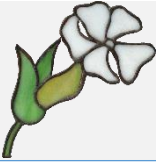


CURRENT METHODOLOGY IN EPIGENETIC ANALYSIS: HISTONE AND GENOMIC DNA MODIFICATIONS

José Luis Rodríguez Lorenzo
23 November 2015



Epigenetic marks

- DNA modifications
- Histone modifications
- Non coding RNA

A timeline illustrating the evolution of DNA methylation analysis methods from 1970 to 2000. The timeline is marked with years from 1970 to 2000 in 5-year increments. Key events are indicated by vertical blue lines with corresponding labels above them:

- 1970:** Sodium Bisulphite reaction described
- 1979:** First use of Methylation Sensitive Restriction enzymes
- 1985:** Anti-5' MeC first used
- 1989:** Bisulphite Sequencing
- 1996:** (Label partially visible: "Bisulphite Sequencing")
- 1997:** (Label partially visible: "Bisulphite Sequencing")
- 1999:** (Label partially visible: "Bisulphite Sequencing")

Logos for Illumina and PacBio are visible in the bottom right corner.

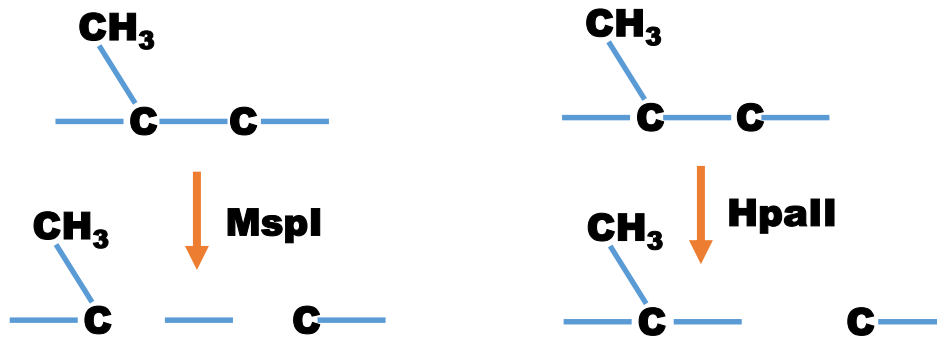


Modified from Harrison et al., 2011

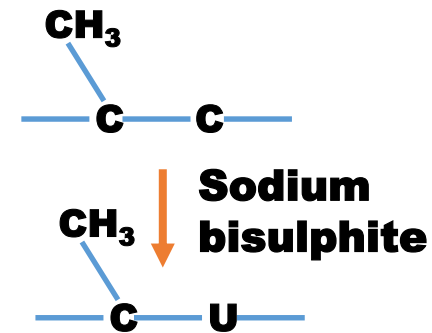


Main approaches for DNA methylation analysis of genomes

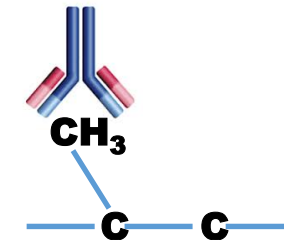
Methyl sensitive Restriction enzyme

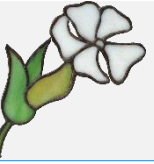


Bisulphite Sequencing

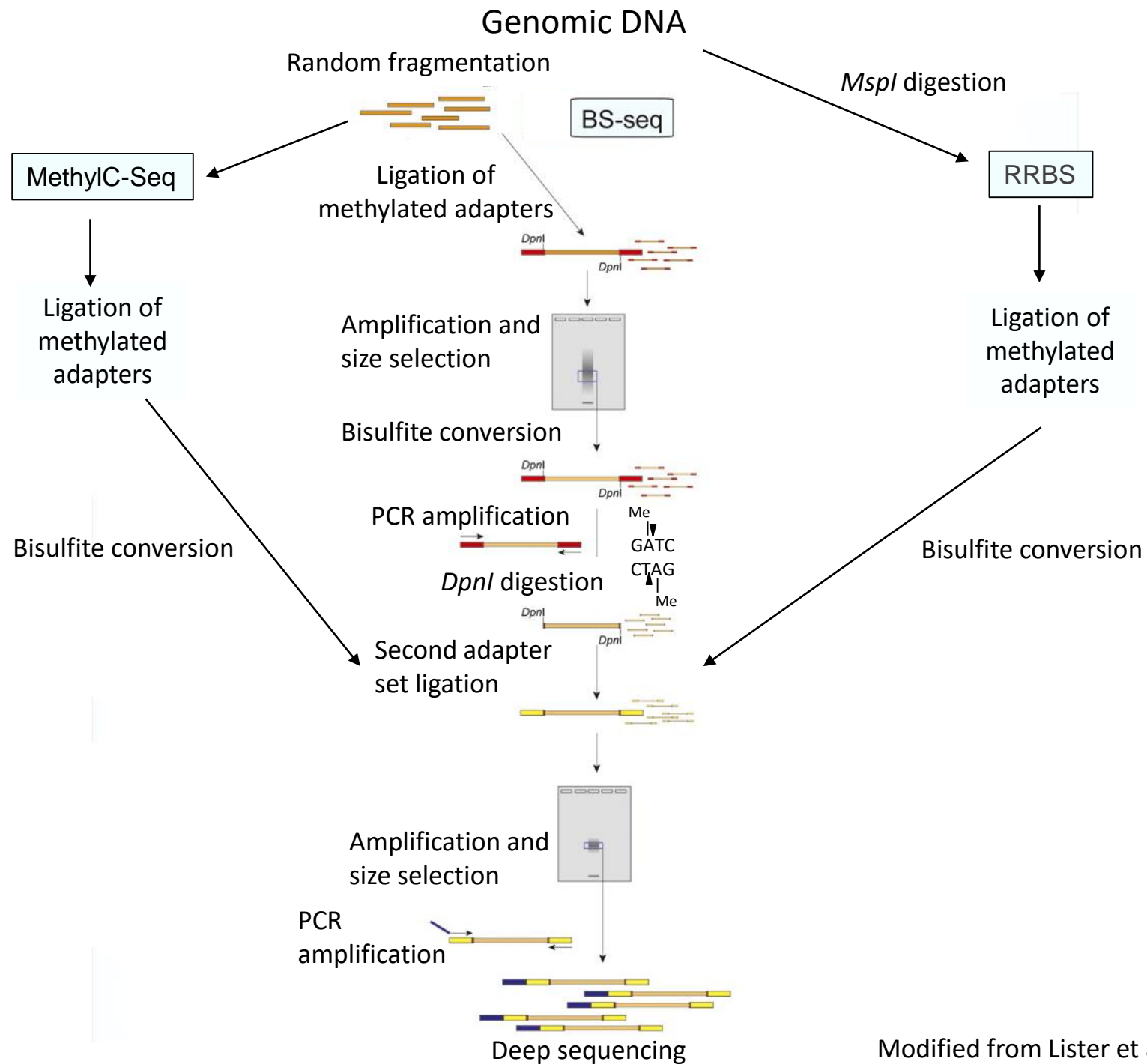


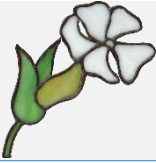
Immunoprecipitation



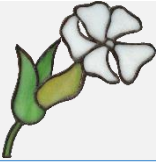


- Bisulphite-based methods:
 - Non-targeted enrichment methods
 - MethylC-seq
 - reduced representation bisulphite sequencing (RRBS)
 - Targeted enrichment methods
 - Bisulphite Padlock Probe (BSPP)
 - Bisulphite Patch PCR
- Enrichment-based techniques:
 - methylated DNA immunoprecipitation sequencing (MeDIP-seq)
 - methylated DNA binding domain sequencing (MBD-seq)

