

On model based clustering of RNA-seq expression data

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Abstract

We propose a model based approach to cluster the reads level expression for bulk RNA and single cell RNA-seq data. It is similar to the topic model in Natural Language Processing and the admixture model in Population Genetics, that assigns grades of cluster membership to each sample. This approach provides us with easily interpretable cluster visualization and detects the underlying structure in the data better than distance based approaches. It also extracts the important genes that drive the clusters and provides measures of model fit to assess the strength of clustering. Further, we show that this method is robust under low coverage of reads. We apply this method on the GTEx tissue level bulk RNA expression data as well as two single cell RNA-seq data. Our methods are implemented in a R package **CountClust**, available at <https://github.com/kkdey/CountClust>.

1 Introduction

Clustering of samples based on gene expression data is a popular exploratory mechanism in bulk RNA-seq and single cell RNA-seq (scRNA-seq) experiments. It aids in quality control and helps in understanding the heterogeneity across tissue samples (bulk RNA-seq) or single cells (scRNA-seq). Usually the clustering techniques more commonly used in RNA-seq literature are distance based, primarily hierarchical and k-means clustering (see Jaitin *et al* 2014 [12], Buettner *et al* 2015 [21], GTEx Consortium paper [4]). However, the data obtained from the RNA sequencing experiments are counts data, representing the number of reads mapping to different genes. and there exist model based clustering methods based on counts which seem to be directly applicable to the RNA-seq reads data.

The clustering model we propose in this paper is similar to the topic model approach, widely used in Natural Language Processing (see Blei, Ng and Jordan 2003 [24], Blei and Lafferty 2009 [25]), which is derived from the Admixture model in population genetics (see Pritchard, Stephens and Donnelly 2000 [6]). This approach models each tissue sample as having some proportion of its reads coming from each cluster. This assumption is biologically sensible since in reality, each tissue sample indeed is a mixture of different cell types and presumably, the clusters under this model could be driven by the cell types. Also, such graded membership approach is capable of representing more continuous cluster patterns.

Deconvolution techniques using marker genes are popularly used to learn about the concentration of different cell types in a cell mixture and cell type signature expression profiles. Our technique is analogous to blind deconvolution approach which estimates the cell type proportions and cell type signatures jointly (see Schwartz *et al* 2010 [29] and Repsilber *et al* 2010 [28]), except that we operate under Poisson model framework to model the counts data. This method can also be extended to semi-supervised deconvolution that assumes some information of cell type signature expression (see Shen-Orr *et al* 2010 [26], Qiao *et al* 2012 [27]).

In this paper, we demonstrate that for RNA-seq (bulk or single cell) data with known structural patterns, such count clustering approach identifies the structure better than hierarchical clustering. It also allows one to interpret each cluster by providing information about genes that are playing a significant role in driving the clusters and these genes may be important from both biological and medical standpoint. Also we show our method to be robust even for low coverage data as might be the case for single cell RNA-seq (scRNA-seq) data. We illustrate the performance of our method on GTEx tissue level bulk-RNA seq data as well as on two single cell data (due to Jaitin *et al* 2014 [12] and Deng *et al* 2014 [13]).

In contrast to traditional clustering methods, the grade of membership (GoM) model allows each sample to have partial membership in each cluster. This idea has been previously used in population genetics to model admixture, where individuals have ancestry from multiple populations, each population being characterized by a vector of allele frequencies at each marker (see Pritchard, Stephens and Donnelly 2000 [6]). It is also used in natural language processing to model documents, where each document has membership in multiple topics, each topic being characterized by a vector of word frequencies (see Blei, Ng and Jordan 2003 [24]). Here we use the GoM model to allow each RNA-seq sample to have membership in

The main idea is that this read could come from hidden subpopulations (may be cell types for tissue level expression study or cell cycle phases for single cell study) and its probability of getting assigned to some gene g may depend on which subpopulation it comes from. Denote the probability that the sample is coming from the k th subpopulation by q_{nk} ($q_{nk} \geq 0$ and $\sum_{k=1}^K q_{nk} = 1$ for each n). Given that the sample is coming from the k th subgroup, the probability of a read being matched to the g th gene is given by θ_{kg} ($\theta_{kg} \geq 0$ and $\sum_{g=1}^G \theta_{kg} = 1$ for k th subgroup). Then one can write

2 Methods and Materials

2.1 Model overview

We assume the RNA-seq data have been summarized by a table of counts $C_{N \times G} = (c_{ng})$, where c_{ng} is the number of reads from sample n mapped to gene (or transcript) g [?]. We remove genes g with all zero counts ($c_{ng} = 0$ for all n). We use the `maptpx` R package [?] to fit the Grade of Membership (GoM) model (or “Latent Dirichlet Allocation” model). This model assumes the

RNA-seq counts for each sample follow a multinomial distribution

$$c_{n\cdot} \sim \text{Mult}(c_{n+}, p_{n\cdot}) \quad (1)$$

where $c_{n\cdot}$ denotes the count vector for the n th sample, $c_{n+} := \sum_g c_{ng}$, and $p_{n\cdot}$ is a probability vector (non-negative entries summing to 1) whose g th element represents the relative expression of gene g in sample n . The model further assumes that

$$p_{ng} = \sum_{k=1}^K q_{nk} \theta_{kg} \quad (2)$$

where $q_{n\cdot}$ is a probability vector whose k th element represents the grade of membership of sample n in cluster k , and $\theta_{k\cdot}$ is a probability vector whose g th element represents the relative expression of gene g in cluster k . The `maptpr` package fits this model using an EM algorithm to perform Maximum a posteriori (MAP) estimation of the parameters q and θ . See [5] for details.

