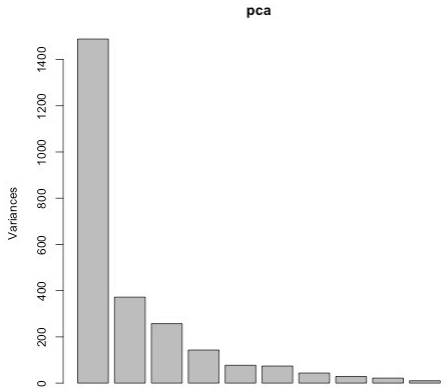
Demonstrating that Histone Modifications are Cell-Type Specific Through Clustering and Identifying Biologically Relevant Domains

Adriana Sperlea & Douglas Arneson

Methods

- PCA analysis across cell-types
- K-means clustering to identify cell type specificity of histone modifications
- Analysis of variance to identify important genomic locations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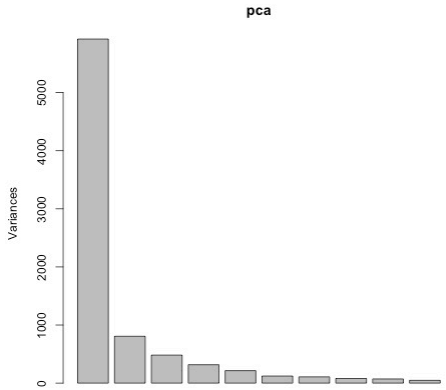
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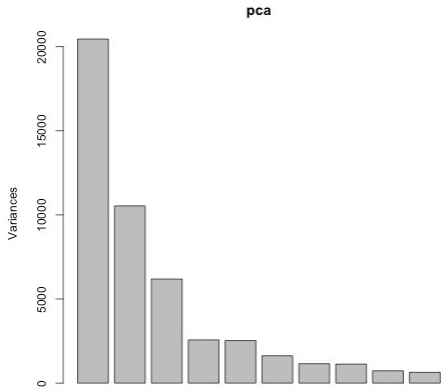
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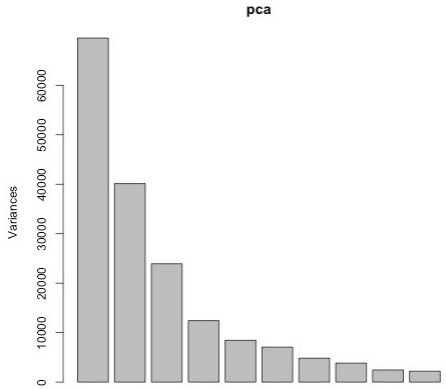
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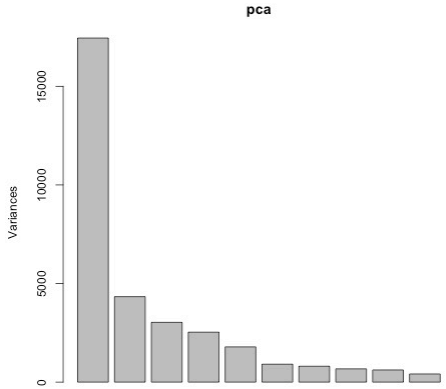
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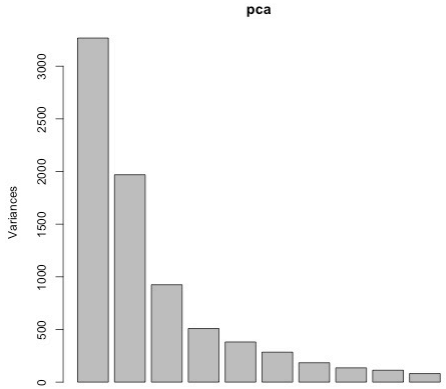
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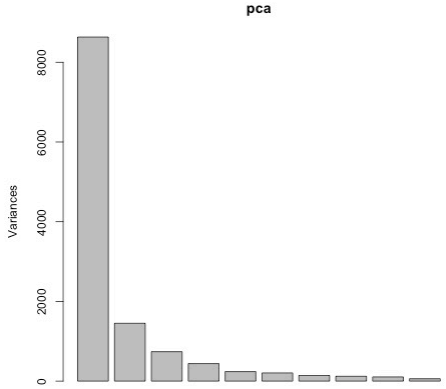
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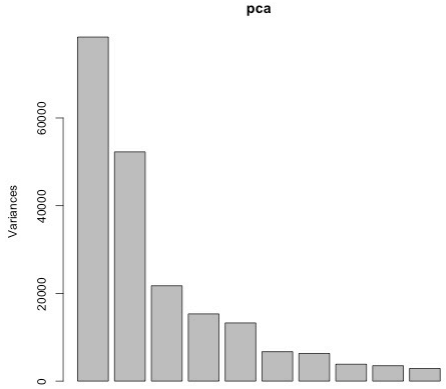
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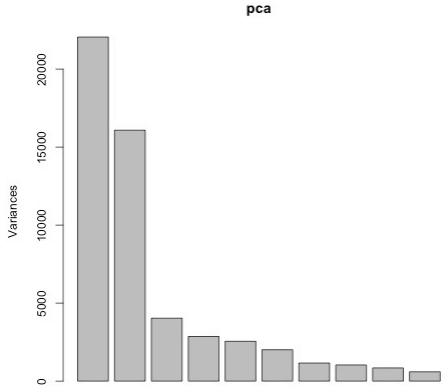
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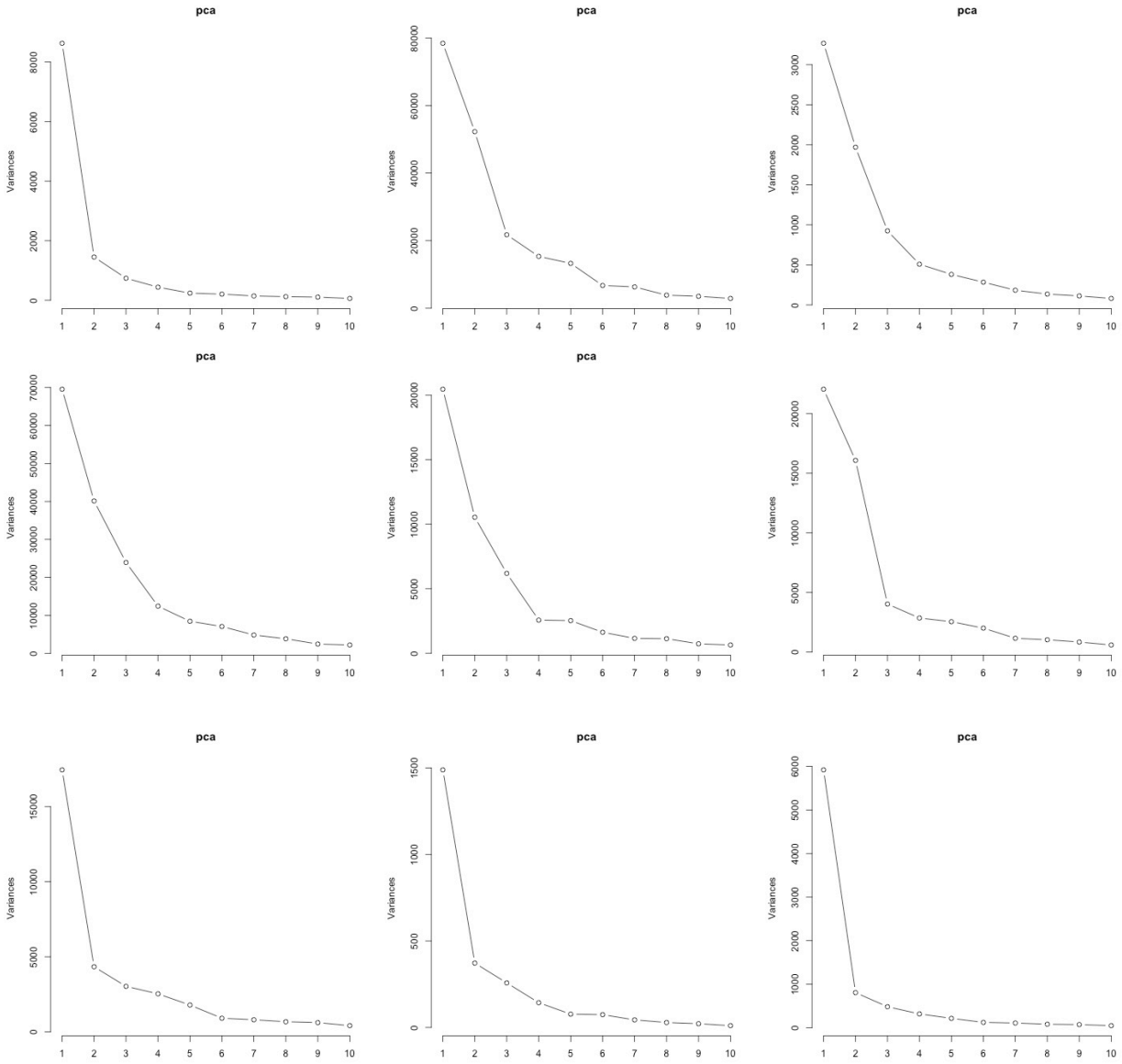
Scree Plots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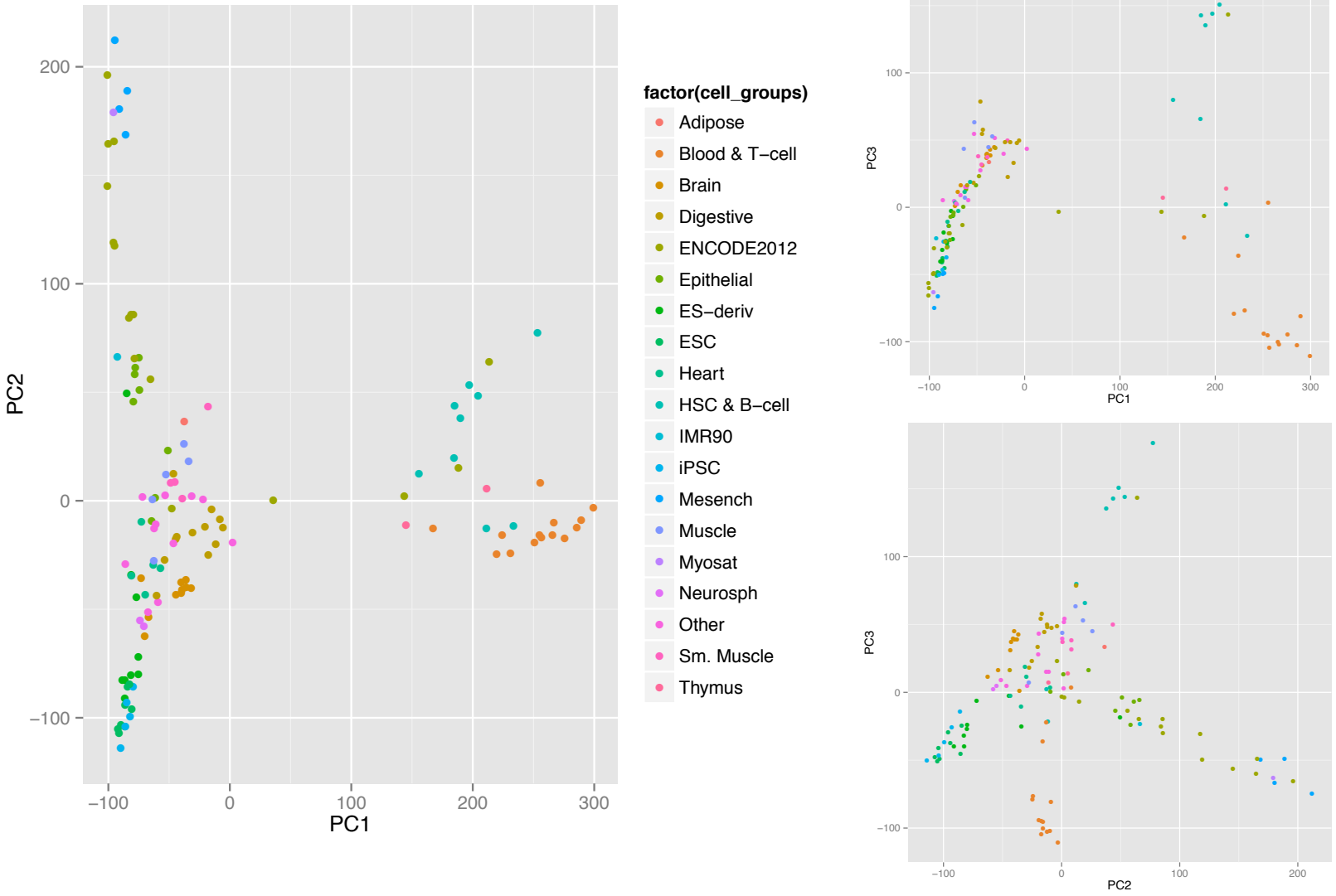
Variances

