ECSEQ BIOINFORMATICS

DNA Methylation Sequencing (Methyl-Seq)

Explore Epigenetic Regulation With Ease.

KEY BENEFITS

Trustable Results
Our Methyl-Seq methods have been tested and validated in many projects, i.a. the ICGC.

Expert Solutions
Rely on in-depth bioinformatics experience and up-to-date scientific methods.[1]

Rapid Results
Our skilled team of professional bioinformaticians delivers results rapidly.

Flexible Analyses
Employ an analysis strategy that is adjusted to your data.

High Data Security
Securely transfer and access your data and the results.

Explore & Share your Results
Comprehensive interactive HTML reports included.

Best-in-class Methods
Get the most biologically relevant information out of your data.

Excellent Support
Always have somebody to discuss your bioinformatics issues.

[1]Discover our epigenetics expertise at ecseq.com/analysis/epigenetics

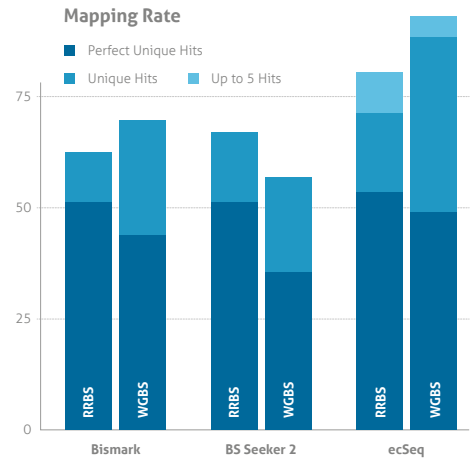
DNA methylation has moved into focus for biomedical researchers, and ecSeq is ready to help – with our new DNA methylation sequencing service, Methyl-Seq. Amongst other applications, Methyl-Seq facilitates the discovery of novel epigenetic biomarkers for the diagnosis and therapy of diseases (e.g. cancer) and can also be used for plant breeding selection. Current methods of next-generation sequencing (NGS) offer the opportunity to investigate this vital layer of epigenetic regulation in an unbiased and genome-wide manner. Sequencing bisulfite-treated DNA yields comprehensive and accurate methylation information at single-nucleotide resolution, and is therefore considered the gold standard. Additionally, DNA methylation sequencing can be integrated with genomic, transcriptomic, and histone modification data to assess their interplay and reveal a complete overview of the involved molecular processes.
Our researchers have proven their expertise in Methyl-Seq analyses in flagship projects such as the International Cancer Genome Consortium (ref. Kretzmer et al., Nature Genetics, 2016).

Bisulfite-specific Read Alignment

The conversions of unmethylated cytosines caused by bisulfite treatment turns NGS alignment into a new algorithmic challenge. We have solved this problem through our specifically designed mapping algorithm. In contrast to commonly used bisulfite aligners such as Bismark or BS Seeker 2, it is not based on pre- and post-processing alignments produced by DNA aligners and is therefore much more powerful (see Figure). In effect, our approach obtains higher mapping rates for uniquely aligned reads, even by limiting it to perfectly aligned ones. This leads to higher precision and robustness in downstream methylation rate estimates. Additionally, in contrast to Bismark and BS Seeker 2, our method optionally provides a portion of the non-uniquely aligned reads, e.g. reads with at most five alignments. This enables a broader view of the methylome by covering more genomic regions.

Methylation Profiling And Differential Methylation Analysis

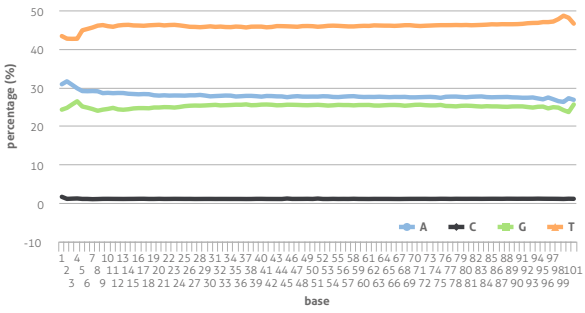
Methylation information is obtained from a set of reliable read alignments that are filtered using



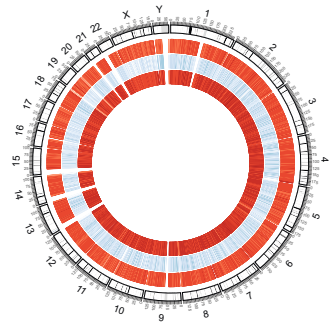
parameters adjusted to your experimental setup. Quality control measures are in place to detect and exclude bisulfite conversion failures, including incompletely converted bases as well as protocol-specific artefacts.
Methylation data can be grouped by treatment or condition to enable intra- and inter-group comparisons of specific methylation sites as well as of methylation in genomic regions such as promoters. Subsequently, a highly efficient and accurate differential methylation analysis method is used to identify differentially methylated regions (DMRs) and positions (DMPs). Methylation variation within groups is factored into the calculation of statistical significance to deliver highly reliable DMRs and DMPs for use as potential biomarkers.

