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The RNA Experts

BC1 Block Module for QuantSeq
User Guide

Catalog Number:
167 (RS-BC1 Block)

167UG346V0101

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1. Overview

This User Guide outlines the protocol for using the BC1 Block Module for QuantSeq, with the QuantSeq 3′ mRNA-Seq Library Prep Kits for Illumina (FWD, Cat. No. 015, 113 - 115, 129 - 131 and REV, Cat. No. 016). QuantSeq uses total RNA as input with oligo(dT) priming to generate first strand cDNA. RNA removal is then performed and second strand synthesis is initiated by random priming. Final library amplification by PCR adds complete Illumina-compatible sequencing adapters and unique indices. For more detailed information about these protocols, please refer to the complete QuantSeq 3′ mRNA-Seq User Guide available at www.lexogen.com/docs/quantseq.

Lexogen's BC1 Block Module blocks the generation of library fragments from abundant BC1 transcripts that are present in mouse brain samples (*Mus musculus*, Mm). The BC1 Block Module consists of a modified RNA Removal Solution containing transcript-specific oligos complimentary to these highly abundant BC1 lncRNAs.

The Removal Solution-BC1 Block (**RS-BC1B ●**) replaces the standard RNA Removal Solution (**RS ○**) at the RNA removal step of the standard QuantSeq 3′ mRNA-Seq Library Prep protocol. The oligos bind to the BC1 first strand cDNA and prevent the generation of amplifiable library fragments from BC1 lncRNAs during second strand synthesis (see Fig. 1).

The BC1 Block Module is provided for 96 reactions and can only be used with the QuantSeq 3′ mRNA-Seq Library Prep Kits from Lexogen (FWD, Cat. No. 015, 113 - 115, 129 - 131 and REV, Cat. No. 016).

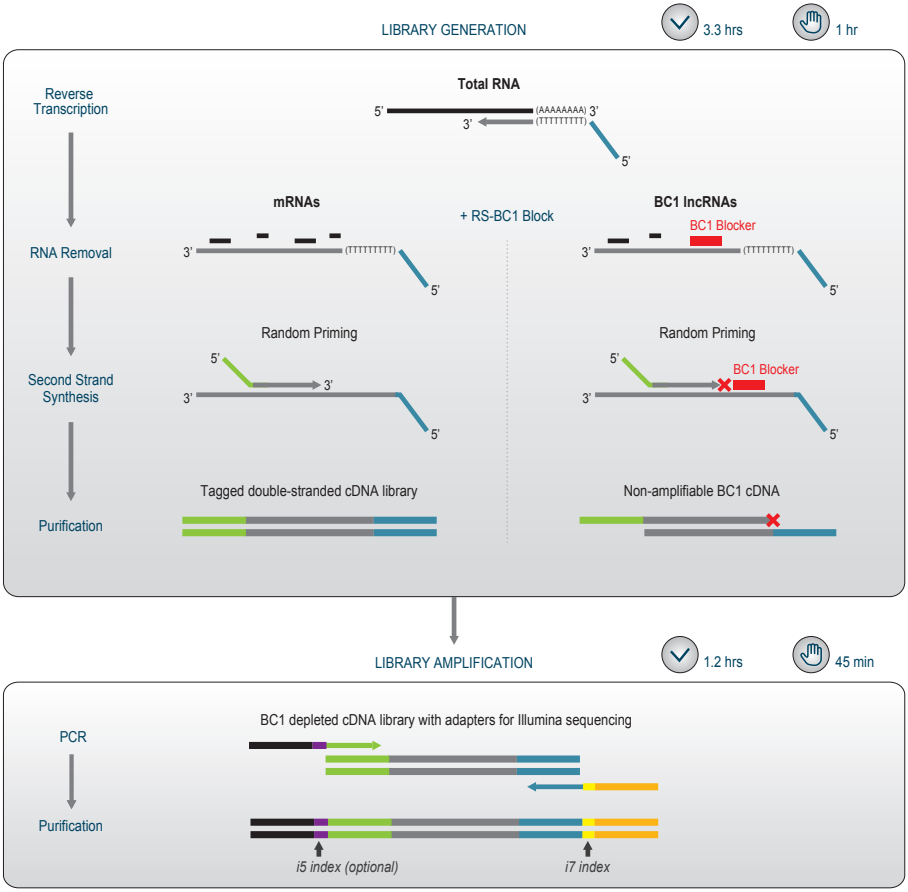

Figure 1. Schematic overview of the QuantSeq workflow using the BC1 Block Module for BC1 depletion. *Mus musculus* (Mm) brain total RNA contains a mixture of both BC1 and non-BC1 mRNAs, which are both reverse transcribed by oligo(dT) priming during first strand synthesis. If RS-BC1 Block (RS-BC1B ●) solution is added instead of the standard RNA Removal Solution (RS ○), the BC1 Blocker oligos bind specifically to BC1 first strand cDNA and block BC1 second strand cDNA synthesis. After second strand synthesis, the sample will contain both non-amplifiable BC1 cDNA fragments and tagged double-stranded cDNA library fragments for non-BC1 mRNAs. During PCR, only the non-BC1 library fragments are amplified to add full-length adapters and indices for Illumina sequencing.

