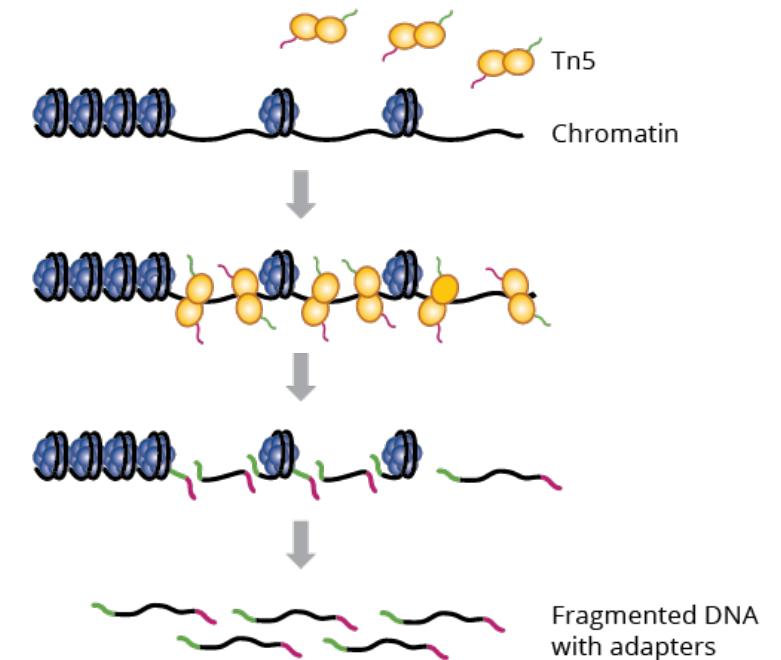
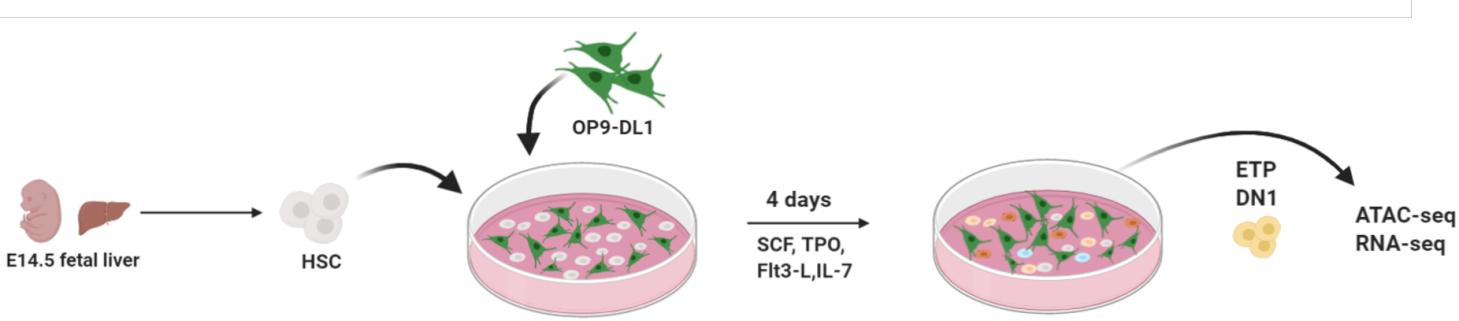
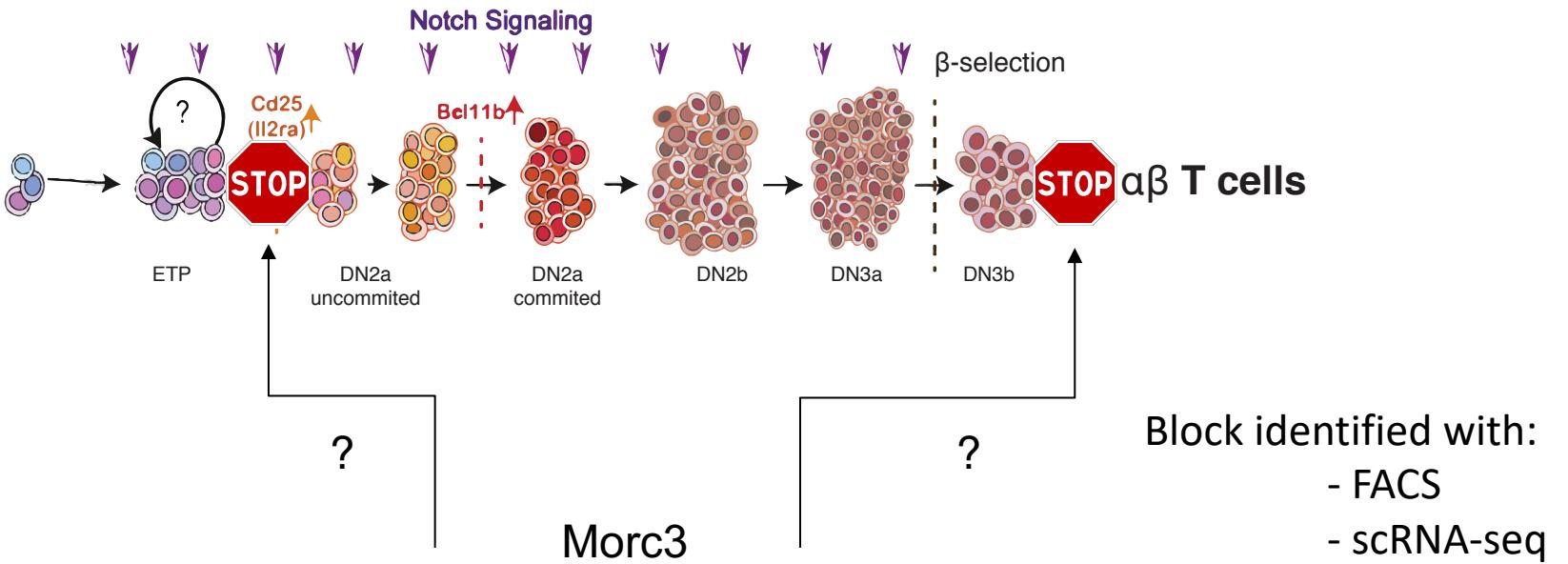


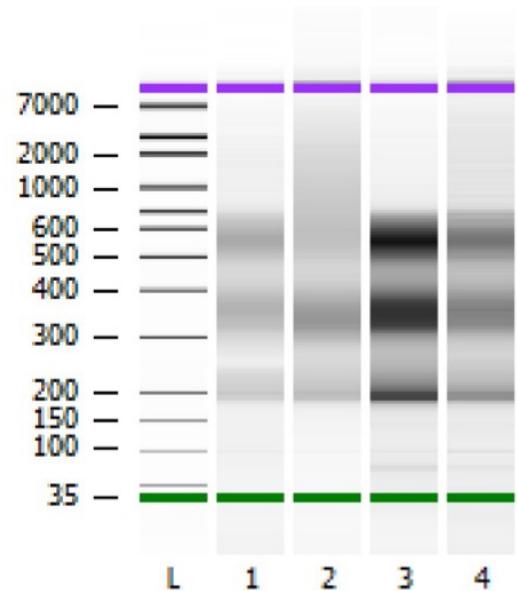
# DN1 ATAC-seq

Veronica

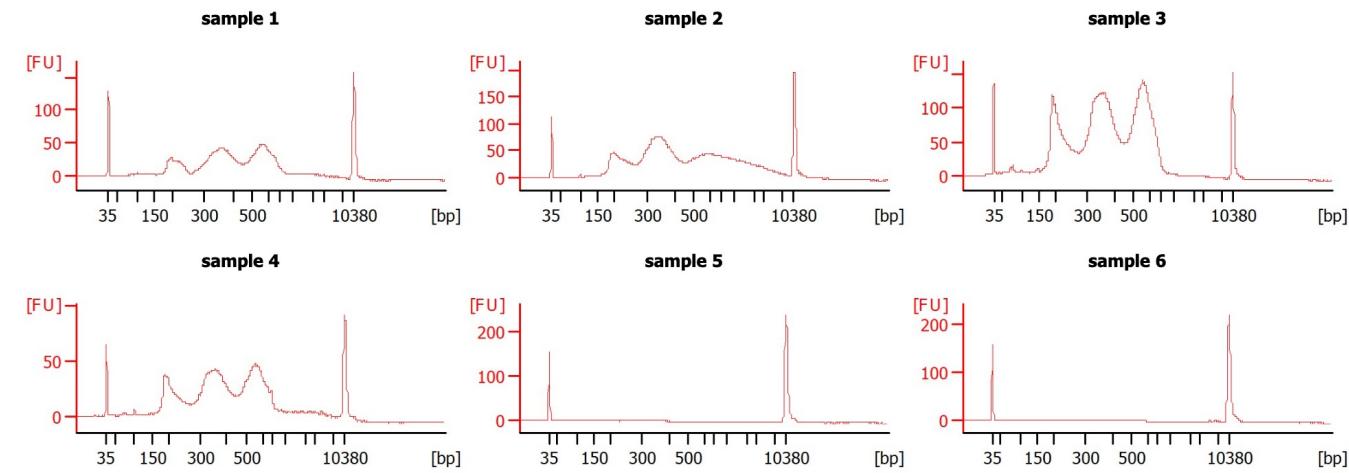
# Background



- 50000 DN1 cells from OP9:
  - 2 x WT
  - 2 x KO

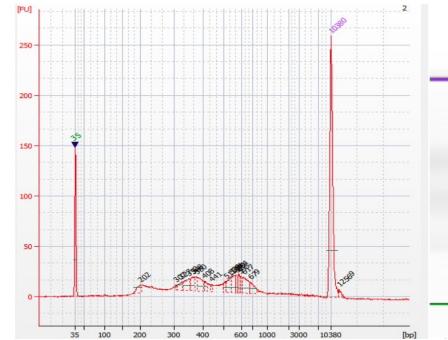


sample nb	name	primer 1	primer 2	Concentration Qubit	Cycles added to second PCR
1	WT1	S507	N702	1,28	12
2	WT2	S507	N704	1,98	13
3	KO1	S507	N705	3,15	10
4	KO2	S507	N707	1,97	8

