Survey of Dimensionality Reduction Techniques and their Applications for Classifying Disease State in Human RNA-seq Data

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Abstract

Biological data pose many challenges for applying machine learning methods, particularly low sample sizes and high dimensionality due to the large number of features being measured. Low sample sizes make the use of recent deep-learning and generative methods infeasible; as such, simpler models are still widely used. Here, we survey the impact of four dimensionality reduction techniques (i.e., *a priori* aggregation, principal component analysis, kernelized principal component analysis, and nonnegative matrix factorization) on improving the accuracy of five different simple classification algorithms (i.e., decision tree, k-nearest neighbors, naïve Bayes, random forest, and support vector machine) across nine different RNA sequencing samples from the Gene Expression Omnibus. We find that the impact of dimensionality reduction is highly variable and likely depends on underlying biological context specific to the data. We note that principal component analysis, kernelized principal component analysis, and nonnegative matrix factorization are nearly identical in their results for these data and that the number of reduced dimensions has a much greater impact than the choice of dimensionality reduction method.

1 Introduction

Machine learning techniques have been successfully applied to answer biological questions by analyzing a wide variety of biological data, such as analyzing sequences to identify molecular interactions between RNA molecules [1], analyzing UV damage patterns to identify transcription factor binding sites [2], and most famously analyzing structural data to predict protein folding [3]. Gene expression data are of particular interest, because they reveal how cells are behaving at a molecular level. Within a cell, the information required to perform cellular activities, such as dividing, taking up nutrients, performing metabolic reactions, etc., is stored in the cell's DNA. DNA is a set of molecules made from series of repeated molecular units called nucleotides. There are four different

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nucleotides used in DNA: adenine (A), cytosine (C), guanine (G), and thymine (T). The information contained in the DNA is encoded in the sequence of nucleotides and is organized into units called genes. Genes are expressed when the cell creates a temporary copy of the gene's nucleotide sequence as a molecule of messenger RNA (mRNA) through a process known as transcription. The RNA molecule is structured similarly to the DNA, consisting of a sequence of the nucleotide bases A, C, G, and U (where U represents uracil; uracil replaces thymine in RNA for evolutionary and chemical reasons). The mRNA transcript is then translated into a sequence of amino acids which folds into a protein. The protein's function ultimately confers traits to the organism. Cells tightly regulate which genes they express and the degree to which these genes are expressed. The exact complement of expressed genes determines the cell's behavior, cell type, etc. Numerous diseases, notably cancers, ultimately result from aberrant gene expression. [4]

Perturbations to the cell (i.e., disease or infection) will alter its gene expression [4]. This begs the converse question of whether disease state can be inferred from the gene expression, which can be neatly phrased as a classification problem and naturally leads to an practical application of machine learning techniques. The goal is to gather gene expression data on both diseased and healthy individuals then train a machine learning model to classify the disease state of individuals based on their gene expression. To measure which genes are being expressed by a population of cells, biologists can perform bulk RNA sequencing (bulk RNA-seq), where the RNA is isolated and the exact nucleotide sequences of the isolated RNA molecules are found. Each RNA molecule corresponds to a gene, the identity of which can be deduced from the sequence. The degree to which any given gene is being expressed can then be inferred from the relative abundance of isolated transcripts which correspond to the gene. [5] The National Institutes of Health has also encouraged researchers to deposit their RNA-seq data on the Gene Expression Omnibus (GEO), available at `https://www.ncbi.nlm.nih.gov/sites/GDSbrowser`.

However, like with any biological data, applying machine learning techniques to RNA-seq data poses many challenges. Particularly, the time and cost of conducting biological experiments leads to low sample sizes, especially if the experiment involves human subjects. Additionally, bulk RNA-seq can only measure gene expression after the selection of a set of genes to measure, and there are many different slight variations on the bulk RNA-seq method (i.e., sequencing platform, data normalization methods, etc.). This results in a repository of observational data using a wide variety of slightly different methodologies and gene sets, which makes it difficult to meaningfully compare data between bulk RNA-seq samples or to sensibly combine data across multiple bulk RNA-seq samples.

Often, the expression of tens of thousands of genes is measured–orders of magnitude more than the typical sample size. This results in data with a high dimensionality which far exceeds the number of observations. The advent of single-cell techniques, particularly single-cell RNA sequencing (scRNA-seq), has partially mitigated this problem. The innovation of scRNA-seq is that instead of isolating RNA from a population of cells, the RNA is sequenced at the level of individual cells, meaning that each cell can act as a separate observation. [6] This allows for the collection of large samples, which have supported the pre-training of deep-learning models such as scGPT [7], Tahoe-x1 [8], and CellFM [9]. However, single-cell techniques are expensive; traditional bulk RNA-seq is still common [10]. Pre-trained deep-learning models for bulk RNA-seq data, namely BulkRNABert and DCNet [11, 12], are intended for specific applications in cancer. These models use data from the cancer genome atlas and expect a fixed set of genes as features for their inputs [11–13], which makes them unsuitable for applications to general bulk RNA-seq samples.

The challenges posed by bulk RNA-seq data, particularly the small sample sizes and the lack of a suitable pre-trained model, often severely limit the practical application of modern generative or deep-learning models. As such, simpler machine learning models that do not require large sample sizes are still commonly used. However, the high dimensionality of the data still poses a challenge, motivating the application of dimensionality reduction. Here, we conduct a survey of four dimensionality reduction techniques–*a priori* aggregation, principal component analysis (PCA), kernelized principal component analysis (kPCA), and nonnegative matrix factorization (NMF)–and assess their impact on the accuracy of five simple machine learning models–the decision tree (DT), k-nearest neighbors (k-NN), the naïve Bayes classifier (NB), the random forest (RF), and the support vector machine (SVM)–in classifying disease state from bulk RNA-seq data across nine samples (Tables 1, 2).

