Barcelona Supercomputing Center
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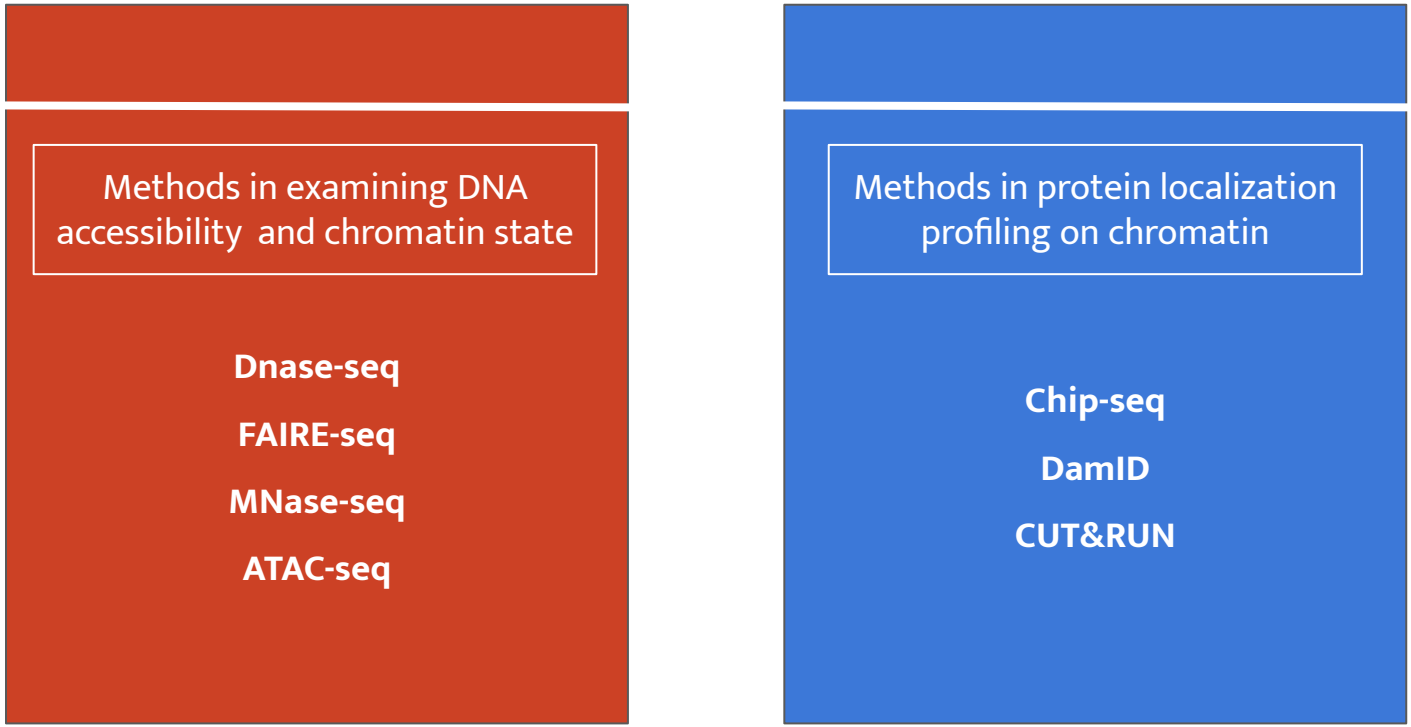
DNA adenine methyltransferase (DamID)

Application case:
Lamin-associated domains (LADs)

