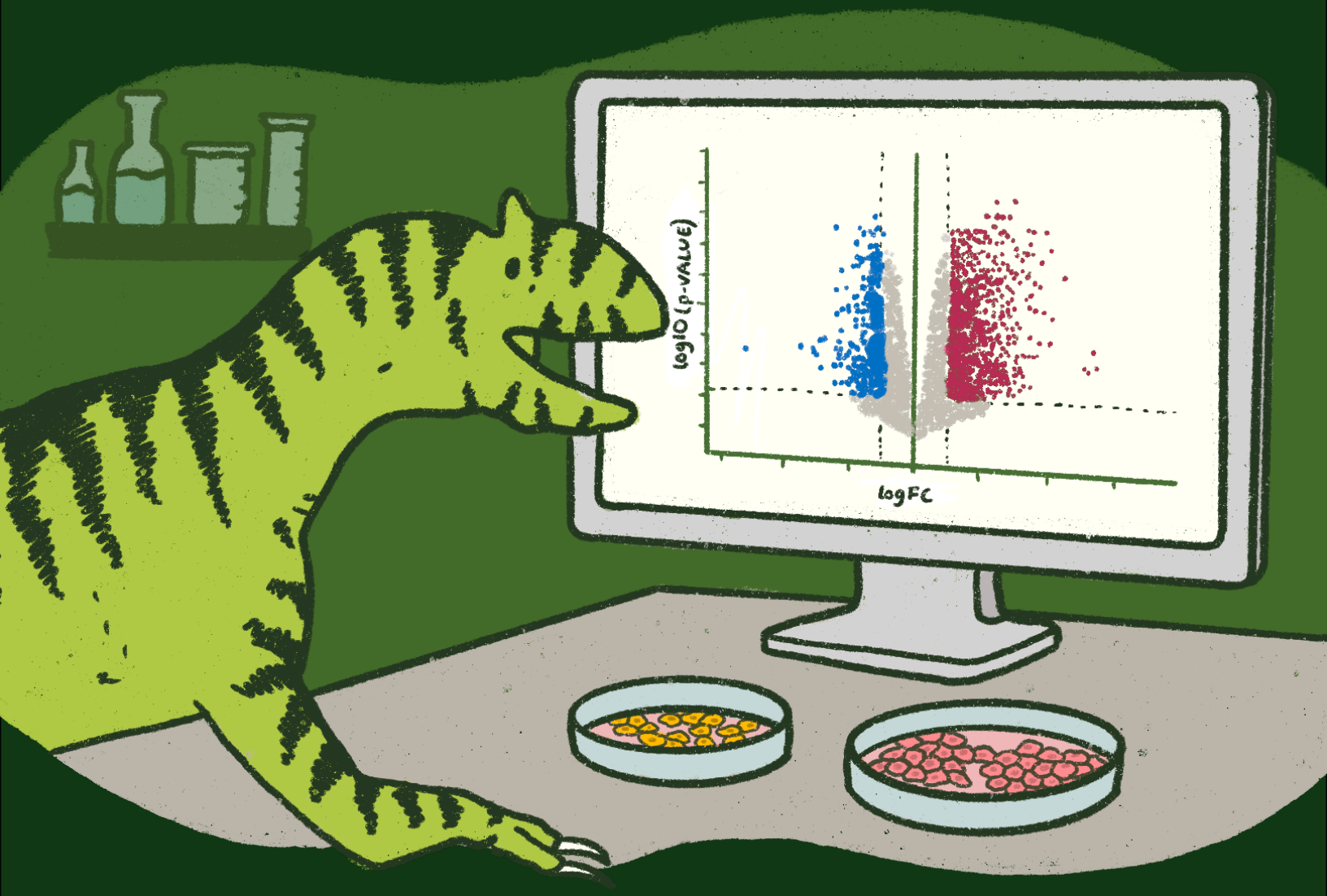
PLASMIDSAURUS

2026

WHITEPAPER

RNA-seq, Optimized for Everyday Discovery

A Comparison of Plasmidsaurus 3′ RNA-seq and Fragmentation-based RNA-seq for Differential Gene Expression