Table 1: Sample details

Abbr.	GEO Accession	Title	Platform	Ref.
UCC	GDS1615	Ulcerative colitis and Crohn's disease comparison: peripheral blood mononuclear cells	GPL96	[16]
SCLC	GDS2373	Squamous cell lung carcinomas	GPL96	[15]
SEC	GDS2771	Large airway epithelial cells from cigarette smokers with suspect lung cancer	GPL96	[17]
MDS	GDS3795	Myelodysplastic syndrome: CD34+ hematopoietic stem cells	GPL570	[18]
ALL	GDS4206	Pediatric acute leukemia patients with early relapse: white blood cells	GPL570	[19]
HIV	GDS4228	HIV infection and Antiretroviral Therapy effects on mitochondria in various tissues	GPL9392	[20]
JIA	GDS4267	Systemic juvenile idiopathic arthritis and non-systemic JIA subtypes: peripheral blood mononuclear cells	GPL570	[21]
GBM	GDS5205	Long-term adult survivors of glioblastoma: primary tumors	GPL570	[22]
MDG	GDS963	Macular degeneration and dermal fibroblast response to sublethal oxidative stress	GPL8300	[14]

Table 2: Sample parameters

			Classes	
Abbr.	Samples	Features	Num.	Description
UCC	127	22283	3	Normal, ulcerative colitis, Crohn's disease
SCLC	130	22283	6	Type Ia, Ib, IIa, IIb, IIIa, IIIb
SEC	192	22283	3	No cancer, suspected cancer, cancer
MDS	200	54675	2	Healthy, myelodysplastic syndrome
ALL	197	54675	3	Early, late, no relapse
HIV	166	4825	2	HIV-negative, HIV-positive
JIA	154	54675	3	No JIA, systemic JIA, non-systemic JIA
GBM	70	54675	3	Short-term, intermediate, long-term overall survival
MDG	36	12625	2	Healthy, macular degeneration

2 Methods

2.1 Data

Nine human bulk RNA-seq samples of varying size from the GEO (`https://www.ncbi.nlm.nih.gov/sites/GDSbrowser`) were surveyed to assess the generalizability of our findings (Tables 1, 2). The samples were chosen to encompass a wide range of sample size, number of class labels, and number of features. The most limiting case is the MDG sample, which has just 36 observations in 12625 dimensions [14]. Additionally, the SCLC sample has 130 observations with six classes that are all similar, each describing a different stage of lung cancer, and poses a challenging classification problem [15]. The samples were downloaded as "full" SOFT files (text files) from the GEO. Gene expression values were extracted and stored in a tab-delimited tabular format. Features corresponding to sequencing platform controls rather than genes were removed from consideration.

2.2 Models

Five simple machine learning strategies were surveyed: DT, k-NN, NB, RF, and SVM. Many combinations of different values for the model hyperparameters were tested for every sample and for every dimensionality reduction strategy. For the DT, the decision boundaries were chosen by optimizing the entropy, and the tree was pruned to maximum depths of 2, 3, and 4. For k-NN, the

Minkowski distance was used with exponents of $p = 1, 2, 3, 4$ and values of $k = 3, 4, 5$. The RF used 100, 200, or 500 estimators, and individual estimators were pruned to maximum depths of 2, 3, and 4. For the SVM, the radial basis function (RBF), linear, and quadratic kernels were tested across regularization parameter values of $C = 10^{-3}, 10^{-2}, 10^{-1}, 10^{0}, 10^{1}, 10^{2}, 10^{3}$. All models were implemented via `scikit-learn` version `1.7.2` with all other settings left at defaults [23].

2.3 Dimensionality Reduction Strategies

As a baseline, no dimensionality reduction was applied. The models were trained on the samples in their full dimensions. This starting point allowed us to explore the practical consequences of the full dimension of each sample and to directly observe the benefit or detriment of each dimensionality reduction technique.

The first dimensionality reduction strategy we applied was aggregation of the data across features based on *a priori* biological knowledge. Genes do not function independently. For instance, some genes encode transcription factors, proteins which directly alter the expression of other genes, and genes whose products all function as part of the same biological pathway or system are often co-regulated. [4] The features of the samples, which correspond to genes, can therefore be grouped together based on their biological functionality. Genes are assigned a gene ontology (GO) category by the GO consortium [24, 25] and the dimensionality of the data is then reduced from the number of individual genes n to the number of GO categories m. The value corresponding to each GO category is taken to be the mean of the values corresponding to each gene which belongs to that category. Three different GO categorization schemes were utilized: component, which corresponds to where the gene product functions (e.g., mitochondria, nucleus); function, which corresponds to what the gene product does (e.g., ATP binding, protein binding); and process, which corresponds to what biological pathway the gene product takes part in (e.g., MAPK cascade, inflammatory response).

The other three dimensionality reduction techniques we applied (i.e., PCA, kPCA, and NMF) are each commonly applied in biological contexts. Briefly, PCA reduces dimensionality by creating new variables which are linear combinations of the original ones, keeping only the new components that capture the most variance. kPCA exploits the fact that PCA can be calculated with only the inner product and utilizes the "kernel trick" (here, the RBF kernel was used). Lastly, NMF decomposes the data matrix into two lower-dimensional matrices whose products approximate the original data matrix in a way which, unlike for PCA and kPCA, does not re-center the data but can only be approximated (up to 200 iterations were allowed for convergence). These three techniques were also implemented via `scikit-learn`. [23] The dimension of the samples were reduced to $d = 2, 3, 4, 5, 6, 7, 8, 9$ with each of these methods to allow for visualization ($d = 2$) and to encompass a range of dimensionalities whilst keeping the dimensionality ~one order of magnitude below the number of observations.

A key disadvantage of many traditional dimensionality reduction strategies (e.g., PCA, kPCA, and NMF) is that they obscure the original context of the features. Here, initially, the features correspond directly to biologically-tractable information: each feature represents a gene. However, the reduced set of features after applying PCA, kPCA, or NMF no longer correspond to biologically meaningful attributes. In contrast, the *a priori* aggregation strategy retains biological tractability, as the reduced set of features correspond to gene categories. However, there are a large number (> 1000) of different GO categories in each categorization scheme (i.e., component, function, and process), meaning that, unlike for PCA, kPCA, and NMF, the dimensionality of the data after *a priori* aggregation will remain high compared to the number of observations. Surveying all four strategies allows us to assess which strategy is more important for the performance of simple classification models: retaining biological context or ensuring the dimensionality is small compared to the number of observations.

2.4 Assessment and Evaluation

Due to limited sample sizes, we utilized leave-one-out validation to assess the models. Models were assessed based on prediction accuracy because the number of classes was variable between samples.

