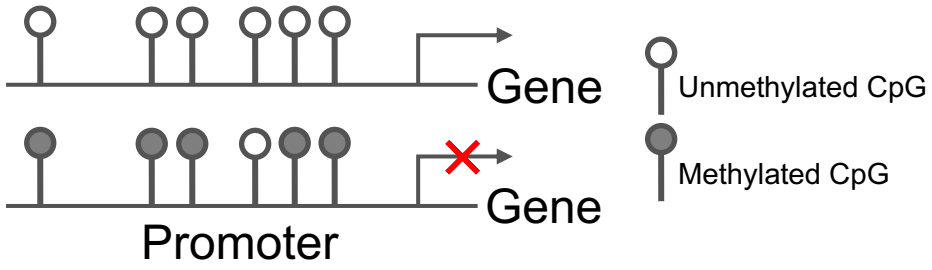
MAX PLANCK INSTITUTE
FOR MOLECULAR GENETICS

Differentially methylated region identification for multi-groups

Zhihan Zhu, Kretzmer Lab at MPI for Molecular Genetics
39th TBI Winterseminar in Bled
15.02.2024

Background - Differentially Methylated Region (DMR)

CpG (a Cytosine followed by a Guanine) methylation:



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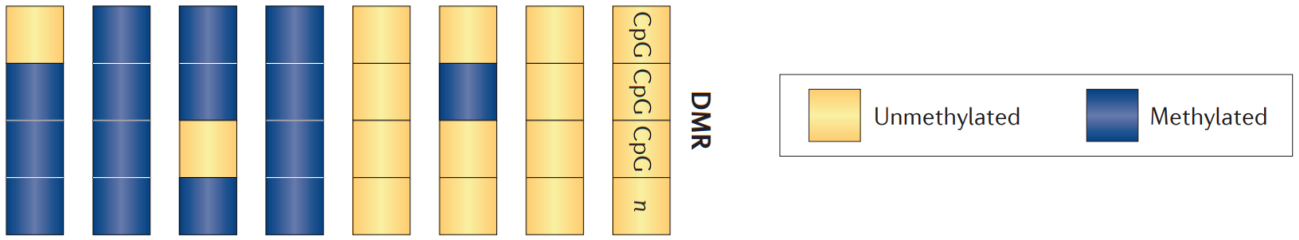

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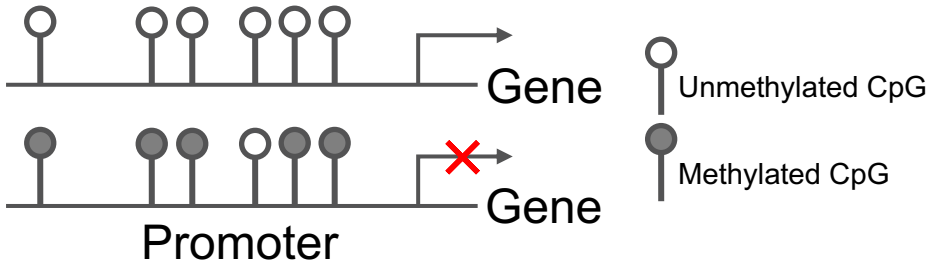

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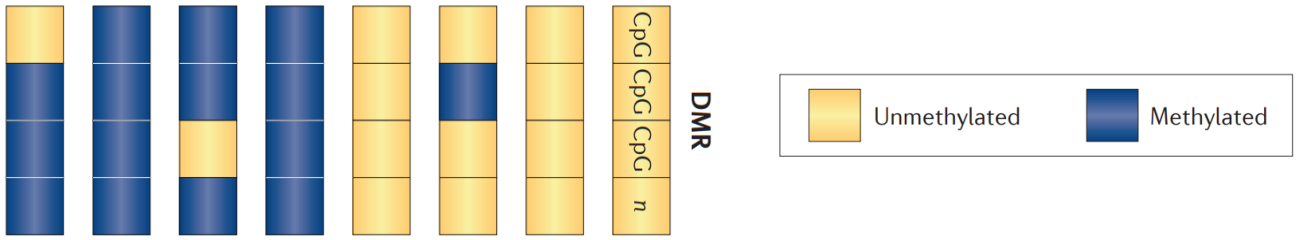
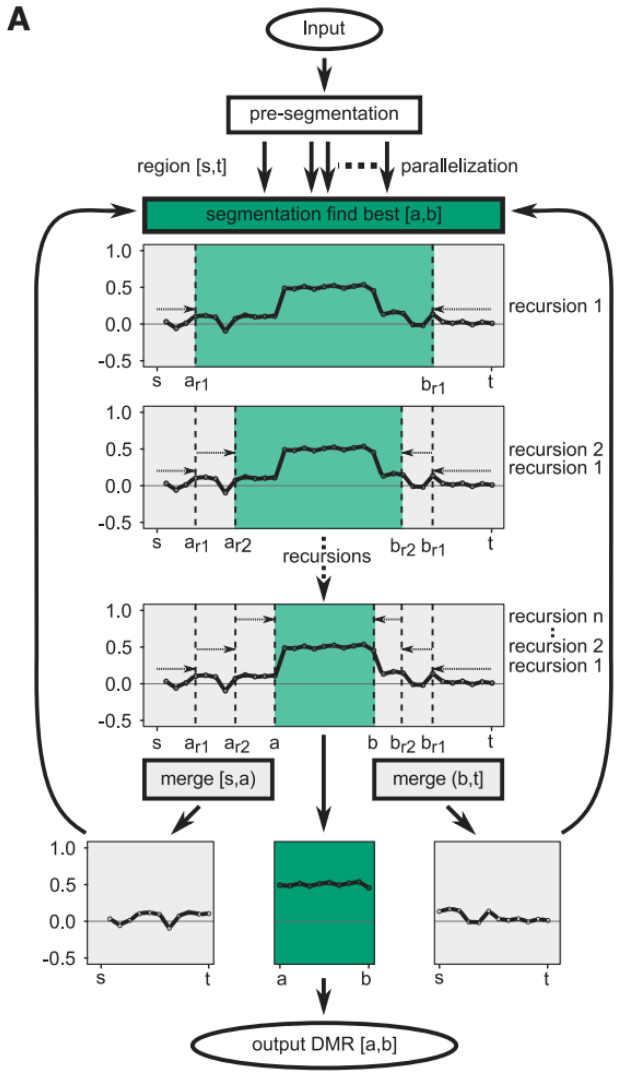


Noisy

Huge #CpGs

Rakyan, Vardhman K., et al. *Nature Reviews Genetics*. 2011.

Background - metilene



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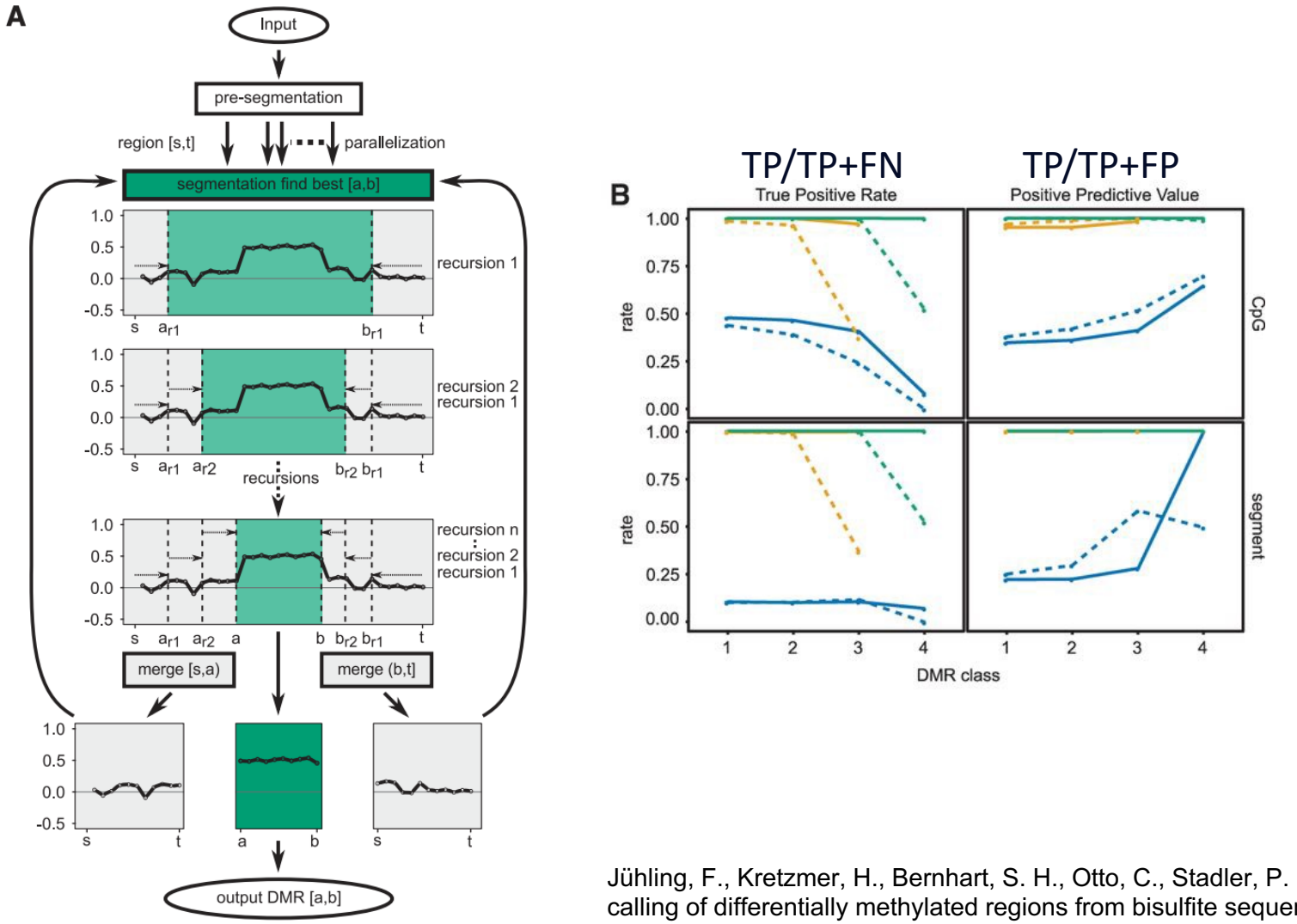

