

Supplementary Information for Pathway Expression Analysis

Nathan Mankovich^{1,*}, Eric Kehoe^{1,o}, Amy Peterson^{1,x}, and Michael Kirby^{1,+}

¹Colorado State University, Mathematics, Fort Collins, 80523, USA

*nathan.mankovich@gmail.com

^oekehoe@colostate.edu

^xapete41lsu@gmail.com

⁺michael.kirby@colostate.edu

ABSTRACT

This is the Supplementary Information for the Pathway Expression Analysis paper.

Results

Visualization

For our PCA (Principle Component Analysis) visualization, we use gene expression data from 9 to 16 hours after infection that have been batch corrected for subject identifier using LIMMA. We select features using 4 studies containing H1N1 and H3N2 strains of influenza. Then we do two PCA plots of the first two principle components of data from 2 HRV test studies. One plot uses all the features for the PCA. The second plot uses only the features found using the 4 H1N1 and H3N2 studies. The feature selection is done with gene expression data and with pathway expression data using the same SSVM feature selector methodology. The object of this experiment is a head to head comparison between gene expression to pathway expression using the linear separation between between the controls (subjects before infection) and shedders (subjects from 9 to 16 hours after infection). These PCA plots are in Figure 1. For this experiment and time bin, we notice better linear separation between the pathway expression data than the gene expression data.

When we produce the same types of PCA visualizations using each train/test split along with each of the different time bins and pathway expression types we notice that the gene expression data appears to linearly separate better than pathway expression data. But, upon further investigation, we find that the explained variance ratio of the first two principle components for pathway expression data in these plots are generally less than 0.5. So most of the variance of pathway expression data is captured in the 3rd to n^{th} principle components. This provides some explanation for the poor linear separation of pathway expression data using the first two principle components of PCA.

0.1 Classification Results

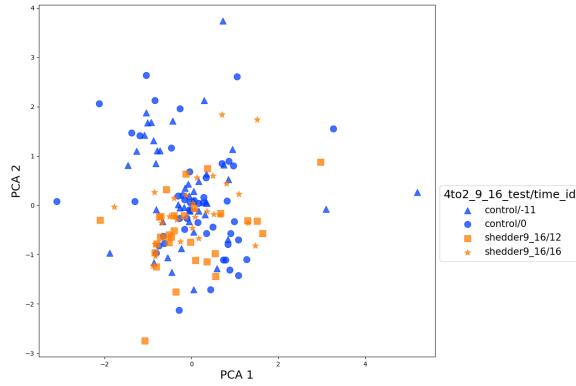
CPE requires two parameters to be set: a pathway gene network edge type and a centrality measure. We test CPE by using either correlation edges or pre-computed (from Reactome) edges, which may be directed or undirected. For centrality measures, we use either PageRank or out-degree centrality. Table 1 is a table of the CPE configuration which produces the highest test BSR. Using this table, we see there is no clear winning parameter combination for maximizing classification rates across all data partitions. However, we do observe that pre-computed edges with PageRank centrality is the most common method across all experiments and time bins to produce the highest BSR. In fact, at the 25 to 32 hour time bin the CPE parameters which produced the highest BSR are all undirected networks with pre-computed edges with PageRank centrality.

We now compare the effects of batch correction via LIMMA on the classification results in Figure 2. We notice that the violins for pathway expression methods are wider than the violins from the gene expression methods. This implies that the BSRs for gene expression methods change less uniformly from batch correction on subject identifier than pathway expression methods. Consequently, we claim that pathway expression methods are more robust to subject differences within a class.

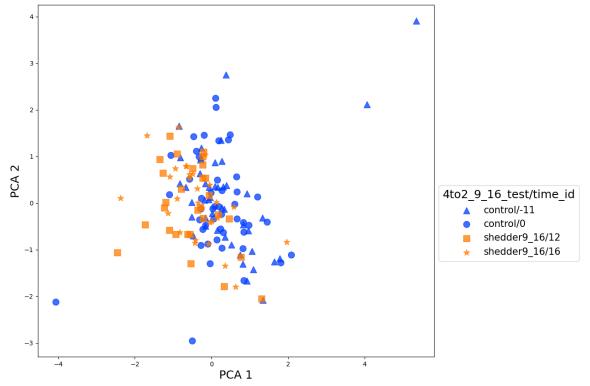
In addition, linear pathway expression methods appear more robust to subject differences within a class than gene expression methods since the hourglass for LPE in Figure 2 is at 0 whereas the widest part of the CPE hourglass is above 0.