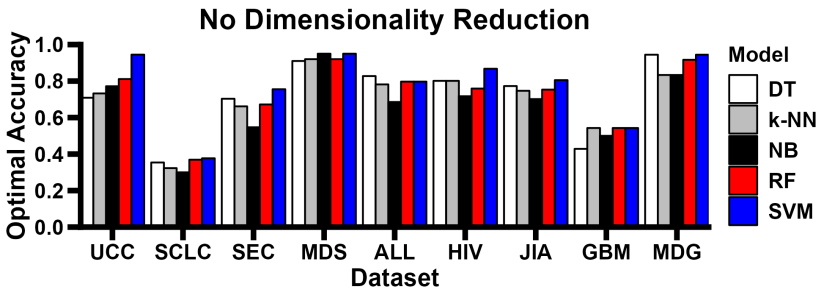

Figure 1: Baseline performance is highly variable. When no dimensionality reduction is applied to the samples, the classification accuracy varies heavily between the nine different samples and between the five different models. For each model, the leave-one-out validation accuracy was computed for every sample across a combination of hyperparameter values. The optimal accuracy for each model on each sample is the leave-one-out validation accuracy for the combination of hyperparameter values which resulted in the highest leave-one-out validation accuracy for that model on that sample.

3 Results

3.1 Baseline Performance is Highly Variable Across Samples

First, we applied no dimensionality reduction and performed leave-one-out validation to assess the baseline accuracy of the five models on all nine samples (Fig 1). When the hyperparameters of the model are optimized for the sample, the performance is highly variable. Unsurprisingly, the accuracy of the models on the SCLC sample was quite low, not exceeding 40% for any model. In contrast, the performance on the MDS sample was very high, with every model scoring above 90%. While the baseline performance was highly variable between samples, the performance between models was more consistent. Generally, the NB model performed worst or near worst while the SVM performed best or near best (Fig 1).

3.2 Impact of *a priori* Aggregation is Highly Variable

We next performed dimensionality reduction by aggregating the features based on the GO categorization of the genes. We trained the models on the modified samples (and computed the leave-one-out validation accuracy as above) then compared the accuracy on the reduced sample to baseline (Fig 2). The impact of this dimensionality reduction strategy was inconsistent across samples and models. For the DT model in particular, this dimensionality reduction strategy exhibited a wide range of impacts: leave-one-out validation accuracy increased by ~10% on the SCLC sample and decreased by ~20% on the MDG sample. In contrast, the performance was largely unaltered on any sample for the RF model.

3.3 Reduced Dimension has a Greater Impact than Choice of Dimensionality Reduction Method

We next applied PCA, kPCA, and NMF dimensionality reduction. If the reduced dimension is fixed, the performance of all three methods are largely similar across most samples (Fig 3). Notable exceptions are: the NB model, for which kPCA generally performed worse than PCA or NMF; the UCC and MDG samples, for which kPCA also caused lower performance in each model when compared to PCA and NMF; and the SCLC sample, for which kPCA generally performed better than PCA and NMF. Figure 3 displays the optimized leave-one-out validation accuracy for reduction to four dimensions, but a similar pattern is true across all dimensions tested (supplementary figures are available on GitHub, see below).

Far more impactful than the choice of dimensionality reduction method was the choice of reduced dimension. For some models, particularly NB and the SVM, the leave-one-out validation accuracy depended strongly on the reduced dimension (Fig 4). Interestingly, the trend was not necessarily monotonic, and peak accuracy for some models on some samples (e.g., the NB model on the HIV

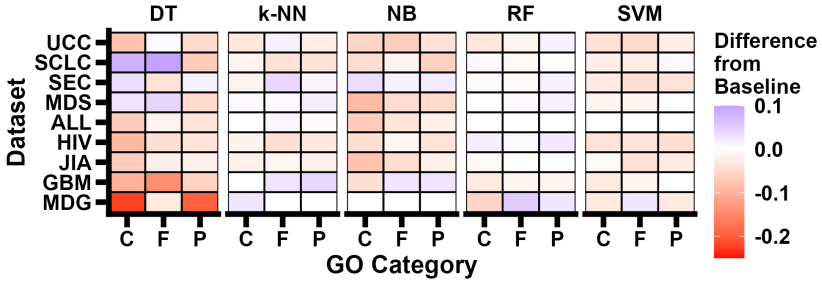

Figure 2: *a priori* aggregation has variable impacts on performance. When the dimensionality of the samples is reduced by aggregating the data based on GO categorization, the change in classification accuracy from baseline varies heavily between the nine different samples and between the five different models. For the GO categorization, "C" stands for component, "F" stands for function, and "P" stands for process.

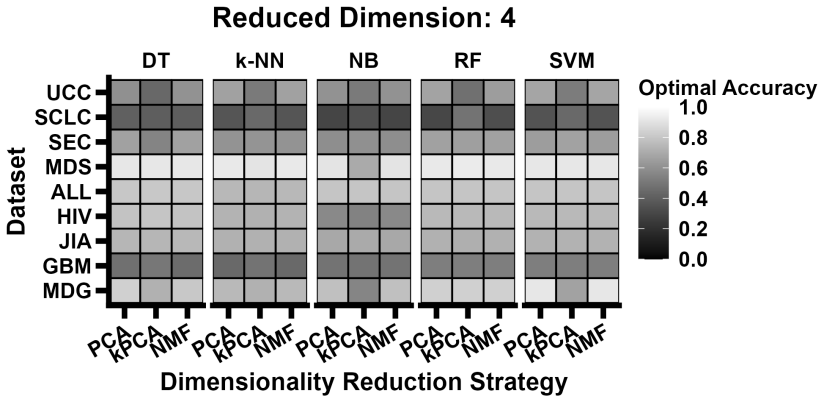

Figure 3: For a fixed dimension ($d = 4$ shown here), the choice of dimensionality reduction method between PCA, kPCA, and NMF has little impact on model performance. Here, the optimal leave-one-out validation accuracy - for models trained on each reduced sample via PCA, kPCA, and NMF - is reported. For most samples, the accuracy is large the same regardless of whether PCA, kPCA, or NMF is used.

sample, Fig 4) was achieved at a dimension less than 9. However, the leave-one-out validation accuracy was generally poor in very low dimensions such as 2 or 3 across all models. Figure 4 shows the performance of all models across all nine samples for the PCA reduction strategy, but the trends were similar for kPCA and NMF (supplementary figures are available on GitHub, see below).