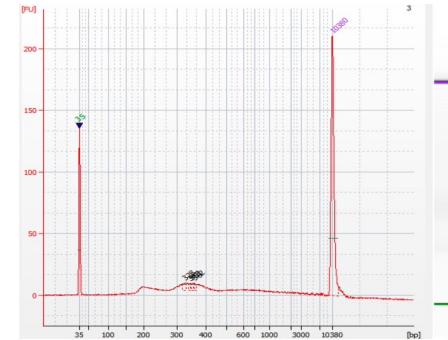


#	Library Name	Library Type	Conc. (ng/uL)	Conc. (nM)	Size (bp)	Result*	
1	DN1_WT_rep1	ETC	0.2	0.72	434	Fail	Low Quantity to (Run or Capture)
2	DN1_WT_rep2	ETC	0.26	0.97	409	Fail	Low Quantity to (Run or Capture)
3	DN1_KO_rep1	ETC	0.85	3.26	403	Fail	Low Quantity to (Run or Capture)
4	DN1_KO_rep2	ETC	0.24	0.88	417	Fail	Low Quantity to (Run or Capture)

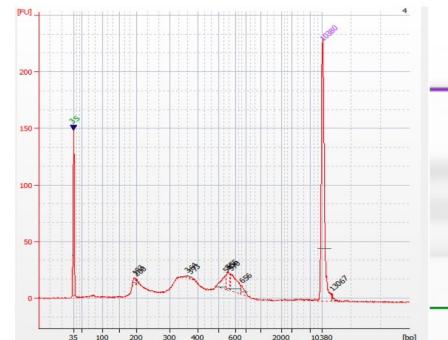
[Click to Enlarge =>1:Library : DN1\\_WT\\_rep1](#)



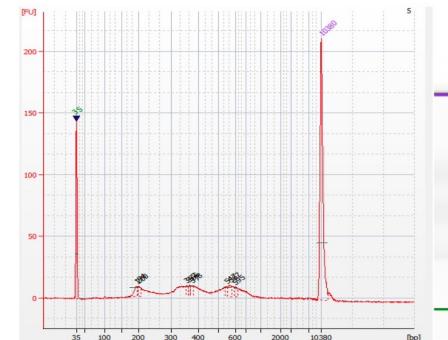
[Click to Enlarge =>2:Library : DN1\\_WT\\_rep2](#)



[Click to Enlarge =>3:Library : DN1\\_KO\\_rep1](#)



[Click to Enlarge =>4:Library : DN1\\_KO\\_rep2](#)



# QC reports provided by Macrogen

Client Name	Macrogen Europe
Company / Institution	Macrogen Europe
Order Number	HN00155645
Type of Read	Paired-end
Read Length	151
Number of Samples	3
Type of Sequencer	Illumina platform

Download link	File size	md5sum
<a href="#">DN1_KO_rep1_1.fastq.gz</a>	1.0G	c2095e9bc993755383be9247d830e2e3
<a href="#">DN1_KO_rep1_2.fastq.gz</a>	991.1M	8d654303f1407119a14ccf671cd4d375
<a href="#">DN1_WT_rep1_1.fastq.gz</a>	347.0M	31260d4e330390984afb1aabf1f39e60
<a href="#">DN1_WT_rep1_2.fastq.gz</a>	322.8M	ff40f5e78e477fc6632206f1cc6ece8f
<a href="#">DN1_WT_rep2_1.fastq.gz</a>	295.6M	339faea0788651328629e0dd3722b12f
<a href="#">DN1_WT_rep2_2.fastq.gz</a>	283.9M	ee417d9b45ea135496b10b6edebf49ce

Sample ID	Total read bases (bp)	Total reads	GC(%)	AT(%)	Q20(%)	Q30(%)
DN1_KO_rep1	5,031,883,230	33,323,730	51.04	48.96	94.66	89.32
DN1_WT_rep1	1,474,346,182	9,763,882	51.46	48.54	94.92	89.57
DN1_WT_rep2	1,013,035,444	6,708,844	51.28	48.72	89.67	77.98

Table 1. Raw data Stats (maximum 20 samples)

Sample ID	Total read bases (bp)	Total reads	GC(%)	AT(%)	Q20(%)	Q30(%)
DN1_KO_rep1	5,031,883,230	33,323,730	51.04	48.96	94.66	89.32
DN1_WT_rep1	1,474,346,182	9,763,882	51.46	48.54	94.92	89.57
DN1_WT_rep2	1,013,035,444	6,708,844	51.28	48.72	89.67	77.98

# Analysis

- Done with our [Snakemake-ATAC-seq pipeline](#)

# Quality controls

## General Statistics

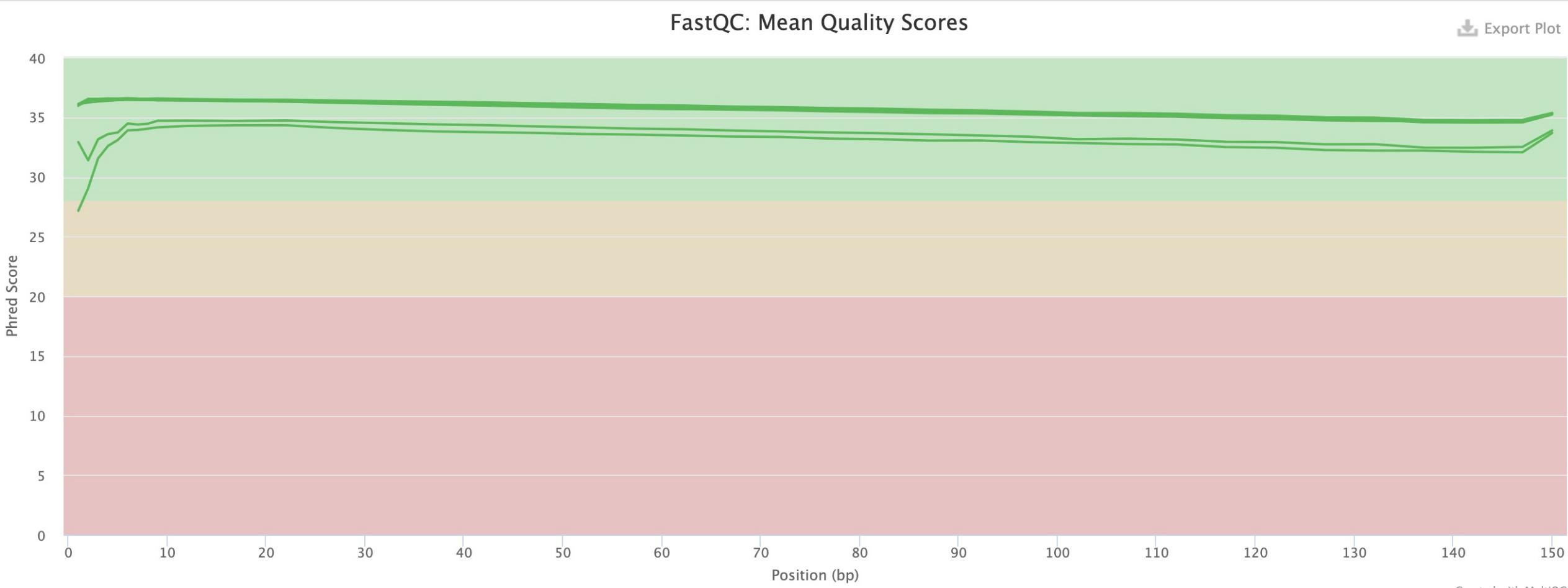
Copy table   Configure Columns   Plot   Showing 9/9 rows and 7/9 columns.

