MeDIP-qPCR or MeDIP-Seq?

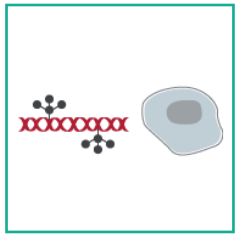
MeDIP-qPCR	MeDIP-Seq
single-locus data requires design of loci-specific primers	genome-wide data suitable for discovery analysis
low-cost fast	high sequencing cost longer workflow (library prep, sequencing, data analysis)

Workflow: Methylated-DNA ImmunoPrecipitation (MeDIP)

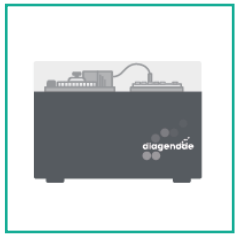
qPCR

NGS

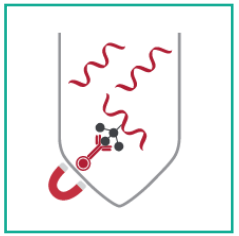
Cell lysis & DNA extraction



DNA shearing

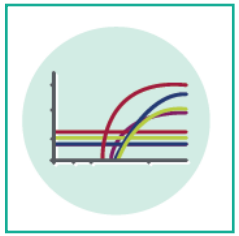


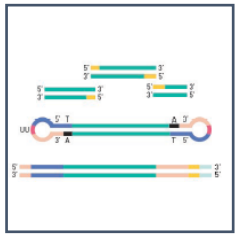
MeDIP



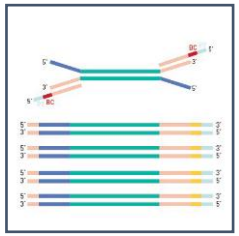
DNA isolation

qPCR analysis





Library preparation



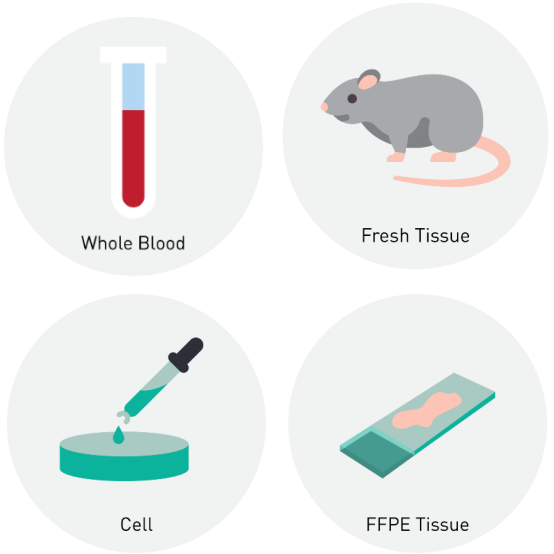
Library amplification

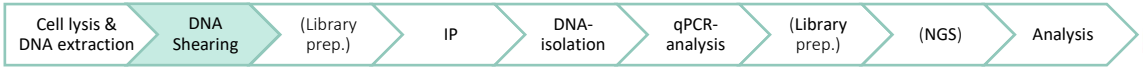
NGS analysis

Cell lysis & gDNA extraction

High integrity gDNA

- **Cultured cells**
  - Recommended kit: XL GenDNA Extraction Module (Diagenode)
  - For large amounts of cultured cells (~1-1.5 million)
- **Other template (e.g. tissue, blood)**
  - Flexibility to use other kits
  - Ensure good gDNA quality and purity
- **Starting amount for MeDIP:** 10ng - 1 µg of sheared gDNA





gDNA shearing

- Shear gDNA to consistent fragment mean size
  - 300-400 bp for qPCR
  - 200 bp for NGS
- Use a good sonicator
  - Recommended: Bioruptor® Pico (Diagenode)
- Shearing TE buffer
- Sample concentration at 2-100 ng/µl

Precise fragmentation

gDNA shearing: Parameters for Bioruptor® Pico

Guide for successful gDNA shearing using the Bioruptor® Pico				
Sample type	**Tube holder**	**Tube**	**Volume of sample**	**Shearing protocol**
Low sample amounts (from 10 ng)	for 0.2ml tubes B01201144	0.2ml Bioruptor® Microtubes C30010020	**50 µl**	13 cycles [30sec ON / 30sec OFF]
Standard sample amounts	for 0.65ml tubes B01201143	0.65ml Bioruptor® Microtubes C30010011	**100 µl**	13 cycles [30sec ON / 30sec OFF]

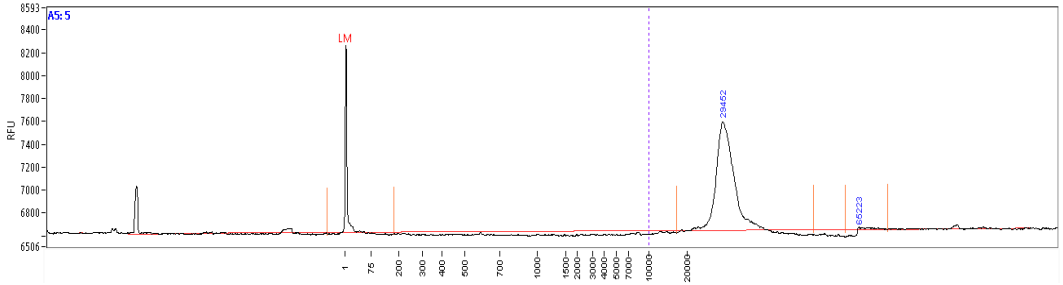
https://www.diagenode.com/files/protocols/protocol-dna-shearing-bioruptor-pico.pdf

Features & Benefits

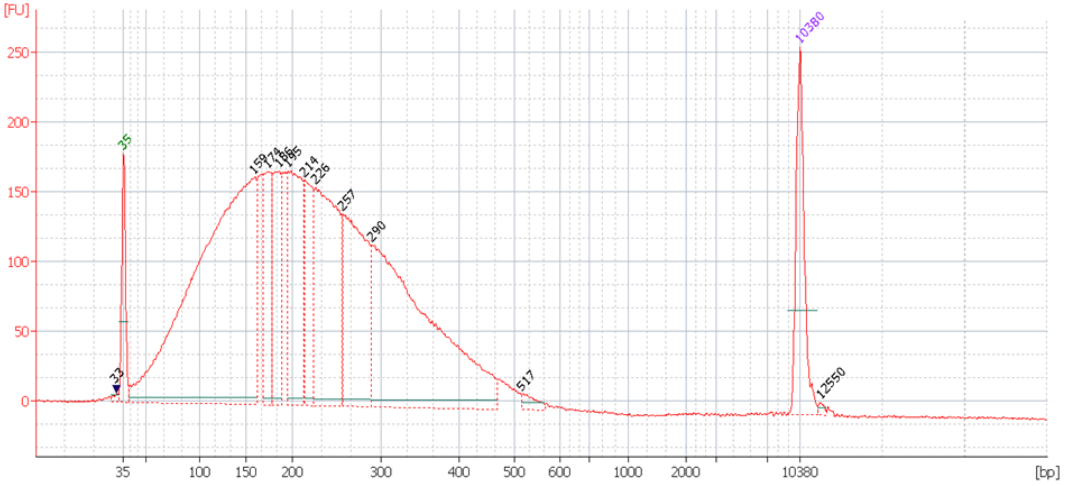
- Best suited for NGS sample preparation
- Superior sample yields
- Consistent fragment size
- High reproducibility