María Morales Martínez
maria.morales@bsc.es

Genomic methods to profile DNA

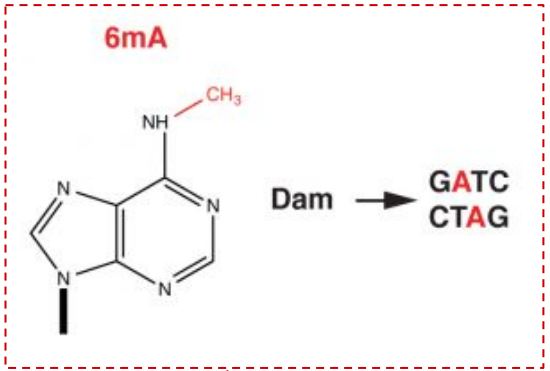


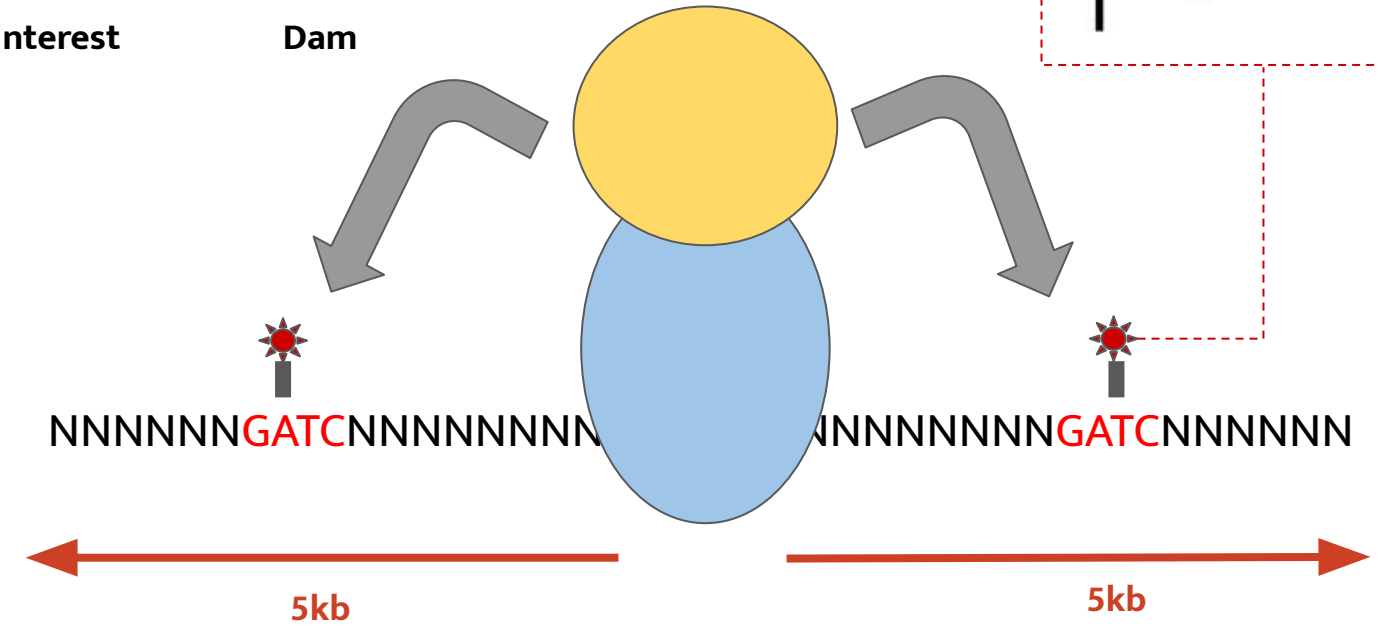
(Klein DC and Hainer SJ, 2020)

What is DamID?