Sample Name	% Aligned	Insert Size	% Dups	% Dups	% GC	Length	M Seqs
DN1KO1	92%	168 bp	23.4%				
DN1KO1_R1				25.3%	47%	111 bp	16.6
DN1KO1_R2				25.7%	47%	111 bp	16.6
DN1WT1	93%	148 bp	19.8%				
DN1WT1_R1				18.2%	48%	110 bp	4.9
DN1WT1_R2				18.6%	48%	110 bp	4.9
DN1WT2	95%	178 bp	11.2%				
DN1WT2_R1				6.9%	49%	123 bp	3.4
DN1WT2_R2				6.9%	49%	123 bp	3.4

# Quality controls

FastQC: Mean Quality Scores

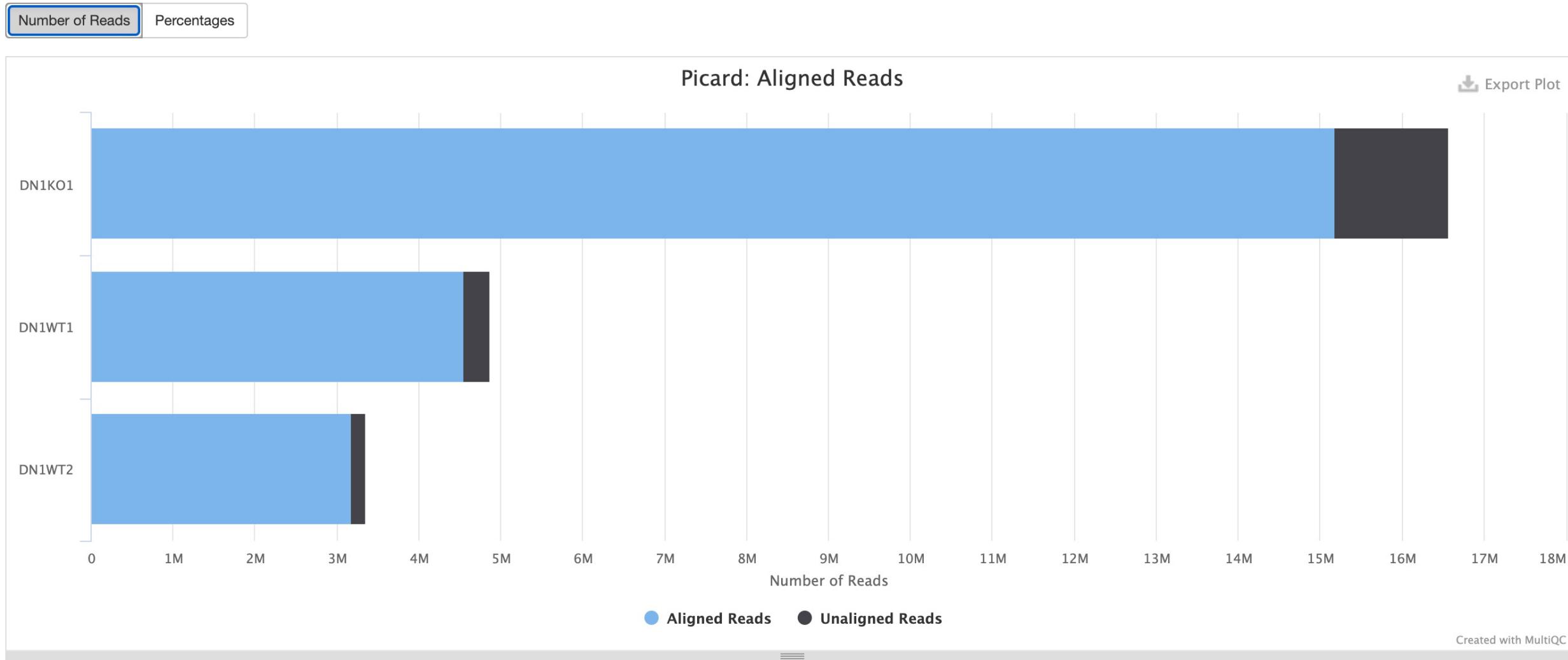
 Export Plot



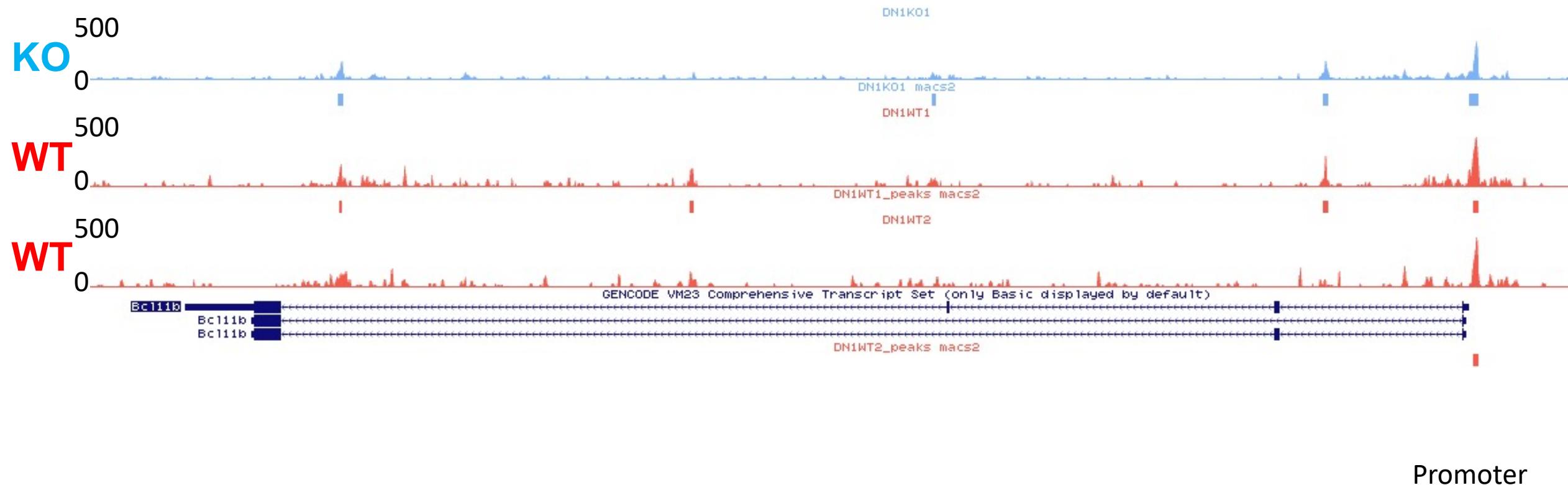
# Quality controls

## Alignment Summary

Please note that Picard's read counts are divided by two for paired-end data.

