Pangenome mapping enhances genotype-phenotype associations in BXD mouse family

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COMPLEX TRAIT COMMUNITY

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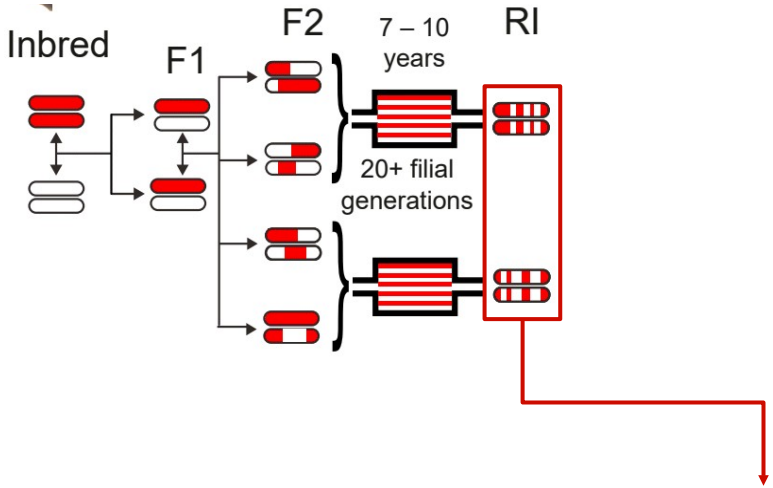
eQTL mapping in the BXD mouse family

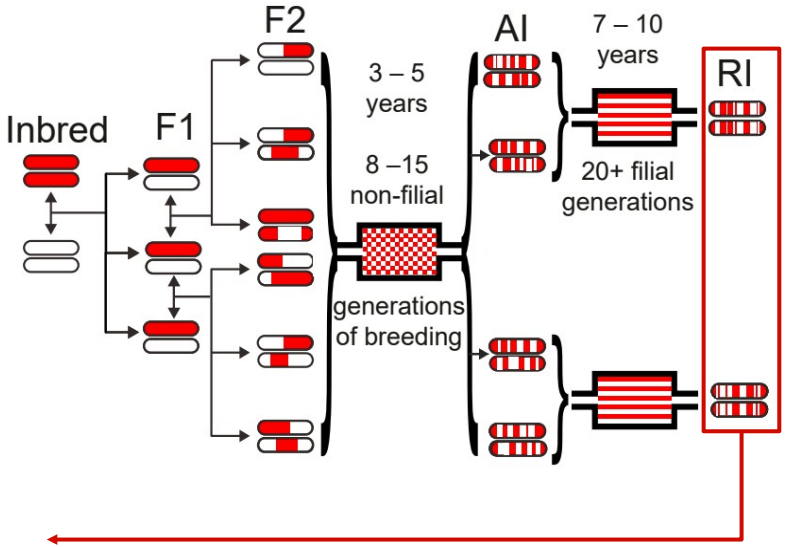
The extended BXD mouse family

C57BL/6J (mother) → **B** X **D** ← DBA/2J (father)

RECOMBINANT INBRED



ADVANCED INTERCROSS



Whole Genome Sequencing
10X Linked reads

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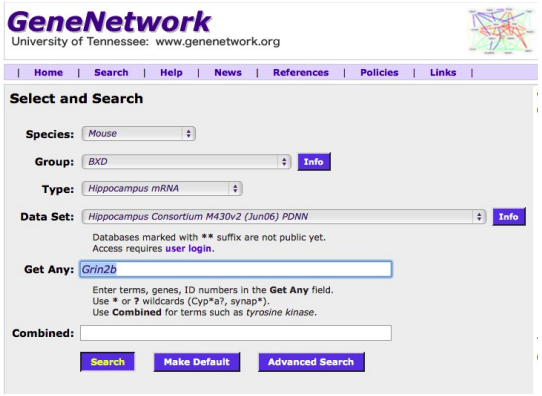
Mulligan, Megan K., et al. *Systems Genetics*. (2017)

Ashbrook, D. G., et al. Cell Systems (2021)

Expression QTL mapping in BXD mice

- 155 BXDs strain
- 21M mapped quantitative traits



Remap eQTL using genetic markers derived from the pangenome graph

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Pangenomics

A pangenome is just a set of sequences…

>ATCTCTCGTAACACTGTCAGTCAGTCA
>ACTCTCATTGTGTCCCTGTACTGTCTG
>CGTGCTGCTGATCGGGGTGCTGGCTAG
>CGTGCTGATTGTCGTGGGCTGTAGGTG

…but pangenomics is a paradigm shift in genomics

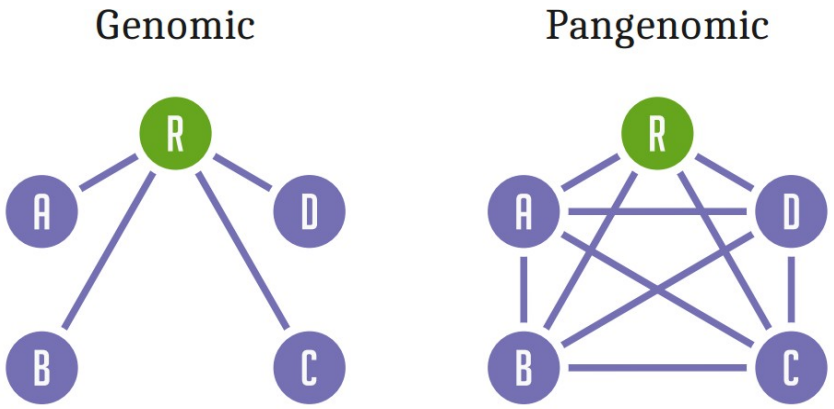


Figure from Eizenga et al., 2020.

Haplotype resolved complete representation of genomes

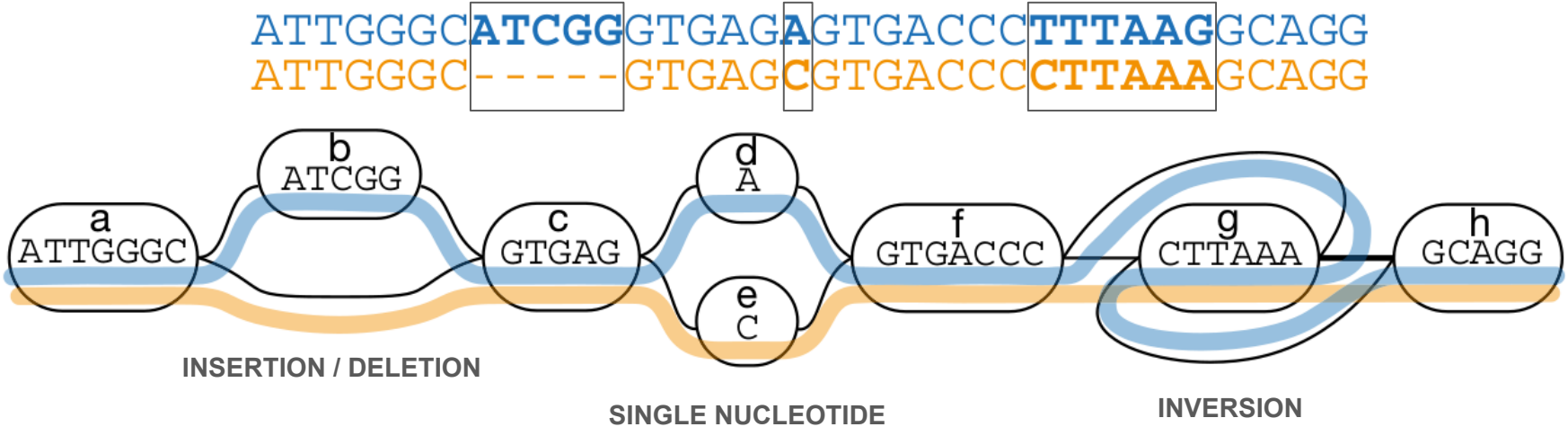
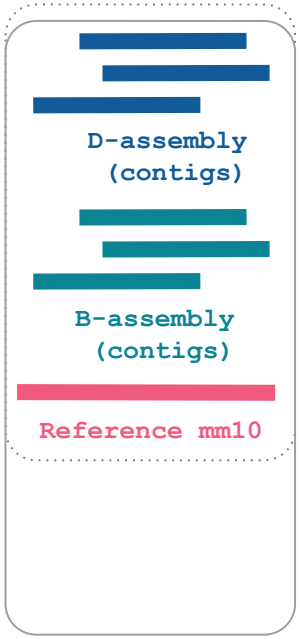