gDNA shearing: Examples of DNA profiles

BEFORE



AFTER



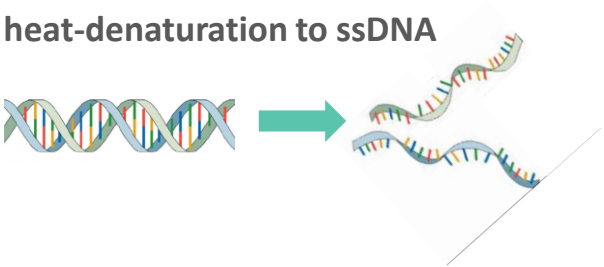
Performing IP

Keep on ice to maintain ssDNA

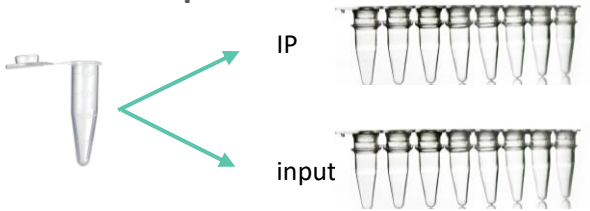
1. **Prepare Beads** (washing and resuspension)

2. **Prepare IP reaction tube**
   - Sheared gDNA
   - Spike-in controls
   - Buffers

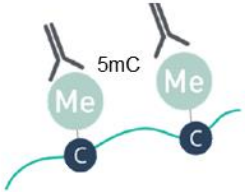
3. **heat-denaturation to ssDNA**



4. **Set aside INPUT samples**



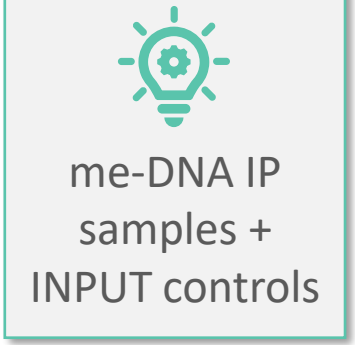
5. **Add 5mC specific antibody**
   Overnight incubation on rotating wheel



6. **Remove unmethylated DNA**
   Wash bead/antibody/DNA complexes

Performing IP: INPUT sample

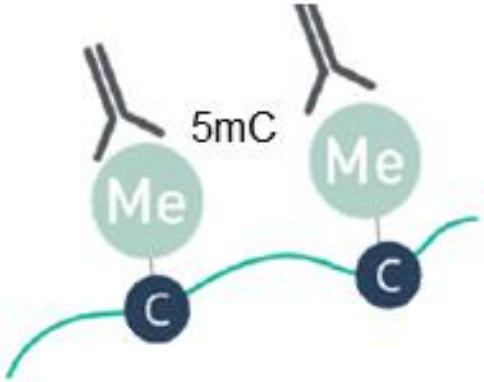
- Fraction of sheared DNA is kept aside as INPUT (~10%)
  - Processed in parallel with IP-samples (without antibody)
  - Recommended: 1 input for each DNA sample
- Key reference for MeDIP-qPCR and MeDIP-seq analysis
- MeDIP-qPCR: used to calculate the IP recovery (% of input)
- MeDIP-seq: mandatory for bio-informatics analysis
  - Normalization for mappability of a region, to avoid duplication bias etc.

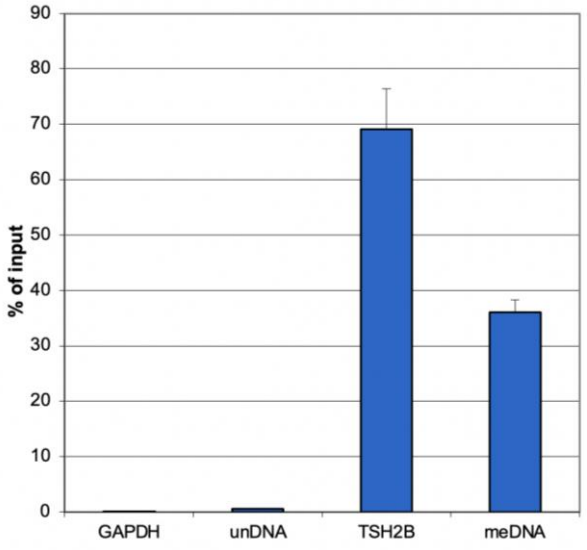


Performing IP: 5-mC antibody

Recommended antibody:
5-methylcytosine (5-mC) Antibody - clone 33D3 (Diagenode)

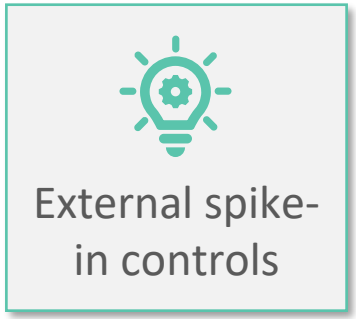
Highly specific antibody





Performing IP: Spike-in controls

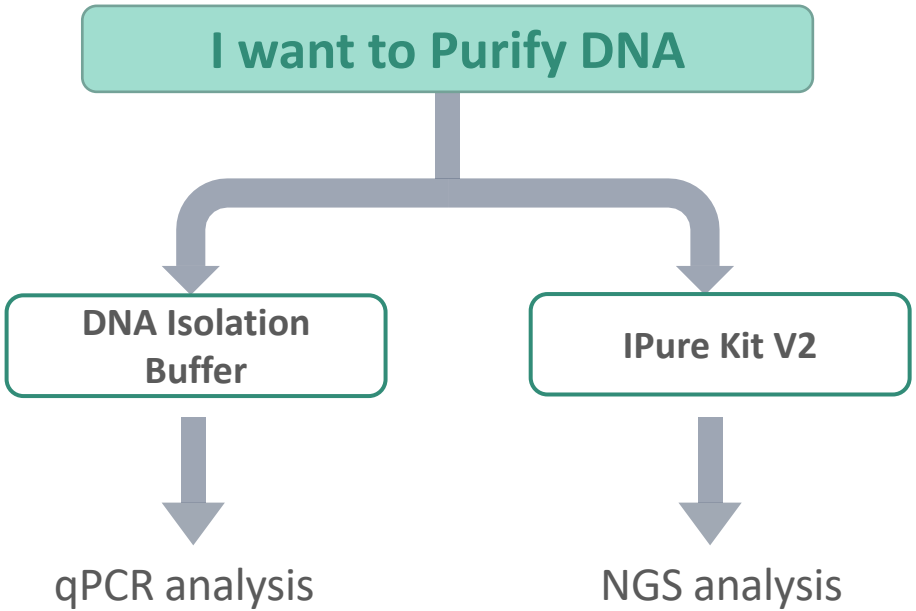
- **External DNA spike-in controls** & primer pairs
  - Synthetic sequence without homology to any model species
  - No interference with DNA sample of interest
  - Add directly to each sheared gDNA sample before IP
  - Not sequenced but use as control during qPCR analysis