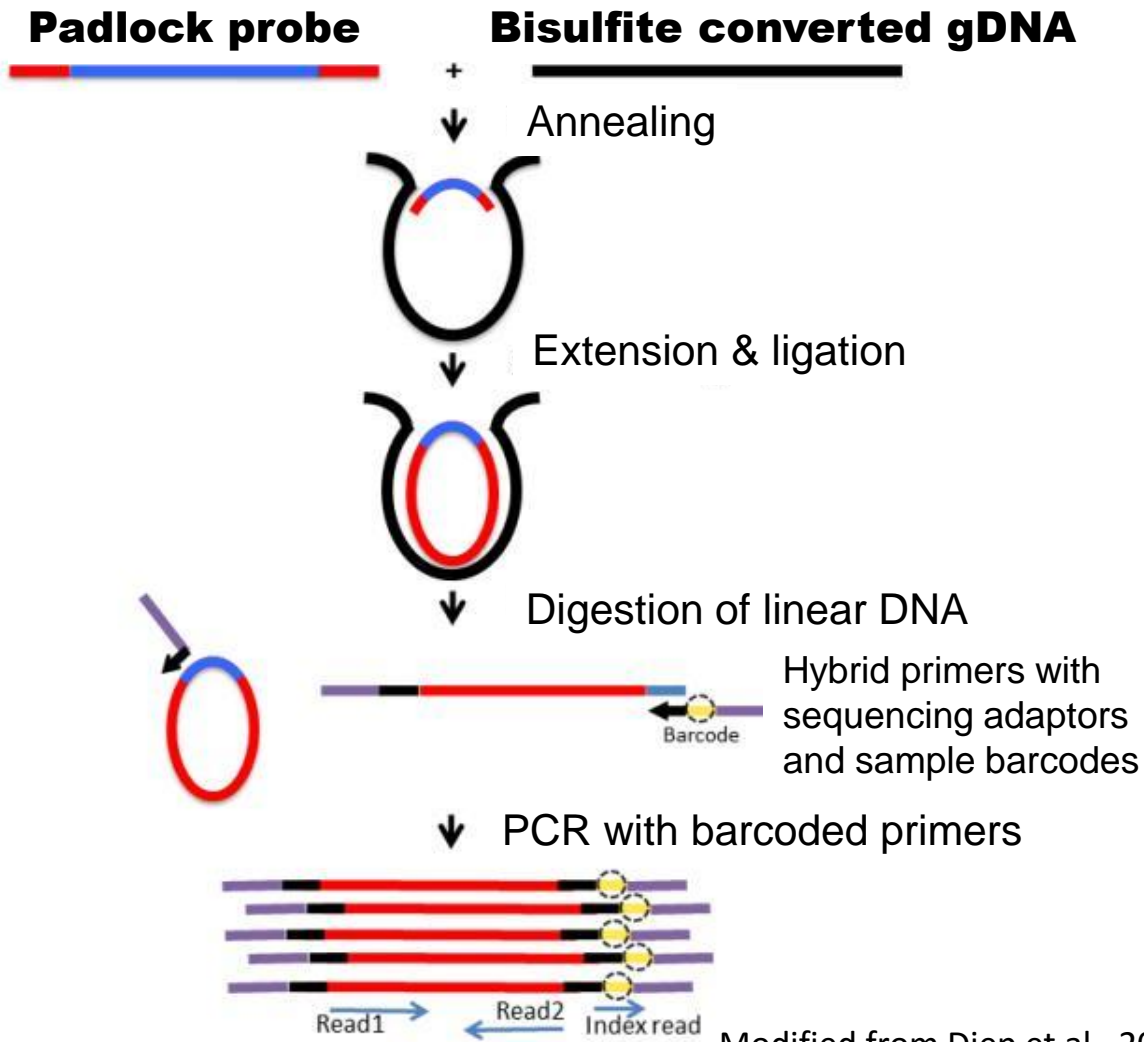




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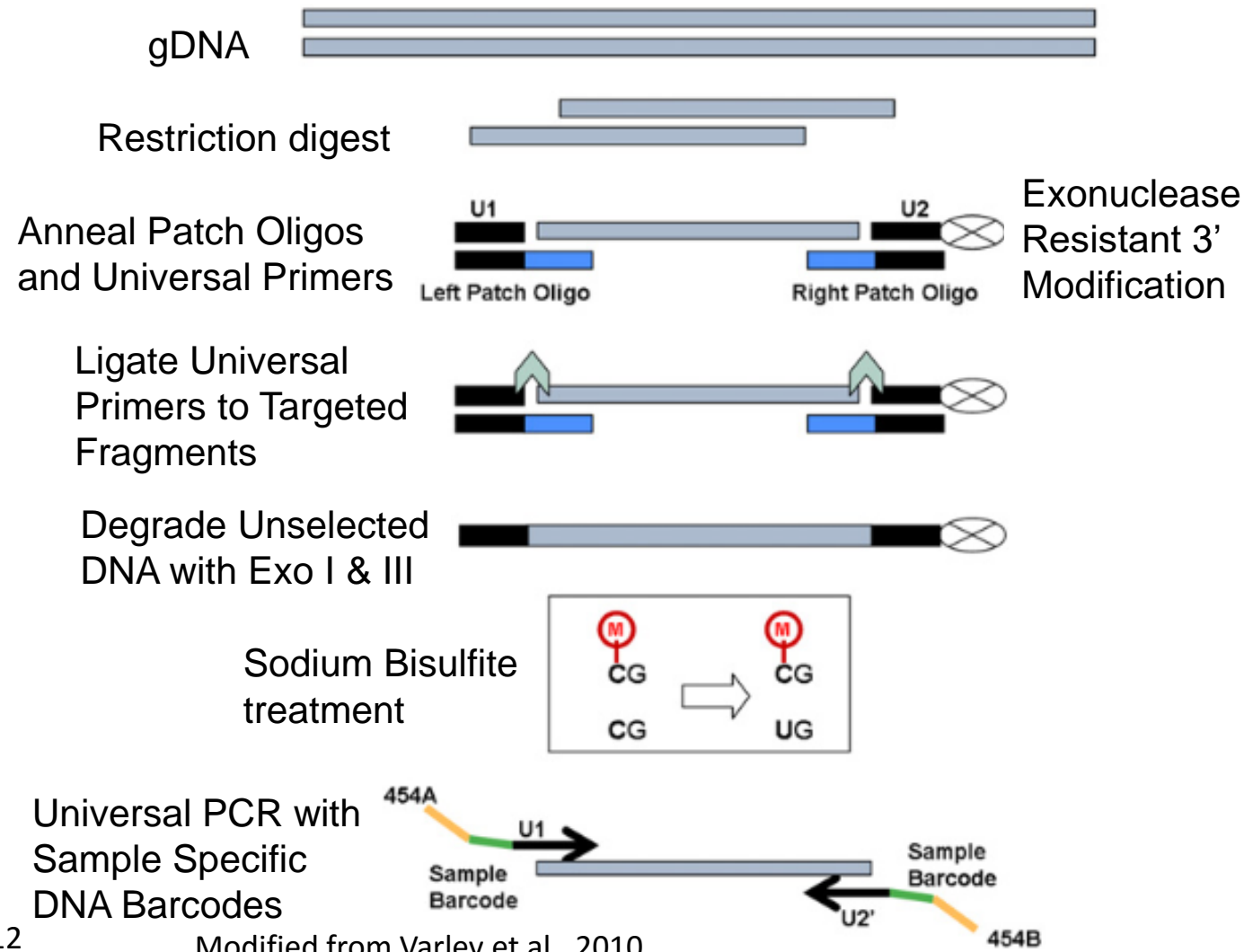


Bisulfite Padlock Probe (BSPP)

