Technical Note

Guidelines for Pooling QuantumScale and 10x Genomics Libraries

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Introduction

Pooling Quantum libraries with 10x Genomics libraries on a single sequencing run is technically possible, but requires careful planning and comes with important caveats. The following guidance is based on an internal assessment by the Scale Bio technical team and is provided for informational purposes only. Performance of this approach has not been fully validated in production settings, and users should proceed cautiously and in close coordination with their bioinformatics and sequencing teams.

Disclaimer: This guidance is **hypothetical** and reflects a feasibility assessment only. It is not an officially supported configuration, and Scale Bio cannot guarantee sequencing or downstream analysis performance when Quantum and 10x libraries are pooled.

Pooling Quantum Libraries with 10x Genomics Libraries

Read Configuration Considerations

When pooling Quantum and 10x libraries on the same flow cell, use a sequencing kit with sufficient read length to accommodate the requirements of both library types. Quantum requires extended Index 1 reads and specific pipeline handling, while 10x requires shorter Index 1 and Read 1 for compatibility with Cell Ranger.

Requirement	**Quantum**	**10x**	**Both**
Read 1	≤82 cycles	28 cycles	82 cycles
Read 2	16 cycles	90 cycles	90 cycles
Index 1	32 cycles	10 cycles	32 cycles
Index 2	8 cycles	10 cycles	10 cycles
			214 cycles

Important:

- Although a 100-cycle kit may be used for Quantum libraries alone, in order to support both Quantum and 10x requirements, a kit with more cycles must be used, as long as Index 1 is at least 32 bases, Index 2 is at least 8 bases, and Read 2 is at least 16 bases.

Generating Separate FASTQ Files

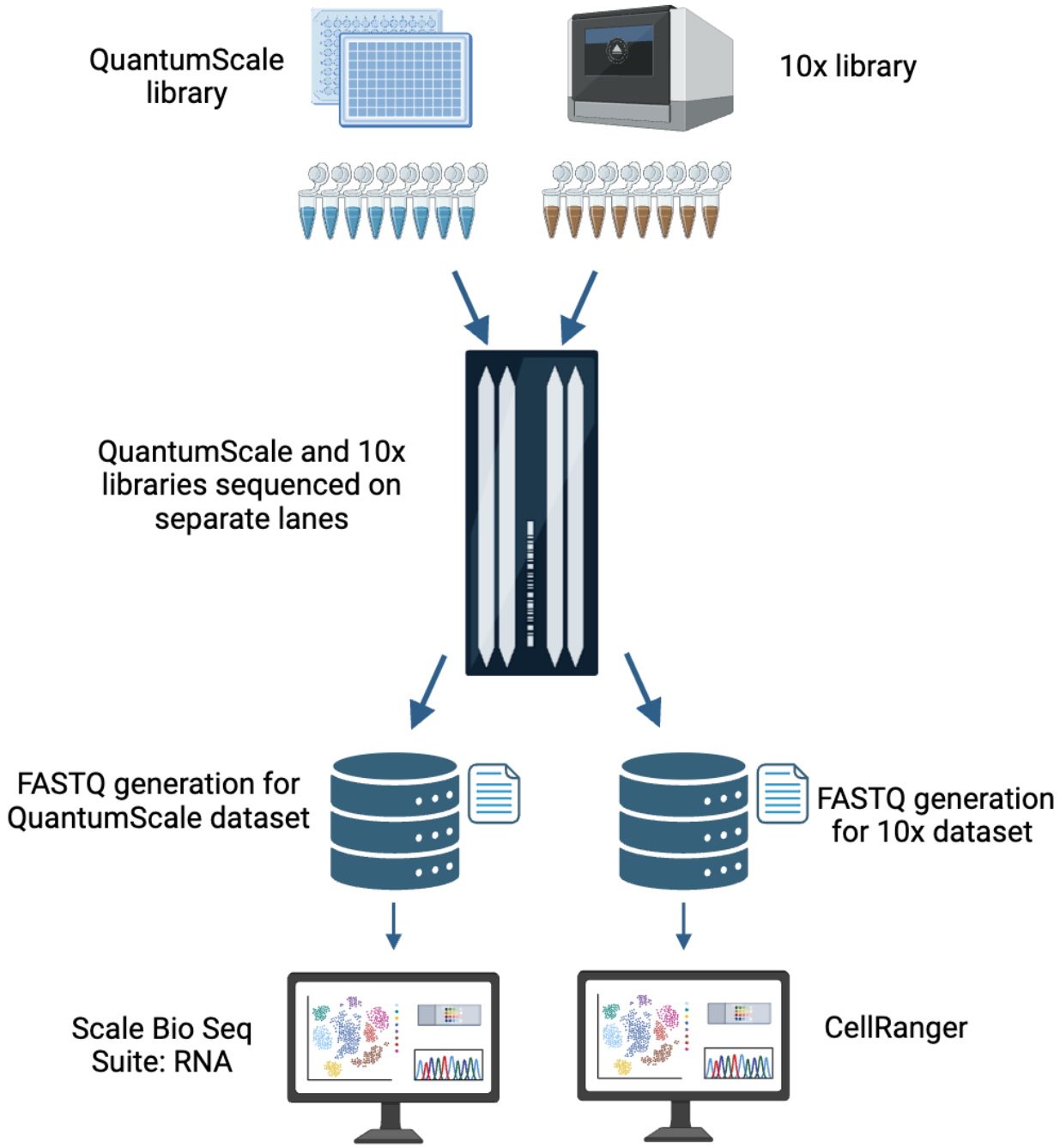
When pooling Quantum and 10x libraries:

- Run FASTQ generation (demultiplexing) twice:
    - Once for Quantum with OverrideCycles:
        - Y82;I8U24;I8N2;Y90
    - Once for 10x with suggested OverrideCycles:
        - Y28N54;I10N22;I10;Y90
- Alternatively, if supported, use BCL Convert with library-specific OverrideCycles settings.
- If possible, sequence libraries on separate flow cell lanes.

Additional tips:

- Work closely with your sequencing core or facility.
- Coordinate with your bioinformatics team to ensure proper demultiplexing and pipeline compatibility.
- Be prepared to handle the demultiplexing and analysis as two distinct workflows.

Figure 1: Parallel library preparation, sequencing, and demultiplexing workflow for QuantumScale and 10x Genomics libraries



Document Revision History

Revision	Revision Date	Document ID	Changes
A	Jun 2025	1352112	Initial release.