Technical Note

QuantumScale Single Cell RNA Sequencing Guidelines

For Research Use Only.

Legal Notices

Document 1352110, Rev A, Jun 2025

5601 Oberlin Dr., Suite 110
San Diego, CA 92121
https://scale.bio/
support@scale.bio

Disclaimer of Warranty:
TO THE EXTENT PERMITTED BY APPLICABLE LAW, SCALEBIO PROVIDES THIS DOCUMENT "AS IS" WITHOUT WARRANTY OF ANY KIND, INCLUDING WITHOUT LIMITATION, ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NONINFRINGEMENT. IN NO EVENT WILL SCALEBIO BE LIABLE TO YOU OR ANY THIRD PARTY FOR ANY LOSS OR DAMAGE, DIRECT OR INDIRECT, FROM THE USE OF THIS DOCUMENT, INCLUDING WITHOUT LIMITATION, LOST PROFITS, LOST INVESTMENT, BUSINESS INTERRUPTION, GOODWILL, OR LOST DATA, EVEN IF SCALEBIO IS EXPRESSLY ADVISED IN ADVANCE OF THE POSSIBILITY OF SUCH LOSS OR DAMAGE. Any warranties applicable to the ScaleBio products are set forth in the Terms and Conditions accompanying such product and such Terms and Conditions are not modified in any way by the terms of this notice.

Trademark Information:
ScaleBio may make reference to products or services provided by other companies using their brand names or company names solely for the purpose of clarity, and does not assert any ownership rights over those third-party marks or names. Images were created with BioRender.com

Patent Information:
ScaleBio products may be covered by one or more patents as indicated at:
https://scale.bio/legal-notice/

Terms and Conditions:
The use of the ScaleBio products described herein is subject to ScaleBio's Terms and Conditions that accompany the product, or such other terms as have been agreed to in writing between ScaleBio and the user.

Intended Use:
All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Introduction

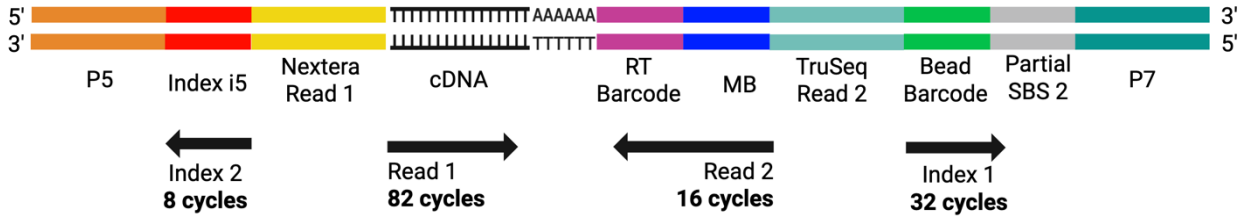
This technical note provides guidance for sequencing Quantum libraries on Illumina platforms, including specific instructions for configuring custom read settings, managing large datasets, and estimating flow cell usage for each library.

Quantum libraries have a unique read structure that requires a manual override of the standard Illumina sequencing recipe to ensure accurate barcode capture and compatibility with the Scale Bio Seq Suite analysis pipeline.

In addition, careful consideration of flow cell usage and demultiplexing strategy is required to optimize data quality and downstream analysis performance.

Overview of Custom Read Configuration for Quantum Libraries

Quantum libraries incorporate several barcode types and require extended Index 1 reads to capture all necessary information:



Read	Cycles	Purpose
Read 1	≤82 cycles	Transcript
Read 2	16 cycles	RT Barcode + Molecular Barcode
Index 1	32 cycles	Bead Barcode (~880K unique barcodes)
Index 2	8 cycles	PCR Index
	138 cycles	Total Cycles Required

This read configuration is compatible with a 100-cycle sequencing kit due to additional overage cycles provided by the kit (up to 138 total cycles). Read 1 is configured to fit within this limit while still capturing the full transcript. Minor reductions to the Read 1 length may have minimal impact on data quality; however, reducing Read 1 to 50 cycles or fewer is *not recommended*.

