Apple
(Malus domestica)

Alligator
(Alligator mississippiensis)

Cow
(Bos taurus)

Barrel Clover
(Medicago truncatula)

Chicken
(Gallus gallus domesticus)

Platypus
(Ornithorhynchus anatinus)

Salmon
(Salmo salar)

Rat
(Rattus norvegicus)

Mouse
(Mus musculus)

Explore Epigenomics

with Next-Gen sequencing services

Shown here are some of the diverse species analyzed by our team

Human
(Homo sapien)

Wheat
(Triticum)

Dog
(Canis lupus familiaris)

Oppossum
(Didelphimorphia)

Soybean
(Glycine max)

Pig
(Sus scrofa domesticus)

Bean
(Phaseolus vulgaris)

Wine Grape
(Vitis vinifera)

Fruit Fly
(Drosophila melanogaster)

Rockcress
(Arabidopsis)

Baboon
(Papio anubis)

Guinea Pig
(Cavia porcellus)

Earthworm
(Lumbricina)

Cod
(Gadus morhua)

Sheep
(Ovis aries)

Zebrafish
(Danio rerio)

Zebra Finch
(Taeniopygia guttata)

ZYMO RESEARCH
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Overview

Epigenetic Analysis

DNA Methylation
Platforms for genome-wide and targeted single-base resolution DNA methylation analysis

MethylCheck™ Bisulfite Sequencing
Validate epigenetic markers from a large sample cohort or specific gene region

Epigenetic Biomarker Discovery Program
Start to finish development for your diagnostic test

DNA Hydroxymethylation: RRHP
Enrichment and single-base resolution platforms for detection of 5-hydroxymethylation in DNA

DNA Hydroxymethylation: Mirror-Seq™
Detect 5-hmC at single-nucleotide resolution with high sensitivity and low background

ChIP-Seq
Genome-wide analysis of protein-DNA interactions

Expression Services

RNA-Seq
Transcriptome-wide analysis of total RNA or small RNA (miRNA)

Epigenetic Aging Clock Service

Epigenetic Aging Clock
Gauge the biological age from a wide vareity of human samples

Additional Services

Mass Spectrometry
Global quantitative analysis of DNA methylation and hydroxymethylation levels

Custom Bioinformatics
Fully customizable bioinformatics solutions for the analysis of raw data from any of your Next-Generation sequencing experiments

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DNA Methylation Services

5-mC

Zymo Research offers four platforms for genome-wide DNA methylation analysis at single nucleotide resolution, each designed to suit your specific coverage needs. The main difference between the platforms is the percentage of the total genome actually being sequenced. All platforms accommodate a wide range of sample types, including any species with a reference genome, low-input (>10 ng), and FFPE samples.

Classic RRBS (Reduced Representation Bisulfite Sequencing) combines restriction enzyme digestion with bisulfite sequencing to enrich for a CpG-dense fraction of the genome. The Classic RRBS platform allows for a maximum amount of methylation data using a minimal amount of sequencing at a significantly reduced cost. This combination makes Classic RRBS the perfect platform for pilot studies. Classic RRBS covers ≥70% all CpG islands, >75% all gene promoters, and detects 1.5-2 million unique CpG sites at 5-10x average minimum coverage*.

Methyl-MiniSeq® is an expanded version of Classic RRBS. The system is extremely robust and the read depth is impressive, making it ideal for biomarker discovery using identification of differentially methylated regions. The low cost of this platform relative to the sequence data it produces also makes Methyl-MiniSeq® a good platform for pilot studies. Methyl-MiniSeq® covers ≥85% all CpG islands, >80% all gene promoters, and captures approximately 4 million unique CpG sites at 5-10x average minimum coverage*.

Methyl-MidiSeq® extends coverage to include a large majority of genetic regulatory elements (enhancers), gene bodies, and repeat DNA sequences that Classic RRBS and Methyl-MiniSeq® do not capture due to low CpG density in those regions. Methyl-MidiSeq® allows for the detection of 8-9 million unique CpG sites at 5-10x coverage.

