Bioinformatics Resources

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Main Principles of DNA methylation analysis

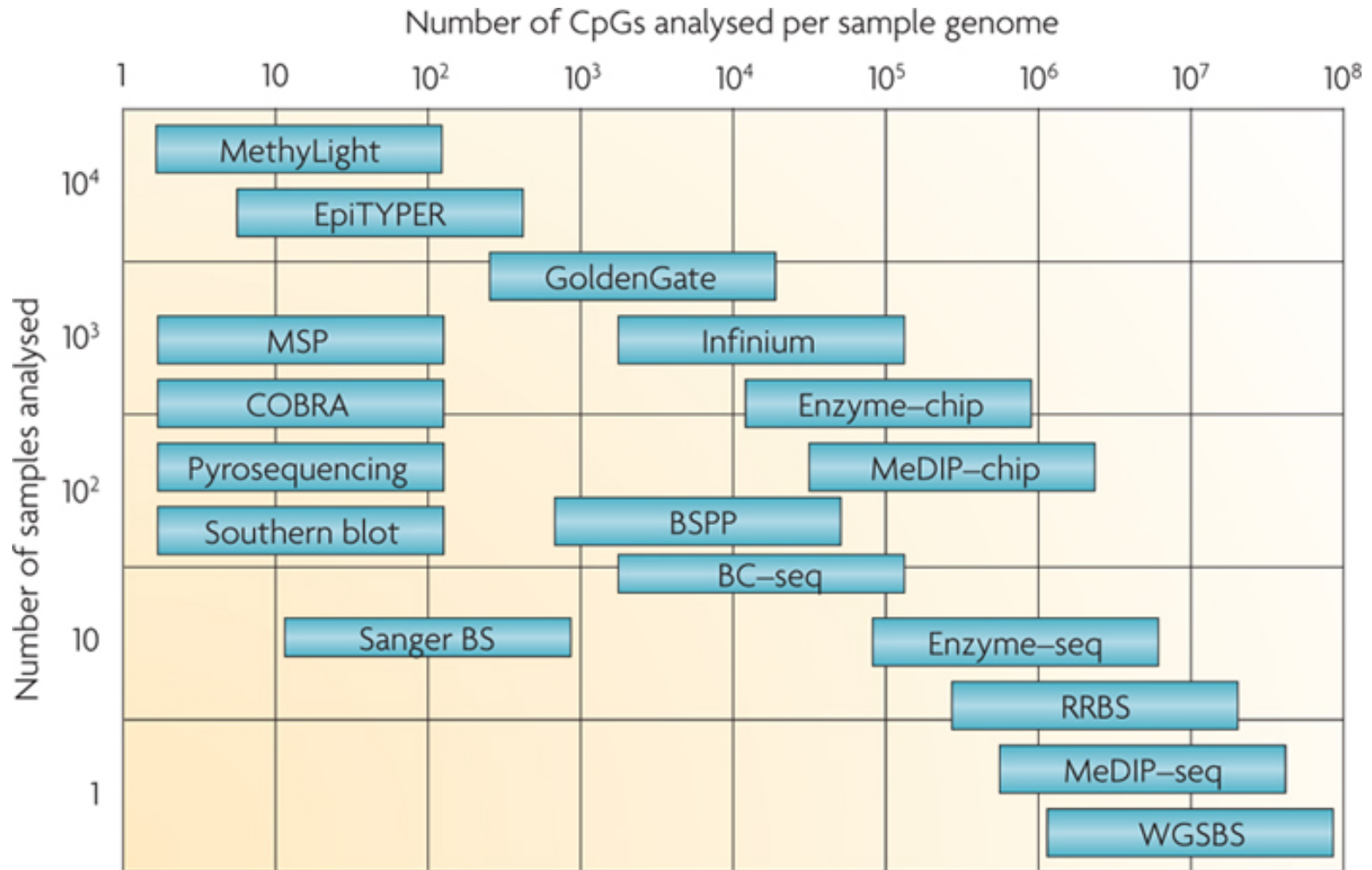| Pretreatment	Analytical step			
	Locus-specific analysis	Gel-based analysis	Array-based analysis	NGS-based analysis
Enzyme digestion	• *Hpa*II-PCR	• Southern blot • RLGS • MS-AP-PCR • AIMS	• DMH • MCAM • HELP • MethylScope • CHARM • MMASS	• Methyl–seq • MCA–seq • HELP–seq • MSCC
Affinity enrichment	• MeDIP-PCR		• MeDIP • mDIP • mCIP • MIRA	• MeDIP–seq • MIRA–seq
Sodium bisulphite	• MethyLight • EpiTYPER • Pyrosequencing	• Sanger BS • MSP • MS-SNuPE • COBRA	• BiMP • GoldenGate • Infinium	• RRBS • BC–seq • BSPP • WGSBS

