**NIH Proposal Outline**

Six Page Limit – For Activity Codes R03, R13, R21, R36, SC2, SC3

1. **Introduction to Application**

The rise in popularity of single-cell RNA sequencing (RNA-Seq) technology [1] in recent years has lead to exciting developments into the investigation of heterogeneity between individual cells in biological samples [2, 3, 4, 5, 6]. Such variability necessitates single cell resolution in a number of investigative endeavors. One area of study that has received relatively little investigation in the light of this new data resolution is Boolean implication network construction and visualization. Previous works have been published on implication network construction using bulk cell population transcriptomics and microarray assays [7, 8]. However, these studies suffer from interpretation issues. The central goal of this proposal is to advance the state of the art in Boolean gene implication network construction taking advantage of the single-cell resolution available via single-cell RNASeq data.

1. **Specific Aims**
2. Development of a novel methodology to infer directed gene regulatory networks from single-cell RNASeq data by combining the resampling methodology presented in *Conditional density-based analysis of T cell signaling in single-cell data* by *Krishnaswamy et al.* [9] with the fuzzy Boolean implication network construction methodology of *Boolean implication networks derived rom large scale, whole genome microarray datasets* by *Sahoo et al* [10]*.*
   1. Apply our novel inference network workflow to various subsets of single-cells of interest according to a pseudo-temporal tree of cell states to better understand the dynamic interactions of genes through a biological process of states and/or differentiation of cell types. Such a partitioning of samples can be seen as a decoupling of binary gene relationships from their dependency on cell state.
3. Visualize for hypothesis generation tool providing an array of organizational and information tools to aid investigators’ navigation.
4. **Research Strategy**
   1. **Significance:** Gene dependence and correlation analyses have long been used to investigate the biological processes underpinning samples of interest. Recent work has been done regarding the susceptibility of traditional transcriptomic technologies to Simpson’s Paradox—the confounding of a mixture of signals that suggests one trend when in fact another is true. With the rise of single-cell RNAseq technologies, transcriptomics can now play a role in answering questions regarding tissue heterogeneity. This methodology promises a glimpse of the ‘rules of the game’ for a given progression across a dynamic cellular biological process [11, 3].

Previous work from our lab performed similar inference of Boolean implication networks with microarray data [12]. However, this methodology suffered, in part, from poor interpretability given the unknown cellular composition of the input datasets. A single-celled perspective in theory does not suffer from the averaging effects of bulk sample transcriptomics data and therefore derived implications in this work will not suffer from the same interpretation issues. Sub-aim 1.a, provides an exciting first look into the dynamics of Boolean implication of a given dataset and biological context. This work can further be used as a starting point for interesting future work, such as the generalization of implication detection in single-celled data from bivariate relationships to, the potentially more-interesting, gene triplet relationships.

* 1. **Innovation:** To our knowledge, no work has attempted to infer Boolean implication networks from single-cell RNASeq data. Furthermore, no other work has attempted to combine the works of Sahoo’s Boolean implication inference methodologies [10, 13] with Pe’er’s DREMI dependency metric [9]. We believe that the combination of these techniques yield the unique potential to discover gene implications that would otherwise be masked do to the rarity of certain cellular states in a given dataset. We build upon this innovation by separating samples according in pseudotemporal space with the help of Monocle presented in Trapnelle et al. [14]. This innovation gives investigators the ability to witness the dynamics of genetic implication through the progression of some biological process captured in a dataset.
  2. **Approach:**

**Aim 1**

Our first goal is to develop a novel methodology to infer Boolean implication gene networks from single-cell RNASeq data. The DREMI metric [Eq. 4] is of particular interest to this investigation because it has been shown to expose functional relationships between variables whose joint probability is dominated by a seemingly independent signature. DREMI is an application of mutual information [15] that measures the decrease in uncertainty of one variable given the value of another. To grasp the metric fully, consider the following definitions:

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| Let and |  |

Namely,andare vectors of random variables whileandare vectors of a sample’s X and Y components from the joint distribution .