Protein of interest

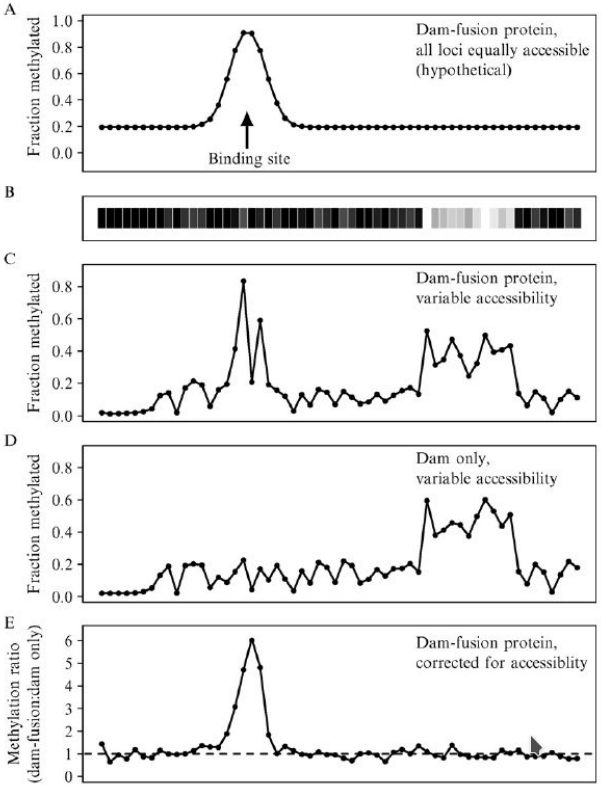
Dam

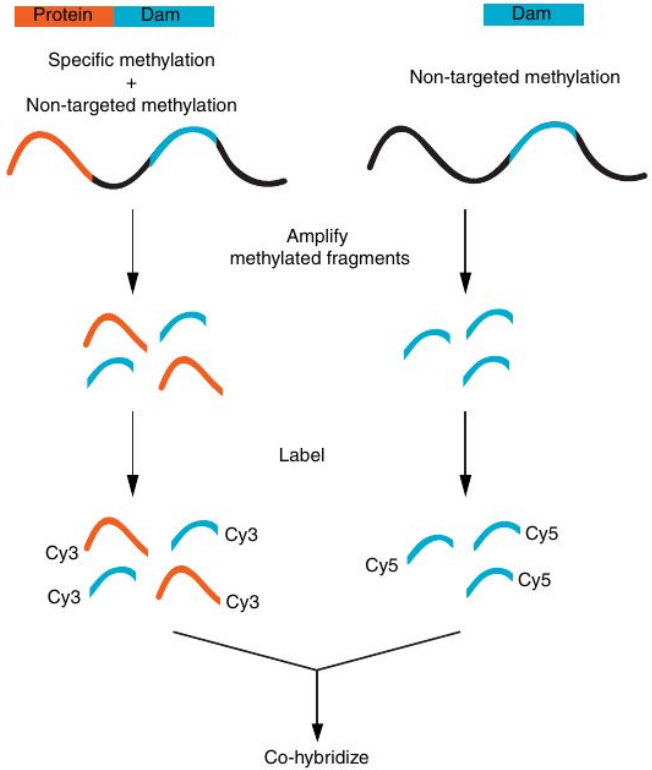




(Mod Greil F et al., 2006)

Correcting for Untargeted Binding of Dam





(Greil F et al, 2006; Maartje JV et al, 2007)

Expression Levels of Dam and Dam Fusion Proteins

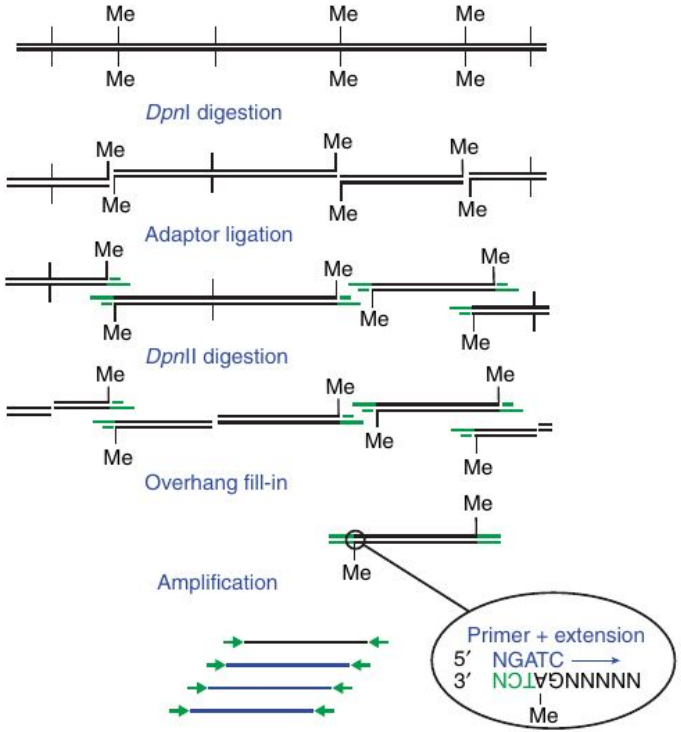
Why is important use weak promoters?

- High levels of m6A has deleterious effects on cell function
- Gene expression saturation could interfere with fundamental process in cell
- Imposible to apply quantitative approach to detect methylation levels

(Gabriel NA et al., 2019; Maartje JV et al, 2007)

Principle of DamID

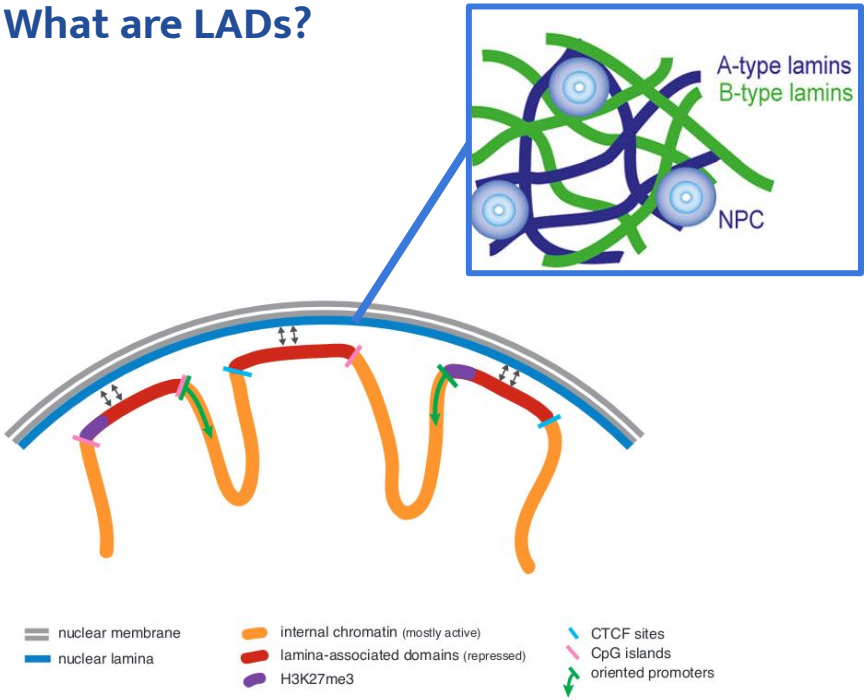
1. Generation of animals or cells with Dam-fusion transgene and Dam-only control.
2. Extraction of genomic DNA from tissue or organism of interest.
3. Digestion of DNA with DpnI (only methylated GATC)
4. Ligation of PCR adapters to digested DNA
5. Digestion of remaining unmethylated DNA with DpnII (not methylated GATC)
6. PCR amplification of adapter ligated GATC fragments
7. Microarray preparation techniques to analysis (depends on protocol Sequencing could be used)