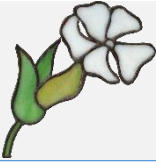


Modified from Diep et al., 2012

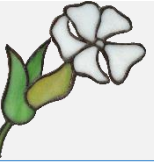
Bisulfite Patch PCR



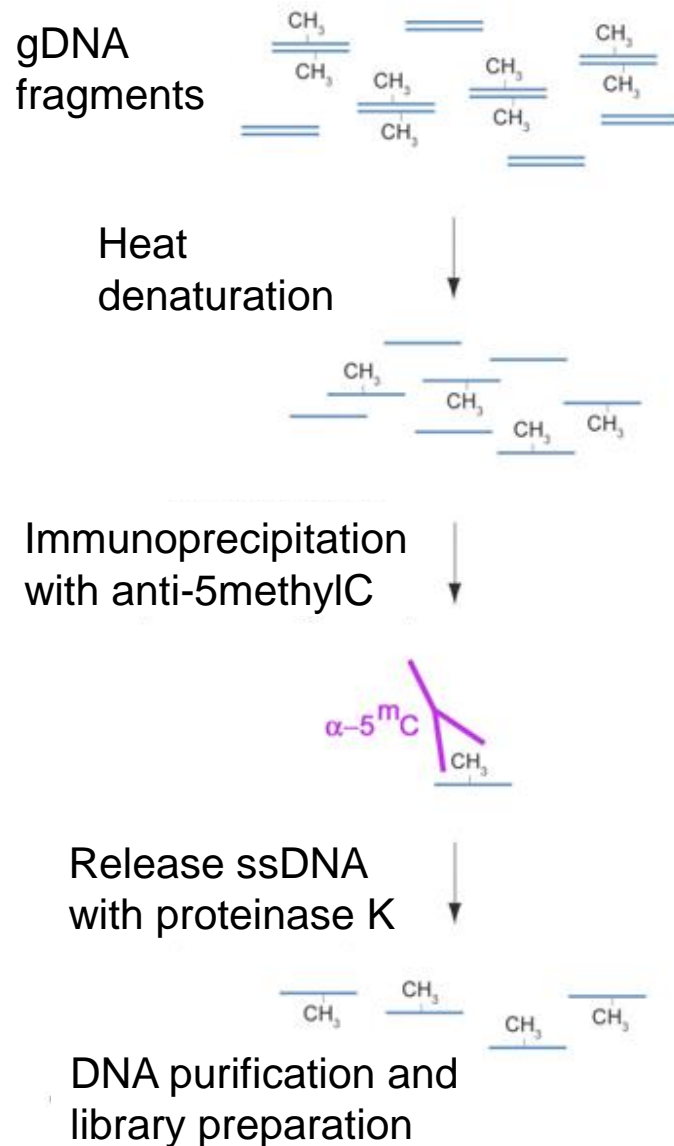
Modified from Varley et al., 2010



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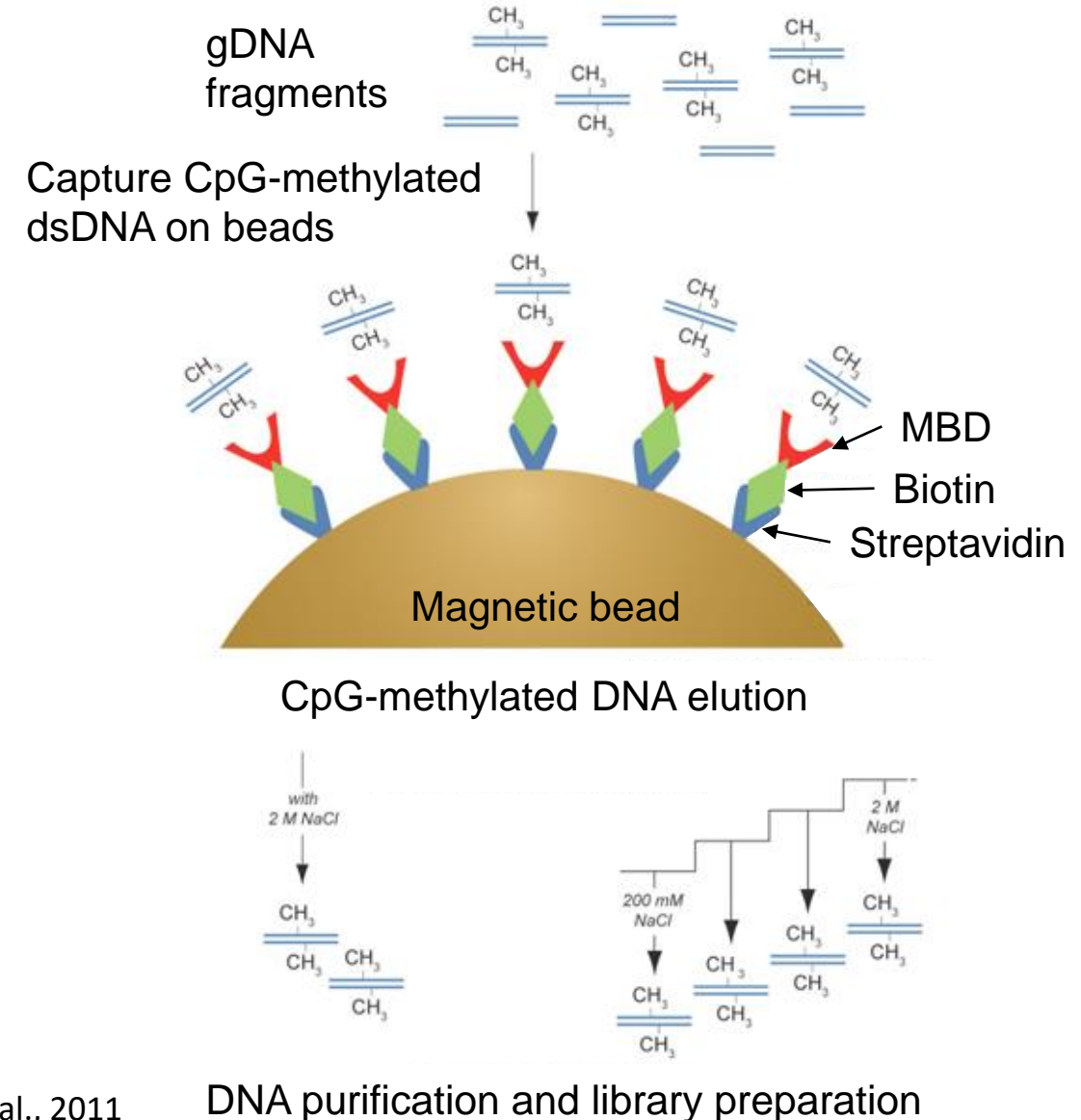


MeDIP



Modified from Kennedy et al., 2011

MBD

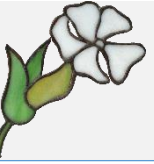


DNA purification and library preparation

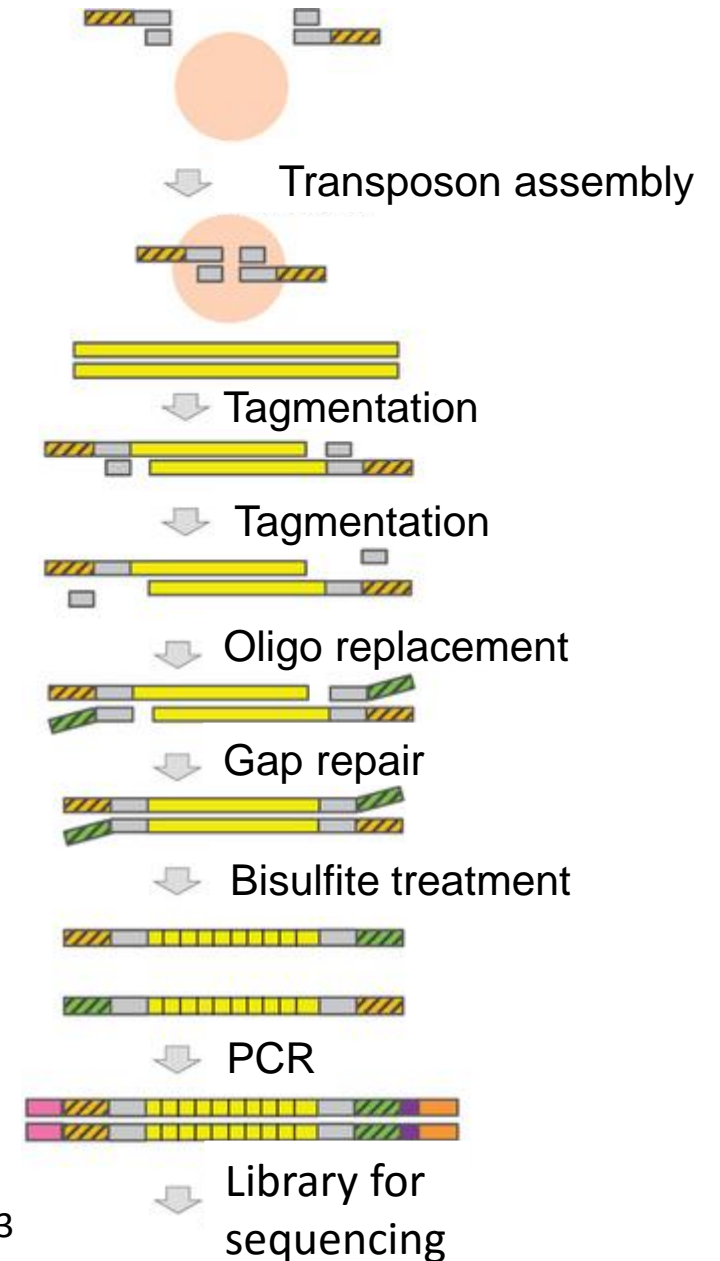
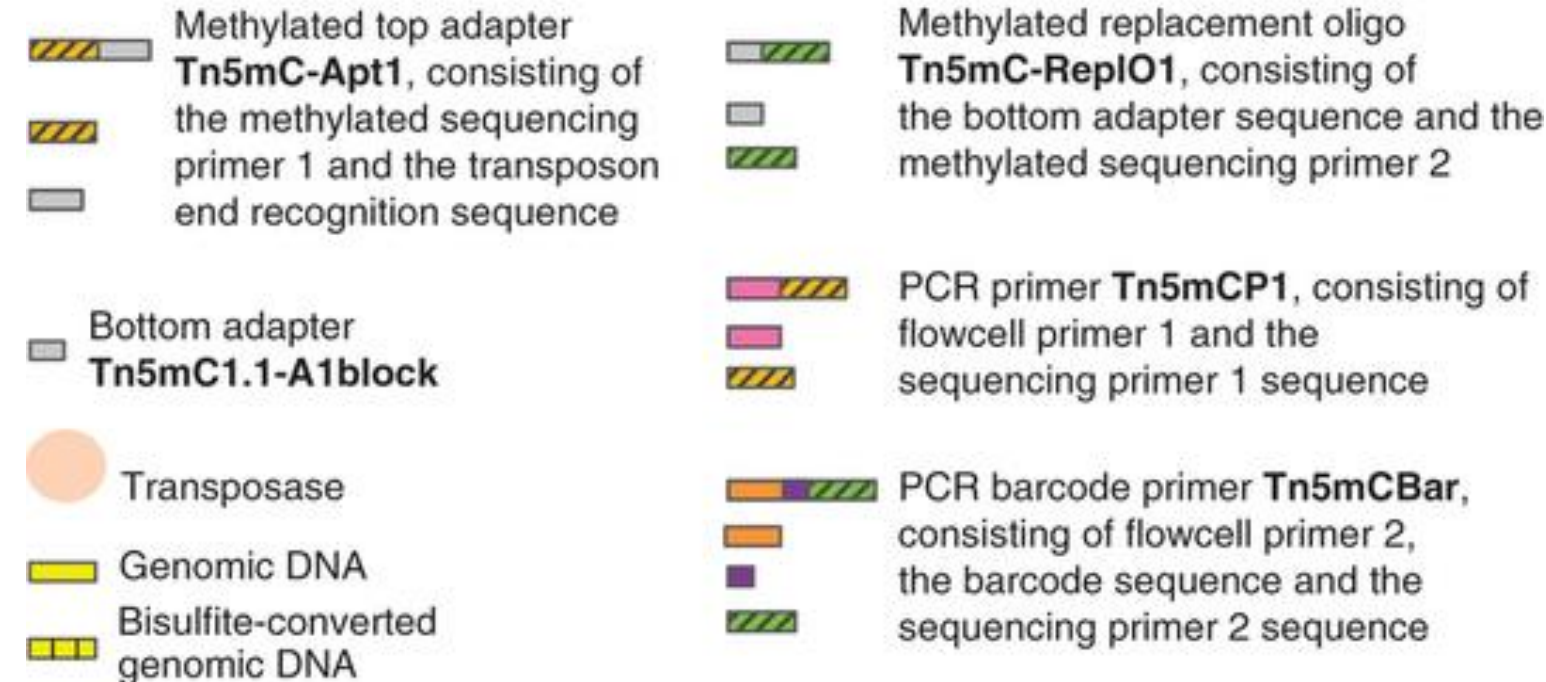