2.2 Visualizing Results

We visualize results using a “Structure plot” [7], which is named for its widespread use in visualizing the results of the “structure” software [?] in population genetics. The Structure plot represents each GoM vector $q_{n\cdot}$ as a vertical stacked barchart, with bars of different colors representing membership proportion in each cluster (e.g. Figure ??). If the colored patterns of two bars are similar, then the two samples have similar membership proportions. The Structure plot is particularly helpful when external information is available on each sample that can be used to order or group the samples in an informative way.

We have also found it useful to visualize results using t-distributed Stochastic Neighbor Embedding (t-SNE), which is a method for visualizing high dimensional datasets by placing them in a two dimensional space, attempting to preserve the relative distance between nearby samples (see L.J.P. van der Maaten [10] and L.J.P. van der Maaten and Hinton [9]). t-SNE tends to place samples with similar membership proportions together in the two-dimensional plot, forming visual “clusters” that can be identified by eye (e.g. Figure ??). This may be particularly helpful in settings where no external information is available to aid in making an informative Structure plot.

2.3 Cluster annotation

A question of considerable biological interest is which genes are significantly differentially expressed across the clusters, or in other words, which genes are driving the clustering. To answer this, we fix one cluster and for each gene, define a distance metric between that cluster and any of the other clusters, based on the cluster expression profile of the gene, namely the θ values. For cluster k , we define the distance from cluster l based on expression profile of gene g to be

$$KL^g[k, l] := \theta_{kg} \log \frac{\theta_{kg}}{\theta_{lg}} + \theta_{lg} - \theta_{kg}$$

This is similar to the Kullback Leibler divergence of the Poisson distribution with parameter θ_{lg} from another Poisson distribution with parameter θ_{kg} . For each cluster k , we define the divergence measure for gene g as

$$Div^g[k] = \min_{l \neq k} KL^g[k, l]$$

The higher the divergence measure, the more significant is the role of the gene in the clustering. We choose a small subset of around 50-100 genes with highest values of $Div^g[k]$ for each k and this set of genes can be viewed to be the most important genes driving the cluster k .

Once the most important driving genes for each cluster k have been extracted, we perform gene annotations on them using **mygene** R Bioconductor package (due to Mark A, Thompson R and Wu C 2014 [19]). We check if the driving genes for a particular cluster are associated with some specific biological functionality. This would validate whether the subgroups are biologically relevant.

2.4 Comparison with hierarchical clustering

Distance based hierarchical clustering methods are the most commonly used clustering techniques in single cell RNA-seq experiments. To compare between the graded membership model and the distance based hierarchical clustering algorithm, we observe which method separates out samples coming from two different tissues better than the other. For each pair of distinct tissues, we draw 50 samples from the pool of all samples coming from these tissues. We first apply hierarchical clustering using both the complete and the average linkage using Euclidean distance metric and cut the dendrogram at $K = 2$. We observe if this cut separates out the samples coming from the two tissues. Next we apply our model based approach on these 50 samples for $K = 2$. We sort the samples based on the decreasing proportional membership in one cluster (say cluster 1) and we partition the samples at the point of the steepest fall in this membership. As a result, we will essentially cluster the samples into two groups- one with high membership in cluster 1 and the other with low membership in cluster 1. Again we check if these clusters correspond to the samples coming from the two tissues. Between the hierarchical approach and the graded membership approach, the method that can separate out the samples from two tissues using the above mechanism more number of times (across all pairs of tissues) is considered to be superior than the other.

3 Results

3.1 Clustering human tissues based on bulk RNA expression

We begin by illustrating our method on bulk RNA expression measurements from the GTEx project (V6 dbGaP accession phs000424.v6.p1, release date: Oct 19, 2015, <http://www.gtexportal.org/home/>). These data consist of per-gene read counts from RNA-seq performed on 8555 samples collected from 450 human donors across 51 tissues and LCL and transformed fibroblast cell-lines. We analyzed 16,069 genes that satisfied filters (e.g. exceeding certain minimum expression levels) that were used during eQTL analyses by the GTEx project (gene list available in https://github.com/stephenslab/count-clustering/blob/master/utilities/gene_names_GTEX_V6.txt).

To assess structure in these data we applied the grade-of-membership model with $K = 10, 12, 15$. Although results differ with K , many of the primary patterns were consistent across K . Here, for brevity, we focus on results for $K = 15$, shown as a Structure plot in **Figure 1(a)** (see also an alternative visualization using a 2-dimensional projection with t-sne [9], [10], in **Supplementary Fig 1** http://stephenslab.github.io/count-clustering/project/src/tissues_tSNE.html). Reassuringly, much of the structure highlighted by these results follows the known division of samples into tissues: that is, samples from the same tissue tend to have similar grades of membership across clusters. Some tissues are represented by essentially a single cluster (e.g. Pancreas, Whole Blood), whereas other tissues are represented as a mixture of multiple clusters (e.g. Thyroid). Furthermore, the results highlight biological similarity among some tissues by assigning samples from those tissues similar membership proportions. For example, samples from different parts of the brain have similar memberships, as do the arteries (Artery-aorta, Artery-tibial and Artery-coronary) and skin (Skin Not Sun Exposed- suprapubic and Skin Sun Exposed- lower leg). Samples from the tibial nerve have small but consistent amounts of membership in common with brain tissues, as well as larger amounts in common with the adipose tissues (Adipose Subcutaneous and Adipose Visceral (Omentum)). The latter may reflect contamination of those samples with adipose cells (CHECK).