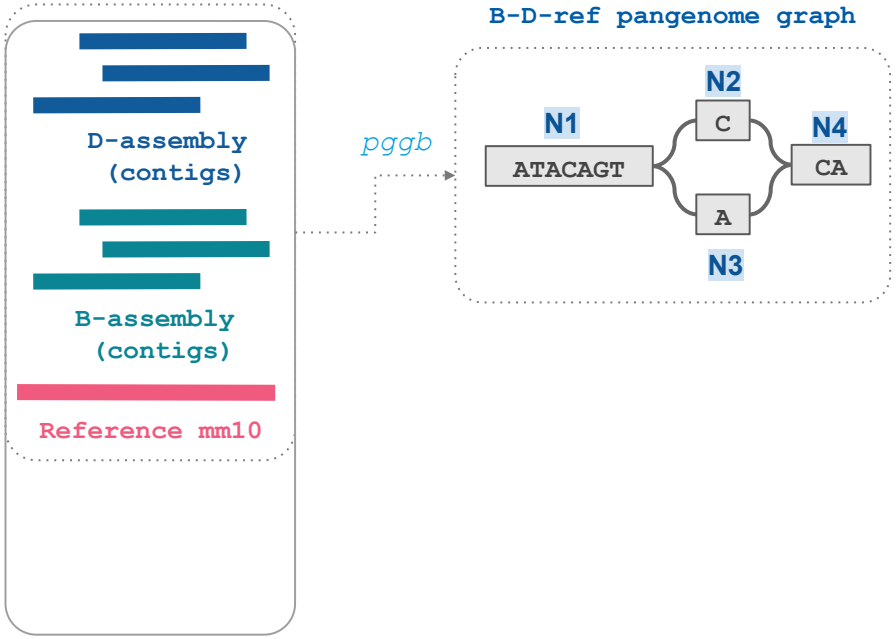
Figure from Liao *et al*, 2023

Mapping short-read *vs* a pangenome

Mapping short-read against a pangenome graph



Mapping short-read against a pangenome graph



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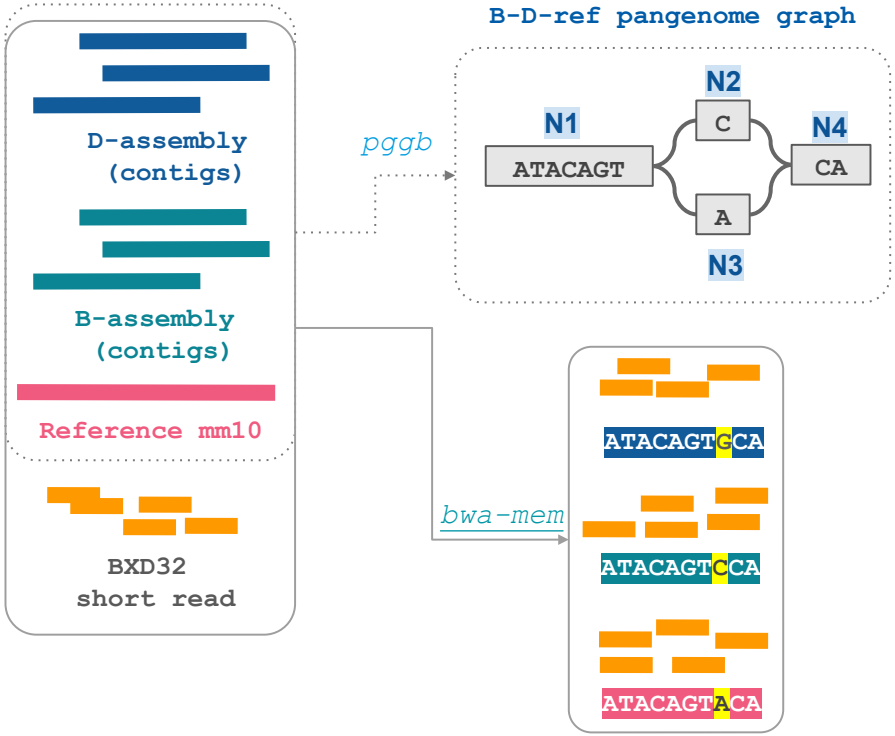