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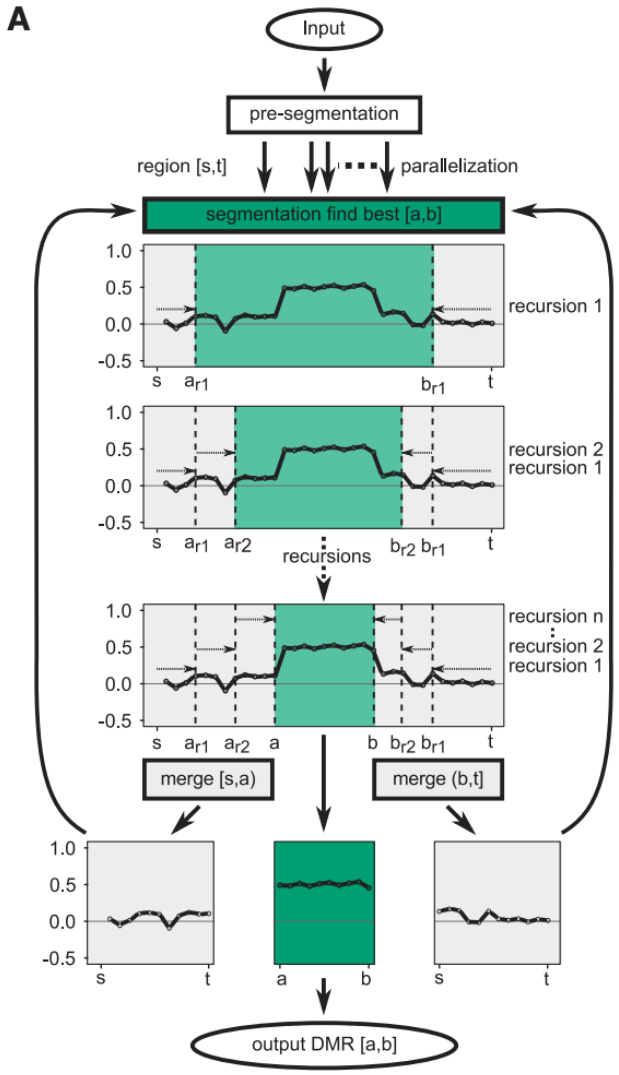

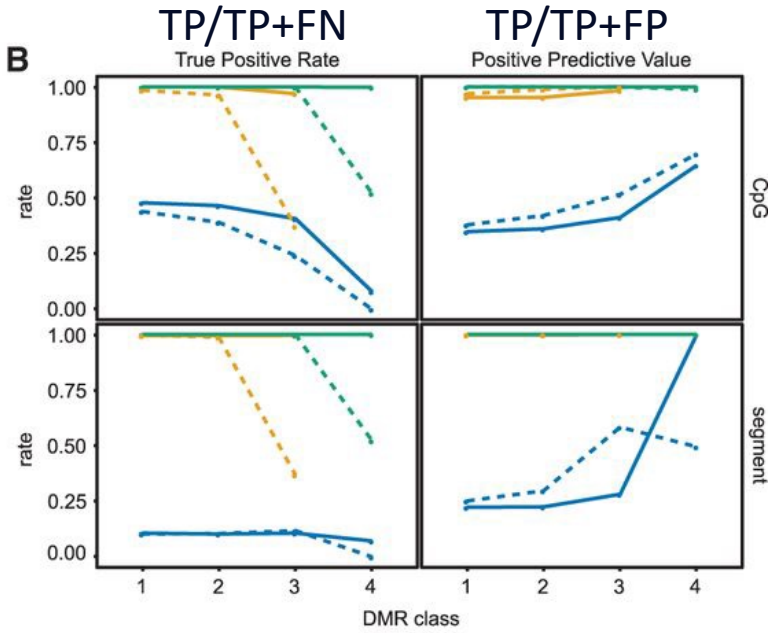

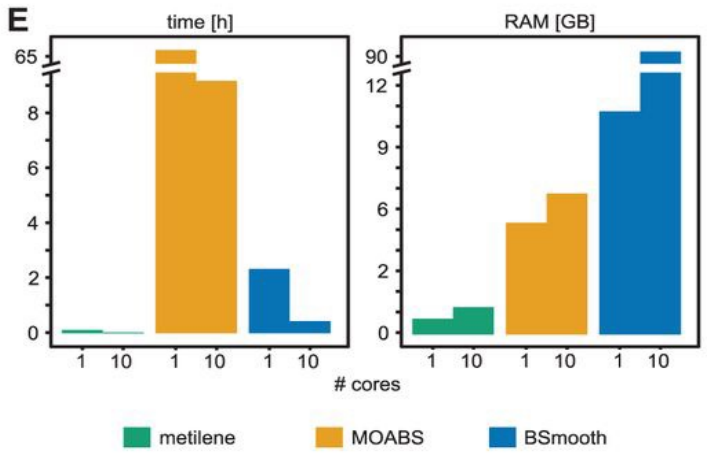

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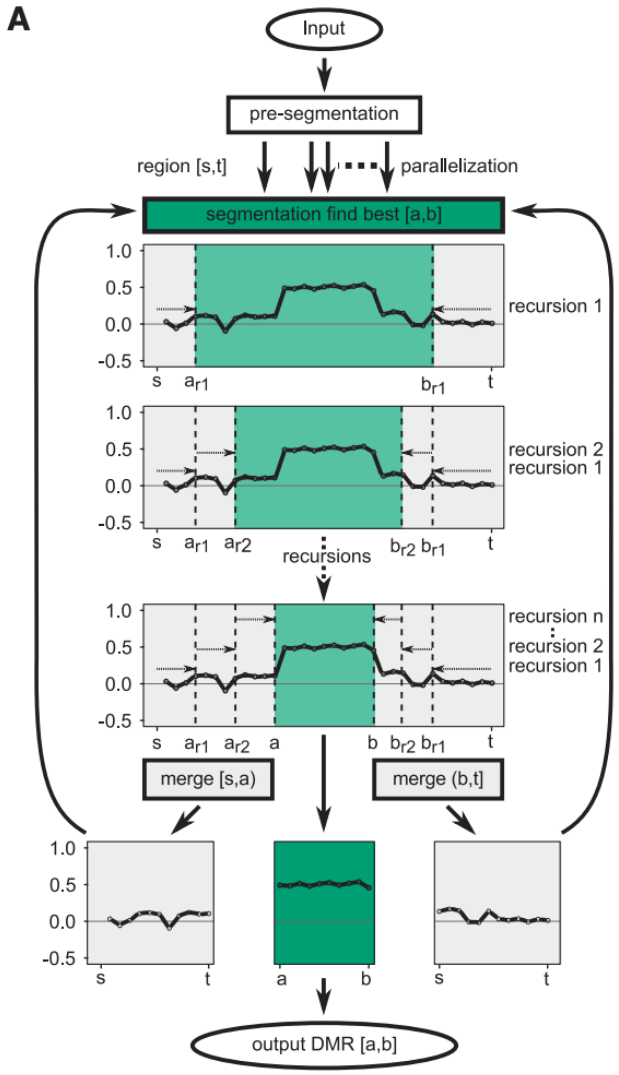

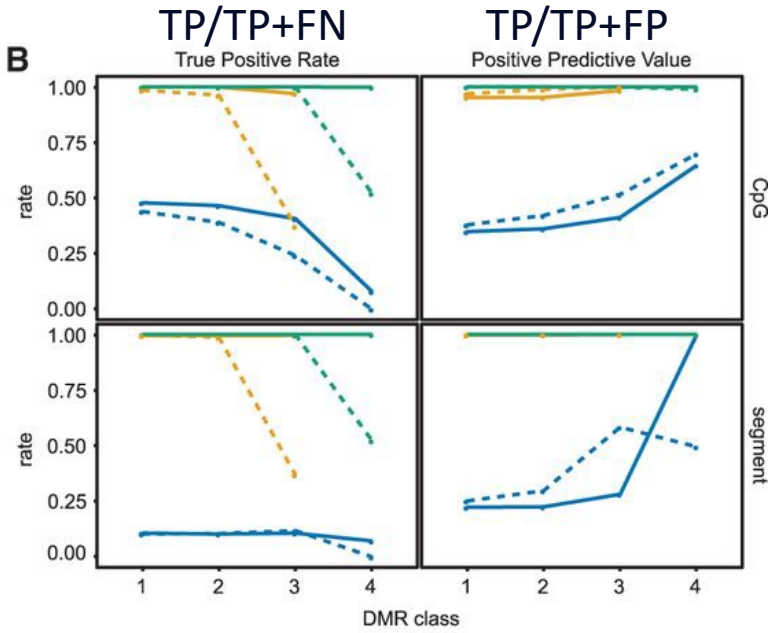

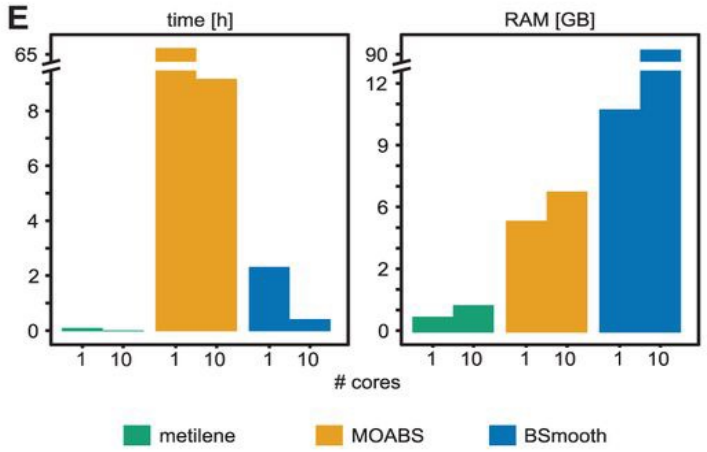

A gap – more than two groups to be compared?

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A gap – more than two groups to be compared?

