BASiCS workflow: a step-by-step analysis of expression variability using single cell RNA sequencing data

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Abstract Cell-to-cell gene expression variability is an inherent feature of complex biological systems. Single-cell RNA sequencing can be used to quantify this heterogeneity, but it is prone to strong technical noise. Here, we describe a step-by-step computational workflow which uses the BASiCS Bioconductor package to robustly quantify expression variability within and between known cell populations (such as experimental conditions or cell types). BASiCS provides an integrated framework for data normalisation, technical noise quantification and downstream analyses, whilst propagating statistical uncertainty across these steps. Within a single seemingly homogeneous cell population, BASiCS can be used identify highly variable genes that drive the heterogeneity within the population as well as lowly variable genes that might exhibit housekeeping-like behavior. BASiCS also provides a probabilistic rule to identify changes in expression variability between cell populations, while avoiding confounding effects related to differences in technical noise or in overall abundance. Using two publicly available datasets, we guide users through a complete pipeline which includes preliminary steps for quality control and data exploration using the scater and scran Bioconductor packages. Data for the first case study was generated using the Fluidigm@ C1 system, in which extrinsic spike-in RNA molecules were added in order to quantify technical noise. The second dataset was generated using a droplet-based system, for which spike-in RNA is not available. The latter analysis provides an example, in which differential variability testing reveals insights regarding a possible early cell fate commitment process.

Keywords

Single-cell RNA sequencing, expression variability, transcriptional noise, differential expression testing

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Introduction

Single-cell RNA-sequencing (scRNA-seq) enables the study of genome-wide transcriptional heterogeneity in cell populations that remains otherwise undetected in bulk experiments [1, 2, 3]. Existing applications range from characterising cell types in immunity [4, 5, 6] and development [7, 8, 9] to dissecting the mechanisms for cell fate commitment [10, 11].

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