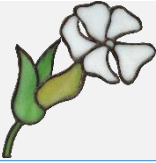
- Low Input and Single-cell methods

- Tn5mC-seq
- Methylation-sensitive restriction enzyme-based assay (RSMA)

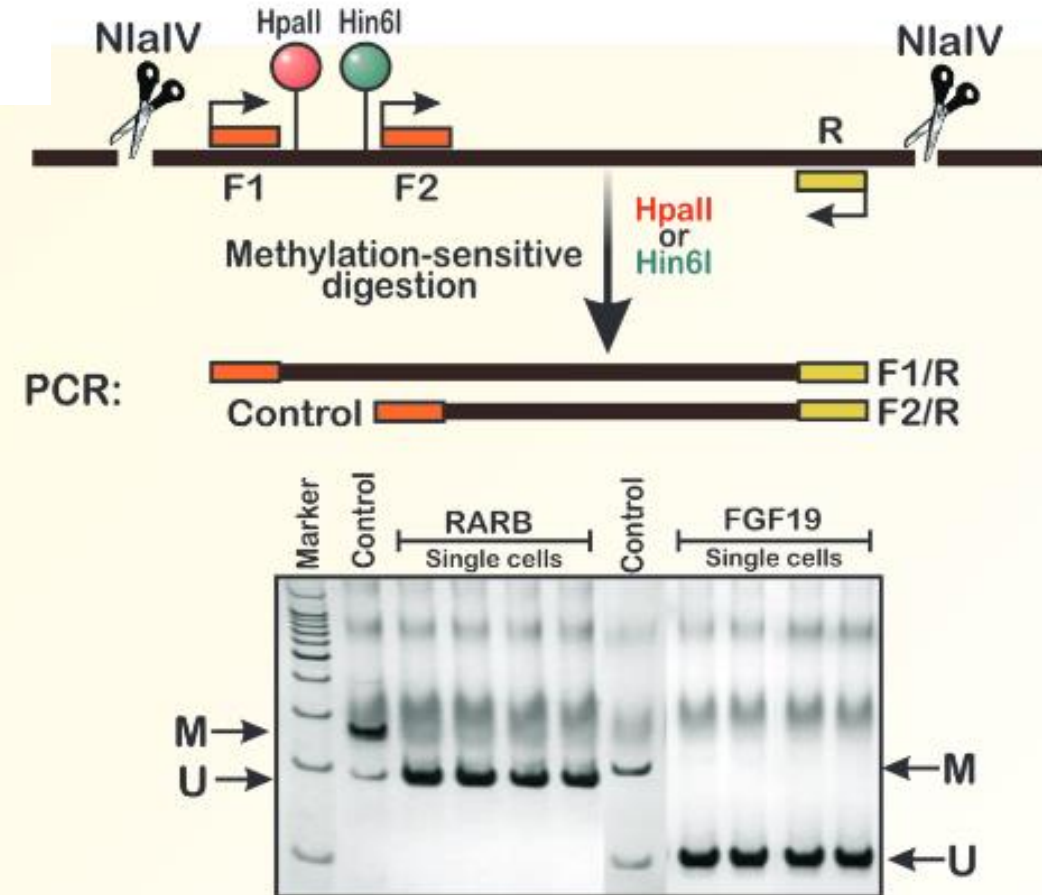
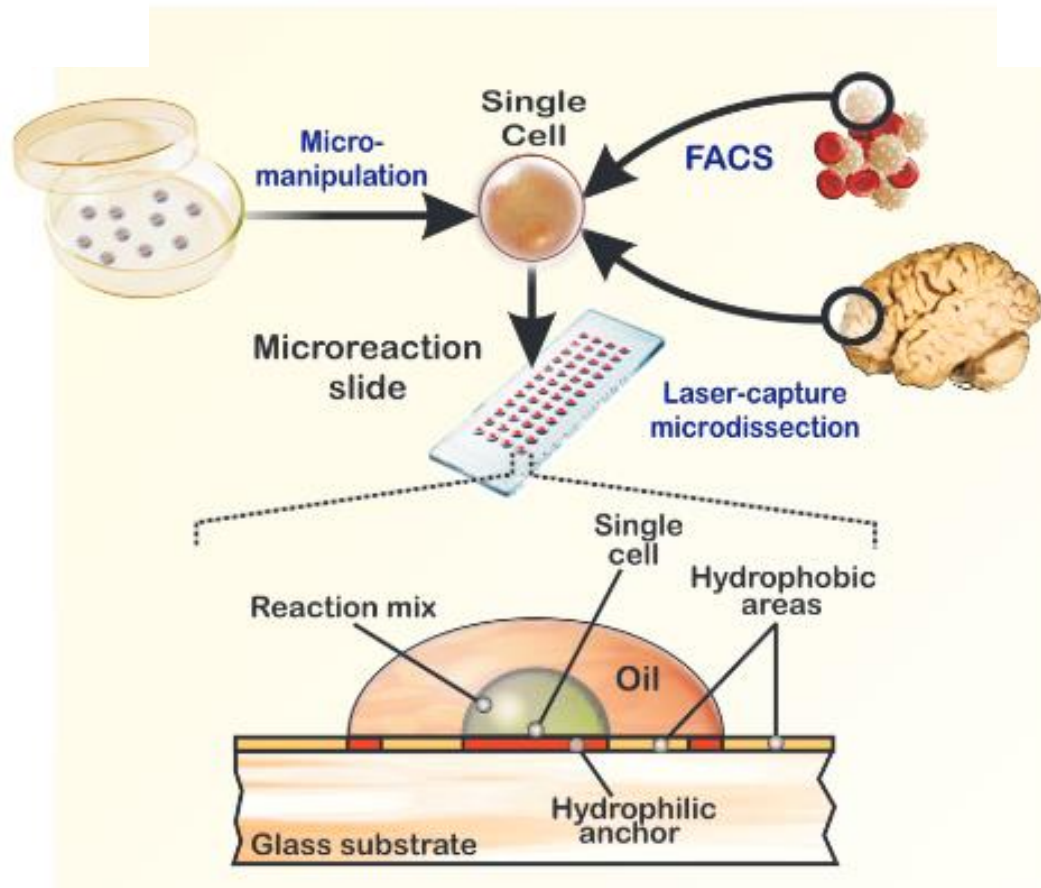