In comparison to the baseline performance, the effect of PCA, kPCA, and NMF dimensionality reduction was highly variable (Fig 5). For the RF model, the performance was not highly different from baseline across most samples, but NB and the SVM were strongly impacted. While the change in performance was mostly negative, some models saw improved leave-one-out validation accuracy with these dimensionality reduction strategies on some samples (i.e., the NB model on the SEC and ALL samples and the DT model on the GBM sample). Figure 5 shows the performance of all models across all nine samples for the PCA reduction strategy, but the trends were similar for kPCA and NMF (supplementary figures are available on GitHub, see below).

4 Discussion

The high variability in the baseline performance of the models (Fig 1) is expected given the wide range of sample sizes and numbers of classes represented across the nine samples. The overall poor performance on the SCLC sample may be due to the large number of classes (6) within the sample

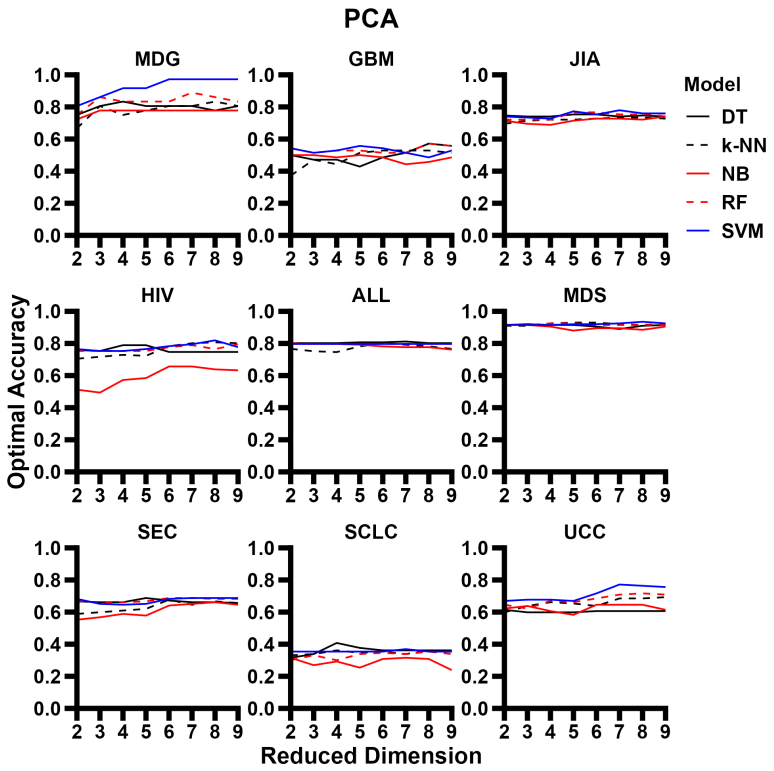

Figure 4: For a fixed dimensionality reduction strategy (PCA shown here), the choice of dimension had a strong impact on model performance across multiple samples. The optimal dimension varied with sample and model. Here, the optimal leave-one-out validation accuracy for models trained on each sample reduced to dimensionality $d = 2 \ldots 9$ is reported.

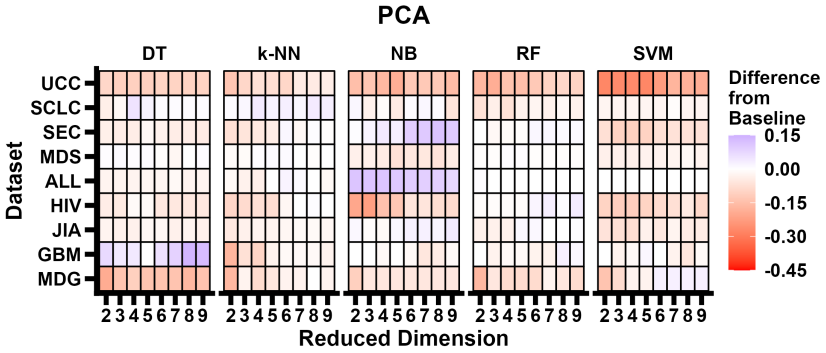

Figure 5: For a fixed dimensionality reduction strategy (PCA shown here), the performance of the model in comparison to the baseline performance is variable. The performance of the RF model was not heavily impacted by this dimensionality reduction for most samples, whereas the NB and SVM were heavily impacted.

and because of how similar the classes are to each other: each class describes a different stage of lung cancer progression [15]. The high performance on the MDS sample is also expected, as the MDS sample has the highest number of observations (200) and only two classes: a disease state (myelodysplastic syndrome) and a healthy state [18]. The NB model assumes that each feature is independent. This naïve assumption may explain the generally poor baseline performance of the NB model (Fig 1). Since each feature here represents the expression level of a gene, and because some genes influence the expression of others, assuming independence makes little biological sense. The contrastingly good baseline performance of the SVM (Fig 1) also makes sense, as SVMs are known to perform well with limited data in high dimension due to the implicit feature mapping of the kernel and regularization [26].

The inconsistencies in the data aggregation strategy (Fig 2) may be due to underlying biological context. In particular, cancer is a complicated disease that arises from mutations that tend to co-occur in genes with similar roles or that belong to the same biological pathway [27], so aggregating the features according to their GO categories may result in a reduced set of features that are more informative for cancer samples, which can aid the DT model. However, for other samples, if a very small number of individual genes are important, then taking the mean in the aggregation process may instead obscure the important fine-grain features and instead harm performance. Future experiments could consider different aggregation strategies (e.g., taking the max, geometric mean, etc. instead of the arithmetic mean) or other gene categorization/annotation schemes to explore other methods to incorporate existing biological knowledge into the dimensionality reduction.

