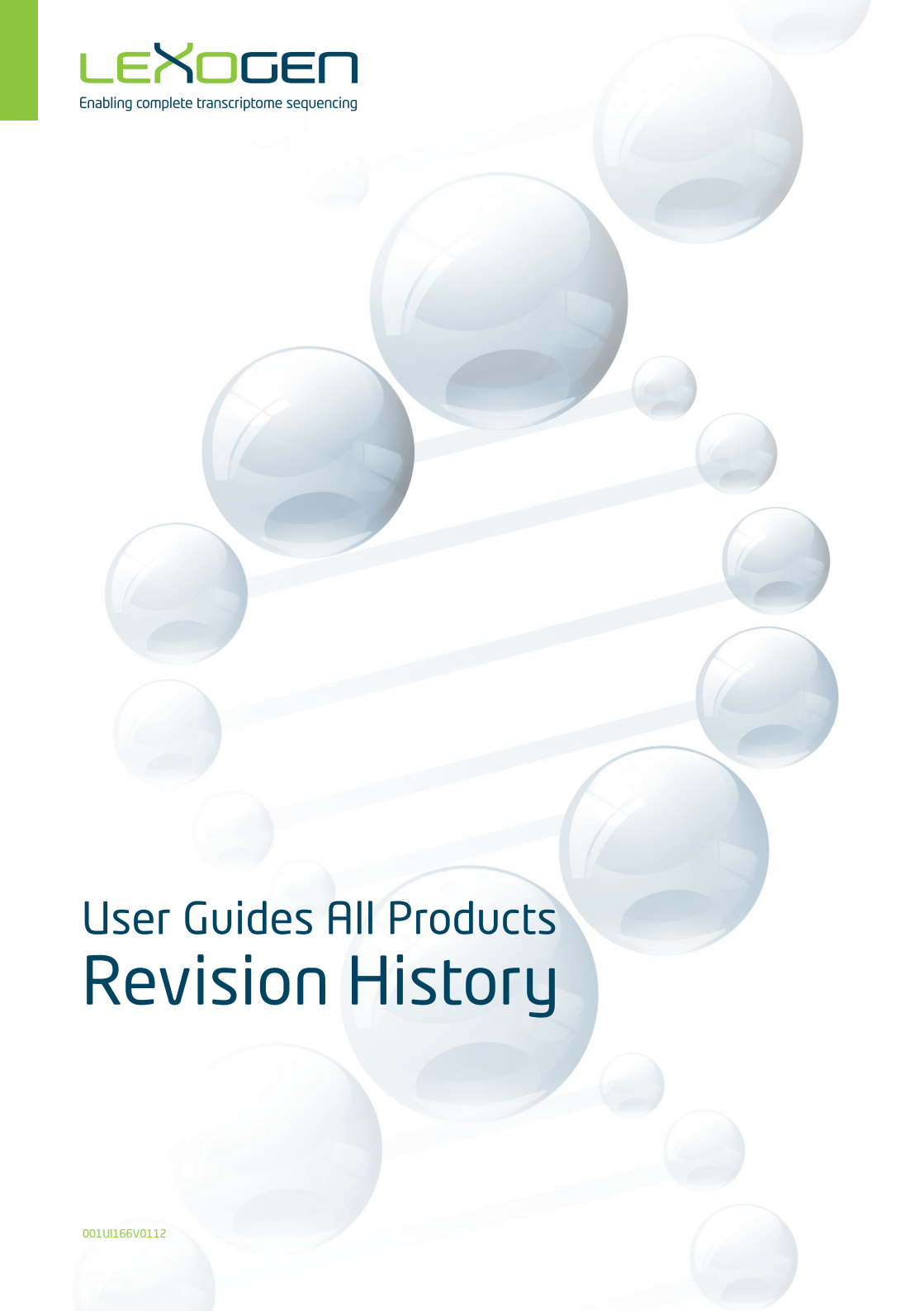
LEXOGEN

Enabling complete transcriptome sequencing



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001UI166V0112

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QuantSeq 3′ mRNA-Seq Library Prep Kit (FWD/REV) for Illumina