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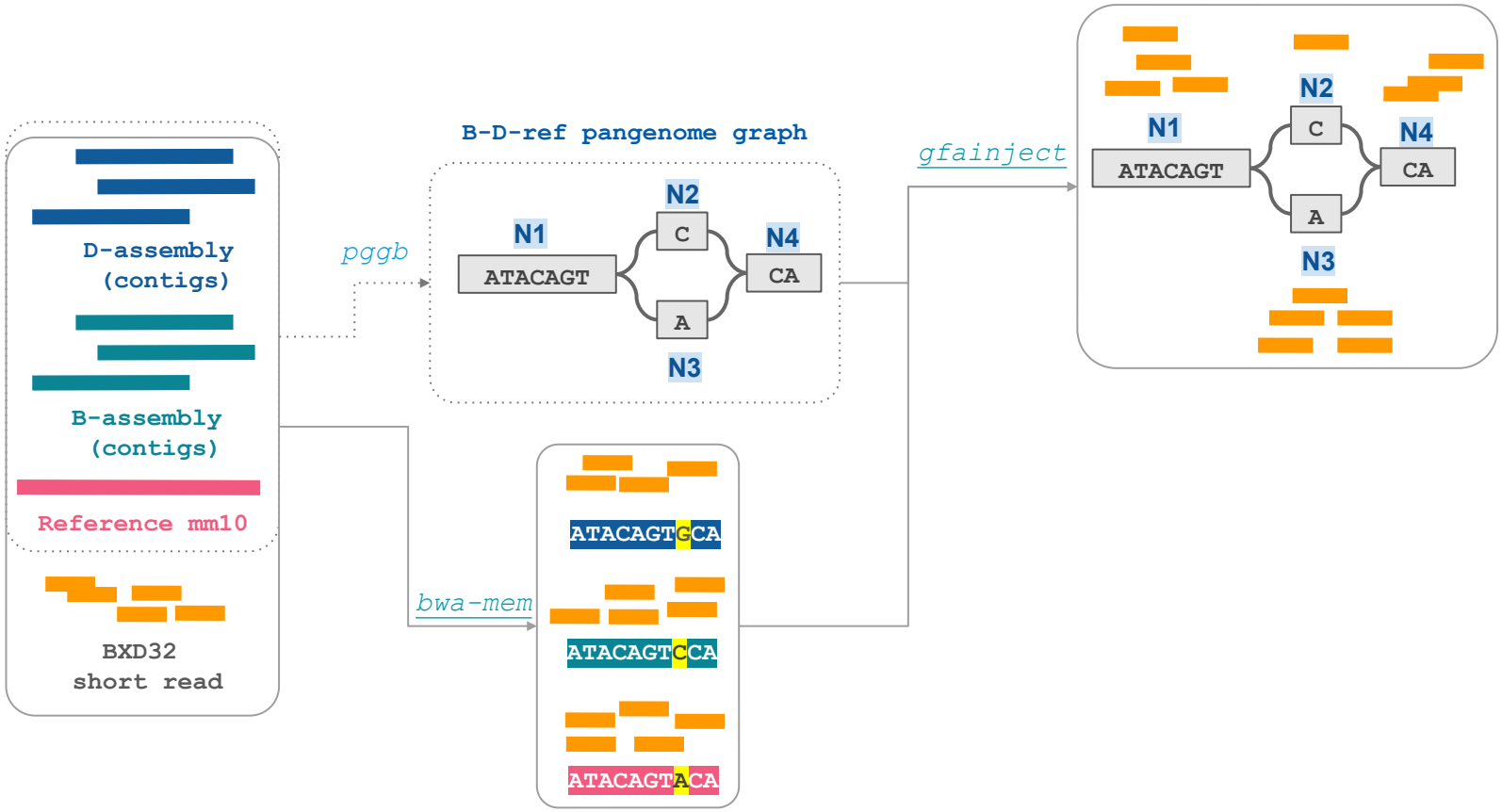

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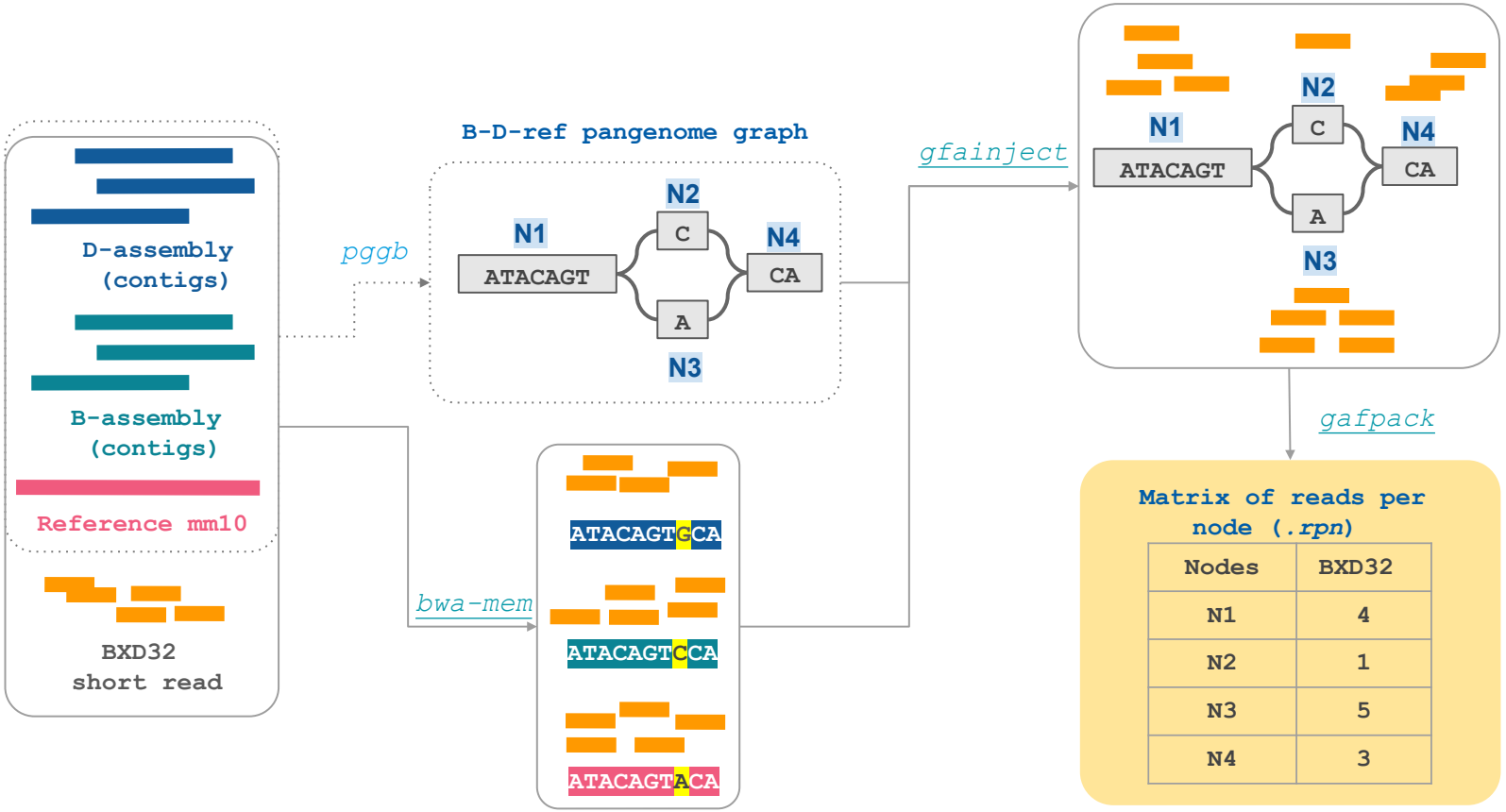


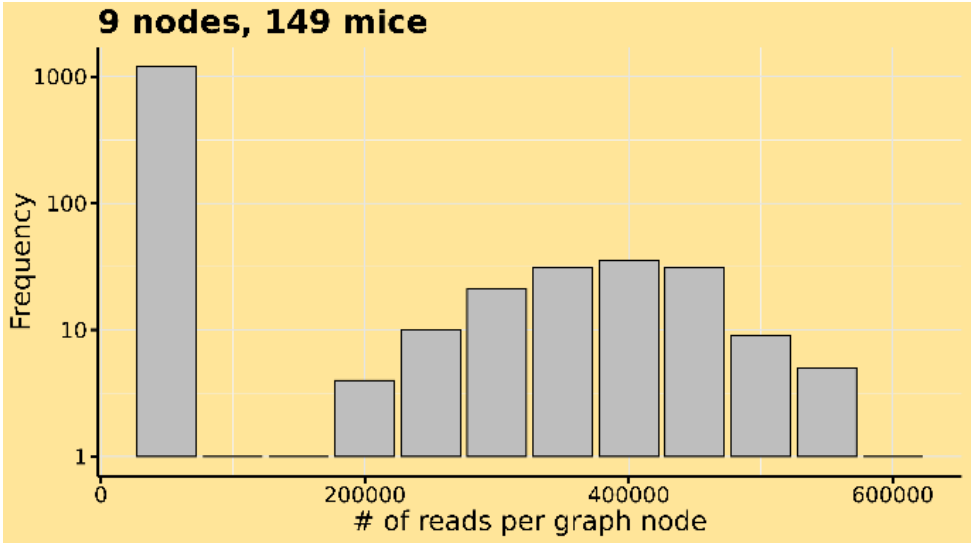
Matrix of reads per node (*.rpn*)