Each cluster in our model is characterized by a vector that contains the mean expression level for each gene. To help biologically interpret each cluster we annotate it by identifying the genes whose expression levels most strongly distinguish that cluster from the others (see Cluster Annotation, in Methods and Materials). **Tab ??** summarizes the results of this cluster annotation (top three genes) for the GTEx analysis in Figure 1a. Again, reassuringly, the results consistently align with known biology. For example, the top three genes driving the light brown cluster, which distinguishes Pancreas from other tissues, are *PRSS1* (protease serine 1), *CPA1* (carboxypeptidase) and *PNLIP* (pancreatic lipase), all of which are intimately involved in pancreatic function. Similarly, the top three genes driving the grey cluster, which distinguishes Whole Blood, are all hemoglobin genes, *HBB* (hemoglobin, beta), *HBA2* (hemoglobin, alpha 2) and *HBA1* (hemoglobin, alpha 1). Similarly, spermatogenesis and sperm-related genes characterize the Testis cluster, Keratin-related genes characterize the skin cluster, Myosin-related

genes characterize the muscle skeletal cluster, etc. In cases where a cluster occurs in multiple tissues these annotations may be particularly helpful for understanding what may be driving this co-membership. For example, the top three genes in the burlywood cluster which is common to Lung, Spleen and Small Intestine - Terminal Ileum , code for surfactant proteins B, A2 and A1.

Although global analysis of all tissues is useful for highlighting major structure in the data, it may be less effective at identifying finer-scale structure within tissues or among similar tissues. For example, here our global analysis allocated similar cluster memberships to all brain tissues, and we suspected that these tissues may exhibit substructure that could be uncovered by analyzing the brain samples separately. **Fig 1(b)** shows the Structure plot for $K = 4$ on only the Brain samples. The results highlight much finer-scale structure compared with the global analysis. Brain Cerebellum and Cerebellar hemisphere are essentially assigned to a separate cluster, whose top 3 defining genes are SNAP25 (synaptosomal-associated protein, 25kDa), ENO2 (enolase 2- gamma, neuronal) and CHGB (chromogranin B), all of which are associated with neuronal activities (**Supplementary Table 1**). The spinal cord samples also show consistently strong membership in a single cluster, whose top 3 defining genes are MBP (involved in myelination [31]) and MYH11 and ACTA2, both of which are .. Genetic mutations in the latter two genes can cause TAAD Thoracic aortic aneurysms/ dissection [?]. The remaining samples all show membership in multiple cluster, with cortex samples being distinguished from other samples by stronger membership in a cluster (blue in figure) whose top 3 defining genes include UCHL1, which is expressed in neurons and is required for normal synaptic and cognitive function [33].

3.2 Quantitative comparison with hierarchical clustering

Since most clustering of gene expression datasets is performed using distance-based hierarchical clustering methods, we compared the accuracy of these distance-based methods with the model-based approach. Specifically, for each pair of tissues in the GTEX data we assessed whether or not each clustering method correctly partitioned samples into the two groups (See methods for details.) We found that the model-based method succeeded in 59% of the comparisons, compared with 29% for the distance-based method.

3.3 Robustness to sequencing depth

RNA-sequencing of single-cell data typically involve substantially lower effective sequencing depth compared with bulk experiments, due to the lower number of molecules available to sequence in a single cell. Therefore, we checked robustness of our results above to sequencing depth. Specifically we repeated analyses using thinned data designed to mimic lower sequencing depth: if c_{ng} is the counts of number of reads mapping to gene g for sample n for the original

data, then the thinned counts are given by

$$t_{ng} \sim \text{Bin}(c_{ng}, p_{thin}) \quad (3)$$

where we used $p_{thin} = 0.0001$. With this value of p_{thin} total sequencing depth is similar to the per-sample depth in the single-cell data from [?] considered below. (See **Fig ??** [check!] and **Fig 5** for results with other values of p_{thin} .)

For the thinned GTEX data the Structure plot for $K = 15$ (**Fig 1(c)**) preserves most of the major features of the original analysis on unthinned data (**Fig 1(a)**). For the comparisons with distance-based methods, both methods suffer reduced accuracy for the thinned data, but the model-based method retains its superior performance (xx vs yy).

3.4 Clustering of single-cell RNA-seq data

To examine the potential for model-based clustering of single cell RNA-seq data we examined two datasets, from Jaitin *et al* [12] and Deng *et al* [13].