Methyl-MaxiSeq® is a whole-genome bisulfite sequencing (WGBS) option that provides DNA methylation information at single nucleotide resolution in CpG, as well as in the less common CHG and CHH contexts, across all regions of the genome.

The Basic Service package for each platform includes sample standardization, library construction, sequencing, and raw data alignment. The Full Service package offers additional downstream bioinformatics processing and statistical analysis.

*Coverage estimates based on the human genome.

Service Option	Classic RRBS	Methyl-MiniSeq®	Methyl-MidiSeq®	Methyl-MaxiSeq®
Capable with low DNA input?	Yes	Yes	Yes	Yes
Single-base Resolution?	Yes	Yes	Yes	Yes
Methylome Coverage*	1.5 - 2 million sites	3 - 4 million sites	8 - 9 million sites	Entire methylome
Quantitative Analysis?	Yes	Yes	Yes	Yes
Genomic Regions covered	Nearly all CpG islands and gene promoters	Twice as many unique CpG sites compared to Classic RRBS	Also includes gene bodies and regulatory regions (90% of enhancers)	Entire methylome
Notes	Efficient genome-wide analysis	Robust biomarker discovery	Expanded methylation analysis	Complete methylation analysis

* calculation based on human genome
** depends on capture efficiency and methylation levels

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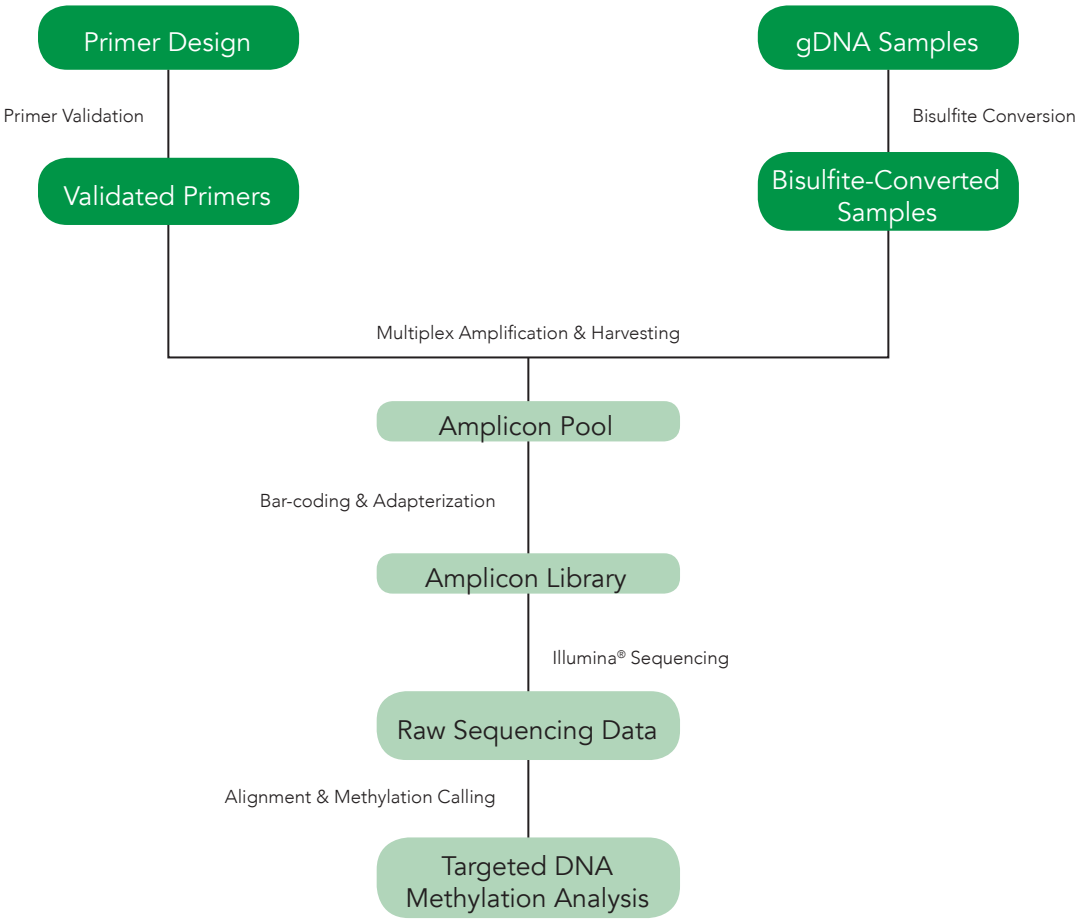
MethylCheck™

