Bioinformatic analysis of complex, high-throughput genomic and epigenomic data in the context of $CD4^+$ T-cell differentiation and diagnosis and treatment of transplant rejection

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Su Lab
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October 24, 2019

Organ transplants are a life-saving treatment

- 36,528 transplants performed in the USA in 2018[1]

[1] organdonor.gov

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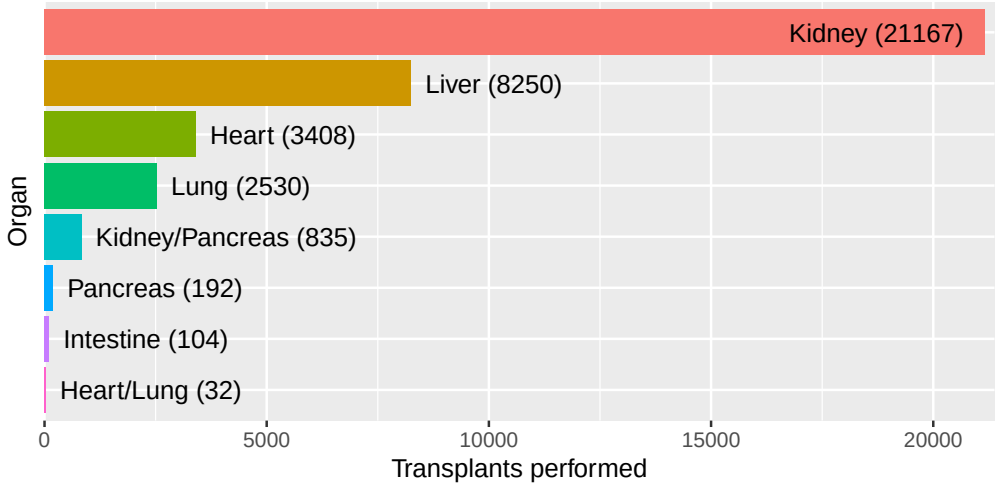
- 100 transplants every day!
- Over 113,000 people on the national transplant waiting list as of July 2019

[1] organdonor.gov

Organ donation statistics for the USA in 2018[2]



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Types of grafts

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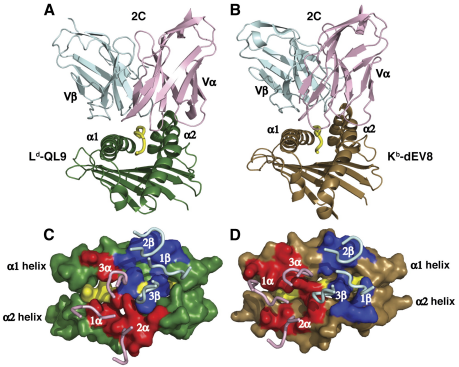
- **Xenograft:** Donor and recipient are *different species*

Recipient T-cells reject allogenic MHCs

- TCR binds to both antigen *and* MHC surface



TCR binding to self (right) and allogenic (left) MHC[3]

[3] Colf, Bankovich, et al. "How a Single T Cell Receptor Recognizes Both Self and Foreign MHC". In: Cell (2007)

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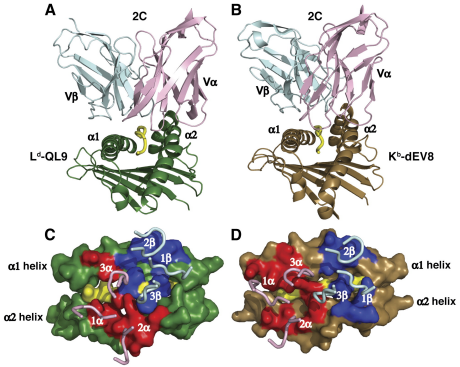

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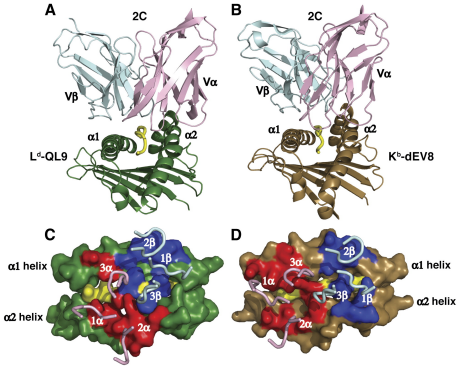
Recipient T-cells reject allogenic MHCs

- TCR binds to both antigen *and* MHC surface
- HLA genes encoding MHC proteins are highly polymorphic
- Variants in donor MHC can trigger the same T-cell response as a foreign antigen



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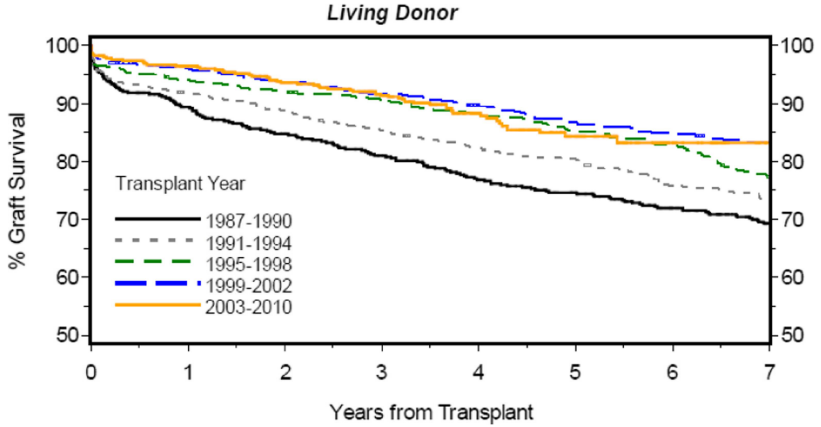
Allograft rejection is a major long-term problem



Kidney allograft survival rates in children by transplant year[4]

[4] Kim & Marks (2014)

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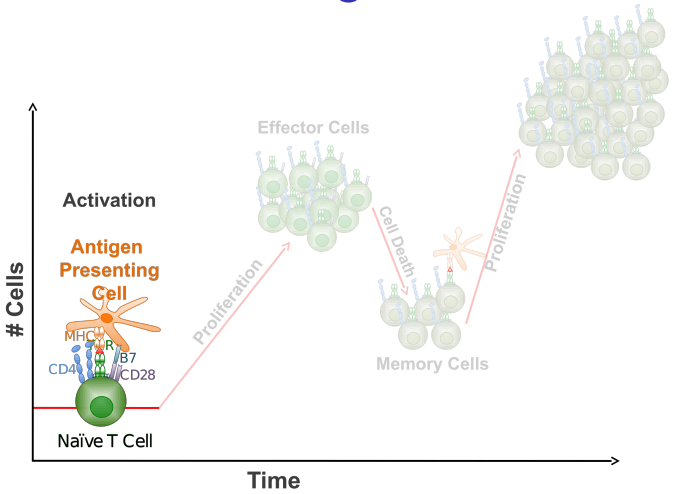
- Graft recipient must take immune suppressive drugs indefinitely
- Graft is monitored for rejection and dosage adjusted over time
- Immune suppression is a delicate balance: too much and too little are both problematic.