AIMS, amplification of inter-methylated sites; BC–seq, bisulphite conversion followed by capture and sequencing; BiMP, bisulphite methylation profiling; BS, bisulphite sequencing; BSPP, bisulphite padlock probes; CHARM, comprehensive high-throughput arrays for relative methylation; COBRA, combined bisulphite restriction analysis; DMH, differential methylation hybridization; HELP, *Hpa*II tiny fragment enrichment by ligation-mediated PCR; MCA, methylated CpG island amplification; MCAM, MCA with microarray hybridization; MeDIP, mDIP and mCIP, methylated DNA immunoprecipitation; MIRA, methylated CpG island recovery assay; MMASS, microarray-based methylation assessment of single samples; MS-AP-PCR, methylation-sensitive arbitrarily primed PCR; MSCC, methylation-sensitive cut counting; MSP, methylation-specific PCR; MS-SNuPE, methylation-sensitive single nucleotide primer extension; NGS, next-generation sequencing; RLGS, restriction landmark genome scanning; RRBS, reduced representation bisulphite sequencing; –seq, followed by sequencing; WGSBS, whole-genome shotgun bisulphite sequencing.

Laird, P. W. Principles and challenges of genome-wide DNA methylation analysis. *Nature Review Genetics*. **11**, 191-203 (2010).

Features and Sources of Bias for Various Techniques

Technology	Features							Potential sources of bias							
	Unambiguous identification of CpG measured	In cis co-methylation information	Non-CpG methylation information	Allele-specific measurement capability	Good coverage of regions with low CpG density	Compatible with low amounts of input DNA	Full repeat-masked genome coverage	Copy-number variation bias	Fragment size bias	Incomplete bisulphite conversion bias	Bisulphite PCR bias	Cross-hybridization bias	DNA methylation status bias	GC content bias	CpG density bias
Infinium	(•)					•				•	•	•			
Enzyme–chip	(•)	(•)			(•)				•			•		•	
MeDIP–chip						•	•					•		•	•
BSPP	•	•	•	•						•	•		•		
BC–seq	•	•	•	•						•	•		•		
RRBS	•	•	•	•		•				•	•				
Enzyme–seq	•	•		•	(•)	•			•						
MeDIP–seq			•			•	•	•						•	•
WGSBS	•	•	•	•	•	•	•			•	•				

'•' indicates that the method has this feature or potentially has this bias; '(•)' indicates that the method has this feature to a limited extent or in some circumstances. BC–seq, bisulphite conversion followed by capture and sequencing; BSPP, bisulphite padlock probes; –chip, followed by microarray; MeDIP, methylated DNA immunoprecipitation; RRBS, reduced representation bisulphite sequencing; –seq, followed by sequencing; WGSBS, whole-genome shotgun bisulphite sequencing.

