Principal component analysis-based unsupervised feature extraction applied to single-cell gene expression analysis¹

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Abstract. Due to missed sample labeling, unsupervised feature selection during single-cell (sc) RNA-seq can identify critical genes under the experimental conditions considered. In this paper, we applied principal component analysis (PCA)-based unsupervised feature extraction (FE) to identify biologically relevant genes from mouse and human embryonic brain development expression profiles retrieved by scRNA-seq. When evaluating the biological relevance of selected genes by various enrichment analyses, the PCA-based unsupervised FE outperformed conventional unsupervised approaches that select highly variable genes as well as bimodal genes in addition to the recently proposed dpFeature.

Keywords: principal component analysis, feature selection, embryonic brain development

1 Introduction

Single-cell analysis is a newly developed high-throughput technology that enables us to identify gene expression profiles of individual genes. There is a critical difference between single-cell analysis and conventional tissue-specific analysis; tissue samples are labeled distinctively (e.g., patients vs healthy controls) while single-cell samples are not always. Inevitably, we need an unsupervised methodology, such as highly variable genes [1, 2] and bimodal genes [3] or recently proposed dpFeature [4]. Highly variable genes are able to select genes that can discriminate the underlying cluster structure depicted by unsupervised clustering, namely tSNE [5]. In contrast, bimodal genes are selected because unimodal genes are unlikely to be expressed distinctly between multiple classes, e.g., healthy controls and patients. While the combination of tSNE and highly variable genes or bimodal genes approach is often employed and empirically successful, biological validation of selected genes is rarely addressed. Generally, very few studies have evaluated multiple gene selection procedures for

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single-cell RNA-seq. The purpose of this paper is to compare multiple gene selection procedures and to identify the best method.

Principal component analysis (PCA)-based unsupervised feature extraction (FE) has been previously shown to be an effective method to investigate tissue-specific gene expression profiles [6–28]. In this paper, PCA-based unsupervised FE was applied to single-cell gene expression analysis. In addition, its effectiveness was evaluated from the biological point of view and compared to conventional approaches, including the highly variable genes approach as well as the bimodal genes approach.

2 Materials and Methods

2.1 Gene expression

Gene expression profiles used in this study were downloaded from the Gene Expression Omnibus (GEO) database under the GEO ID GSE76381. Specifically, the files named “GSE76381_EmbryoMoleculeCounts.cef.txt.gz” (for human) and “GSE76381_MouseEmbryoMoleculeCounts.cef.txt.gz” (for mouse) were downloaded. Gene expression profiles were standardized such that each sample had zero mean and unit standard deviation. That is, when x_{ij} represented the expression of i th gene in j th sample, $\sum_i x_{ij} = 0$ and $\sum_i x_{ij}^2 = N$ where N represented the number of genes. These two gene expression profiles were generated from single-cell RNA-seq datasets that represented the following: human embryo ventral midbrain cells between 6 and 11 weeks of gestation, mouse ventral midbrain cells at six developmental stages between E11.5 to E18.5, Th+ neurons at P19–P27, and FACS-sorted putative dopaminergic neurons at P28–P56 from Slc6a3-Cre/TdTomato mice.

2.2 PCA-based unsupervised FE

Suppose that matrix X has element x_{ij} representing the gene expression of i th gene of j th sample, then the k th PC score attributed to i th gene u_{ki} can be computed as i th element of k th eigen vector of Gram matrix XX^T as

$$XX^T \mathbf{u}_k = \lambda_k \mathbf{u}_k$$

k th PC loading attributed to j th sample, v_{kj} , can be obtained by $\mathbf{v}_k = X^T \mathbf{u}_k$ since

$$X^T X \mathbf{v}_k = X^T X X^T \mathbf{u}_k = X^T \lambda_k \mathbf{u}_k = \lambda_k \mathbf{v}_k$$