Multi-groups DMR identification:

We can have ~200 cell types

Cancers can have even more subtypes

…

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Multi-groups DMR identification:

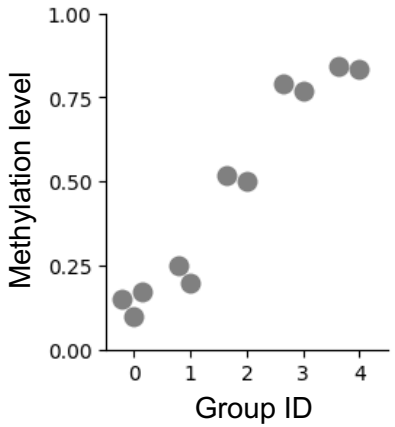
We can have ~200 cell types

Cancers can have even more subtypes

…

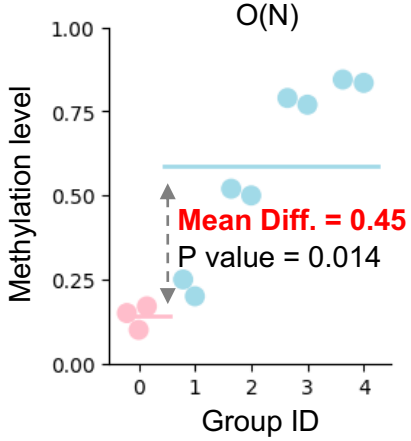
Possible ways of comparing multiple groups – 5-groups example:

Data:

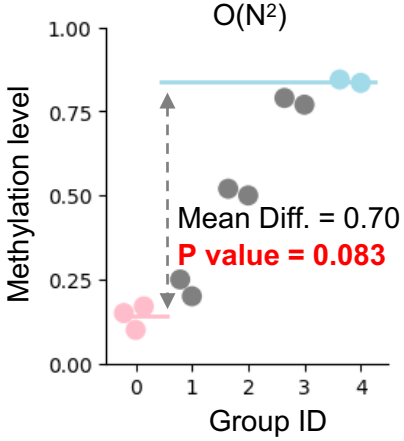


Solutions:

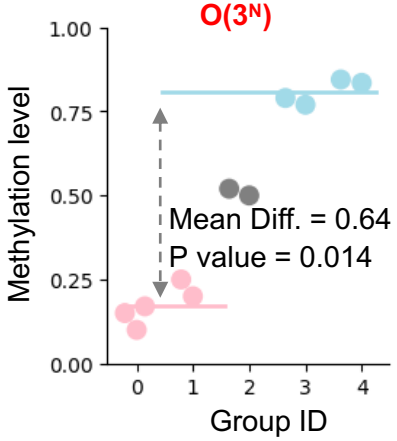
One vs All others
O(N)



One vs One
O(N^2)



All possible comparisons
O(3^N)



$$\frac{3^N - (2\times 2^N - 1)}{2}$$

e.g., N = 20 → 1,742,343,625

Multi-groups metilene – algorithm

Segmentation based on 1 group vs 1 group comparisons
$O(N^2)$

↓

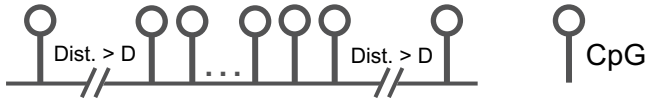
Groups clustering on segments
$O(N)$

↓

Circulation (recursion) based on clustering results
$O(N^2)$

Multi-groups metilene – algorithm

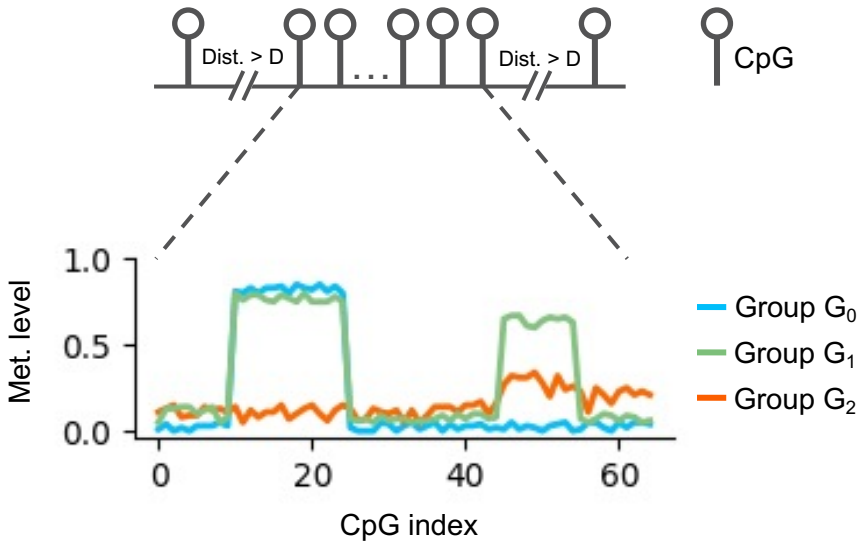
Pre-segmentation



Multi-groups metilene – algorithm

Pre-segmentation

Mean methylation levels M: O(N)

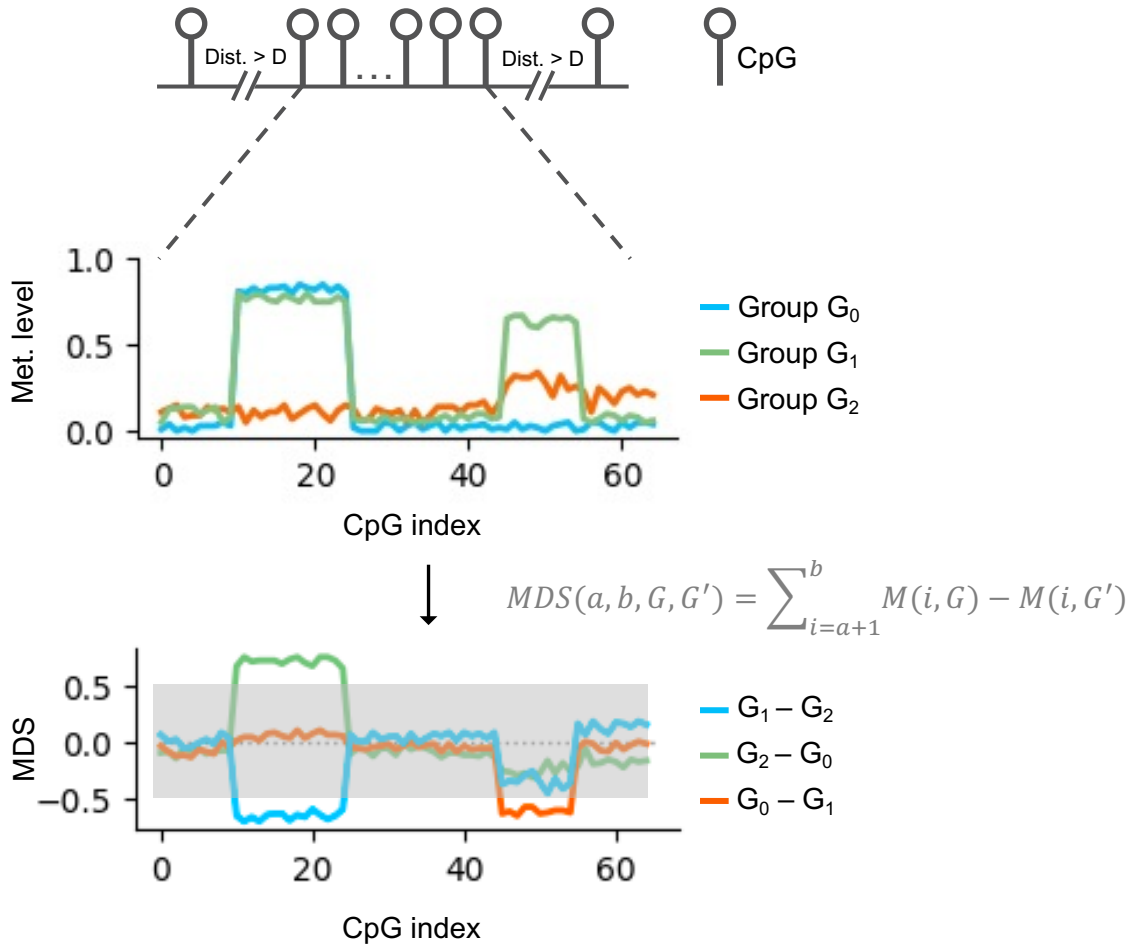


Multi-groups metilene – algorithm

Pre-segmentation

Mean methylation levels M: O(N)

Mean difference signals MDS: O(N^2)



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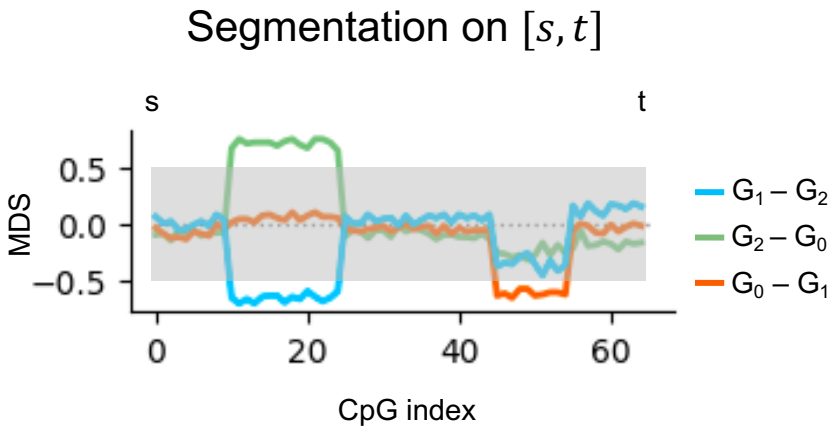
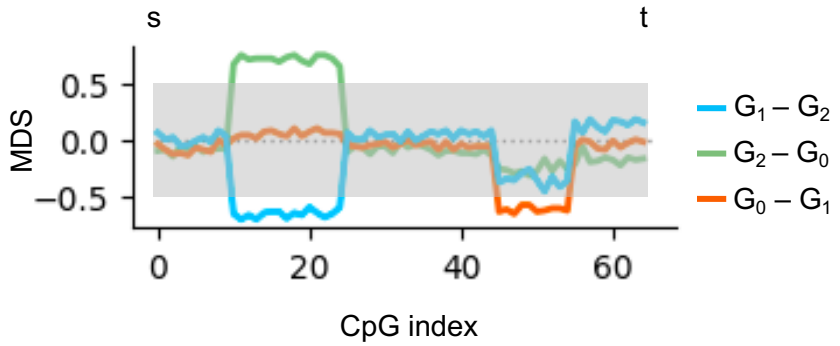