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Sample Throughput versus Genome Coverage

Bismark

BS-Seeker

B-SOLANA

BRAT

MethylCoder

RMAP-BS

SOCS-B

And others

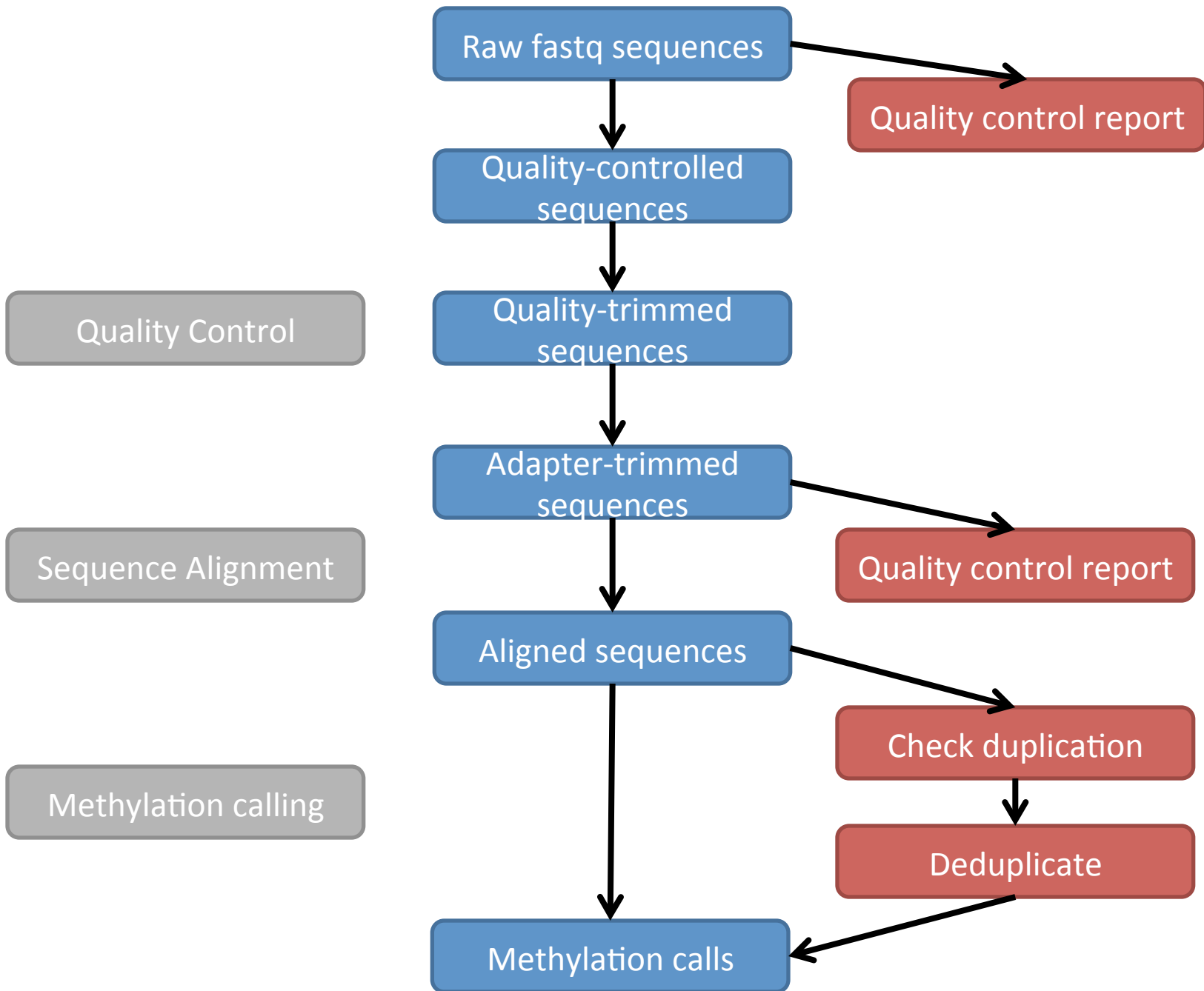| Resource	Purpose	URL	Refs
Batman	MeDIP DNA methylation analysis tool	http://td-blade.gurdon.cam.ac.uk/software/batman	88
BDPC	DNA methylation analysis platform	http://biochem.jacobs-university.de/BDPC	153
BSMAP	Whole-genome bisulphite sequence mapping	http://code.google.com/p/bsmap	154
CpG Analyzer	Windows-based program for bisulphite DNA	-	155
CpGcluster	CpG island identification	http://bioinfo2.ugr.es/CpGcluster	156
CpGFinder	Online program for CpG island identification	http://linux1.softberry.com	-
CpG Island Explorer	Online program for CpG Island identification	http://bioinfo.hku.hk/cpgieintro.html	157
CpG Island Searcher	Online program for CpG island identification	http://cpgislands.usc.edu	158
CpG PatternFinder	Windows-based program for bisulphite DNA	-	159
CpG Promoter	Large-scale promoter mapping using CpG islands	http://www.cshl.edu/OTT/html/cpg_promoter.html	160
CpG ratio and GC content Plotter	Online program for plotting the observed:expected ratio of CpG	http://mwsross.bms.ed.ac.uk/public/cgi-bin/cpg.pl	-
CpGviewer	Bisulphite DNA sequencing viewer	http://dna.leeds.ac.uk/cpgviewer	161
CyMATE	Bisulphite-based analysis of plant genomic DNA	http://www.gmi.oeaw.ac.at/en/cymate-index/	162
EMBOSS CpGPlot/ CpGReport/Isochore	Online program for plotting CpG-rich regions	http://www.ebi.ac.uk/Tools/emboss/cpgplot/index.html	-
Epigenomics Roadmap	NIH Epigenomics Roadmap Initiative homepage	http://nihroadmap.nih.gov/epigenomics	-
Epinexus	DNA methylation analysis tools	http://epinexus.net/home.html	-
MEDME	Software package (using R) for modelling MeDIP experimental data	http://espresso.med.yale.edu/medme	163