Zymo Research makes epigenetic biomarker validation simple with our MethylCheck™ platform. Whether you have methylation array (27K/450K/850K) data that you would like to validate in a large sample cohort or have a specific gene region in mind, our scientists are available to design, validate, and evaluate site-specific DNA methylation changes. Simply send us your samples and regions of interest, and we will perform every step through data analysis, sending you back publication-quality graphs and figures.

The Targeted Bisulfite Sequencing Service Includes:

- Primer Design and Validation
- Targeted Amplification
- Adapterization and Barcoding
- Sequencing with Illumina® Technology
- Sequence Alignment to Reference Genome
- DNA Methylation Analysis



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Epigenetic Biomarker Discovery Program

From Collection to Conclusion

Zymo Research offers a new Epigenetic Biomarker Discovery Program for the development of epigenetic lab diagnostic tests. Whether you are interested in developing epigenetic tests for cancer, developmental disorders, autoimmune diseases, obesity and other anomalies, Zymo Research provides a solution for sample collection through to commercial development. The experts at Zymo Research can help you at any step in the development pipeline by offering a portfolio of products and services for sample collection and purification, biomarker discovery, biomarker validation, platform selection and commercial development.

Sample Collection & Purification

Zymo Research offers specialized collection devices and purification kits for tissues, feces, urine, blood and other biological specimens. Sample collection begins with DNA/RNA Shield™ which is an innovative stabilization reagent that allows samples to be stored and transported at ambient temperatures. DNA/RNA Shield™ does not require the need for refrigeration or specialized equipment and makes shipping your precious specimens to Zymo Research easy.

Biomarker Discovery: Epigenetic NGS Services

With the latest Next-Generation sequencing technologies for DNA methylation analysis, Zymo Research provides comprehensive services and bioinformatics analysis to help discover epigenetic biomarkers in your specific sample set. Zymo Research's Illumina® certified MethylSeq® platforms are each designed to suit your specific coverage needs.

Epigenetic Biomarker Validation

Zymo Research offers the simplest way to validate epigenetic biomarkers with our MethylCheck™ sequencing platform. Whether you have genome-wide DNA methylation (450K/850K array or RRBS) data or a particular gene region in mind, our scientists will design, validate, and evaluate site-specific DNA methylation changes.

Platform Selection

Once you have your specific biomarkers narrowed down and validated, Zymo Research will help you select the most sensitive and cost-effective platform for your lab diagnostic test. A wide range of citation-leading bisulfite and bisulfite-free methods are available to implement your test.

Commercial Development

Zymo Research's associates, Pangea™ CLIA-certified lab, will help you to bring your lab diagnostic test to the market.

PANGEA LABORATORY

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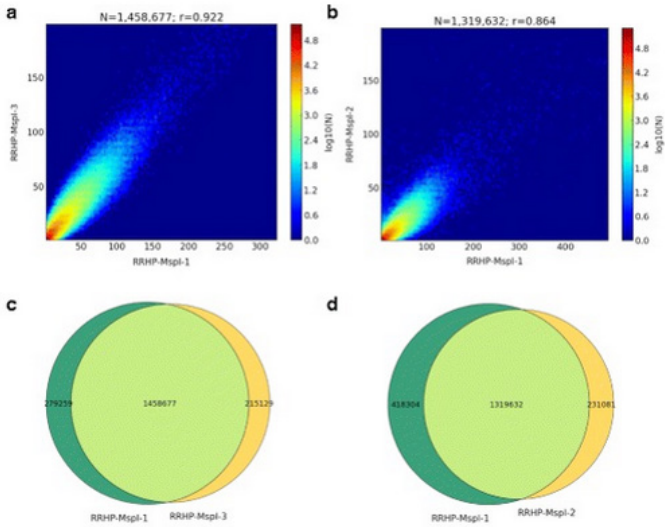
DNA Hydroxymethylation

