A high throughput RNA-seq method to enable efficient drug screening

Samantha Langer, Wenqi Zhu, Ji Li
Singleron Biotechnologies, Cologne, Germany

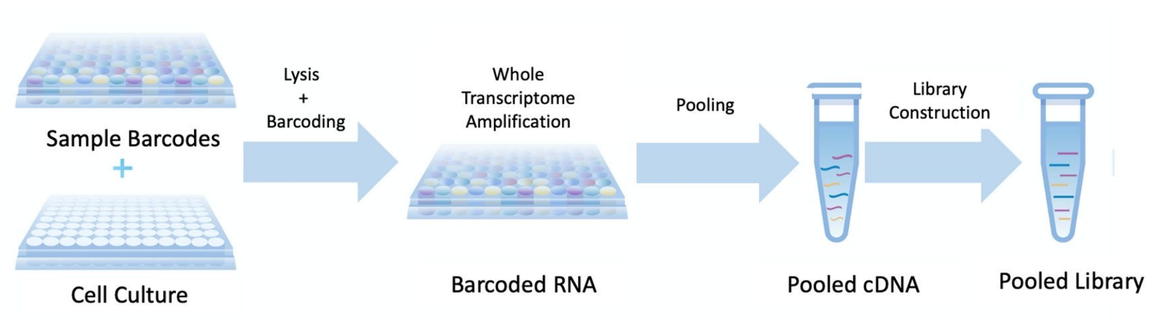
Contact: samantha.langer@singleronbio.de www.singleronbio.com

Singleron Biotechnologies GmbH, Gottfried-Hagen-Straße 60, 51105 Cologne, Germany

Introduction

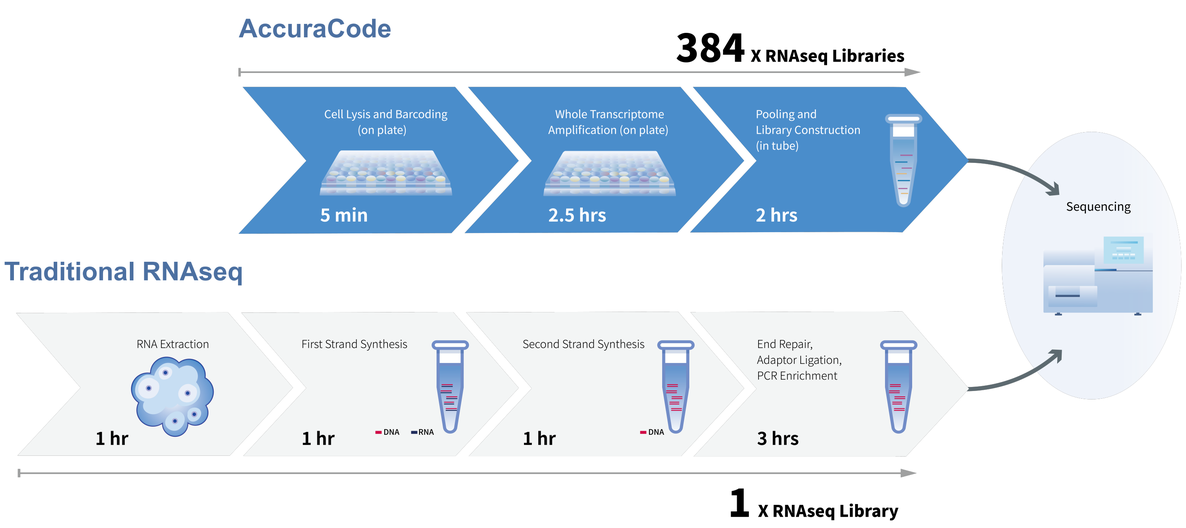
High-throughput screen is an essential part of drug discovery and development. RNA-seq can be used to generate comprehensive information on drug effects on the transcriptome, which could be used to determine the mechanism as well as effectiveness of various drug compounds. However, the RNA-seq library preparation procedure is costly and tedious, making it difficult to use large-scale RNA-seq in drug screening. To overcome these challenges, we have developed AccuraCode, a high-throughput RNA-seq library construction method that drastically reduces time and costs compared to traditional RNA-seq. Cell cultures from 384-well plates are barcoded on-plate, followed by a one-step whole transcriptome amplification (WTA) to yield the cDNA with unique sample barcodes. Since only one library preparation reaction is carried out using the pooled cDNA from up to 384 samples, hands-on time and expenses are reduced by 90%. Thus, AccuraCode provides a fast, cost-effective high throughput method which could greatly facilitate the drug discovery process.

