Showtime: scientist shatters single-cell sequencing sensitivity ceiling with SMART-Seq Single Cell Kit

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BIO VIEW

Our bodies are composed of trillions of cells, each of which possesses a unique, specialized function. Comprehending each cell type will be vital to fully understanding our biology, but given the sheer scale and diversity of the human body, it can seem like a challenge of an insurmountable scale.

Fortunately, some researchers think 'insurmountable' is just a matter of opinion. Dr. Holger Heyn (Team Leader at the National Centre for Genomic Analysis in Barcelona, Spain) is one such person. Up until recently, he extensively used Smart-seq2 (SS2). However, like many SS2 users, he wanted something that was off the shelf, validated, reproducible, and sensitive—so we were excited when he decided to test out our SMART-Seq Single Cell Kit (SSsc).

We'll just spoil the ending: we came out ahead.

Setting the stage

Library preparations were run side by side with both B and T cells, which were specifically chosen due to their low RNA content. Both SS2 and SSsc reactions were similarly miniaturized for this study. Dr. Heyn tested two different PCR amplification conditions (23 and 25 cycles) for each chemistry.

Lights. Camera. Action!

Following preparation, libraries were quantified and analyzed on a Bioanalyzer 2100 instrument. While electropherogram profiles were consistent between SSsc and SS2, Dr. Holger Heyn noted the higher cDNA yield generated by the SSsc chemistry.

Following sequencing, both of the chemistries exhibited comparable performance when it came to total reads generated per library. However, while SSsc demonstrated high reproducibility in the percentage of mapped reads across all PCR and workflow conditions, SS2 displayed a marked difference between libraries prepared with 23 versus 25 PCR cycles (Figure 1).

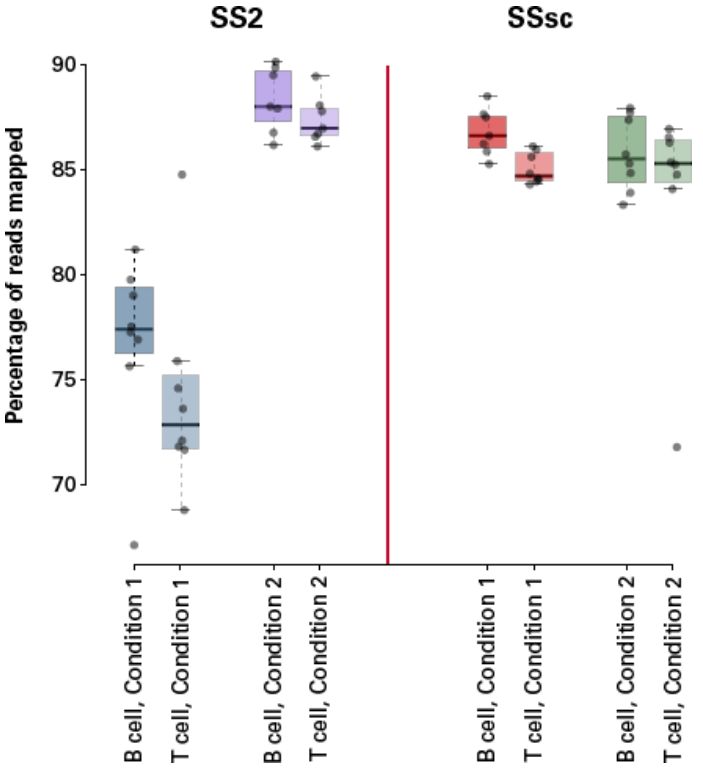

Figure 1. SSsc demonstrates a more robust performance compared to SS2. Libraries were prepared from both B and T cells with either 23 (Condition 1) or 25 (Condition 2) cycles of PCR amplification. While SSsc demonstrated a negligible difference in the percentage of mapped reads between the two conditions, SS2 exhibited marked differences in 23 versus 25 PCR cycles. This indicated that small variations in workflow have a significant impact on the reproducibility of SS2 libraries.

In addition to mapped read percentage, SSsc also outperformed SS2 in another key metric: the number of genes identified. SS2 identified an average of ~400 genes per library across all conditions, while SSsc identified at least five times as many (~2,000–2,750) genes per library (Figure 2), speaking to the unprecedented sensitivity afforded by our new SSsc chemistry.

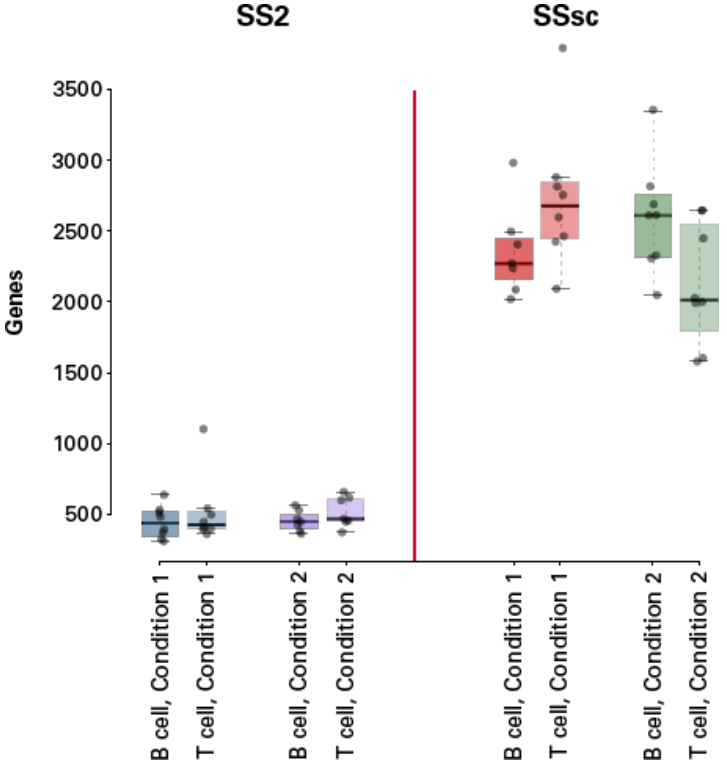

Figure 2. SSsc outperforms SS2 in gene identification. In all sample types and conditions (identical to Figure 1, above), SSsc identified five times as many genes as SS2, exhibiting unprecedented sensitivity compared to SS2.

And the award goes to...

These data demonstrate how Dr. Heyn leveraged the strengths of our new SMART-Seq Single-Cell Kit to break performance barriers. The optimized chemistry allowed him to achieve unparalleled sensitivity and improved cDNA yield over SS2—all while using a validated kit, which is made under stringent quality standards for superior lab-to-lab reproducibility.

Fill out the form to the left to be the first to know when we release additional SSsc data, or to reach our expert technical support team and learn how we can help you get more out of your single-cell research workflows. If you are on a mobile device, click on the hamburger icon (☰) on the top left of your screen, then scroll down to access the registration form.

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