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Differential Expression vs. Differential Transcript Usage: Sleuth and DRIMSeq

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Abstract: ***This paper provides a comparative evaluation of two computational tools-Sleuth and DRIMSeq-for analyzing single-cell RNA sequencing data at the transcript level. Sleuth focuses on identifying differential expression in transcript abundance, integrating quantification uncertainty through bootstrapping methods. In contrast, DRIMSeq targets differential transcript usage by modeling isoform proportions using a Dirichlet-multinomial framework. The review outlines the statistical foundations, application contexts, and practical limitations of each tool, emphasizing their suitability for different research questions. By clarifying when and why to use each approach, this article supports informed decision-making in transcriptomic studies.***

Keywords: Single-cell RNA-seq, Sleuth, DRIMSeq, transcript analysis, differential expression

1. Introduction

Single-cell RNA sequencing (scRNA-seq) has revolutionized the study of gene expression by enabling transcriptome analysis at the resolution of individual cells. This technology provides unprecedented insights into cellular heterogeneity, gene regulation and disease mechanisms. However, extracting meaningful biological information from scRNA-seq data remains challenging, particularly when examining expression beyond the gene level. Many genes produce multiple transcript isoforms through alternative splicing, which may have distinct regulatory roles or produce distinct protein products. Simply analyzing gene expression can overlook important shifts in isoform usage or expression.

To address this challenge, computational methods have been developed to analyze gene expression at the transcriptome level. Two such methods, Sleuth and DRIMSeq, offer unique approaches for interpreting transcriptomic data. Sleuth focuses on identifying transcripts whose overall expression levels change across conditions (differential expression), whereas DRIMSeq detects changes in the relative proportions of different isoforms produced by a gene (differential transcript usage). By capturing different aspects of gene regulation, these tools provide complementary insights into the dynamics of the transcriptome. Given the increasing complexity of transcriptomic datasets and the rising use of scRNA-seq, understanding the distinctions between Sleuth and DRIMSeq is critical for designing accurate and biologically meaningful analyses.

This review adopts a comparative approach to examine two computational methods, Sleuth and DRIMSeq, for analyzing transcript-level gene expression in single-cell RNA sequencing (scRNA-seq) data. It introduces the importance of transcript-level analysis, compares the statistical frameworks of both methods, and contrasts their biological applications: Sleuth for identifying differentially expressed transcripts and DRIMSeq for detecting changes in relative isoform proportions. This review discusses the applications of these tools in scRNA-seq analysis, evaluates their respective strengths and limitations while highlighting the potential benefits of integrating multiple analytical methods for a more comprehensive understanding of transcriptional regulation. This comparative approach enables readers to understand the distinct features and applications of Sleuth and DRIMSeq, facilitating the selection of the most appropriate tool for specific research questions. This article aims to compare the capabilities, assumptions, and applicability of Sleuth and DRIMSeq for transcript-level analysis of single-cell RNA sequencing data.

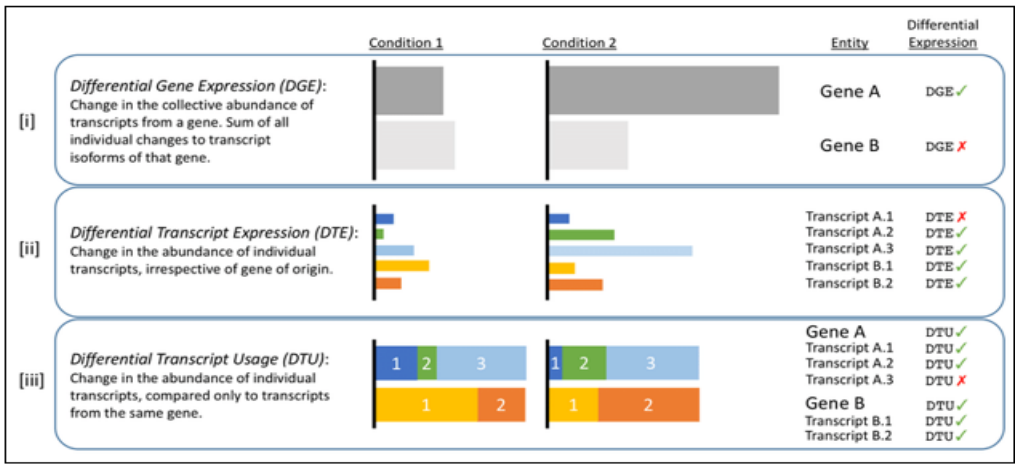

Figure 1: Differences between DGE, DTE, and DTU (Froussios et. al, 2019)