5-hmC

Zymo Research's platforms for the analysis of DNA hydroxymethylation have unparalleled sensitivity and coverage of 5-hydroxymethylcytosine (5-hmC). With traditional bisulfite-conversion methods, 5-hmC cannot be distinguished from 5-mC. Therefore, Zymo Research has developed two platforms, Mirror-Seq™ 5-hmC and Reduced Representation Hydroxymethylcytosine Profiling (RRHP®), combined with Next-Generation sequencing to ensure high coverage and sensitivity for the detection of 5-hmC at single-base resolution. Mirror-Seq™ allows single-base quantification of 5-hmC sites while RRHP® allows genome-wide profiling for 5-hmC with reduced sequencing requirements.

Reduced Representation Hydroxymethylation Profiling - RRHP®

This service is for genome-wide profiling of 5-hydroxymethylcytosine in DNA at single-nucleotide resolution. RRHP® also allows strand-specific determination of the location of the 5-hmC modification as well as relative quantification of 5-hmC levels. Data from RRHP® can be combined with DNA methylation data from Methyl-MiniSeq®, allowing for direct comparison of DNA methylation and hydroxymethylation in the same sample. RRHP® is compatible with low DNA inputs and has the added advantage of providing read data for simultaneous SNP detection.



Replicate sample 5-hmC levels show very strong correlation when assessed using the RRHP® platform.
(Petterson A, Chung TH, Tan D, Sun X, Jia XY. Genome Biol. 2014 Sep 24;15(9):456.)

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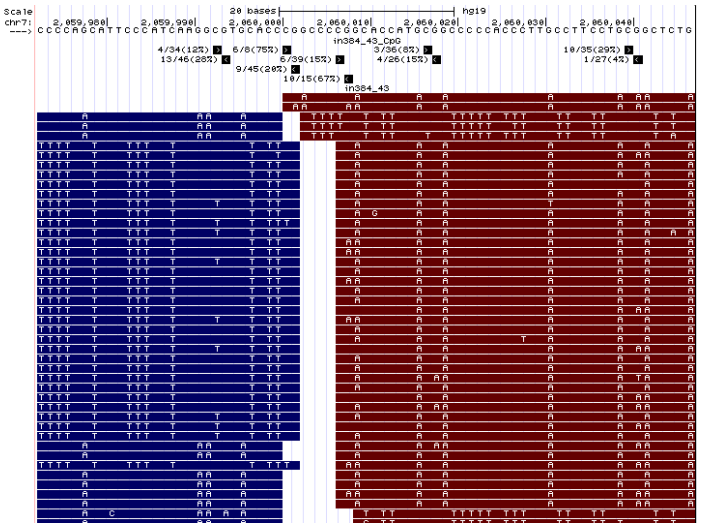
Mirror-Seq™

This innovative platform allows for the detection of 5-hmC at single-nucleotide resolution with high sensitivity and low background. The method consists of three main steps: (1) synthesis of a new mirroring strand to generate a semi-conservative duplex, (2) enzymatic treatment, and (3) bisulfite conversion. The glucosyltransferase enzyme specifically glucosylates 5-hmC residues in the parental strand. As a result, methylation of the mirroring CpG site is inhibited, making the cytosine susceptible to bisulfite conversion. Other CpG sites mirroring a non-5-hmC site are efficiently methylated and resistant to bisulfite conversion. After sequencing of the synthesized mirroring strand, any converted cytosine in a CG context detected indicates the presence of a 5-hmC in the original parental strand.