AccuraCode® - High-Throughput RNA-seq Library Construction Kit

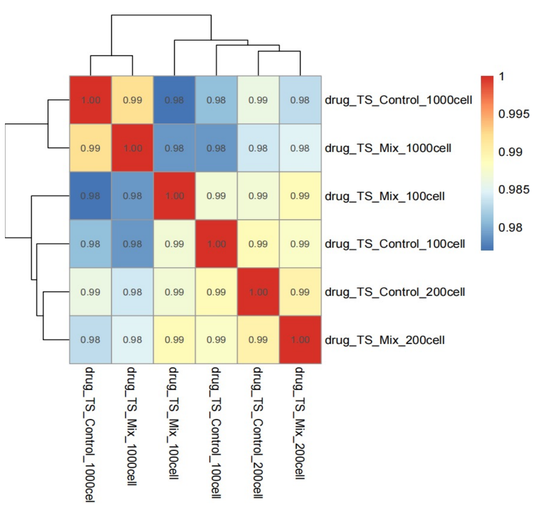


This bulk RNA-sequencing, ultra-high-throughput library construction technology enables drug discovery and screening of drug responses at large scale. Cell lysis, barcoding, and one-step whole transcriptome amplification, are all performed on-plate in 384-well plates. Then cDNA from 384-well plates are pooled and one library construction is conducted. This method massively reduces labor, time and cost by more than 90%.

Easy and Fast Workflow



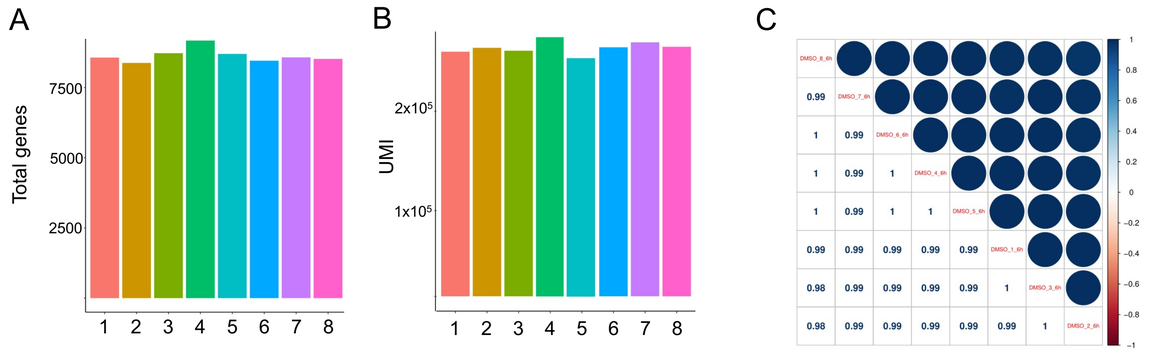
High Sensitivity



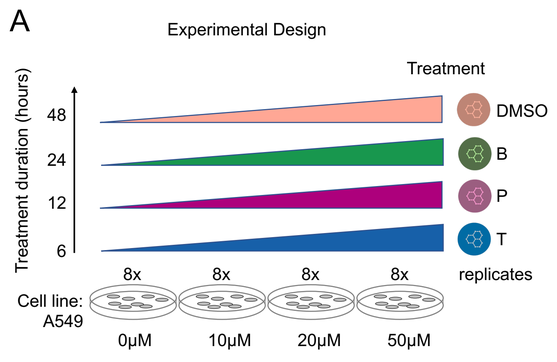
To test whether low cell input has an impact on data reproducibility, we performed library preparation and sequencing of 100, 200, and 1000 cells originating from the same sample in duplicates. Correlation analysis revealed excellent reproducibility independent of the number of cells processed within one well. Thus, AccuraCode provides an ultra-high-throughput solution with high sensitivity.