- **Methylated (positive) spike-in**: confirm overall efficiency of IP workflow
- **Unmethylated (negative) spike-in**: measure non-specific IP background

  Recommended kit: DNA methylation control package V2 (Diagenode)

DNA isolation



qPCR analysis: QC check

IP QC check using qPCR on the spike-in controls

1. **% recovery**

$$= 2^{[Ct\,(INPUT) - 3.32 - Ct(IP)]} * 100$$

$= \log 10 / \log 2 =$, where 10 is dilution factor IP/INPUT

2. **Enrichment**

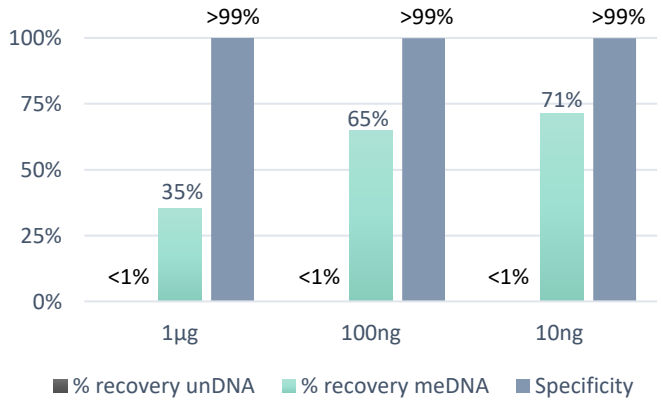
$$= \frac{\%\ recovery\ meDNA}{\%\ recovery\ unDNA}$$

3. **Specificity**

$$= 1 - \frac{1}{Enrichment}$$

Success criteria

- Methylated spike-in recovery > 20%
- Unmethylated spike-in recovery < 1%
- Spike-in IP specificity > 99%



qPCR analysis: QC check & analysis

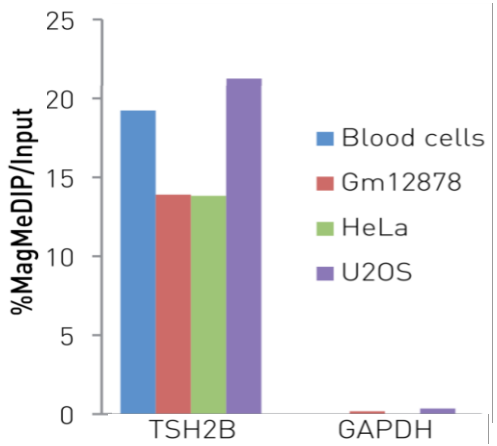
qPCR quality controls

- Primer pairs for **Positive/Negative control regions** within the DNA sample of interest

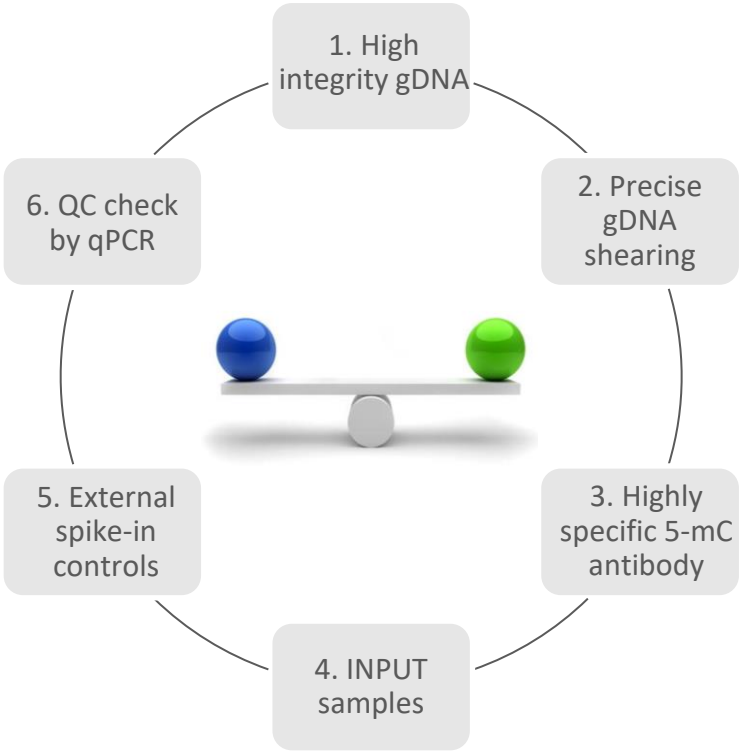
  QC check during qPCR & NGS analyses

- Available primer pairs for human/mouse/rat
  - **Positive TSH2B locus:** methylated region
  - **Negative GAPDH locus:** unmethylated region

- Or design your own qPCR primers



Summary – Tips for Good MeDIP-qPCR Assay

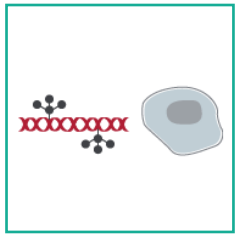


Workflow: Methylated DNA ImmunoPrecipitation (MeDIP)