methBLAST	Similarity search program for bisulphite-modified DNA	http://medgen.ugent.be/methBLAST	164
MethDB	Database for DNA methylation data	http://www.methdb.de	165–168
MethPrimer	Primer design for bisulphite PCR	http://www.urogene.org/methprimer	169
methPrimerDB	PCR primers for DNA methylation analysis	http://medgen.ugent.be/methprimerdb	164
MethTools	Bisulphite sequence data analysis tool	http://www.methdb.de	170
MethyCancer Database	Database of cancer DNA methylation data	http://methycancer.psych.ac.cn	171
Methyl Primer Express	Primer design for bisulphite PCR	http://www.appliedbiosystems.com/methylprimerexpress	-
Methylumi	Bioconductor package for analysing DNA methylation data from Illumina platforms	http://www.bioconductor.org/packages/bioc/html/methylumi.html	-
Methylyzer	Bisulphite DNA sequence visualization tool	http://ubio.bioinfo.cnio.es/Methylyzer/main/index.html	-
mPod	Genome-wide DNA methylation viewer integrated with the Ensembl genome browser	http://www.compbio.group.cam.ac.uk/Projects/p4meth.html	172
PubMeth	Database of DNA methylation literature	http://www.pubmeth.org	173
QUMA	Quantification tool for methylation analysis	http://quma.cdb.riken.jp	174
TCGA Data Portal	Database of TCGA DNA methylation data	http://cancergenome.nih.gov/dataportal	-

BDPC, Bisulphite Sequencing Data Presentation and Compilation; BSMAP, Bisulphite Sequence Mapping Program; CyMATE, Cytosine Methylation Analysis Tool for Everyone; EMBOSS, European Molecular Biology Open Software Suite; MeDIP, methylated DNA immunoprecipitation; MEDME, Modelling Experimental Data with MeDIP Enrichment; NIH, US National Institutes of Health; QUMA, Quantification Tool For Methylation Analysis; TCGA, The Cancer Genome Atlas.

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