TECHNICAL NOTE

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USING THE SWIFT NORMALASE® KIT WITH 10x GENOMICS FOR RAPID, LOW COST NORMALIZATION OF SINGLE-CELL NGS LIBRARIES

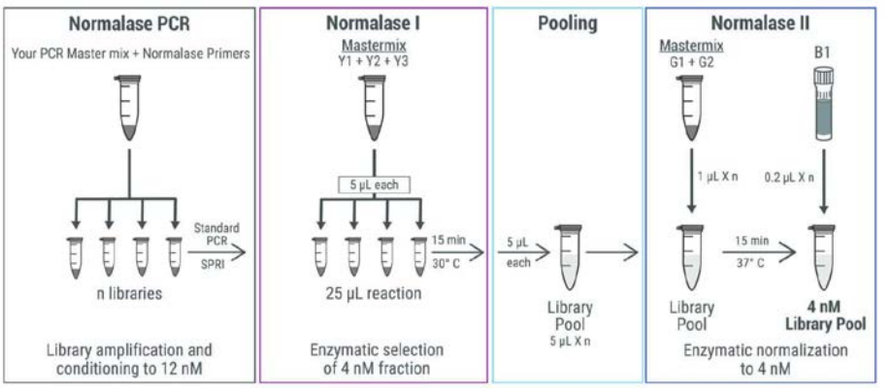
SUMMARY

This technical note illustrates how the Swift Normalase Kit can be used to normalize 10x Genomics libraries, providing an alternative to labor-intensive qPCR-based library quantification.

- We present a method for integrating Normalase into the 10x Genomics workflow, based on a customer use case at Cedars-Sinai Applied Genomics, Computation, and Translational (AGCT) Core.
- We present customer sequencing data for the usage of Normalase to normalize 10x Genomics scRNA-Seq libraries and the expected results to normalized yields and clustering distribution and standard deviation between libraries.

INTRODUCTION

The Swift Normalase Kit is a novel enzymatic NGS library normalization technology that streamlines library balancing and pooling for ease of loading on Illumina sequencing platforms. The Normalase workflow eliminates the need for using library quantification and insert size determination to individually adjust concentrations prior to library pooling, enabling equal volume pooling for mid- to high-throughput sequencing labs. This fast, robust and automatable workflow results in optimal cluster density and library balance and can easily be integrated into your standard DNA and RNA library preparation protocols to improve turnaround time, increase efficiency and reduce cost for NGS laboratories.



In this technical note, we present a method and data for saving time and steps with 10x Genomics libraries by using Normalase to complete the library normalization step. Rather than qPCR quantification, Normalase balances and pools libraries ready for sequencing at the appropriate concentration, as well as providing optimized CV, clustering, and coverage data.

WORKFLOW*

Where do you integrate Normalase in the 10x Genomics library amplification step?	Indexing PCR is performed with 10x Genomics primers followed by the recommended double-sided clean-up. We then run a BioAnalyzer to look at the peak and to make sure that we have at least 12nM. So far, we have not had any problems as our samples are above 12 nM even though fewer indexing PCR cycles are performed (explained in the next section). Then we incorporate the Normalase PCR, and we follow the Normalase library purification and subsequent steps.
How many cycles of PCR do you perform?	We subtract 3 PCR cycles at the indexing PCR step so that when we perform Normalase PCR, we do the 3 PCR cycles as indicated in the Normalase protocol. There are 14 PCR cycles total (11 indexing PCR + 3 Normalase PCR with a clean-up step in between).
Which Normalase primers do you use?	We use the Normalase primers included in the kit (Reagent R5) from Swift Cat No. 66096*.
What were the results from the Normalase treated libraries?	Based on our data, there is no significant difference when using Normalase vs. the standard 10x workflow. We got more or less the same results. We also tried doing 3 extra PCR cycles of the Normalase PCR to see if duplicate rate was affected. For example, we needed 12 indexing PCR cycles and we did 12 indexing PCR cycles and 3 cycles in the Normalase PCR step for a total of 15 cycles. There was no difference in our data, however, this is not necessary to do and not recommended. We incorporate Normalase when we have more than three 10x samples because we use the NovaSeq and it requires a large volume for sequencing. With only 3 samples we use all 25 µl for pooling to continue to Normalase II which can be challenging especially if for any reason samples need to be re-sequenced.

*An alternative streamlined workflow can be incorporated where Swift Normalase Indexing primers (Cat. No. 68096 or custom) can be used in a single library amplification step that combines library indexing and conditioning required for Normalase compatibility. Please contact TechSupport@swiftbiosci.com for more information.

RESULTS

Libraries were prepared with the 10x Genomics scRNA-Seq and scCNV workflows and samples were treated with Normalase. Libraries were amplified for 3 cycles less than the 10x protocol recommendation. Then Normalase PCR was performed for 3 cycles using KAPA HiFi HotStart ReadyMix and the cycle conditions listed in the Normalase protocol. As size was already selected for during 10x's post-PCR cleanup, a 1:1 PCR cleanup was performed per the Normalase protocol. The NovaSeq was loaded at 400-450 pM. Using the Normalase workflow, an index balance with a CV < 10 % was observed for all runs.

Coefficient of variation (CV) for six runs using 10x Genomics scRNA-Seq and scCNV workflow with Normalase

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Libraries in Normalase pool	9	4	8	3	8	3
Average reads per library (x10^6)	449	210	232	385	502	338
CV of library reads within pool	**4.6%**	**4.8%**	**8.9%**	**2.3%**	**6.6%**	**6.2%**

CONCLUSION

"We are happy with the results we obtain when incorporating Normalase as our normalization method. We get an even distribution of reads across the samples. ***Also, it is definitely a time saver because there is no need to do qPCR especially in our case.*** *We used to do two independent qPCR quants to make sure our molarities and loading concentrations were the most accurate. We like Normalase because it saves us from doing the qPCR-based quantification of every 10x library, gives tight CVs between libraries, optimal clustering, and good coverage."*

– Esther Guerrido and Chintda Santiskulvong, Cedars-Sinai Applied Genomics, Computation, and Translational (AGCT) Core.

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