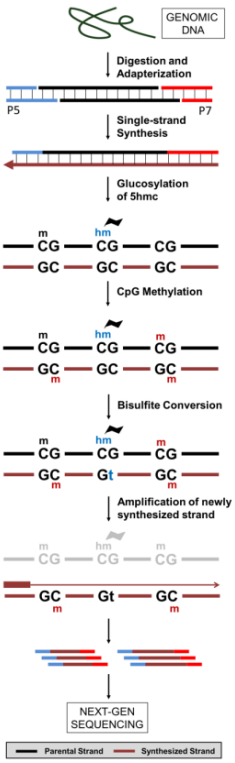
With Zymo Research's Mirror-Seq™ service, you simply submit purified DNA, and we will perform the library preparation, pre-sequencing quality controls, Next-Generation sequencing, and bioinformatics analysis. Using our bioinformatics pipeline, we can efficiently map the bisulfite-converted sequence and quantitate strand-specific 5-hmC levels. Also, we can perform additional analysis to help identify differentially hydroxymethylated sites. Mirror-Seq™ service is available in different genome-wide scales as well as locus-specific analysis.

Advantages of Mirror-Seq™ services:

- Single-base quantification of 5-hmCs for all species with a reference genome
- Compatible with inputs as low as 100 ng
- Pre-sequencing QC ensures efficient library preparation and accurate detection of 5-hmCs



UCSC genome browser tracks for hydroxymethylation calling and sequencing reads using Mirror-Seq™. The presence of 5-hmC at CpG sites are indicated by the presence of a thymine on the positive strand (blue) or adenine on the negative strand (red). Tracks on top indicate the percentage of 5-hmC at specific CG dinucleotides for both DNA strands.



Workflow for Mirror-seq™ library preparation.

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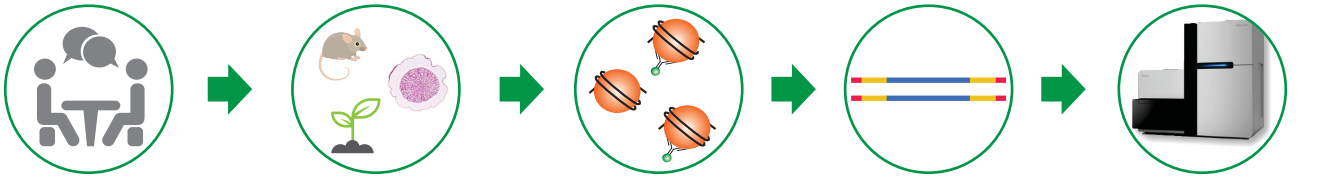
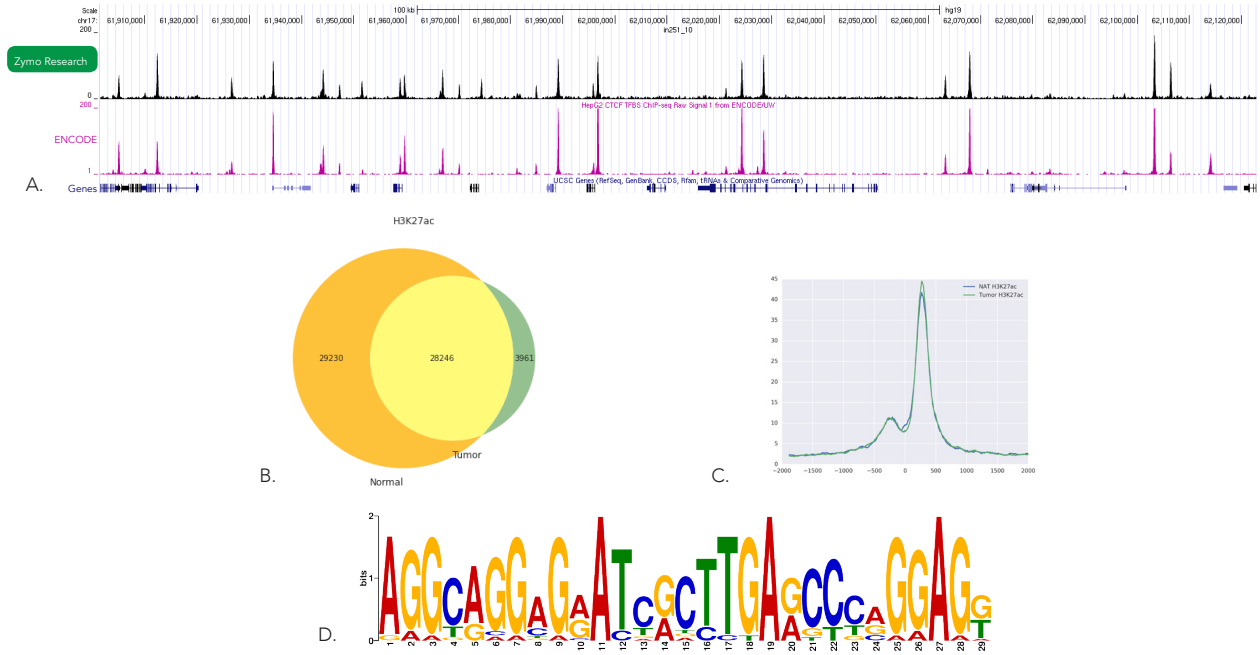
ChIP Seq