K-Means Clustering



Subset Data: Remove “Other” and “ENCODE” Cell Types

GROUP					
IMR90	Blood & T-cell	Mesench	Brain	Digestive	ENCODE2012
ESC	Blood & T-cell	Mesench	Brain	Digestive	ENCODE2012
ESC	Blood & T-cell	Mesench	Brain	Digestive	ENCODE2012
ESC	Blood & T-cell	Mesench	Brain	Digestive	ENCODE2012
ESC	Blood & T-cell	Myosat	Adipose	Digestive	ENCODE2012
ESC	Blood & T-cell	Epithelial	Muscle	Digestive	ENCODE2012
ESC	Blood & T-cell	Epithelial	Muscle	Digestive	ENCODE2012
ESC	Blood & T-cell	Epithelial	Muscle	Digestive	ENCODE2012
ESC	Blood & T-cell	Epithelial	Muscle	Other	ENCODE2012
iPSC	Blood & T-cell	Epithelial	Muscle	Other	ENCODE2012
iPSC	Blood & T-cell	Epithelial	Heart	Other	ENCODE2012
iPSC	Blood & T-cell	Epithelial	Heart	Other	ENCODE2012
iPSC	Blood & T-cell	Epithelial	Heart	Other	
iPSC	Blood & T-cell	Neurosph	Heart	Other	
ES-deriv	HSC & B-cell	Neurosph	Heart	Other	
ES-deriv	HSC & B-cell	Thymus	Sm. Muscle	Other	
ES-deriv	HSC & B-cell	Thymus	Sm. Muscle	Other	
ES-deriv	HSC & B-cell	Brain	Sm. Muscle	Other	
ES-deriv	HSC & B-cell	Brain	Sm. Muscle	Other	
ES-deriv	HSC & B-cell	Brain	Digestive	ENCODE2012	
ES-deriv	HSC & B-cell	Brain	Digestive	ENCODE2012	
ES-deriv	HSC & B-cell	Brain	Digestive	ENCODE2012	
ES-deriv	HSC & B-cell	Brain	Digestive	ENCODE2012	

Subset Data: Remove “Other” and “ENCODE” Cell Types

		GROUP		
IMR90	Blood & T-cell	Mesench	Brain	Digestive
ESC	Blood & T-cell	Mesench	Brain	Digestive
ESC	Blood & T-cell	Mesench	Brain	Digestive
ESC	Blood & T-cell	Mesench	Brain	Digestive
ESC	Blood & T-cell	Myosat	Adipose	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	
iPSC	Blood & T-cell	Epithelial	Muscle	
iPSC	Blood & T-cell	Epithelial	Heart	
iPSC	Blood & T-cell	Epithelial	Heart	
iPSC	Blood & T-cell	Epithelial	Heart	
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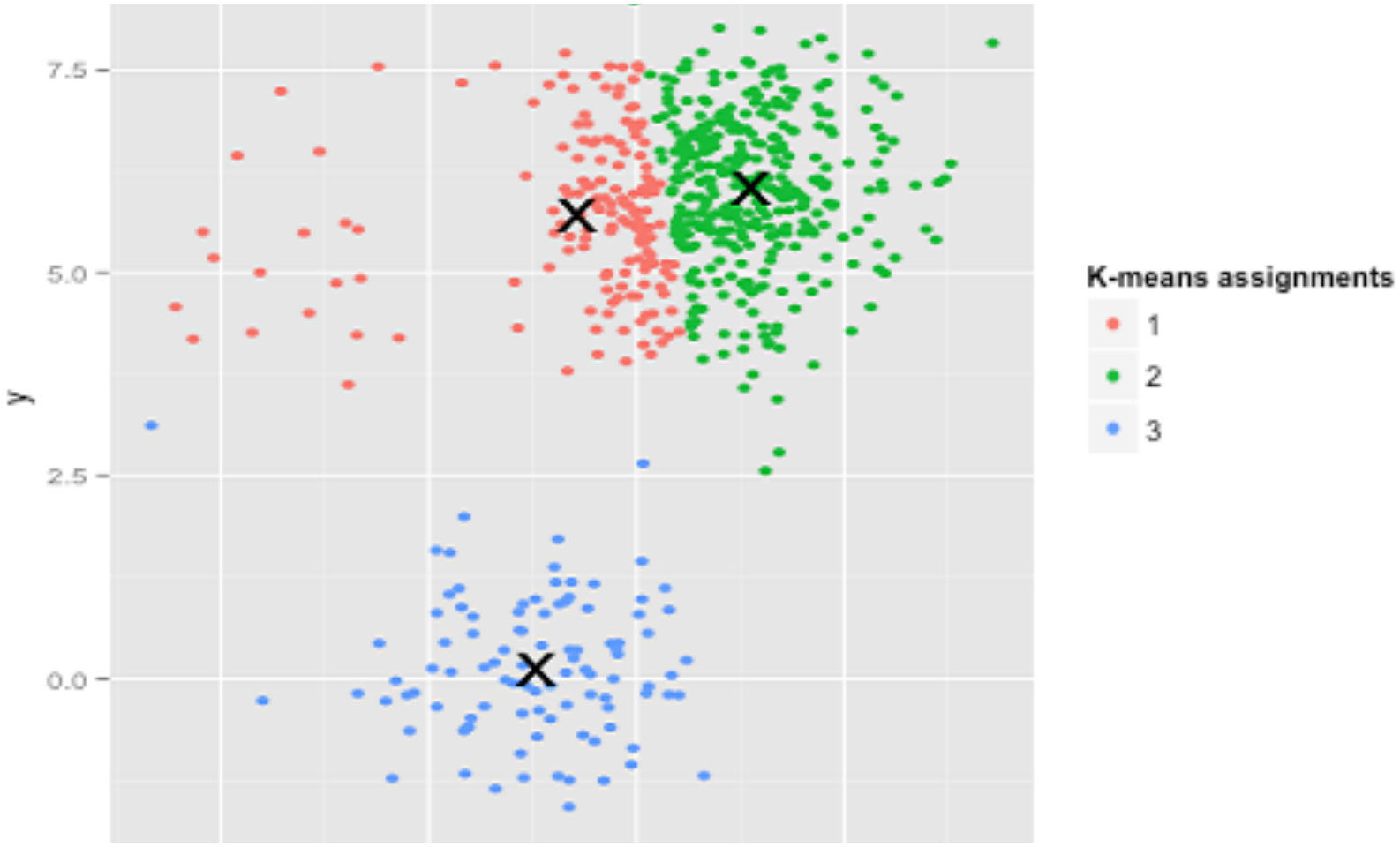
ES-deriv	HSC & B-cell	Neurosph	Heart	
ES-deriv	HSC & B-cell	Thymus	Sm. Muscle	
ES-deriv	HSC & B-cell	Thymus	Sm. Muscle	
ES-deriv	HSC & B-cell	Brain	Sm. Muscle	
ES-deriv	HSC & B-cell	Brain	Sm. Muscle	
ES-deriv	HSC & B-cell	Brain	Digestive	
ES-deriv	HSC & B-cell	Brain	Digestive	
ES-deriv	HSC & B-cell	Brain	Digestive	
ES-deriv	HSC & B-cell	Brain	Digestive	

Problem with K-means Algorithm

$$J = \sum_{j=1}^{k} \sum_{i=1}^{n} \left\| x_i^{(j)} - c_j \right\|^2$$

- K-means attempts to minimize the objective function J
- The term inside the summations gives the distance measure between the chosen point and the cluster center
- An issue with this algorithm is by minimizing the sum of squares within clusters, more weight is given to larger clusters

Problem with K means Algorithm



http://stats.stackexchange.com/questions/133656/how-to-understand-the-drawbacks-of-k-means

Further Subset Data: Remove Cell Types with Less than Five Entries

		GROUP		
IMR90	Blood & T-cell	Mesench	Brain	Digestive
ESC	Blood & T-cell	Mesench	Brain	Digestive
ESC	Blood & T-cell	Mesench	Brain	Digestive
ESC	Blood & T-cell	Mesench	Brain	Digestive
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ESC	Blood & T-cell	Epithelial	Muscle	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	
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ES-deriv	HSC & B-cell	Thymus	Sm. Muscle	
ES-deriv	HSC & B-cell	Thymus	Sm. Muscle	
ES-deriv	HSC & B-cell	Brain	Sm. Muscle	
ES-deriv	HSC & B-cell	Brain	Sm. Muscle	
ES-deriv	HSC & B-cell	Brain	Digestive	
ES-deriv	HSC & B-cell	Brain	Digestive	
ES-deriv	HSC & B-cell	Brain	Digestive	
ES-deriv	HSC & B-cell	Brain	Digestive	

Further Subset Data: Remove Cell Types with Less than Five Entries

	GROUP			
	Blood & T-cell		Brain	Digestive
ESC	Blood & T-cell		Brain	Digestive
ESC	Blood & T-cell		Brain	Digestive
ESC	Blood & T-cell		Brain	Digestive
ESC	Blood & T-cell			Digestive
ESC	Blood & T-cell	Epithelial	Muscle	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	Digestive
ESC	Blood & T-cell	Epithelial	Muscle	
iPSC	Blood & T-cell	Epithelial	Muscle	
iPSC	Blood & T-cell	Epithelial	Heart	
iPSC	Blood & T-cell	Epithelial	Heart	
iPSC	Blood & T-cell	Epithelial	Heart	
iPSC	Blood & T-cell		Heart	
ES-deriv	HSC & B-cell		Heart	
ES-deriv	HSC & B-cell			
ES-deriv	HSC & B-cell			
ES-deriv	HSC & B-cell	Brain		
ES-deriv	HSC & B-cell	Brain		
ES-deriv	HSC & B-cell	Brain	Digestive	
ES-deriv	HSC & B-cell	Brain	Digestive	
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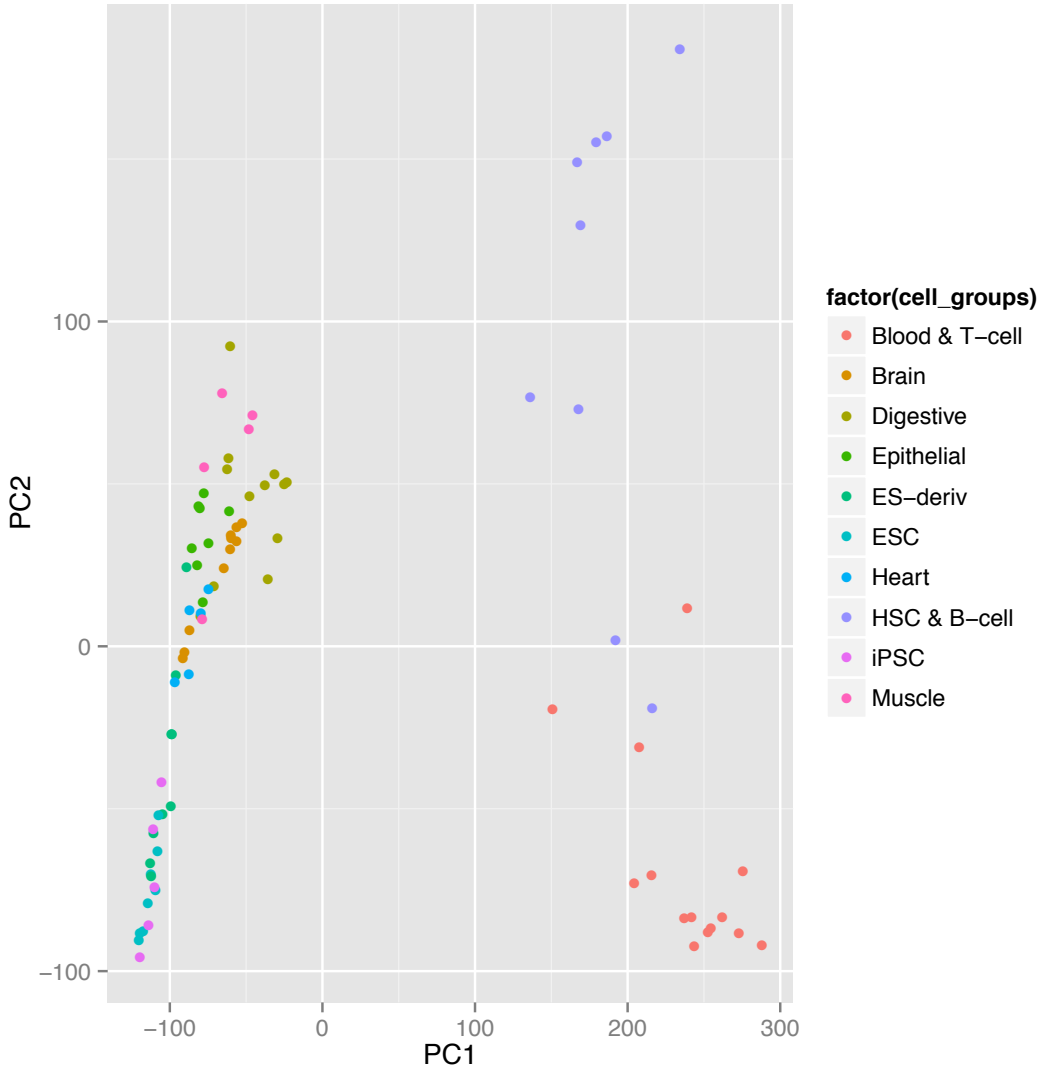
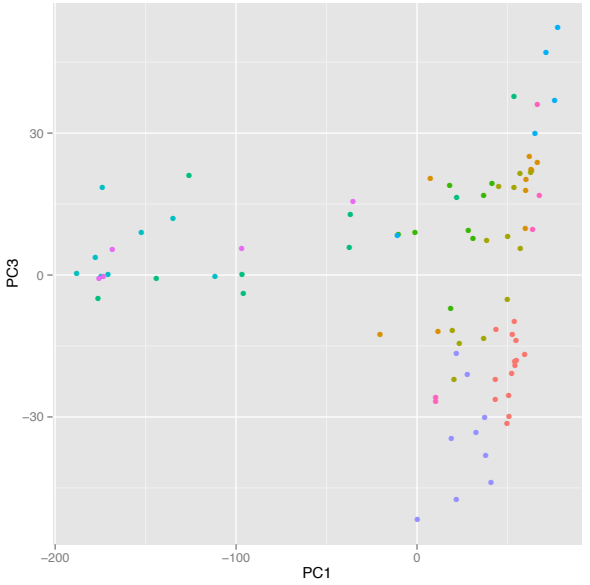
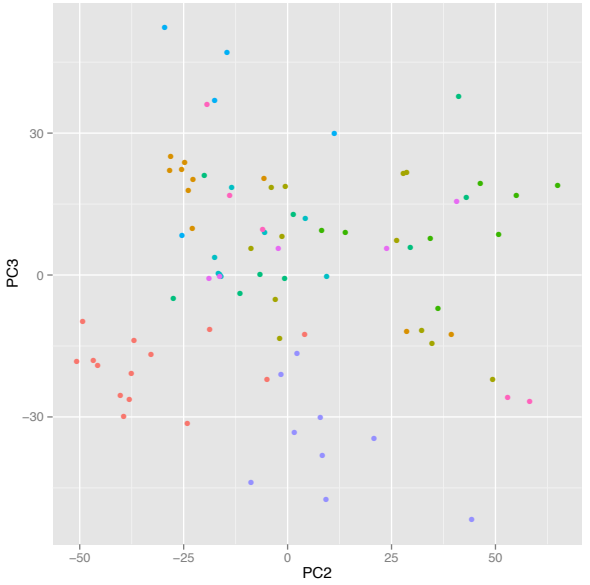
ES-deriv	HSC & B-cell	Brain	Digestive	