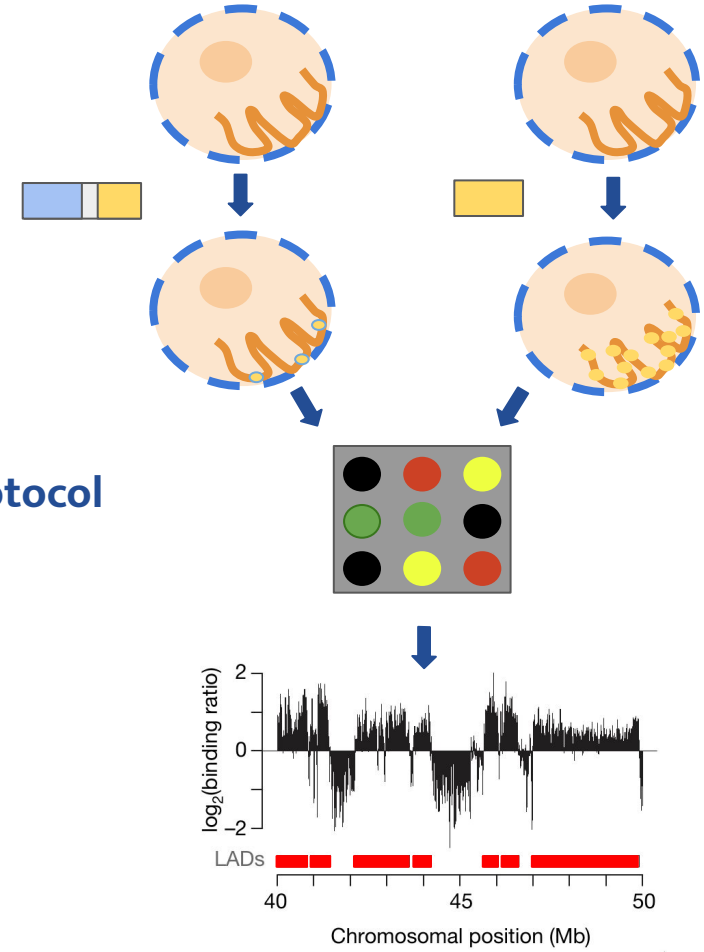
(Greil F et al., 2006; Gabriel NA et al.,2019)

Case of study: Detection of LADs

What are LADs?



Protocol



(Biand N and Collas P, 2019; Mod Guelen L et al., 2018)

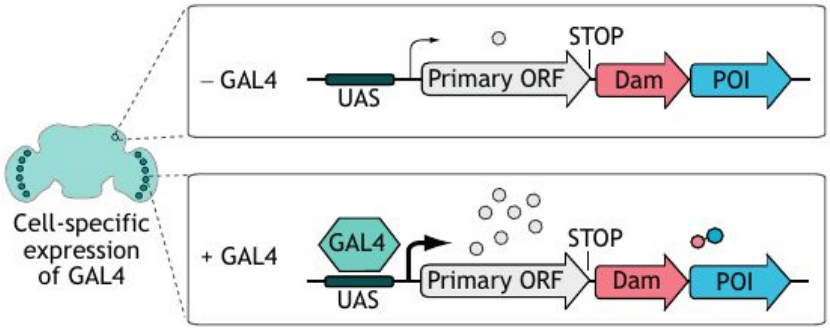
Chip-seq vs DamID vs CUT&RUN

Technique	Typical cell input	Minimal cell input	Approximate sequencing coverage necessary for mammalian genome	Advantages	Disadvantages	References
Chip-seq	>500,000	100-10,000	20-40 M reads	Most common profiling technique Numerous protocols and comparative datasets available	Mapping resolution limited by chromatin shearing efficiency Limited by quality of antibody Snapshot in single point of time After cell/tissue processing	(Albert et al.2007; Cao et al.2015)
DamID	>10,000	1	10-40 M reads	Not antibody or other affinity reagents dependent What occurs over several hours Events *in vivo*	Dependent on GATC presence Does not profile endogenous protein Low base-pair resolution because of extensive Dam range of action Transgenic cells	(Kind et al. 2015; van Steensel and Heinikoff 2000)
CUT&RUN	>100,000	1	10 M	HIgh signal noise ratio Low celular input necessary Native conditions	Limited by quality of antibody	(Hainer et al. 2019; Skene and Henikoff 2017)