Initially, PC loading attributed to samples were identified, which were coincident with distinction between considered class labels attributed to the samples. Since single-cell RNA-seq (scRNA-seq) lacks sample labeling, the first k PCs were employed. Subsequently, assuming multiple Gaussian distribution to PC scores, P -values were attributed to gene i using χ^2 distribution,

$$P_i = P_{\chi^2} \left[> \sum_{k=1}^K \left(\frac{u_{ki}}{\sigma_k} \right)^2 \right]$$

where σ_k represented standard deviation and $P_{\chi^2}[> x]$ represented the cumulative probability of χ^2 distribution that the argument was larger than x . The summation was taken over the selected first k PC scores. Obtained P -values were adjusted using Benjamini and Hochberg (BH) criterion [29] and genes associated with adjusted P -values less than 0.01 were selected.

2.3 Enrichment analysis

In order to perform enrichment analyses, selected genes were uploaded to Enrichr [30], which included various enrichment analysis.

2.4 Highly variable genes

The procedure was performed as previously described [1], and a brief description is provided below. Suppose that x_{ij} represented gene expression of i th gene of j th sample, then the mean expression of i th gene was defined as

$$\mu_i = \frac{\sum_j x_{ij}}{M}$$

where M represented the number of samples. The standard deviation σ_i was defined as

$$\sigma_i^2 = \sum_j \frac{(x_{ij} - \mu_i)^2}{M}$$

Following, the regression relation between σ_i and μ_i was assumed as

$$\log_{10} \left(\frac{\sigma_i}{\mu_i} \right) = \frac{1}{2} \log_{10} \left(\frac{\beta}{\mu_i} + \alpha \right) + \epsilon_i$$

where α and β were the regression coefficients. Subsequently, P -value P'_i was attributed to ϵ_i assuming χ^2 distribution as

$$P'_i = P_{\chi^2} \left[> \left(\frac{\epsilon_i}{\sigma'} \right)^2 \right]$$

where σ' was the standard deviation. Finally, genes i with an adjusted P -value less than 0.01 by BH criterion were selected as highly variable genes.

2.5 Bimodal genes

P-values attributed to genes that reject the null hypothesis (unimodal genes) were computed by the `dip.test` function in R using the default setting. Obtained P-values were adjusted by BH criterion, and genes associated with adjusted P-values less than 0.01 were selected.

2.6 dpFeature

dpFeature was performed using `monocle` package in R. More detailed instruction was in Supplementary Document.

3 Results

Applying PCA-based unsupervised FE to human and mouse embryonic brain developmental gene expression profiles, 116 genes for human ($k = 2$, i.e., the first two PC scores were used for gene selection) and 118 genes for mouse ($k = 3$, i.e., the first three PC scores were used for gene selection) were selected, respectively. Interestingly, 53 genes of the selected genes were common both in human and mouse samples. The large overlap between the two genes sets with highly restricted numbers of genes is not plausible to occur by chance; therefore, it is very likely that these selected genes play critical roles in embryonic midbrain development.

To validate the biological relevance of the selected genes, various enrichment analyses were applied using Enrichr. Table 1 shows an Enrichment analysis by Enrichr, “MGI Mammalian Phenotype 2017”, of the 118 genes selected in mice. Among the top five ranked terms, four were brain-related terms. As all terms described abnormal morphology, this is an expected result since fetal gene expression is often distinct from adults that lack fetal-specific gene expression. Table 2 and Table 3 show another Enrichment analysis by Enrichr, “Allen Brain Atlas down”, of the 116 genes selected in humans and 118 genes selected in mice, respectively. All genes were downregulated in brain regions. This is again reasonable since fetal genes expressed in the embryo is unlikely to be expressed in adult tissue. The lack of fetal brain-specific gene expression in the selected genes can also be seen in Table 4 and Table 5.