Memory cells: faster, stronger, and more independent



Naïve T-cell activated by APC

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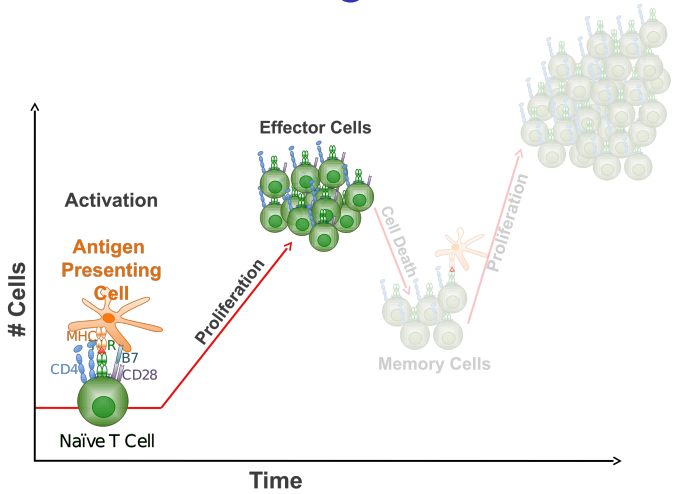

Naïve T-cell differentiates and proliferates into effector T-cells

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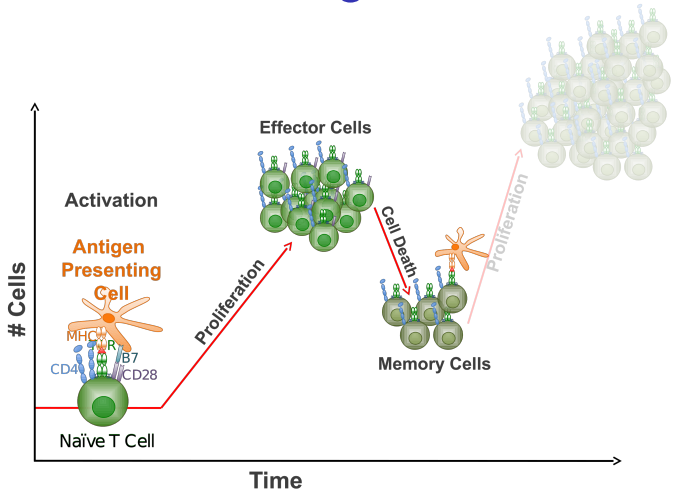

Post-infection, some effectors cells remain as memory cells

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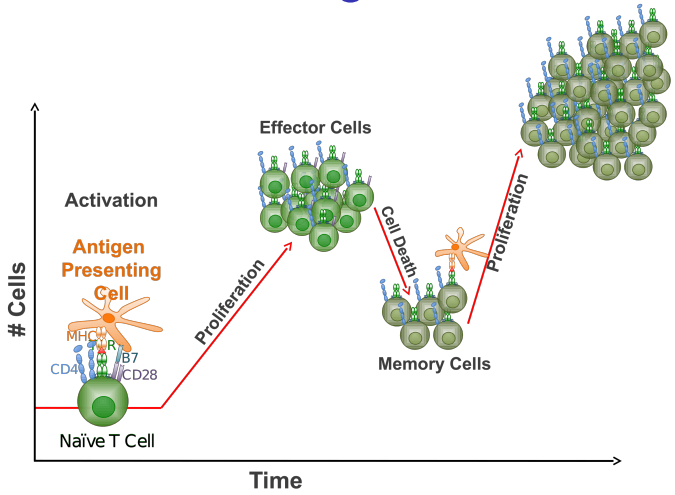

Memory T-cells respond more strongly to activation

3 problems relating to transplant rejection

1. How are memory cells different from naïve?

2. How can we diagnose rejection noninvasively?

3. How can we evaluate effects of a rejection treatment?

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Genome-wide epigenetic analysis of H3K4 and H3K27 methylation in naïve and memory $CD4^+$ T-cell activation

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Globin-blocking for more effective blood RNA-seq analysis in primate animal model for experimental graft rejection treatment

Today's focus

1. How are memory cells different from naïve?

Genome-wide epigenetic analysis of H3K4 and H3K27 methylation in naïve and memory $CD4^+$ T-cell activation

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- Cell surface markers fairly well-characterized
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Hypothesis: Epigenetic regulation of gene expression through histone modification is involved in $CD4^+$ T-cell activation and memory.

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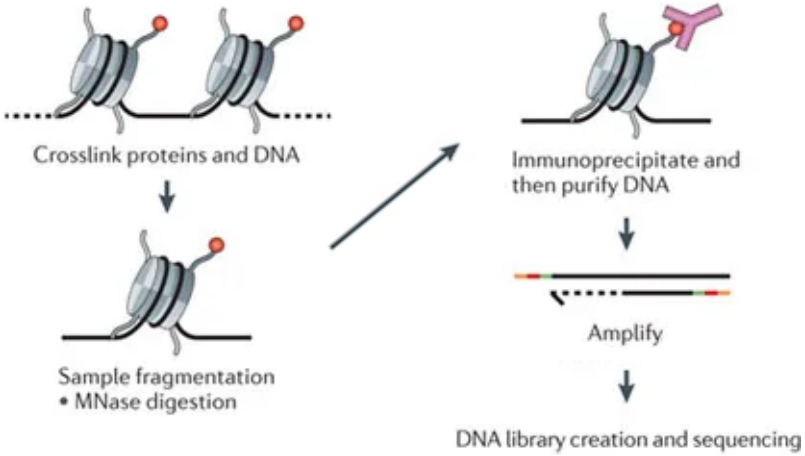
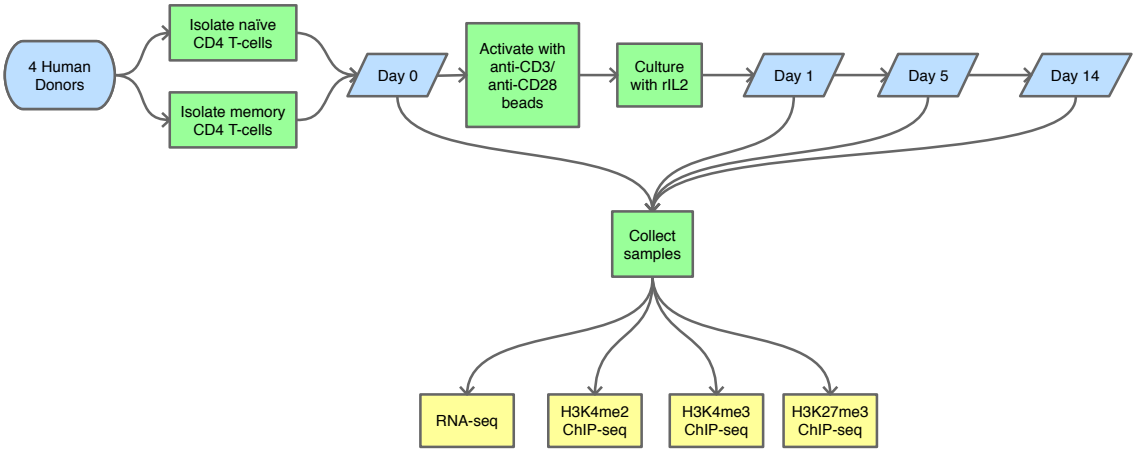
All involved in T-cell differentiation, but activation dynamics unexplored