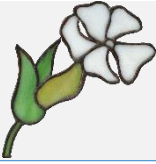
Tn5mC-seq





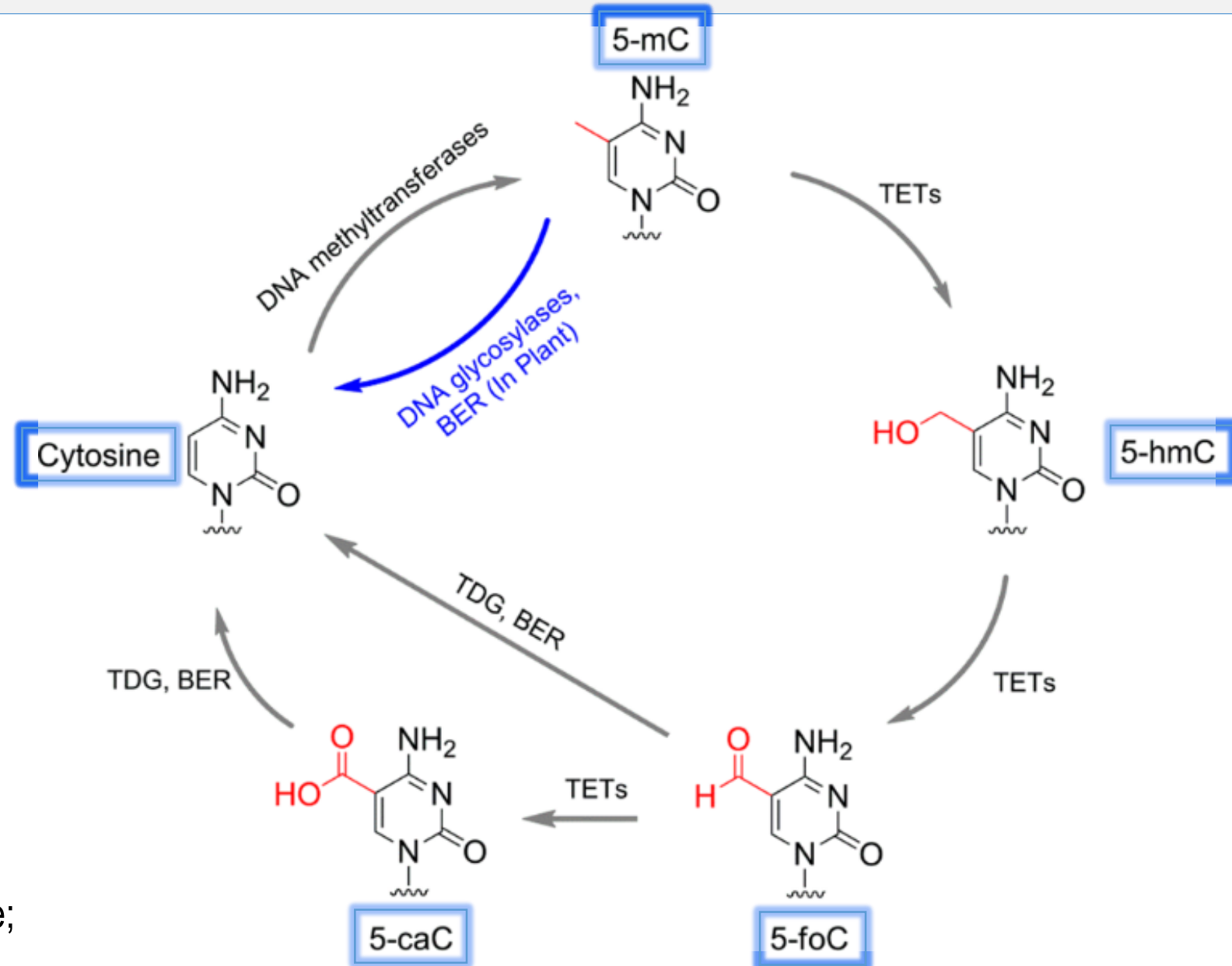
Methylation-sensitive restriction enzyme-based assay (RSMA)



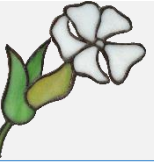


DNA Modifications

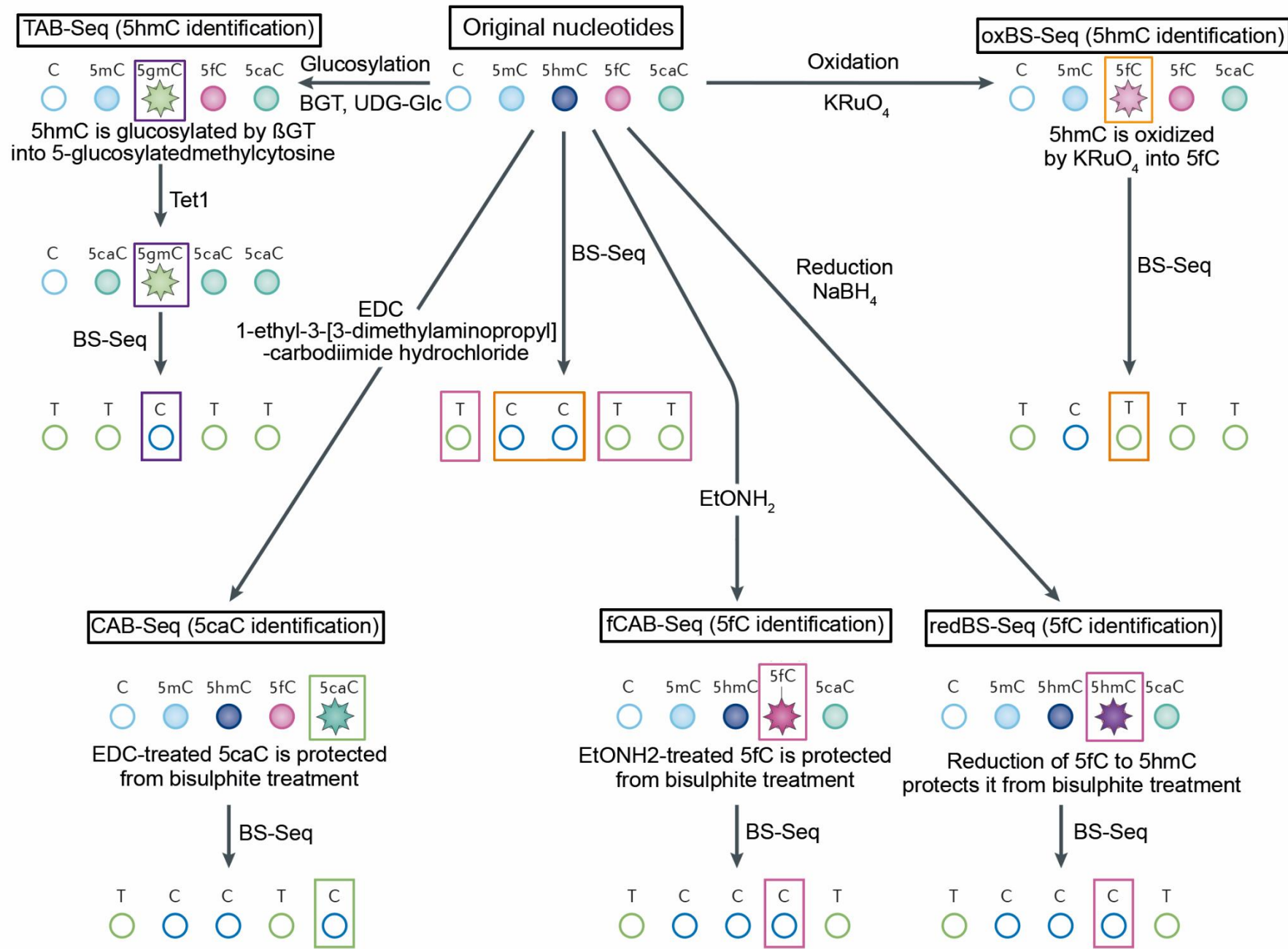
- 5-methylcytosine (5mC)
- 5-hydroxymethylcytosine (5hmC)
- 5-formylcytosine (5fC)
- 5-carboxycytosine (5caC)

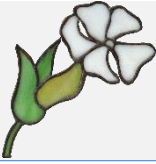


BER, base excision repair;
TDG, thymine-DNA glycosylase;
TET, Ten-eleven translocation.



Assays for mapping 5-methylcytosine (5mC)
oxidation derivatives at single base resolution



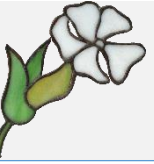


Base	Bs-seq	Oxbs-seq	TAB-seq	fCAB-Seq	redBS-Seq	CAB-Seq
C	T	T	T	T	T	T
5mC	C	C	T	C	C	C
5hmC	C	T	C	C	C	C
5fC	T	-	-	C	C	T
5caC	T	-	-	-	-	C
C read out	5mC+5hmC	5mC	5hmC	5mC+5hmC+5fC	5mC+5hmC+5fC	5mC+5hmC+5caC