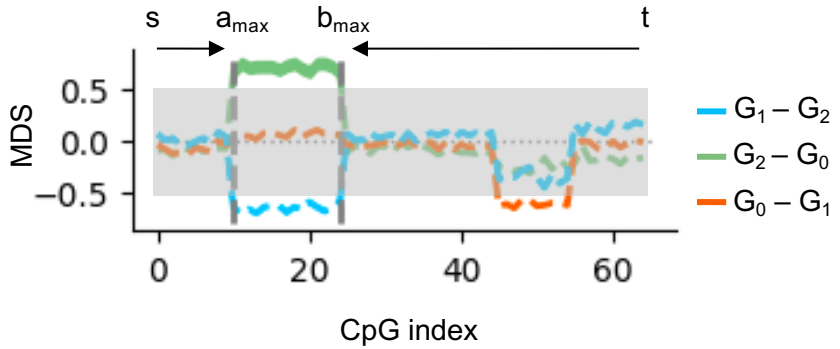
Multi-groups metilene – algorithm

Segmentation on $[s, t]$



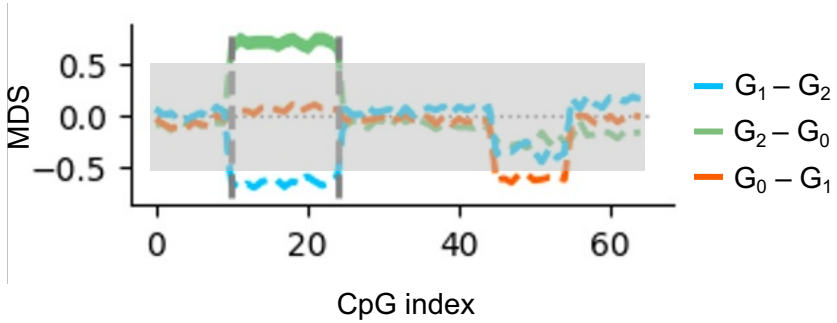
$$Z(s,t,a,b,G,G') = \frac{\left[|MDS(a,b,G,G')| - \frac{b-a}{t-s} \cdot |MDS(s,t,G,G')|\right]^2}{(b-a)\left[1 - \frac{b-a}{t-s}\right]}$$

$$a_{max}, b_{max}, G_{max}, G'_{max} = \underset{s \le a < b \le t, G, G'}{argmax} Z(s,t,a,b,G,G')$$



Multi-groups metilene – algorithm

Clustering based on G_{max}, G'_{max} :



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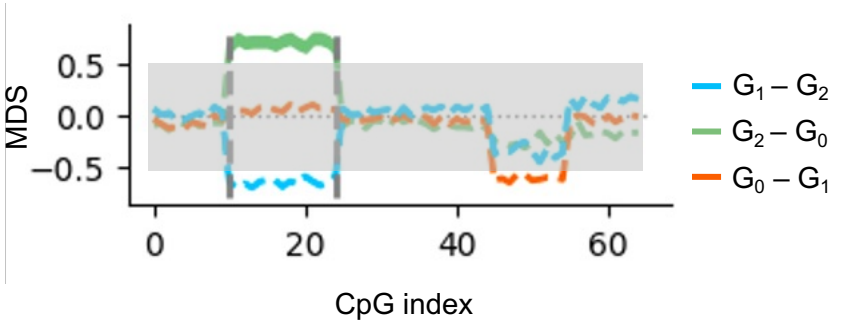
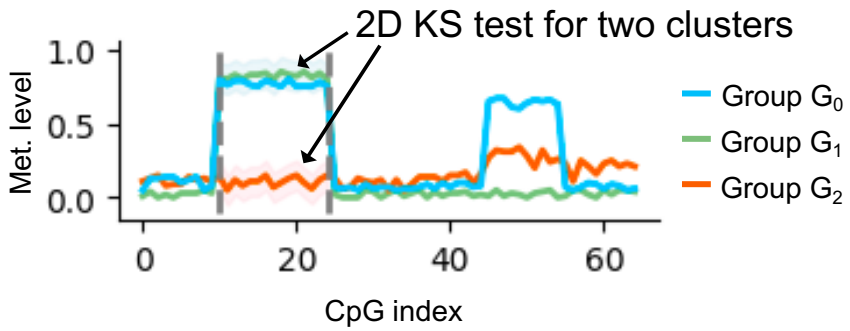


$$Distance(a,b,n,m) = \frac{\sum_{i=a+1}^{b}(|M(i,n) - M(i,m)| < \varepsilon)}{(b\ -a)}$$



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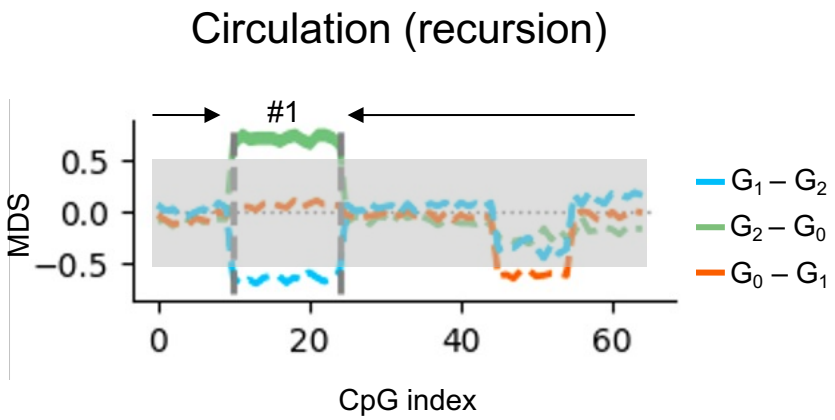
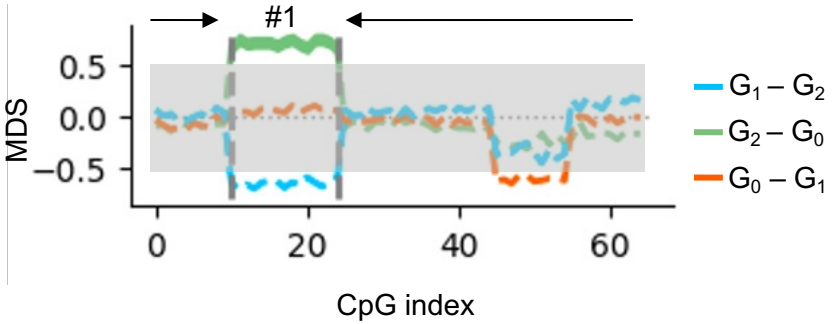
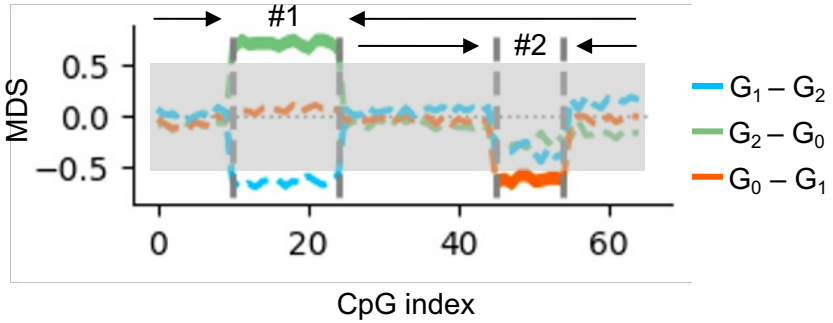


Multi-groups metilene – algorithm

Circulation (recursion)



- # CpGs $> \omega$ and
- $\exists P(a, b, A', B') < P(s, t, A, B),\ s \leq a < b \leq t$



Multi-groups metilene – De novo mode

DMR identification between **TWO** groups

Multi-groups metilene – De novo mode

DMR identification between **TWO** groups

DMR identification among **>=3** groups

Multi-groups metilene – De novo mode

DMR identification between **TWO** groups

DMR identification among **>=3** groups

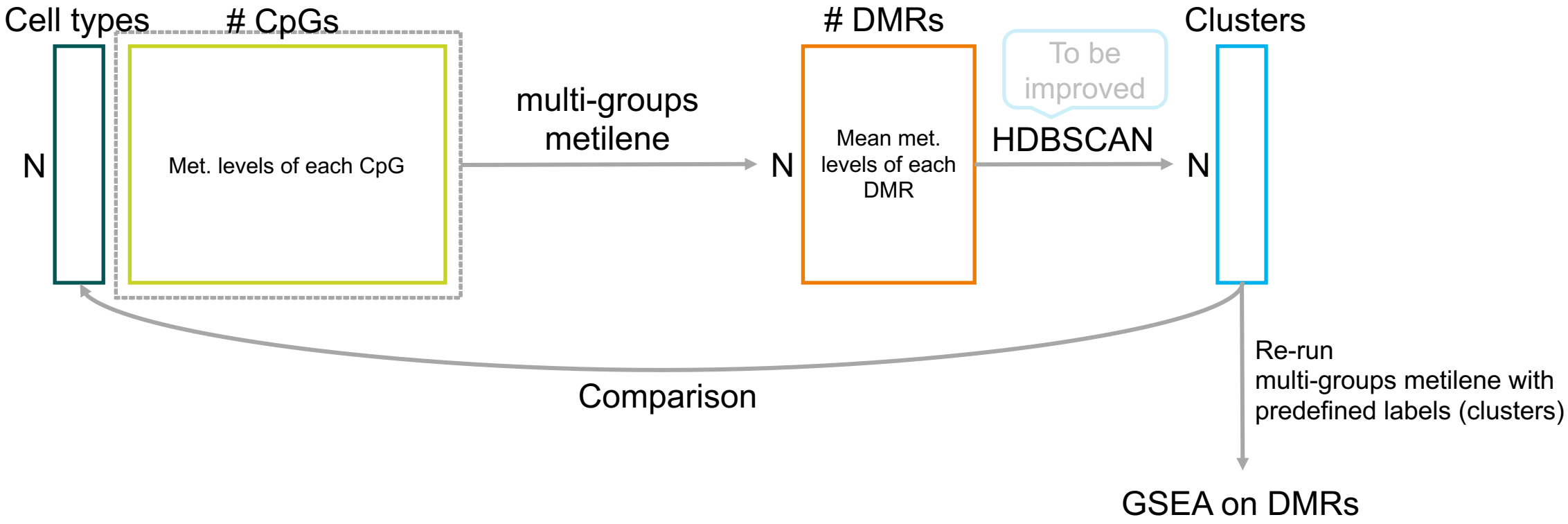
DMR identification without group information (label) - De novo mode
(One group one sample)

Multi-groups metilene – application

DMR identification (de novo mode) on N=21 WGBS samples from pancreas

Loyfer, Netanel, et al. *Nature*. 2023.