ChIP-seq measures DNA bound to marked histones[5]



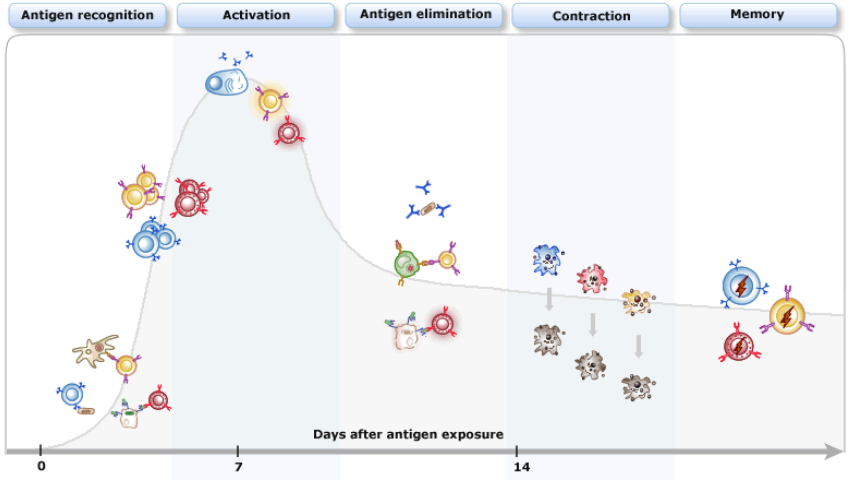
[5]Furey (2012)

Experimental design

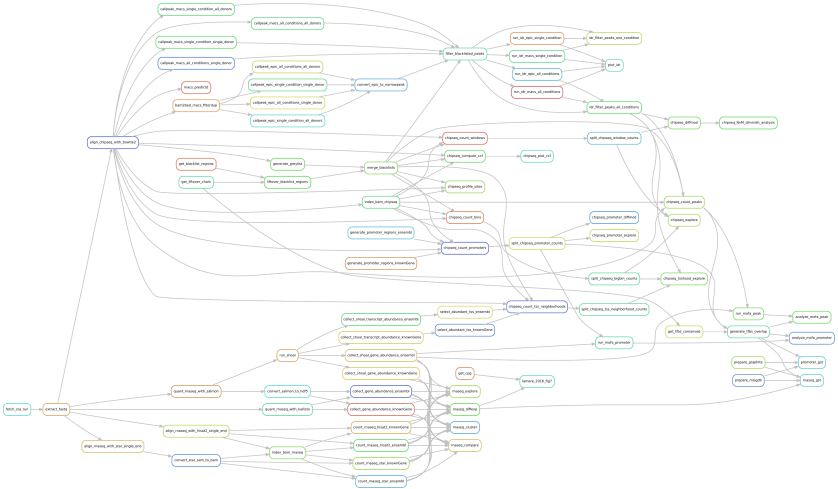


Data generated by Sarah Lamere, published in GEO as GSE73214

Time points capture phases of immune response



A few intermediate analysis steps are required



Questions to focus on

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3. What can these histone marks tell us about T-cell activation and differentiation?

First question

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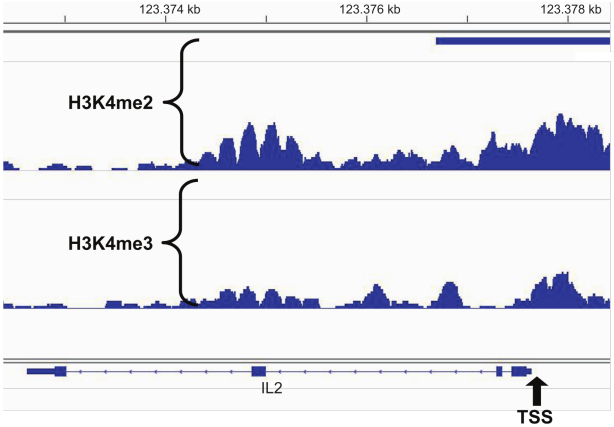
Histone modifications occur on consecutive histones



ChIP-seq coverage in IL2 gene[6]

[6]Sarah LaMere. Ph.D. thesis (2015).

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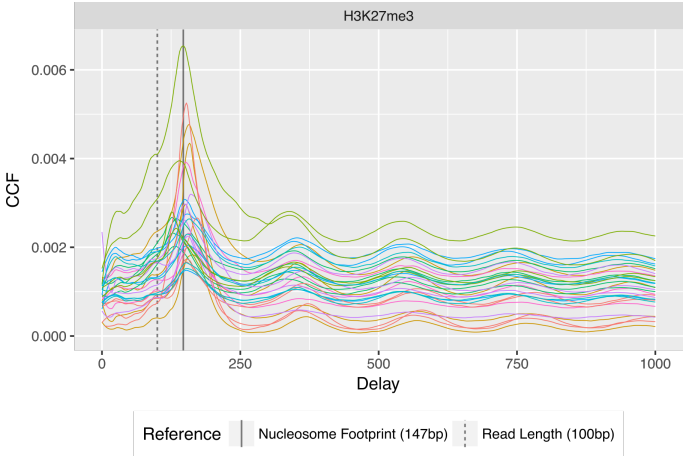

Strand cross-correlation plots show histone-sized wave pattern

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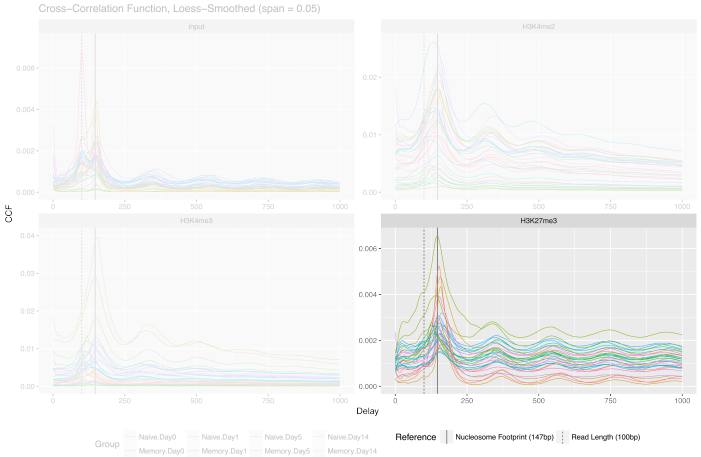