Imagine that there may exist cell populations that are dominated by a particular cellular state or subtype (we’ll just say ‘state’ here for simplicity). Values in and may be dominated by a specific sub-range of the spans of and. If values in the dominant cell state are centralized around some (x, y) value, then many metrics may conclude that no relationship exists between genes X and Y. However, rare signatures for X and Y may offer discerning information regarding the functional dependence of genes X and Y. DREMI addresses this issue by subsampling according to an estimation of rather than . We’ll refer to this subsampling as along with the following definitions:

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DREMI deconvolution is achieved by estimating via a non-parametric diffusion kernel [Eq. 1] applied over bins of X values. Data is resampled according to this estimated conditional probability. And finally, mutual information [Eq. 2] is calculated for the down-sampled data. They show that this is equivalent to mutual information [Eq. 3] where every sample is weighted by [Eq. 4].

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The DREMI metric gives a reasonable measure for the strength of functional relationships between gene X and gene Y. The next step is to extract inference wherever possible. In other words, we want to turn our undirected network into a directed one. A number of previous works attempt to infer directionality in transcriptome data (Sahoo 2008). Previous work from our lab have successfully utilized the approach used by Sahoo et al. for the ‘fuzzification’ and directionality inference of relationships extracted from microarray data [12]. We intend to use the same basic methodology applied to the down-sampled technique described in Pe’er et al.

For a scatterplot of , we start by discretizing the resampled by labeling a given gene expression value as either ‘high’ or ‘low’. This is done by first ordering all observed values of from smallest to largest and fitting a step function to those ordered values using StepMiner [13]—an algorithm presented in Sahoo et al. These step functions aim to minimize the mean squared error (MSE) using an adaptive regression process. Next, the average of the high and low steps in a fitted step function serve to be the decision threshold between fuzzy ‘high’ and ‘low’ labels for gene X.

To discretize , we may be able to take advantage of the P(Y|X) normalization effects that occur in the subsampling proposed by Pe’er et al. In our lab’s original work, step function fitting was chosen, in part, because the minimization of MSE can still find a suitable boundary decision line in the presence of outliers. However, Pe’er’s conditional probability normalization resampling methodology can serve to remove outliers. We propose that a simpler boundary decision can be found:

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After discretization, we continue Sahoo’s workflow to test for sparsity amongst the quadrants formed by the decision boundaries for X and Y. This is done by testing sample counts in a quadrant against the null hypothesis of uniformity in the distribution of samples. For instance, consider the test for the quadrant corresponding to low values of both X and Y. Letting , , , and be the number of samples categorized with X and Y labels ‘low’ and ‘low’, ‘low’ and ‘high, ‘high and ‘low’, and ‘high and ‘high respectively. Sparsity of the low-low quadrant is determined as follows:

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|  |  | (8) |

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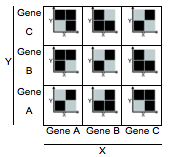
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| , for thresholds and | () |

Analogous calculations are performed to determine , , and . Now let, , , , and be Boolean variables that are true when, for a particular biological context, gene X or Y is highly expressed or not highly expresses (note that and ). Depending on the sparsity profile of the quadrants we propose that the following implications can be drawn.

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|  |  |  |  | Class | Conclusion |
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| 1 | 0 | 1 | 0 | Macintosh HD:Users:Brian:Documents:Research:CandidacyProposal:Figures:1010.png | No implication |
| 0 | 1 | 0 | 1 | Macintosh HD:Users:Brian:Documents:Research:CandidacyProposal:Figures:0101.png | No implication |
| 1 | 0 | 0 | 1 | Macintosh HD:Users:Brian:Documents:Research:CandidacyProposal:Figures:1001.png |  |
| 0 | 1 | 1 | 0 | Macintosh HD:Users:Brian:Documents:Research:CandidacyProposal:Figures:0110.png |  |

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The set of resulting, gene pair-wise Boolean implications constitute a Boolean implication network between ‘fuzzified’ expression values in a given biological context. An example of one such network containing 3 genes—A,B, and C—is illustrated in Figure 1.



**Figure 1|** Example implications (Left), and implication network (Right) for a set of three genes A, B, and C.

Note that the conditional probability subsampling methodology proposed does not guarantee symmetric implications about X and Y. Non-symmetric patterns may be of interest biologically—potentially indicating the presence of a rare expression pattern.