- 6 cell types: endothelium, acinar, alpha, beta, delta, duct



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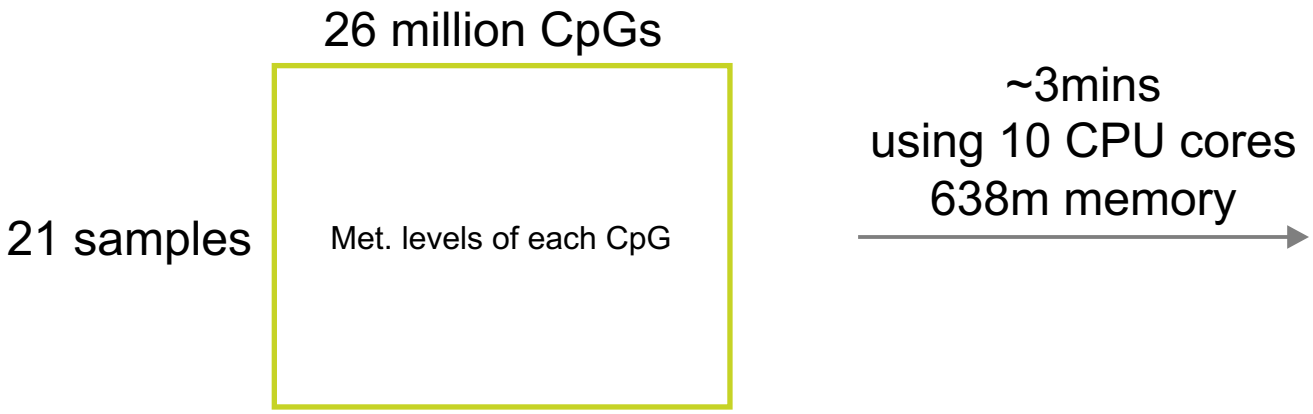
Multi-groups metilene with (default) parameters -



14,995 DMRs

chr	start	stop	P-value	means	clusters
Chr?	1	100	0.01	1|0.5|…	1|0|…
…	…	…	…	…	…

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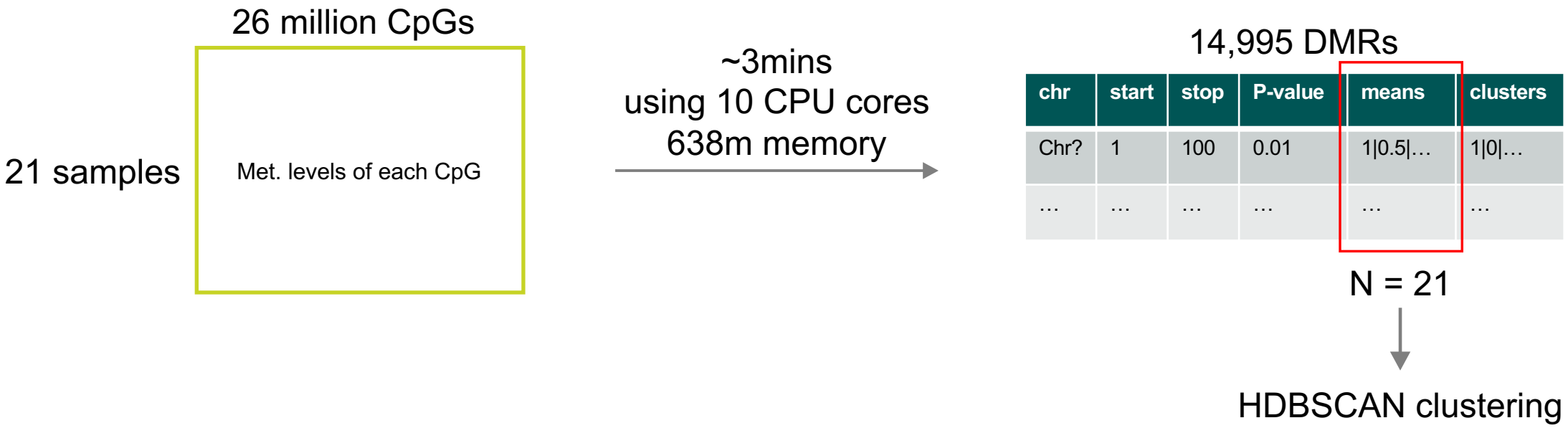
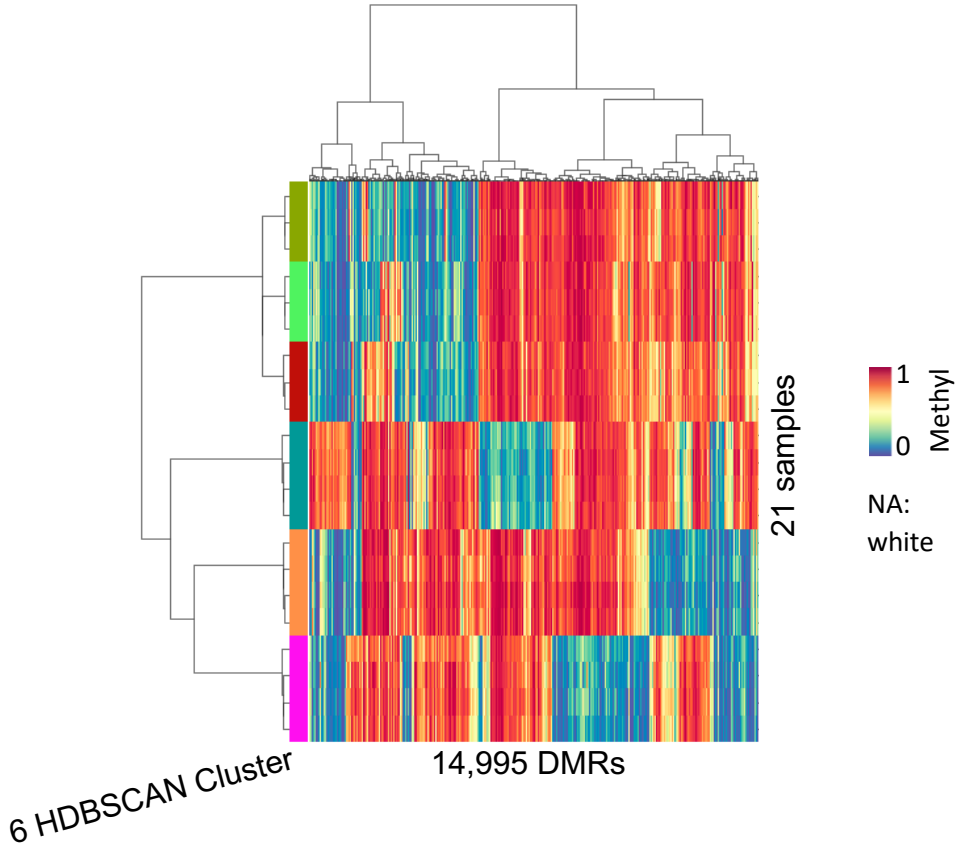