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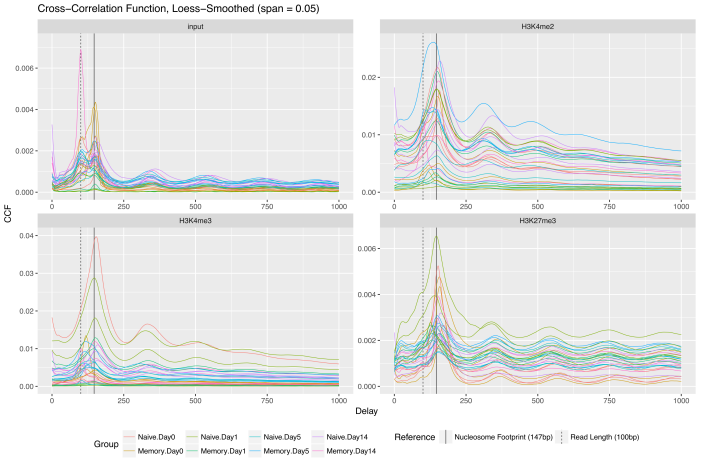

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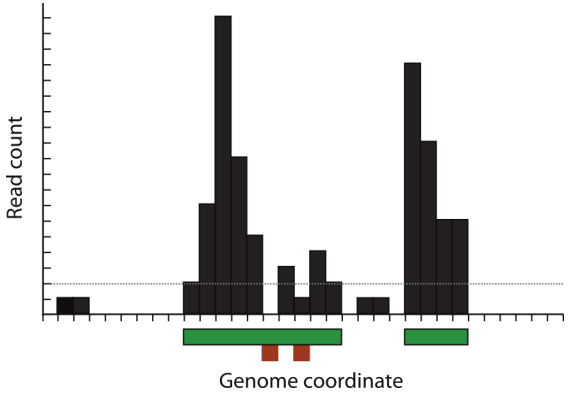
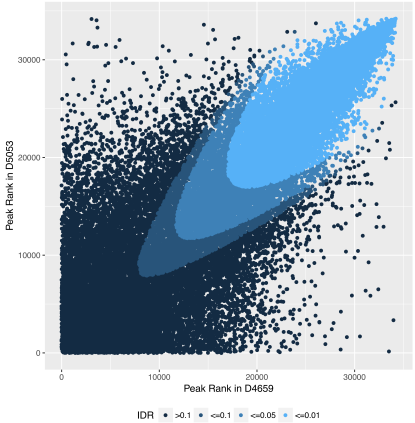
SICER identifies enriched regions across the genome



Finding "islands" of coverage with SICER[7]

[7] Zang et al. (2009)

IDR identifies *reproducible* enriched regions



Example irreproducible discovery rate[8] score consistency plot

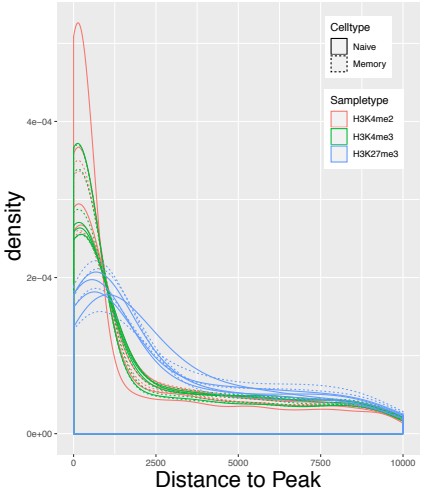
[8] Li et al. (2011)

Finding enriched regions across the genome

Histone Mark	# Peaks	Mean peak width	genome coverage	FRiP
H3K4me2	14,965	3,970	1.92%	14.2%
H3K4me3	6,163	2,946	0.588%	6.57%
H3K27me3	18,139	18,967	11.1%	22.5%

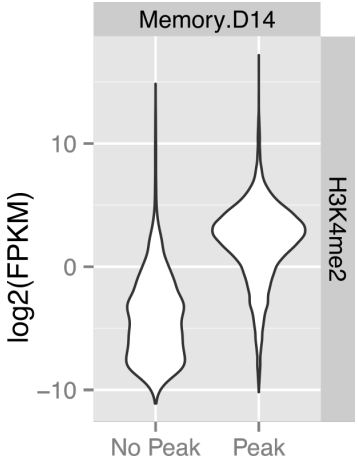
Peak-calling summary statistics

Each histone mark has an “effective promoter radius”



Enrichment of peaks near promoters

Peaks in promoters correlate with gene expression



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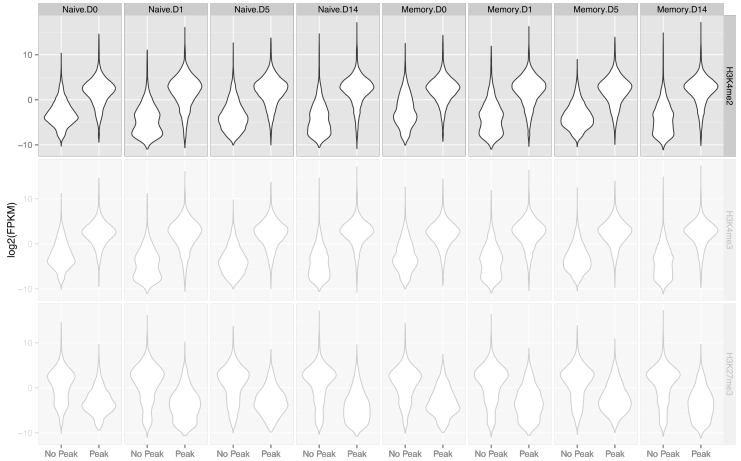

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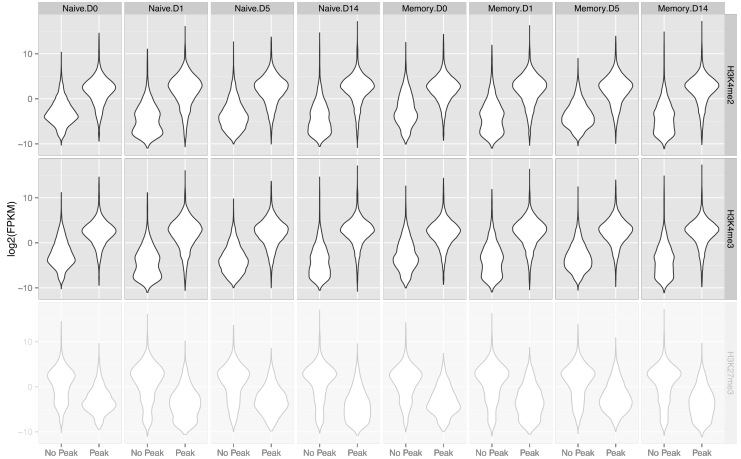

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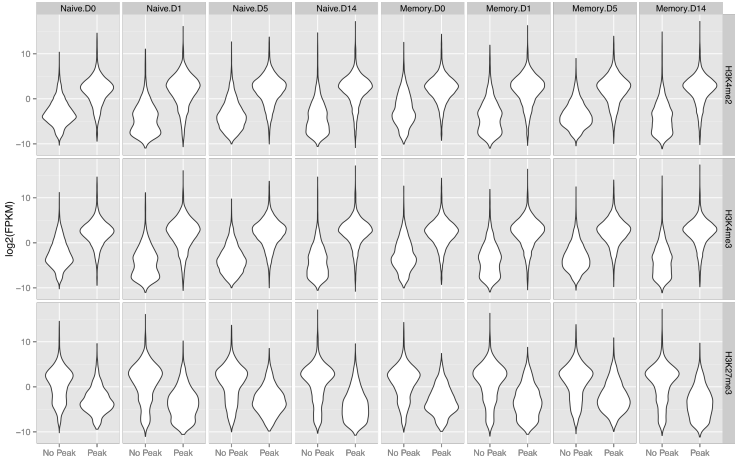