Table 1. Enrichment analysis by Enrichr, “MGI Mammalian Phenotype 2017”, of 118 selected genes in mice (Top 5 ranked terms)

Term	Overlap	P-value	Adjusted P-value
MP:0000788_abnormal_cerebral_cortex_morphology	7/145	2.45×10^{-5}	4.55×10^{-5}
MP:0003651_abnormal_axon_extension	5/48	9.18×10^{-6}	4.55×10^{-3}
MP:0000812_abnormal_dentate_gyrus_morphology	5/58	2.34×10^{-5}	4.55×10^{-3}
MP:0000807_abnormal_hippocampus_morphology	5/86	1.56×10^{-4}	2.04×10^{-2}

MP:0000819_abnormal_olfactory_bulb_morphology	4/48	1.83×10^{-4}	2.04×10^{-2}
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Table 2. Enrichment analysis by Enrichr, “Allen Brain Atlas down”, of 116 selected genes in humans (Top 5 ranked terms)

Term	Overlap	P-value	Adjusted P-value
periventricular stratum of cerebellar vermis	18/300	1.33×10^{-13}	3.72×10^{-11}
Simple lobule	18/300	1.33×10^{-13}	3.72×10^{-11}
Simple lobule, molecular layer	18/300	1.33×10^{-13}	3.72×10^{-11}
Simple lobule, granular layer	18/300	1.33×10^{-13}	3.72×10^{-11}
white matter of cerebellar vermis	18/300	1.33×10^{-13}	3.72×10^{-11}

Table 3. Enrichment analysis by Enrichr, “Allen Brain Atlas down”, of 118 selected genes in mice (Top 5 ranked terms)

Term	Overlap	P-value	Adjusted P-value
Pyramus (VIII), granular layer	18/300	1.81×10^{-13}	4.66×10^{-11}
Pyramus (VIII)	18/300	1.81×10^{-13}	4.66×10^{-11}
Pyramus (VIII), molecular layer	18/300	1.81×10^{-13}	4.66×10^{-11}
Paraflocculus, molecular layer	18/300	1.81×10^{-13}	4.66×10^{-11}
Cerebellar cortex	18/300	1.81×10^{-13}	4.66×10^{-11}

Table 4. Enrichment analysis by Enrichr, “GTEx Tissue Sample Gene Expression Profiles down”, of 116 selected genes in humans (Top 5 ranked terms)

Term	Overlap	P-value	Adjusted P-value
GTEx-Q2AG-0011-R10A-SM-2HMLA_brain_female_40-49_years	51/1467	1.47×10^{-27}	3.29×10^{-24}
GTEx-TSE9-3026-SM-3DB76_brain_female_60-69_years	49/1384	1.06×10^{-26}	1.19×10^{-23}
GTEx-S7SE-0011-R10A-SM-2XCDF_brain_male_50-59_years	44/1278	3.20×10^{-23}	1.43×10^{-20}
GTEx-QMR6-1426-SM-32PLA_brain_male_50-59_years	41/1066	2.57×10^{-23}	1.43×10^{-20}
GTEx-RNOR-2326-SM-2TF4I_brain_female_50-59_years	47/1484	2.02×10^{-23}	1.43×10^{-20}

Table 5. Enrichment analysis by Enrichr, “GTEx Tissue Sample Gene Expression Profiles down”, of 118 selected genes in mice (Top 5 ranked terms)

Term	Overlap	P-value	Adjusted P-value
GTEx-U8XE-0126-SM-	15/376	6.13×10^{-9}	3.45×10^{-6}

4E3I3_testis_male_30-39_years			
GTEX-X4XX-0011-R10B-SM-46MWO_brain_male_60-69_years	23/938	5.25×10^{-9}	3.45×10^{-6}
GTEX-U4B1-1526-SM-4DXSL_testis_male_40-49_years	13/282	1.23×10^{-8}	3.71×10^{-6}
GTEX-Q2AG-0011-R10A-SM-2HMLA_brain_female_40-49_years	29/1467	5.11×10^{-9}	3.45×10^{-6}
GTEX-RNOR-2326-SM-2TF4I_brain_female_50-59_years	29/1484	6.62×10^{-9}	3.45×10^{-6}

While these above results are highly significant, they are also negative results. As such, we speculated whether these results could provide enough support and confidence for the selected genes. Therefore, in order to show positive results, gene expression in the embryonic brain was assessed. Subsequently, it was found that these genes were enriched in “Jensen TISSUES” by Enrichr. Table 6 shows that selected genes are enriched in the embryonic brains of humans and mice, respectively. Thus, confidence of the selected genes is supported by both negative and positive selection.