Multi-groups metilene – application

HDBSCAN clustering on mean methylation levels of DMRs



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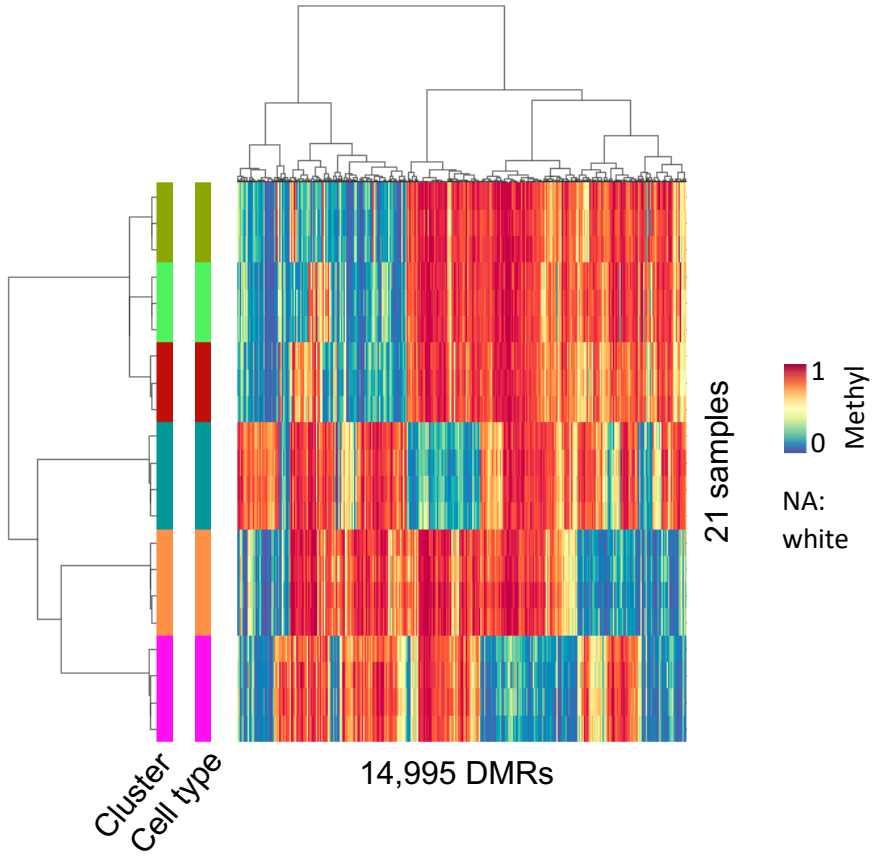
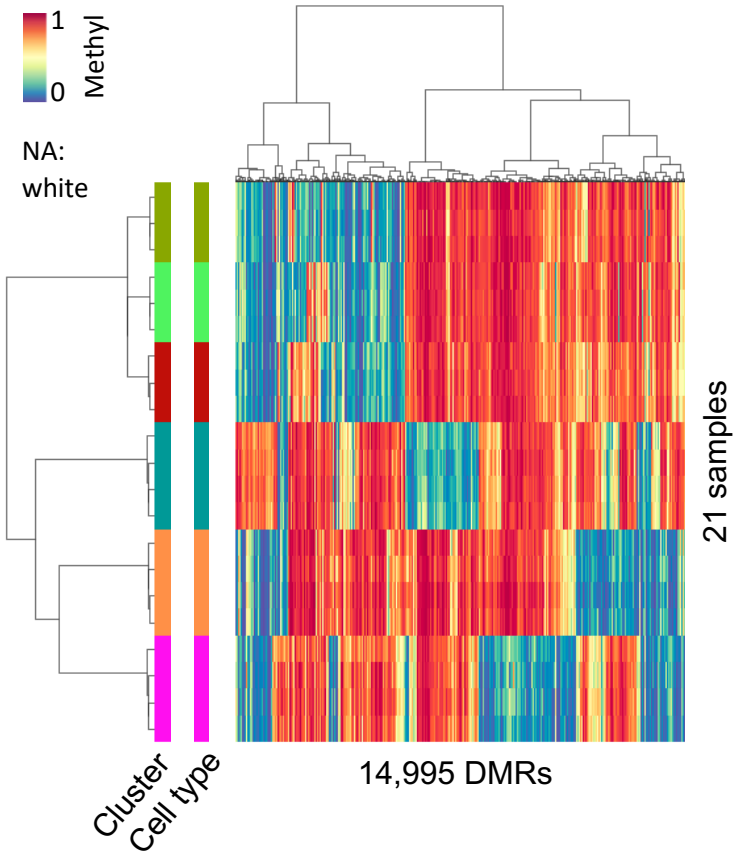
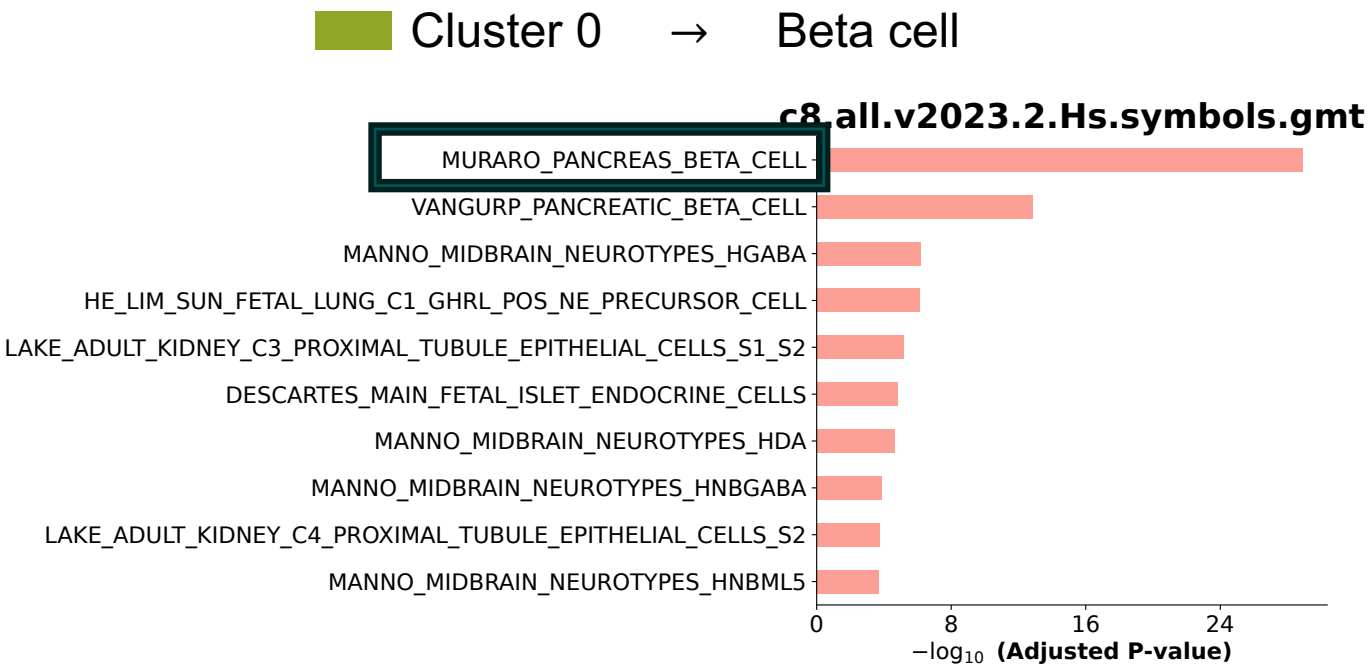


- Cluster 0 → Beta cell
- Cluster 1 → Alpha cell
- Cluster 2 → Delta cell
- Cluster 3 → Endothelium cell
- Cluster 4 → Duct cell
- Cluster 5 → Acinar cell

Multi-groups metilene – application

GSEA on cell type-specific expression gene sets from single-cell RNA sequencing:





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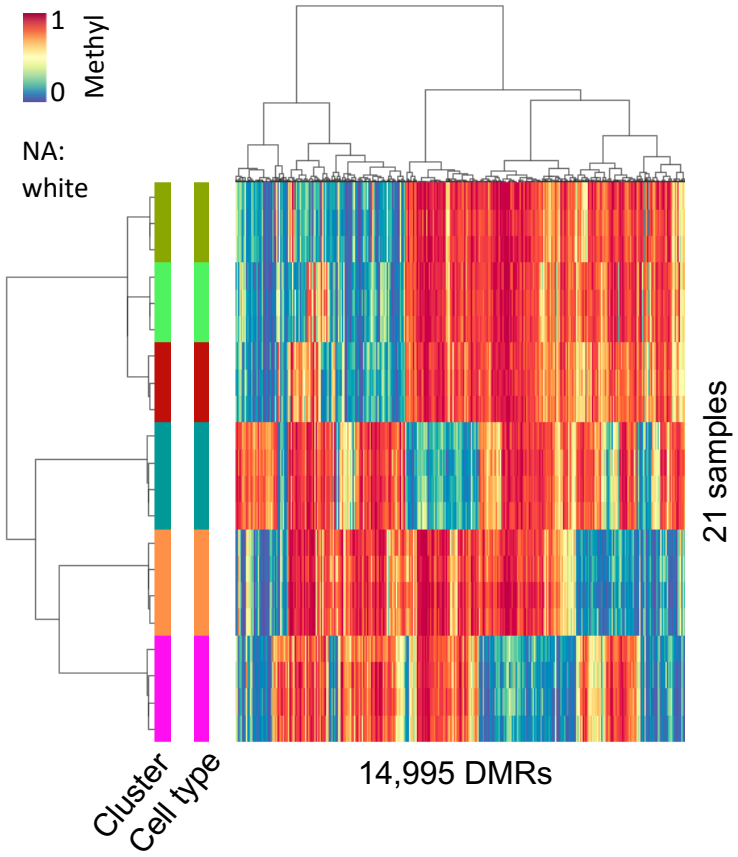