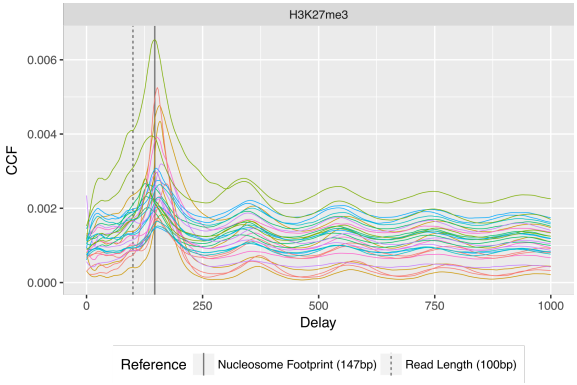
Expression distributions of genes with and without promoter peaks

First question

How do we define the “promoter region” for each gene?

Answer: Define the promoter region empirically!

- H3K4me2, H3K4me3, and H3K27me3 occur in broad regions across the genome
- Enriched regions occur more commonly near promoters
- Each histone mark has its own "effective promoter radius"
- Presence or absence of a peak within this radius is correlated with gene expression



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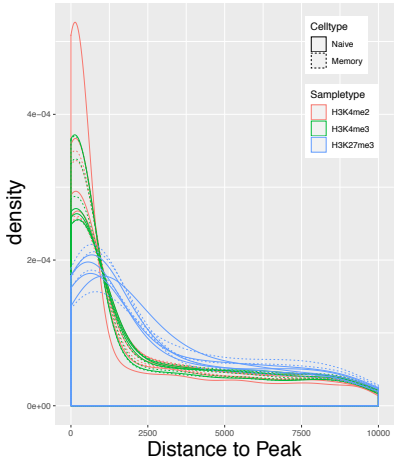

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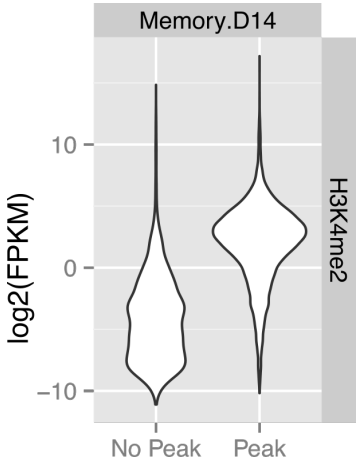
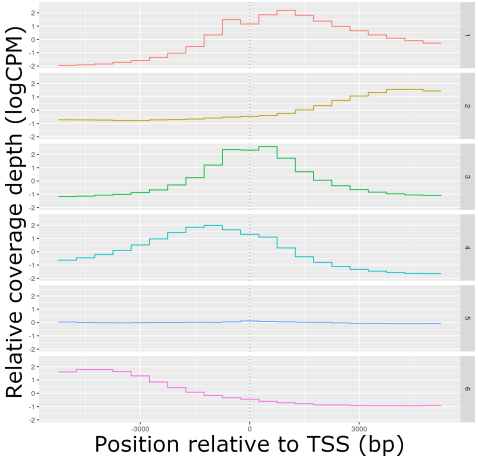


Next question

How do these histone marks behave in promoter regions?

H3K4me2 promoter neighborhood K-means clusters



Cluster means for H3K4me2

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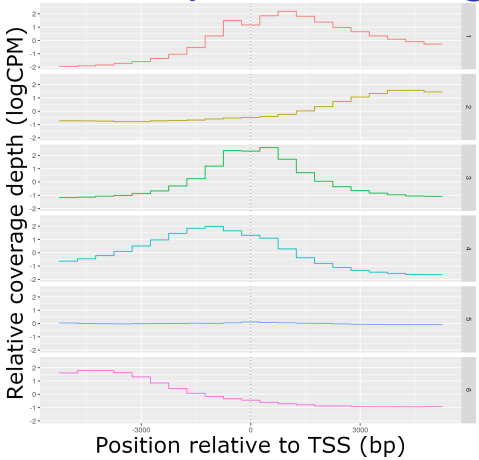
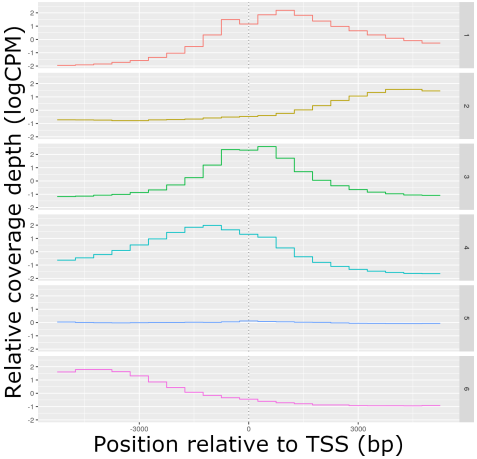


Cluster means for H3K4me2

H3K4me2 cluster PCA shows a semicircular “fan”



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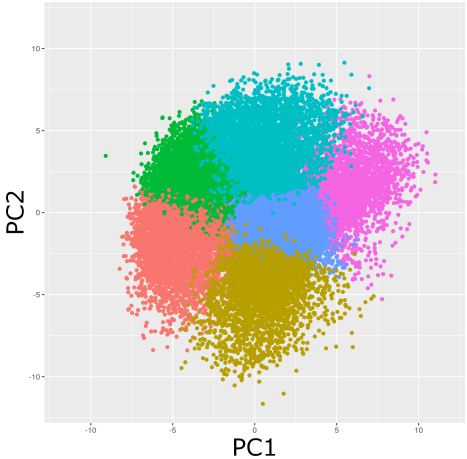
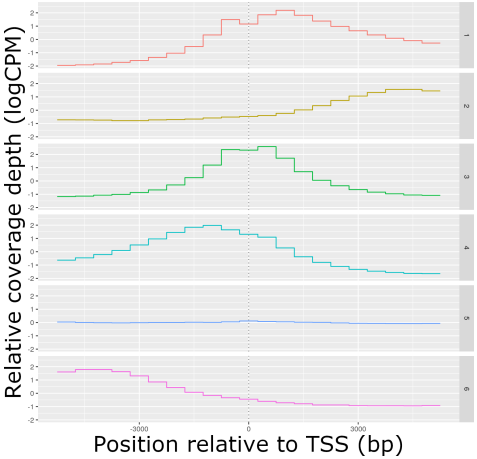