Chromatin Immunoprecipitation Sequencing (ChIP-Seq) is a technique that combines chromatin immunoprecipitation with the quantitative power and genome-wide coverage of Next-Generation sequencing. It is a powerful tool for genome-wide mapping of DNA interactions with transcription factors, histone modifications, and chromatin binding proteins and is essential for understanding the effect of DNA-protein interaction on gene regulation.

With the ChIP-Seq service from Zymo Research, you can either perform the ChIP assay yourself and send us the enriched DNA for library construction and Next-Gen Sequencing, or we can process your samples using our proprietary chromatin shearing and enrichment procedures. We also perform the bioinformatics and statistical analyses, and send you the publication-ready results.

Simply send us your samples and we will handle the rest!





Example of Zymo Research's ChIP-Seq Services Data Output: A. Browser tracks for visualization of peak regions. B. Venn diagram showing sample comparison data. C. Peak density profile to analyze peak locations relative to transcriptional start sites. D. Motif analysis to analyze bound genomic regions.

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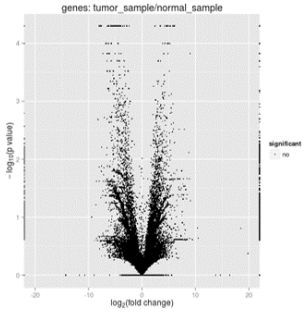
Expression Services: RNA-Seq

Zymo Research's RNA-Seq service makes Next-Generation transcriptome analysis available to every researcher, without the need for expensive equipment or bioinformatics expertise. Now you can achieve transcriptome-wide coverage of total RNA, or small RNA with the latest Next-Generation sequencing technology.

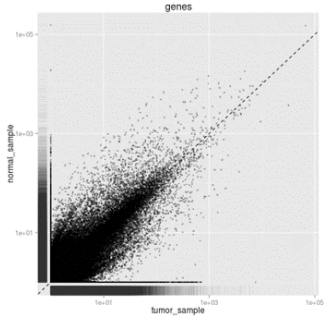
Useful for:

- Gene expression studies
- miRNA analysis
- Non-coding RNA investigations
- Discovering splice variants, SNPs, and RNA editing sites
- And much more!

A.



B.



A) Volcano plot showing the relationship between expression fold change and significantly different expression. B) Scatterplot for expression bias identification. C) The Database for Annotation, Visualization and Integrated Discovery (DAVID) pathway analysis was used to identify enrichment of biological processes amongst the top 100 significantly differentially methylated genes. Metabolic pathways were highly represented.

C.