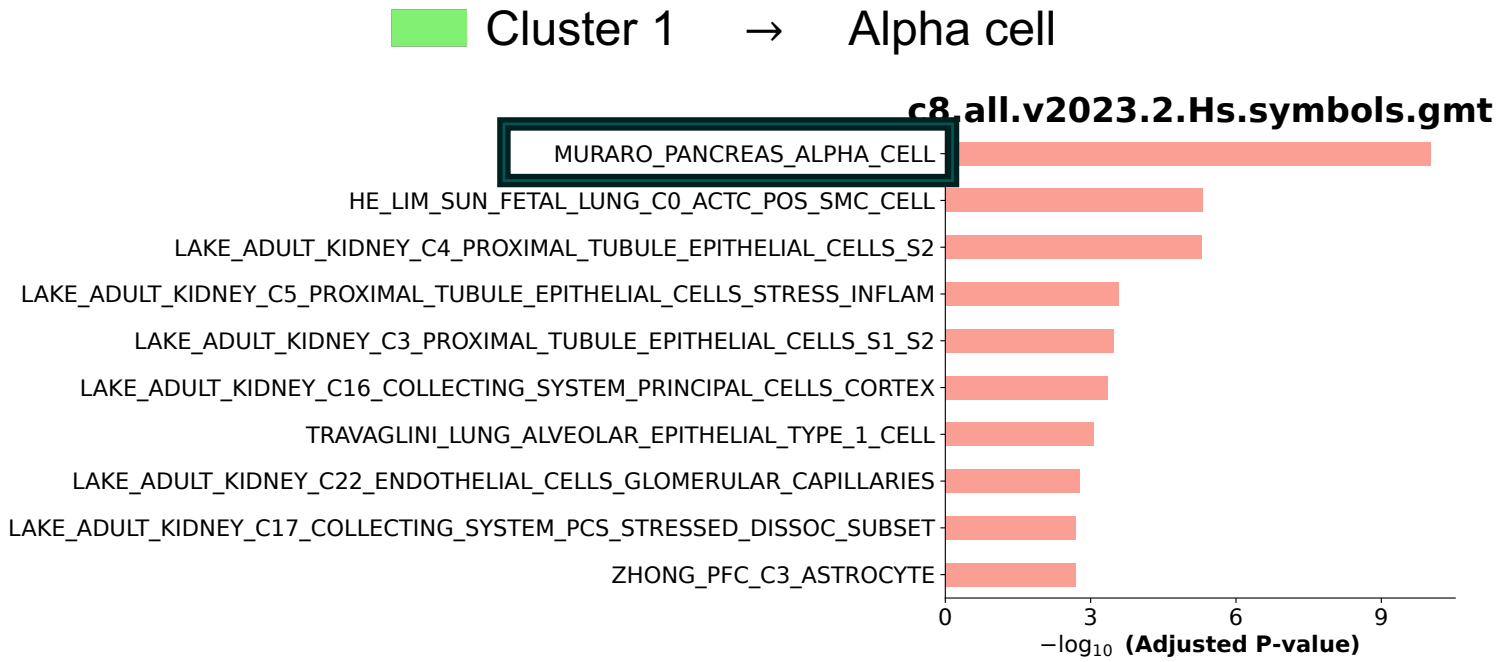

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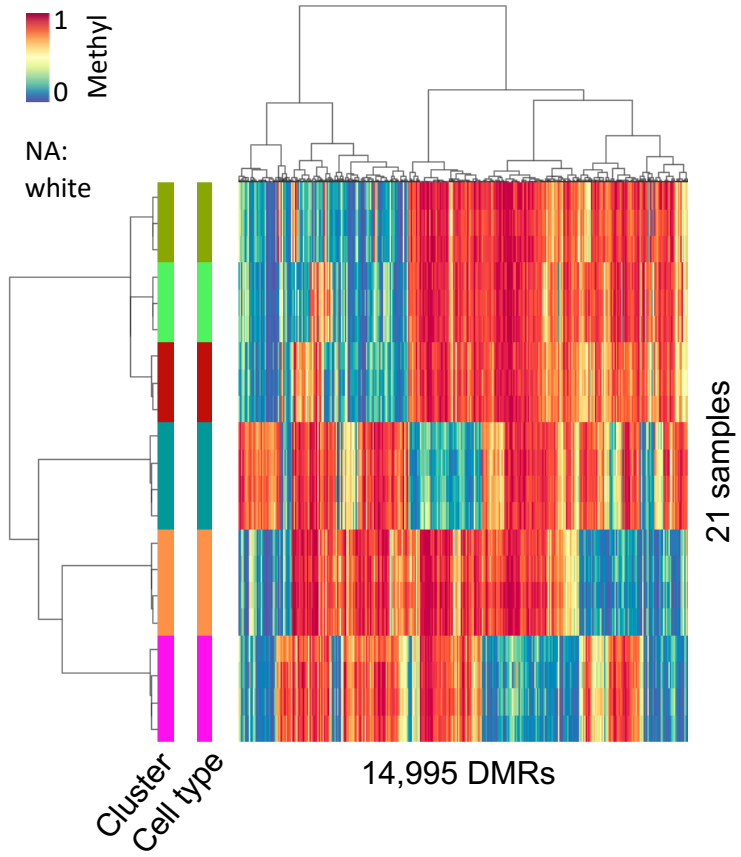

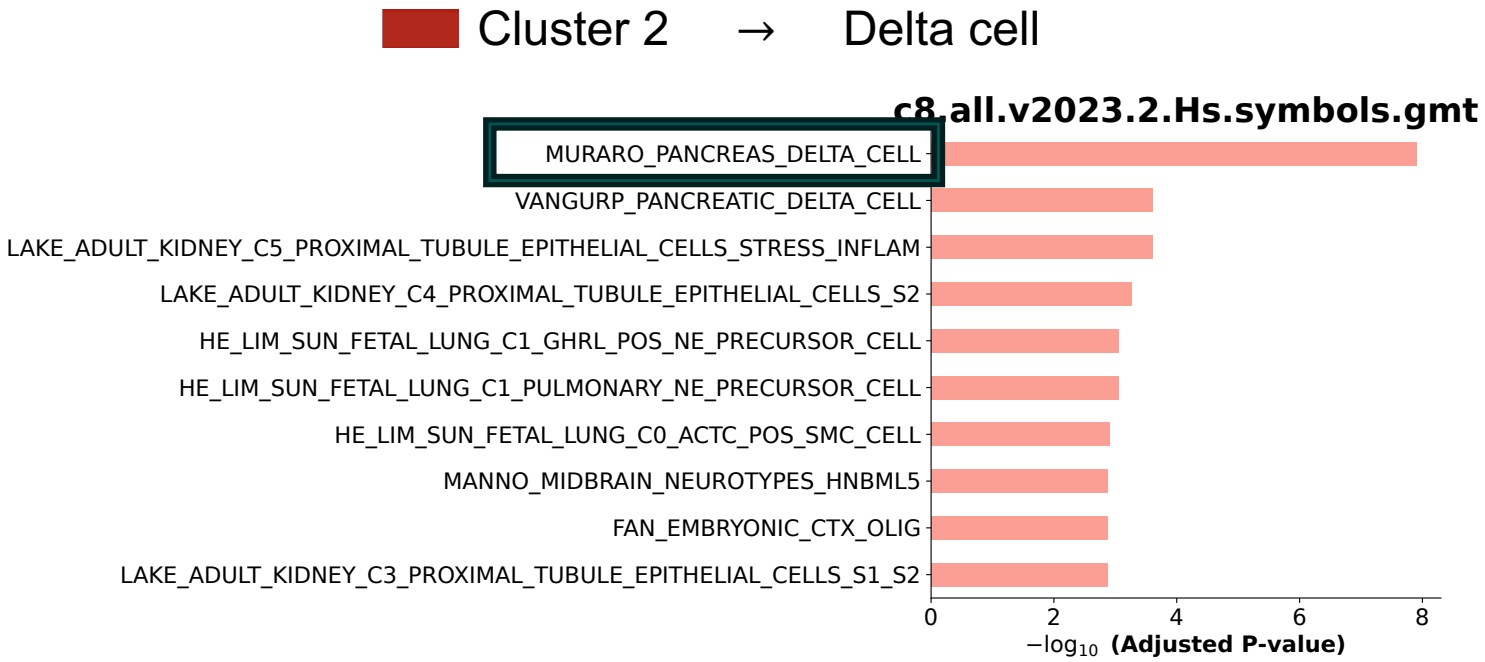

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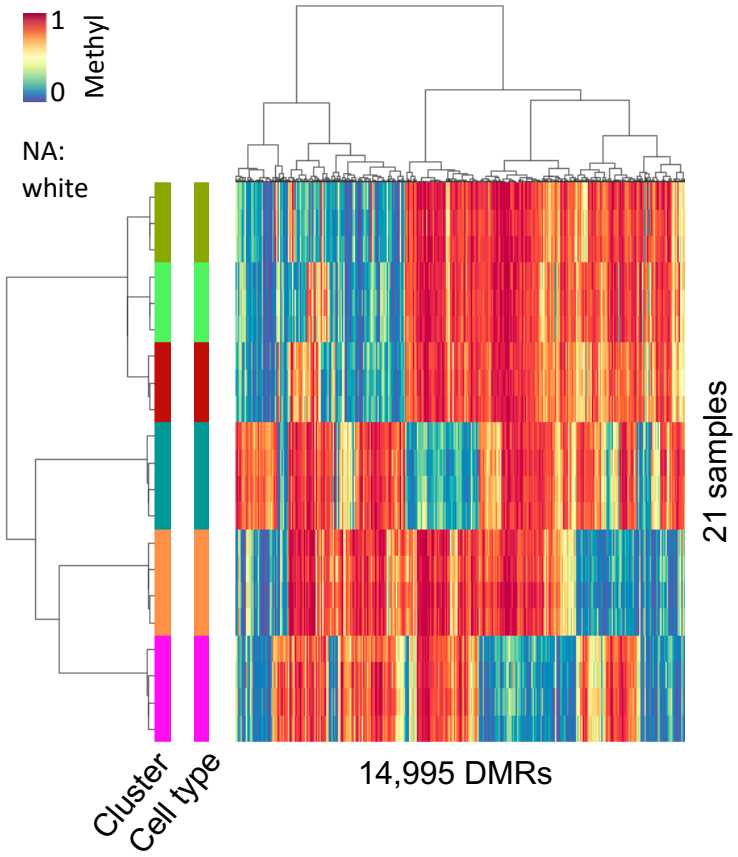