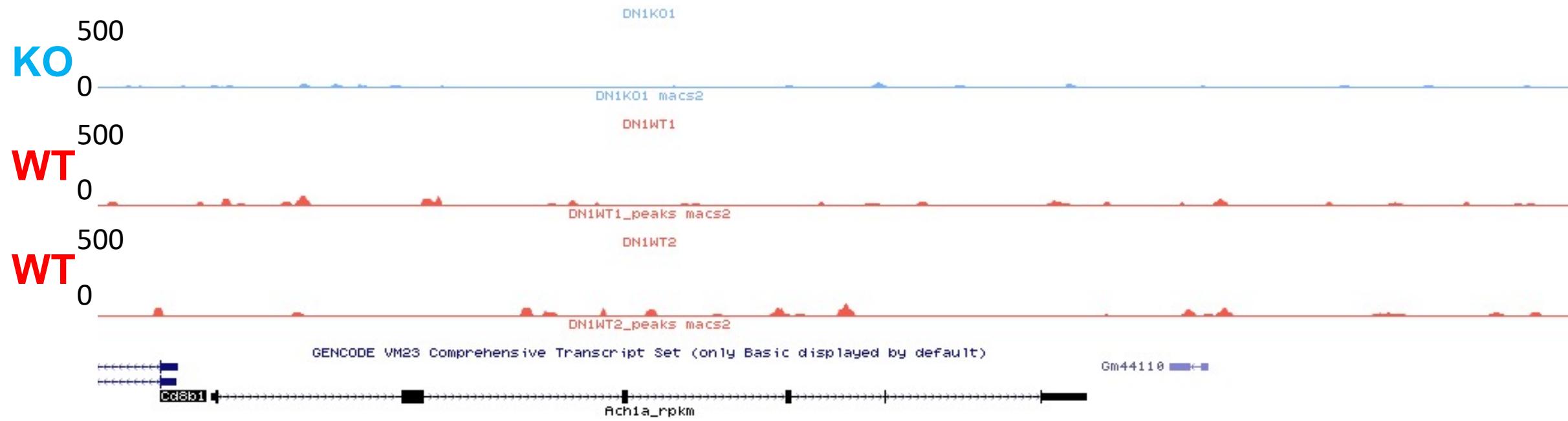


# *Bcl11b*



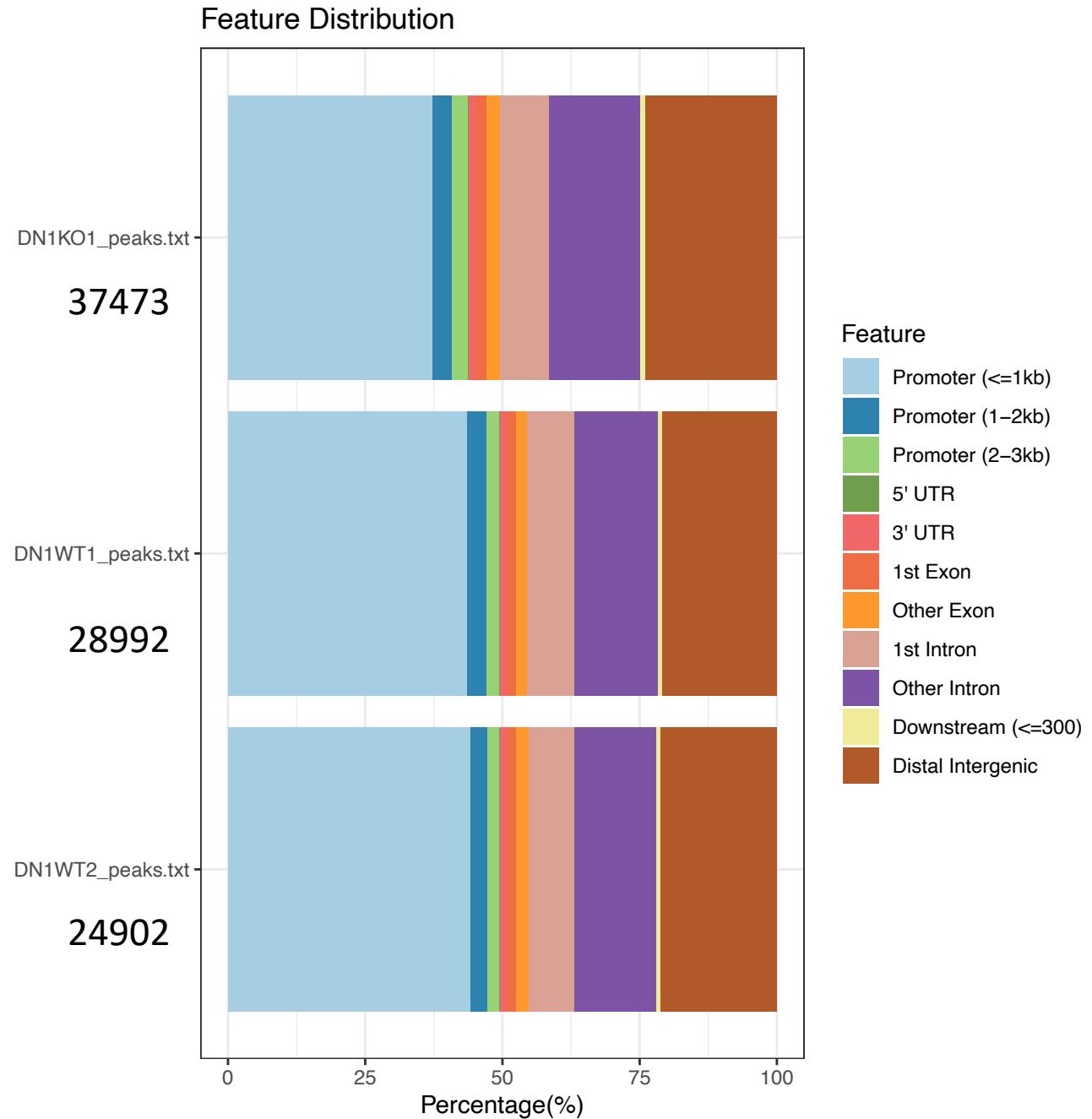
Promoter

# *Cd8b1*



# Peak calling: MACS2

name	peaks
WT1	28992
WT2	24902
KO1	37473



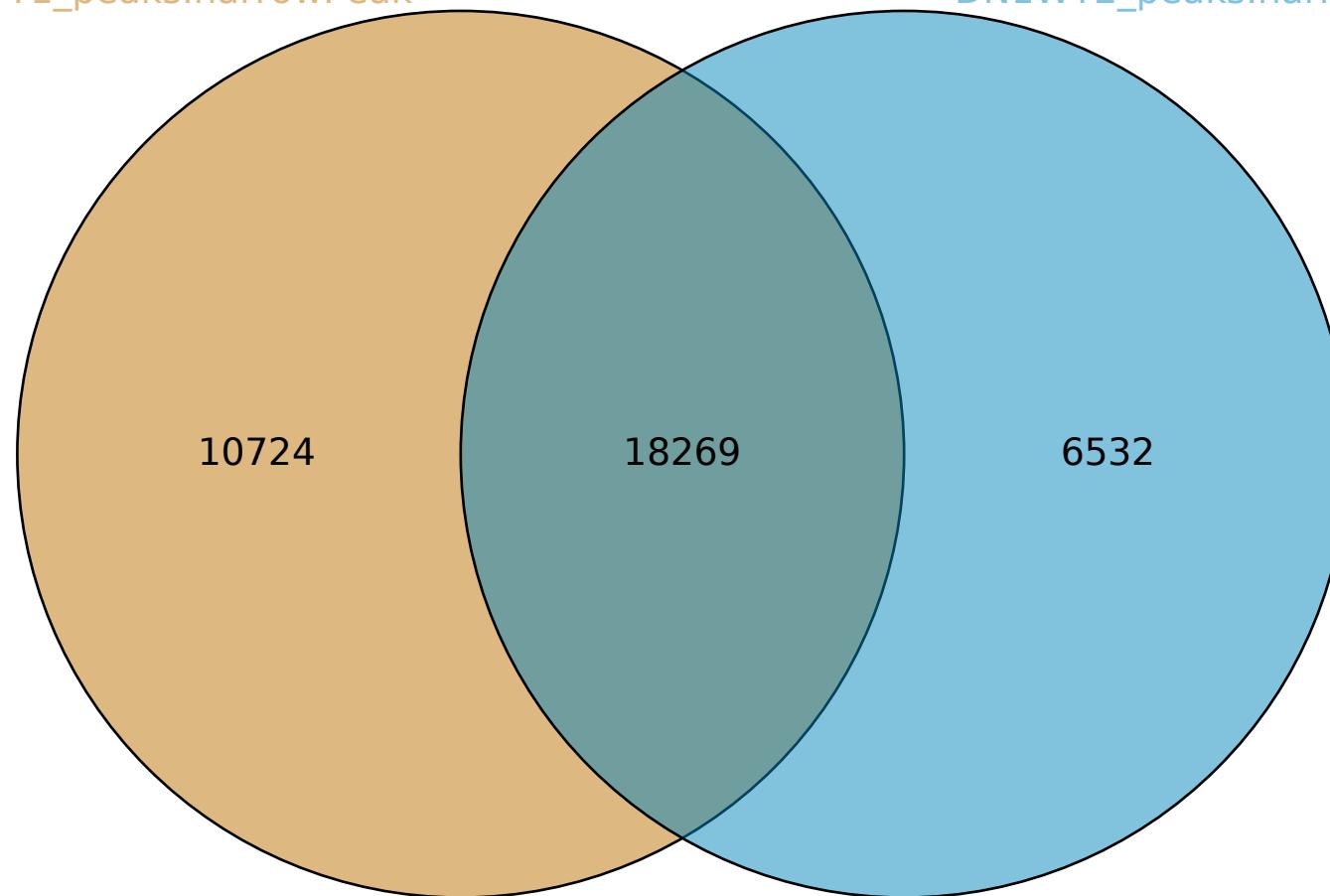
# Overlap WT1 and WT2

DN1WT1\_peaks.narrowPeak  
DN1WT2\_peaks.narrowPeak

DN1WT1\_peaks.narrowPeak      DN1WT2\_peaks.narrowPeak

63% of WT1 peaks overlap  
with WT2

73% of WT2 peaks overlap  
with WT1



# Differential peak analysis

2xWT versus 1xKO

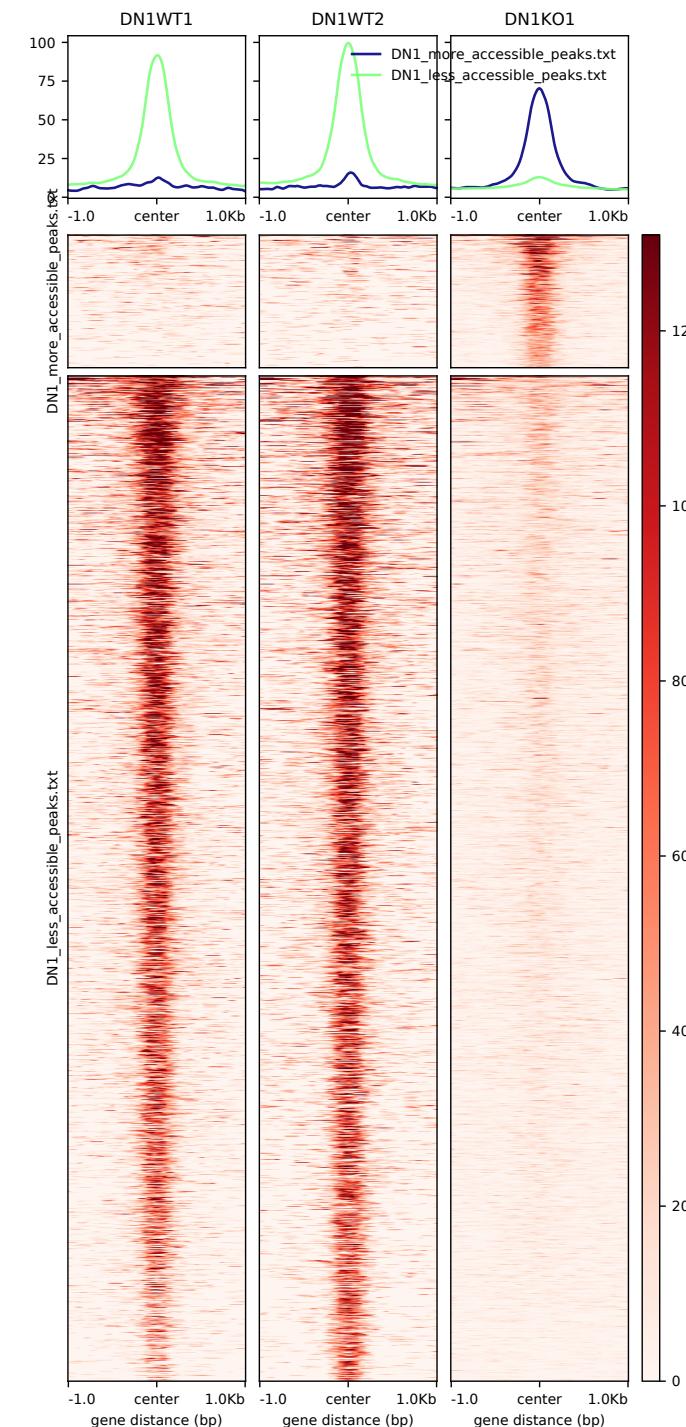
Homer **getDifferentialPeaks FC > 4 / p-value <0.05**