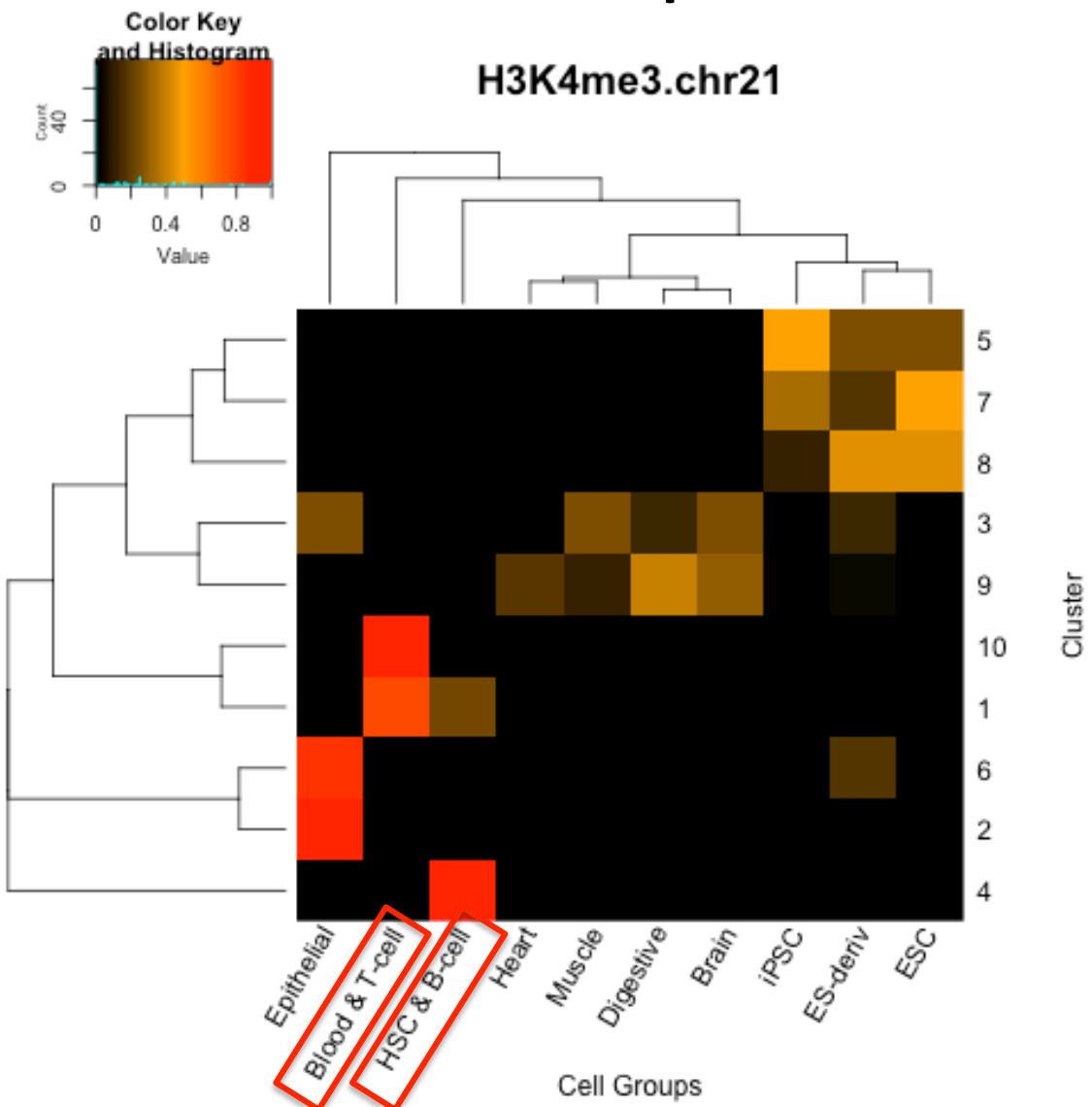
K-Means Clustering: Refined



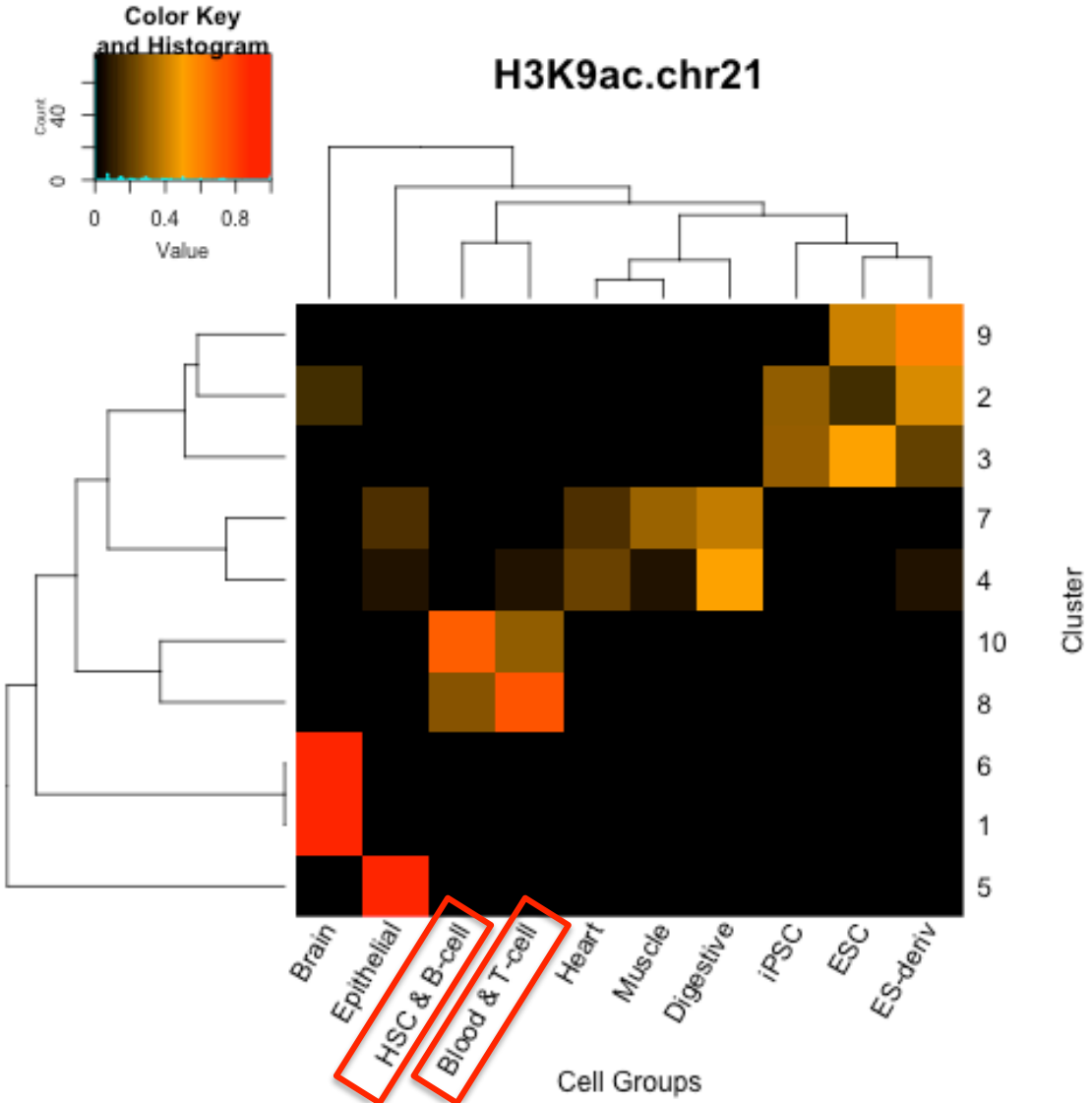




Visualizing Clustering Efficacy: Heatmaps



Visualizing Clustering Efficacy: Heatmaps