Interestingly, the leave-one-out validation accuracy of models trained on samples reduced by PCA, kPCA, and NMF were similar (Fig 3). The results for PCA and NMF in particular were nearly identical across all models and all samples. One important difference between PCA and NMF is that NMF does not re-center the data. These similarities may then indicate that re-centering the data has little impact on classification problems in RNA-seq data, and that the relative values in gene expression are more important than the absolute values. kPCA did impact the performance of some samples and some models, namely the SCLC, UCC, and MDG samples and the NB model (Fig 3). However, these impacts were found in the most limiting cases of the samples surveyed: the MDG sample had the fewest observations (36) [14], the SCLC sample had the most classes (6) and overall lowest baseline performance [15]), and the NB model performed the worst at baseline out of all models surveyed. This may indicate that for more limiting cases (i.e., when data is especially scarce or the models perform especially poorly), simpler and more direct dimensionality methods (i.e., PCA or NMF) may be more appropriate than kPCA, but future research can be directed at testing different kernels for kPCA to assess the impact of kernel choice on model performance in these limiting cases. Nonetheless, for most of the samples, the choice between PCA, kPCA, and NMF had little impact on model performance for any fixed choice of reduced dimension.

In contrast to the choice between PCA, kPCA, and NMF, the choice of reduced dimension had a large impact on the leave-one-out validation accuracy (Fig 4), particularly for the NB and SVM models. The maximal leave-one-out validation accuracy was not necessarily achieved on the highest dimension (e.g., the NB model on the HIV sample or the SVM model on the MDG or UCC samples), which may be indicative of overfitting, even with as few as $d \leq 9$ dimensions. Given the high levels of noise in RNA-seq data [28], this is not surprising–including too many principal components/features may reintroduce some of the noise present in the original sample; the models may then overfit to this noise. However, for some samples (i.e., ALL, SEC, and MDS), the choice of reduced dimension had only a very small impact on the leave-one-out validation accuracy of every model tested (Fig 4). This may be due to the sample size; the MDS, ALL, and SEC samples were the largest, with 200, 197, and 192 observations, respectively (Table 2). Perhaps, a stronger effect may be seen if the number of reduced dimensions is increased beyond $d = 9$. Future studies could look into the impact of sample size on model performance after applying PCA, kPCA, and NMF, perhaps also extending the number of reduced dimensions beyond 10.

The highly variable impact of dimensionality reduction via PCA, kPCA, or NMF on the leave-one-out validation accuracy (Fig 5) may also be due to the underlying rationales of the classification models. The NB model showed improved performance on the SEC and ALL samples under PCA, kPCA, and NMF, perhaps because, unlike the individual genes, the principal components or reduced features may behave independently. PCA, kPCA, and NMF also improved the performance of the DT on the GBM sample, which may be because reducing the dimension to a small number via PCA, kPCA, and NMF reduces the noise present in bulk RNA-seq data [28]. Reducing the amount of noise may allow

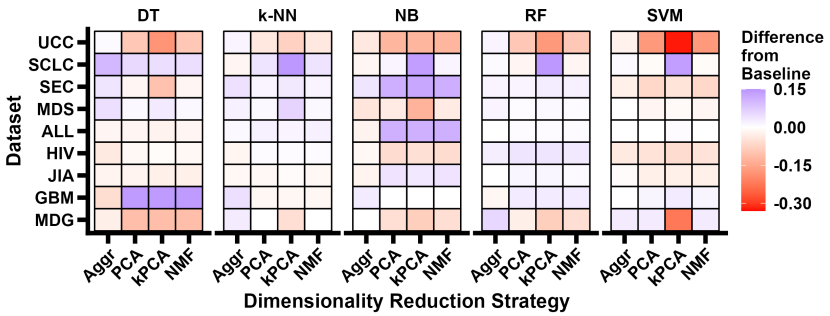

Figure 6: Impact of dimensionality reduction on model performance is variable and specific. The change in classification accuracy from baseline varies heavily between the nine different samples, the five different models, and the four different dimensionality reduction strategies. Here, "Aggr" refers to the *a priori* aggregation strategy, and the accuracies are reported as the optimal accuracy across all reduced dimensions/GO categories for each dimensionality reduction strategy.

the DT to choose more genuinely informative features for splitting the data as opposed to overfitting on noise. In contrast, the SVM saw generally lowered performance under dimensionality reduction via PCA, kPCA, or NMF across most samples, which may be because reducing the dimension results in transformed samples which are more difficult to separate linearly, especially for samples with greater than two classes. Consistent with this notion, the SVM generally saw improved performance as the dimension increased towards $d = 9$.

5 Conclusions

Classifying disease state from human bulk RNA-seq data is a difficult challenge. The lack of large samples and suitable pre-trained models requires the use of older, simpler models (e.g., DT, k-NN, NB classifier, RF, and SVM). Adding to the challenge is the high dimensionality of bulk RNA-seq data, which can hinder the performance of these models. Here, we surveyed four different dimensionality reduction strategies across nine human bulk RNA-seq samples to assess the impact of dimensionality reduction on the performance of five simple machine-learning models commonly used in biology. The vast heterogeneity of the samples made it difficult to draw general conclusions. The impact of dimensionality reduction appears to be highly specific to the sample, the model being used, and the underlying biological context (Fig 6).

The performance of the *a priori* aggregation strategy is highly variable across samples and is likely strongly influenced by underlying biological context, while the PCA, kPCA, and NMF methods had mostly similar impacts on model performance across most samples. By contrast, kPCA may have a different impact on samples in limiting cases with few observations but many classes. The choice of reduced dimension had a much stronger impact than the choice between PCA, kPCA and NMF; however, this impact was variable across samples and models. Together, these results indicate that dimensionality reduction can improve model performance and is a valuable tool for classifying disease states from bulk RNA-seq data. However, it must be used with caution, and a strong understanding of the underlying biological context, limitations of the data (e.g., small sample size, noisy observations, etc.), and nature of the classification problem (e.g., number of data classes and similarities between classes that may make it difficult to distinguish between them) is needed to inform the choice of dimensionality reduction strategy, classification model, and reduced dimension.

6 Data/Code Availability and Supporting Information

All the data and code used in the developing this report - as well as supplementary figures and supporting information - are available at `https://github.com/nolan-middleton/CPT_S-Group-Project-Fall-2025`.

References

[1] Tingpeng Yang, Yonghong He, and Yu Wang. Introducing tec-lncmir for prediction of lncrna-mirna interactions through deep learning of rna sequences. *Brief Bioinform*, 26(1):bbaf046, 02 2025. ISSN 1477-4054. doi: 10.1093/bib/bbaf046. URL https://doi.org/10.1093/bib/bbaf046.