Comparing Pathway Selection Methodologies

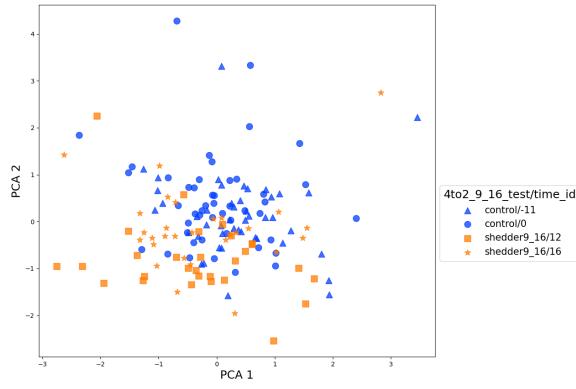
We perform two pathway selection experiments using two data sets: 1) 4 studies (the training data for the 4 to 2 and the 4 to 3 experiments) and 2) 6 studies (the training data for the 6 to 1 experiments).



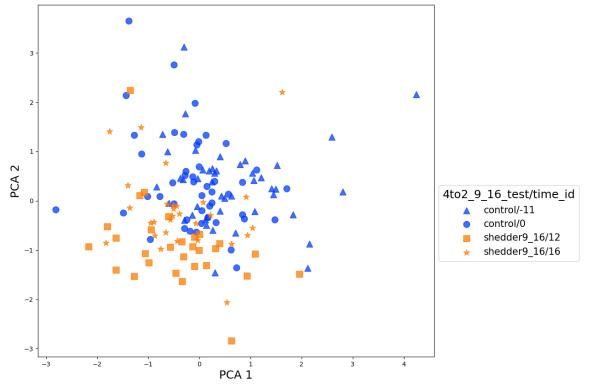
(a) GE test data with all the features.



(b) GE test data with the selected features.



(c) CPE test data with all the features.



(d) CPE test data with the selected features.

Figure 1. A PCA embedding of the 2 HRV test studies using GE and CPE in the time bin 9 to 16 hours after infection. The first column is an embedding with all the features and the second is with the selected features. These CPE data are generated using pre-computed, undirected edge with out-degree centrality.

Experiment	Time Bin	Centrality	Similarity	Directed
4 to 2	1 to 8	PageRank	pre-computed	True
4 to 2	9 to 16	out-degree	pre-computed	False
4 to 2	17 to 24	out-degree	correlation	False
4 to 2	25 to 32	PageRank	pre-computed	False
4 to 3	1 to 8	out-degree	correlation	False
4 to 3	9 to 16	PageRank	pre-computed	False
4 to 3	17 to 24	out-degree	pre-computed	True
4 to 3	25 to 32	PageRank	pre-computed	False
6 to 1	1 to 8	PageRank	pre-computed	True
6 to 1	9 to 16	PageRank	correlation	False
6 to 1	17 to 24	PageRank	pre-computed	True
6 to 1	25 to 32	PageRank	pre-computed	False

Table 1. The CPE centrality and similarity configurations which resulted in the highest test BSR for CPE given each data partition and time bin. The test BSR for each of these configurations are in Table ??.