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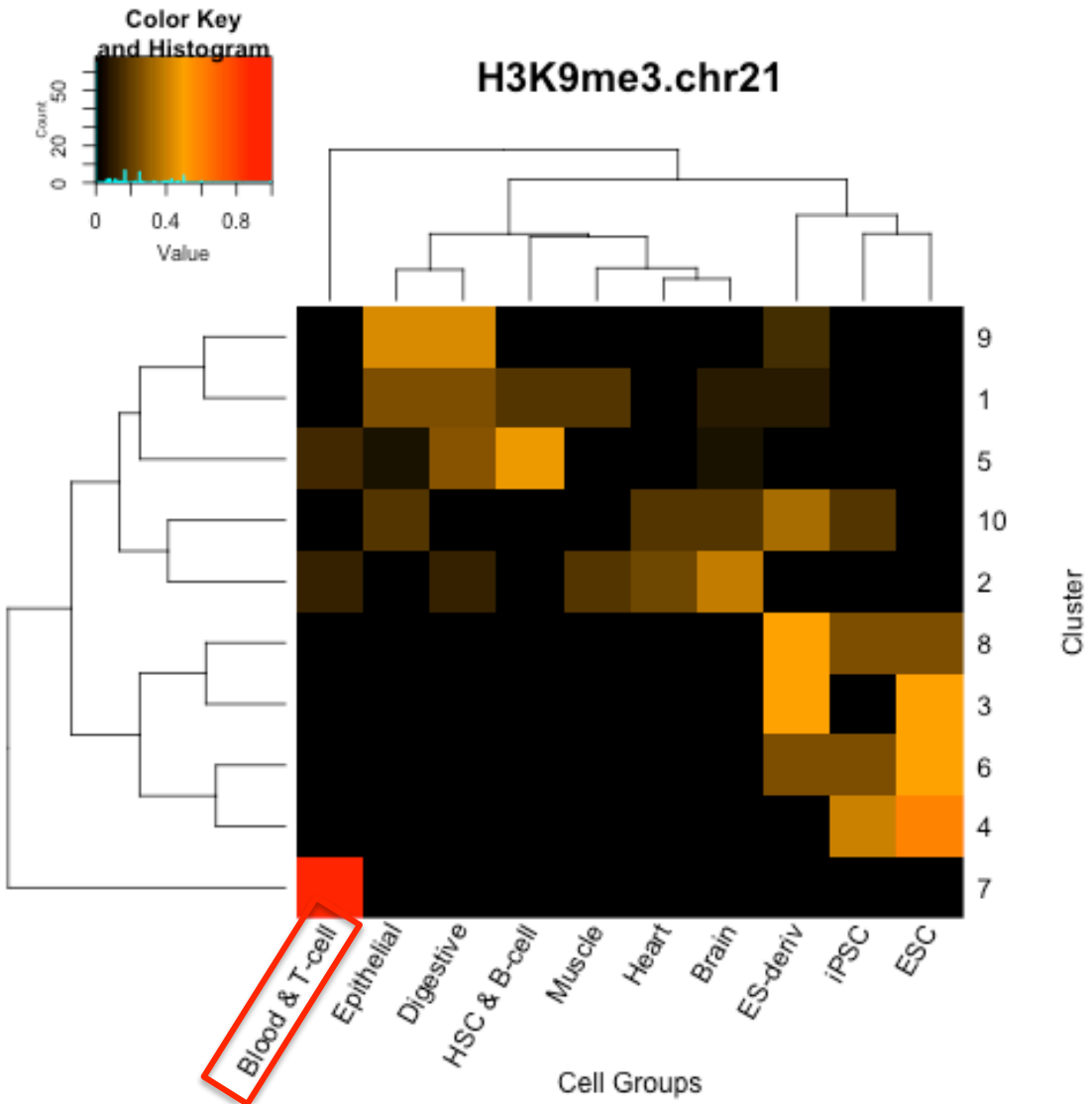

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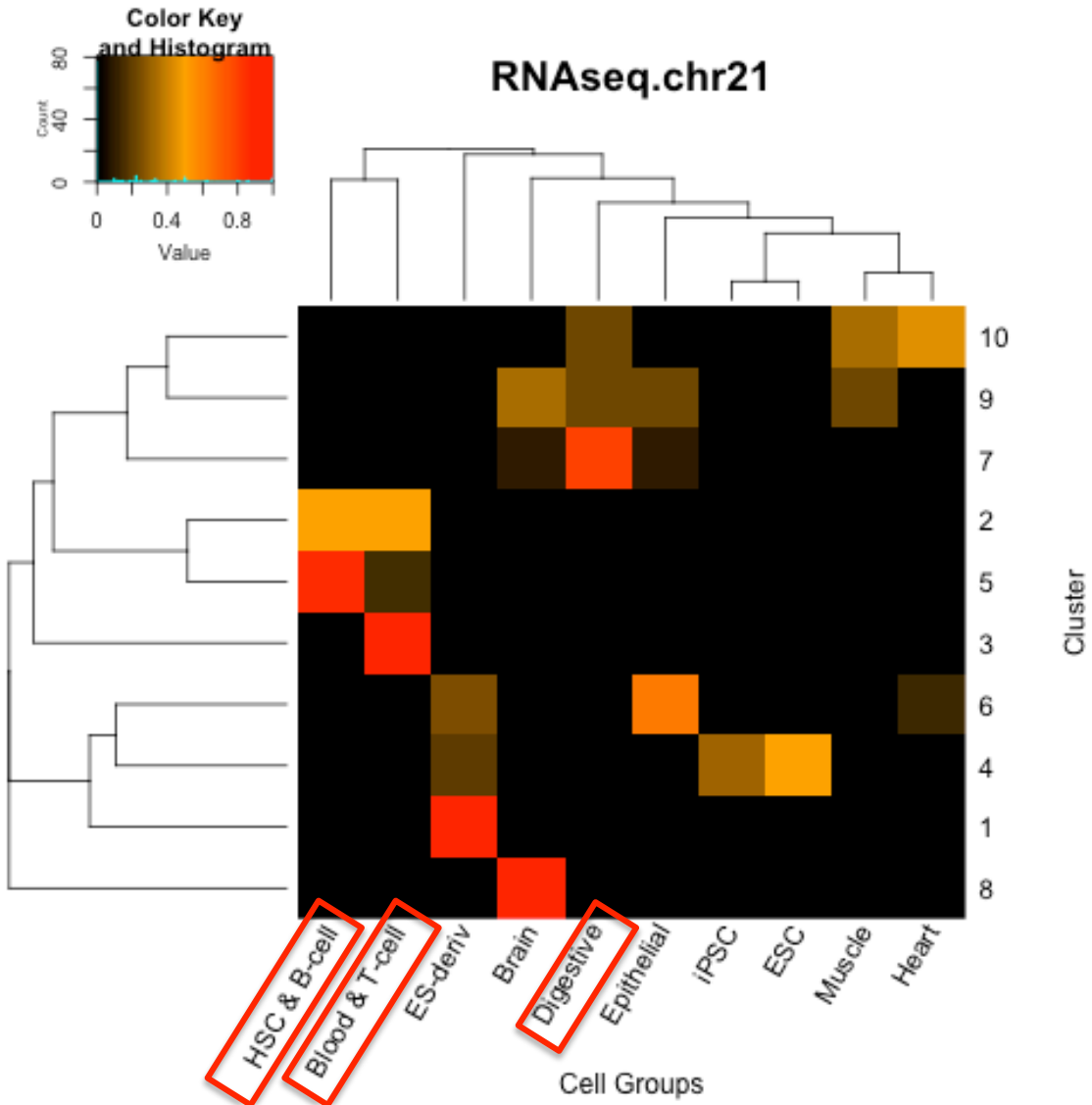
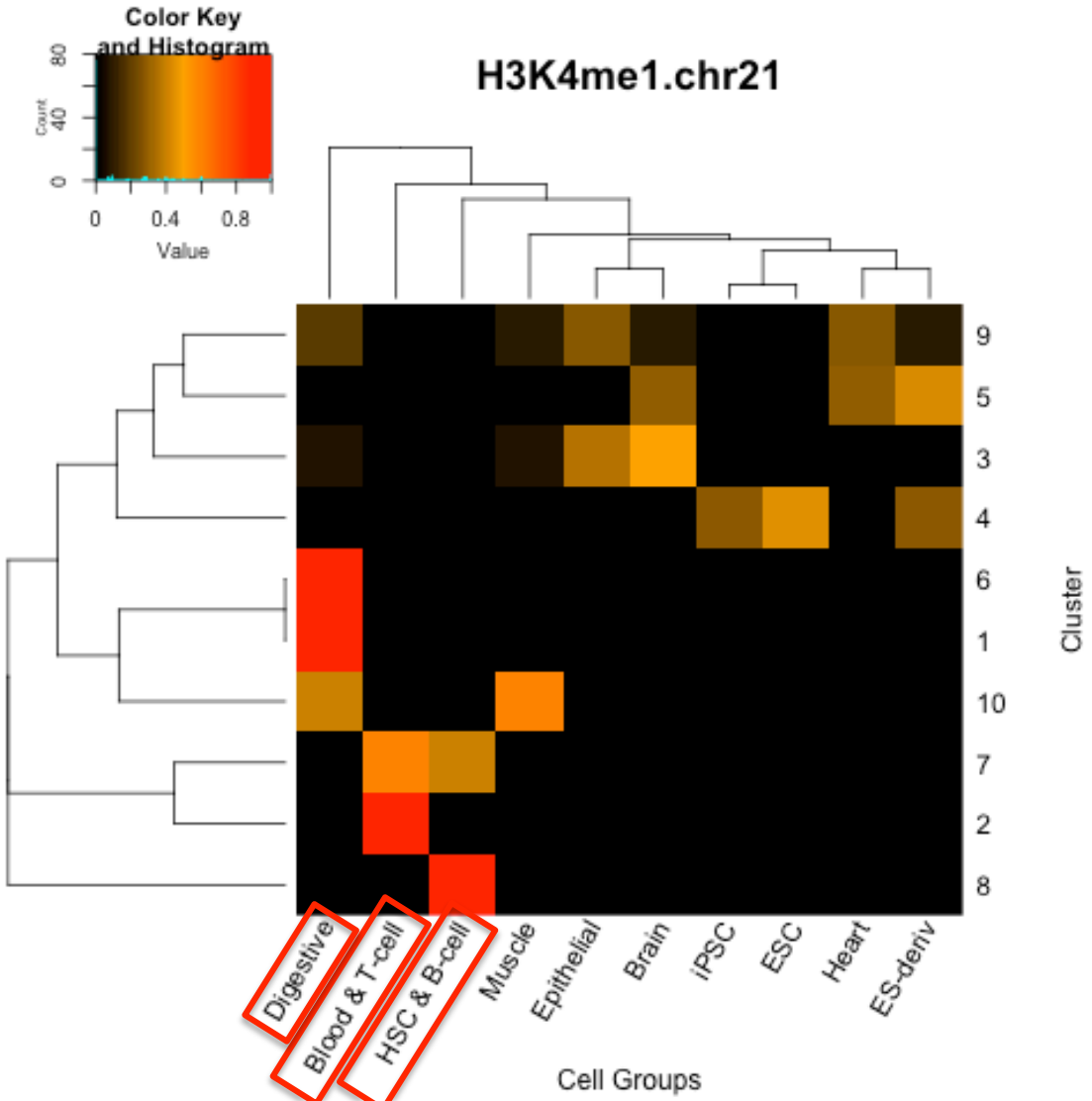
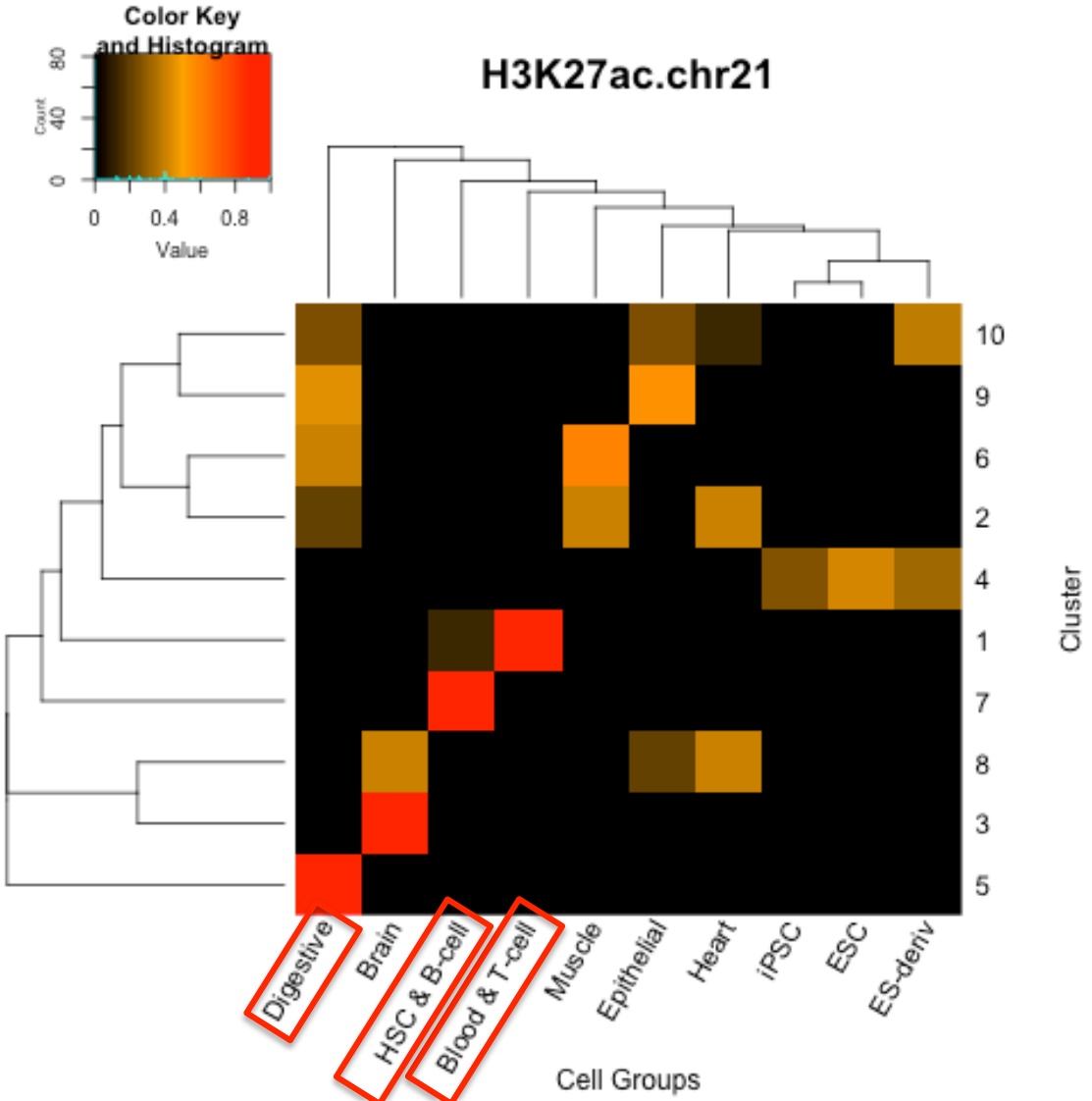