Annotation Cluster 1	Enrichment Score: 7.492493953533086				
Category	Term	Count	P-Value	Fold Enrichment	FDR
GOTERM_BP_FAT	GO:0016054~organic acid catabolic process	12	1.68E-12	23.9751883	2.51E-09
GOTERM_BP_FAT	GO:0046395~carboxylic acid catabolic process	12	1.68E-12	23.9751883	2.51E-09
GOTERM_BP_FAT	GO:0009063~cellular amino acid catabolic process	9	5.55E-10	29.35197686	8.31E-07
GOTERM_BP_FAT	GO:0009063~cellular amino acid catabolic process	9	1.70E-09	25.5889029	2.55E-06
GOTERM_BP_FAT	GO:0006575~cellular amino acid derivative metabolic process	6	8.30E-04	8.015800909	1.236062
GOTERM_CC_FAT	GO:0005829~cytosol	8	0.5042612	1.182834008	99.97043

Let Zymo Research's scientists do the work, starting with RNA purification and sample prep all the way through bioinformatic analyses with the delivery of a report with publication-ready figures directly to you. Each project is fully customizable to ensure your needs are met!

Many types of analyses are available including total RNA-Seq, small RNA-Seq (miRNA), polyadenylated RNA-Seq, and non-polyadenylated RNA-Seq.

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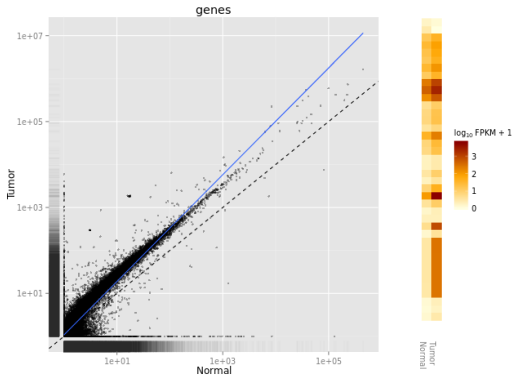

Scatterplot and heatmap showing expression bias and gene expression, respectively.

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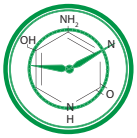
Epigenetics Aging Clock



Highlights

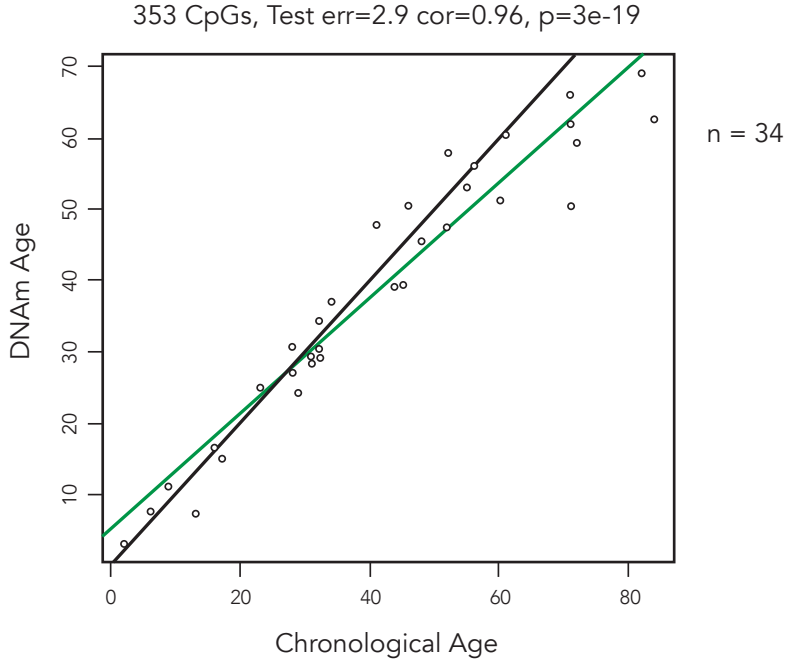
- Reliably determine the true biological age of any human sample.
- Quantify changes in biological age following lifestyle interventions or drug treatments.
- Identify disease associated aging alterations.