Introduction

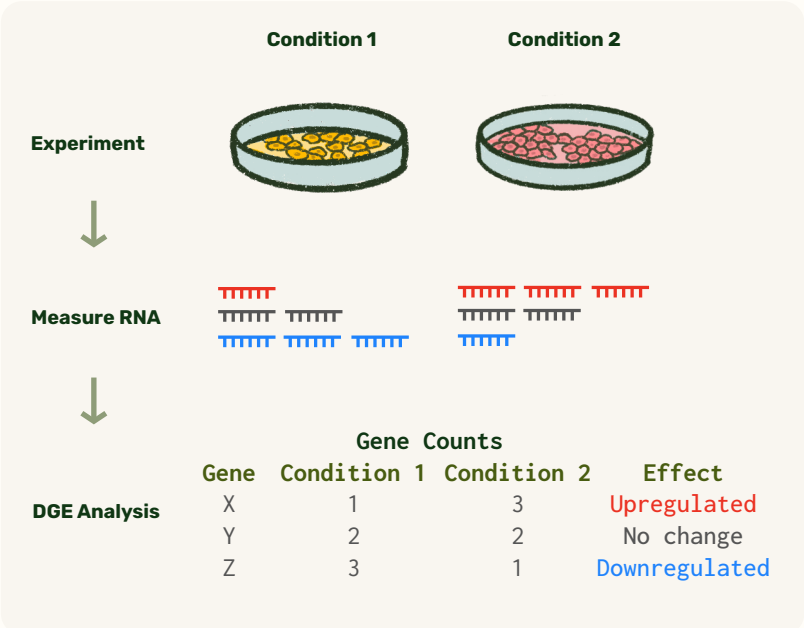
RNA-seq is the gold-standard tool for measuring differential gene expression (DGE). By capturing genome-wide expression patterns, RNA-seq can reveal pathway-level responses, uncover unexpected biology, and show how cells respond to perturbation. But fragmentation-based RNA-seq is often too slow, expensive, and operationally complex to use routinely. As a result, RNA-seq is reserved for major project milestones rather than using it as part of the experimental feedback loop.

For day-to-day experiments, many scientists rely on RT-qPCR because it is fast, familiar, and accessible. But RT-qPCR only measures changes in a small set of genes selected in advance, making it useful for targeted validation but poorly suited for discovery. Researchers are left choosing between speed and scope: a targeted assay that can be run quickly, or a genome-wide assay that provides deeper insight but is harder to use routinely.

Plasmidsaurus 3′ RNA-seq was built to close that gap. Using a 3′ end counting approach optimized for gene-level expression analysis, Plasmidsaurus delivers analyzed RNA-seq results in 3 days for $50 per academic sample and $80 per commercial sample, including bioinformatics analysis through an interactive portal. The goal is to make RNA-seq practical not only as a final experiment, but as a routine tool for guiding the next one.

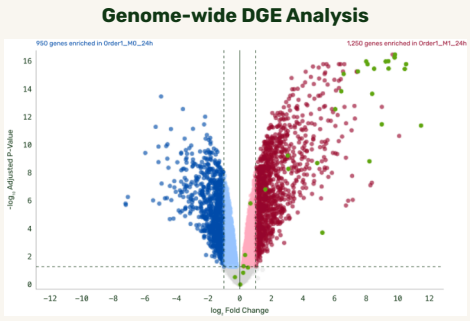
Plasmidsaurus 3′ RNA-seq is not simply a faster version of traditional RNA-seq. It is a different approach, optimized for a specific and common use case: measuring gene-level expression across many samples. The method is still unfamiliar to many researchers, who assume it answers the same questions, in the same way, as fragmentation-based RNA-seq. This whitepaper compares Plasmidsaurus 3′ RNA-seq with fragmentation-based RNA-seq to clarify where the methods overlap, where they differ, and how each fits into experimental design. For gene-level