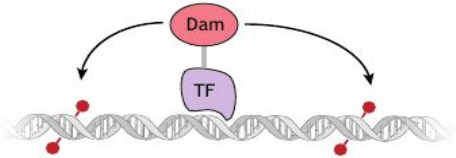
(Klein DC and Hainer SJ, 2020)

Alternative techniques:

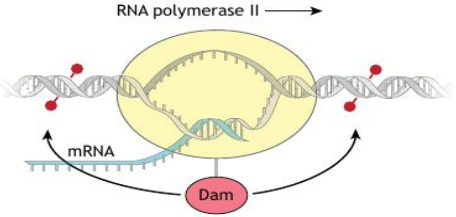
Targeted DamID (TaDa)



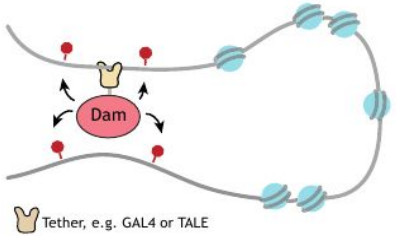
Transcription factor and chromatin modifier profiling



Transcriptional state



Long range DNA interactions

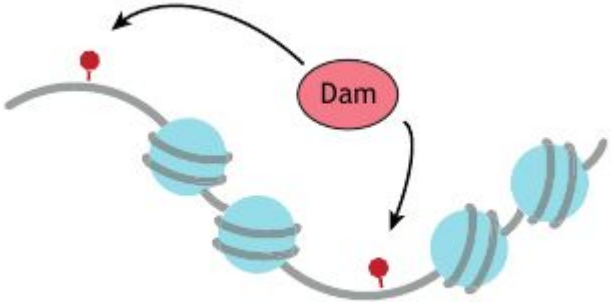


(Gabriel NA et al., 2019)

Alternative techniques:

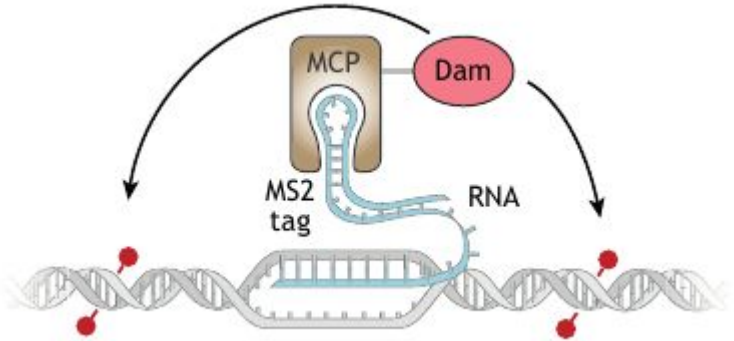
Chromatin Accessibility TaDa (CANTaDa)

Chromatin accessibility profiling



RNA- DamID

RNA-DNA interactions

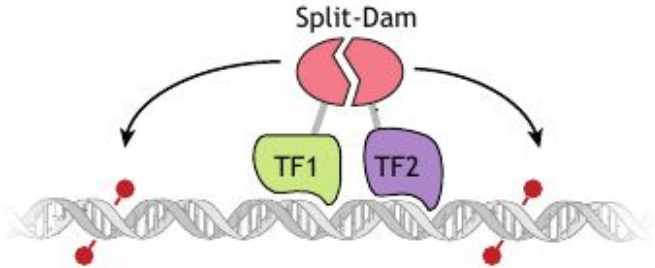


(Gabriel NA et al., 2019)

Alternative techniques:

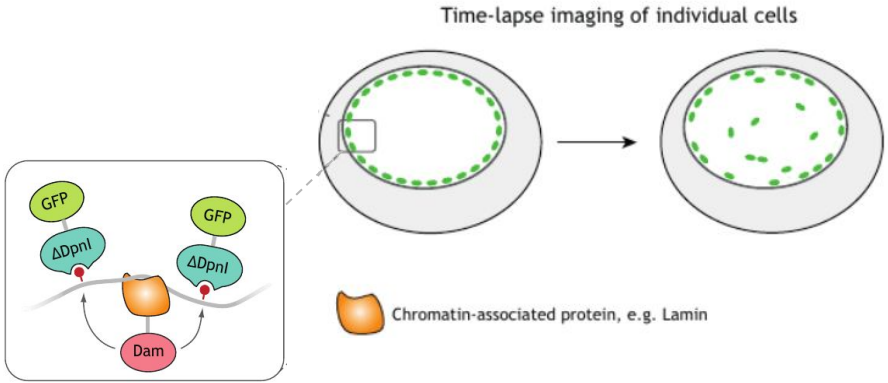
Split Damid (SpDamID)