Visualizing Clustering Efficacy: Heatmaps



Visualizing Clustering Efficacy: Heatmaps



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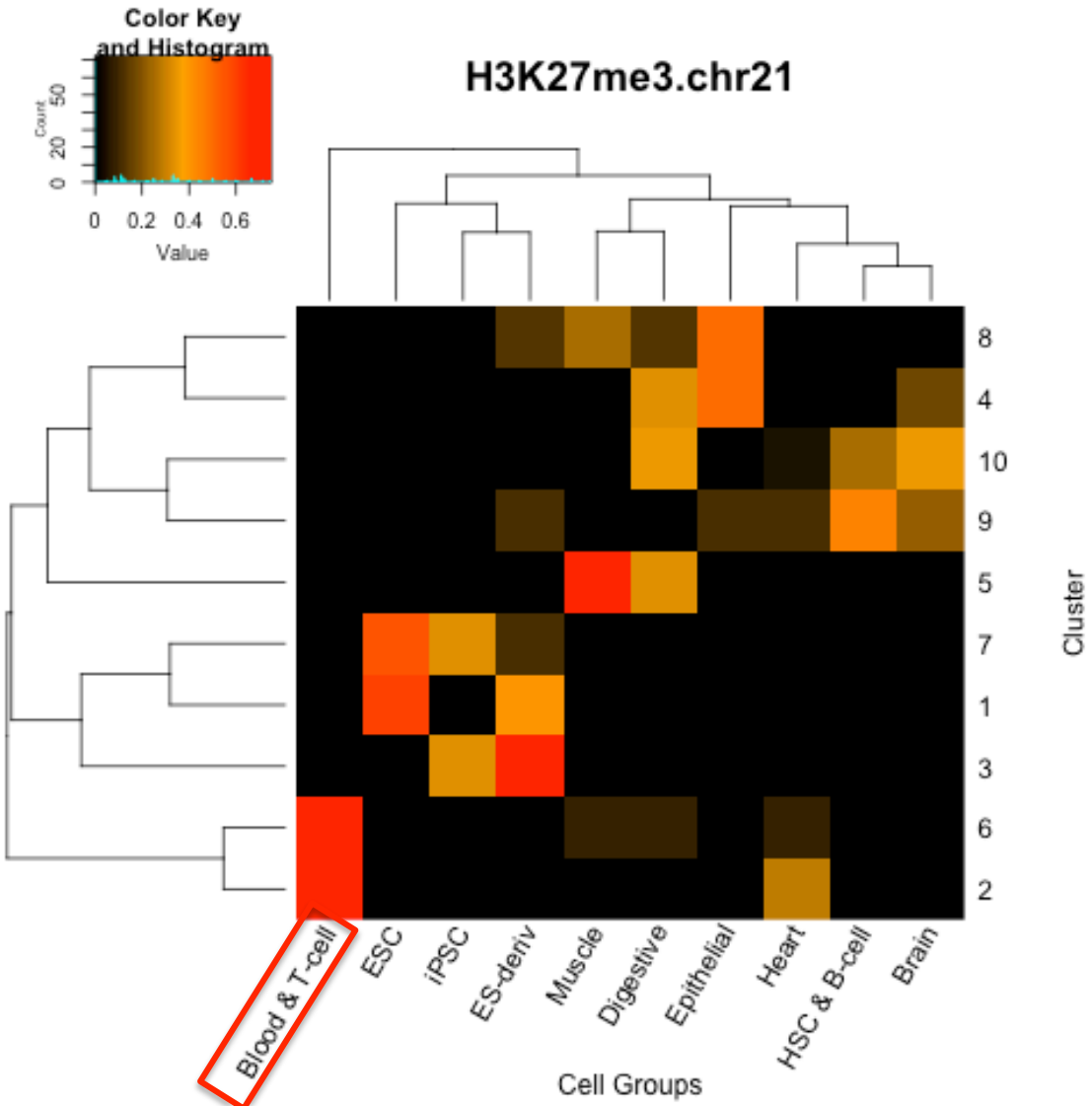
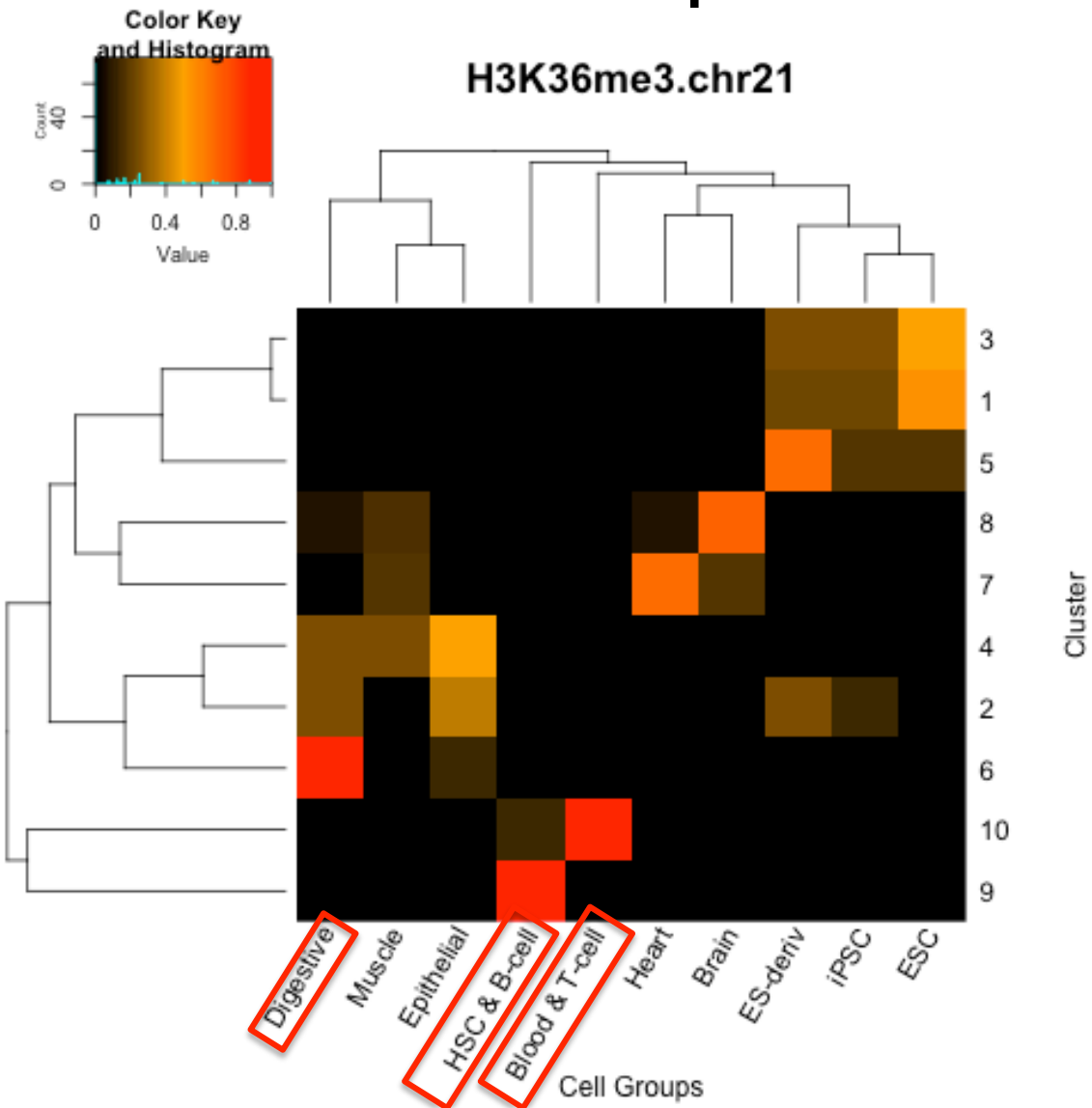
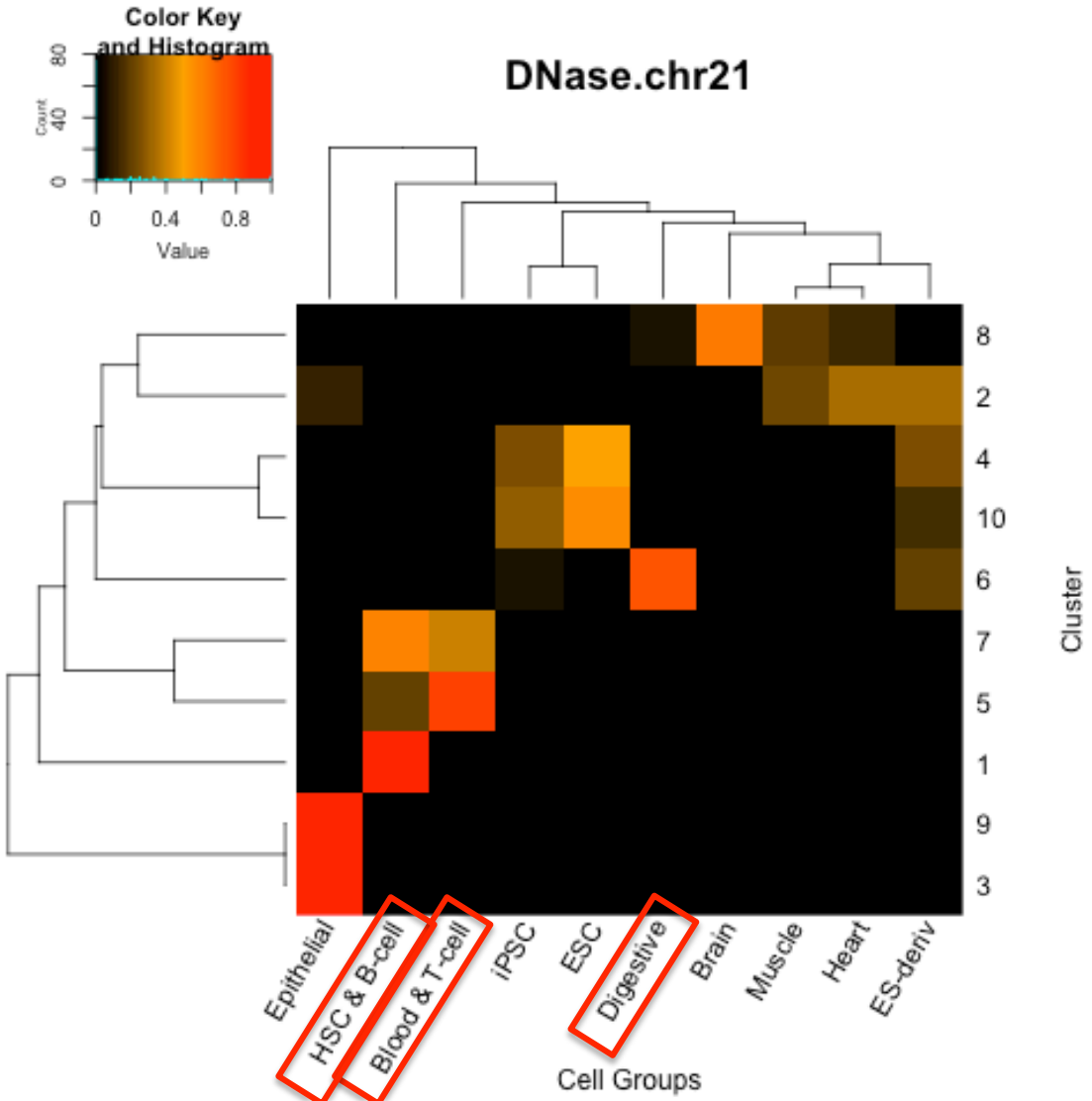