2. Kit Components and Storage Conditions

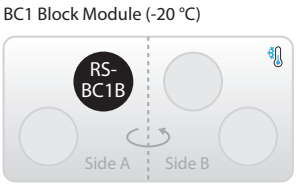

Figure 2. Location of kit components.

RS-BC1 Block, 96 rxn (Cat. No. 167)	Tube Label	Volume*	Storage
		96 rxn	
Removal Solution-BC1 Block	**RS-BC1B ●**	528 µl	-20 °C

*including ≥10 % surplus

ATTENTION: Upon receipt the solution should be stored in a -20 °C freezer.

NOTE: The **BC1 Block Module for QuantSeq is not a stand-alone kit**. It is an Add-on Module for the QuantSeq FWD (Cat. No. 015, 113 - 115, 129 - 131) and REV (Cat. No. 016) kits and requires the components therein for functionality.
The Removal Solution-BC1 Block (**RS-BC1B ●**) replaces the standard RNA Removal Solution (**RS ○**) at the RNA removal step of the standard QuantSeq 3′ mRNA-Seq Library Prep protocol.

NOTE: For user-supplied consumables and equipment needs, please refer to the QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina User Guide.

3. Protocol

ATTENTION: QuantSeq generated first strand cDNA (FWD, Cat. No. 015, 113 - 115, 129 - 131 and REV, Cat. No. 016) is required as input for RNA removal using the Removal Solution-BC1 Block (**RS-BC1B ●**, Cat. No. 167). BC1 blocker oligos hybridize specifically to BC1 first strand cDNA and block the extension of second strand cDNA in subsequent library preparation steps. This results in non-amplifiable, single-stranded BC1 cDNA fragments.

RNA Removal - BC1 Block

During this step the RNA template is degraded, which is essential for efficient second strand synthesis, and BC1 blockers are added. Before removing the sealing foil after the first strand synthesis reaction, quickly spin down the plate to make sure all liquid is collected at the bottom of the wells.

ATTENTION: The Removal Solution-BC1 Block (**RS-BC1B ●**) replaces the RNA Removal Solution (**RS ○**) from the standard QuantSeq 3′ mRNA-Seq Library Prep Kits for Illumina.

NOTE: RS-BC1 Block (**RS-BC1B ●**) should be used for mouse brain RNA samples.

➡	First Strand cDNA Synthesis is carried out according to the standard QuantSeq User Guide (015UG009 or 113UG227) steps 1 to 4.
5	Add 5 µl of Removal Solution-BC1 Block (**RS-BC1B ●**) directly to the first strand cDNA synthesis reaction. Mix well, reseal the plate using a fresh foil, and spin down. **REMARK:** Use a pipette set to 15 µl for efficient mixing.
6	Incubate for 10 minutes at 95 °C, then cool down to 25 °C. Spin down the plate at room temperature and carefully remove the sealing foil.
➡	Proceed to Second Strand Synthesis (step 7) as described in QuantSeq User Guide (015UG009 or 113UG227).

4. Appendix A: Input RNA and PCR Cycles

Total RNA from mouse brain is the intended input for QuantSeq library preps using RS-BC1 Block Module for BC1 depletion. No prior depletion of BC1 lncRNA, poly(A) enrichment, or ribosomal RNA is required. The minimum recommended input for this protocol is 10 ng of total RNA (RIN ≥6). It is important to ensure that total RNA is of high purity: Ideal values for A260/280 and A260/230 absorbance ratios should be 1.8 - 2.1, and approximately 2, respectively. For low quality RNA samples, please follow the protocol modification listed at the bottom of the page.

We recommend the use of Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008) for isolation of total RNA. The table below shows an example of PCR cyle numbers used for input amounts of mouse total RNA from brain samples.

Species	Sample Type	Input Amount	PCR Cycles	
			Undepleted	+ RS-BC1 Block
Mouse	Brain	500 ng	13	13
		50 ng	16	16
		10 ng	19	20

BC1 lncRNAs constitute >34 % of the total mRNA pool. Therefore, when blocking these lncRNAs from the final step of library generation, the cycle numbers may need to be increased to achieve the same library yield as non-depleted samples. Adding one additional PCR cycle compared to non-depleted blood samples is typically sufficient. However, as the mRNA content, purity, and quality of total RNA from brain samples may vary depending on the origin of the sample, **we strongly recommend performing the qPCR assay to determine the optimal number of cycles for the library amplification** (see the respective Appendix in the QuantSeq User Guides (015UG009 or 113UG227)).

ATTENTION: **For low quality RNA** we recommend: skipping step 2, reducing the amount of **PS** added in step 16 (48 µl instead of 56 µl), and reducing the amount of **PB** in step 29 (31.5 µl **PB** instead of 35 µl **PB** for dual indexed libraries and 27 µl **PB** instead of 30 µl **PB** for single indexed libraries, respectively). For information regarding the use of input amounts <50 ng please contact support@lexogen.com, or see the QuantSeq FWD online FAQs at www.lexogen.com.