Nodes	BXD32
N1	4
N2	1
N3	5
N4	3

Genotyping from pangenome

Matrix of reads per node

Nodes	BXD28	BXD22	BXD34	BXD40	BXD44	…
N1	348940	439853	384809	342335	232189	…
N2	2940	5277	6911	5884	4951	…
N3	125	218	377	392	211	…
N4	3	8	5	8	6	…
N5	0	3	7	5	2	…
N6	0	24	9	48	58	…
…	…	…	…	…	…	…



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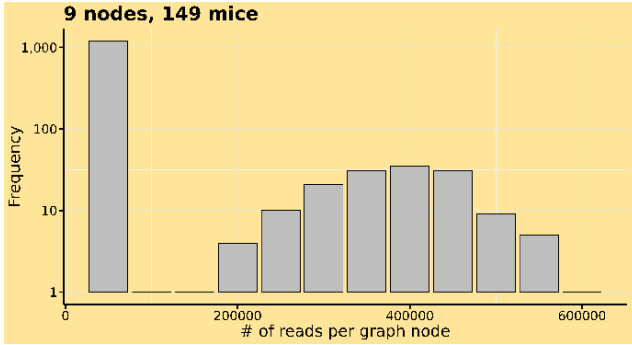

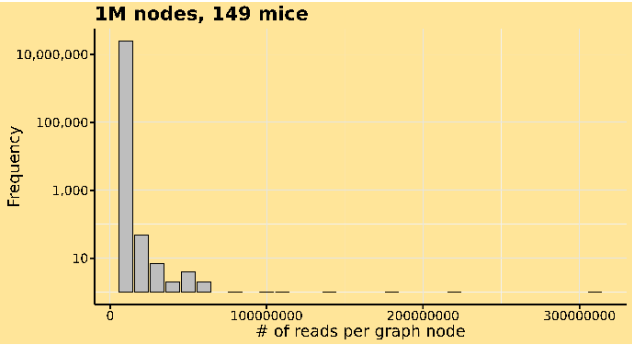

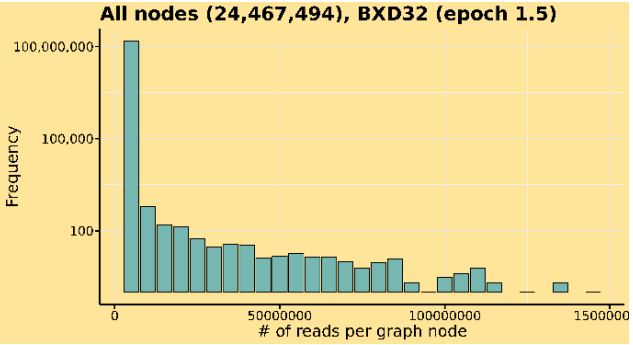

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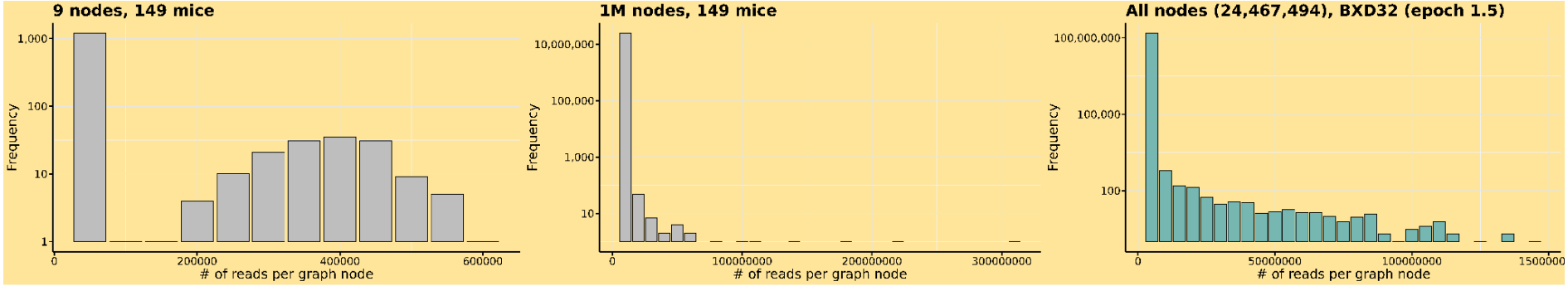