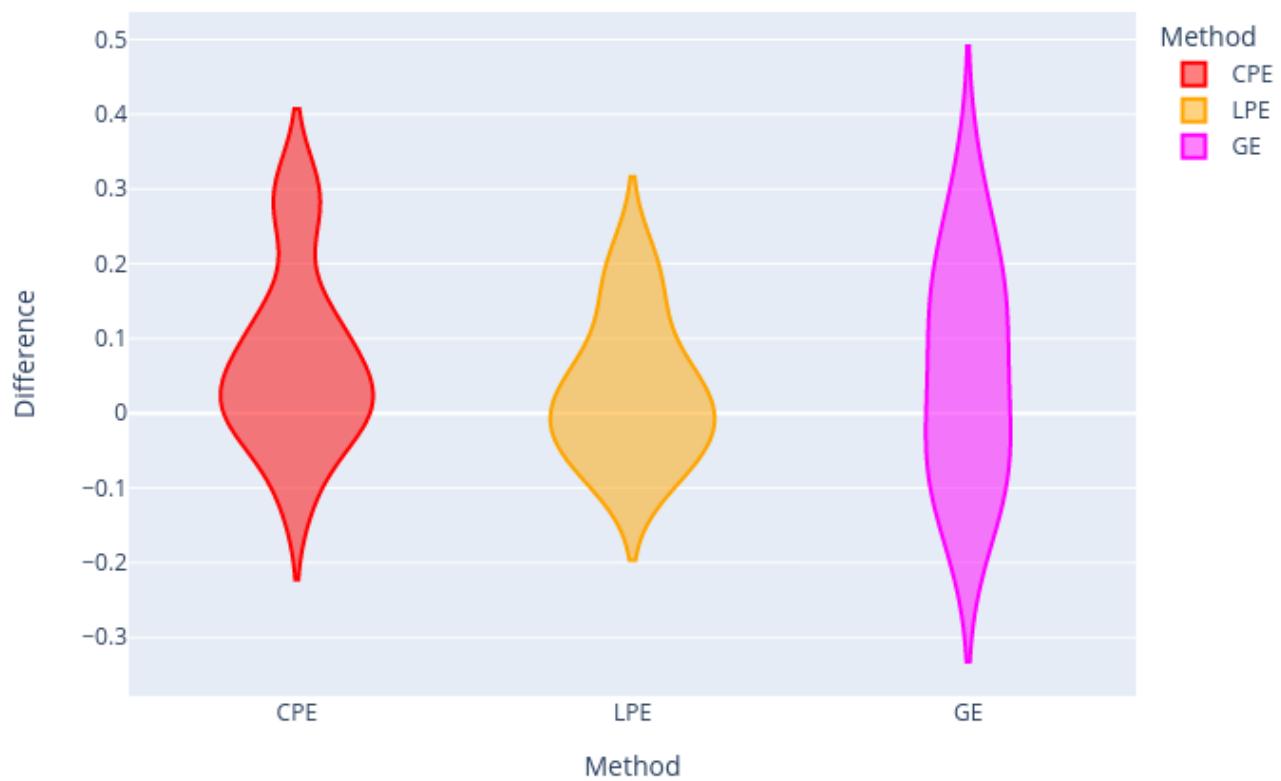


Figure 2. The difference between test BSR with LIMMA normalization using subject identifier and test BSR without LIMMAs. Each violin represents the distribution of these differences across experiment time bin and data partition for each method. A positive difference in BSR indicates that LIMMA normalization using subject identifier increased the classification accuracy. For CPE, we use pre-computed, directed edges with PageRank centrality for CPE.