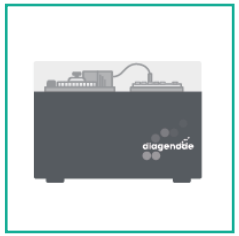
qPCR

NGS

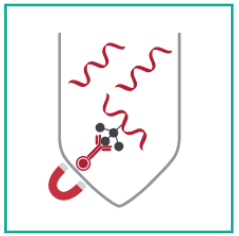
Cell lysis & DNA extraction



DNA shearing

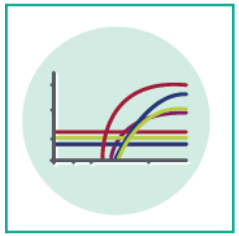


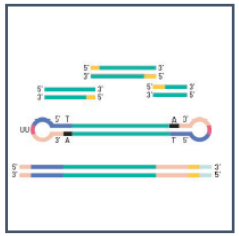
MeDIP



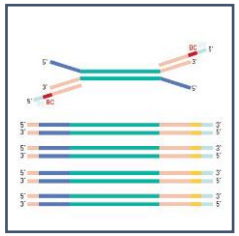
DNA isolation

qPCR analysis





Library preparation



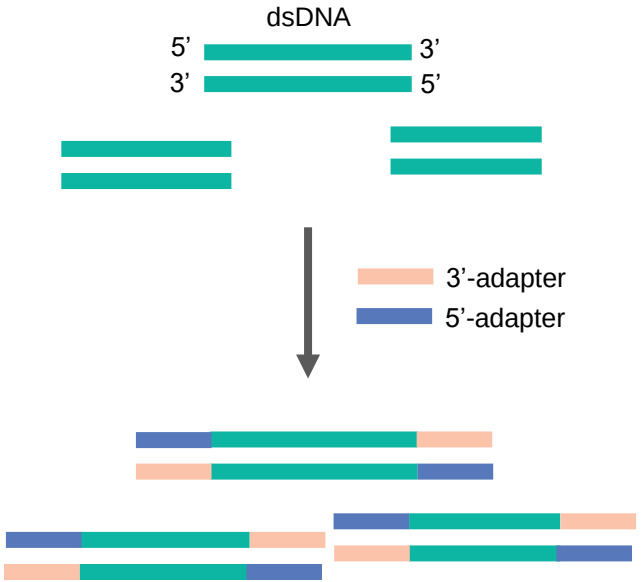
Library amplification

NGS analysis

Library preparation: Adaptor ligation before IP

- Critical step for successful sequencing
- Adaptor ligation on dsDNA before denaturation and IP

- Recommended kit: iDeal DNA IP Library Preparation Kit
  - Fully optimized & validated for MeDIP-seq
  - Low input (from 10ng DNA)
  - High library yield



MeDIP-seq: Performing IP

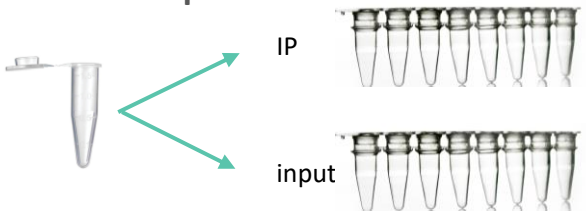
Keep on ice to maintain ssDNA

1. **Prepare Beads** (washing and resuspension)

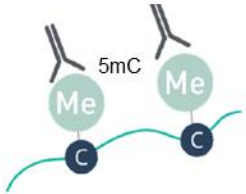
2. **Prepare IP reaction tube**
   - Adaptor-ligated DNA
   - Spike-in controls
   - Buffers

3. **Heat-denaturation to ssDNA**

4. **Set aside INPUT samples**

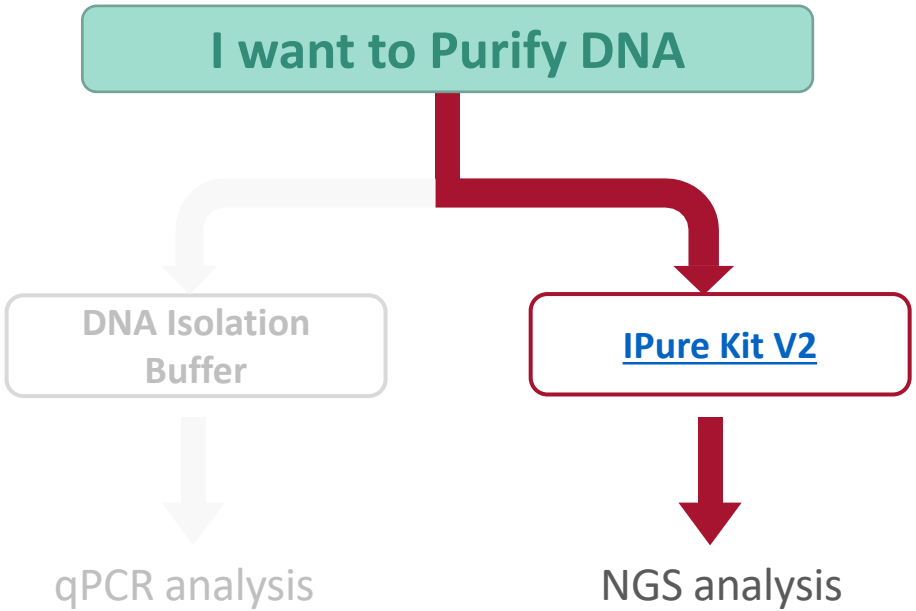


5. **Add 5mC specific antibody**
   Overnight incubation on rotating wheel



6. **Remove unmethylated DNA**
   Wash bead/antibody/DNA complexes

MeDIP-seq: DNA isolation



Library preparation: Optimal PCR cycles

Minimization of PCR cycles

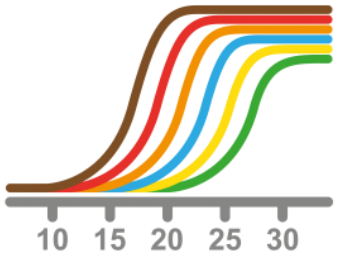
- Limits the risk of introducing bias during PCR
- Minimizes # of PCR duplicates

Minimize # PCR cycles

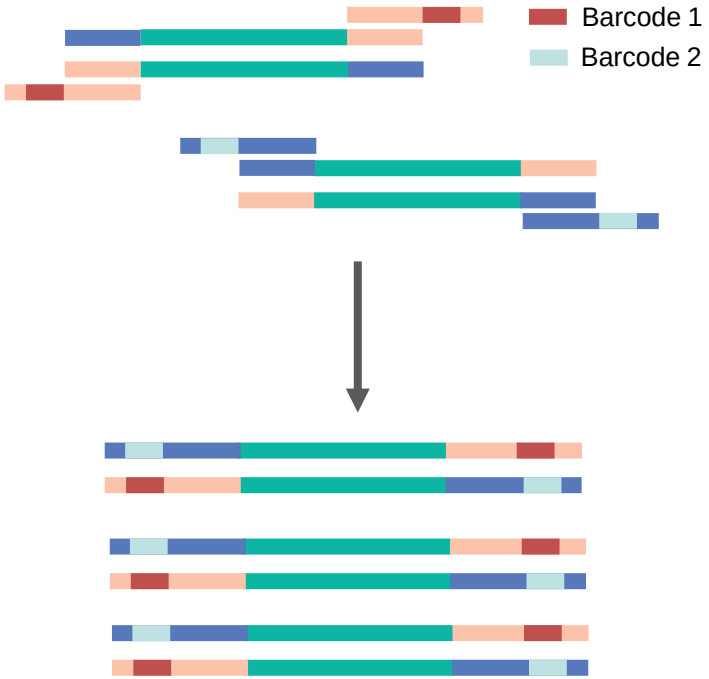
Determine optimal # of PCR cycles for each sample and input on a small aliquot