PCA plot of promoters

H3K4me2 near TSS correlates with expression



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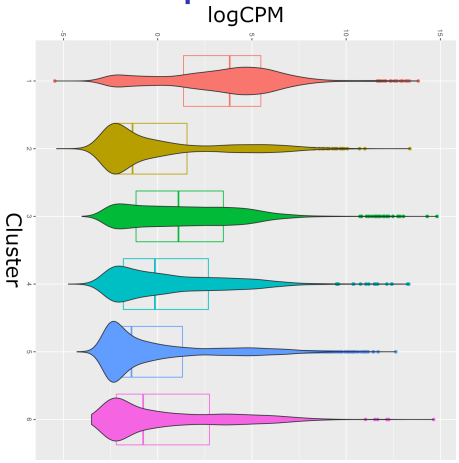
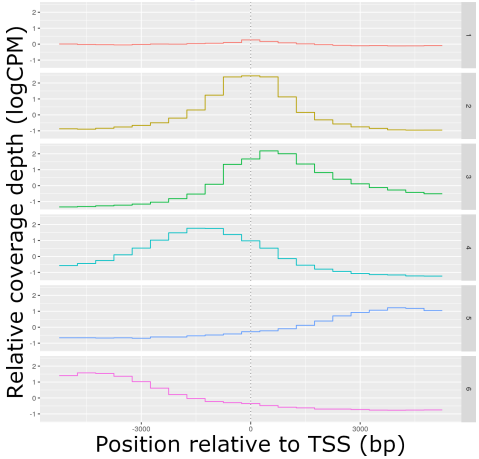


Cluster expression distributions

H3K4me3 pattern is similar to H3K4me2



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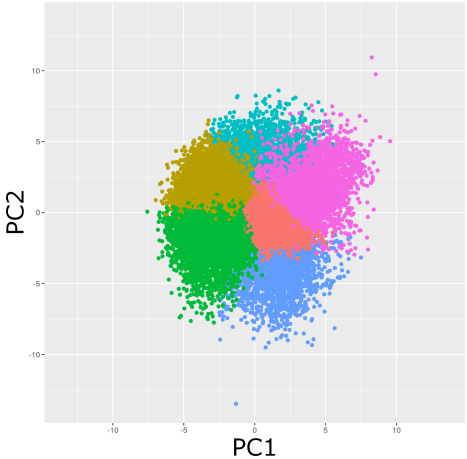

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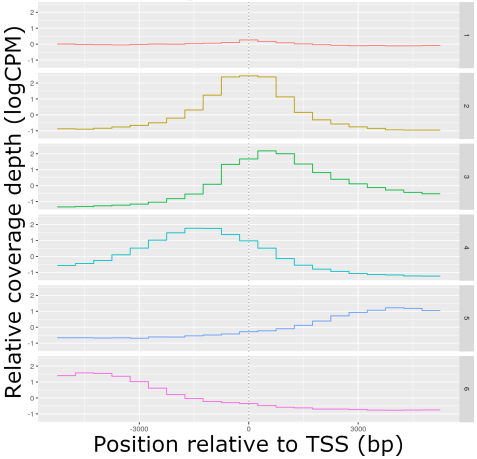
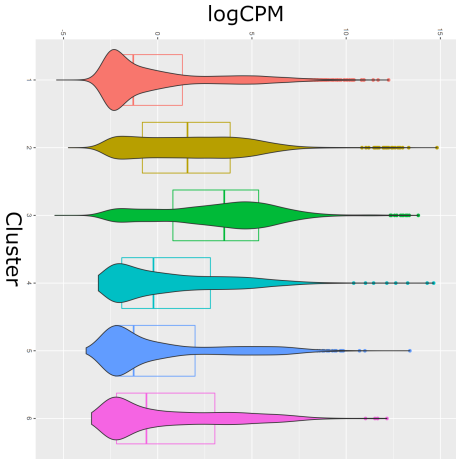
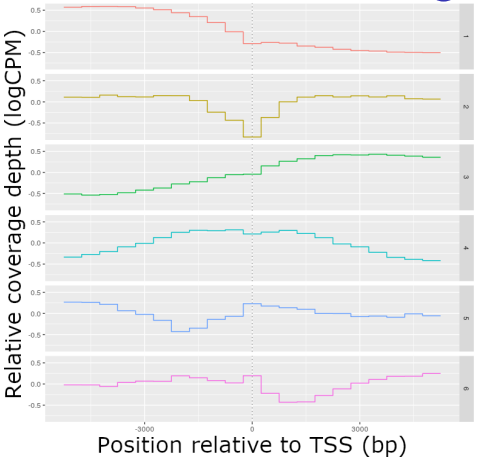


Cluster means for H3K4me3



Cluster expression distributions

H3K27me3 clusters organize into 3 opposed pairs



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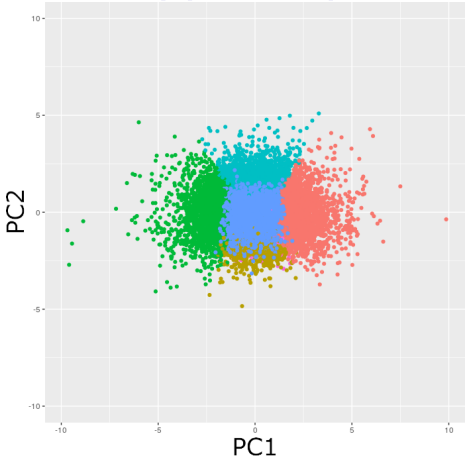
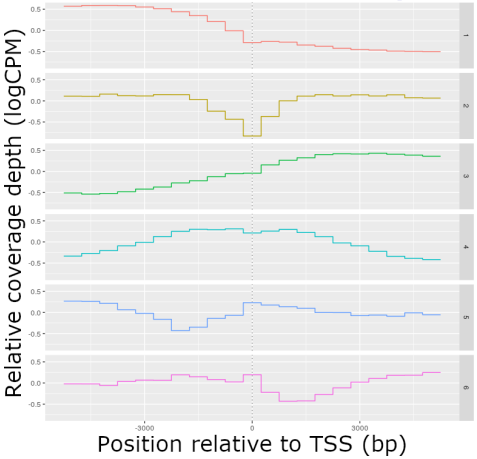
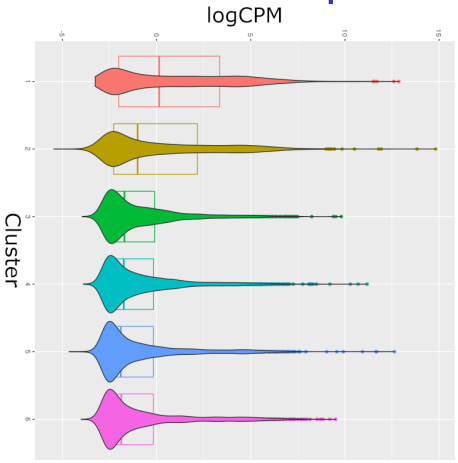


PCA plot of promoters

Specific H3K27me3 profiles show elevated expression



Cluster means for H3K27me3



Cluster expression distributions

Current question

How do these histone marks behave in promoter regions?

Answer: Presence and position both matter!