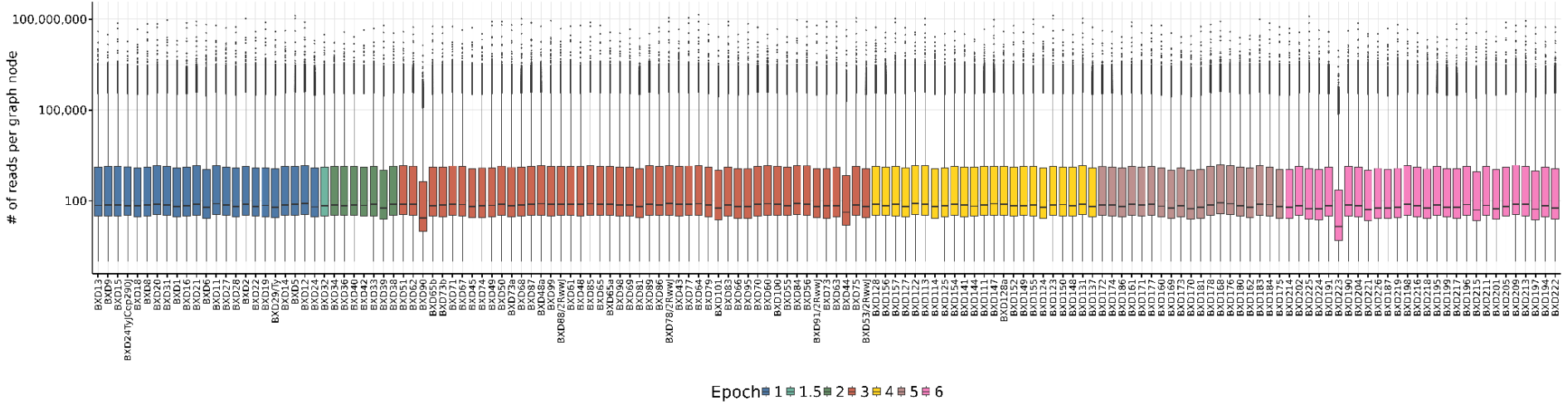

Application to eQTL mapping

Standard approach

Genotype matrix

rsID	A1	A2	Ind1	Ind2
rs3144	A	T	1	0

Phenotype matrix

ID	Value
Ind1	1.2
Ind2	2.3

Marker map

rsID	Pos	Chr
rs3144	1200	1

GEMMA: Genome-wide Efficient Mixed Model Association

Standard approach

Genotype matrix

rsID	A1	A2	Ind1	Ind2
rs3144	A	T	1	0

Phenotype matrix

ID	Value
Ind1	1.2
Ind2	2.3

Marker map

rsID	Pos	Chr
rs3144	1200	1

Pangenomic approach

Matrix of mapped reads

Node	A1	A2	Ind1	Ind2
N1	A	A	1	2

Phenotype matrix

ID	Value
Ind1	1.2
Ind2	2.3

Marker map

Node	Pos	Chr
N1	10	1

GEMMA: Genome-wide Efficient Mixed Model Association

eQTL mapping

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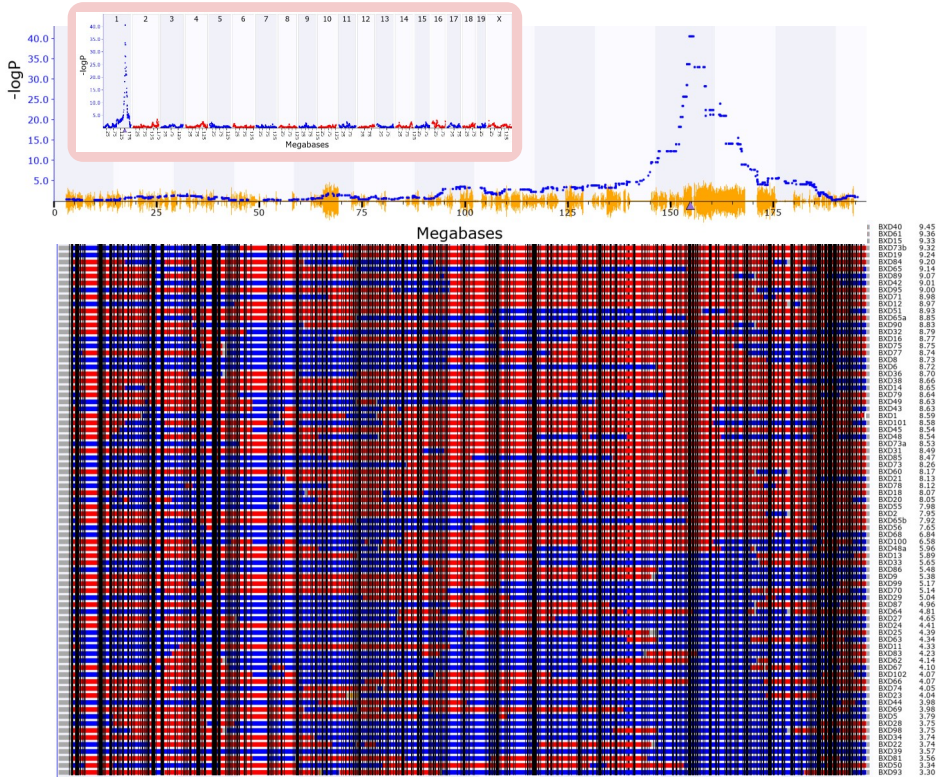
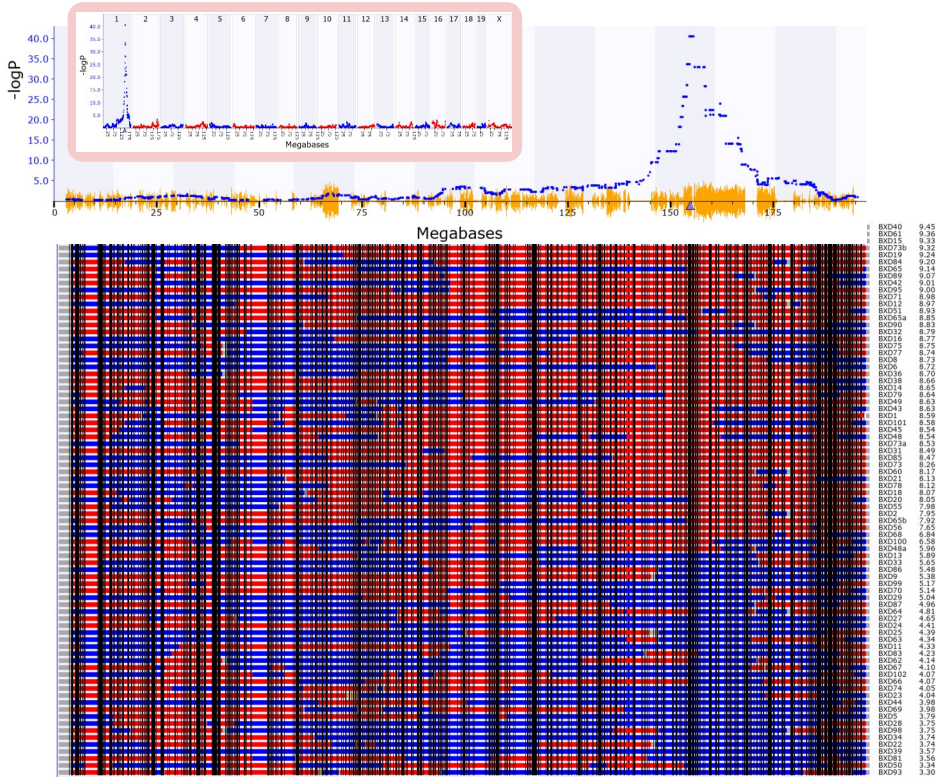
A very strong signal as a positive control

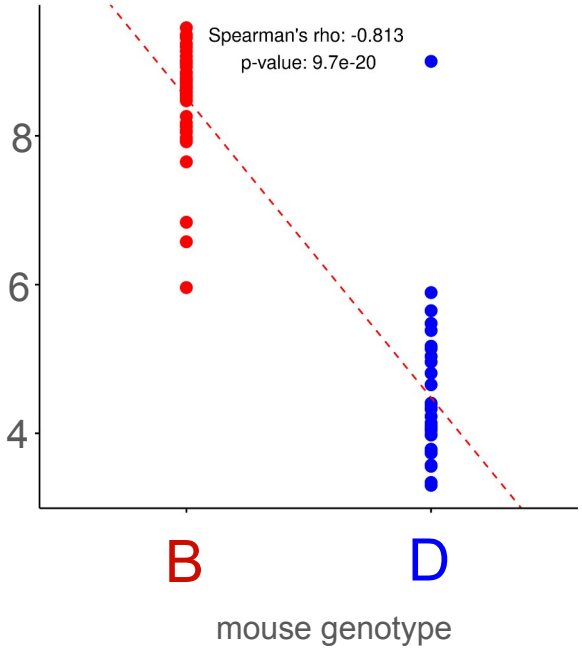


https://genenetwork.org/show_trait?trait_id=10358973&dataset=UTHSC_SPL_RMAEx_1210