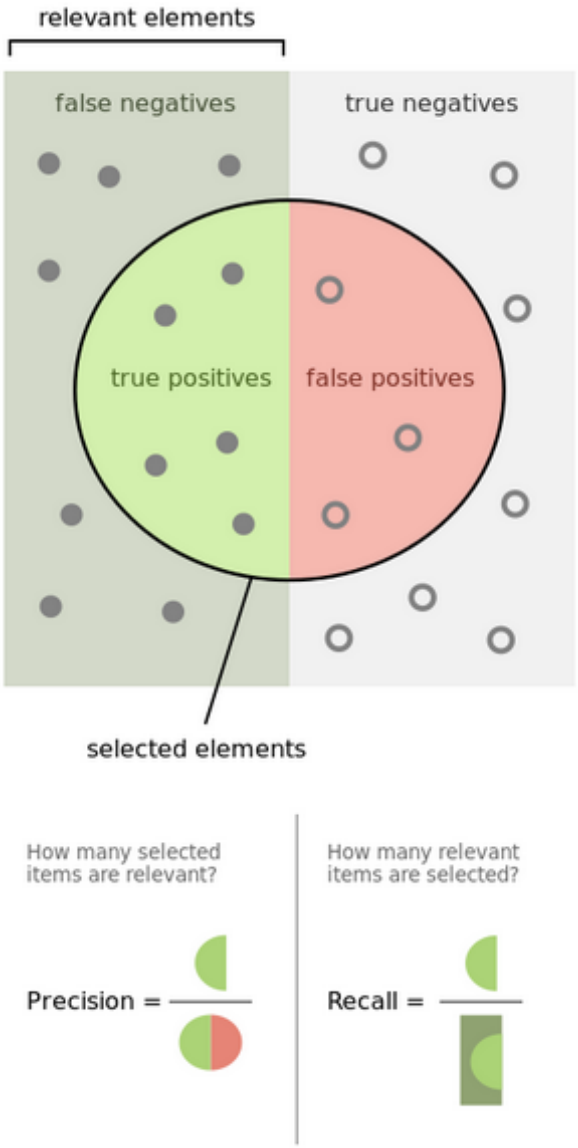
Visualizing Clustering Efficacy: Heatmaps



Visualizing Clustering Efficacy: Heatmaps



Precision and Recall



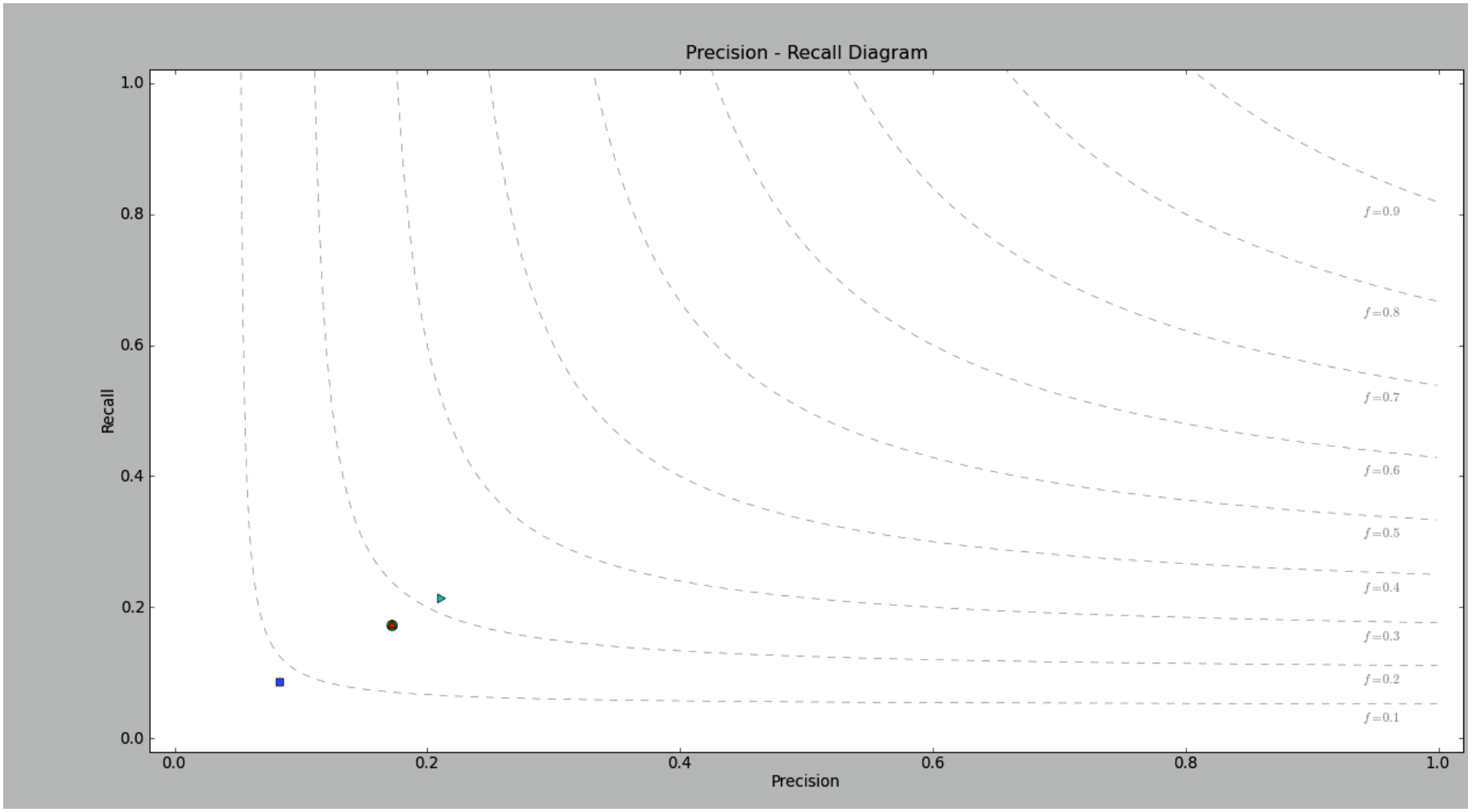
http://upload.wikimedia.org/wikipedia/commons/thumb/2/26/Precisionrecall.svg/2000px-Precisionrecall.svg.png

Evaluating Clustering Efficacy: F-Measure (F-Beta Score)

$$P = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FP}} \qquad R = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FN}} \qquad F_\beta = \frac{(\beta^2 + 1)PR}{\beta^2 P + R}$$

- P – precision
- R – recall
- TP – True Positives
- FP – False Positives
- FN – False Negatives
- β – weights recall (for values >1, penalizes false negatives more strongly than false positives)

Precision & Recall vs F Score



http://aimotion.blogspot.com/2011/05/evaluating-recommender-systems.html

Evaluating Clustering Efficacy: F-Measure (F-Beta Score)

- **DNase.chr21 - 0.5944444**
- **H3K27ac.chr21 - 0.6277778**
- H3K27me3.chr21 - 0.3944444
- **H3K36me3.chr21 - 0.5805556**
- H3K4me1.chr21 - 0.5166667
- H3K4me3.chr21 - 0.5416667
- H3K9ac.chr21 - 0.4555556
- H3K9me3.chr21 - 0.3555556
- RNAseq.chr21 - 0.5083333

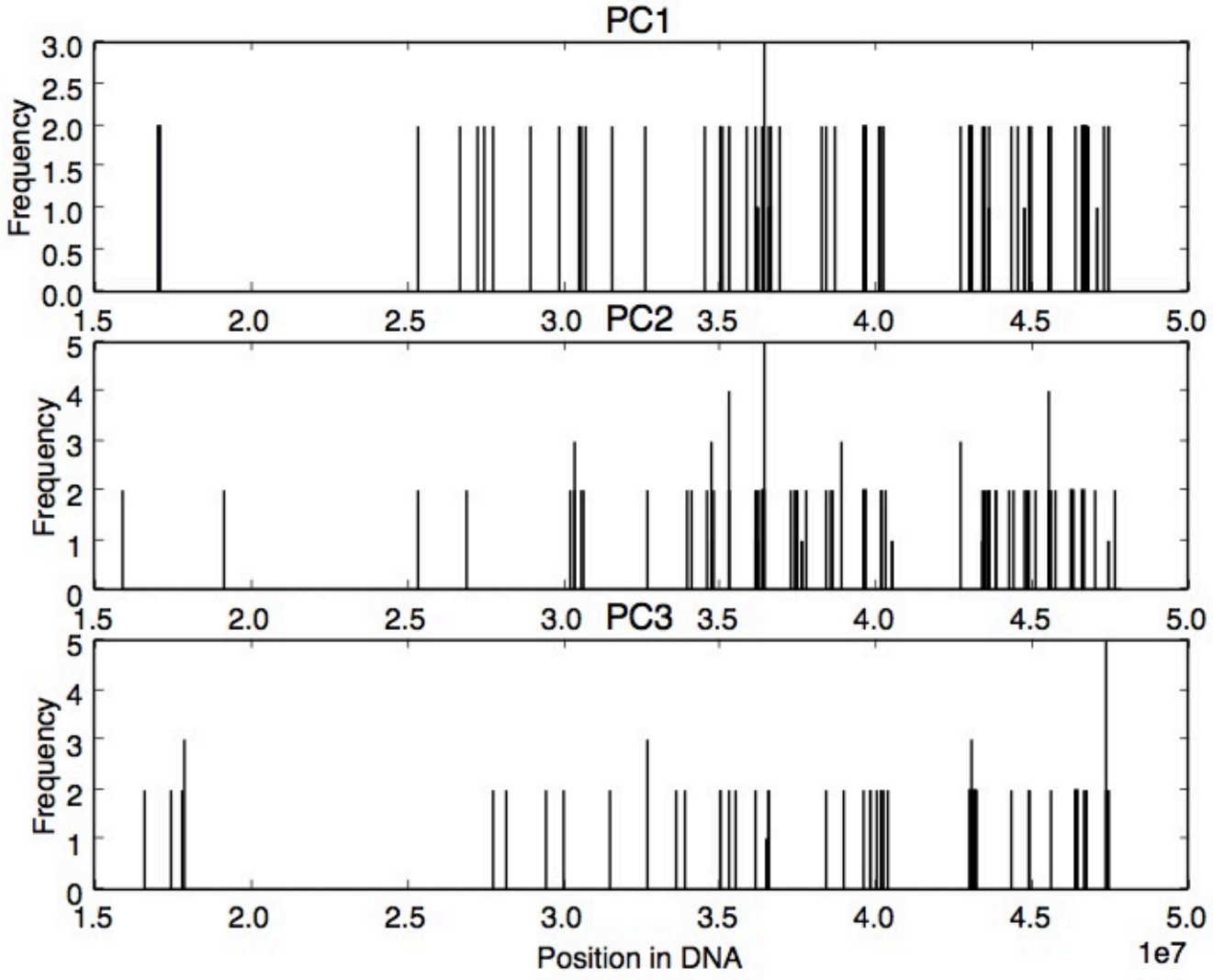
Analysis of Variance

- Question: Are there certain locations in the genome that explain the PCs and to these locations have an important biological function?