Figure 1. Differential Gene Expression



Gene Counts

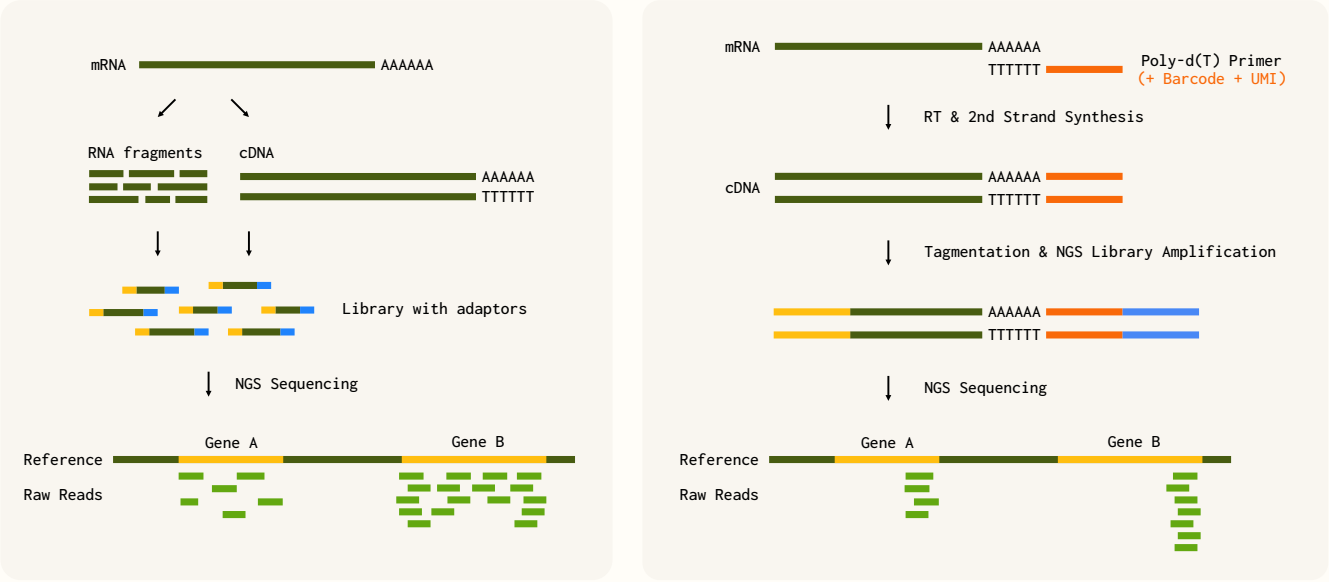
Gene	Condition 1	Condition 2	Effect
X	1	3	Upregulated
Y	2	2	No change
Z	3	1	Downregulated



Pathway-Level Insights

Gene set	Pathway	Effect
A,B,C	Hypoxia	Upregulated
D,E,F	Apoptosis	Downregulated
G,H,I	Glycolysis	Upregulated

Figure 2. Comparison of RNA-seq Workflows



Fragmentation-based RNA-seq **3' end counting RNA-seq**

expression quantification, Plasmidsaurus 3′ RNA-seq delivers performance comparable to fragmentation-based RNA-seq while making transcriptome-wide analysis faster, more scalable, and easier to use.

Why Use a 3′ End Counting Workflow?

Fragmentation-based RNA-seq has long been the standard for genome-wide DGE analysis. As the name implies, in these approaches transcripts or their cDNA are broken into smaller fragments before sequencing, resulting in coverage across the length of all genes (Figure 2, left). This makes the data useful not only for measuring gene expression, but also for gathering information about transcript structure, including splice isoforms, alternative transcription start sites, and novel transcripts. However, because short-read RNA-seq sequences fragmented transcripts or cDNA, it can be difficult or impossible to fully reconstruct transcript structures, especially when genes produce multiple overlapping isoforms. Long-read, full-length transcript sequencing approaches, such as PacBio Iso-Seq or Oxford Nanopore RNA/cDNA sequencing, are better suited to answer structural questions (Pardo-Palacios et al. 2024).

In many DGE experiments, the goal is not to reconstruct every transcript, but to measure how gene expression changes between conditions. Plasmidsaurus 3′ RNA-seq is built for that use case. By focusing reads at the 3′ end of polyadenylated transcripts, it captures gene-level expression information efficiently, without requiring full transcript coverage (Figure 2, right).

The 3′ end counting design also enables a more efficient workflow. In fragmentation-based RNA-seq, samples must be fragmented and processed separately through key library preparation steps, which makes the workflow difficult to streamline

Table 1. Comparison of DGE Methods

Method	Best for	Limitations	Speed	Cost
RT-qPCR	Fast measurement of a small number of known genes	Limited to pre-selected genes, requires PCR optimization, not discovery-friendly	Hours-Days	Scales per gene and sample
Plasmidsaurus 3′ RNA-seq	Fast, efficient, transcriptome-wide gene-level expression	No isoforms, limited to polyadenylated RNA (no bacteria, no small RNAs, fewer lncRNAs)	Days	$
Fragmentation-based RNA-seq	Transcriptome-wide gene-level expression and limited transcript structure characterization	Higher cost, slower turnaround, more complex analysis	Weeks	$$$
Long-read sequencing (PacBio, Oxford Nanopore)	Isoform characterization and quantitation	Prohibitive expense, typically means low quantitative sensitivity	Weeks	$$$$

and scale. Because sample-specific barcodes are introduced early in the Plasmidsaurus 3′ RNA-seq workflow, samples can be pooled earlier and processed together through downstream library preparation steps. That reduces hands-on processing, improves throughput, and helps reduce both turnaround time and cost.