**Aim 1.a- Pseudotemporal Model**

Next, we attempt to decouple functional relationships between gene X and gene Y from conditional dependence upon a factor that Trapnell et al. refer to as ‘pseudotime’—“a quantitative measure of progress through a biological process” [14]. The interplay of genes is known to change as a cell progresses through dynamic biological processes such as differentiation, cell cycle, or oncogenic transformation. Our approach may not be able to identify strong relationships between X and Y when considering cells from multiple cellular states if the true, underlying relationships between X and Y in those states differ. For example, consider the example illustrated by Figure 2. Clusters and , when considered together, yield no implication. However, partitioning of the samples gives rise to separate implications. Moreover, if these clusters represent cellular states along a biological progression, we may gain valuable information by analyzing the difference in the class of implications yielded by each partition.

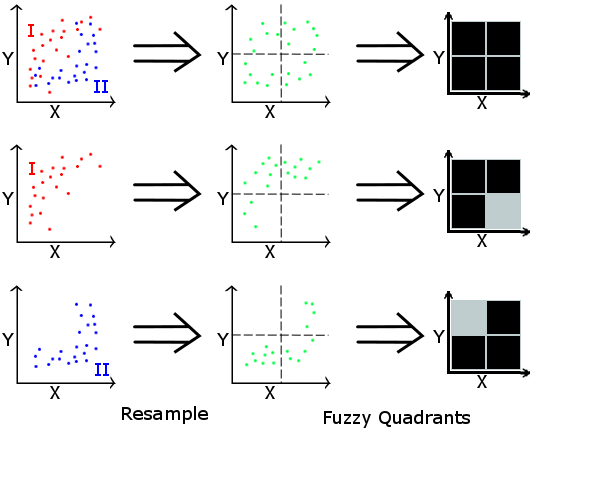


Figure 2| Somewhat contrived example of the convolution of signal that may exist in a dataset due to the combination of biologically differentiable cellular states.

To overcome this convolution of signals through pseudotime, we combine sample clustering with the temporal ordering output of an algorithm presented in Trapnell et al.—Monocle [14]. We plan to cluster samples in 2-dimensional independent component analysis (ICA) [16] space using hierarchical clustering. Let sets define these output clusters such that . And then check for agreement between our clustering and Monocle’s output as described below.

First, an overview of the algorithm in question: Monocle orders single-cells along an inferred smooth transition function in Euclidean gene expression space. Here represent the true set of expression values between states, and captures biological and technical noise. Monocle estimates by adapting a methodology that was first introduced by Magwene et al [7]. First, independent component analysis (ICA) [16] is performed on the data, and fitted with a minimum spanning tree (MST) in 2-dimensional independent component space. The diameter of the MST is taken as an estimate of . The algorithm goes on to find potential orderings of samples in the data relative to using a PQ tree. A PQ tree defines a family of orderings of discrete elements in a set; it is a tree with two types of nodes—a Q node whose children are ordered (although reversible), and a P type node where children are permutable. So, a PQ tree is created with a single Q node denoted QMain. All vertices along the diameter of the MST with degree greater than 2 are deemed ‘indecisive’, and ‘decisive’ otherwise. The ‘indecisive backbone’ of the diameter is located—the longest sequence of vertices for which the endpoints are indecisive. All decisive vertices along the indecisive backbone are added to QMain in an ordered fashion. Then for any indecisive vertex along the indecisive backbone, a P node is appended to the tree and the indecisive vertex is added as a child of that P node. This same approach is then applied recursively to each branch of the indecisive vertex. Possible orderings of samples in pseudotime are given by those orders extractable from the final PQ tree.

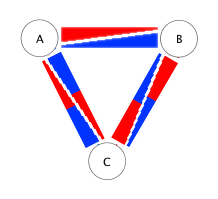
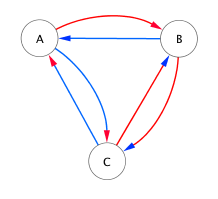
Using the ordering proposed by the PQ tree with the shortest total distance in component space to define our samples’ ordering in pseudotime, we can define an order between sample clusters. We’ll let be the ordered set of indices output by Monocle that orders our samples in pseudotime. Clusters can then be ordered by We will say that a given clustering agrees with the MST output by Monocle if the variance of per cluster is sufficiently small. We purposely supply this crude definition of ‘agree’ because clustering can be a highly customizable process by method selection and parameterization therein. Furthermore, we believe that the clustering achieved by hierarchical clustering and the ordering of individual samples by Monocle will largely agree because, if two samples are assigned to the same cluster, they are relatively close to each other and Monocle will therefore likely assign indices that are relatively close to each other as well.