[2] Hannah E Wilson, Scott Stevison, Levi Lamprey, and John J Wyrick. Mapping transcription factor binding sites by learning uv damage fingerprints. *Nucleic Acids Res*, 53(19):gkaf1014, 10 2025. ISSN 1362-4962. doi: 10.1093/nar/gkaf1014. URL https://doi.org/10.1093/nar/gkaf1014.

[3] John Jumper, Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger, Kathryn Tunyasuvunakool, Russ Bates, Augustin Žídek, Anna Potapenko, Alex Bridgland, Clemens Meyer, Simon A A Kohl, Andrew J Ballard, Andrew Cowie, Bernardino Romera-Paredes, Stanislav Nikolov, Rishub Jain, Jonas Adler, Trevor Back, Stig Petersen, David Reiman, Ellen Clancy, Michal Zielinski, Martin Steinegger, Michalina Pacholska, Tamas Berghammer, Sebastian Bodenstein, David Silver, Oriol Vinyals, Andrew W Senior, Koray Kavukcuoglu, Pushmeet Kohli, and Demis Hassabis. Highly accurate protein structure prediction with AlphaFold. *Nature*, 596(7873):583–589, August 2021. doi: 10.1038/s41586-021-03819-2. URL https://doi.org/10.1038/s41586-021-03819-2.

[4] Jocelyn Krebs, Elliott Goldstein, and Stephen Kilpatrick. *Lewin's Genes XII*. Jones & Bartlett Learning, 5 Wall Street, Burlington, MA 01803, 12 edition, 2018. ISBN 9781284104493.

[5] Zhong Wang, Mark Gerstein, and Michael Snyder. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*, 10(1):57–63, January 2009. doi: 10.1038/nrg2484. URL https://doi.org/10.1038/nrg2484.

[6] Fuchou Tang, Catalin Barbacioru, Yangzhou Wang, Ellen Nordman, Clarence Lee, Nanlan Xu, Xiaohui Wang, John Bodeau, Brian B Tuch, Asim Siddiqui, Kaiqin Lao, and M Azim Surani. mRNA-Seq whole-transcriptome analysis of a single cell. *Nat Methods*, 6(5):377–382, May 2009.

[7] Haotian Cui, Chloe Wang, Hassaan Maan, Kuan Pang, Fengning Luo, Nan Duan, and Bo Wang. scGPT: toward building a foundation model for single-cell multi-omics using generative AI. *Nat Methods*, 21(8):1470–1480, August 2024. doi: 10.1038/s41592-024-02201-0. URL https://doi.org/10.1038/s41592-024-02201-0.

[8] Shreshth Gandhi, Farnoosh Javadi, Valentine Svensson, Umair Khan, Matthew G. Jones, Johnny Yu, Daniele Merico, Hani Goodarzi, and Nima Alidoust. Tahoe-x1: Scaling perturbation-trained single-cell foundation models to 3 billion parameters. *bioRxiv*, 2025. doi: 10.1101/2025.10.23.683759. URL https://www.biorxiv.org/content/10.1101/2025.10.23.683759v1.

[9] Zhuoyi Wei Yun Su Ningyuan Shangguan Shuangyu Yang Chengyang Zhang Wenbing Li Jinbo Zhang Nan Fang Hongyu Zhang Huiying Zhao Yutong Lu Jue Fan Weijiang Yu Yuansong Zeng, Jiancong Xie and Yuedong Yang. Cellfm: a large-scale foundation model pre-trained on transcriptomics of 100 million human cells. *Nat Commun*, 16(4679), May 2025. doi: 10.1038/s41467-025-59926-5. URL https://doi.org/10.1038/s41467-025-59926-5.

[10] Tracy Boakye Serebour, Adam P. Cribbs, Mathew J. Baldwin, Collen Masimirembwa, Zedias Chikwambi, Angeliki Kerasidou, and Sarah J. B. Snelling. Overcoming barriers to single-cell rna sequencing adoption in low- and middle-income countries. *Eur J Hum Genet*, 32(10):1206–1213, October 2024. doi: 10.1038/s41431-024-01564-4. URL https://doi.org/10.1038/s41431-024-01564-4.

[11] Maxence Gélard, Guillaume Richard, Thomas Pierrot, and Paul-Henry Cournède. Bulkrnabert: Cancer prognosis from bulk rna-seq based language models. In Stefan Hegselmann, Helen Zhou, Elizabeth Healey, Trenton Chang, Caleb Ellington, Vishwali Mhasawade, Sana Tonekaboni, Peniel Argaw, and Haoran Zhang, editors, *Proceedings of the 4th Machine Learning for Health Symposium*, volume 259 of *Proceedings of Machine Learning Research*, pages 384–400. PMLR, 15–16 Dec 2025. URL https://proceedings.mlr.press/v259/gelard25a.html.

[12] Liu D Wang N He D Wu Z Zhu X Wen X Li X Li J Wang Z Wang X, Wang H. Deep learning using bulk rna-seq data expands cell landscape identification in tumor microenvironment. *Oncoimmunology*, 25(11), February 2022. doi: 10.1080/2162402X.2022.2043662.