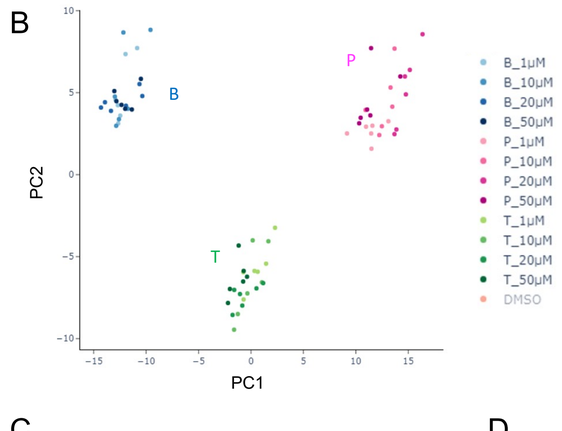
High Reproducibility

Eight samples were processed in parallel and sequenced at 1M reads per sample to examine reproducibility of data output. Total genes (A) and UMI (B) were consistent among the eight replicates. Correlation of the overall gene expression patterns (C) between those eight replicates revealed high reproducibility among replicates.

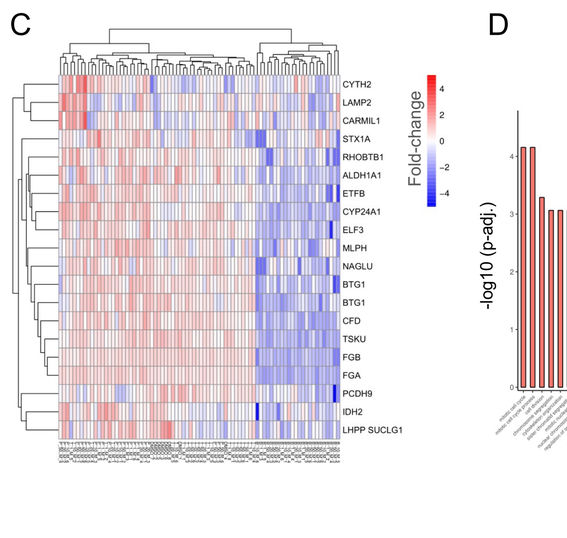


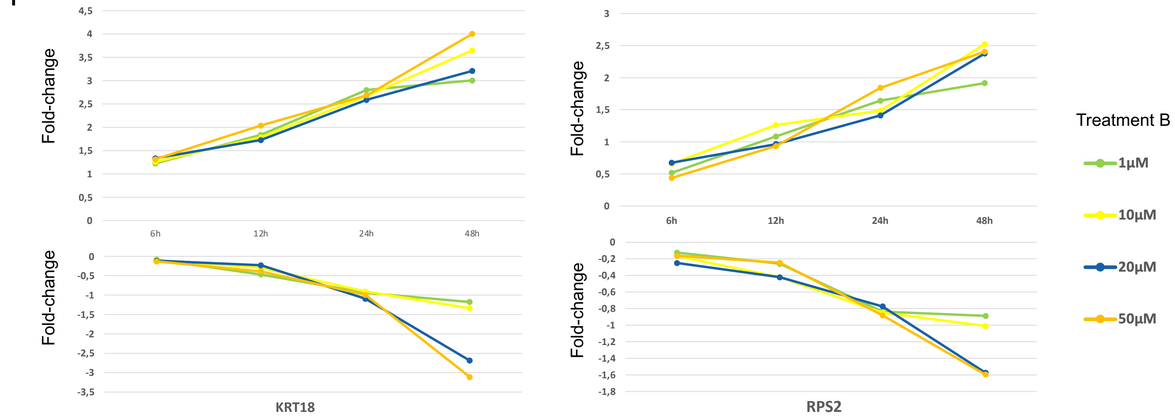
Drug screening and drug responses study





(A) To examine the performance of AccuraCode, A549 cells were treated in 8 replicates with different concentrations of 3 drugs (B, P, and T) for a time of 6, 12, 24, or 48h. Principal component analysis (B) revealed clustering of samples treated with the same drug. (C) Drug treatment resulted in sample specific changes of various transcripts allowing to decipher the exact molecular mechanisms induced upon drug treatment. (D, E) Further pinpointing transcriptional changes GO-Term enrichment analysis revealed significant changes of biological processes and cellular components. (F) Ultimately, differentially expressed genes could be tracked in a time-resolved manner.





Conclusion

AccuraCode:

- is an easy high-throughput RNA-seq method to prepare 384 sample libraries simultaneously within 5 hours.
- shows high sensitivity, reproducibility, and accurate transcriptome profiles with as low as 100 cells.
- can be applied to drug screening and drug discovery to enable efficient transcriptome profiling, and to identify optimal treatment regarding choice of drug, doses, and time-points.