Sleuth

Sleuth is a transcript-level statistical method for analyzing differential expression in RNA-seq data, with a specific focus on transcript-level changes. What makes Sleuth unique is that it does not just look at how much RNA is expressed; it also asks how confident one can be in that number. Unlike most tools that start with simplified count tables and make assumptions about gene expression, Sleuth is tested using more realistic simulations that begin with raw sequencing reads (Pimentel et. al, 2017). This better reflects the messy reality of RNA-seq data, such as reads that map ambiguously or to multiple isoforms that are difficult to tell apart. The authors of Sleuth, Pimentel et. al, benchmarked it against other tools and found that it consistently outperformed them, especially in situations where other methods claimed to be highly confident but actually had high error rates. Sleuth was shown to be cautious, even overly cautious, but this meant that the transcripts it identified as significantly different were far more likely to be truly different.

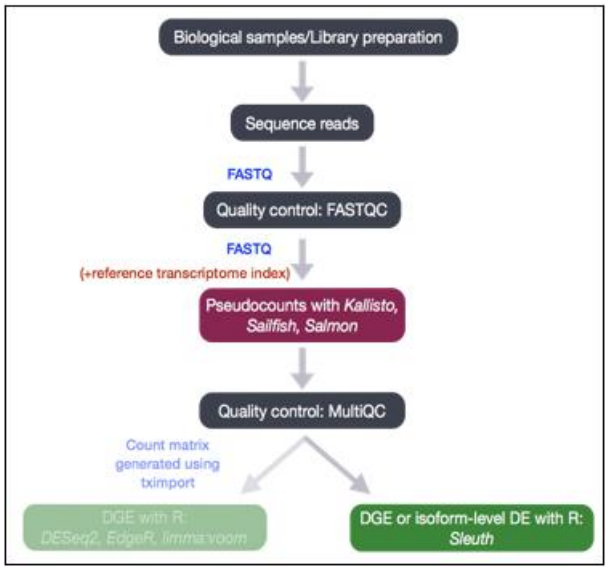

Figure 2: Sleuth pipeline (*Differential expression of transcripts using Sleuth.,* n.d.).

At the core of Sleuth is the additive response-error model, which separates the total variation in expression into two components: biological variance (real differences between samples) and inferential variance (measurement uncertainty). Inferential variance happens when it's hard to measure transcript levels accurately, like when sequences are very similar or there aren't many reads. Sleuth estimates this using a method called bootstrapping via kallisto, a fast tool that quantifies RNA and repeats the measurement many times to see how much it varies. To avoid overreacting to noisy data, Sleuth also applies shrinkage, which pulls extreme or unstable variance values toward more average ones (Pimentel et. al, 2017). This helps reduce false positives caused by random fluctuation. To test if a transcript is truly differentially expressed, Sleuth uses a likelihood-ratio test. In order to find transcripts that are truly different between conditions, Sleuth compares two models. One model assumes that the experimental condition (like treated vs. untreated) affects expression, and the other model assumes it doesn't. Then, it checks which model fits the data better. If the model that includes the condition fits much better, even after accounting for noise, Sleuth decides that the transcript is likely differentially expressed.

This method of separating measurement error from real biological change is what makes Sleuth so powerful. Most RNA-seq tools don't account for uncertainty; they just assume the expression values are solid and proceed with statistical testing. But Sleuth asks: how shaky is this number, and would the same value be obtained if the analysis is run again? This approach leads to more trustworthy results, especially when working with small sample sizes or with transcripts that have ambiguous reads. In simulations performed by Pimentel et. al, where the true differentially expressed genes were known, Sleuth had higher sensitivity and a lower false discovery rate (FDR) than other tools. While most tools underestimated how often they make mistakes, Sleuth was honest, and even conservative, about its confidence. This transparency is crucial in studies where a false positive could send researchers down the wrong path.