Publication No. / Revision Date	Change	Page
015UG009V0251 Feb. 26, 2019	Naming changes for: "i7 Index Plate" to "Lexogen i7 6 nt Index Set", and "i5 Dual Indexing Add-on Kit" to "Lexogen i5 6 nt Dual Indexing Add-on Kit".	6-48
	New CSP Version (V5) included in REV kits - CSPV5 is supported for use on HiSeq, MiSeq, MiniSeq, and NextSeq instruments.	39-41
	"REMARK" notes in steps 1 - 4 edited to refer only to specific instructions for low input/low quality/FFPE samples.	11-12
	Instructions for use of RS-Globin Block and UMI modules in place of RS and SS1, respectively, were added to detailed protocol steps 5 and 7.	12-13
	Reformatting of attention notes in detailed protocol	11-17
	Short protocol format updated to include clear steps for low quality / low input / FFPE protocol modifications, and to include qPCR assay steps.	18-19
	Text and figures updated for Appendices A - N.	20-45
	Revision history table includes 2017 onwards only	46-47
015UG009V0241 Aug. 27, 2018	Updated sequencing guidelines for Lexogen libraries.	39
015UG009V0240 Jun. 5, 2018	Safe stopping point removed after step 4: Proceed immediately to RNA removal.	12
	Updated text for RNA input and PCR cycles in Appendix B.	22-23
	Updated text and added table of protocol modifications for low input in Appendix C.	24
	Modified format and text for Appendix E: qPCR and F: Library Reamplification.	26-27
	Added new Appendix I: Globin Block Modules.	32-33
	Added new Appendix J: Unique Molecular Identifiers.	34-35
	Added information on dual indexing, lane mix preparation, and repurification to Appendix K.	37-38
	Reduced Revision history table to show updates from 2016 onwards.	46-47
015UG009V0230 Nov. 22, 2017	Added remark: Pre-warm mastermixes for first strand synthesis (step 3).	11
	Added sample handling guidelines for critical steps during first strand synthesis, for low input / low quality samples.	11-12
	Revised attention text for PCR cycle numbers.	14
	Minor revision of text in appendices B, C, D, and E.	21-24
	Link for https://www.bluebee.com/lexogen updated.	35, 38

015UG009V0223 Sep. 5, 2017	Correction of figure labels for Appendices F and G.	26-28
015UG009V0222 Jul. 6, 2017	After denaturing (step 2), leave the reactions on the thermocycler at 42 °C until step 4.	11
	Dry the beads only at room temperature after ethanol washes.	13, 16
	Short procedure reformatted over 2 pages, font size increased and Attention notes added for First Strand cDNA Synthesis steps.	17, 18
	Page numbers for appendices were changed and SIRVs text updated.	19-34
	Updated machine-specific instructions for sequencing.	30, 33
015UG009V0221 Mar. 8, 2017	Do not cool the FS2/E1 mastermix! Safe stopping point. Typos fixed.	11, 15-17, 22
015UG009V0220 Feb. 7, 2017	RS1, renamed to RS. No more RS2 solution, hence one step less in the protocol.	12, 17
	A new SS1 solution (now only 10 µl have to be added not 15 µl as previously).	12, 17
	The total volume of the second strand synthesis reaction is now 40 µl (previously it was 50 µl).	12, 17
	Post second strand synthesis only 16 µl PB have to be added (previously it was 20 µl).	13, 17
	In step 16 (was previously step 17) only 56 µl PS have to be added (previously it was 72 µl).	13, 17
	Barcode Plate (BC) was rearranged for improved balance and renamed to i7 Index Plate (7001-7096). Previous BC05: TAATCG replaced by 7025: TTTATG to avoid overlap with Illumina-specific indices.	4-6, 14-30
	Barcode 00 (BC00) in PCR Add-on Kit renamed to P7 Primer 7000.	23
	qPCR endpoint determination using only 1.7 µl template and set to 50 % FU (previously 33 %). Subtract 3 cycles from determined endpoint (EP) when using 10x as much template (17 µl in EP, 1.7 µl in qPCR).	23
	Evaluation tool to check the color balance of index subsets.	28
015UG009V0215 Nov. 22, 2016	In short procedure: remove from magnet after EB addition.	16
015UG009V0214 Oct. 17, 2016	Rinse with RNAse-free water after RNaseZap usage!	8
	QuantSeq-Flex First Strand Synthesis Module for longer insert size. Removed SS1 dilution.	11, 12
	Thaw SS1 at 37 °C. Check for precipitates. Visually inspect fill levels of BC Plate.	12, 14
	Lowest recommended input RNA amount set to 10 ng for QuantSeq REV.	17, 21
	Changes to Input RNA amount Table, Figure 3, and 4.	21, 24
015UG009V0213 Jun. 10, 2016	Indication of safe stopping points.	11-13, 15-16