We further sought the regulatory elements that can regulate the selected genes, as there are likely common regulatory elements if the selected genes are truly co-expressed. In order to perform this, we investigated “ENCODE and ChEA Consensus TFs from ChIP-X” by Enrichr for both human and mouse, respectively. Specifically, 42 TFs for humans and 23 TFs for mice were associated with adjusted *P*-values less than 0.01 (Table 7). Thus, they are likely co-regulated by these TFs. Moreover, most mouse TFs were also identified in humans (bold faces in Table 7). Therefore, it is very likely that we successfully identified common (species non-specific or conserved) TFs that regulate genes expression during embryonic brain development.

Additionally, we assessed whether the TF constructs functioned cooperatively. These TFs were uploaded to regnetwork server [31], and TF networks were identified as shown in Fig. 1. It is evident, even partially, that these TFs interact with each other.

We also investigated whether the identified TFs were related to fetal brain development. TAF7 was reported to play a critical role in embryonic development [32]. KAT2A, ATF2 and TAF1 were also suggested to be included in brain development [33]. BRCA1 has been shown to play critical roles in brain development [34]. Additionally, CEBPD and CREB were reported to be related to brain disease [35, 36]. E2F1 was reported to be related to postnatal brain development [37] while functional EGR1 was found in the embryonic rat brain [38]. PML and SIN3A were also reported to be involved in brain development [39][40]. TCF3 has been shown to play a role in zebrafish brain development [41]. YY1 was also reported in brain development [42]. In conclusion, most of the selected genes in common (bold faces in Table 7) between humans and mice are related to brain development. Thus, the selection of genes is possibly reasonable.

Table 6. Selected gene enrichment in the embryonic brain of “Jensen TISSUES” by Enrichr

Term	Overlap	P-value	Adjusted P-value
Human			
Embryonic_brain	71/4936	2.52×10^{-16}	4.07×10^{-15}
Mouse			
Embryonic_brain	75/4936	8.90×10^{-20}	1.06×10^{-18}

Table 7. TF enrichment in “ENCODE and ChEA Consensus TFs from ChIP-X” by Enrichr for human and mouse (Bold TFs are common)

human	ATF2, BCL3, BCLAF1, BHLHE40, BRCA1, CEBPB, CEBPD, CHD1, CREB1, CTCF, E2F1, E2F4, EGR1, ELF1, ETS1, FLI1, GABPA, KAT2A, KLF4, MAX, MYC, NANOG, NELFE, NFYA, NFYB, NR2C2, PBX3, PML, RELA, SALL4, SIN3A, SIX5, SOX2, SP1, SPI1, TAF1, TAF7, TCF3, USF2, YY1, ZBTB33, ZMIZ1
mouse	ATF2, BCL3, BRCA1, CEBPB, CEBPD, CHD1, CREB1, E2F1, EGR1, KAT2A, KLF, MAX, MYC, NELFE, PBX3, PML, RELA, SIN3A, TAF1, TAF7, TCF3, YY1, ZMIZ1

In addition to the unconventional PCA-based unsupervised FE, we applied the widely used highly variable genes approach to the present data set and determined which strategy was more consistent with the enrichment analysis [1]. After applying the highly variable genes strategy, we obtained 168 genes for human and 171 genes for mouse. The numbers of selected genes were similar to those selected by PCA-based unsupervised FE. Additionally, there were 44 commonly selected genes between human and mouse. Thus, the highly variable genes method shows some efficacy.