However, Sleuth is not perfect. One of its limitations is that it's tightly coupled to algorithms that use bootstrap sampling, such as Sailfish, Salmon, and Kallisto. This makes it less flexible if a researcher prefers a different pipeline. Also, because it operates at the transcript level, it may not be the best fit for experiments that are designed only for gene-level analysis. Still, its speed, thanks to kallisto, and its ability to visualize results, give Sleuth an advantage.

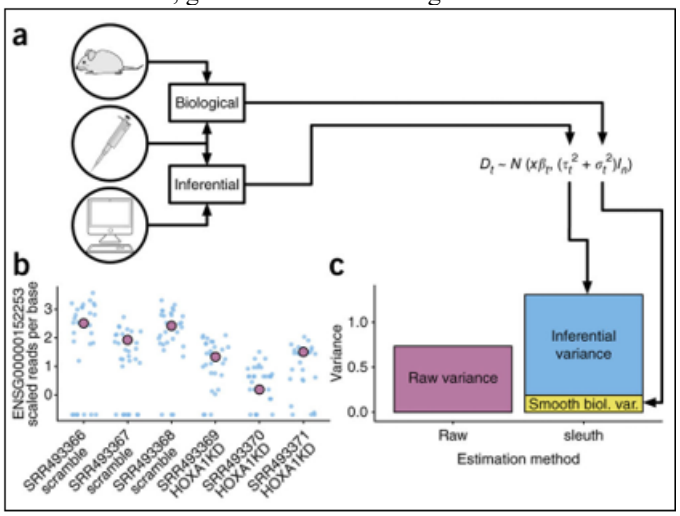

Figure 3: Sleuth variance determination (Pimentel et. al, 2017).

DRIMSeq

When studying RNA-seq data, each gene can produce multiple transcripts or isoforms. DRIMSeq was developed to test whether the relative proportions of these transcripts differ between conditions; this type of analysis is called differential transcript usage (DTU). Instead of asking if a transcript's expression changes on its own, DRIMSeq looks at whether the distribution of isoforms within a gene changes (Nowicka and Robinson, 2016). For example, if a healthy sample mostly expresses one isoform and a diseased sample expresses a different combination, DRIMSeq detects that change. This kind of data is often messy and shows more variation than expected, which is called overdispersion. Basic statistical models like the Poisson or multinomial distribution cannot account for this extra variability, so DRIMSeq employs a Dirichlet-multinomial (DM) model instead . This makes it

well suited for isoform-level analysis, especially in cases where transcript counts vary widely or when sample sizes are small.

DRIMSeq looks at the full set of transcripts for each gene as a single group rather than analyzing each one on its own. It treats the transcript counts like a vector—a list of isoform-specific counts—and uses a Dirichlet-multinomial model to study the overall proportions within that group. Since all the transcript proportions in a gene must add up to one, DRIMSeq models them together, which captures how a change in one isoform affects the others. To deal with noisy data or small sample sizes, DRIMSeq uses a method called empirical-Bayes shrinkage, which helps make the estimates more stable and less sensitive to random variation (Papastamoulis and Rattray, 2017). This is especially helpful when there are only a few replicates. In contrast to other tools that test each transcript independently, DRIMSeq understands that isoforms within a gene are related, which makes it more accurate when studying changes in transcript usage. Another strength of DRIMSeq is that it works with count data from any RNA-seq quantification tool, like Salmon, kallisto, and others. Unlike Sleuth, which only works with kallisto outputs and performs separate tests for each transcript, DRIMSeq does one test per gene, which means fewer comparisons and enhances the likelihood of identifying biologically meaningful changes.

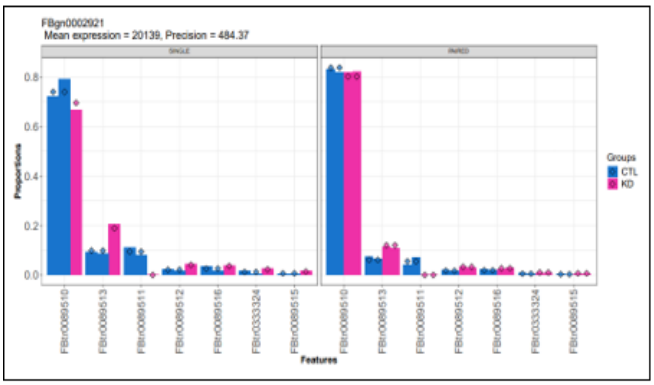

Figure 4: Gene expression proportions expressed as a whole via DRIMSeq analysis (Nowicka and Robinson, 2025)