- **Optimal cycle number** $= Ct \quad (rounded \quad up) + 1$

  e.g. $Ct = 6.82$ → 8 amplification cycles



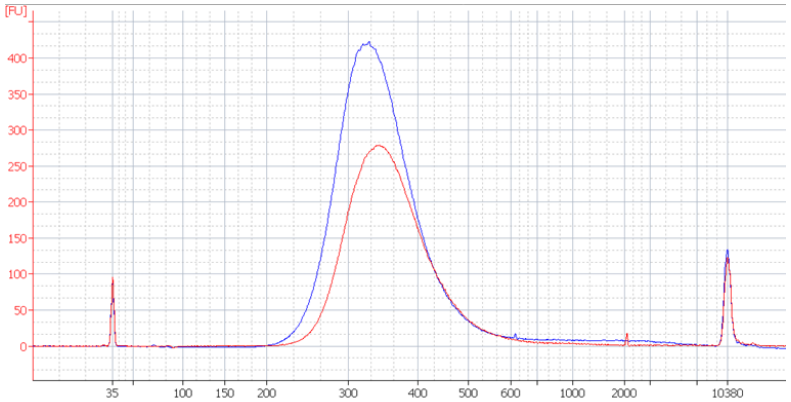
Library preparation: Amplification



- Recommended kit: [iDeal Unique Dual Indexes for Illumina]
  - Library multiplexing
  - Unique i5 and i7 barcodes
  - Filtering of index-hopping events (especially on NovaSeq)

Library preparation: Pooling & quality controls

- **Determine library size**
  - Bioanalyzer or Fragment Analyzer
- **Quantify**
  - Qubit
  - Convert ng/µl to nM by average library size
- **Dilute and pool normalized libraries**
  - Same size for best clustering



MeDIP-seq: Sequencing Settings

Read length

- Paired-end 50 bp

Sequencing depth

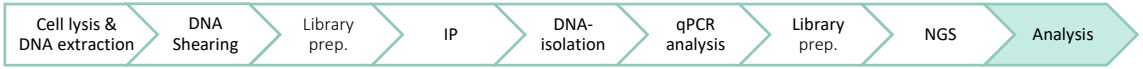
- Mainly set by genome size, samples and flow cell type
- 50 M for genome-wide DNA methylation analysis (human & mouse)
- Use same depth for input

Replicates

- Biological duplicates are minimum for most bioinformatic analyses
- Increased replicate number will improve sensitivity of the downstream analysis

Input sequencing

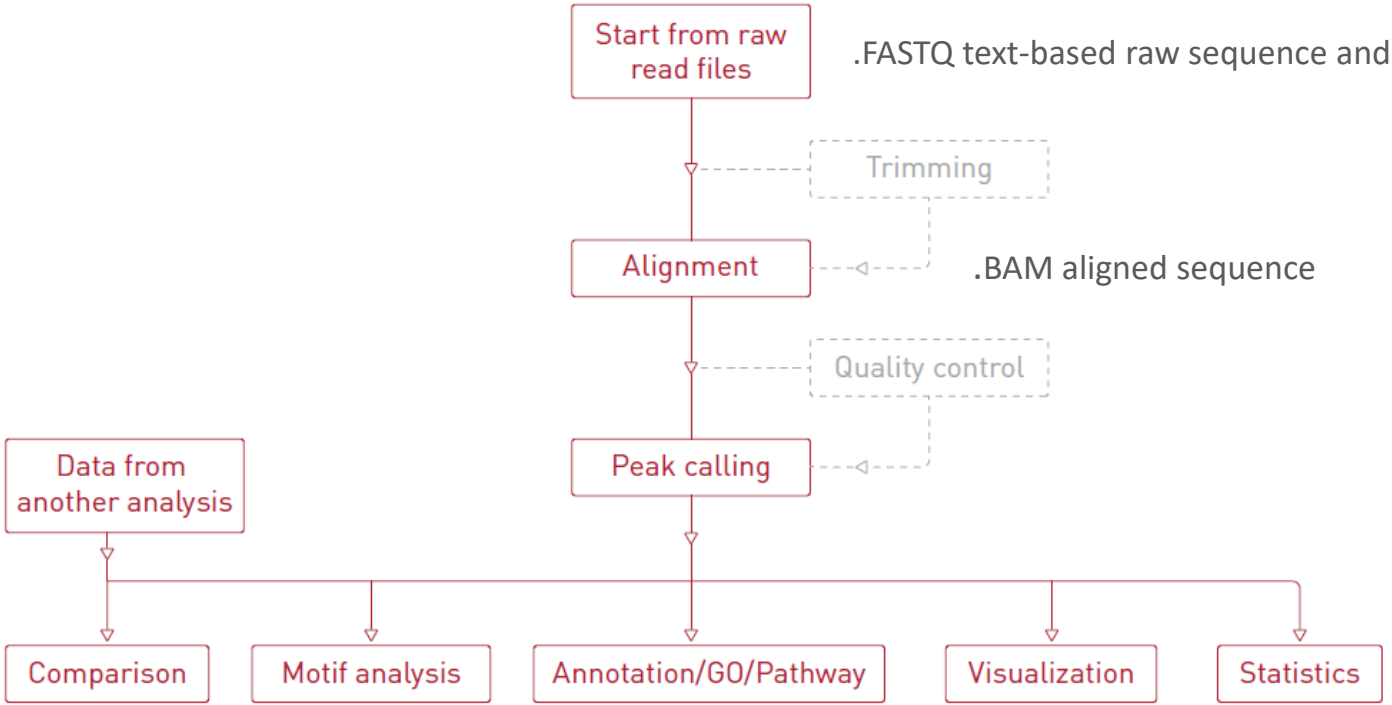
- One input per sample is gold standard
- Pooling inputs from replicates can often be considered