At this point, we will assume that constitute different cellular states separated by different progressions through pseudotime. By constructing Boolean implication networks by the approach outlined for aim 1 for each individual cluster, we are, in a sense, looking at relational dependencies between genes at different slices of pseudotime. More interestingly, maybe, are the Boolean implication networks that result from considering samples that belong to pairs of adjacent clusters, where adjacency is defined according to and Monocle’s MST. Effectively we can construct the rules of genetic interplay for both major cellular states in a dynamic biological process as well as for the transitions between those states.

**Aim 2 –Visualization**

We will build an interactive visualization that will allow investigators to navigate the networks constructed by the approaches outlined in aim 1—complete with appropriate organizational and informational tools that can help the user with gene regulation oriented hypothesis generation.

First we wish to develop a concise visual encoding capable of conveying all classes of Boolean implication between gene pairs possible as output of aim 1 of this proposal. A traditional Boolean implication network visualization, as seen in Figure 1, requires nodes (‘high’ expression and ‘low’ expression labels per gene). Such a separation can complicate hypothesis generation. For this reason, we strive for a visualization consisting of a one-to-one mapping between genes and nodes. However, because traditional Boolean implication network nodes capture the state of Boolean variables, the proposed consolidation requires a translation of this information into another encoding. We propose capturing the state of a given variable in an implication in the directed edge between nodes as seen in the center panel of Figure 3. Finally we propose to further consolidate the information contained in this graph by consolidating implication classes between two genes using edge glyphs as seen in the right panel of Figure 3.



**Figure 3|** Example Boolean implication network from Figure 1 (Left). A node-consolidated visualization of the same example (Middle). Colors denote high (red) and low (blue) labels of genes in an implication relationship. Colors of arrow stems map to the upstream gene in an implication while arrowheads map to the downstream gene in an implication. And our proposed glyph encoding of edges (Right) which captures the same information.

Additional visual encodings:

* Edge thickness or transparency will be proportional to DREMI .
* Node color will denote user-specified gene groupings.
* Pairs of directed Boolean implication classes will be encoded according to the following chart:

Our application will be provided as an R package developed using the web interface framework Shiny [17]. Using a web interface framework like Shiny that can also be run locally provides the flexibility of future extension into a web service without sacrificing benefits of local applications (latency, data security, etc…). Scatterplots and heatmaps will be realized using the ggplots [18] package and network data structures, manipulations (such as layout), and visualizations will be handled using the igraphs package [19].

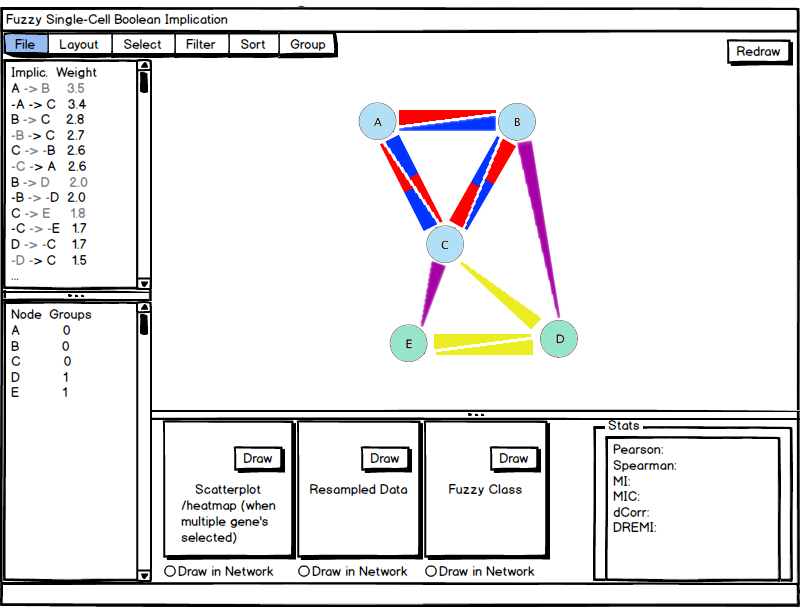
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Figure 4| Mockup of proposed interactive visualization application.