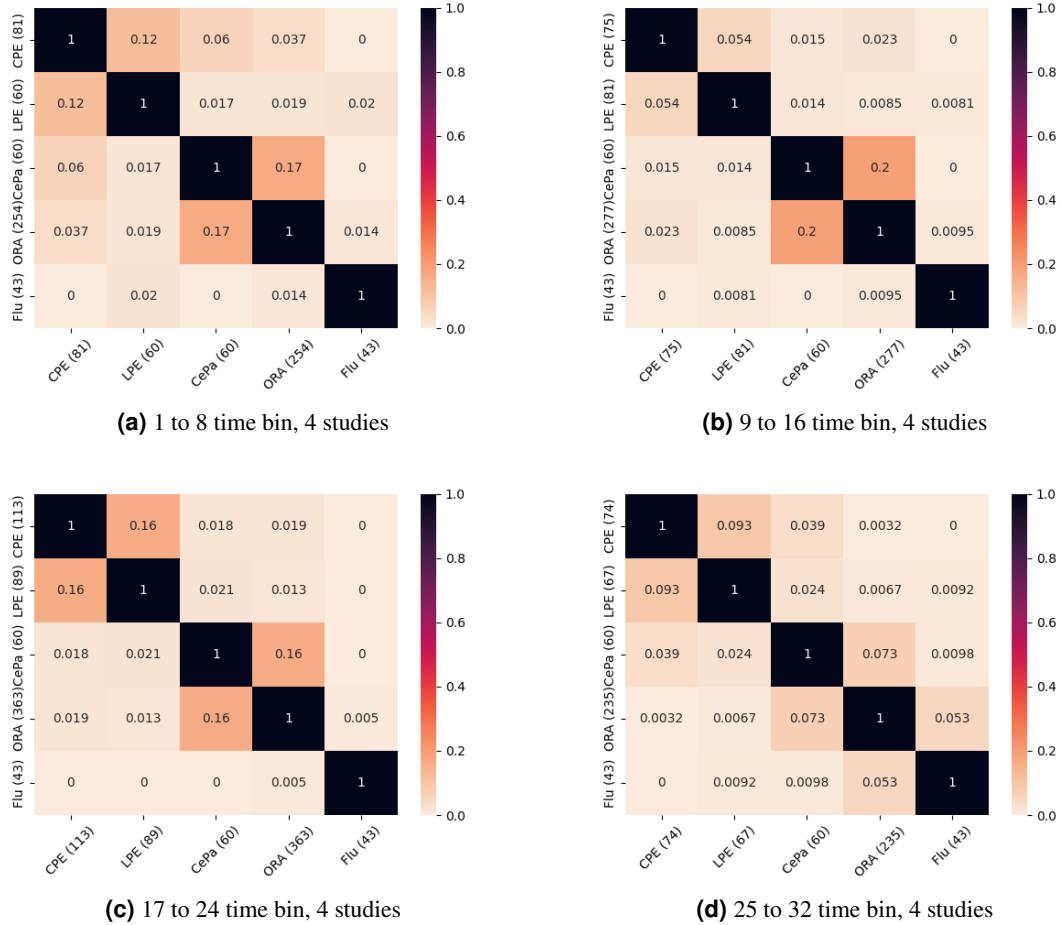


Figure 3. Jaccard overlap between the selected pathways for different methodologies. Pathways are selected using the 4 training studies. Each plot is for a different train/test experiment with LIMMA using subject identifier. The CPE configuration is pre-computed, directed edges with PageRank centrality.

In these experiments, we compare the pathways that are selected by the pathway expression methods to the pathways selected by standard pathway ranking algorithms, ORA and CePA, as well as a list of influenza related pathways (labeled Flu) from Reactome. We find this list of influenza related pathways by simply searching for influenza on the Reactome website. For methodological consistency in these Jaccard plots, we use the same edge and centrality methods for CePa and CPE, namely pre-computed, directed edges with PageRank centrality.

For comparison, we use the Jaccard/Tanimoto similarity coefficient as a measure of overlap between these two sets of pathways. The Jaccard similarity plot for the features from the 4 training study experiments are in Supplementary Information Fig 3.

Methods

This is the supplementary material for the Methods section.

Data Set (GSE73072)

We break our data sets down into 4 sets of shippers from evenly spaced time bins within 32 hours after infection. Our train/test splits are described in Table 2 using the format: study identifier (virus).

Pathway Ranking Using Gene Feature Sets

Let G be the set of all genes in the data set and $F \subseteq G$ be a feature set of genes. In this paper, these gene feature sets are calculated using SSVM feature selection (detailed in ??). In this subsection we will introduce two pathway ranking methods

Partition Name	Train	Test
4 to 2	DEE2 (H3N2), DEE3 (H1N1), DEE4 (H1N1), DEE5 (H3N2)	UVA (HRV), Duke (HRV)
4 to 3	DEE2 (H3N2), DEE3 (H1N1), DEE4 (H1N1), DEE5 (H3N2)	UVA (HRV), Duke (HRV), DEE1 (RSV)
6 to 1	UVA (HRV), Duke (HRV), DEE1 (RSV) DEE3 (H1N1), DEE4 (H1N1), DEE5 (H3N2)	DEE2 (H3N2)

Table 2. The train/test splits by study ID for the 4 to 2, 4 to 3 and 6 to 1 experiments. The parenthetical after each study ID is it's associated virus.

using a feature set of genes (or probe IDs): 1) ORA and 2) CePa. Let $P \subseteq G$ be the set of genes in a given pathway. These methods, ORA and CePa, assign a score to each pathway by leveraging the genes in F . The higher the score, the more important the pathway. In summary, each of these methods are a map $\phi : P \rightarrow \mathbb{R}$ where P is the set of all pathways.

ORA is one of the most simple and widely used pathway scoring methods, so it is an ideal ground-truth pathway ranking method. Generally, ORA is a methodology for investigating the statistical significance of the overlap of genes in the feature set with known pathways. Using the hypergeometric distribution, ORA determines the p -value of the significance of the overlap, which is the score of the pathway, by

$$p = 1 - \sum_{k=0}^{f-1} \frac{\binom{\hat{F}}{k} \binom{N-\hat{F}}{n-k}}{\binom{N}{n}}.$$

In this formula, f is the number of genes in the overlap of feature set and pathway, \hat{F} is the number of genes in the feature set, N is the total number of genes possible, and n is the number of genes in the pathway. This is the significance of the representation of the pathway in the feature set. We implement this method using the python package reactome2py to use the ORA analysis tools on the Reactome website. The github page for ReactomePY is <https://github.com/reactome/reactome2py>.