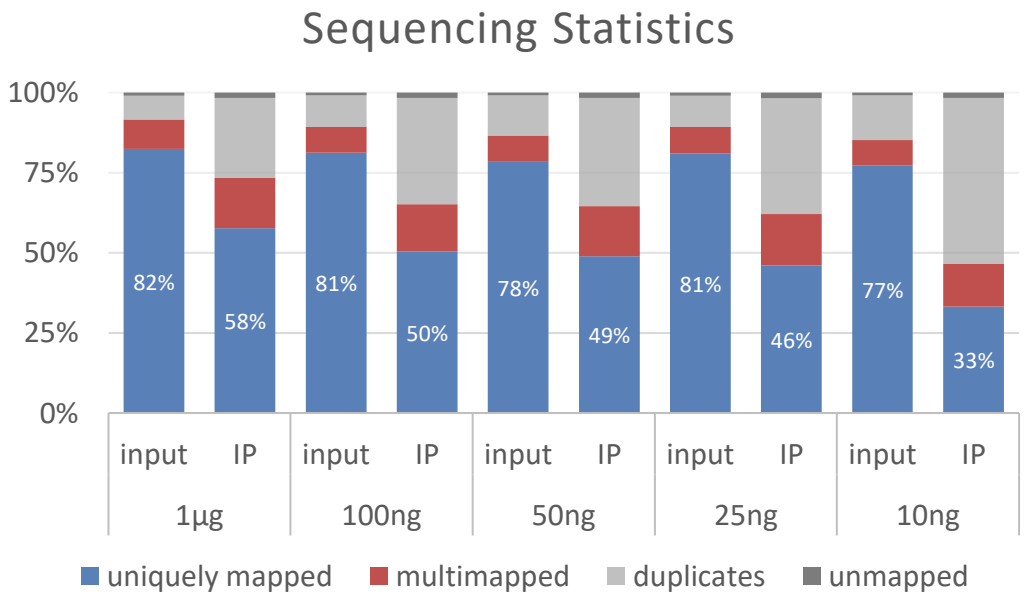
MeDIP-seq: Analysis

Bioinformatician	R	https://bioconductor.org/packages/release/bioc/html/MEDIPS.html
	free-ware and online tool kits	www.bioinformatics.babraham.ac.uk/projects/seqmonk/ biit.cs.ut.ee/gprofiler/gost
Wet-lab expert with free time	standard bioinformatic services	https://www.diagenode.com/en/categories/Services Comprehensive DNA methylation and bioinfo services
	free-ware and online tool kits	www.bioinformatics.babraham.ac.uk/projects/seqmonk/ Initial & advanced data analysis, genome browser, graphical presentation of data https://biit.cs.ut.ee/gprofiler/gost Functional profiling tool
No expertise or no free time	advanced bioinformatic services	https://www.diagenode.com/en/categories/Services Comprehensive DNA methylation and bioinfo services

MeDIP-seq: Analysis



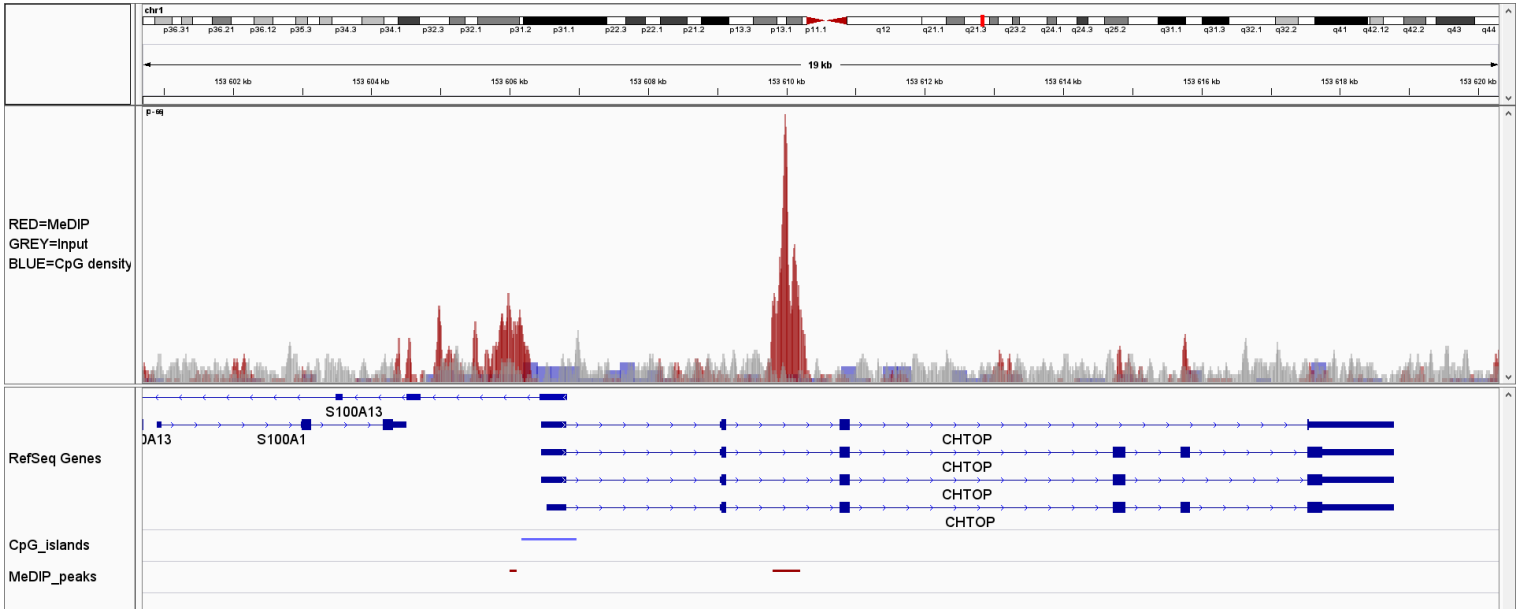
MeDIP-seq: Example of results



Uniquely mapped
= reads after mapping
+ duplicates removal
+ multi-mappers removal
= reads for methylation analysis