Jaitin *et al* sequenced over 4000 single cells from mouse spleen. Following the original authors protocol, we also filtered out 16 genes that they found to show significant batch-specific expression. Here we analyze 1041 of these cells that were categorized as *CD11c+* in the *sorting markers* column of their data (http://compgenomics.weizmann.ac.il/tanay/?page_id=519), and which had total number of reads mapping to non-ERCC genes greater than 600. (We believe these cells correspond roughly to the 1040 cells in their Figure S7.) Our hope was that applying our method to these data would identify, and perhaps refine, the cluster structure evident in Figures 2A and 2B of [12]. However, our method yielded rather different results (Figure 3). Each cell was assigned to multiple clusters, and the cluster membership vectors strongly correlated with amplification batch (which in turn is strongly correlated with sequencing batch). The fact that batch effects are detectable in data like these is not particularly surprising. There is a growing recognition of the importance of batch effects in high-throughput data generally [16] and in single cell data specifically [17]. And indeed, dimension reduction methods such as the ones we use here can be helpful in controlling for such effects [14] [15]. However, why these batch effects are not visible in the original analyses [12], is unclear.

Deng *et al* collected expression data from individual cells from zygote to blastocyst stages of mouse preimplantation development [13]. Deng *et al*'s analysis focussed particularly on allele-specific expression from the two contributing mouse strains (CAST/EiJ and C57BL/6J). Here we analyze the counts of the two alleles combined. Visual inspection of the Principal Components Analysis in [13] suggested 6-7 clusters, so we fit the cluster model with $K = 6$. The results (Figure 4) clearly highlight the structure in the different development stages starting from zygote, through early/mid/late 2 cells, 4 cells, 8 cells, 16 cells, and early/mid blastocyst to finally late blastocyst. Specifically, cells that are from the same stage show similar cluster membership proportions. Further, many of the clusters show notable trends through the stages. For example, membership in the red cluster is non-existent in early stages, starts in the 4-cell stage, becomes

more prominent in the 8-16 cell stages, drops substantially in the early and mid-blastocyte stages, and is essentially absent in the late blastocytes. More generally, cluster memberships for cells from adjacent stages tend to be more similar to one another than those for cells from distant stages.

Examining the clustering results by embryo highlights apparent embryo-level effects in the early stages (Figure 4): that is, cells from the same embryo sometimes showed distinctive differences from other embryos. For example, the two cells from one of the 2-cell embryos (check) shows much stronger membership in the magenta cluster than other 2-cell embryos, and four cells from one of the 4-cell embryos (embryo 4) shows consistently more yellow membership than the other 4-cell embryos.

Finally, the results indicate a few samples that appear to be outliers - for example, a cell from a 16-cell embryo that looks like a very early stage cell (zygote or early 2-cell), and a cell from an 8-stage embryo that looks rather different from any of the others.

Notably, for both these single-cell data sets, most cells are assigned to a combination of more than one cluster, rather than a single cluster (the exception being the very early-stage cells in data from Deng et al). This highlights the potential utility for grade-of-membership models to capture structure in single cell data that might be missed by simpler cluster-based approaches.

4 Discussions

We have presented a model based clustering approach for RNA-seq (bulk or single cell) read counts data which models each sample as having a mixed membership in different clusters and also helps identifying genes driving the clusters, which may be of significant bio-medical importance. Our approach is an alternative to the distance based methods of clustering, for instance hierarchical clustering, and it seems to outperform the latter in separating biologically meaningful groups in our tests.

Since our method is model based, it provides an optimal K for the model fit. However, one has to run the model on the data for a range of K 's and that is not always practical when running the model on large datasets as in RNA-seq reads data. For a single run with the algorithm running till the successive log posterior increase is less than 0.01, the computation time for the algorithm was approximately 33.4 mins for Jaitin *et al* [12] single cell experiment ($K = 7$), 16.2 mins for Deng *et al* single cell experiment ($K = 6$) and 3297.4 mins for GTEx V6 data ($K = 15$).

So far, in our studies, we fitted the model on the entire RNA-seq reads data, comprising of all the genes. In reality, most of the genes will not be informative about the clusters and an efficient variable selection algorithm, if incorporated with the clustering algorithm, can lead to significant speed up without much loss of information. This is a future direction to this work we are interested in. Another point of biological interest would be to perform cluster annotation of

genetic pathways which would be more meaningful as genes often act together with other genes in pathways related to different activities.

The methods discussed in this paper are implemented in the package **CountClust** available on Github (<https://github.com/kkdey/CountClust>) which is a wrapper package of **maptpx** due to Matt Taddy [5].