Transcription factor co-binding



m6A-tracer

Live imaging



(Gabriel NA et al., 2019)

Summary of DamID variants

Table 1. Summary of DamID variants used for studying genome biology

Method	Summary	Cell-type specific?	Figure	Reference(s)
DamID	Identification of protein-DNA interactions		Fig. 2A	van Steensel and Henikoff, 2000
Targeted DamID (TaDa)	Cell-type-specific identification of protein-DNA interactions	Yes	Fig. 1A	Southall et al., 2013
FLP-out cell-specific DamID	Cell-type-specific identification of protein-DNA interactions	Yes	Fig. 1B	Pindyurin et al., 2016
Mammalian targeted DamID (MaTaDa)	Inducible identification of protein-DNA interactions			Cheetham et al., 2018
RNA-DamID	Identification of RNA-DNA interactions	Yes	Fig. 2D	Cheetham and Brand, 2018
Chromatin accessibility TaDa (CATaDa)	Identification of accessible chromatin	Yes	Fig. 2C	Aughey et al., 2018
Split DamID (SpDamID)	Identification of protein-DNA interactions (two proteins)		Fig. 2E	Hass et al., 2015
Long-range DamID	Identification of long-range chromatin interactions	Yes	Fig. 2F	Lebrun et al., 2003
Single cell DamID	Only one cell required	Yes		Kind et al., 2015
DamIP	DamID with immunoprecipitation and DamK9A methylation			Xiao et al., 2010
MadID	DamID with immunoprecipitation and M.EcoGII methylation			Sobecki et al., 2018
m6A-tracer	Visualisation of chromatin dynamics	Yes	Fig. 2G	Kind et al., 2013

A technique is marked as 'cell-type specific' if it has been shown to work in a cell-specific manner. However, it should be noted that the remainder do have the potential to be adapted for such use. The references refer to the first description of the particular technique.

(Gabriel NA et al., 2019)

Future perspectives

- Useful **alternative** when samples are difficult to obtain in **large quantities**
- Several DamID-based methods **could be combined** to deal with a broader range of biological problems
- Future **combination with CRISPR** to study functional chromatin interactions and regulatory elements
- Great expectations in **synthetic biology**:
    - Dam combined with new synthetic binding proteins
    - Generate novel biological properties

(Gabriel NA et al., 2019;)

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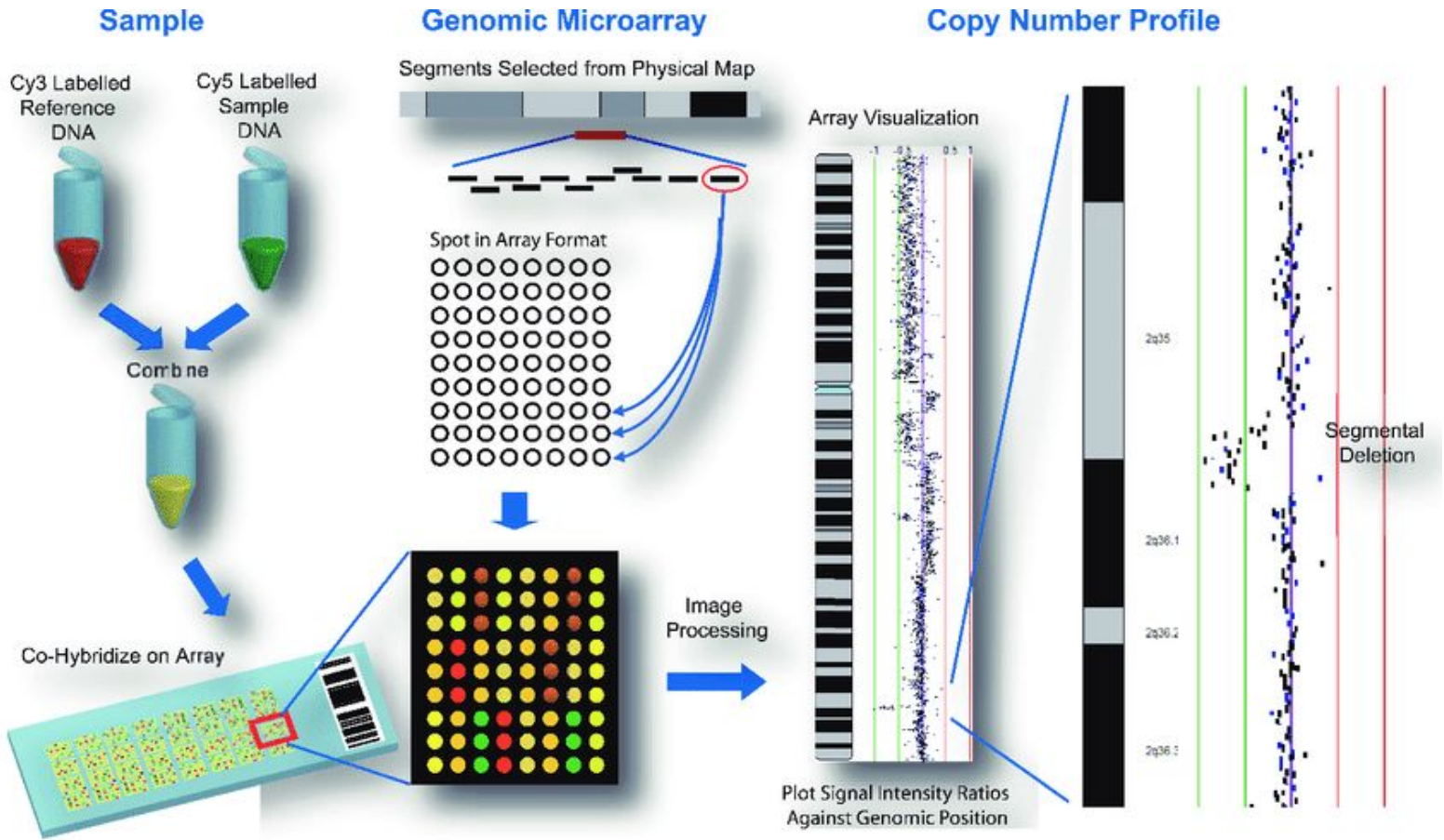
QUESTIONS?