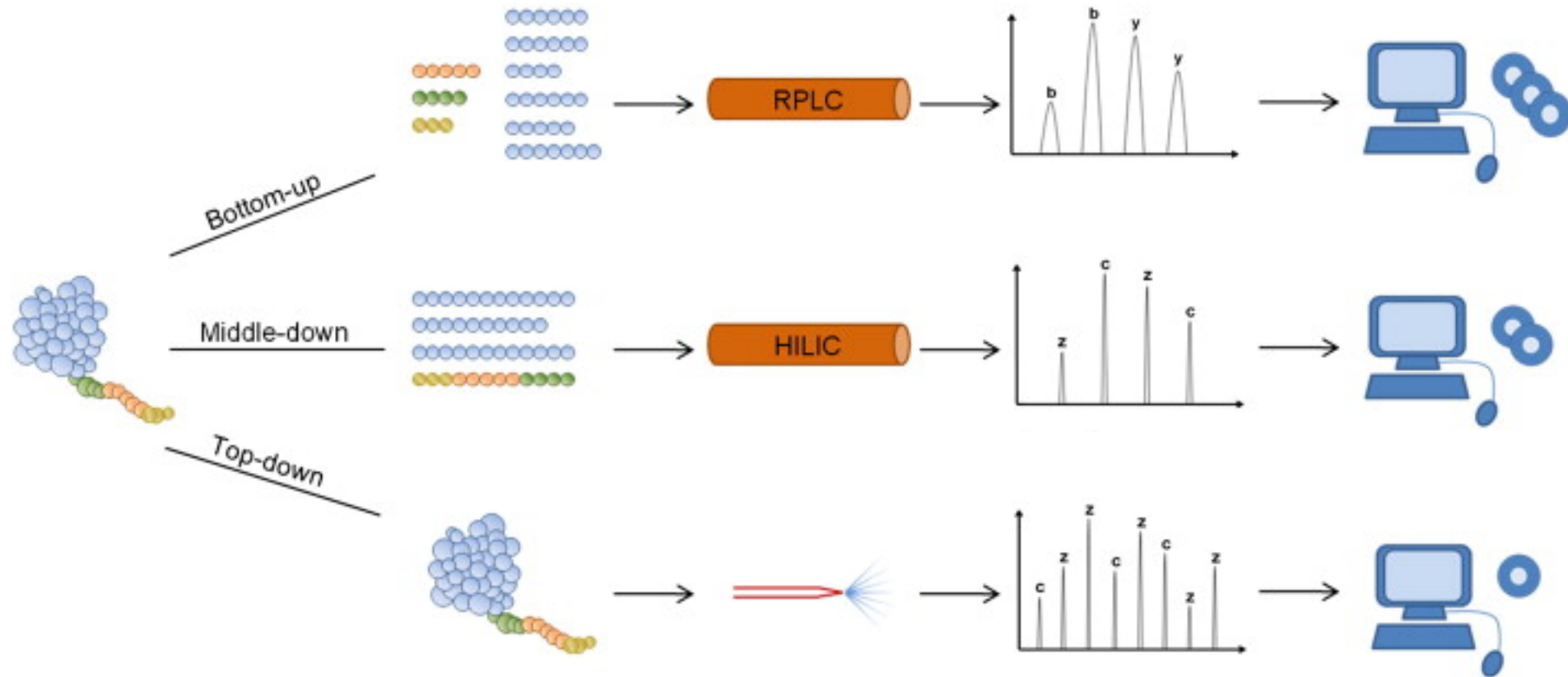


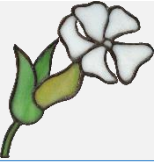
Histones and transcription factors

- Global quantification
- DNA-protein interaction
 - Chromatin Immunoprecipitation (ChIP)
 - Protein based (ChIP-like methods)
 - DNA based
- Chromosome Conformation Capture

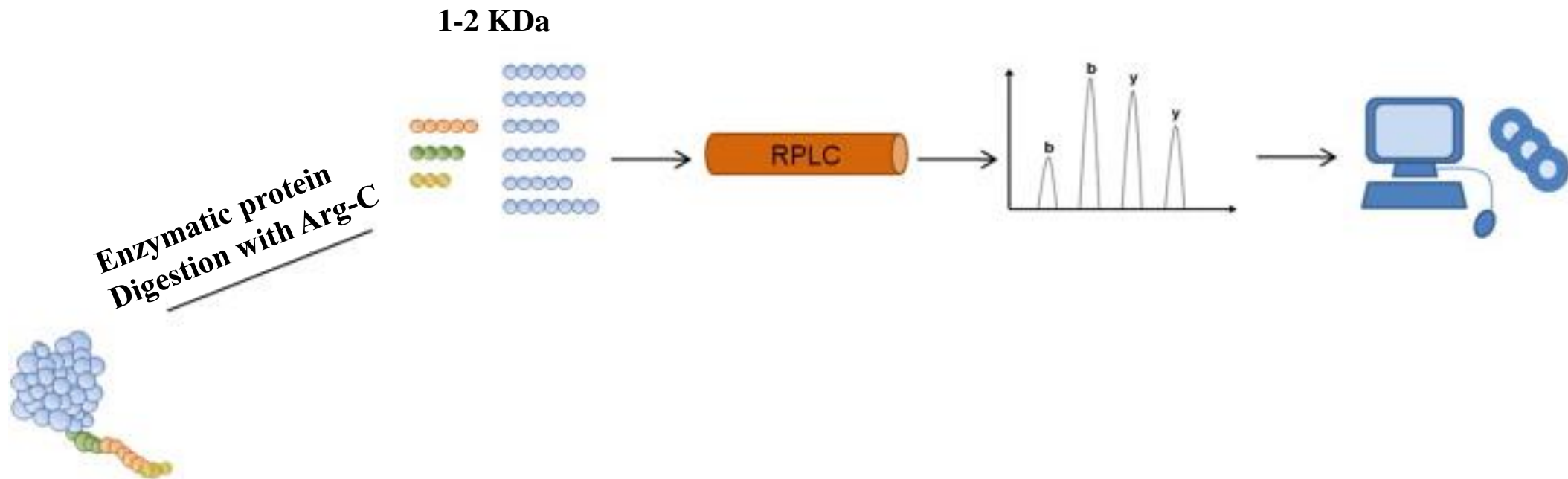


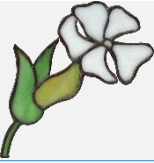
Global quantification by Mass Spectrometry



