, PCR Reagent And SpectroCHIP Kit | 3840 | 384 |
| **Bisulfite Conversion Kits** | | | |
| 10131 | EZ 96 DNA Bisulfite Treatment Kit (96-well Plate Format) | 192 | N/A |
| 10132 | EZ DNA Bisulfite Treatment Kit (Tube Format) | 50 | N/A |

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

## References


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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