TABLE 1 | Comparison of the Relative Advantages and Disadvantages of DNA adenine methyltransferase identification (DamID) Compared with chromatin immunoprecipitation (ChIP)

	ChIP	DamID
Specific reagents required	Antibody with good specificity and high affinity.	Transgenic cells expressing Dam-fusion protein of interest.
Resolution	High resolution.	Methylation depends on the distribution of GATC in the genome. Resolution still comparable to ChIP.
Applicable organism	Any organism for which high-affinity antibody can be obtained.	Any genetically tractable animal or cell type.
Detection of post-translational modifications	Possible with appropriate antibody.	Not possible.
Tissue-specific profiling	Requires physical separation of cells or nuclei.	Dam-fusions can be expressed in a tissue-specific manner.
Detection of long range or transient interactions	Not possible due to specific binding required.	Methylation of nearby or transiently Dam-associated sequences is possible.
Requires 'fixing' of samples	*In vitro* technique. Requires formaldehyde crosslinking of samples.	Methylation occurs *in vivo*. DNA can be extracted from unfixed or even live cells.
Temporal resolution	Limited only by time taken for fixing (minutes).	Dam must be expressed for several hours.
Isoform specificity	ChIP antibodies may bind to multiple isoforms of the same protein.	A specific sequence must be expressed; therefore, binding of only one isoform is assayed.
Proteins expressed at low levels	May be difficult to purify low expressed proteins with ChIP antibody.	Dam concentration is independent of endogenous protein levels. (Dam-fusions have to be expressed at very low levels).


Sample
Genomic Microarray
Copy Number Profile
Cy3 Labelled Reference DNA
Cy5 Labelled Sample DNA
Combine
Co-Hybridize on Array
Segments Selected from Physical Map
Spot in Array Format
Image Processing
Array Visualization
Plot Signal Intensity Ratios Against Genomic Position
2q35
2q36.1
2q36.2
2q36.3
Segmental Deletion