The workflow difference matters because it makes RNA-seq practical earlier and more often in the experimental feedback loop. It moves RNA-seq from a slow, expensive milestone experiment toward a routine tool for everyday experimental feedback.

Experimental Methods

Plasmidsaurus 3′ RNA-seq and Illumina TruSeq RNA-seq were compared by performing a differential gene expression experiment using HEK cells treated with IFN-β, a cytokine known to induce broad transcriptional changes (Der et al. 1998). This well-established perturbation model was used to evaluate whether the two approaches recover concordant biological signatures. Four technical replicate libraries were generated from untreated and IFN-β-treated HEK cells. Purified RNA (100 ng per sample) was used for library preparation with either the Plasmidsaurus 3′ RNA-seq workflow or Illumina TruSeq Stranded mRNA Library Prep Kit (20020594), and sequenced on an Illumina NovaSeq X Plus.

The Plasmidsaurus 3′ RNA-seq service targets 20 million raw reads per sample, while fragmentation-based RNA-seq services commonly sequence 20 to 30 million raw reads per sample for fast expression profiling and 30 to 60 million raw reads per sample for standard whole-transcriptome experiments. To compare performance across this range, each method was sequenced to 50 million raw reads per sample and then downsampled across read depths.

Results and Discussion

We investigated the capacity of both methods to detect genes across major annotation classes, including protein-coding genes, long non-coding RNAs (lncRNAs), and processed pseudogenes. A gene was counted as detected if at least one read from one sample mapped to that gene. We chose not to filter low counts in order to present the most inclusive view of gene detection across read depths.

Across downsampling thresholds, Plasmidsaurus 3′ RNA-seq detected a comparable number of protein-coding genes and processed pseudogenes as TruSeq. For lncRNA transcripts, the apparent number of genes detected diverged between the two methods as read count increased (Figure 3). This pattern is consistent with prior comparisons of stranded fragmentation-based RNA-seq and 3′ end counting RNA-seq: protein-coding gene measurements tend to agree strongly between methods, while non-coding RNA detection and differential expression calls are more variable and more dependent on the read-mapping approach used (Corley et al. 2019). More broadly, because both methods compared here rely on poly(A) enrichment, neither is optimized for studies focused on comprehensive lncRNA profiling. Long non-coding RNAs may be polyadenylated or non-polyadenylated, so poly(A)-dependent workflows can underrepresent the full diversity of lncRNA transcripts.

These results show why Plasmidsaurus sequences samples to a standard depth of 20 million reads. Beyond this point, additional sequencing provides diminishing returns for gene detection, making 20 million reads a practical balance between broad transcriptome coverage and cost efficiency.