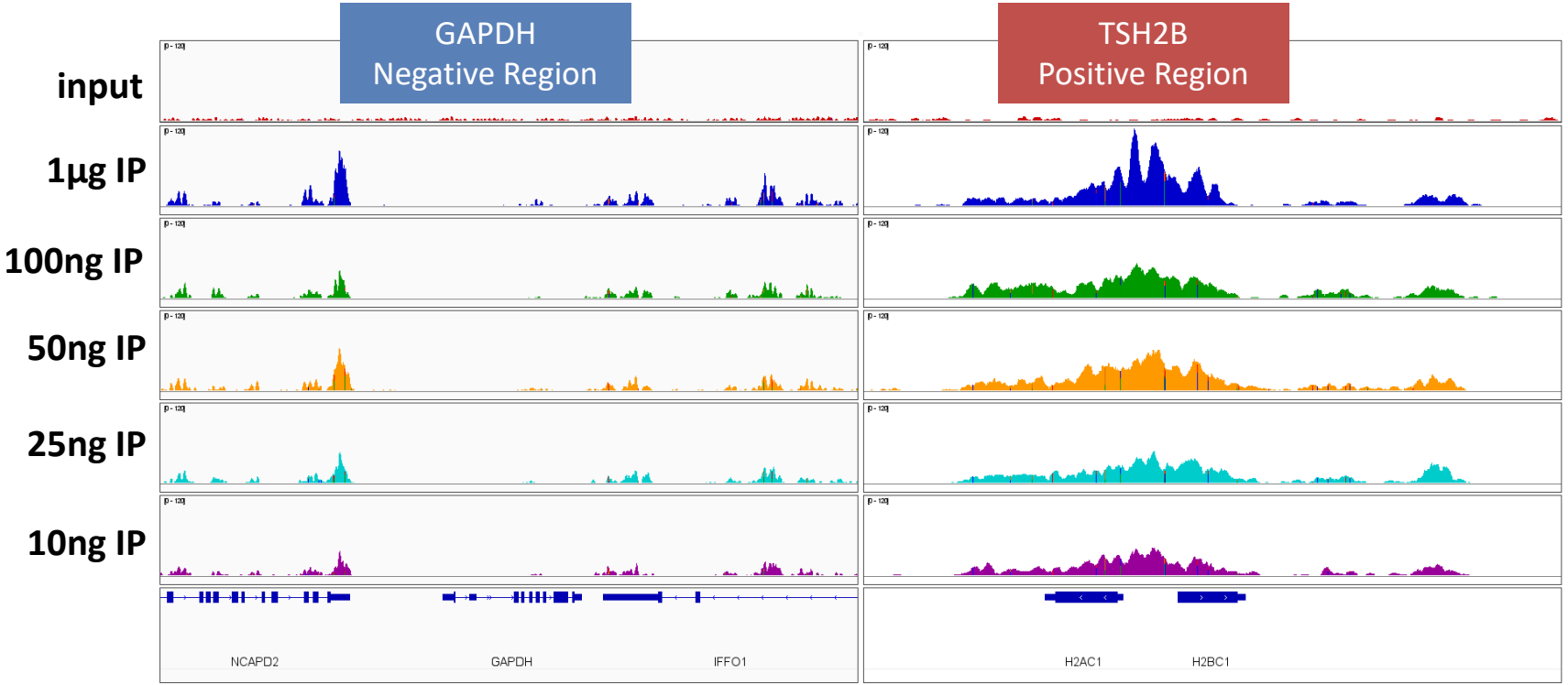
Sample: Human whole blood genomic DNA
Starting amount: 1 µg; 100 ng; 50 ng; 25 ng; 10 ng
Sequencing: PE 50 bp on Illumina Novaseq
Bioinformatics analysis: MeDIPS