H3K4me2 & H3K4me3

- Peak closer to promoter $\Rightarrow$ higher gene expression
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H3K27me3

- Depletion of H3K27me3 at TSS $\Rightarrow$ elevated gene expression
- Enrichment of H3K27me3 upstream of TSS $\Rightarrow$ *more* elevated expression
- Other coverage profiles: no association

Last question

What can these histone marks tell us about T-cell activation and differentiation?

Differential modification disappears by Day 14

	Number of significant promoters			Est. differentially modified promoters		
Time Point	H3K4me2	H3K4me3	H3K27me3	H3K4me2	H3K4me3	H3K27me3
Day 0	4553	927	6	9967	4149	2404
Day 1	567	278	1570	4370	2145	6598
Day 5	2313	139	490	9450	1148	4141
Day 14	0	0	0	0	0	0

Differential modification between naïve and memory samples at each time point

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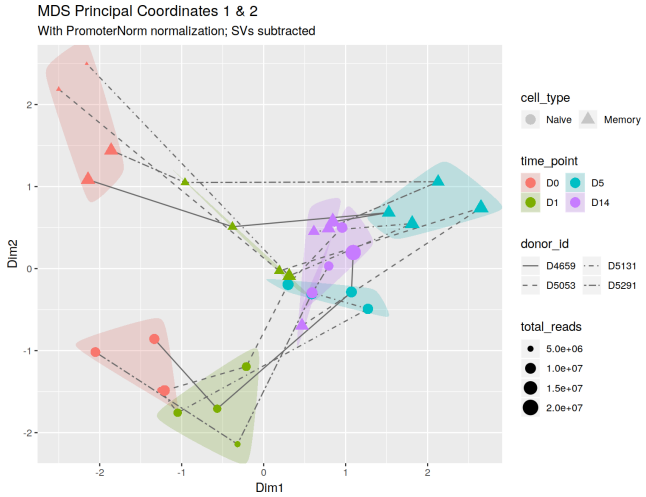

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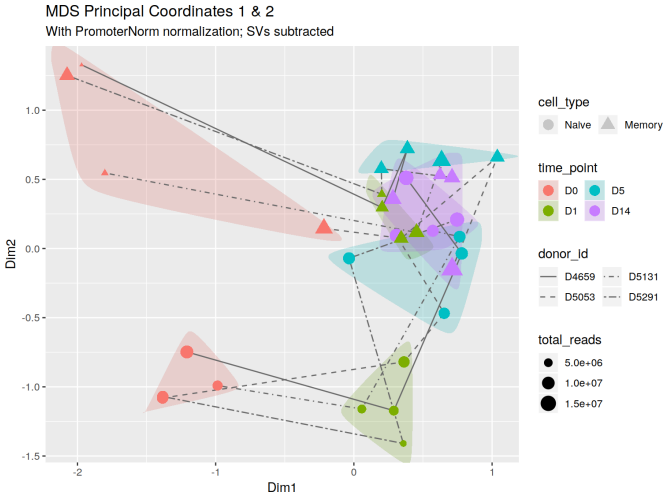
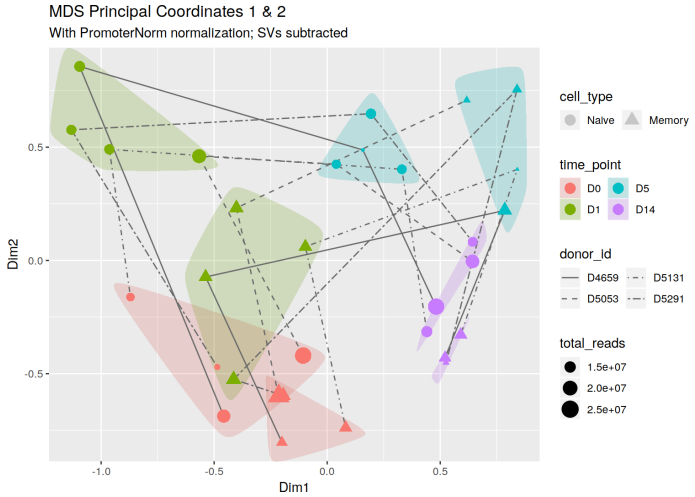
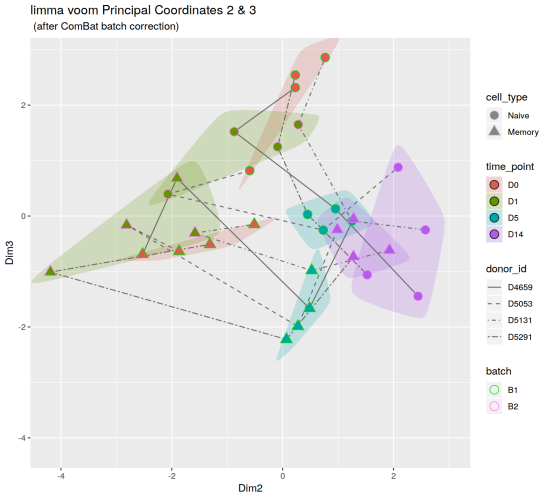


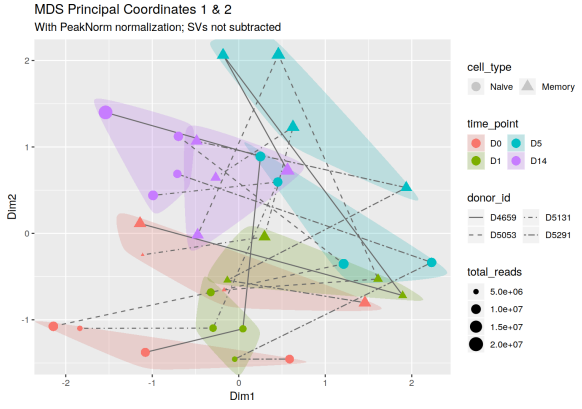
Promoter H3K27me3 levels converge at Day 14?



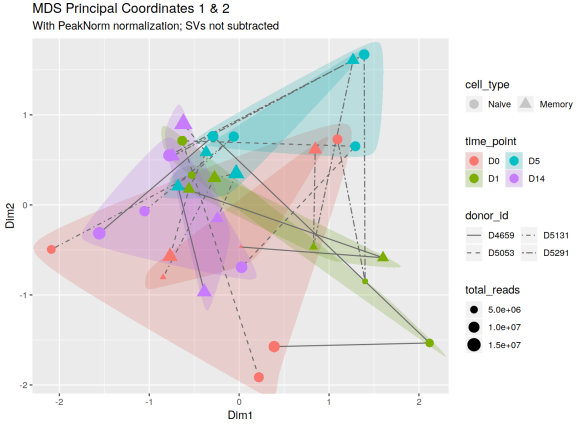
Expression converges at Day 14 (in PC 2 & 3)



But the data isn't really that clean...