Figure 3. Unique Genes Identified

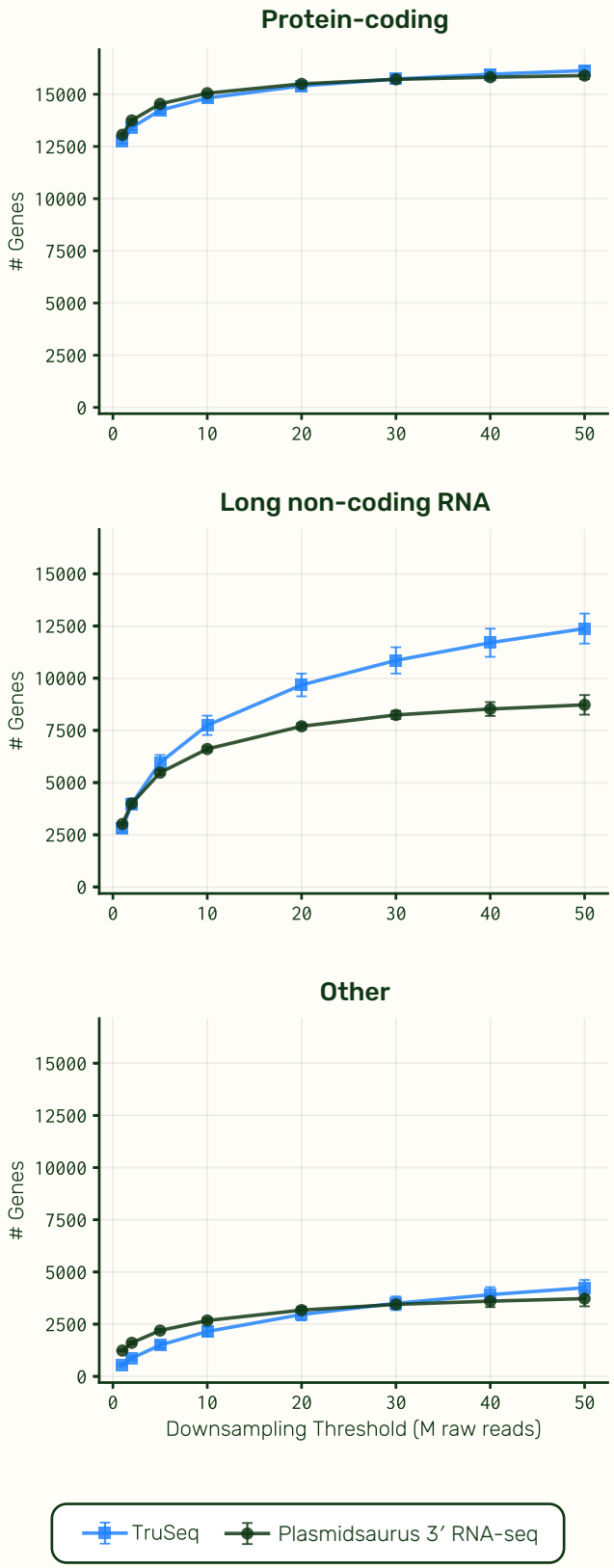
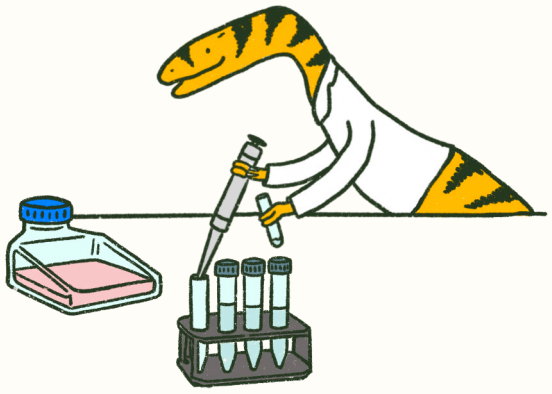


Figure 4. Differential Gene Expression Analysis Results

We next compared the ability of both methods to detect changes in gene expression between the untreated and IFN-β-treated HEK cells. We performed differential expression analysis using the Plasmidsaurus on-platform bioinformatics pipeline on each of the downsampled datasets, using a 2-fold-change cutoff and a false discovery rate (FDR) threshold of <0.05.

The total number of differentially expressed genes found in each downsampled dataset is shown in Figure 4A. At all read depths, Plasmidsaurus found more differentially expressed genes than TruSeq. In both methods, protein-coding genes accounted for the majority of differentially expressed genes (Figure 4B). Although Plasmidsaurus detected more differentially expressed genes overall, the difference was largely driven by non-coding biotypes (Figure 4B).

Finally, we carried out a pairwise comparison at each downsampling threshold and calculated the number of differentially expressed genes shared between the two methods (Figure 4C). The two methods shared >70% of DE genes at every downsampling level.



The Plasmidsaurus Difference

Together, these results show that Plasmidsaurus 3′ RNA-seq delivers robust gene-level differential expression data that is comparable to fragmentation-based RNA-seq.

But sequencing speed is only part of the challenge. Even rapid RNA-seq can leave researchers bottlenecked if raw data still has to be converted into interpretable results. Plasmidsaurus pairs rapid sequencing with expert-built automated analysis, so researchers receive interpretable results rather than raw data alone. After sequencing, results are processed through standardized QC, statistical analysis, and visualization steps, then presented in intuitive, interactive figures. We take care of the data wrangling, statistical analysis, and visualization so researchers can focus on interpretation: evaluating results, identifying patterns, and deciding what experiment to run next.