Typical Results

Figures 3 and 4 show examples of QuantSeq libraries prepared from mouse brain samples with and without the Removal Solution-BC1 Block (RS-BC1B ●). Distinct peaks in bioanalyzer traces between 230 and 280 bp, which correspond to abundant BC1 lncRNA library fragments, are present in standard libraries but are no longer present in **RS-BC1B ●** libraries.

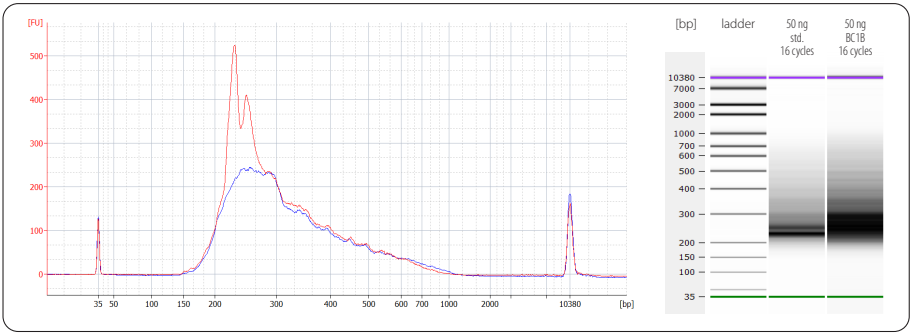

Figure 3. Bioanalyzer traces for mouse QuantSeq FWD libraries prepared with and without the RS-BC1 Block solution (RS-BC1B, Cat. No. 167). Libraries were prepared from 50 ng of mouse brain RNA with the Standard QuantSeq FWD protocol (RS Standard, red trace), versus QuantSeq with BC1 Block (+RS-BC1B, blue trace). Commercially available mouse brain RNA (BioCat MR-201) was used. Both libraries were amplified with 16 cycles. The two dominant peaks between 230 and 280 bp that are visible in the standard libraries are no longer visible after BC1 blocking.

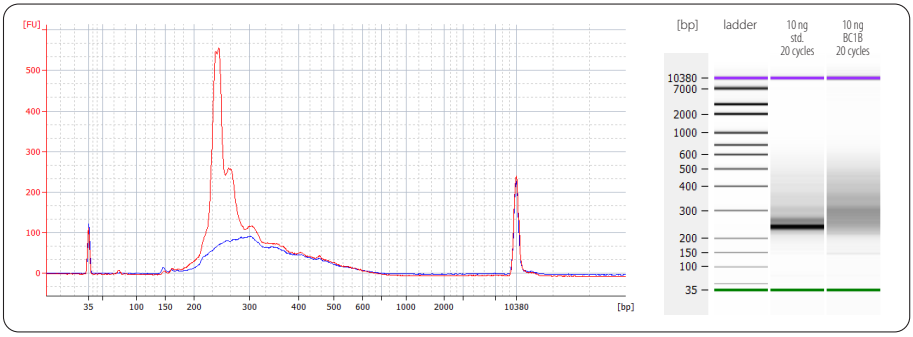

Figure 4. Bioanalyzer traces for mouse QuantSeq FWD libraries prepared with and without the RS-BC1 Block solution (RS-BC1B, Cat. No. 167). Libraries were prepared from 10 ng of mouse brain RNA with the Standard QuantSeq FWD protocol (RS Standard, red trace), versus QuantSeq with BC1 Block (+RS-BC1B, blue trace). RNA was isolated using the SPLIT RNA Extraction Kit (Lexogen). Both libraries were amplified with 20 cycles. Dominant BC1 peaks that are visible in the standard libraries are no longer visible after BC1 blocking.

Overcycling

A peak in high molecular weight regions (between 1,000 - 9,000 bp) is an indication of overcycling. This could occur if cycle numbers are increased too much to compensate for lower input material. Prevent overcycling by using the qPCR assay as described in the respective Appendix of the QuantSeq User Guides (015UG009 or 113UG227).

5. Appendix B: Revision History

Publication No. / Revision Date	Change	Page
167UG346V0101 Jan. 25, 2023	Updated Kit Components Figure 2 and Table to reflect current packaging and storage requirements.	6
167UG346V0100 May 19, 2021	Initial Release.	

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Associated Products:

008 (SPLIT RNA Extraction Kit)
015 (QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina (FWD))
016 (QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina (REV) with Custom Sequencing Primer)
020 (PCR Add-on Kit for Illumina)
022 (Purification Module with Magnetic Beads)
025, 050, 051, 141 (SIRVs Spike-in RNA Variant Control Mixes)
080 (Reamplification Add-on Kit for Illumina)
081 (UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1))
113 - 115, 129 - 131 (QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set A1, A2, A3, A4, A1-A4, or B1)

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