CePa is a network centrality informed method for pathway ranking on a given feature set, developed by Gu et al.¹. This method combines similar notions of statistical that are used in ORA with biological pathway network information, specifically network centrality. For a given pathway P , we generate a pathway network where genes are the nodes and edges. Let $c_P : G \rightarrow \mathbb{R}$ be a pathway centrality map from the gene space to the real numbers. Given gene $g \in G$, $c_P(g)$ is the centrality of g within it's pathway network. Then the rank for pathway P is just the sum of the centralities of the genes in both P and the feature set F as in Equation 1.

$$\text{CEPA}(P) = \sum_{n \in P \cap F} c_P(n) \quad (1)$$

We perform six CePa experiments where we use pre-computed directed, pre-computed undirected or correlation edges with either out-degree (normalized by maximum out-degree) or PageRank centrality methods. To the best of our knowledge, this is the first time CePa has been used with PageRank centrality.

The final step in CePa is determining the pathway significance score relative to a collection of null feature sets. To do this, a large number of $m \in \mathbb{N}$ null trials are run with $|F|$ genes selected from a uniform distribution over all the genes. Then CePa is run for each of these null trials resulting in a set of null pathway rankings. Let the vector of the set of null pathway rankings for pathway P be denoted \mathbf{np} and ordered by null feature set. Define the null value indicator map, denoted I , in Equation 2.

$$I(\mathbf{np}) = \begin{cases} 1 & \text{if } \mathbf{np} > \text{CEPA}(P) \\ 0 & \text{otherwise.} \end{cases} \quad (2)$$

We then use the indicator map to find the “null value” for pathway P as the mean of the entries in $I(\mathbf{np})$ in Equation 3.

$$\text{null value}(P) = \frac{\sum_{i=1}^m I(\mathbf{np})_i}{m} \quad (3)$$

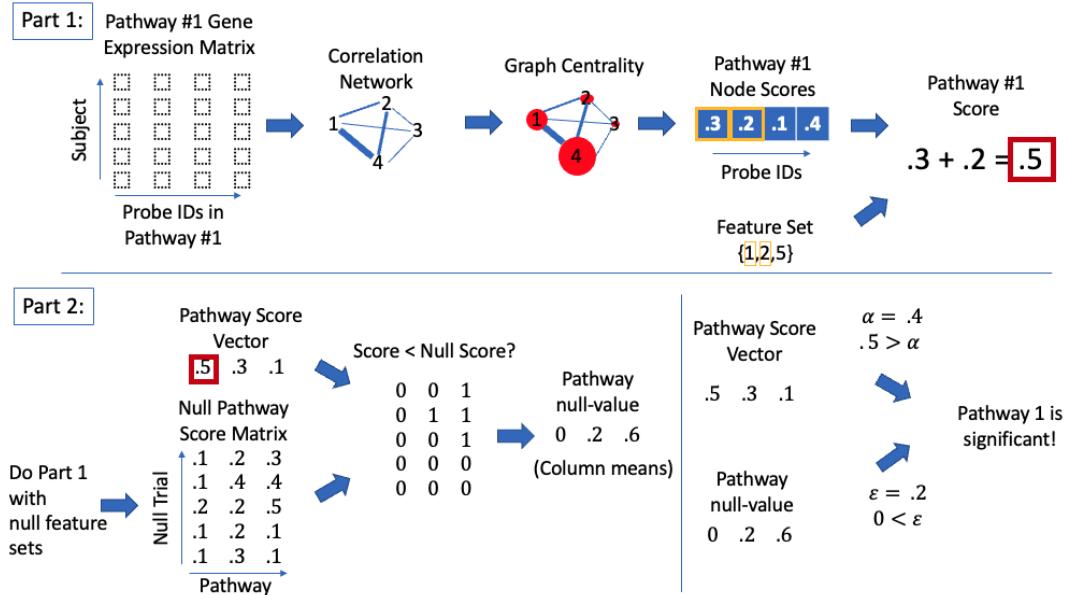


Figure 4. The workflow for CePa.

Finally, the significant pathways from CePa are the pathways with the highest CePa scores and lowest null values. So given a pathway score threshold $\alpha \in \mathbb{R}$ and null value threshold $\varepsilon \in \mathbb{R}$, we say a pathway P is significant if $CEPA(P) > \alpha$ and $\text{null value}(P) < \varepsilon$. For this paper, we take the top 60 pathways with null value less than .05.

Overall, CePa consists of three steps. First determine pathway scores, then find null values and select pathways using pathway score and null value thresholds. An overview of the CePa algorithm workflow is provided in Figure 4.

References

- Gu, Z., Liu, J., Cao, K., Zhang, J. & Wang, J. Centrality-Based Pathway Enrichment: a Systematic Approach for Finding Significant Pathways Dominated by Key Genes. *BMC Syst. Biol.* **6**, 1–13 (2012).

Acknowledgements

This paper is based on research partially supported by the National Science Foundation under Grant No. NSF-1830676.