MeDIP-seq: Enrichment visualization

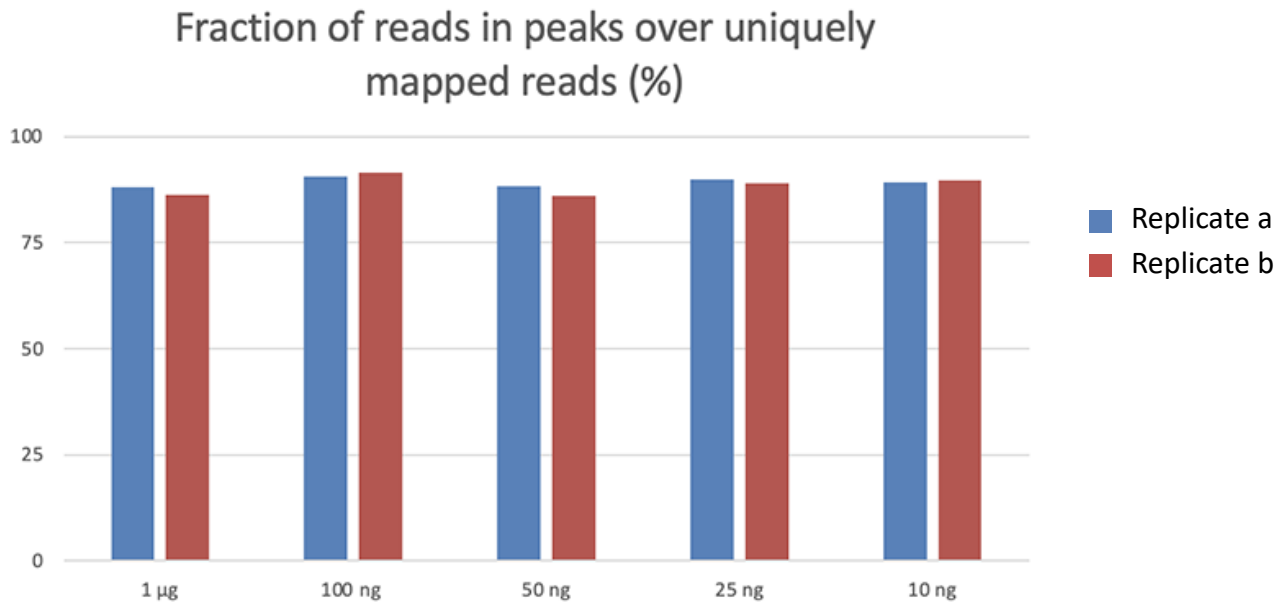


Enrichment of me-DNA compared to INPUT sample

MeDIP-seq: Coverage profiles

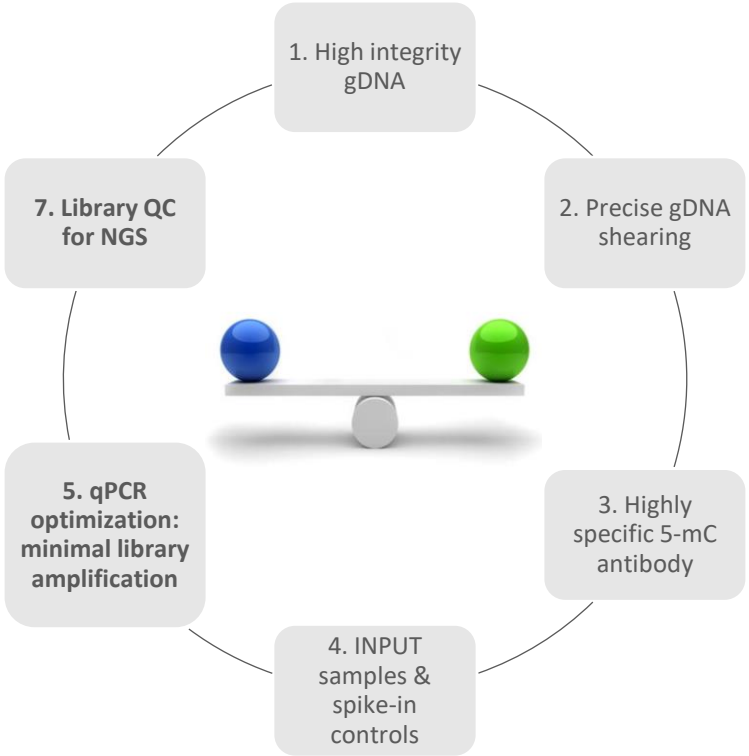


MeDIP-seq: Peak calling

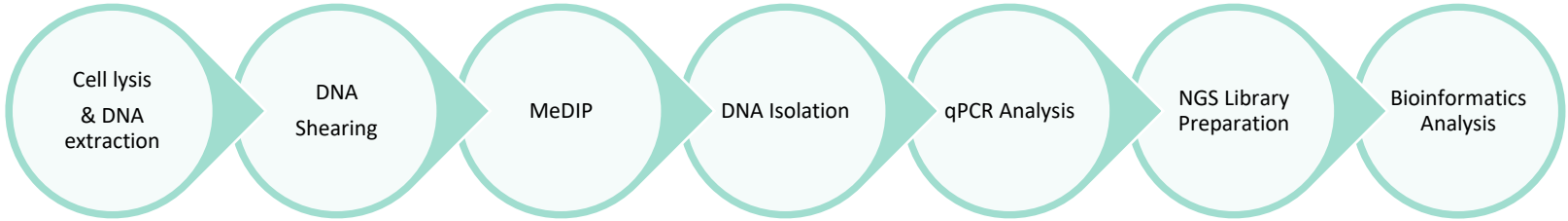


Specific me-DNA enrichment compared to reference genome

Summary – Tips for Good MeDIP-seq Assay



Summary – Diagenode Support



Hardware:		Bioruptor				
Reagents:	XL GenDNA Extraction Module		5-mC antibody Spike-in controls	IPure kit	Primer Pairs	
Kits:	MagMeDIP qPCR Kit					
	MagMeDIP-seq Package V2					
Services:	Epigenomic Profiling Services					
						Data Analysis Service

www.diagenode.com

Applications

MeDIP-qPCR on FFPE samples

Mol Neurobiol
DOI 10.1007/s12035-016-0345-x

Evaluating the Feasibility of DNA Methylation Analyses Using Long-Term Archived Brain Formalin-Fixed Paraffin-Embedded Samples

Stine T. Bak[1] · Nicklas H. Staunstrup[1,2,3,4] · Anna Starnawska[1,3,4] · Tina F. Daugaard[1] · Jens R. Nyengaard[5,6] · Mette Nyegaard[1,3] · Anders Borglum[1,3,4,7] · Ole Mors[3,4,7] · Karl-Anton Dorph-Petersen[2,6,8] · Anders L. Nielsen[1,3]

Received: 3 August 2016 / Accepted: 5 December 2016

MeDIP-qPCR on cfDNA

IJMCM
Winter 2017, Vol 6, No 1

MeDIP Real-Time qPCR has the Potential for Noninvasive Prenatal Screening of Fetal Trisomy 21

Mohammad Kazemi[1,2,3], Mansoor Salehi[1,2,3*], Majid Kheirollahi[3]

1. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Medical Genetic Center of Genome, Isfahan, Iran.
3. Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Noncommunicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran.

MeDIP-seq on plants

The methylation pattern of DNA and complex correlations with gene expressions during *TuMV* infection in Chinese cabbage

J. YU[1], L.-W. GAO[1], Y. YANG[1], C. LIU[1], R.-J. ZHANG[1], F.-F. SUN[2], L.-X. SONG[3], D. XIAO[1], T.-K. LIU[1], X.-L. HOU[1], and C.-W. ZHANG[1]*

State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Horticulture, Nanjing Agricultural University, Nanjing, 210095, P.R. China[1]
Nanjing Vegetable Science Research Institute, Nanjing, 210095, P.R. China[2]
Jiangsu Key Laboratory for Horticultural Crop Genetic Improvement, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, 210014, P.R. China[3]

MeDIP-seq on cfDNA from plasma…

LETTER

https://doi.org/10.1038/s41586-018-0703-0

Sensitive tumour detection and classification using plasma cell-free DNA methylomes

Shu Yi Shen[1,12], Rajat Singhania[1,12], Gordon Fehringer[2,12], Ankur Chakravarthy[1,12], Michael H. A. Roehrl[1,3,4], Dianne Chadwick[1], Philip C. Zuzarte[5], Ayelet Borgida[2], Ting Ting Wang[1,4], Tiantian Li[1], Olena Kis[1], Zhen Zhao[1], Anna Spreafico[1], Tiago da Silva Medina[1], Yadon Wang[1], David Roulois[1,6], Ilias Ettayebi[1,4], Zhuo Chen[1], Signy Chow[1], Tracy Murphy[1], Andrea Arruda[1], Grainne M. O'Kane[1], Jessica Liu[4], Mark Mansour[4], John D. McPherson[7], Catherine O'Brien[1], Natasha Leighl[1], Philippe L. Bedard[1], Neil Fleshner[1], Geoffrey Liu[1,4,8], Mark D. Minden[1], Steven Gallinger[9,10], Anna Goldenberg[11], Trevor J. Pugh[1,4], Michael M. Hoffman[1,4,11], Scott V. Bratman[1,4], Rayjean J. Hung[2,8*] & Daniel D. De Carvalho[1,4*]

… and urine

nature medicine | BRIEF COMMUNICATION

https://doi.org/10.1038/s41591-020-0933-1

Check for updates

Detection of renal cell carcinoma using plasma and urine cell-free DNA methylomes

Pier Vitale Nuzzo[1,2,3,16], Jacob E. Berchuck[1,2,16], Keegan Korthauer[4,5,16], Sandor Spisak[2,16], Amin H. Nassar[1,2], Sarah Abou Alaiwi[1,2], Ankur Chakravarthy[6], Shu Yi Shen[6], Ziad Bakouny[1], Francesco Boccardo[3,7], John Steinharter[1], Gabrielle Bouchard[1], Catherine R. Curran[1], Wenting Pan[1], Sylvan C. Baca[1,2,8], Ji-Heui Seo[1,2], Gwo-Shu Mary Lee[1,2], M. Dror Michaelson[9], Steven L. Chang[10], Sushrut S. Waikar[11,12], Guru Sonpavde[1], Rafael A. Irizarry[13,14], Mark Pomerantz[1,2], Daniel D. De Carvalho[6,15,17] ✉, Toni K. Choueiri[1,8,17] ✉ and Matthew L. Freedman[1,2,8,17] ✉

MeDIP-seq on oysters

PLOS GENETICS

RESEARCH ARTICLE

Dynamics of DNA methylomes underlie oyster development

Guillaume Riviere[1,2]*, Yan He[3]*, Samuele Tecchio[1,2]*, Elizabeth Crowell[1,2], Michaël Gras[1,2], Pascal Sourdaine[1,2], Ximing Guo[3], Pascal Favrel[1,2]

1 Normandy University, Caen, France, 2 Université de Caen Normandie, UMR BOREA MNHN, UPMC, UCBN, CNRS-7208, IRD-207, Caen, France, 3 Ministry of Education Key Laboratory of Marine Genetics and Breeding, College of Marine Life Sciences, Ocean University of China, Qingdao, Shandong, China, 4 Haskin Shellfish Research Laboratory, Department of Marine and Coastal Sciences, Rutgers University, Port Norris, NJ, United States of America

These authors contributed equally to this work.
* guillaume.riviere@unicaen.fr