Analysis of Variance

- Sorted components of each PC in descending order of absolute value
- Looked at patterns in the components that contributed more than 5% to the variance
- Binned regions into 5kb segments

DNase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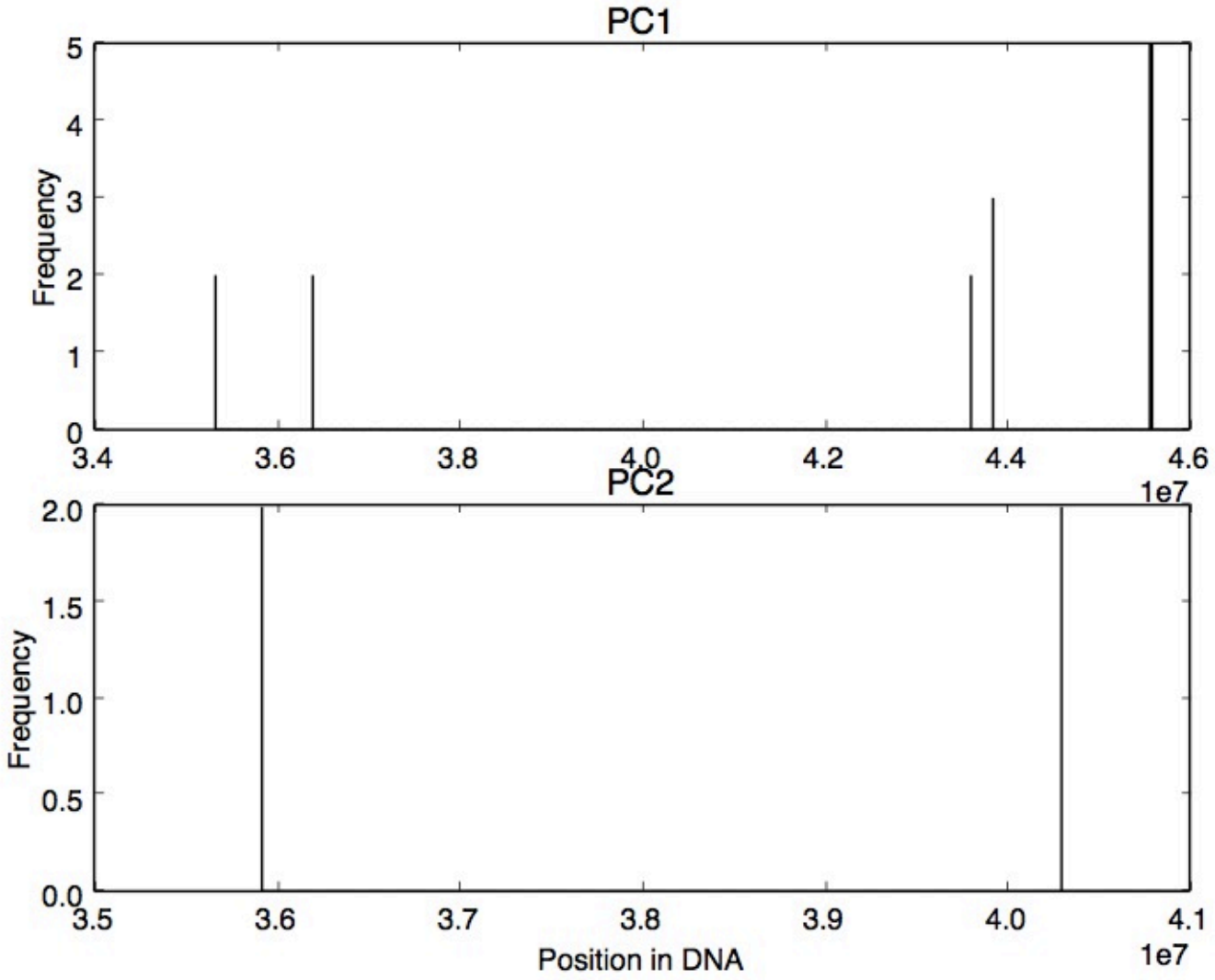
2.5
2.0
1.5
1.0
0.5
0.0
1.5
2.0
2.5
3.0
PC2
3.5
4.0
4.5
5.0
Frequency
5
4
3
2
1
0
1.5
2.0
2.5
3.0
PC3
3.5
4.0
4.5
5.0
Frequency
5
4
3
2
1
0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Position in DNA
1e7

H3K4me1


PC1
Frequency
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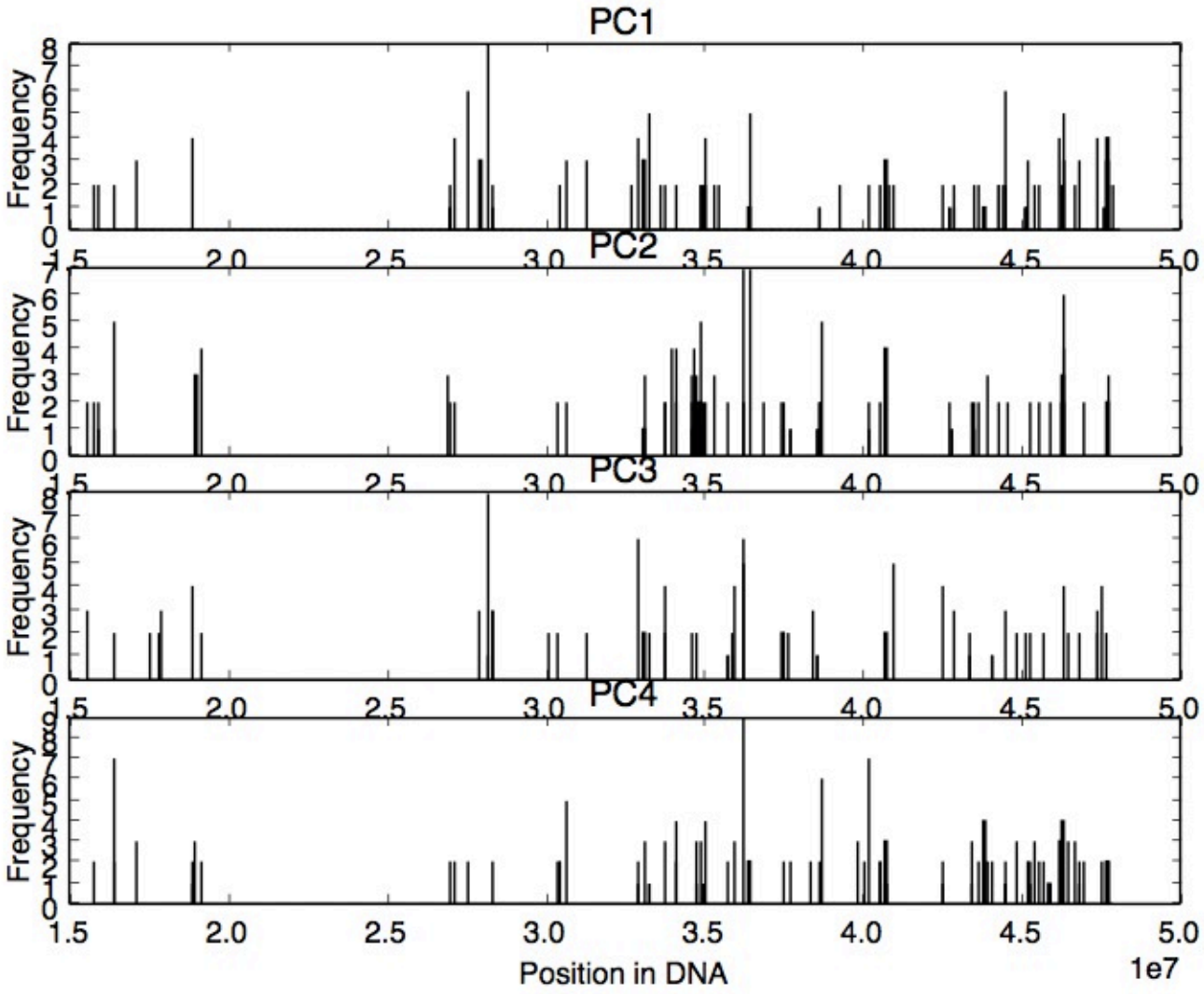
4
3
2
1
0
3.4
3.6
3.8
4.0
4.2
4.4
4.6
1e7
PC2
Frequency
2.0
1.5
1.0
0.5
0.0
3.5
3.6
3.7
3.8
3.9
4.0
4.1
1e7
Position in DNA

H3K4me3


PC1
PC2
PC3
PC4
Frequency
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2.0
2.5
3.0
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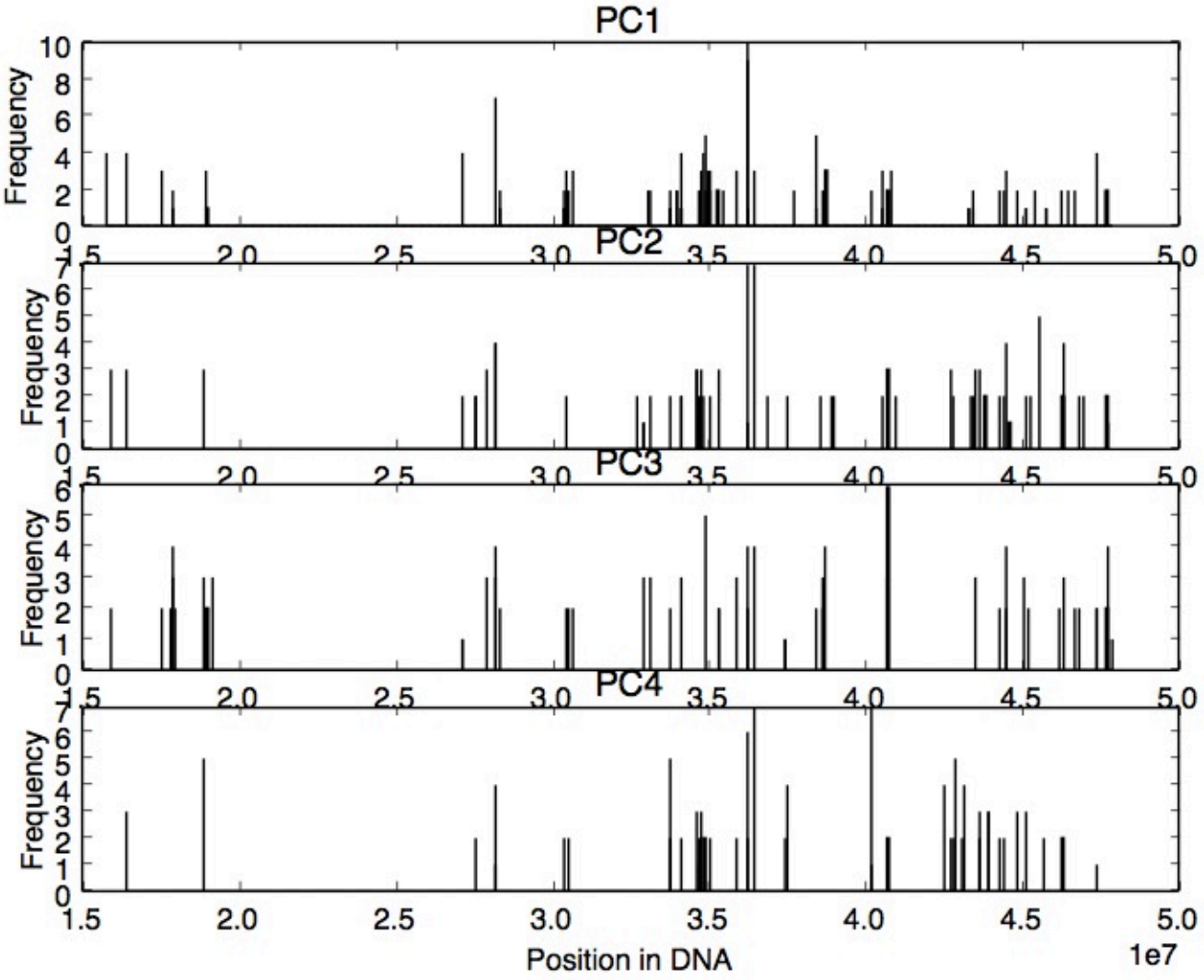
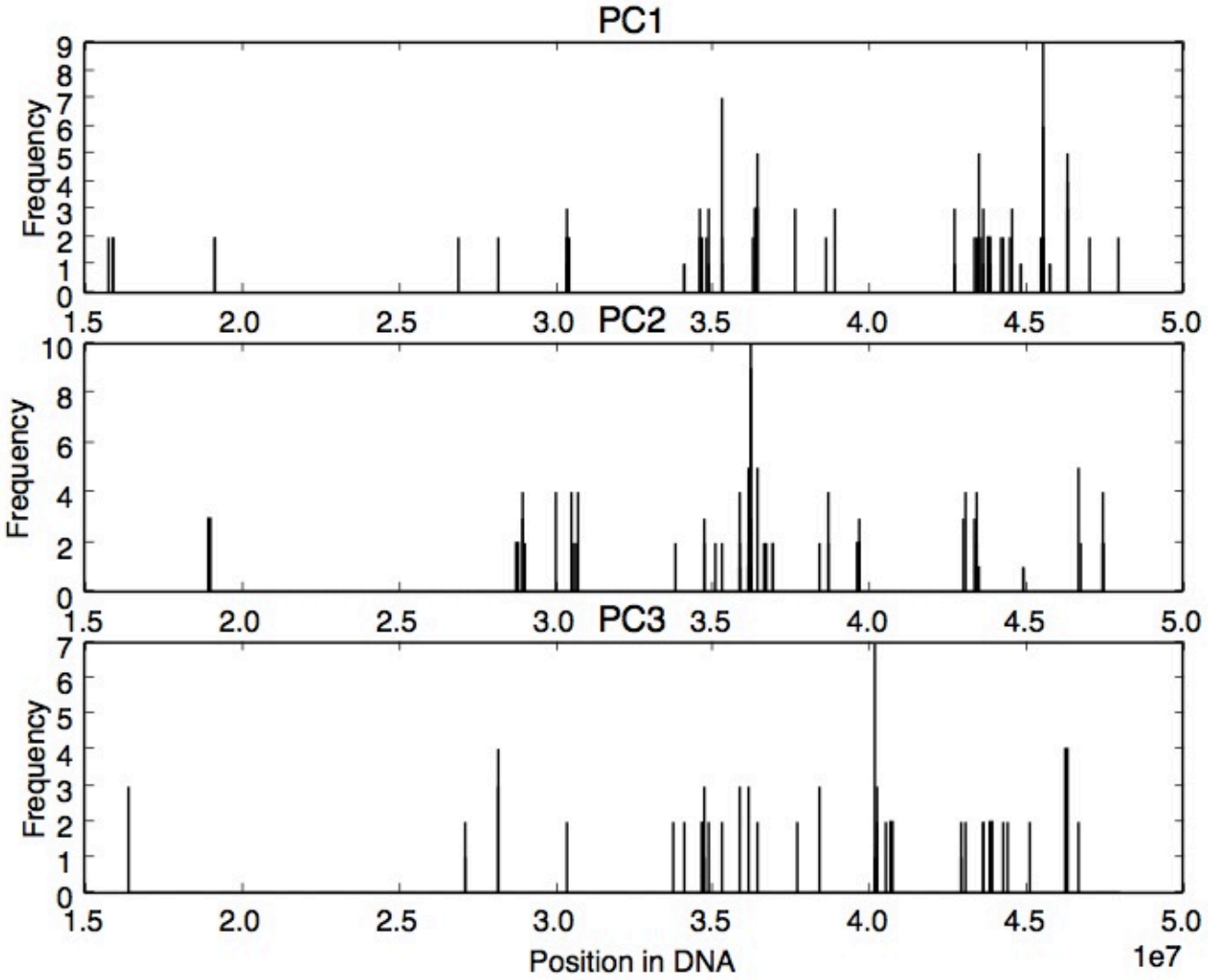
4.0
4.5
5.0
Position in DNA
1e7