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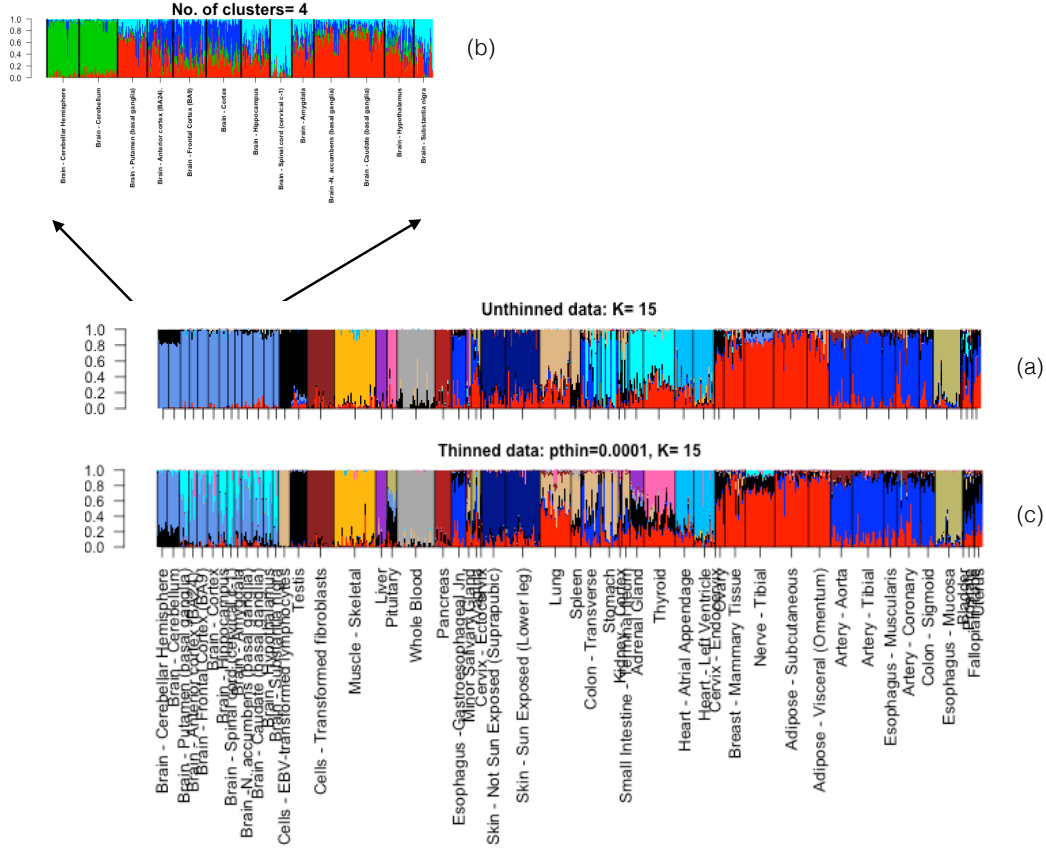
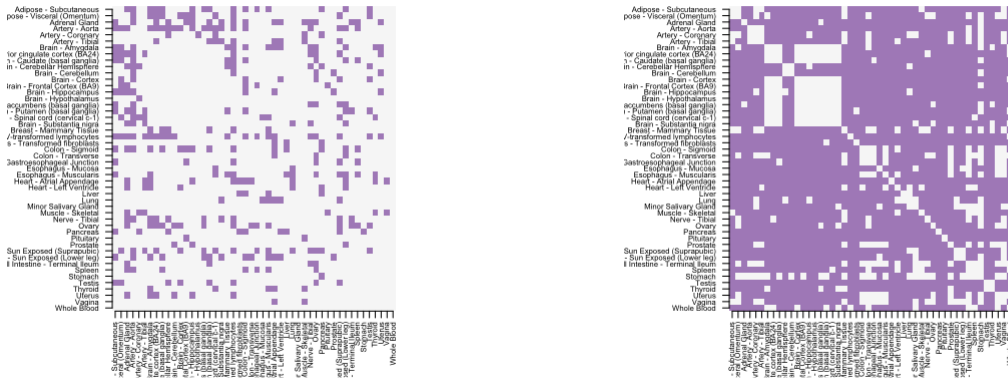


Figure 1. (a): Structure plot of the admixture proportions from model fit with 15 topics/clusters for the 8555 tissue samples coming from 53 tissues in GTEx V6 data based on 16069 cis genes derived using a quality assessment procedure (check Results) . Note that the samples coming from the same tissue have similar admixing patterns. Tissues of same origin, for instance all the Brain tissues, all the arteries seem to cluster together. Also, some other tissues, presumably not of same origin, show markedly similar clustering patterns - for instance Breast mammary tissue, Nerve Tibial and Adipose tissues are very similar in clustering patterns. (b): Structure plot of the cluster memberships from model fit with 4 clusters on just the brain tissue samples. We see more clustering patterns across the Brain tissues in this plot, that was masked by inter tissue variation in (a). Brain cerebellum and cerebellar hemisphere seem to be represented by the green cluster while the Spinal cord and Substantia nigra have high grades of membership in cyan cluster. (c): Structure plot of all tissue samples in GTEx V6 data thinned data with $p_{thin} = 0.0001$ for $K = 15$. The thinning parameter has been chosen so that the GTEx RNA-seq data can be interpreted at the same scale as a scRNA-seq data. The clustering patterns are slightly more noisy compared to the non-thinned version in (a), but overall, the similarity patterns across the tissues are retained.



(a) hierarchy thin 0.1

(b) admixture thin 0.1

Figure 2. A comparison of the hierarchical method with the admixture method. For each pair of tissues, we selected randomly 50 samples and then on the reads data for these 50 samples, we applied the hierarchical clustering method with complete linkage and Euclidean distance and then cut the tree at $K = 2$. We then observed if it separates out the samples coming from the two tissues, in case it does, we color the cell corresponding to that pair of tissues. We apply admixture model on the same data for $K = 2$. Then we fixed one cluster, observed the proportions for that cluster, sorted the samples based on the proportions for that cluster and separated out the samples at the point of maximum jump/fall in the proportions for that cluster. If that separates out the two tissues, we color the cell, else keep it blank. From the graph it seems that the admixture model has been far more successful in separating out different tissues compared to the hierarchical method.