However, after a detailed investigation, there were very few overlaps between genes selected by PCA-based unsupervised FE and highly variable genes (only four genes were commonly selected by PCA-based unsupervised FE and highly variable genes for both human and mouse). This result did not provide much confidence since genes selected by PCA-based unsupervised FE were demonstrated to be biologically reliable. However, there was still a possibility that highly variable genes were coincident to the enrichment analysis without significant overlap with genes selected by PCA-based unsupervised FE.

tween human and mouse by PCA-based unsupervised FE and highly variable genes, respectively. Furthermore, there were no commonly selected genes between bimodal genes and PCA-based unsupervised FE. Enrichment analyses by Enricher for bimodal genes were also inferior to that of PCA-based unsupervised FE. “MGI Mammalian Phenotype 2017” of 200 bimodal genes selected for mouse included no terms associated with adjusted P-values less than 0.01 (Table S1). Top five ranked terms by “Allen Brain Atlas down” (Tables S2 and S3) were less significant than PCA-based unsupervised FE (Tables 2 and 3) as P-values were generally larger for bimodal genes. In addition, top five ranked terms by “GTEx Tissue Sample Gene Expression Profiles down” included no brain-related terms (Tables S4 and S5). Taken together, all of these suggests that bimodal genes are unlikely to be biologically more reliable than genes selected by PCA-based unsupervised FE.

Nevertheless, bimodal genes are slightly better than highly variable genes. Specifically, “Jensen TISSUES” by Enrichr included Embryonic_brain as a significant term (Table S6). In addition, “GTEx Tissue Sample Gene Expression Profiles down” identified 40 TFs associated with adjusted P-values less than 0.01 for human and mouse, among which as many as 30 TFs were commonly selected (Table S7). These TFs were also highly connected in the regnetworkweb (Figure S1).

Interestingly, among the 30 commonly selected TFs between human and mouse in Table S7, 11 TFs were also commonly selected between human and mouse in Table 7. When considering that no genes were commonly selected between top ranked 200 bimodal genes and genes selected by PCA-based unsupervised FE, the high number of commonly selected TFs between PCA-based unsupervised FE and bimodal genes suggests the robustness of TFs selected. Thus, as an overall evaluation PCA-based unsupervised FE is better than the other two.

Although we have also compared with the newly proposed approach, dpFeature, because of lack of space, it was included in Supplementary document. dpFeature could not select biologically more reliable genes than PCA based unsupervised FE, either.

While PCA is not a new technology and highly variable genes and bimodal genes are also not new concepts, the application to scRNA-seq is innovative and valuable. Therefore, even if methods themselves applied are not new, their application to new technology, scRNA-seq, can be innovative, especially if they have never been applied to scRNA-seq or were successful. Especially if PCA is more successful than even newly proposed approach, e.g., dpFeature.

4 Conclusions

We applied PCA-based unsupervised FE to gene expression profiles retrieved by scRNA-seq analysis. Since scRNA-seq primarily lacks the labeling of sample (each cell), an unsupervised approach is necessary. Genes selected by PCA-based unsupervised FE for human and mouse embryonic brain development were not only associated with numerous significant biological terms enrichment but also highly coincident between mice and humans. In contrast, the frequently employed highly variable genes

approach as well as the bimodal genes approach or recently proposed dpFeature could not identify as many genes associated with significant biological terms enrichment as the PCA-based unsupervised FE achieved. Thus, PCA-based unsupervised FE is more favorable than the highly variable genes approach, the bimodal genes approach or dpFeature from the biological point of view.

5 Supplementary materials

Supplementary materials are available at <https://github.com/tagtag/SC>.

Full list of genes, enrichment analyses for genes selected by PCA-based unsupervised FE, bimodal genes and dpFeature, supplementary document that includes supplementary tables and figure for bimodal gene analyses and dpFeature, R codes that identify genes selected by PCA-based unsupervised FE, highly variable genes, bimodal genes and dpFeature.

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