H3K4me2



H3K4me3

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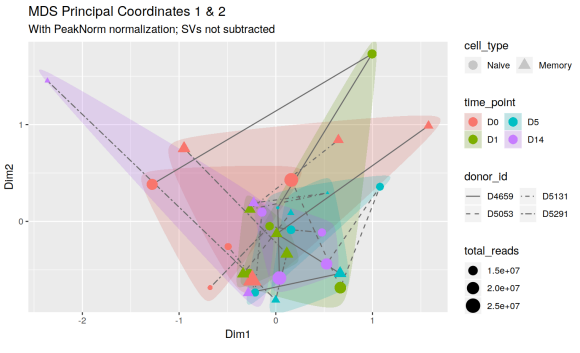
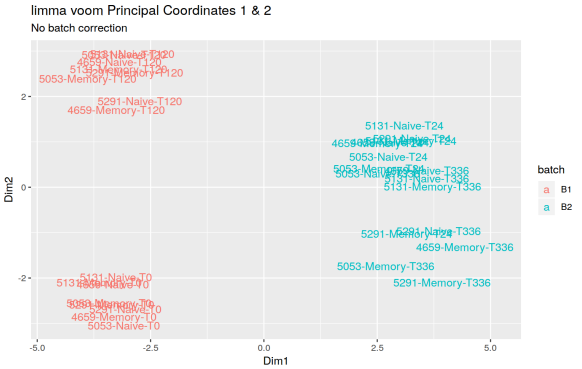
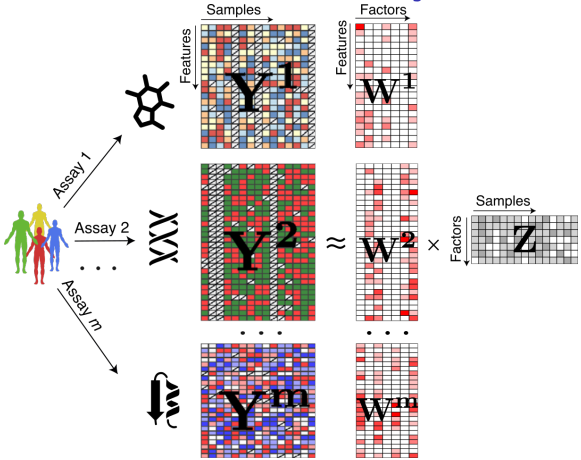


H3K27me3



RNA-seq

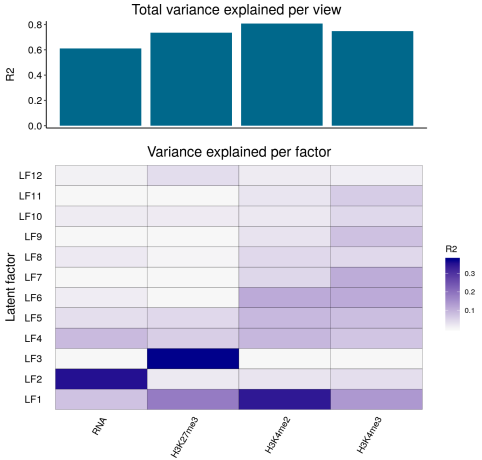
MOFA: cross-dataset factor analysis



MOFA factor analysis schematic[9]

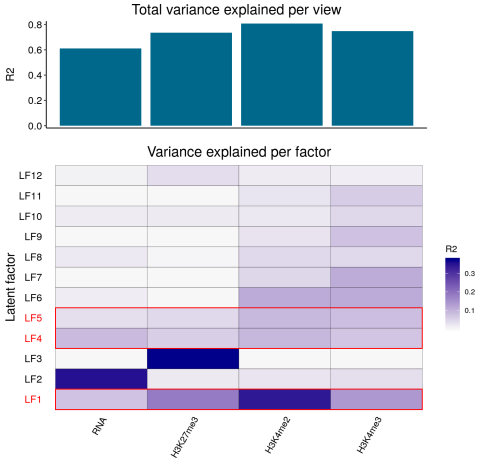
[9] Argelaguet, Velten, et. al. (2018)

Some factors are shared while others are not



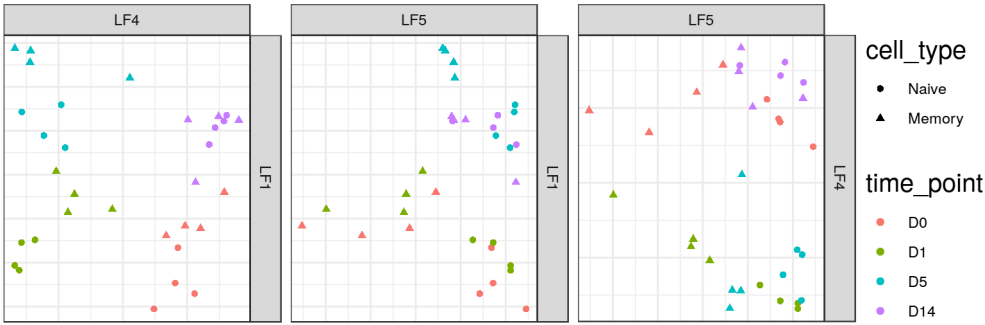
Variance explained in each data set by each LF

3 factors are shared across all 4 data sets



LFs 1, 4, and 5 explain variation in all 4 data sets

MOFA LF5 captures convergence pattern



LF1 & LF4: time point effect; LF5: convergence

Last question

What can these histone marks tell us about T-cell activation and differentiation?

Answer: Epigenetic convergence between naïve and memory!

- Almost no differential histone modification observed between naïve and memory at Day 14, despite plenty of differential modification at earlier time points.
- Expression and 3 histone marks all show "convergence" between naïve and memory by Day 14 in the first 2 or 3 principal coordinates.
- MOFA captures this convergence pattern in a single latent factor, indicating that this is a shared pattern across all 4 data sets.

Answers to key questions

How do we define the "promoter region" for each gene?

Define empirically using peak-to-promoter distances; validate by correlation with expression.

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Define empirically using peak-to-promoter distances; validate by correlation with expression.

How do these histone marks behave in promoter regions?

Location matters! Specific coverage patterns correlated with elevated expression.

What can we learn about T-cell activation and differentiation?

Epigenetic & expression state of naïve and memory converges late after activation, consistent with naïve differentiation into memory.

Further conclusions & future directions

- "Effective promoter region" is a useful concept but "radius" oversimplifies: seek a better definition
- Coverage profiles were only examined in naïve day 0 samples: further analysis could incorporate time and cell type
- Coverage profile normalization induces degeneracy: adapt a better normalization from peak callers like SICER
- Unimodal distribution of promoter coverage profiles is unexpected

Further conclusions & future directions

- Experiment was not designed to directly test the epigenetic convergence hypothesis: future experiments could include cultured but un-activated controls
- High correlation between H3K4me3 and H3K4me2 is curious given they are mutually exclusive: design experiments to determine the degree of actual co-occurrence

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- Can we stop memory cells from forming by perturbing histone methylation?
- Can we disrupt memory cell function during rejection by perturbing histone methylation?
- Can we suggest druggable targets for better immune suppression by looking at epigenetically upregulated genes in memory cells?

Acknowledgements

- My mentors, past and present: Drs. Terry Gaasterland, Daniel Salomon, and Andrew Su
- My committee: Drs. Nicholas Schork, Ali Torkamani, Michael Petrascheck, and Luc Teyton.
- My many collaborators in the Salomon Lab
- The Scripps Genomics Core
- My parents, John & Chris Thompson

Questions?