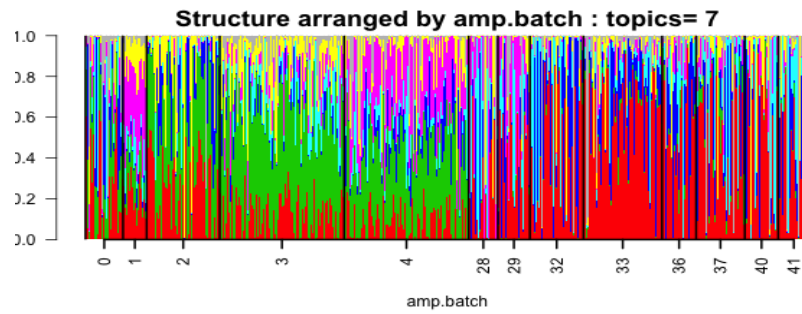


Figure 3. Structure plot of the 1041 single cells for $K=7$ of the Jaitin *et al* data [12] arranged by the amplification batch. It is observed that the clustering patterns within each batch are similar and so, either the amplification batch is driving the clustering or it is confounded with the actual biological effects, making it difficult to interpret these clusters.

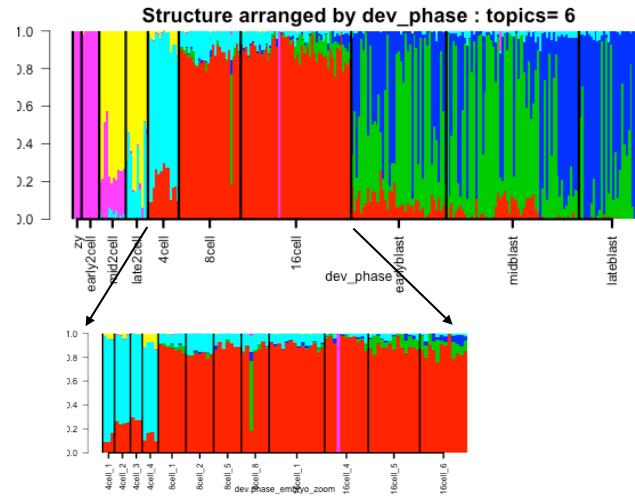
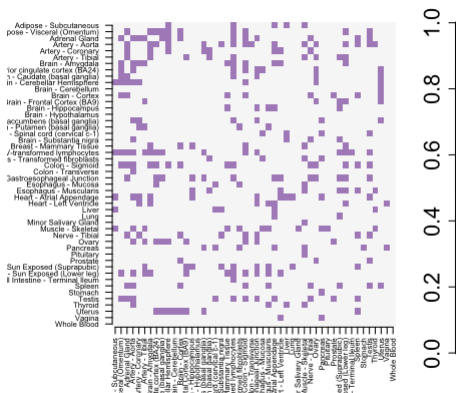
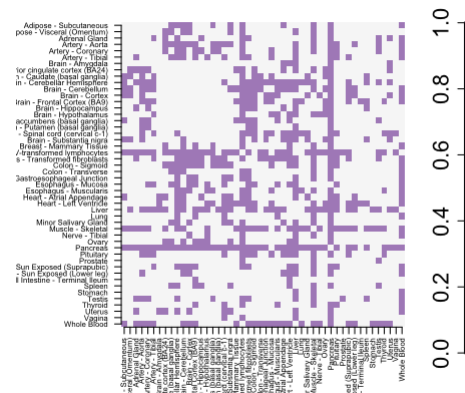


Figure 4. Structure plot of all samples for $K = 6$ of Deng et al data [13], arranged by the preimplantation development phases of the cells and within each phase, arranged in the same order as in the data. Some developmental phases are represented by a single cluster, for example- *zygote/early2cell* while some developmental phases can be written as a mix of two clusters or more clusters.

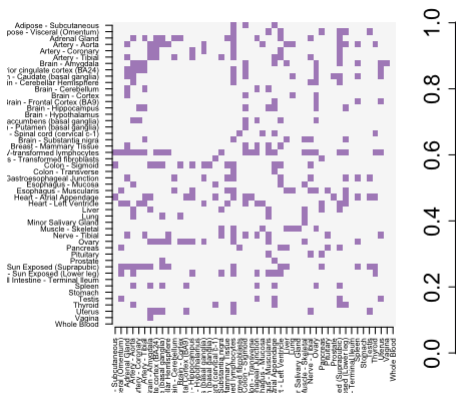
4.1 Supplemental figures



(a) hierarchy thin 0.01



(b) admixture thin 0.01



(c) hierarchy 0.001



(d) admixture thin 0.001

Figure 5. In this graph, we compare the hierarchical clustering method with the admixture method for thinned data with thinning parameters being $p_{thin} = 0.001$ and $p_{thin} = 0.0001$. The color coding scheme is similar to **Fig 3**. Note that the performance of the admixture indeed deteriorates from **Fig 3** in separating out the clusters as is expected. But it still outperforms the hierarchical clustering.