A growing number of studies have highlighted the strong correlation of DNA methylation changes with aging. Additionally, accelerated biological aging, as determined by DNA methylation profiling, has been associated with disease phenotypes including Down Syndrome and HIV-1-infection. DNA methylation-based biological age is a valuable surrogate biomarker of molecular aging.

The Epigenetic Aging Clock Service allows you to effectively gauge the biological age of any human tissue sample. With this easy to use service, the only thing you have to do is provide us with the sample. Starting with DNA purification all the way through bioinformatics analysis, Zymo scientists will do the work for you and provide you with an accurate biological age estimate along with a comprehensive report. Enhance any aging study or satisfy your intellectual curiosity with this multi-tissue age predictor.

This profiler is designed to:

- Detect methylation changes at highly informative CpG sites
- Use optimized data analysis workflow to provide an accurate biological age



Predicted epigenetic age of urine samples from healthy donors.

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Additional Services

Mass Spectrometry

Zymo Research offers DNA composition analysis with LC/MS analysis. Please inquire for more information.

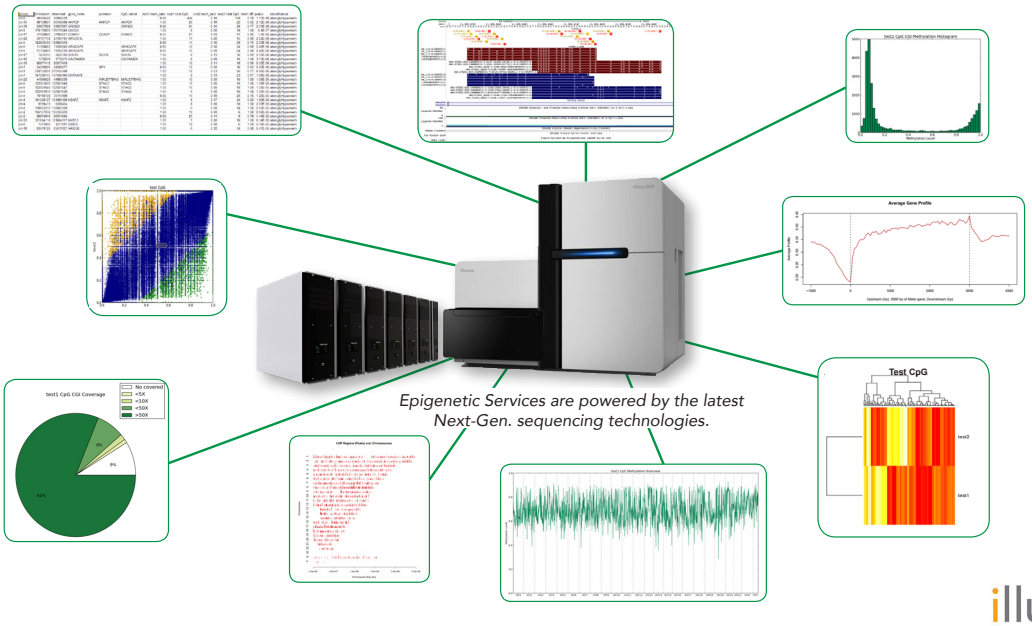
Custom Bioinformatics

Do you have Next-Generation sequencing data that you need analyzed? Zymo Research offers complete bioinformatics solutions to fulfill your needs. Whether it is whole-genome bisulfite sequencing data or ChIP-Seq data, we can help make sense of your overwhelming data sets. We use established as well as customizable bioinformatic pipelines to transform raw sequence data into manageable and interpretable figures and data sets. Simply provide the raw (FASTQ) or aligned (SAM or BAM) data and we will provide you with your desired downstream analyses.

Service Packages

Basic Service Packages for all of the platforms include sample standardization, library construction, Next-Generation sequencing, and raw data alignment.

Full Service Packages offer additional down-stream bioinformatic processing and statistical analysis specifically tailored to fit your needs.



Epigenetic Services are powered by the latest Next-Gen. sequencing technologies.

illumina CSPro® CERTIFIED SERVICE PROVIDER

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