Proposed features:

* Import/export of data matrices.
* Boolean implication network with aforementioned visual encodings.
  + Click and drag interaction support.
  + Automatic node layout algorithm support.
* Node organization tools: criteria/manual selection, filter, sort, and grouping.
* Edge organization tools: criteria/manual selection, filter, and sorting.
* Sample organization tools: criteria/manual filtering.
* Node information tools: histogram
* Node group information tools:
  + Color-coding user specified
  + Use biomaRt [20] to query gene selections against public genetics databases.
  + Heatmap
* Edge information tools:
  + Visualization of underlying scatterplots, resampled data, and implication class progression.
  + Various statistics that can be calculated on the fly for a given relationship.
    - Pearson, Spearman, mutual information (MI) [15], maximum information coefficient (MIC) [21], distance correlation (dCorr) [22], and DREMI.
* Sample information tools:
  + Color-coding (to be viewed in scatterplots).
* Computationally intensive operations should be user initiated—never automatic.

**Datasets**

To take full advantage of the resolution of this technology, the dataset(s) should include samples of likely different cellular subtypes. Therefore, tissue whose cells are suspected of going through a dynamic biological process such as proliferation, differentiation, or transition into malignancy may be of interest. Such a dataset would also be well suited for evaluation of aim 2. Furthermore, the number of samples per subtype will impact the accuracy of estimated gene dependency metrics.

We will focus on stem cell differentiation datasets with a large number of samples to test our analytics. GSE60749 is an examination of pluripotent stem cells (PSCs) in mus musculus brain tissues [2]. Kumar et al. find that there were two clusters 1 composed of 98% of the samples and the other only 8% or 14 individual cells. This breakdown may constitute a rare cellular state.

GSE64016 provides 460 human embryonic stem cells (hESC)—213 H1 single cells and 247 H1-Fucci labeled single cells.

GSE65525 provides ~3000 UMI-barcoded, differentiating, embryonic mouse stem cells from a massively parallelized microfluidics-based single-cell sequencing technology called DropSeq [23].