Higher cycle kits (e.g., 300-cycle kits) can still be run with the same read configuration and OverrideCycles. If read configuration is different, the OverrideCycles parameters for FASTQ generation will need to be updated accordingly.

To properly sequence Quantum libraries, the following OverrideCycles parameter is needed:
OverrideCycles: Y82;I8U24;I8;Y16

- The first 8 cycles of Index 1 and all of Index 2 are used for FASTQ demultiplexing.
- The remaining 24 cycles of Index 1 are retained for Bead Barcode extraction by the Scale Bio Seq Suite pipeline.

Manual Override Configuration on Sequencers

1. Download the appropriate Scale Bio v2 samplesheet from the Scale Bio support website.
2. If required during Run Setup (on-instrument or BaseSpace), enter the OverrideCycles.
3. Verify the total read cycles match the OverrideCycles specification.
4. Start run as normal.

Sequencing Large Quantum Libraries

When sequencing large libraries on multiple flowcells (e.g., 2M cell Quantum kit with 8 sublibraries across two NSX 25B flow cells), **avoid pooling all sublibraries into a single sequencing run/lanes** and instead follow the recommended strategy below:

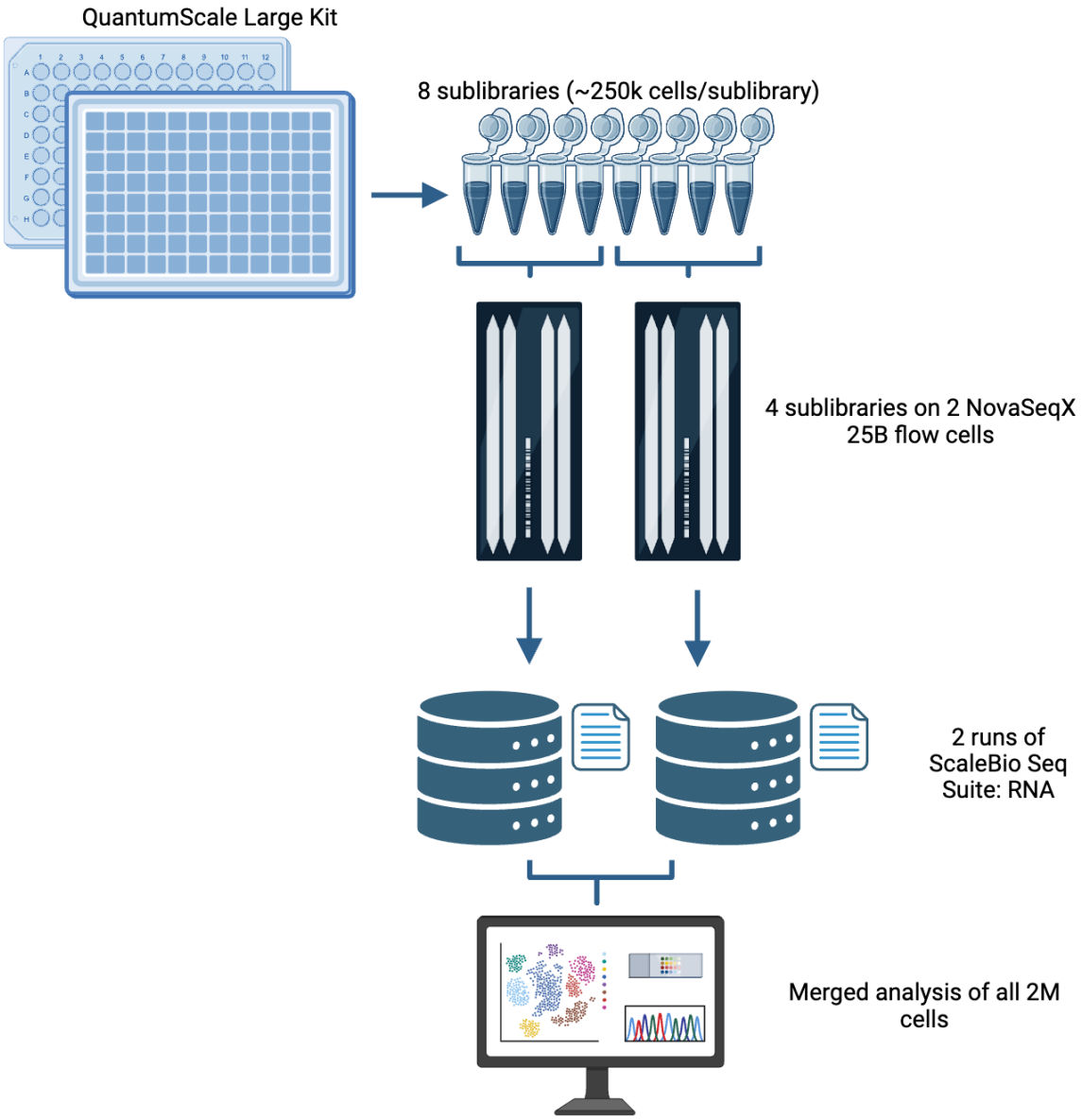
Recommended strategy:

- Run 4 sublibraries on one NovaSeq X 25B flow cell.
- Run the remaining 4 sublibraries on a second NovaSeq X 25B flow cell.

Rationale:

- Facilitates finer control over sequencing read depth per sublibrary
- Avoids excessive compute and memory requirements.
- Allows parallel analysis of sublibraries, avoids cumbersome FASTQ file manipulation

Figure 1: Example workflow for QuantumScale Single Cell RNA – Large Kit



Sequencing Usage for Quantum Libraries

Selecting the appropriate flow cell or wafer configuration is necessary to achieve optimal sequencing performance and data quality for Quantum libraries. The choice of flow cell depends on multiple factors, including:

- The size of the Quantum kit (Small, Medium, Large, Extra Large)
- The target sequencing depth (reads per cell/nucleus)
- The number of sublibraries being sequenced

It is advised to augment the total flow cell reads targeted based on the reads required by 20%.

To assist users in planning their sequencing runs, the following matrixes provide recommended flow cell and wafer configurations for each kit size and sequencing depth. These recommendations balance capacity, cost efficiency, and run time considerations based on typical platform performance.

Table 1: Recommended reads based on sequencing depth targeted and 20% overage

	Kit Size			
Reads per Cell	**Small**	**Medium**	**Large**	**Extra Large**
10,000	1,008,000,000	2,016,000,000	24,000,000,000	48,000,000,000
20,000	2,016,000,000	4,032,000,000	48,000,000,000	96,000,000,000
30,000	3,024,000,000	6,048,000,000	72,000,000,000	144,000,000,000
40,000	4,032,000,000	8,064,000,000	96,000,000,000	192,000,000,000
50,000	5,040,000,000	10,080,000,000	120,000,000,000	240,000,000,000

Table 2: Recommended Illumina Flow Cell based on sequencing depth targeted

	Kit Size			
Reads per Cell	**Small**	**Medium**	**Large**	**Extra Large**
10,000	1 x NS2K P4 FC	2 x NS2K P4 FC	1 x NSX 25B FC	2 x NSX 25B FC
20,000	2 x NS2K P4 FC	3 x NS2K P4 FC	2 x NSX 25B FC	4 x NSX 25B FC
30,000	2 x NS2K P4 FC	1x NSX 10B FC	3 x NSX 25B FC	6 x NSX 25B FC
40,000	3 x NS2K P4 FC	1x NSX 10B FC	4 x NSX 25B FC	8 x NSX 25B FC
50,000	3 x NS2K P4 FC	1x NSX 10B FC	5 x NSX 25B FC	10 x NSX 25B FC

Document Revision History

Revision	Revision Date	Document ID	Changes
A	Jun 2025	1352110	Initial release.