Gene expression

Normalization of single-cell RNA-seq counts by $\log(x + 1)^\dagger$ or $\log(1 + x)^\dagger$

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1 Results

The ACE2 receptor, which facilitates entry of SARS-Cov-2 into cells (Zhang et al., 2020), has become one of the most studied genes in the history of genomics over the past two months. There are already hundreds of preprints about the gene (Google Scholar), and it is currently the default gene displayed on the UCSC genome browser (Lee et al., 2020). Several studies have reported on the expression of ACE2 at single-cell resolution, and papers have been rife with speculation about implications of differential ACE2 mRNA abundance for severity of disease. As is common in single-cell RNA-seq, the expression estimates of ACE2 are derived from counts that are filtered and normalized. Figure 1a shows an analysis of ACE2 mRNA in mice lungs (data from (Angelidis et al., 2019)). The expression is computed from cells containing at least one copy of the gene. While single-cell RNA-seq expression data has been modeled with many different distributions (Dadaneh et al., 2020; Van den Berge et al., 2018), for simplicity in illustrating our points we model this count data with a simple Poisson random variable $X$ with parameter $\lambda$ in order to demonstrate the implications of this restriction. Application of the filter amounts to computing

$$E[X|X > 0] = \frac{\lambda}{1 - e^{-\lambda}}. \quad (1)$$

While this is approximately $\lambda$ when $\lambda$ is large, it is close to 1 when $\lambda$ is small (de L’Hospital, 1768). Figure 1b shows the fraction of cells containing at least one copy of ACE2 (Booeshaghi and Pachter, 2020). Evidently, Figure 1a creates a misleading impression. While it may appear that average ACE2 expression is similar between young and old mice, when comparing the fraction of cells with non-zero expression of ACE2 it is clear that ACE2 has significantly lower mRNA expression in the lungs of aged mice than young mice.

The fraction $f$ of cells with non-zero expression of a gene has a useful statistical interpretation. We leave it as an exercise for the reader to show that the following estimator for the Poisson rate is consistent:

$$\hat{\lambda} = -\log(1 - f). \quad (2)$$

Since $f$ is approximately equal to this expression when $f$ is small, this provides an interpretation of the fraction of cells with at least one copy of a low-abundance gene as an estimate of the rate parameter $\lambda$ in a Poisson distribution.

Another mistake that we’ve found to be common in reporting ACE2 expression has to do with the log transformation, frequently used as part of a normalization of counts. Counts are log transformed for two reasons: the first is to stabilize the variance, as the log transform has the property that it stabilizes the variance for random variables whose variance is in the mean (Bartlett, 1947; Yeo and Johnson, 2000). There are two main considerations for this step: first when performing PCA on the gene expression matrix to find a reduced-dimensional representation that captures the variance, it is desirable that all genes contribute equally. The second consideration for the log transform is that it converts multiplicative relative changes to additive differences. In the context of PCA, this allows for interpreting the projection axes in terms of relative, rather than absolute, abundances of genes.

A seemingly minor technical issue in log transforming counts is that zero counts cannot be ‘logged’, as $\log(0)$ is undefined. To circumvent this problem, it is customary to add a ‘pseudocount’, e.g. $+1$, to each gene count prior to log transforming the data (Innes and Badger, 2018). We denote $\log(1 + x)$ by $\log_{1p}$ in accordance with nomenclature standard in scientific computing (Liu, 1988). For a gene with an average of $\lambda$ counts where $\lambda$ is large, it is intuitive that the average of the $\log_{1p}$ transformed counts is approximately $\log(\lambda)$. However, this is not true for small $\lambda$. An understanding of the result of applying the $\log_{1p}$ transform begins with the observation that for a random variable $X$, $E[f(X)]$ is not, in general, equal to $f(E[X])$. For example, if $X$ is a Poisson random variable with parameter $\lambda$, it is not true that $E[\log(1 + X)] = \log(\lambda + 1)$. By Taylor approximation,

$$E[\log(X + 1)] \approx \log(E[X] + 1) - \frac{E[X]}{2(E[X] + 1)^2} \quad (3)$$

$$= \log(\lambda + 1) - \frac{\lambda}{2(\lambda + 1)^2}. \quad (4)$$

This shows that for low-expressed genes, the average $\log_{1p}$ expression can differ considerably from $\log(\lambda)$, with the maximum difference according to the Taylor approximation at $\lambda \approx 1$. (see
Fig. 1. (a) Changes in ACE2 expression in the lungs of eight 3-month old mice and seven 24-month old mice after log1p transformation of the raw counts on the cells with non-zero ACE2 expression. The P-value was computed using a t-test. (b) Changes in ACE2 expression as determined by the fraction of ACE2 positive cells. The P-value was computed using a t-test. (c) A comparison of the naive estimate of the expectation of log1p (red) to the Taylor approximation of the expectation of log1p (blue), with the dashed black line of slope 1 showing the asymptotic behavior log(1 + x) ≈ log(x).

Conflict of Interest: none declared.

References
Angelidis, I. et al. (2019) An atlas of the aging lung mapped by single cell transcriptomics and deep tissue proteomics. Nat. Commun., 10, 963.
Bacher, R. et al. (2017) SCnorm: robust normalization of single-cell RNA-seq data. Nat. Methods, 14, 584–586.
Bartlett, M.S. (1947) The use of transformations. Biometrics, 3, 39–52.
Booeshaghi, A.S. and Pachter, L. (2020) Decrease in ACE2 mRNA expression in aged mouse lung. https://www.biorxiv.org/content/10.1101/2020.04.02.021451v1.
Dadaneh, S.Z. et al. (2020) Bayesian gamma-negative binomial modeling of single-cell RNA sequencing data. BMC Genomics, 21, 1–10.
de L’Hospital, G.F.A. (1768) Analyse des infiniment petits Moutard. Berkeley elementary function test suite. 2010-1O 10]. http://citeseerx.ist.psu.edu/viewdoc/download.
Hafemeister, C. and Satija, R. (2019) Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. Genome Biol., 20, 1–15.
Innes, B.T. and Bader, G.D. (2018) scClustViz—single-cell RNAseq cluster assessment and visualization. FI000Research, 7, 1522.
Lee, C.M. et al. (2020) UCSC Genome Browser enters 20th year. Nucleic Acids Res., 48, D756–D761.
Liu, Z.A. (1988) Berkeley Elementary Functions Test Suite. Lun,A. (2018) Overcoming systematic errors caused by log-transformation of normalized single-cell RNA sequencing data. https://www.biorxiv.org/content/10.1101/404962v1.
Tian, L. et al. (2019) Benchmarking single cell RNA-sequencing analysis pipelines using mixture control experiments. Nat. Methods, 16, 479–487.
Van den Berge, K. et al. (2018) Observation weights unlock bulk RNA-seq tools for zero inflation and single-cell applications. Genome Biol., 19, 17.
Warton,D.I. (2018) Why you cannot transform your way out of trouble for small counts. Biometrics, 74, 362–368.
Yeo, I.J. and Johnson, R.A. (2000) A new family of power transformations to improve normality or symmetry. Biometrika, 87, 954–959.
Zhang, H. et al. (2020) Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med., 46, 586–590.