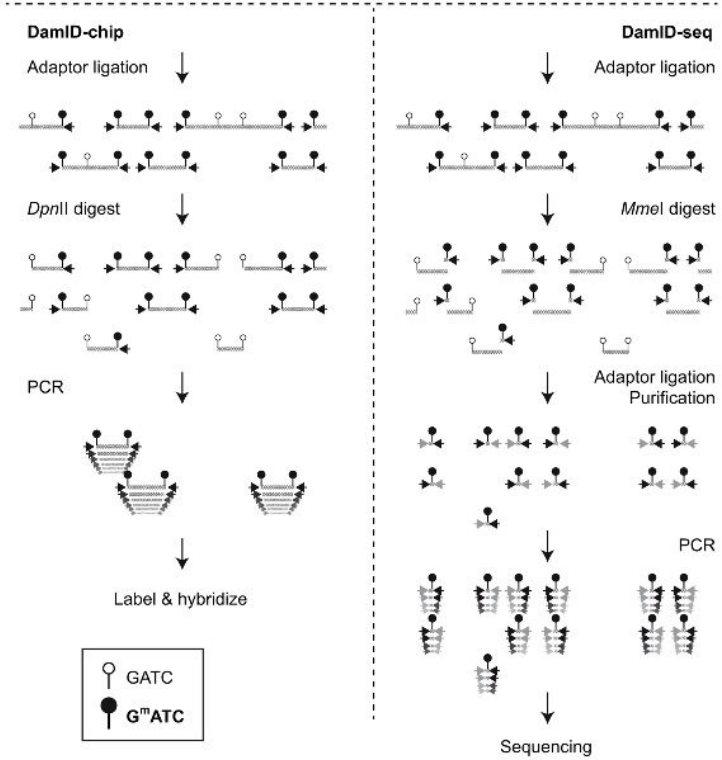
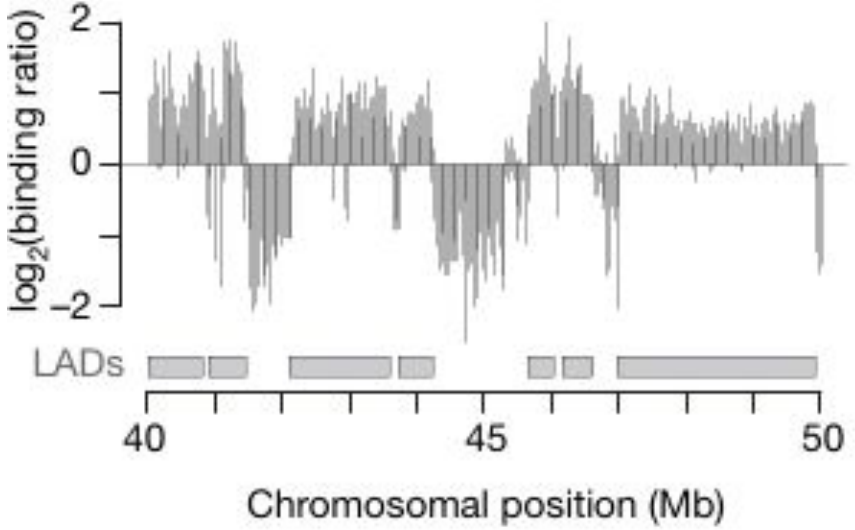
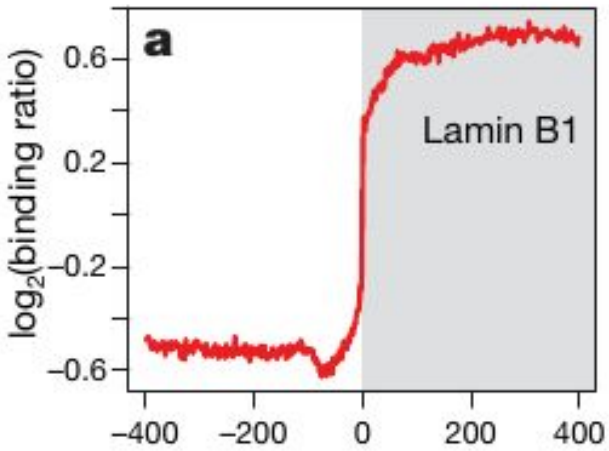


Figure 2. Principles of DamID analysis of protein-DNA interactions. *In vivo* expression of trace amounts of DNA adenine methyltransferase (Dam) fused to a chromatin-associated protein ("gene-of-interest") generates specific methylation marks (G^mATC) normally not found in *C. elegans*. As control for DNA accessibility, a strain expressing GFP-Dam is analyzed in parallel. Genomic DNA (gDNA) is purified and incubated with *Dpn*I restriction enzyme to specifically cleave Dam-methylated G^mATC sites. Depending on whether DNA arrays (DamID-chip; left) or high-throughput sequencing (DamID-seq; right) is used to identify methylated DNA, different oligonucleotide adaptors are ligated to the cleaved ends. For DamID-chip both samples are digested with *Dpn*II enzyme to cut all non-methylated GATC sequences (for simplicity only one sample is shown). This generates a pool of DNA fragments, of which only the ones that are flanked by two G^mATC sequences without intervening non-methylated sites can serve as PCR templates. Finally, amplified DNA fragments from the two strains are identified and quantified by co-hybridization to DNA arrays. Alternatively, for DamID-seq short tags for sequencing are generated by using adaptors with a recognition site for a restriction enzyme that cuts 19-27 nt from the recognition site (e.g., *Mme*I). After digestion, a second adaptor is ligated to the tags, which are then amplified by PCR. One or several purification steps are included to isolate ~120 bp DNA fragments, which are identified by high-throughput sequencing.

Case of study: Detection of LADs

What are LADs?





(Mod Guelen L et al., 2018)