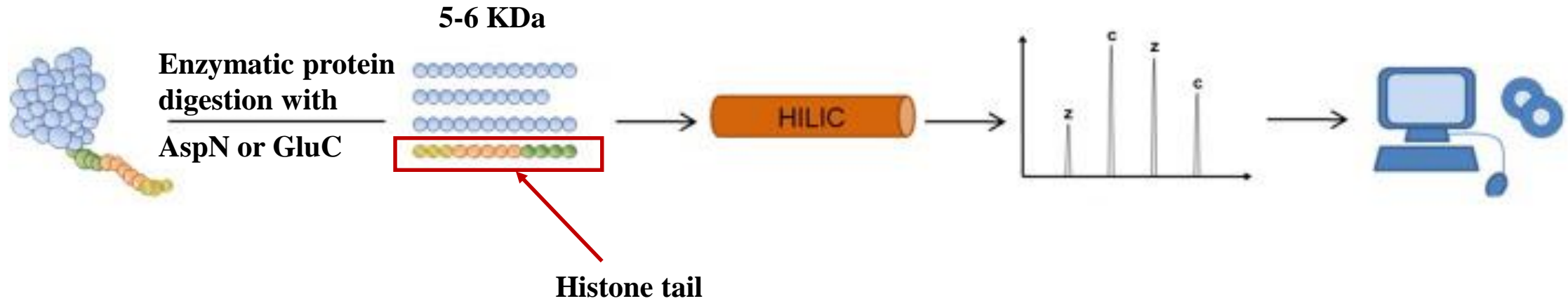


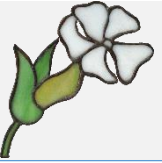
Bottom-up analysis



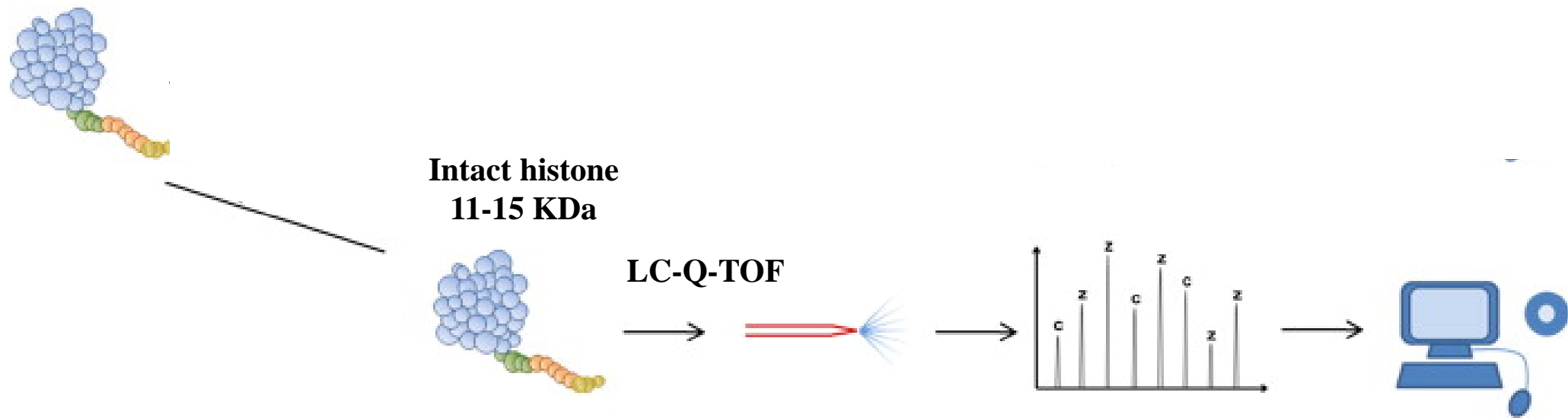


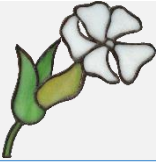
Middle-down analysis





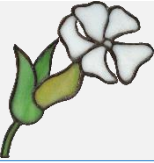
Top-down analysis



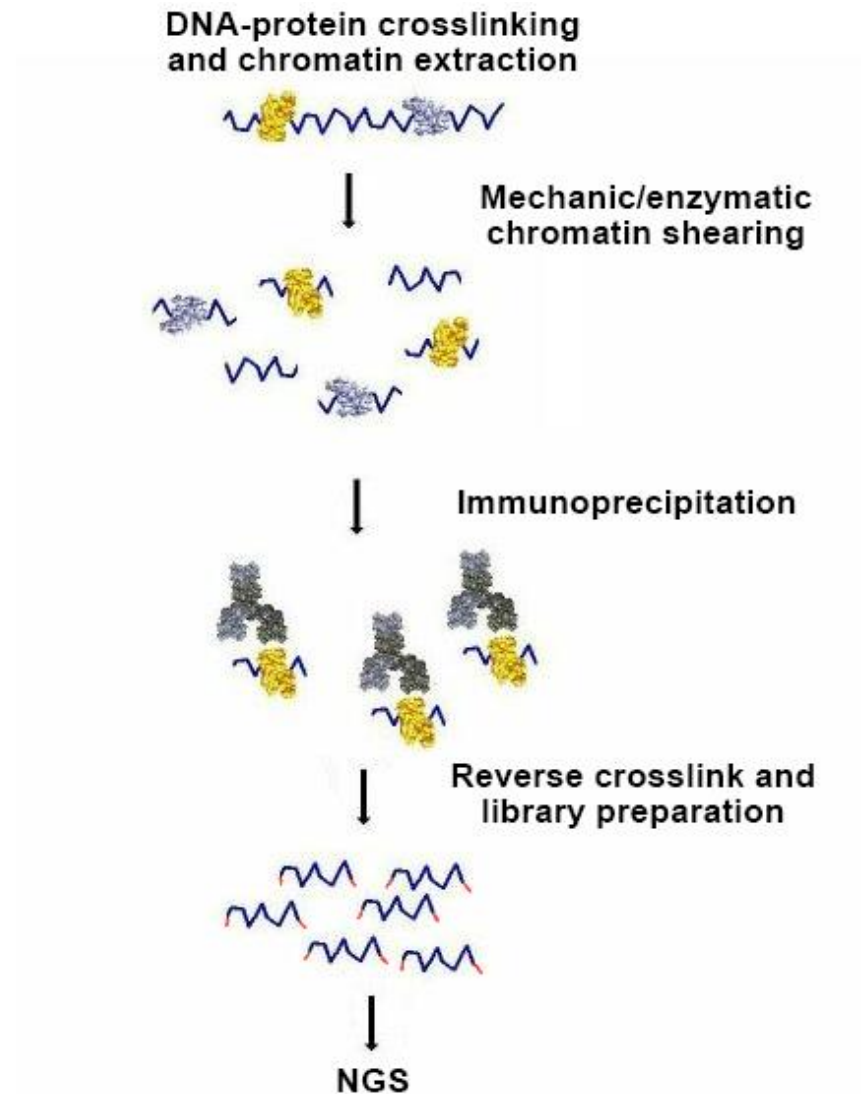


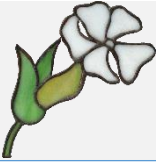
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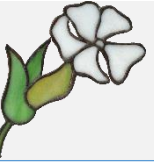
Chromatin Immunoprecipitation (ChIP)





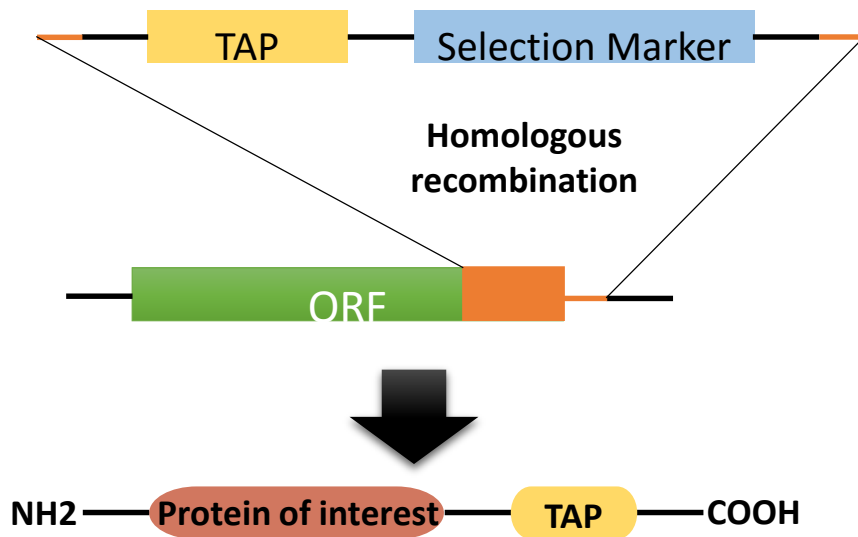
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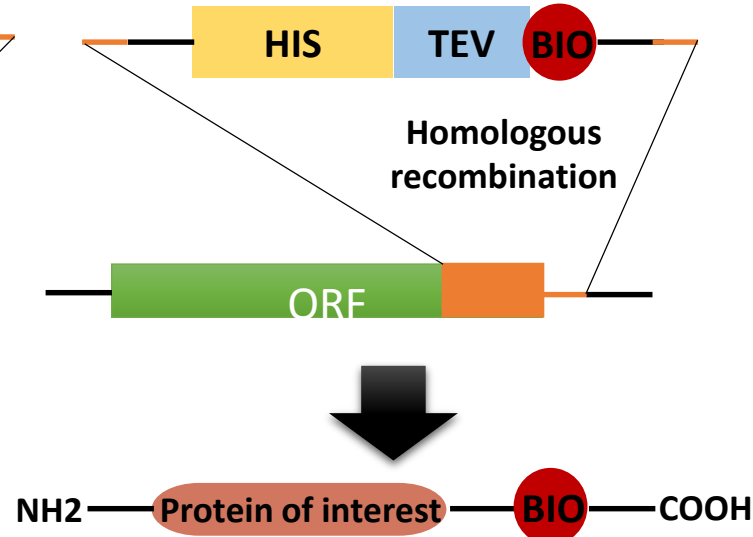
Modified Chromatin Immuno Purification

TAP-tagged histone



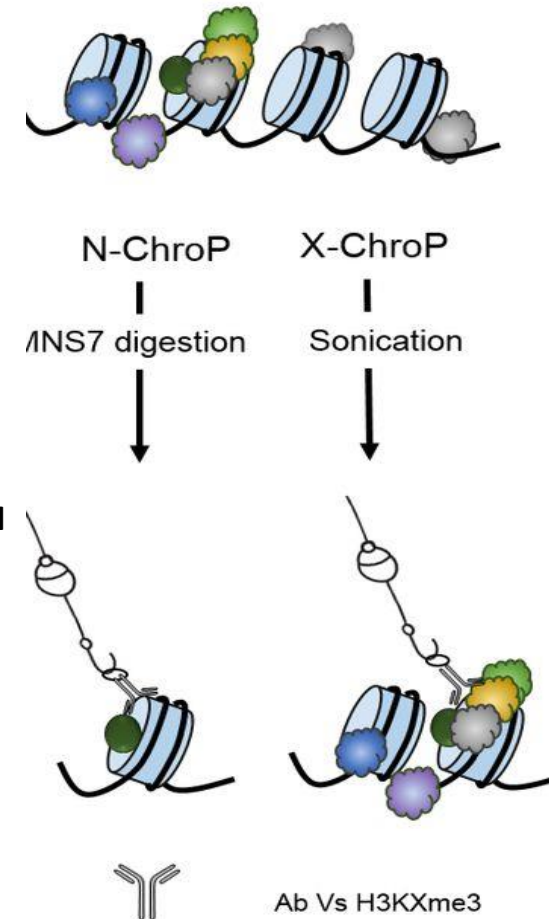
Chromatin-Interacting Protein Mass Spectrometry

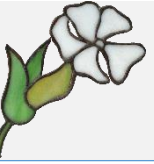
HTB tag



Chromatin Proteomics

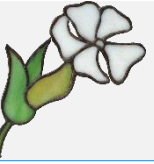
ChroP





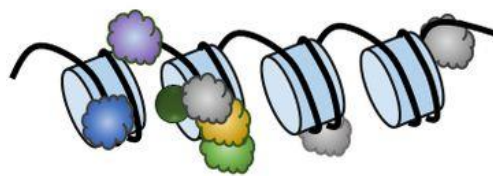
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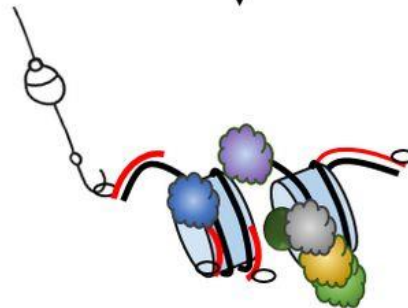