DRIMSeq is especially valuable in studies focusing on alternative splicing or splicing quantitative trait loci (sQTLs). It can be used in many types of analyses, including PolyA-seq, differential methylation, and allele-specific expression, showing its broad range of applications. DRIMSeq is also more conservative than some other tools, meaning it identifies fewer genes with significant changes. However, the genes it does detect are more likely to be biologically meaningful, particularly in relation to splicing. DRIMSeq looks at how the proportions of different isoforms change, rather than just the overall expression level of a gene (Nowicka and Robinson, 2016). This allows it to detect complex cases where one isoform becomes more or less dominant, as long as that isoform is included in the reference file. It performs best when given transcript-level counts, although it can also work with exon-level counts, which tend to be noisier.

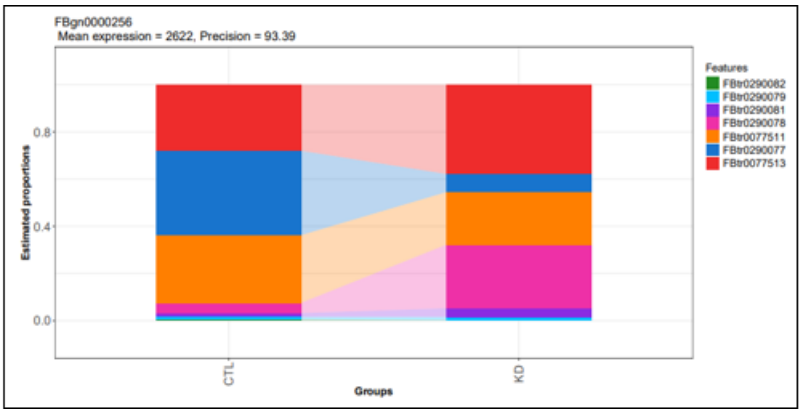

Figure 5: Gene expression proportions expressed in a bar chart via DRIMSeq analysis (Nowicka and Robinson, 2025)

One current limitation of DRIMSeq is that it treats transcript counts as fixed and does not take into account quantification uncertainty from upstream tools. For example, Sleuth handles this using kallisto's bootstraps, which help measure the confidence in each count. DRIMSeq does not currently include this kind of uncertainty modeling. However, future versions may improve this by incorporating observation weights or bootstrapped estimates. Like other transcript-level tools, DRIMSeq also depends on a good reference annotation. It cannot detect novel isoforms that are not already included in the dataset. Still, DRIMSeq's ability to model transcript proportions accurately, its flexible input requirements, and its support for a variety of analyses make it a powerful and widely applicable tool for understanding transcript-level changes in gene expression.

Comparing Sleuth and DRIMSeq

Both Sleuth and DRIMSeq are powerful tools for transcript-level analysis of RNA-seq data, developed to capture changes in the transcriptome. Each tool helps reveal subtle yet meaningful shifts in transcript expression or usage, particularly in contexts like alternative splicing or isoform regulation. They are statistically rigorous and conservative in calling results, aiming to reduce false positives while extracting biologically relevant patterns. However, while they share the broader goal of analyzing transcriptomic variation across conditions, Sleuth and DRIMSeq differ in the specific questions they ask, the statistical models they apply, and the kinds of biological insights they offer.

Table 1: A comparison of Sleuth and DRIM-Seq based on various categories of operation