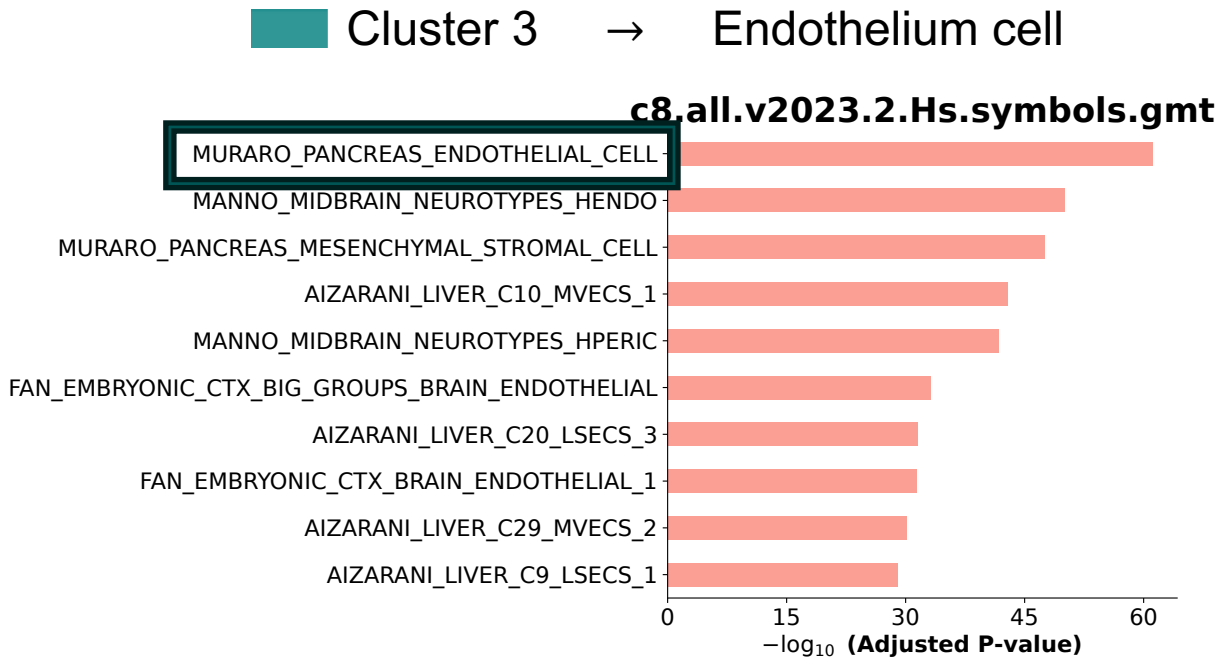

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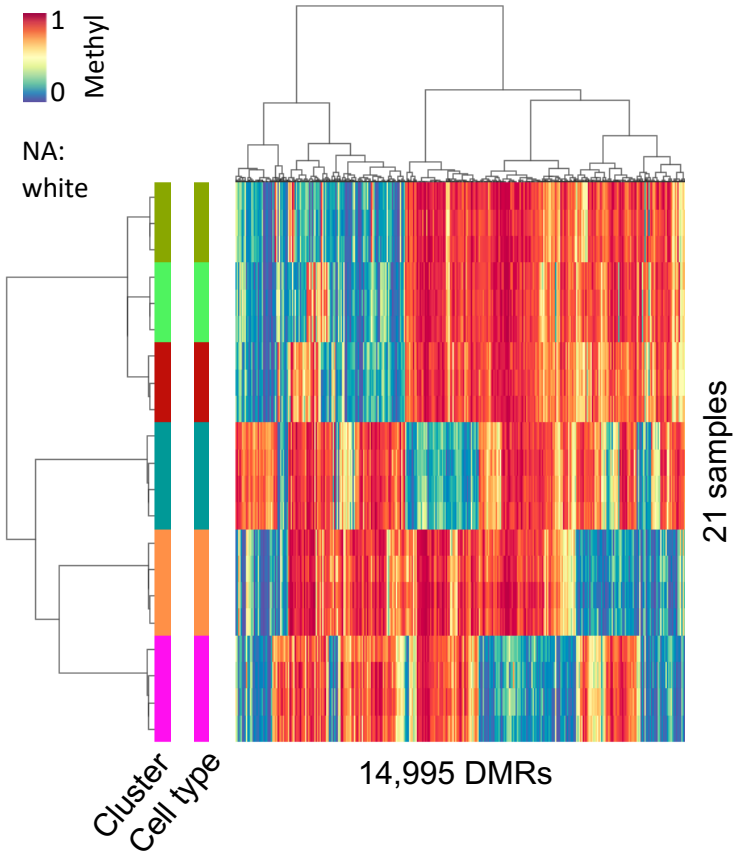

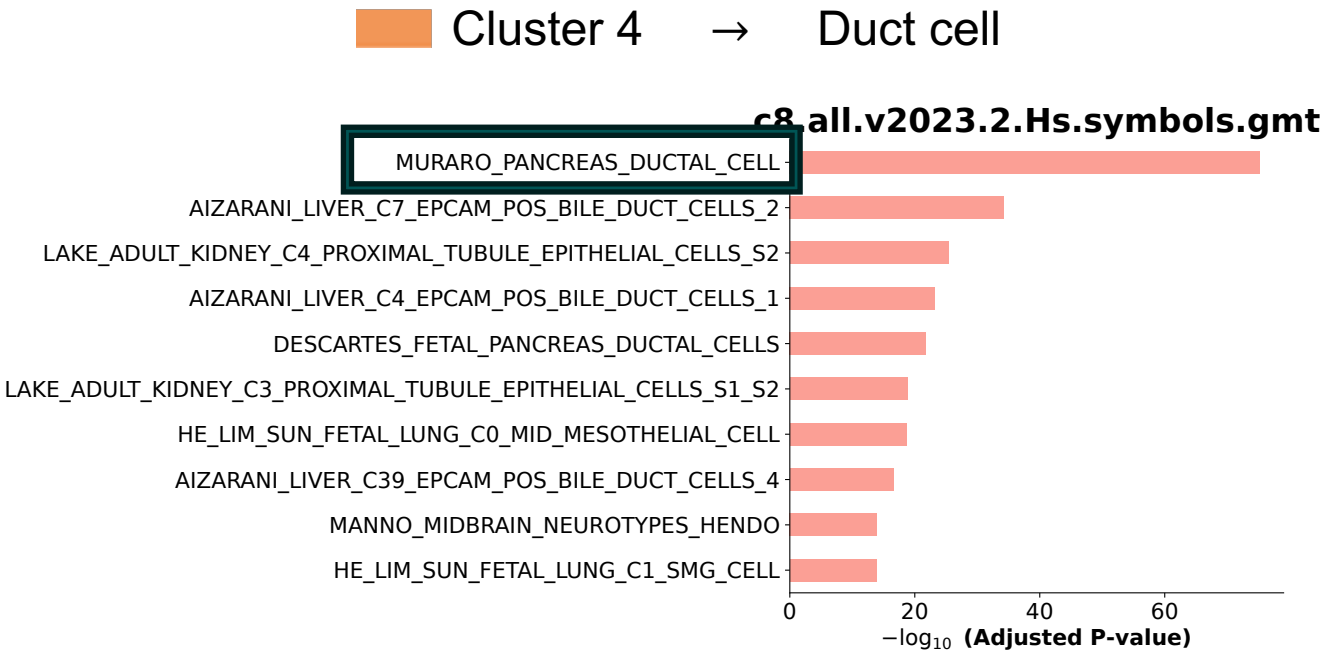

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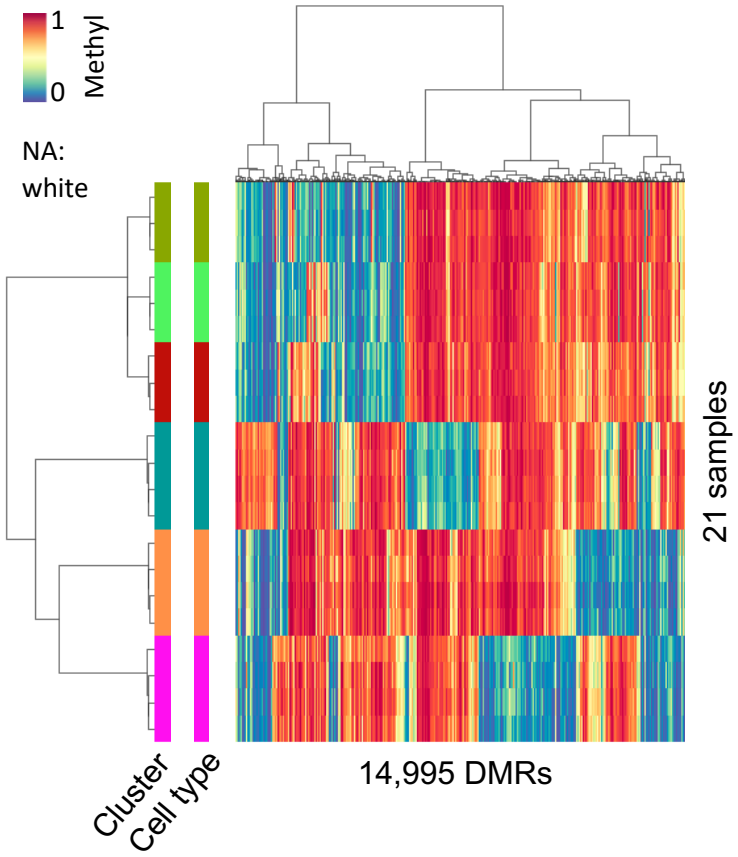

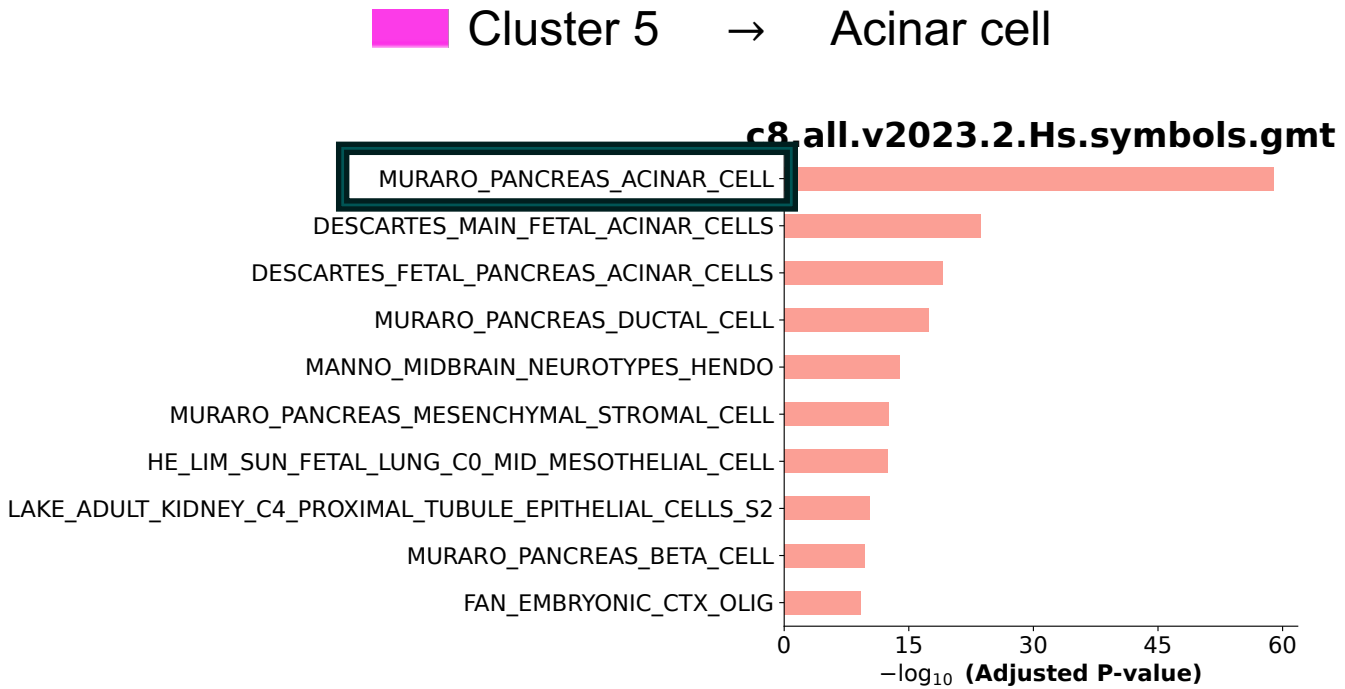

Summary

- Multi-groups metilene: integrating segmentation and clustering leads to fast and accurate DMR identification among multiple groups.

- DMRs identified by multi-groups metilene (de novo mode) could be used to find biological meaningful clusters.

- Downstream clustering (currently HDBSCAN) might be further improved.

Acknowledgement

Supervision:

Helene Kretzmer

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Lab members:

Sara Hetzel

Rosaria Tornisiello

Mara Steiger

Isabelle Kraus

Thank you!