Cluster	Gene names	Proteins	Summary
cluster red (Nerve, Adipose)	FABP4	fatty acid binding protein 4, adipocyte	FABP4 encodes the fatty acid binding protein found in adipocytes, roles include fatty acid uptake, transport, and metabolism
	APOD	apolipoprotein D	encodes a component of high density lipoprotein that has no marked similarity to other apolipoprotein sequences, closely associated with lipoprotein metabolism.
	PLIN1	perilipin 1	coats lipid storage droplets in adipocytes, thereby protecting them until they can be broken down by hormone-sensitive lipase.
cluster blue (Arteries, Esophagus)	MYH11	myosin, heavy chain 11, smooth muscle	functions as a major contractile protein, converting chemical energy into mechanical energy through the hydrolysis of ATP.
	ACTA2	actin, alpha 2, smooth muscle, aorta	protein encoded by this gene belongs to the actin family of proteins, which are highly conserved proteins that play a role in cell motility, structure and integrity, defects in this gene cause aortic aneurysm familial thoracic type 6.
	ACTG2	actin, gamma 2, smooth muscle, enteric	encodes actin gamma 2; a smooth muscle actin found in enteric tissues, involved in various types of cell motility and in the maintenance of the cytoskeleton.
cluster corn-flowerblue (Brain)	MBP	myelin basic protein	major constituent of the myelin sheath of oligodendrocytes and Schwann cells in the nervous system
	GFAP	glial fibrillary acidic protein	encodes one of the major intermediate filament proteins of mature astrocytes, mutations causes Alexander disease.
	SNAP25	synaptosomal-associated protein, 25kDa	this gene product is a presynaptic plasma membrane protein involved in the regulation of neurotransmitter release.
cluster black (Testis)	PRM2	protamine 2	Protamines are the major DNA-binding proteins in the nucleus of sperm
	PRM1	protamine 1	Protamines are the major DNA-binding proteins in the nucleus of sperm
	PHF7	PHD finger protein 7	This gene is expressed in the testis in Sertoli cells but not germ cells, regulates spermatogenesis.
cluster cyan (Thyroid, Stomach)	TG	thyroglobulin	thyroglobulin produced predominantly in thyroid gland, synthesizes thyroxine and triiodothyronine, associated with Graves disease and Hashimoto's thyroiditis.
	LIPF	lipase, gastric	encodes gastric lipase, an enzyme involved in the digestion of dietary triglycerides in the gastrointestinal tract, and responsible for 30 % of fat digestion processes occurring in human.
	PGC	progastricsin (pepsinogen C)	encodes an aspartic proteinase that belongs to the peptidase family A1. The encoded protein is a digestive enzyme that is produced in the stomach and constitutes a major component of the gastric mucosa, associated with susceptibility to gastric cancers.
cluster dark blue (Skin)	KRT10	keratin 10, type I	encodes a member of the type I (acidic) cytokeratin family, mutations associated with epidermolytic hyperkeratosis.
	KRT1	keratin 1, type II	specifically expressed in the spinous and granular layers of the epidermis with family member KRT10 and mutations in these genes have been associated with bullous congenital ichthyosiform erythroderma.
	KRT2	keratin 2, type II	expressed largely in the upper spinous layer of epidermal keratinocytes and mutations in this gene have been associated with bullous congenital ichthyosiform erythroderma.
cluster brown4 (Cells fibroblasts)	FN1	fibronectin 1	Fibronectin is involved in cell adhesion, embryogenesis, blood coagulation, host defense, and metastasis.
	COL1A1	collagen, type I, alpha 1	Mutations in this gene associated with osteogenesis imperfecta types I-IV, Ehlers-Danlos syndrome type and Classical type, Caffey Disease.
	COL1A2	collagen, type I, alpha 2	Mutations in this gene associated with osteogenesis imperfecta types I-IV, Ehlers-Danlos syndrome type and Classical type, Caffey Disease.