# References

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| [1] | K. AA, K. JK, S. V, M. JC and T. SA, *The technology and biology of single-cell RNA sequencing.,* European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK; Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK. Electronic address: saraht@ebi.ac.uk.,, pp. --. |
| [2] | K. RM, C. P, S. AK, S. R, D. AJ, L. H, Z. J, P. K, G. D, T. JJ, F. TC, R. A, D. GQ and C. JJ, *Deconstructing transcriptional heterogeneity in pluripotent stem cells.,* 1] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, USA [2] Howard Hughes Medical Institute, Department of Biomedical Engineering, Center of Synthetic Biology, Boston University, Boston, Massachusetts 02215, USA., pp. --. |
| [3] | A. el AD, M. D. Davis KL FAU Tadmor, E. F. Tadmor MD FAU Simonds, J. H. Simonds EF FAU Levine, S. C. Levine JH FAU Bendall, D. K. Bendall SC FAU Shenfeld, S. Shenfeld DK FAU Krishnaswamy, G. P. Krishnaswamy S FAU Nolan, D. Nolan GP FAU Pe'er and P. D, *viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia.,* Department of Biological Sciences, Columbia Initiative for Systems Biology, Columbia University, New York, New York, USA. FAU - Davis, Kara L, pp. --. |
| [4] | A. K. Shalek, R. Satija, X. Adiconis, R. S. Gertner, J. T. Gaublomme, R. Raychowdhury, S. Schwartz, N. Yosef, C. Malboeuf, D. Lu, J. J. Trombetta, D. Gennert, A. Gnirke, A. Goren, N. Hacohen, J. Z. Levin, H. Park and A. Regev, "Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells," *Nature,* vol. 498, no. 7453, pp. 236-240, #jun# 2013. |
| [5] | M. GK, K. W. B. F. McCue, G. P. M. K. F. Schroth, J. S. G. F. Gertz, R. M. G. J. F. Myers, B. J. M. R. F. Wold and W. BJ, *From single-cell to cell-pool transcriptomes: stochasticity in gene expression and RNA splicing.,* Division of Biology, California Institute of Technology, Pasadena, California 91125, USA; FAU - Williams, Brian A,, pp. --. |
| [6] | A. P. Patel, I. Tirosh, J. J. Trombetta, A. K. Shalek, S. M. Gillespie, H. Wakimoto, D. P. Cahill, B. V. Nahed, W. T. Curry, R. L. Martuza, D. N. Louis, O. Rozenblatt-Rosen, M. L. Suvà, A. Regev and B. E. Bernstein, "Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma," *Science,* 2014. |
| [7] | M. PM, J. Lizardi P FAU Kim and K. J, *Reconstructing the temporal ordering of biological samples using microarray data.,* Department of Ecology and Evolutionary Biology, Yale University School of Medicine, New Haven, CT, USA. FAU - Lizardi, Paul, pp. --. |
| [8] | Y. Cai, B. Fendler and G. S. Atwal, "Utilizing RNA-Seq data for cancer network inference," in *Genomic Signal Processing and Statistics,(GENSIPS), 2012 IEEE International Workshop on*, 2012. |
| [9] | K. S, S. MH, M. M, B. SC, L. O, S. E, P. D and N. GP, *Systems biology. Conditional density-based analysis of T cell signaling in single-cell data.,* Baxter Laboratory in Stem Cell Biology, Department of Microbiology and Immunology, Stanford University, Stanford, CA, USA., pp. --. |
| [10] | S. D, A. J. Dill DL FAU Gentles, R. Gentles AJ FAU Tibshirani, S. K. Tibshirani R FAU Plevritis and P. SK, *Boolean implication networks derived from large scale, whole genome microarray datasets.,* Department of Computer Science, Stanford University, Stanford, CA 94305, USA. FAU - Dill, David L, pp. --. |
| [11] | H. L, B. F and T. FJ, *Diffusion maps for high-dimensional single-cell analysis of differentiation data. LID - btv325 [pii],* Institute of Computational Biology, Helmholtz Zentrum Munchen 85764 Neuherberg, Germany and Department of Mathematics, Technische Universitat Munchen 85748 Garching, Germany Institute of Computational Biology, Helmholtz Zentrum Munchen 85764 Neuherberg, Germany and Department of Mathematics, Technische Universitat Munchen 85748 Garching, Germany., pp. --. |
| [12] | A. W. M. S. H. C. K. H. A. Yates and R. Machiraju, "Visualizing Multidimensional Data with Glyph SPLOMs," *Eurographics Conference on Visualization (EuroVis),* 2014. |
| [13] | S. D, R. Dill DL FAU Tibshirani, S. K. Tibshirani R FAU Plevritis and P. SK, *Extracting binary signals from microarray time-course data.,* Department of Electrical Engineering, Stanford University, USA. FAU - Dill, David L, pp. --. |
| [14] | C. Trapnell, D. Cacchiarelli, J. Grimsby, P. Pokharel, S. Li, M. Morse, N. J. Lennon, K. J. Livak, T. S. Mikkelsen and J. L. Rinn, "The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells," *Nat Biotech,* vol. 32, no. 4, pp. 381-386, #apr# 2014. |
| [15] | C. Shannon, "A mathematical theory of communication," *Bell System Technical Journal, The,* vol. 27, no. 3, pp. 379-423, July 1948. |