Relative to WT:

Less accessible in KO	2046
More accessible in KO	269

Average size less accessible peaks: 320bp  
(SD=113bp)

Average size more accessible peaks: 284bp  
(SD=145bp)



# The *cis*-Regulatory Atlas of the Mouse Immune System

2x DN1 analyzed

99% Aligned reads (10M reads)

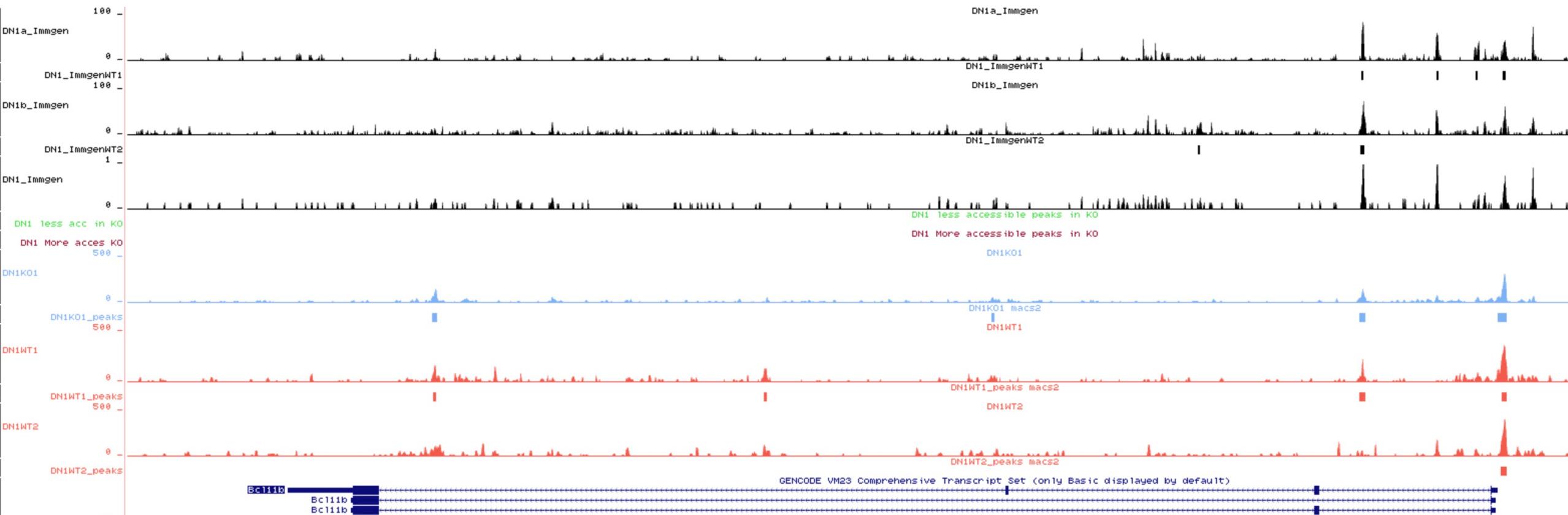
Phred > 35

Samples	Peaks
ImmgenWT1	58066
ImmgenWT2	42862

## Authors

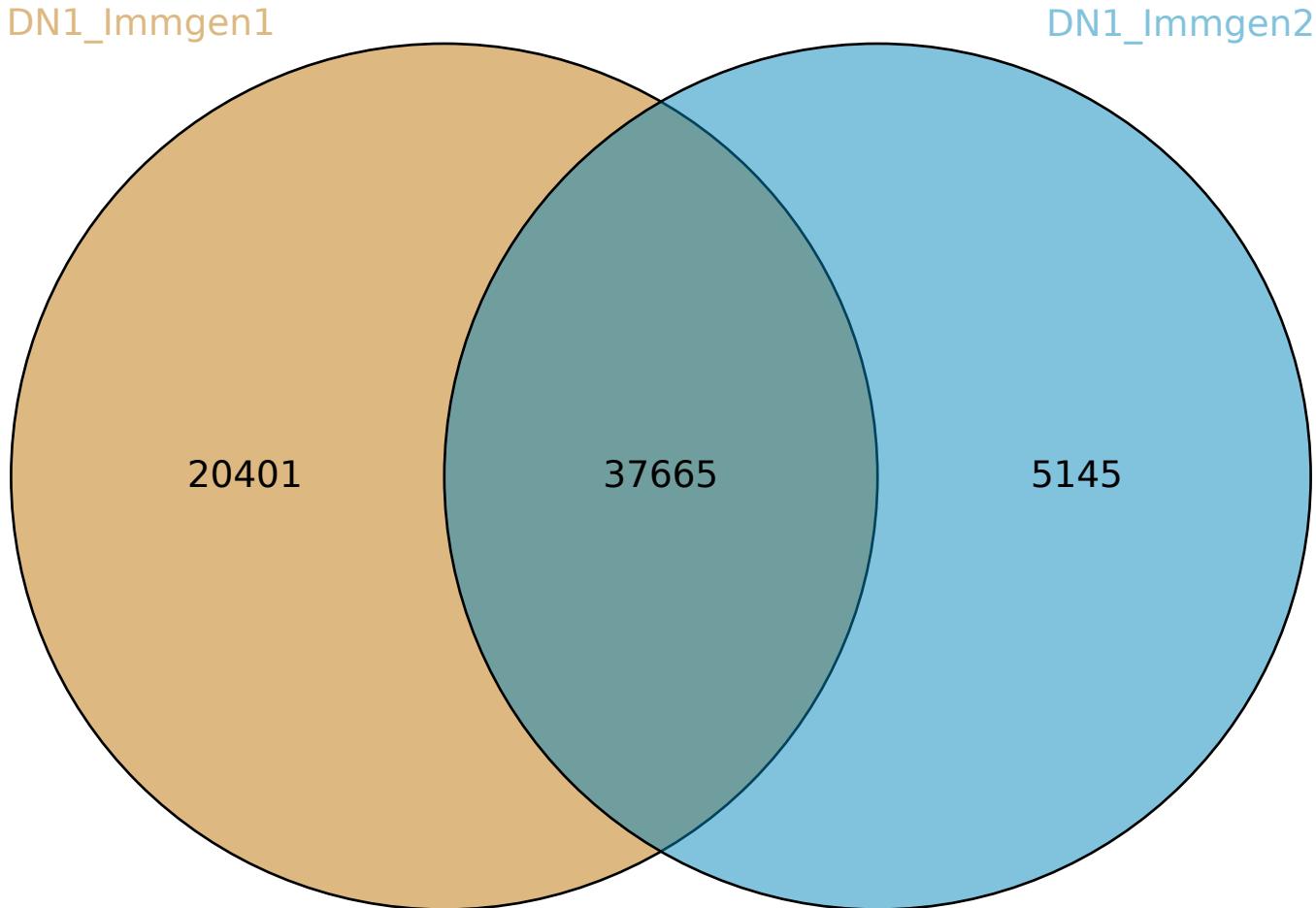
Hideyuki Yoshida, Caleb A. Lareau,  
Ricardo N. Ramirez, ...,  
Jason D. Buenrostro, Christophe Benoist,  
the Immunological Genome Project