[13] Allison P. Heath, Vincent Ferretti, Stuti Agrawal, Maksim An, James C. Angelakos, Renuka Arya, Rosita Bajari, Bilal Baqar, Justin H. B. Barnowski, Jeffrey Burt, Ann Catton, Brandon F. Chan, Fay Chu, Kim Cullion, Tanja Davidsen, Phuong-My Do, Christian Dompierre, Martin L. Ferguson, Michael S. Fitzsimons, Michael Ford, Miyuki Fukuma, Sharon Gaheen, Gajanan L. Ganji, Tzintzuni I. Garcia, Sameera S. George, Daniela S. Gerhard, Francois Gerthoffert, Fauzi Gomez, Kang Han, Kyle M. Hernandez, Biju Issac, Richard Jackson, Mark A. Jensen, Sid Joshi, Ajinkya Kadam, Aishmit Khurana, Kyle M. J. Kim, Victoria E. Kraft, Shenglai Li, Tara M. Lichtenberg, Janice Lodato, Laxmi Lolla, Plamen Martinov, Jeffrey A. Mazzone, Daniel P. Miller, Ian Miller, Joshua S. Miller, Koji Miyauchi, Mark W. Murphy, Thomas Nullet, Rowland O. Ogwara, Francisco M. Ortuño, Jesús Pedrosa, Phuong L. Pham, Maxim Y. Popov, James J. Porter, Raymond Powell, Karl Rademacher, Colin P. Reid, Samantha Rich, Bessie Rogel, Himanso Sahni, Jeremiah H. Savage, Kyle A. Schmitt, Trevar J. Simmons, Joseph Sislow, Jonathan Spring, Lincoln Stein, Sean Sullivan, Yajing Tang, Mathangi Thiagarajan, Heather D. Troyer, Chang Wang, Zhining Wang, Bedford L. West, Alex Wilmer, Shane Wilson, Kaman Wu, William P. Wysocki, Linda Xiang, Joseph T. Yamada, Liming Yang, Christine Yu, Christina K. Yung, Jean Claude Zenklusen, Junjun Zhang, Zhenyu Zhang, Yuanheng Zhao, Ariz Zubair, Louis M. Staudt, and Robert L. Grossman. The nci genomic data commons. *Nat Genet*, 53(3):257–262, March 2021. doi: 10.1038/s41588-021-00791-5. URL `https://doi.org/10.1038/s41588-021-00791-5`.

[14] Nataly Strunnikova, Sara Hilmer, Jessica Flippin, Michael Robinson, Eric Hoffman, and Karl G Csaky. Differences in gene expression profiles in dermal fibroblasts from control and patients with age-related macular degeneration elicited by oxidative injury. *Free Radic Biol Med*, 39(6): 781–796, September 2005.

[15] Mitch Raponi, Yi Zhang, Jack Yu, Guoan Chen, Grace Lee, Jeremy M G Taylor, James Macdonald, Dafydd Thomas, Christopher Moskaluk, Yixin Wang, and David G Beer. Gene expression signatures for predicting prognosis of squamous cell and adenocarcinomas of the lung. *Cancer Res*, 66(15):7466–7472, August 2006.

[16] Michael E Burczynski, Ron L Peterson, Natalie C Twine, Krystyna A Zuberek, Brendan J Brodeur, Lori Casciotti, Vasu Maganti, Padma S Reddy, Andrew Strahs, Fred Immermann, Walter Spinelli, Ulrich Schwertschlag, Anna M Slager, Monette M Cotreau, and Andrew J Dorner. Molecular classification of crohn's disease and ulcerative colitis patients using transcriptional profiles in peripheral blood mononuclear cells. *J Mol Diagn*, 8(1):51–61, February 2006.

[17] Adam M Gustafson, Raffaella Soldi, Christina Anderlind, Mary Beth Scholand, Jun Qian, Xiaohui Zhang, Kendal Cooper, Darren Walker, Annette McWilliams, Gang Liu, Eva Szabo, Jerome Brody, Pierre P Massion, Marc E Lenburg, Stephen Lam, Andrea H Bild, and Avrum Spira. Airway PI3K pathway activation is an early and reversible event in lung cancer development. *Sci Transl Med*, 2(26):26ra25, April 2010.

[18] Petra Gorombei, Fabien Guidez, Saravanan Ganesan, Mathieu Chiquet, Andrea Pellagatti, Laure Goursaud, Nilgun Tekin, Stephanie Beurlet, Satyananda Patel, Laura Guerenne, Carole Le Pogam, Niclas Setterblad, Pierre de la Grange, Christophe LeBoeuf, Anne Janin, Maria-Elena Noguera, Laure Sarda-Mantel, Pascale Merlet, Jacqueline Boultwood, Marina Konopleva, Michael Andreeff, Robert West, Marika Pla, Lionel Adès, Pierre Fenaux, Patricia Krief, Christine Chomienne, Nader Omidvar, and Rose Ann Padua. BCL-2 inhibitor ABT-737 effectively targets leukemia-initiating cells with differential regulation of relevant genes leading to extended survival in a NRAS/BCL-2 mouse model of high risk-myelodysplastic syndrome. *Int J Mol Sci*, 22(19):10658, September 2021.

[19] Lüder Hinrich Meyer, Sarah Mirjam Eckhoff, Manon Queudeville, Johann Michael Kraus, Marco Giordan, Jana Stursberg, Andrea Zangrando, Elena Vendramini, Anja Möricke, Martin Zimmermann, Andre Schrauder, Georgia Lahr, Karlheinz Holzmann, Martin Schrappe, Giuseppe Basso, Karsten Stahnke, Hans Armin Kestler, Geertruy Te Kronnie, and Klaus-Michael Debatin. Early relapse in ALL is identified by time to leukemia in NOD/SCID mice

and is characterized by a gene signature involving survival pathways. *Cancer Cell*, 19(2): 206–217, February 2011.

[20] Caryn G Morse, Joachim G Voss, Goran Rakocevic, Mary McLaughlin, Carol L Vinton, Charles Huber, Xiaojun Hu, Jun Yang, Da Wei Huang, Carolea Logun, Robert L Danner, Zoila G Rangel, Peter J Munson, Jan M Orenstein, Elisabeth J Rushing, Richard A Lempicki, Marinos C Dalakas, and Joseph A Kovacs. HIV infection and antiretroviral therapy have divergent effects on mitochondria in adipose tissue. *J Infect Dis*, 205(12):1778–1787, June 2012.

[21] Claas H Hinze, Ndate Fall, Sherry Thornton, Jun Q Mo, Bruce J Aronow, Gerlinde Layh-Schmitt, Thomas A Griffin, Susan D Thompson, Robert A Colbert, David N Glass, Michael G Barnes, and Alexei A Grom. Immature cell populations and an erythropoiesis gene-expression signature in systemic juvenile idiopathic arthritis: implications for pathogenesis. *Arthritis Res Ther*, 12(3):R123, June 2010.