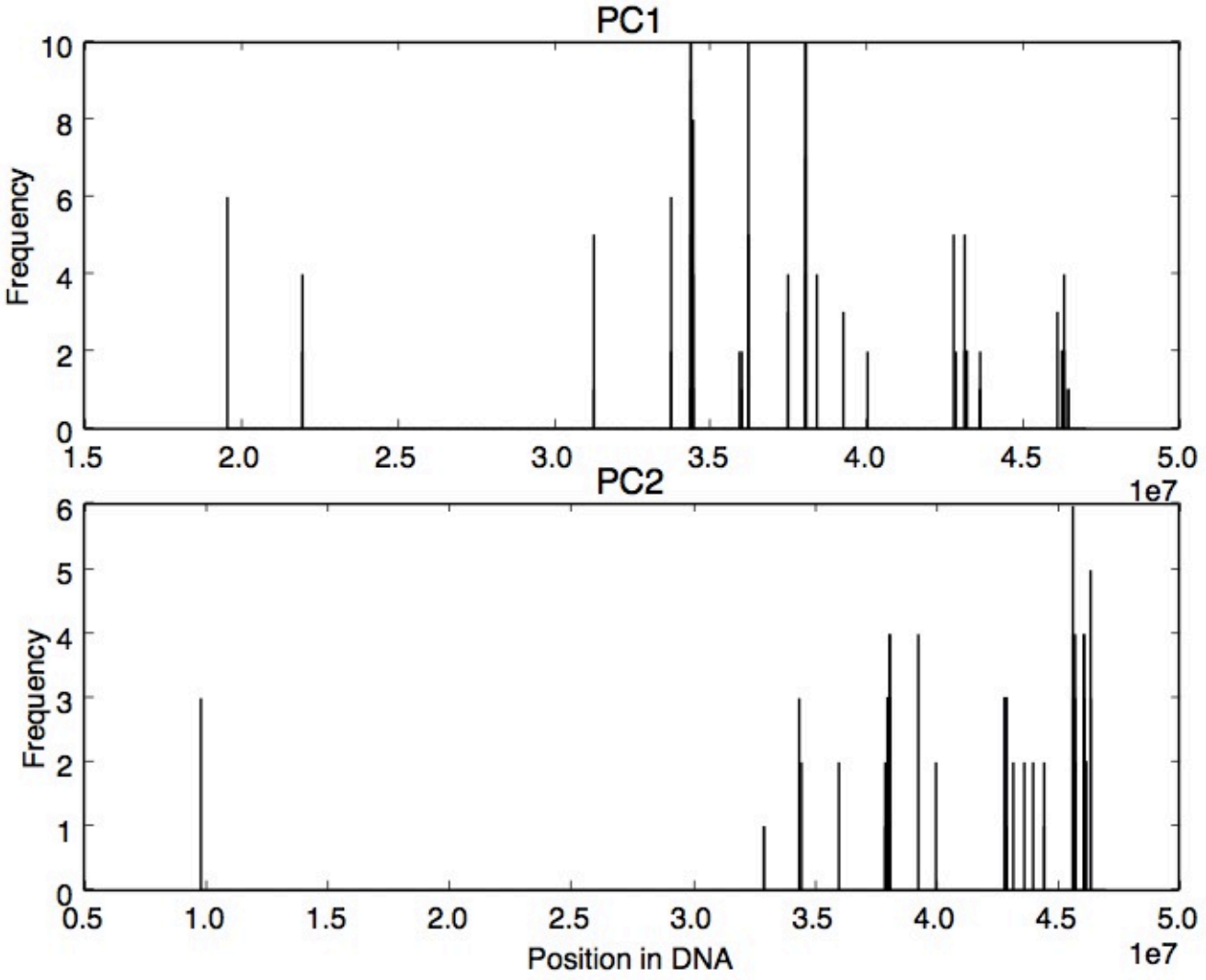
H3K9ac


PC1
PC2
PC3
PC4
Frequency
Position in DNA
1e7
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0

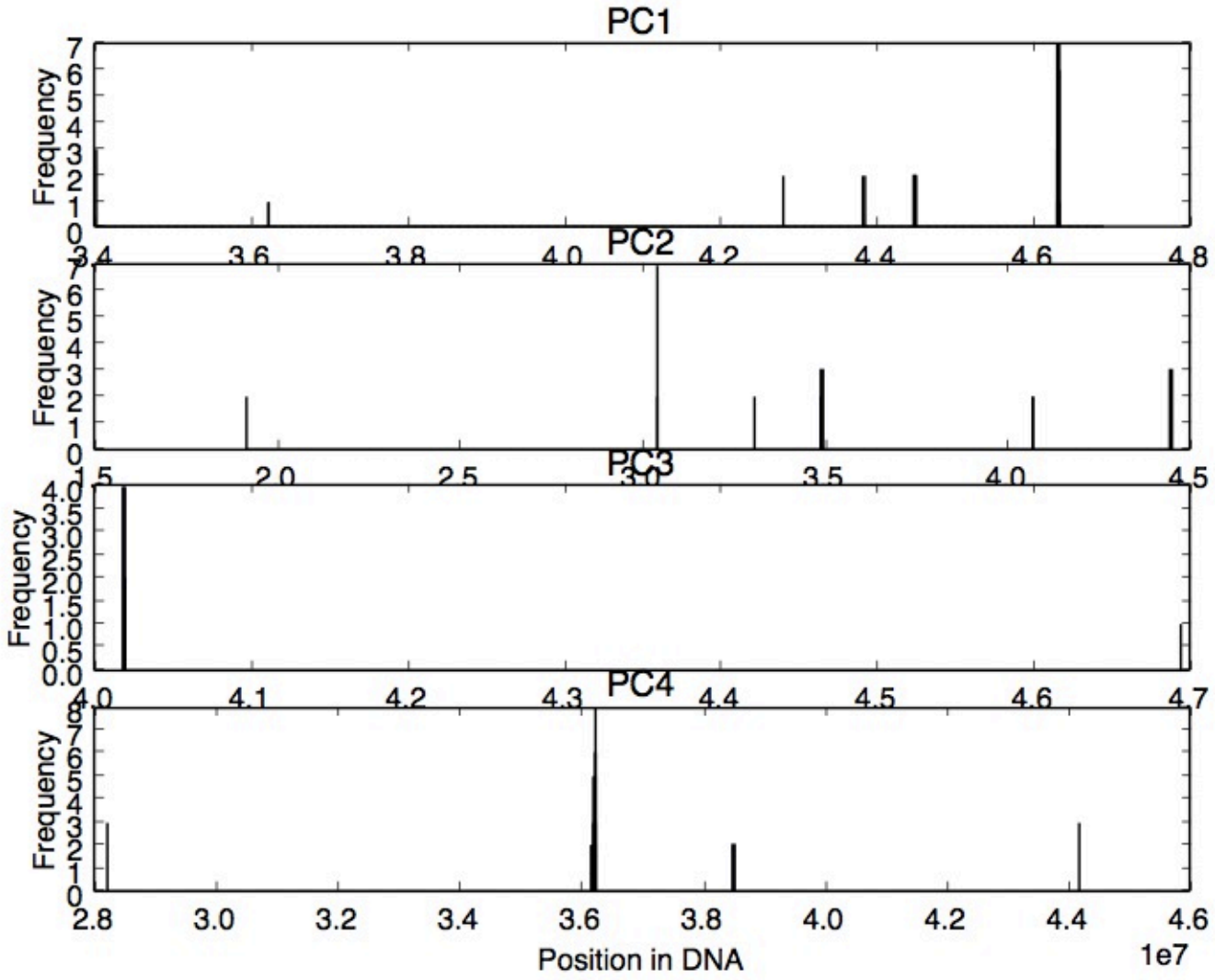
H3K27ac


PC1
Frequency
0 1 2 3 4 5 6 7 8 9
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
PC2
Frequency
0 2 4 6 8 10
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
PC3
Frequency
0 1 2 3 4 5 6 7
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
Position in DNA
1e7

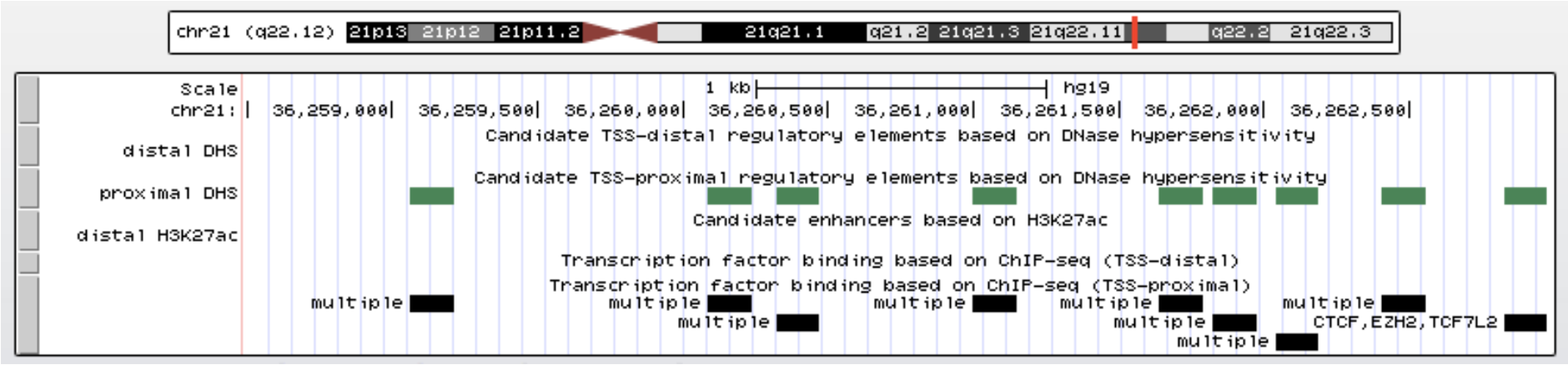
H3K27me3


PC1
Frequency
10
8
6
4
2
0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
1e7
PC2
Frequency
6
5
4
3
2
1
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
1e7
Position in DNA

H3K36me3



Comparison to annotation



- Conclusions:
  - Histone modifications showed the most cell-type specificity in Blood & T-cell, HSC & B-cell and Digestive cells
  - Certain genomic locations contribute more to the variance in cell types
- Future directions:
  - Use a Gaussian mixture model for clustering
  - Use higher resolution data to predict the biological function of important locations in the genome