# *Bcl11b*



# Overlap Immgen Replicates

DN1\_Immgen1  
DN1\_Immgen2

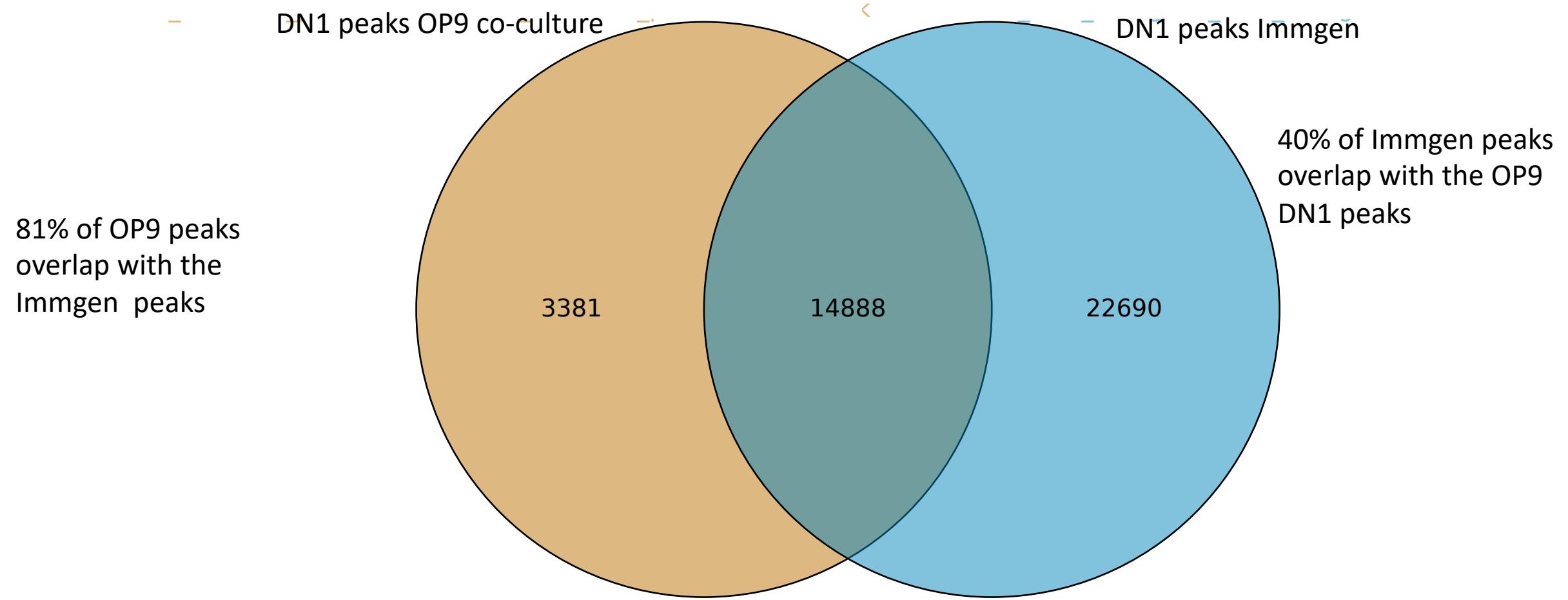


64% of WT1 overlaps with WT2

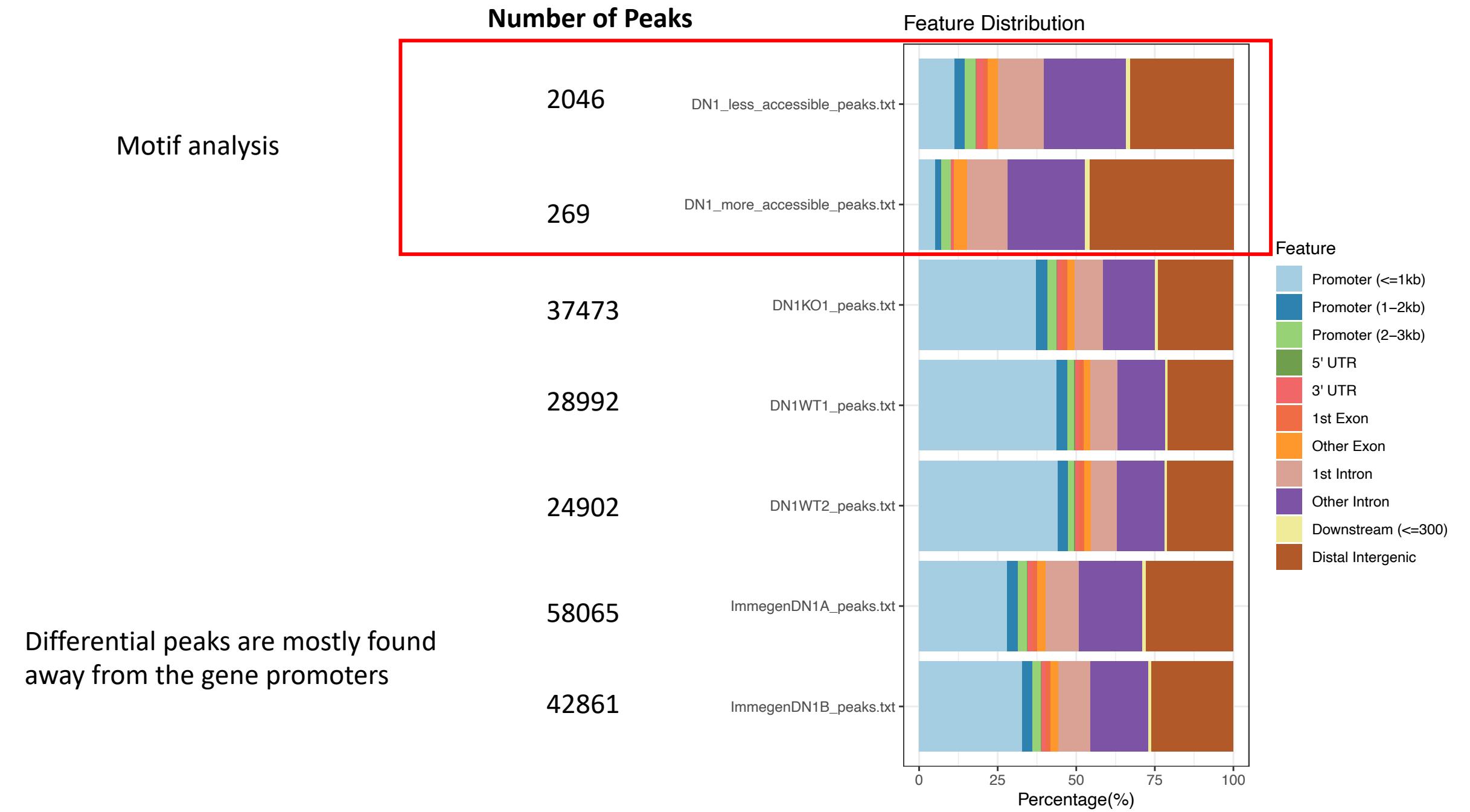
88% of WT2 overlaps with WT1

# Overlap Immgen and OP9 peaks

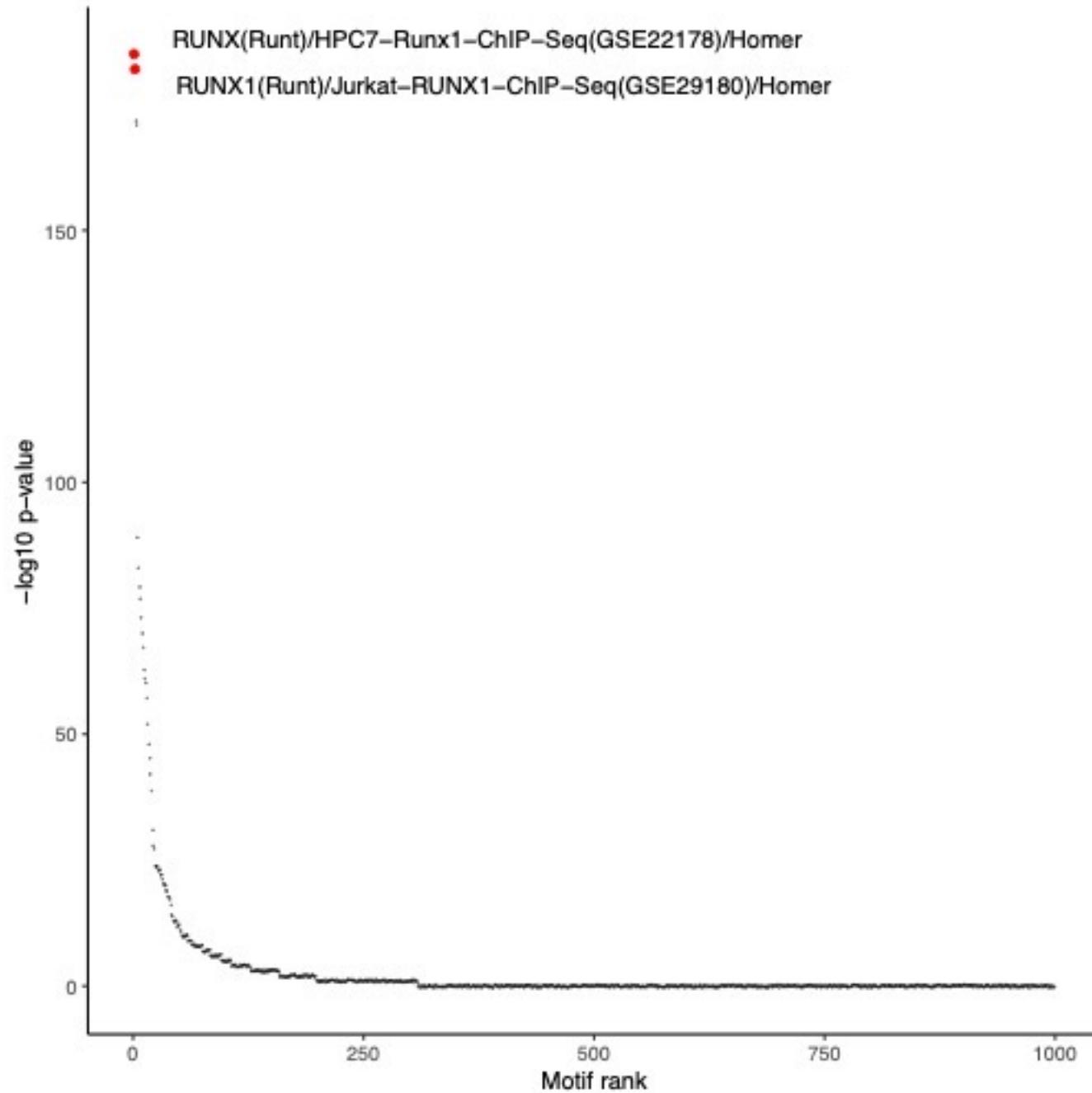