[22] Guido Reifenberger, Ruthild G Weber, Vera Riehmer, Kerstin Kaulich, Edith Willscher, Henry Wirth, Jens Gietzelt, Bettina Hentschel, Manfred Westphal, Matthias Simon, Gabriele Schackert, Johannes Schramm, Jakob Matschke, Michael C Sabel, Dorothee Gramatzki, Jörg Felsberg, Christian Hartmann, Joachim P Steinbach, Uwe Schlegel, Wolfgang Wick, Bernhard Radlwimmer, Torsten Pietsch, Jörg C Tonn, Andreas von Deimling, Hans Binder, Michael Weller, Markus Loeffler, and German Glioma Network. Molecular characterization of long-term survivors of glioblastoma using genome- and transcriptome-wide profiling. *Int J Cancer*, 135(8):1822–1831, October 2014.

[23] F. Pedregosa, G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, V. Dubourg, J. Vanderplas, A. Passos, D. Cournapeau, M. Brucher, M. Perrot, and E. Duchesnay. Scikit-learn: Machine learning in Python. *Journal of Machine Learning Research*, 12:2825–2830, 2011.

[24] M Ashburner, C A Ball, J A Blake, D Botstein, H Butler, J M Cherry, A P Davis, K Dolinski, S S Dwight, J T Eppig, M A Harris, D P Hill, L Issel-Tarver, A Kasarskis, S Lewis, J C Matese, J E Richardson, M Ringwald, G M Rubin, and G Sherlock. Gene ontology: tool for the unification of biology. the gene ontology consortium. *Nat Genet*, 25(1):25–29, May 2000.

[25] The Gene Ontology Consortium, Suzi A Aleksander, James Balhoff, Seth Carbon, J Michael Cherry, Harold J Drabkin, Dustin Ebert, Marc Feuermann, Pascale Gaudet, Nomi L Harris, David P Hill, Raymond Lee, Huaiyu Mi, Sierra Moxon, Christopher J Mungall, Anushya Muruganugan, Tremayne Mushayahama, Paul W Sternberg, Paul D Thomas, Kimberly Van Auken, Jolene Ramsey, Deborah A Siegele, Rex L Chisholm, Petra Fey, Maria Cristina Aspromonte, Maria Victoria Nugnes, Federica Quaglia, Silvio Tosatto, Michelle Giglio, Suvarna Nadendla, Giulia Antonazzo, Helen Attrill, Gil dos Santos, Steven Marygold, Victor Strelets, Christopher J Tabone, Jim Thurmond, Pinglei Zhou, Saadullah H Ahmed, Praoparn Asanitthong, Diana Luna Buitrago, Meltem N Erdol, Matthew C Gage, Mohamed Ali Kadhum, Kan Yan Chloe Li, Miao Long, Aleksandra Michalak, Angeline Pesala, Armalya Pritazahra, Shirin C C Saverimuttu, Renzhi Su, Kate E Thurlow, Ruth C Lovering, Colin Logie, Snezhana Oliferenko, Judith Blake, Karen Christie, Lori Corbani, Mary E Dolan, Harold J Drabkin, David P Hill, Li Ni, Dmitry Sitnikov, Cynthia Smith, Alayne Cuzick, James Seager, Laurel Cooper, Justin Elser, Pankaj Jaiswal, Parul Gupta, Pankaj Jaiswal, Sushma Naithani, Manuel Lera-Ramirez, Kim Rutherford, Valerie Wood, Jeffrey L De Pons, Melinda R Dwinell, G Thomas Hayman, Mary L Kaldunski, Anne E Kwitek, Stanley J F Laulederkind, Marek A Tutaj, Mahima Vedi, Shur-Jen Wang, Peter D'Eustachio, Lucila Aimo, Kristian Axelsen, Alan Bridge, Nevila Hyka-Nouspikel, Anne Morgat, Suzi A Aleksander, J Michael Cherry, Stacia R Engel, Kalpana Karra, Stuart R Miyasato, Robert S Nash, Marek S Skrzypek, Shuai Weng, Edith D Wong, Erika Bakker, Tanya Z Berardini, Leonore Reiser, Andrea Auchincloss, Kristian Axelsen, Ghislaine Argoud-Puy, Marie-Claude Blatter, Emmanuel Boutet, Lionel Breuza, Alan Bridge, Cristina Casals-Casas, Elisabeth Coudert, Anne Estreicher, Maria Livia Famiglietti, Marc Feuermann, Arnaud Gos, Nadine Gruaz-Gumowski, Chantal Hulo, Nevila Hyka-Nouspikel, Florence Jungo, Philippe Le Mercier, Damien Lieberherr, Patrick Masson, Anne Morgat, Ivo Pedruzzi, Lucille Pourcel, Sylvain Poux, Catherine Rivoire, Shyamala Sundaram, Alex Bateman, Emily Bowler-Barnett, Hema Bye-A-Jee, Paul Denny, Alexandr Ignatchenko, Rizwan Ishtiaq, Antonia Lock, Yvonne Lussi, Michele Magrane, Maria J Martin, Sandra Orchard,

Pedro Raposo, Elena Speretta, Nidhi Tyagi, Kate Warner, Rossana Zaru, Alexander D Diehl, Raymond Lee, Juancarlos Chan, Stavros Diamantakis, Daniela Raciti, Magdalena Zarowiecki, Malcolm Fisher, Christina James-Zorn, Virgilio Ponferrada, Aaron Zorn, Sridhar Ramachandran, Leyla Ruzicka, and Monte Westerfield. The gene ontology knowledgebase in 2023. *Genetics*, 224(1):iyad031, 03 2023. ISSN 1943-2631. doi: 10.1093/genetics/iyad031. URL https://doi.org/10.1093/genetics/iyad031.

[26] Derek A. Pisner and David M. Schnyer. Chapter 6 - support vector machine. In Andrea Mechelli and Sandra Vieira, editors, *Machine Learning*, pages 101–121. Academic Press, 2020. ISBN 978-0-12-815739-8. doi: https://doi.org/10.1016/B978-0-12-815739-8.00006-7. URL https://www.sciencedirect.com/science/article/pii/B9780128157398000067.

[27] Musalula Sinkala. Mutational landscape of cancer-driver genes across human cancers. *Sci Rep*, 13(1), August 2023. doi: 10.1038/s41598-023-39608-2. URL https://doi.org/10.1038/s41598-023-39608-2.

[28] Ales Varabyou, Steven L Salzberg, and Mihaela Pertea. Effects of transcriptional noise on estimates of gene and transcript expression in RNA sequencing experiments. *Genome Res*, 31 (2):301–308, February 2021.