A very strong signal as a positive control

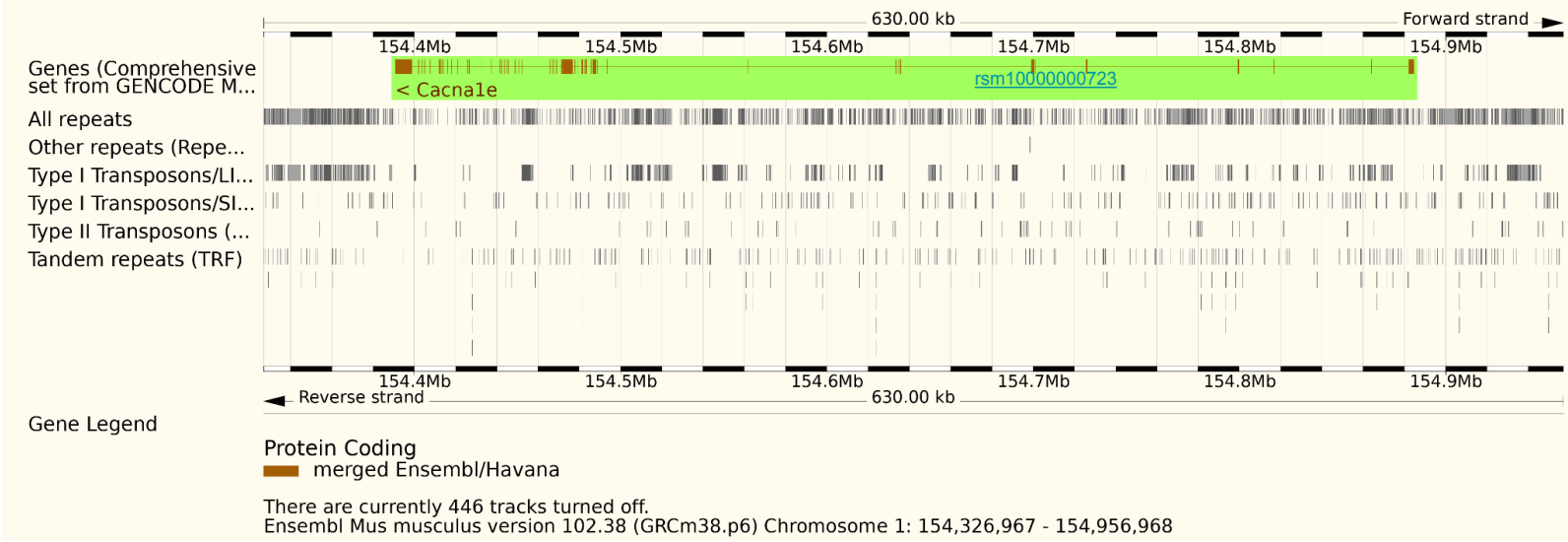


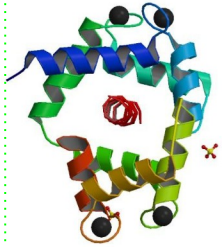
Cacna1e
gene expression
(spleen)



https://genenetwork.org/show_trait?trait_id=10358973&dataset=UTHSC_SPL_RMAEx_1210

Cacna1e gene (chr1)

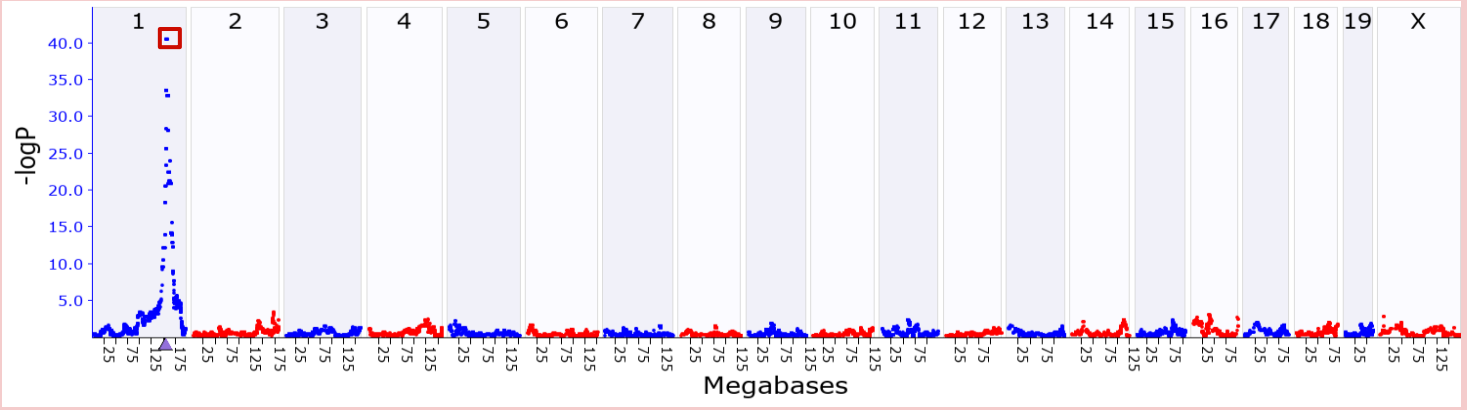




Cacna1e encodes an integral membrane protein that belongs to the calcium channel alpha-1 subunits family.

Signal replication for *Cacna1e* on chromosome 1

Standard approach



Signal replication for *Cacna1e* on chromosome 1

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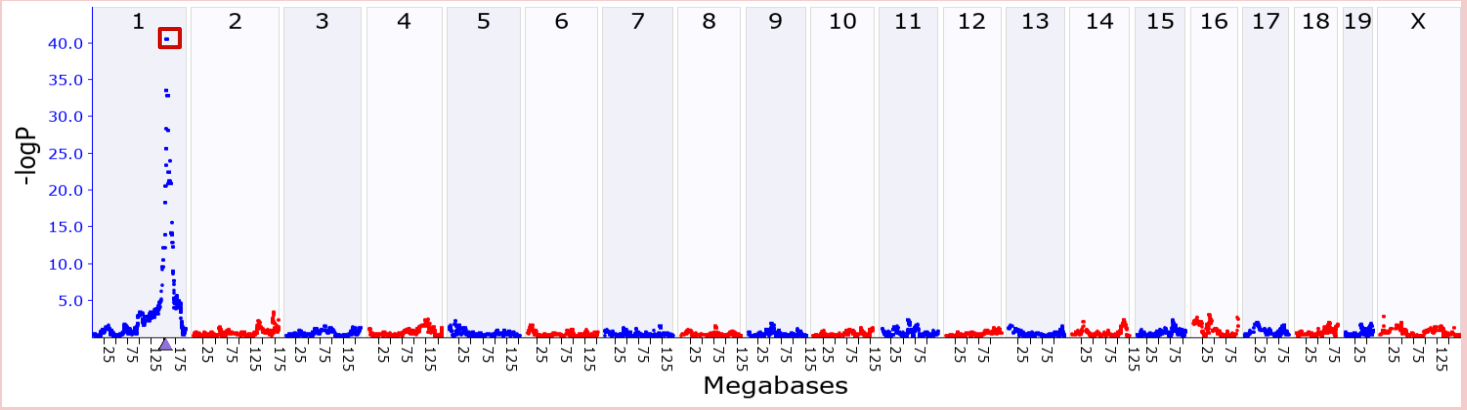
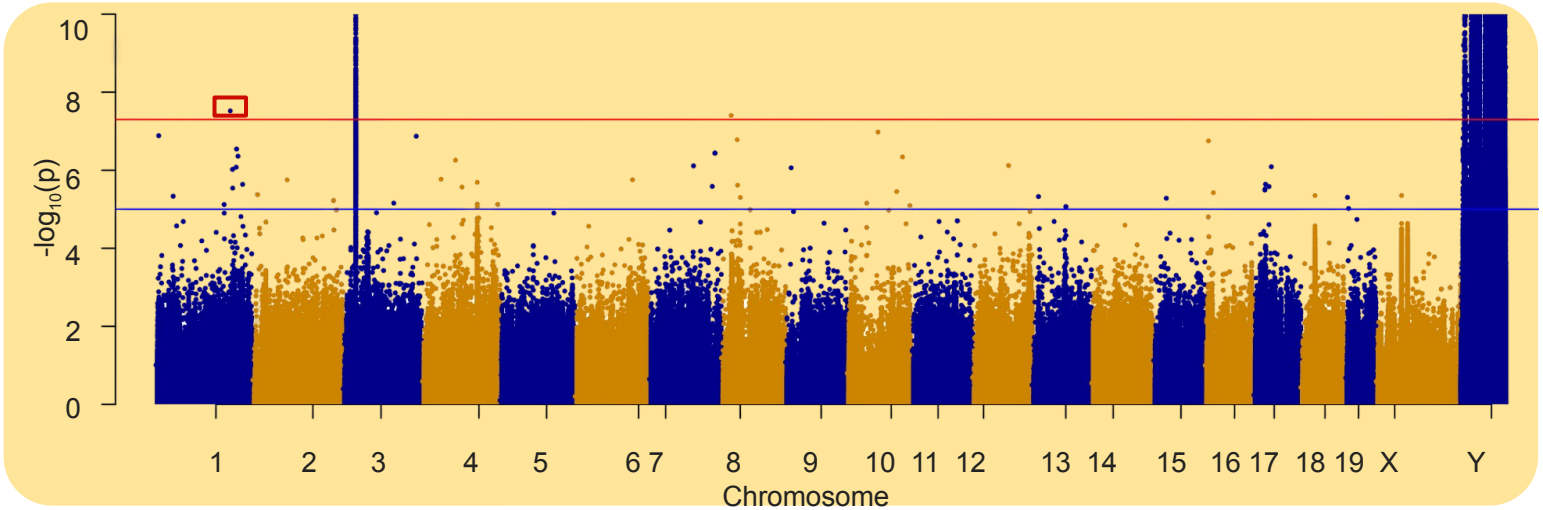