Cluster	Gene names	Proteins	Summary
cluster burly-wood (Lung)	SFTPB	surfactant protein B	an amphipathic surfactant protein essential for lung function and homeostasis after birth, mutations cause pulmonary alveolar proteinosis, fatal respiratory distress in the neonatal period.
	SFTPA2	surfactant protein A2	Mutations in this gene and a highly similar gene located nearby, which affect the highly conserved carbohydrate recognition domain, are associated with idiopathic pulmonary fibrosis.
	SFTPA1	surfactant protein A1	encodes a lung surfactant protein that is a member of C-type lectins called collectins, associated with idiopathic pulmonary fibrosis.
cluster darkgold-enrod (Muscle skeletal)	MYH1	myosin, heavy chain 1, skeletal muscle, adult	a major contractile protein which converts chemical energy into mechanical energy through the hydrolysis of ATP.
	NEB	nebulin	encodes nebulin, a giant protein component of the cytoskeletal matrix that coexists with the thick and thin filaments within the sarcomeres of skeletal muscle, associated with recessive nemaline myopathy.
	MYH2	myosin, heavy chain 2, skeletal muscle, adult	encodes a member of the class II or conventional myosin heavy chains, and functions in skeletal muscle contraction.
cluster darkgray (Whole Blood)	HBB	hemoglobin, beta	mutant beta globin causes sickle cell anemia, absence of beta chain/reduction in beta globin leads to thalassemia.
	HBA2	hemoglobin, alpha 2	deletion of alpha genes may lead to alpha thalassemia.
	HBA1	hemoglobin, alpha 1	deletion of alpha genes may lead to alpha thalassemia.
cluster deepsky-blue (Heart)	NPPA	natriuretic peptide A	protein encoded by this gene belongs to the natriuretic peptide family, associated with atrial fibrillation familial type 6.
	MYH6	myosin, heavy chain 6, cardiac muscle, alpha	encodes the alpha heavy chain subunit of cardiac myosin, mutations in this gene cause familial hypertrophic cardiomyopathy and atrial septal defect 3.
	ACTC1	actin, alpha, cardiac muscle 1	protein encoded by this gene belongs to the actin family, associated with idiopathic dilated cardiomyopathy (IDC) and familial hypertrophic cardiomyopathy (FHC).
cluster dark khaki (Esophagus mucosa)	KRT13	keratin 13, type I	protein encoded by this gene is a member of the keratin gene family, associated with the autosomal dominant disorder White Sponge Nevus.
	KRT4	keratin 4, type II	protein encoded by this gene is a member of the keratin gene family, associated with White Sponge Nevus, characterized by oral, esophageal, and anal leukoplakia.
	CRNN	cornulin	may play a role in the mucosal/epithelial immune response and epidermal differentiation.
cluster firebrick (Pancreas)	PRSS1	protease, serine 1	secreted by pancreas, associated with pancreatitis
	CPA1	carboxypeptidase A1	secreted by pancreas, linked to pancreatitis and pancreatic cancer
	PNLIP	pancreatic lipase	encodes a carboxyl esterase that hydrolyzes insoluble, emulsified triglycerides, and is essential for the efficient digestion of dietary fats. This gene is expressed specifically in the pancreas.
cluster dark orchid (Liver)	MUC7	mucin 7, secreted	encodes a small salivary mucin, thought to play a role in facilitating the clearance of bacteria in the oral cavity and to aid in mastication, speech, and swallowing, associated with susceptibility to asthma.
	ALB	albumin	functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones and plays a role in stabilizing extracellular fluid volume.
	HP	haptoglobin	encodes a preproprotein, which subsequently produces haptoglobin, linked to diabetic nephropathy, Crohn's disease, inflammatory disease behavior and reduced incidence of Plasmodium falciparum malaria.
cluster hotpink (Pituitary)	PRL	prolactin 2	encodes the anterior pituitary hormone prolactin. This secreted hormone is a growth regulator for many tissues, including cells of the immune system.
	GH1	growth hormone 1	expressed in the pituitary, play an important role in growth control, mutations in or deletions of the gene lead to growth hormone deficiency and short stature.
	POMC	proopiomelanocortin	synthesized mainly in corticotroph cells of the anterior pituitary, mutations in this gene have been associated with early onset obesity, adrenal insufficiency, and red hair pigmentation.

4.2 Supplementary Table 1

Cluster	Gene names	Proteins	Summary
cluster 1, red	ATP1A2	ATPase, Na ⁺ /K ⁺ transporting, alpha 2 polypeptide	responsible for establishing and maintaining the electrochemical gradients of Na and K ions across the plasma membrane, mutations in this gene result in familial basilar or hemiplegic migraines, and in a rare syndrome known as alternating hemiplegia of childhood.
	CLU	clusterin	protein encoded by this gene is a secreted chaperone that can under some stress conditions also be found in the cell cytosol, also involved in cell death, tumor progression, and neurodegenerative disorders.
	DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	encodes a member of the DnaJ or Hsp40 (heat shock protein 40 kD) family of proteins, that stimulates the ATPase activity of Hsp70 heat-shock proteins to promote protein folding and prevent misfolded protein aggregation.
cluster 2, green	SNAP25	synaptosomal-associated protein, 25kDa	Synaptic vesicle membrane docking and fusion is mediated by SNAREs located on the vesicle membrane (v-SNAREs) and the target membrane (t-SNAREs), involved in the regulation of neurotransmitter release.
	ENO2	enolase 2 (gamma, neuronal)	encodes one of the three enolase isoenzymes found in mammals, is found in mature neurons and cells of neuronal origin.
	CHGB	chromogranin B	encodes a tyrosine-sulfated secretory protein abundant in peptidergic endocrine cells and neurons. This protein may serve as a precursor for regulatory peptides.
cluster 3, blue	CALM3	calmodulin 3 (phosphorylase kinase, delta)	is a calcium binding protein that plays a role in signaling pathways, cell cycle progression and proliferation.
	FBXL16	F-box and leucine-rich repeat protein 16	Members of the F-box protein family, such as FBXL16, are characterized by an approximately 40-amino acid F-box motif.
	UCHL1	ubiquitin carboxyl-terminal esterase L1	specifically expressed in the neurons and in cells of the diffuse neuroendocrine system. Mutations in this gene may be associated with Parkinson disease.
cluster 4, cyan	MBP	myelin basic protein	protein encoded is a major constituent of the myelin sheath of oligodendrocytes and Schwann cells in the nervous system.
	MYH11	glial fibrillary acidic protein	encodes major intermediate filament proteins of mature astrocytes, a marker to distinguish astrocytes during development, mutations in this gene cause Alexander disease, a rare disorder of astrocytes in central nervous system.
	ACTA2	secreted protein, acidic, cysteine-rich (osteonectin)	encodes a cysteine-rich acidic matrix-associated protein, required for the collagen in bone to become calcified, in extracellular matrix synthesis and cell shape promotion, associated with tumor suppression.