By combining rapid 3′ end counting, integrated analysis, and accessible pricing, Plasmidsaurus brings transcriptome-wide DGE closer to the pace of everyday experimentation. Instead of choosing between broad discovery and practical turnaround, researchers can use RNA-seq to guide the next experiment, support replicate-rich study designs, and capture biology they would have missed with a targeted assay.

No weeks-long wait for raw data. No bioinformatics bottleneck before interpretation. Just fast, accessible gene expression data built for the way experiments actually move.

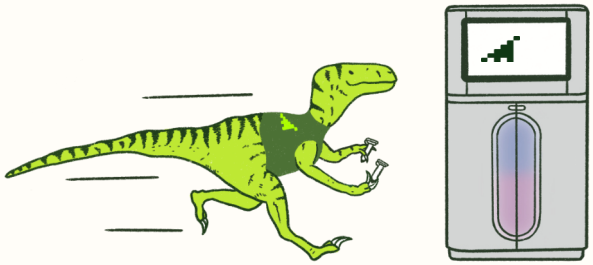
Try Plasmidsaurus 3′ RNA-seq and start learning more from every experiment.

Figure 5. Results in 3 days

Sample submission

Drop off samples at any of our 1,000+ dropbox locations worldwide, or ship straight to our lab.

Skip the dry ice!
Ambient shipping with SEQguard™ Dino Preserve.



Hang tight as we Sequence Everything

Analyzed results delivered in less than 3 days if shipped within the US or Europe. (~1 week from everywhere else.)

Keep moving forward!

Instantly view your analyzed results on our online platform with intuitive, interactive plots.

Scan here for a Live Demo of the Plasmidsaurus 3' RNA-seq analysis features

Citations:

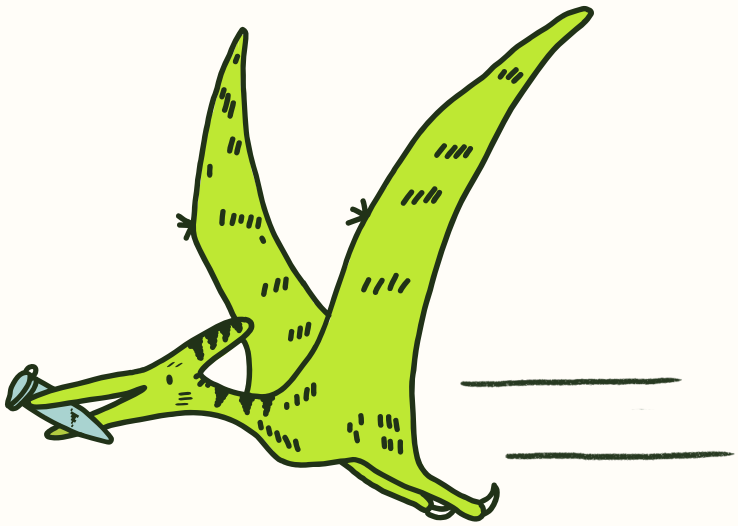
Pardo-Palacios, F. J., Wang, D., Reese, F., Diekhans, M., Carbonell-Sala, S., Williams, B., ... & Brooks, A. N. (2024). Systematic assessment of long-read RNA-seq methods for transcript identification and quantification. *Nature Methods, 21(7),* 1349-1363. https://doi.org/10.1038/s41592-024-02298-3

Corley, S. M., Troy, N. M., Bosco, A., & Wilkins, M. R. (2019). QuantSeq 3′ Sequencing combined with Salmon provides a fast, reliable approach for high throughput RNA expression analysis. *Scientific Reports, 9(1),* 18895. https://doi.org/10.1038/s41598-019-55434-x

Der, S. D., Zhou, A., Williams, B. R., & Silverman, R. H. (1998). Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. *Proceedings of the National Academy of Sciences of the United States of America*, 95(26), 15623–15628. https://doi.org/10.1073/pnas.95.26.15623

About Plasmidsaurus

Plasmidsaurus is the sequencing infrastructure for modern biology. Founded by scientists, for scientists, it was built around a simple idea: fast sequencing changes how research gets done. With global drop-off locations and labs, round-the-clock operations, purpose-built automation, and integrated analysis tools, Plasmidsaurus delivers results researchers can trust and act on. From routine validation to complex DNA sequencing projects, scientists rely on Plasmidsaurus to keep discovery moving.



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