Pangenomic approach



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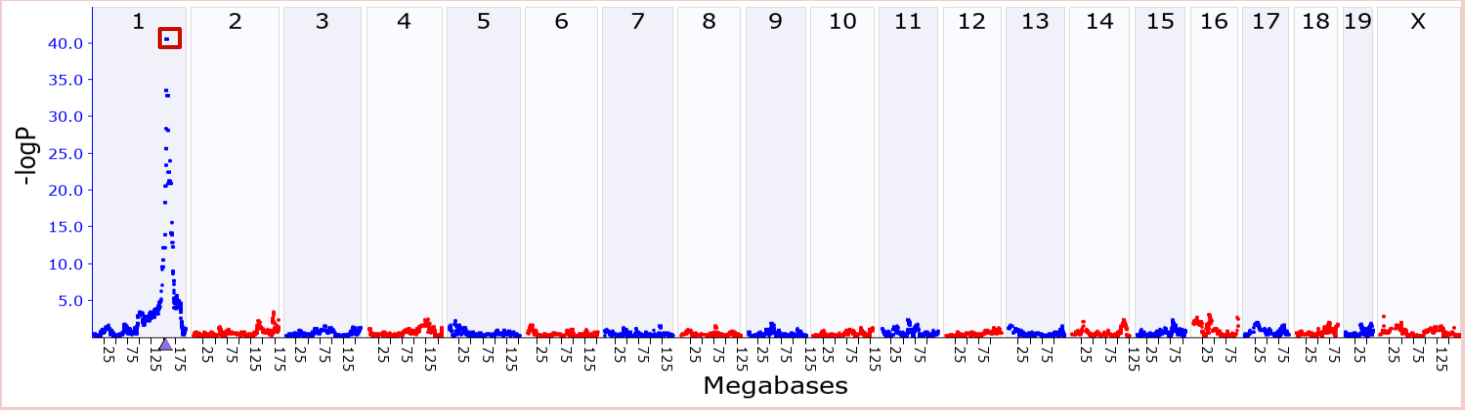
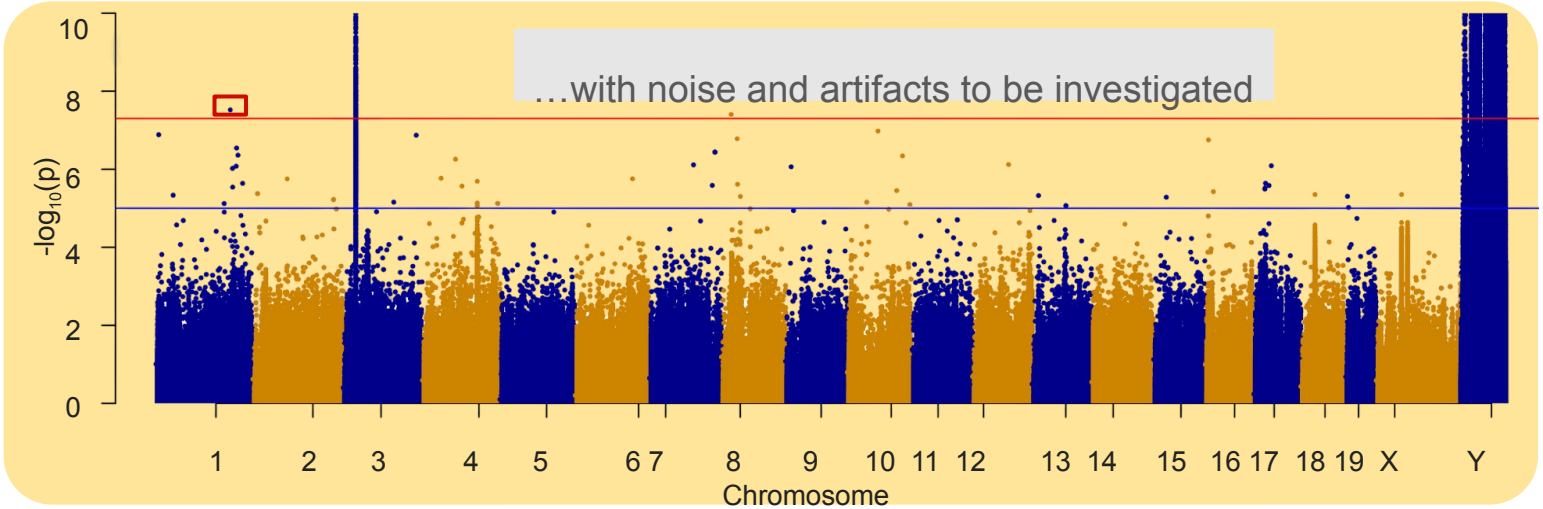
Signal replication for *Cacna1e* on chromosome 1