Proteomics of Isolated Chromatin

PICh



Sonication



LNA capture probes

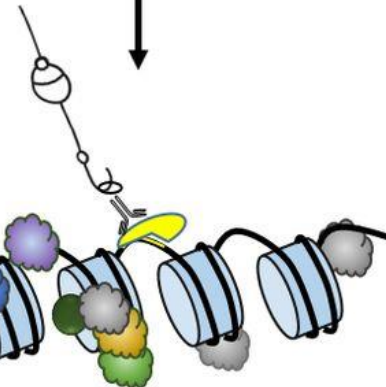
Chromatin Affinity with Mass Spectrometry

ChAP-MS

Engineered LexA
binding site



Sonication



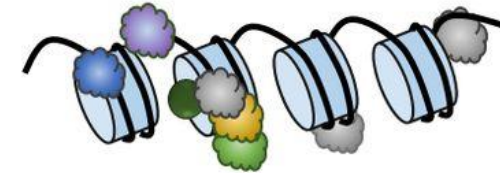
LexA-PrA



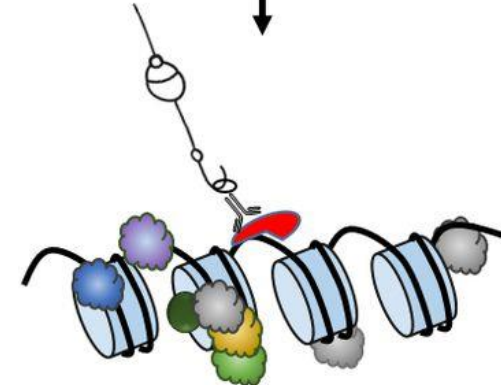
Affinity purification

TALEN/CRISPR-based Chromatin Affinity Purification with Mass Spectrometry

CRISPR-ChAP-MS TALEN-ChAP-MS



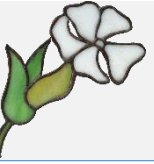
Sonication



TAL Pr

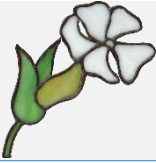


Affinity purification

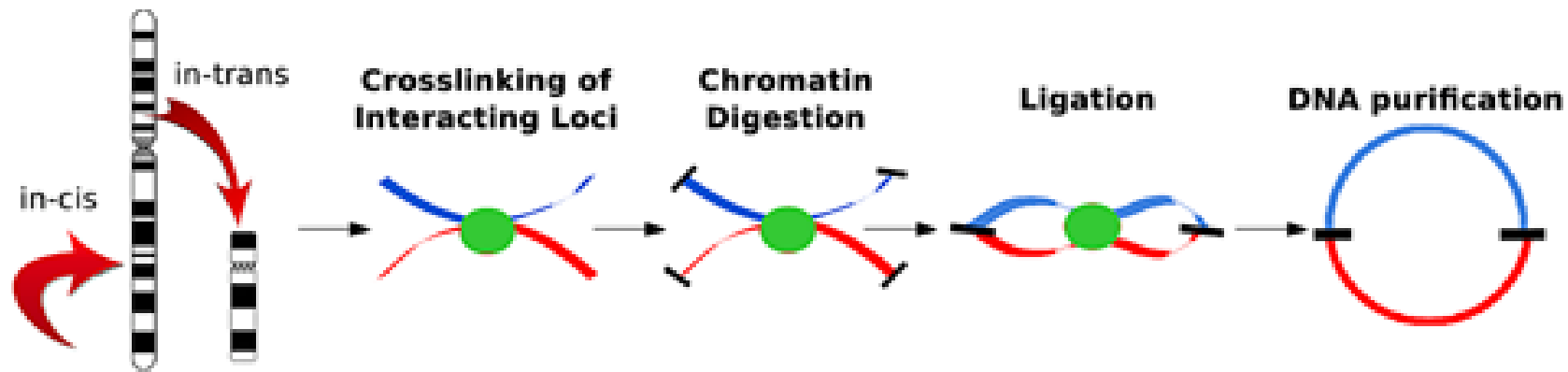


Histones and transcription factors

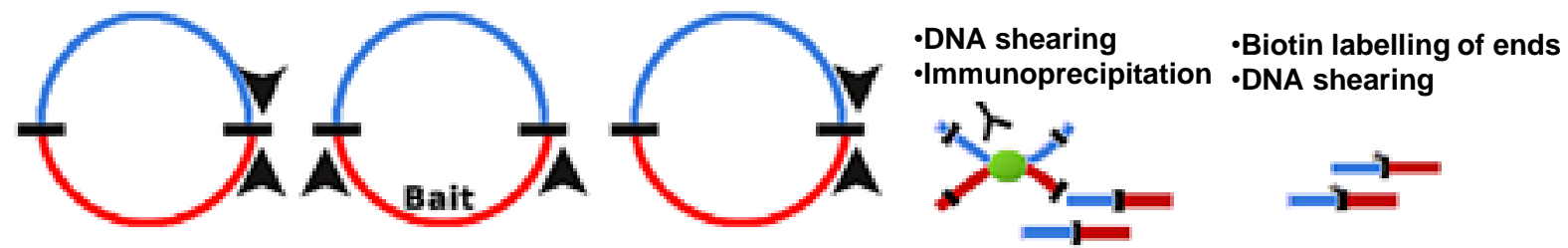
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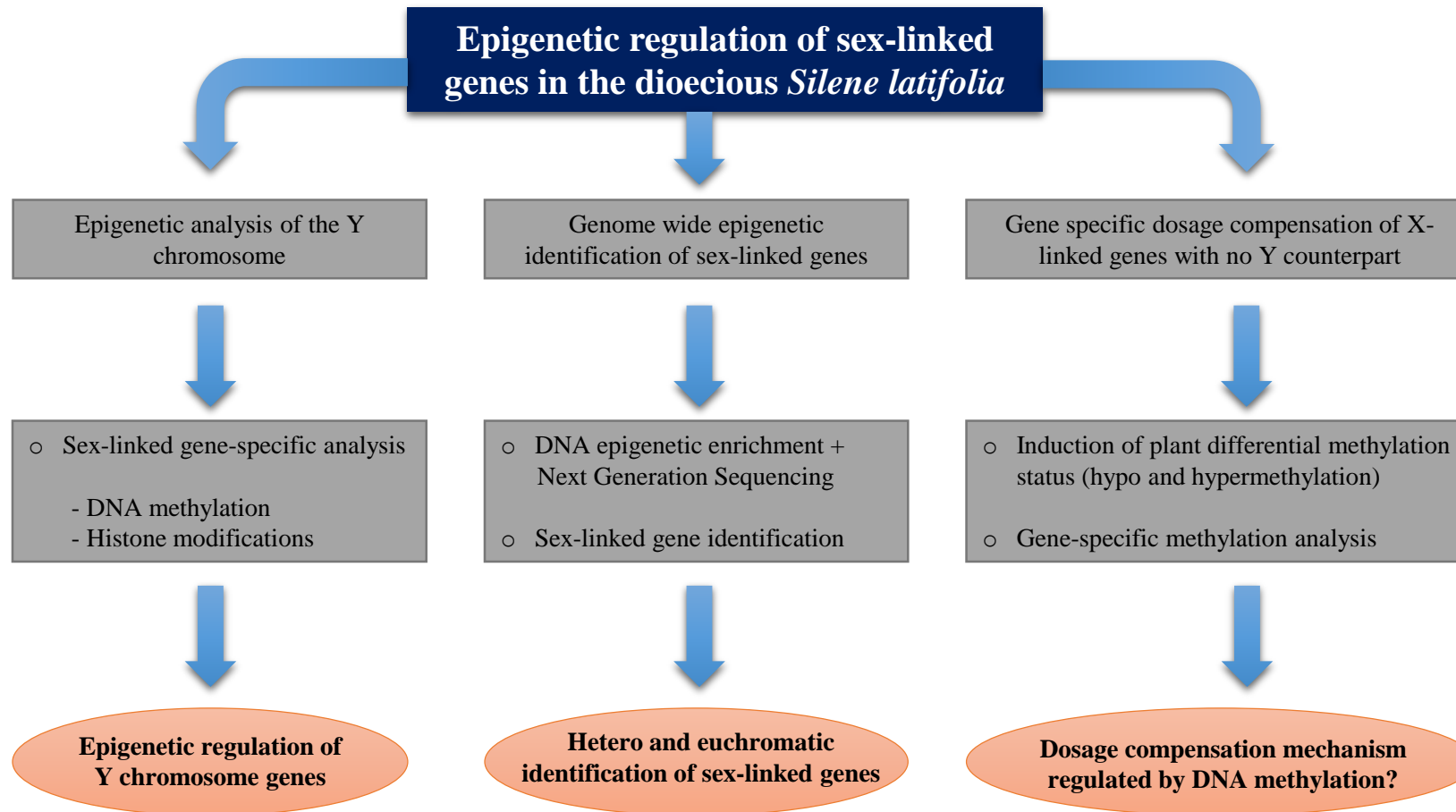
Chromosome Conformation Capture



Method:	3C	4C	5C	ChIA-PET	Hi-C
Type of Data:	One by One All by All	One by All	Many by Many	Many by Many	All by All



Readout:	PCR or sequencing	Inverse PCR Sequencing	Multiplexed LMA Sequencing	Sequencing	Sequencing
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Thank you for your attention