11\_DN1WT1\_peaks.narrowPeak\_DN1WT2\_peaks.narrowPeak  
11\_DN1\_Immgen1\_DN1\_Immgen2



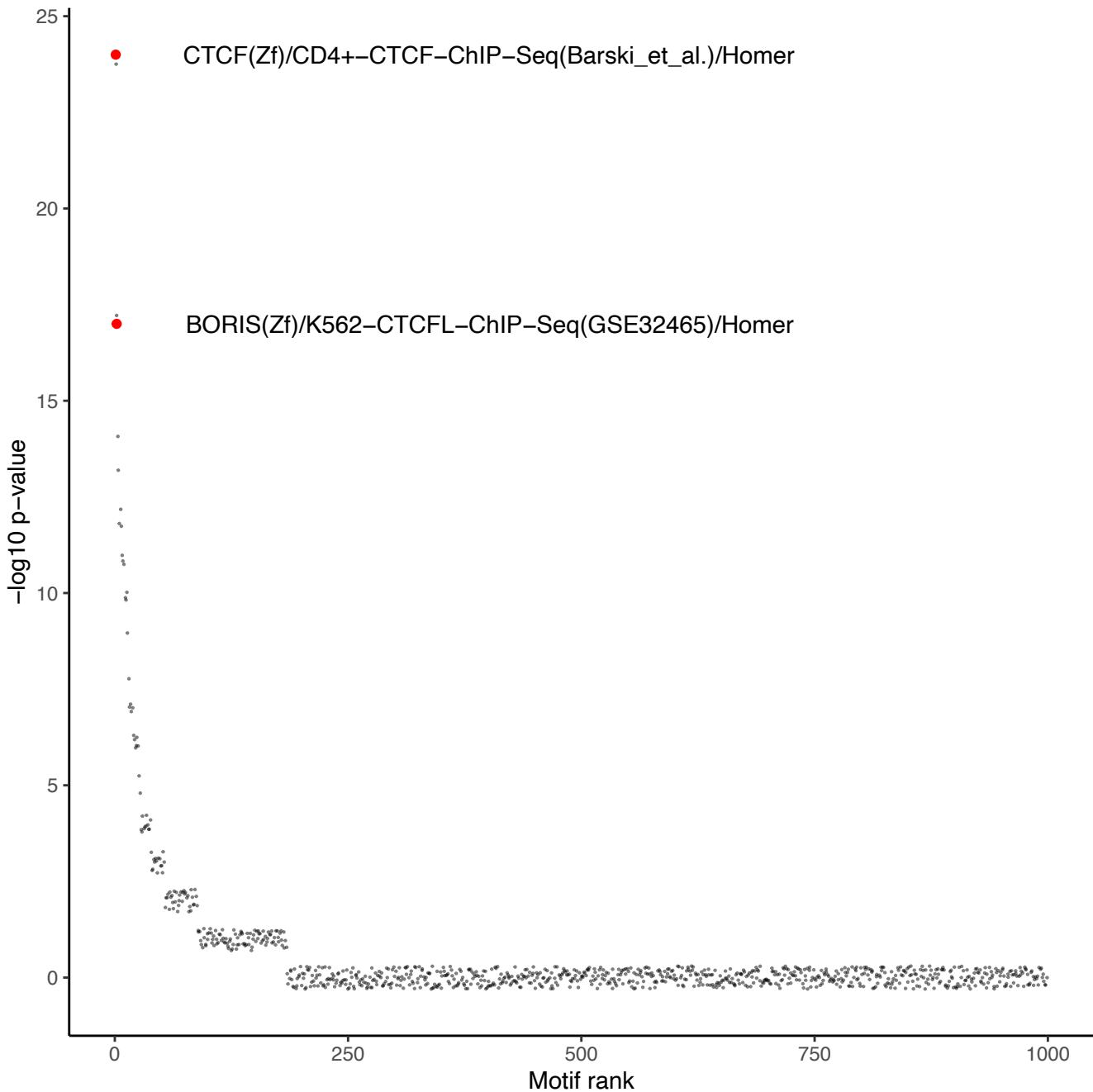
The OP9 ATAC-seq detects less peaks than the Immgen DN1 but most of them are overlap. With our dataset we might be missing some change in accessibility at certain loci.