Standard approach



Pangenomic approach



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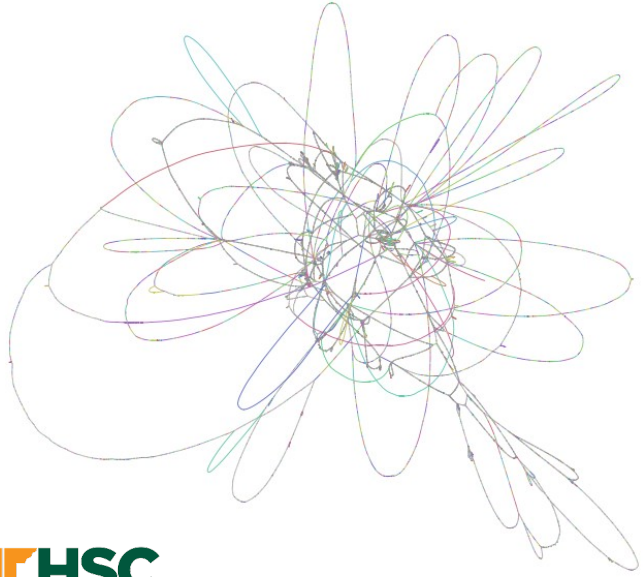
Conclusions

- Pipeline for deriving genotype markers mapping short-read against pangenome graphs
- Application to eQTL mapping in BXD



Future directions

- Apply quality filters
- Manage repeats
- Extend the analyses to all traits in GN

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Acknowledgement

Vincenza Colonna
Erik Garrison
David Ashbrook
Rob Williams
Hao Chen
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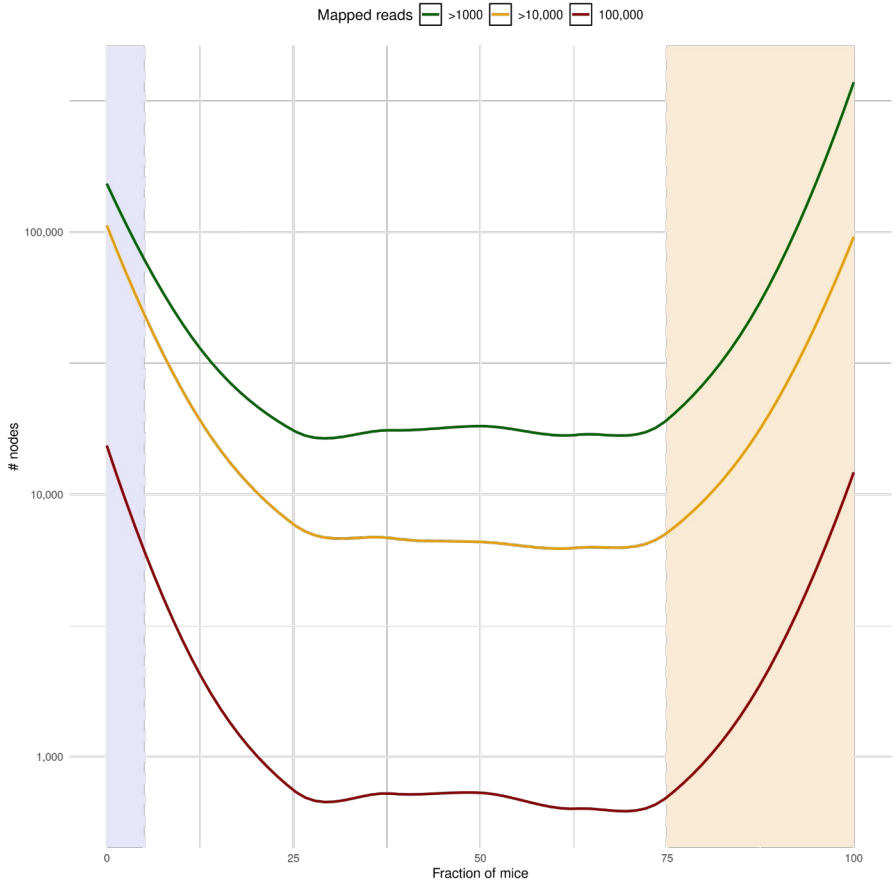
Andrea Guarracino
Farnaz Salehi

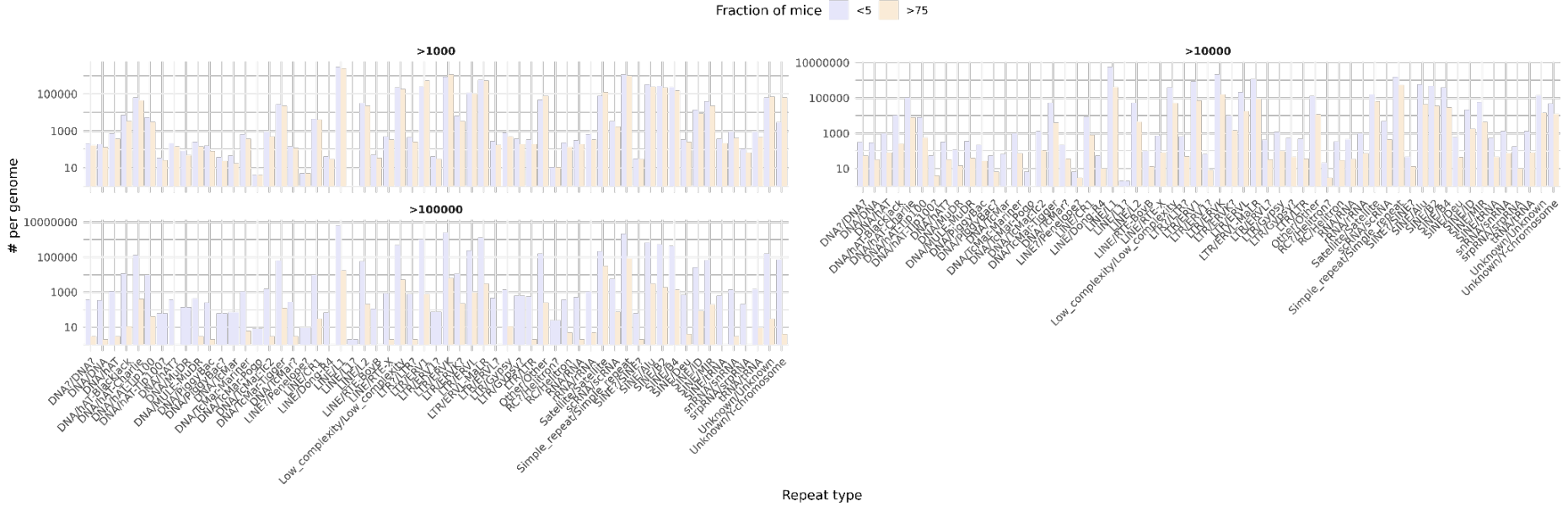
Colonna's lab
Pangenome group
and many others!!

THE UNIVERSITY OF TENNESSEE HEALTH SCIENCE CENTER

THANKS!


Mapped reads
>1000
>10,000
100,000
nodes
100,000
10,000
1,000
0
25
50
75
100
Fraction of mice

Repeat overlap
1000.non-overlap
100000.non-overlap
10000.notinmm10
1000.overlap
100000.overl
10000.non-overlap
1000.notinmm10
100000.notinmm10
10000.overlap
0-5%
75-100%
Count
40000000
30000000
20000000
10000000
0
>1000
>10,000
>100,000
>1000
>10,000
>100,000
Reads mapped


Fraction of mice
<5
>75
>1000
>10000
>100000
per genome
Repeat type