| [16] | H. A and O. E, *Independent component analysis: algorithms and applications.,* Neural Networks Research Centre, Helsinki University of Technology, Finland. aapo.hyvarinen@hut.fi FAU - Oja, E,, pp. --. |
| [17] | W. C. et al., "Package 'shiny': Web Application Framework for R," https://cran.r-project.org/web/packages/shiny/shiny.pdf, 2015. |
| [18] | W. W, S.-C. F and R. M, *GOplot: an R package for visually combining expression data with functional analysis. LID - btv300 [pii],* Department of Cardiovascular Development and Repair and Bioinformatics Unit, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.,, pp. --. |
| [19] | G. Csardi and T. Nepusz, "The igraph software package for complex network research," *InterJournal,* vol. Complex Systems, p. 1695, 2006. |
| [20] | K. RJ, S. K. A. F. Haider, J. H. S. F. Zamora, G. Z. J. F. Proctor, G. P. G. F. Spudich, J. S. G. F. Almeida-King, D. A.-K. J. F. Staines, P. S. D. F. Derwent, A. D. P. F. Kerhornou, P. K. A. F. Kersey, P. K. P. F. Flicek and F. P, *Ensembl BioMarts: a hub for data retrieval across taxonomic space.,* European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UK. rhoda@ebi.ac.uk FAU - Kahari, Andreas,, pp. --. |
| [21] | D. N. Reshef, Y. A. Reshef, H. K. Finucane, S. R. Grossman, G. McVean, P. J. Turnbaugh, E. S. Lander, M. Mitzenmacher and P. C. Sabeti, "Detecting Novel Associations in Large Data Sets," *Science,* vol. 334, no. 6062, pp. 1518-1524, 2011. |
| [22] | G. J. Sz{\'e}kely, M. L. Rizzo and N. K. Bakirov, "Measuring and testing dependence by correlation of distances," *The Annals of Statistics,* vol. 35, no. 6, pp. 2769-2794, 2007. |
| [23] | K. AM, M. L, A. I, T. N, V. A, L. V, P. L, W. DA and K. MW, *Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells.,* Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA. Electronic address: marc@hms.harvard.edu.,, pp. --. |
| [24] | J. Zhang, L. Yu, H. Zhu and G. Sun, "Density and Non-Grid based Subspace Clustering via Kernel Density Estimation," *dicode-project.eu,* 2015. |
| [25] | W. PJ and W. Y, *A fuzzy logic approach to analyzing gene expression data.,* Bioinformatics, Department of Molecular Biology, Parke-Davis Pharmaceutical Research, Warner-Lanbert, Ann Arbor 48105, USA. FAU - Wang, Y, pp. --. |
| [26] | Q. P, S. C. Simonds EF FAU Bendall, K. D. J. Bendall SC FAU Gibbs, R. V. Gibbs KD Jr FAU Bruggner, M. D. Bruggner RV FAU Linderman, K. Linderman MD FAU Sachs, G. P. Sachs K FAU Nolan, S. K. Nolan GP FAU Plevritis and P. SK, *Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE.,* Department of Radiology, Stanford University, Stanford, CA, USA. pqiu@mdanderson.org FAU - Simonds, Erin F, pp. --. |
| [27] | D. P, E. Gong J FAU Syrkin Wurtele, J. A. Syrkin Wurtele E FAU Dickerson and D. JA, *Modeling gene expression networks using fuzzy logic.,* Virtual Reality Applications Center, Iowa State University, Ames 50011-3060, USA. FAU - Gong, Jian, pp. --. |
| [28] | S. I and K. SA, *Activities and sensitivities in boolean network models.,* Cancer Genomics Laboratory, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, USA. FAU - Kauffman, Stuart A, pp. --. |
| [29] | R. H, R. S. Reynolds R FAU Varghese and V. RS, *Increasing the efficiency of fuzzy logic-based gene expression data analysis.,* Intelligent Systems Laboratory, Department of Electrical and Computer Engineering, University of Maine, Orono, Maine 04469, USA. ressom@eece.maine.edu FAU - Reynolds, Robert, pp. --. |
| [30] | B. F, N. KN, C. FP, P. V, S. A, T. F. A.-O. 0000000224191943, T. SA, M. JC and S. O. A.-O. 0000000288187193, *Computational analysis of cell-to-cell heterogeneity in single-cell RNA-sequencing data reveals hidden subpopulations of cells.,* European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK., pp. --. |
| [31] | Z. I. Botev, J. F. Grotowski, D. P. Kroese and others, "Kernel density estimation via diffusion," *The Annals of Statistics,* vol. 38, no. 5, pp. 2916-2957, 2010. |
| [32] | M. AA, K. Nemenman I FAU Basso, C. Basso K FAU Wiggins, G. Wiggins C FAU Stolovitzky, R. Stolovitzky G FAU Dalla Favera, A. Dalla Favera R FAU Califano and C. A, *ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context.,* Department of Biomedical Informatics, Columbia University, New York, NY 10032, USA. adam@dbmi.columbia.edu FAU - Nemenman, Ilya, pp. --. |
| [33] | Z. A, M.-M. AB, C. S, L. P, L. M. G, J. A, M. S, M. H, H. L, B. C, R. C, C.-B. G, H.-L. J and L. S, *Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq.,* Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden. sten.linnarsson@ki.se jens.hjerling-leffler@ki.se.,, pp. --. |