Integrative Methylome Analysis

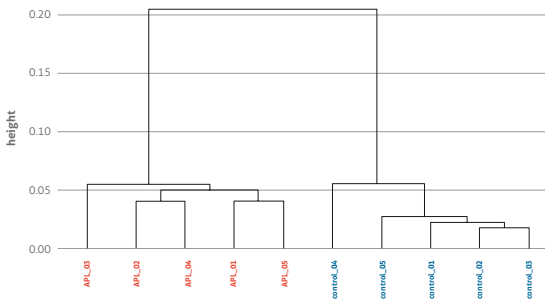
Interesting insights can be gained by integrating DNA methylation data with other (epi-)genomic or transcriptomic information. Correlation of promoter methylation data with gene expression data can help to reveal direct epigenetic mechanisms of gene regulation, while correlation with differential methylation of binding sites for specific transcription factors can help to reveal indirect epigenetic mechanisms. Moreover, the incorporation of histone modification data can give insights into the interplay between the two major categories of epigenetic markers, DNA methylation and histone modification. In addition, histone modification profiles can be used to classify regions (e.g., into active promoters, enhancers, etc.) which may give hints as to the functional impact of a differentially methylated region on the associated gene.



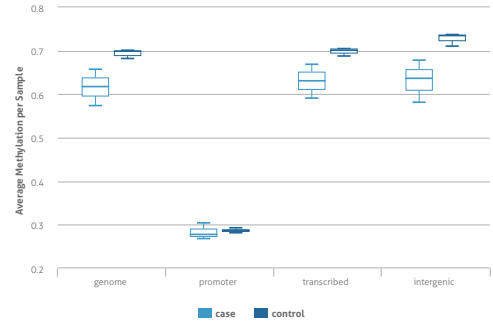
Comprehensive preprocessing options and quality control specific for bisulfite-treated sequence data



Read alignment and extraction of position-wise methylation information of samples and groups as well as group-to-group methylation differences.



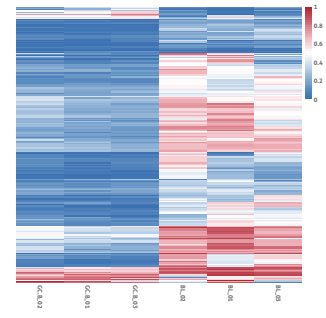
Inspect methylation and its variation among samples and groups using many visualization options.



Detection and annotation of differential methylated regions (DMRs) and positions (DMPs) among groups for detailed comparisons.

Position	Q-Value	Methylation Difference	Length (in Cs)	Length (in nt)	Annotation	Visualization
chr1:870,442-870,629	6.9e-10	0.68	10	187	SAMD11	UCSC / IGV
chr1:1,475,194-1,475,476	2.3e-11	0.60	10	282	TMEM240	UCSC / IGV
chr1:1,564,506-1,564,899	7.1e-22	0.38	33	393	MIB2	UCSC / IGV
chr1:2,706,395-2,706,841	4.4e-53	0.40	48	446	TTC34	UCSC / IGV
chr1:3,567,808-3,567,904	1.4e-11	0.45	13	96	TP73, WRAP73	UCSC / IGV
chr1:6,187,691-6,188,234	9.2e-12	0.46	19	543	CHD5	UCSC / IGV
chr1:10,698,816-10,699,407	6.1e-14	0.43	36	591	CASZ1	UCSC / IGV
chr1:11,714,385-11,714,538	0.0022	0.40	14	153	FBXO2, FBXO44	UCSC / IGV
chr1:13,909,647-13,909,677	6.8e-09	0.45	10	30	PDPN	UCSC / IGV

Work directly with DMRs and DMPs to quickly get to biological insights.



Readily integrate methylome data with information from other sources such as transcriptome, transcription-factor binding sites, or histone modifications.

Additional Analyses

- Correlation with gene expression data
- Integrate transcription factor binding and histone modification information
- Non-CpG Analysis
- Hydroxymethylation analysis
- Detection of partially methylated domains and allele-specific methylation (e.g. imprinting)
- Variation analysis
- GO Term Analysis

End-to-end NGS Solutions

For Methyl-Seq applications we also offer end-to-end analysis, including

- Sample Preparation
- Next-Generation Sequencing
- Bioinformatics Data Analysis

Contact one of our scientists and discuss your project!

Use the callback service on our website **www.ecseq.com** or write to support@ecseq.com

About Us

ecSeq Bioinformatics GmbH is a bioinformatics solution provider focusing on next-generation sequencing technologies. Since 2012 ecSeq Bioinformatics GmbH provides data analysis services and bioinformatics training for various NGS applications.

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