Category	Sleuth	DRIMSeq
Biological Question	Detects differential expression (DE) - changes in the total expression level of individual transcripts across conditions.	Detects differential transcript usage (DTU) - changes in the relative proportions of isoforms within a gene.
Statistical Framework	Uses an additive response-error linear model that separates variation into biological and inferential (technical) noise.	Uses a Dirichlet-multinomial model that captures isoform proportions as a single distribution and models overdispersion.
Handling Uncertainty	Directly models uncertainty using bootstrap replicates from kallisto, allowing Sleuth to estimate confidence in transcript values.	Does not model quantification uncertainty; treats transcript counts as fixed, which may limit reliability in noisy datasets.
Test Level	Performs independent tests for each transcript. This provides fine-tuned resolution but increases the number of comparisons.	Performs one test per gene, improving statistical power and reducing the multiple testing burden.
Transcript Relationships	Treats transcripts independently, ignoring relationships between isoforms of the same gene.	Models transcript proportions jointly, recognizing that changes in one isoform affect the proportions of others.
Quantification Compatibility	Works only with kallisto, as it relies on kallisto's bootstrapping for variance estimation.	Compatible with multiple quantifiers (such as kallisto and Salmon), offering more flexibility in RNA-seq pipelines.
Use Case Strengths	Ideal for detecting transcripts or genes that are upregulated or downregulated due to a condition or treatment.	Best for detecting changes in isoform usage, especially in cases of alternative splicing or splicing QTLs.
Conservativeness	Cautious in calling DE because it accounts for inferential variance; avoids false positives but may miss subtle changes.	Conservative in DTU detection, often reporting fewer significant genes, but those reported tend to be biologically meaningful.
Annotation Dependency	Requires a well-annotated transcript reference; cannot detect unannotated or novel isoforms.	Same as Sleuth; performance depends on the completeness of transcript annotations.

A research team is studying alternative splicing changes in a rare neurological disorder using single-cell RNA-seq data from patient and control brain samples. The sample size was small because of the rarity of the condition.

Sleuth limitations: The team wants to use Sleuth for differential expression analysis but realizes that it only works with kallisto quantification. Their data were previously quantified using Salmon, which they preferred because of its handling of sequence bias. Rerunning quantification with Kallisto would require significant computational resources and time.

DRIMSeq limitations: When using DRIMSeq to detect differential transcript usage, researchers have noticed high variability in their results. They suspect that this is partly due to DRIMSeq not accounting for transcript quantification uncertainty, which is particularly problematic with their limited sample size and the inherent noise in single-cell data.

These limitations force the team to make compromises.

1) Alternatively, time and resources can be invested to requantify with kallisto to enable Sleuth usage, which may introduce inconsistencies with other analyses.
2) Alternatively, DRIMSeq can be used, knowing that the results may be less reliable due to unaccounted quantification uncertainty.

This scenario highlights how tool-specific limitations can complicate analysis workflows and potentially impact the robustness of results, especially in challenging experimental contexts, such as rare diseases with limited samples.

2. Conclusion

As transcript-level analysis gains importance in single-cell RNA-seq, tools such as Sleuth and DRIMSeq offer complementary approaches to understanding gene regulation and expression. Sleuth excels at identifying the differential expression of individual transcripts using kallisto bootstraps to account for quantification uncertainty. This makes it particularly reliable for detecting changes in expression in noisy data. In contrast, DRIMSeq employs a Dirichlet-multinomial model to analyze shifts in relative transcript usage, capturing isoform-level changes, such as alternative splicing.

Although both tools are statistically rigorous and aim to reduce false positives, they have distinct strengths and limitations. Sleuth's ability to model quantification uncertainty is offset by its restriction to kallisto outputs and its inability to model isoform relationships. DRIMSeq offers flexibility in input sources but currently lacks the incorporation of quantification uncertainty.

The choice of tool depends on the specific biological question. Researchers focusing on significant expression changes in individual transcripts may find Sleuth's statistical methods and visualizations more suitable. Those investigating shifts in isoform composition or splicing regulation may prefer the DRIMSeq approach. Together, Sleuth and DRIMSeq represent the growing sophistication of RNA-seq analysis tools, offering deeper insights into transcriptome dynamics than their predecessors. As these tools continue to evolve, addressing current limitations, such as improved uncertainty modeling in DRIMSeq or broader quantifier support for Sleuth, our ability to interpret transcript-level variations will become more refined and effective.

Key Takeaways

1) Sleuth excels at identifying transcripts with overall expression changes (differential expression), while DRIMSeq is designed to detect shifts in isoform proportions (differential transcript usage), making each tool suited to different aspects of gene regulation and alternative splicing.
2) Sleuth is closely integrated with specific quantification tools and accounts for variance using bootstrapping, whereas DRIMSeq is more flexible with input data and

uses a Dirichlet multinomial model. Ultimately, the choice depends on the biological question.

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