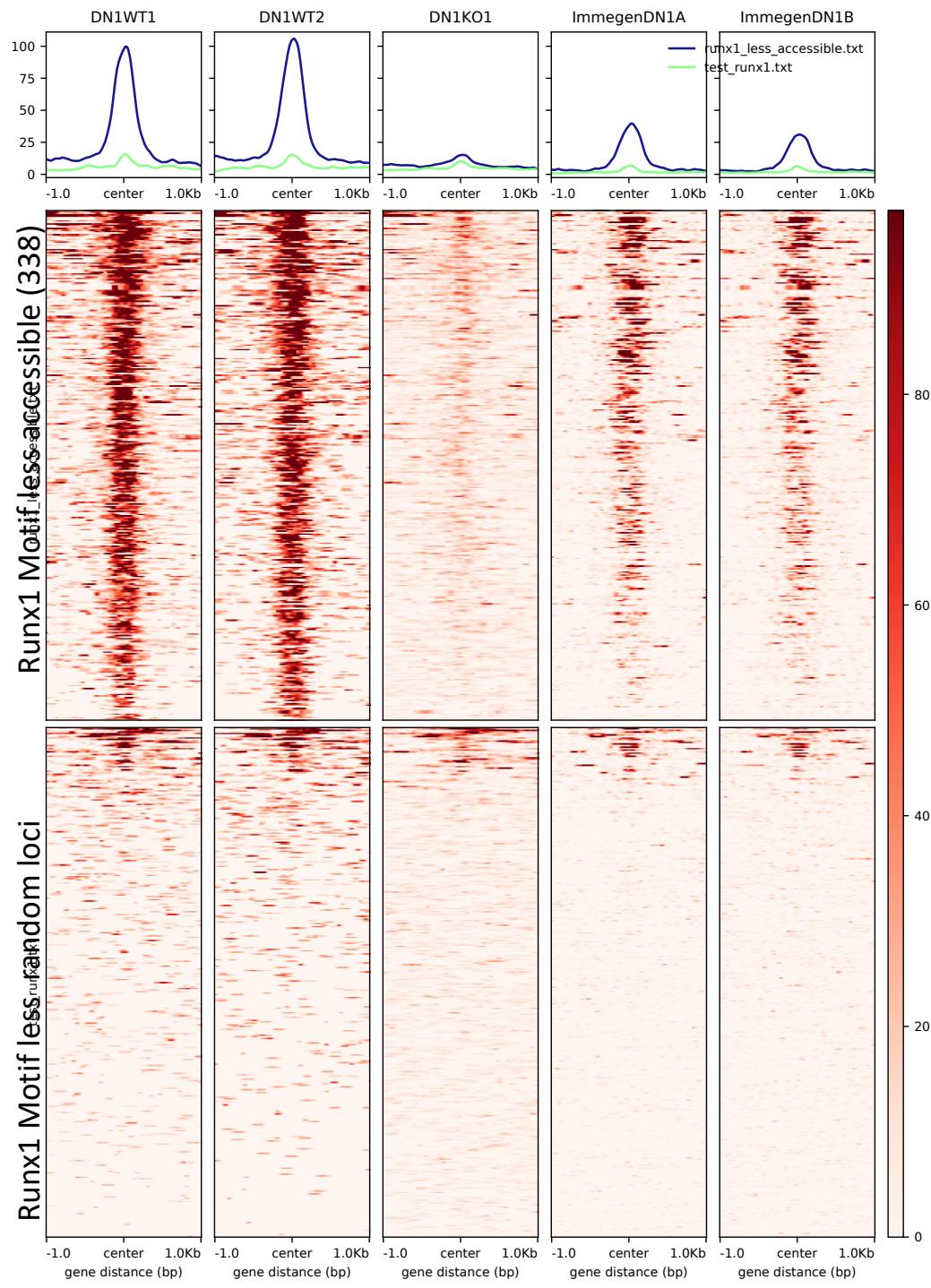


**2046 LESS accessible loci in the mutant compared to the WT**



**269 MORE accessible loci in the mutant compared to the WT**

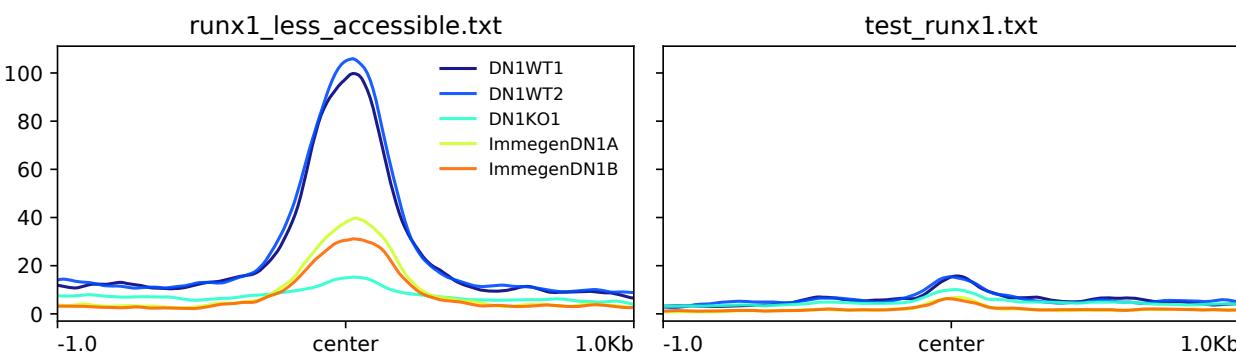




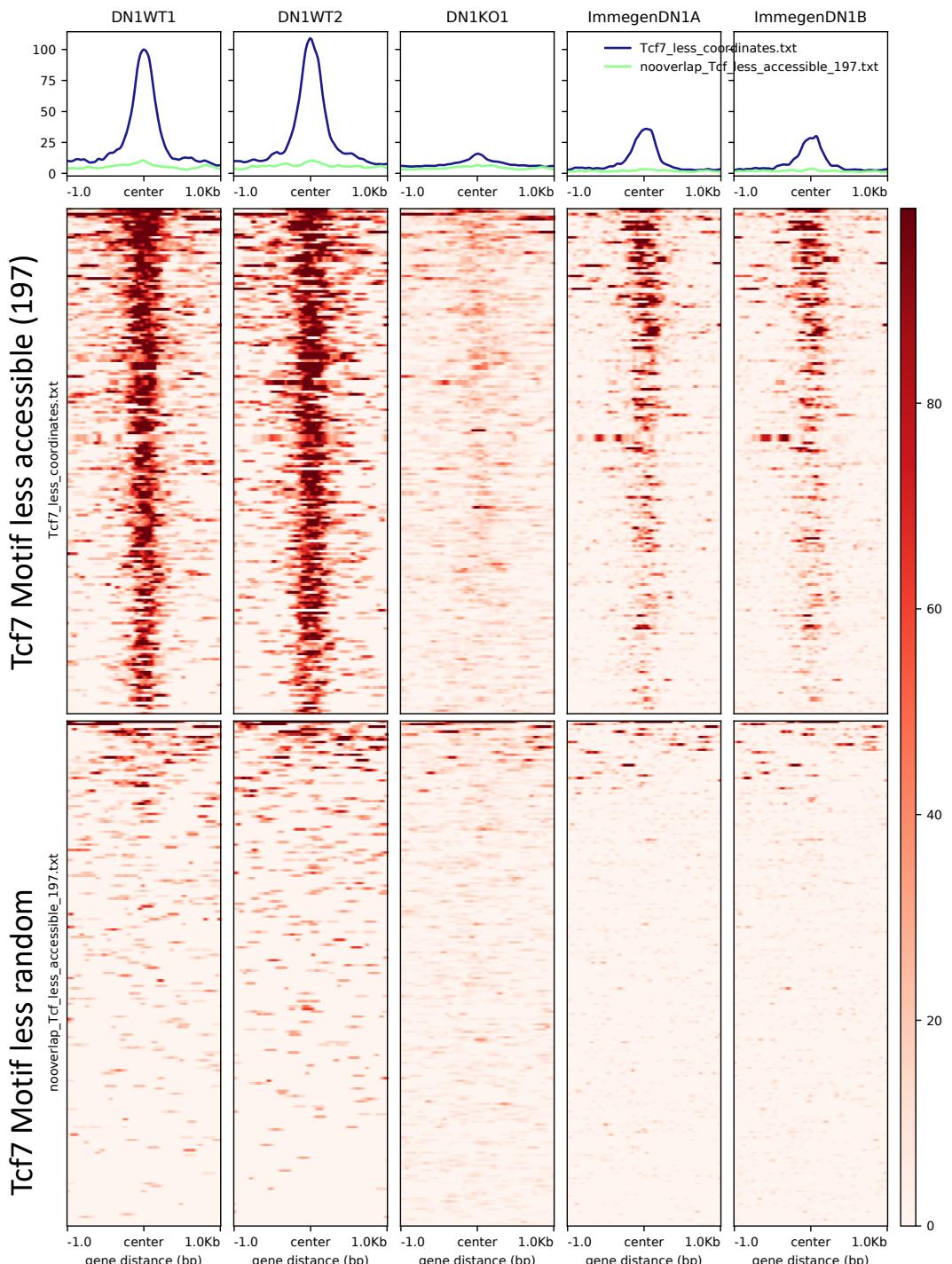
Runx1 motif: CWAACCACAR

Occurrence genome-wide :85689

Overlap with Less accessible peaks: 338 (17%)



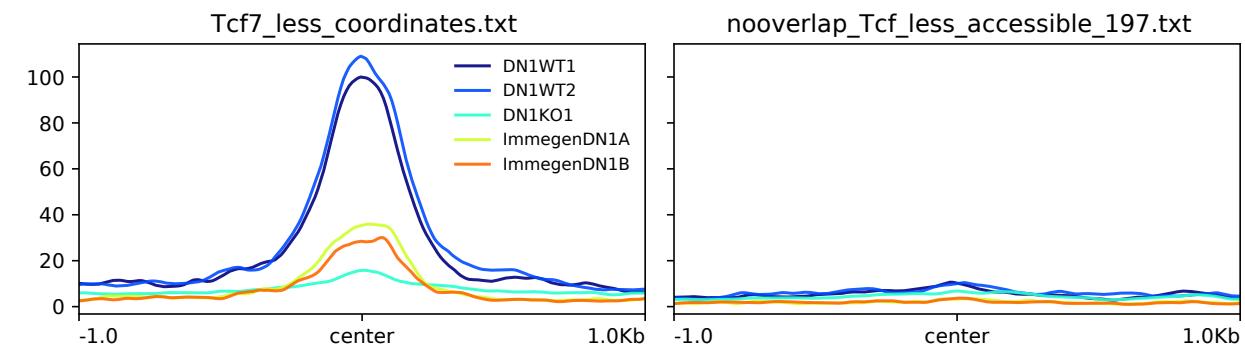
Runx1 motif are more accessible in WT than in mutant.  
Also true from Immgen



Tcf7 motif: CCACATCAAAGG

Occurrence genome-wide : 70110

Overlap with Less accessible peaks: 197 (10%)



# Summary

- Despite low coverage, we can identify accessible chromatin in OP-9 ATAC-seq
- Most peaks identified are located in promoter region
- Identification of Differentially accessible loci (DALs):
  - 2046 are less accessible in the mutant than in WT
  - 269 are more accessible in the mutant than in WT
- The motif analysis shows that potential binding sites for important TF regulators of T-cell commitment are impacted
- Are those DALs enhancers?
- To do: Differential peak analysis between WT OP9 and WT Immgen.