015UG009V0212 Feb. 22, 2016	Optional: Step 4 (RT) may be increased to 1 h @ 42 °C for low input RNA.	11, 16, 21
	Reduce step 6 to 5 minutes at 95 °C for RNA inputs below 1 ng total RNA.	12, 16, 21
	Pierce or cut open new barcode sealing. Reseal after usage!	14
	2000 ng input RNA example and SS1 dilution added to input RNA table and Figure 3.	21, 24
	Link to www.lexogen.com/quantseq-data-analysis.	29
015UG009V0211 Nov. 24, 2015	CSP concentration changed to 100 µM. Adjusted usage instructions.	6, 28
	Increased RT temperature from 37 °C to 42 °C to prevent internal oligodT priming.	11, 16
	Changes to RS1/RS2 for increased stability. SS1 dilution for increased insert size.	12, 16
	Endpoint PCR at 33 % of the maximum qPCR fluorescence. Re-Amplification primer in PCR Add-on Kit.	18, 24
015UG009V0210 Jun. 23, 2015	Changes to Logo, Figures, and listing of kits, and Kit Contents Table. Consistency changes.	1, 5, 6, 14
	Recommendation on SYBR Green I, and more details on dilutions for qPCR.	7, 18
	Removal of CSP recommendations for NextSeq500 and NextSeq550.	27
015UG009V0200 Feb. 17, 2015	Renaming of QuantSeq T-fill to QuantSeq REV, and QuantSeq 015 to QuantSeq FWD.	1, 4
	Figure updates. Explicit instructions to centrifuge at room temperature.	5, 11, 12
	Recommendations for Low Quality RNA - FFPE and exemplary data in Appendix C.	11, 13, 15, 21
	Usage of the Custom Sequencing Primer for QuantSeq REV and alignment details.	27, 29
015UG009V0112 Dec. 22, 2014	PCR Add-on Kit and Instructions for PCR endpoint determination.	17
015UG009V0111 Sep. 16, 2014	Minor User Guide changes (mainly semicolons). Capital letters on Reference Card.	
015UG009V0110 Jun. 11, 2014	Updates of Figures and Tables. Feasibility of QuantSeq for low quality RNA input. Revision History.	5, 17, 20, 22, 27
015UG009V0100 Mar. 24, 2014	Initial Release 015UG009V0100.	

QuantSeq 3′ mRNA-Seq Library Prep Kit FWD HT for Illumina

Publication No. / Revision Date	Change	Page
015UG110V0111 Sep. 7, 2018	Updated sequencing guidelines for Lexogen libraries.	41
015UG110V0110 Jun. 14, 2018	PCR Mix was renamed to Dual PCR Mix (no change to buffer composition) and kit component figure updated.	6, 17-18
	Added note to pre-warm the FS2 / E1 mastermix for 2 -3 minutes at 42 °C at step 2.	11
	Safe stopping point removed after step 4: Proceed immediately to RNA removal.	12
	Updated text for Appendices B and C and added table of protocol modifications for low input.	24-26
	Modified format and text for Appendix E: qPCR, and F: Library Reamplification.	27-28
	Added new Appendices I: Globin Block Modules, and J: Unique Molecular Identifiers.	34-37
	Updated information on dual indexing, multiplexing, and sequencing workflows in Appendices K and L.	38-44
	Reduced Revision History table to show updates from 2016 onwards.	47
015UG110V0102 Jul. 31, 2017	Front page and SIRVs text updated.	0, 22
	After denaturing (step 2), leave the reactions on the thermocycler at 42 °C until step 4.	11, 19-20
	Dry beads only at room temperature after ethanol washes.	13, 16, 18-20
	Added note that up-to-date recommendations can be found on the QuantSeq FAQ webpage.	26
	Consistency changes.	
015UG110V0101 Mar. 8, 2017	Do not cool the FS2/E1 mastermix.	11
	Safe stopping points. Fixed typo.	16, 18 - 20, 26
015UG110V0100 Feb. 7, 2017	Release of QuantSeq FWD HT Cat. No. 015.384 with included (optional) i5 Dual Indexing Add-on Kit.	
	Barcode Plate (BC) was rearranged for improved balance and renamed to i7 Index Plate (7001-7096). Previous BC05: TAATCG replaced by 7025: TTTATG to avoid overlap with Illumina-specific indices.	4-6, 14-17, 19-20, 31-35
	RS1, renamed to RS. No more RS2 solution, hence one step less in the protocol.	12, 19, 20
	A new SS1 solution (now only 10 µl have to be added not 15 µl as previously).	12, 19, 20
	The total volume of the second strand synthesis reaction is now 40 µl (previously it was 50 µl).	12, 19, 20
	Post second strand synthesis only 16 µl PB have to be added (previously it was 20 µl).	13, 19, 20
	In step 16 (was previously step 17) only 56 µl PS have to be added (previously it was 72 µl).	13, 19, 20

	For dual indexing the PCR buffer from the basic kit (yellow cap) has to be exchanged with the PCR buffer (purple cap) included in the i5 Dual Indexing Add-on Kit.	16, 20
	Dual indexing PCR has a final volume of 35 µl (i7 single indexing PCR has a final volume of 30 µl).	17, 20
	Post dual indexing PCR 35 µl PB added (or 31.5 µl PB for low input, low quality FFPE RNA samples).	17, 20
	Table with reference values for 500 ng input RNA from different species.	25
	Barcode 00 (BC00) in PCR Add-on Kit renamed to P7 Primer 7000.	27
	qPCR endpoint determination using only 1.7 µl template and set to 50 % FU (previously 33 %). Subtract 3 cycles from determined endpoint (EP) when using 10x as much template (17 µl in EP, 1.7 µl in qPCR).	27
	Evaluation tool to check the color balance of index subsets.	32
	015.384 includes i5 Dual Indexing Add-on Kit (four i5 indices). Information on i5 and dual indexing.	33 - 36
Mar. 24, 2014	Initial Release of QuantSeq 3' mRNA-Seq Kit (015UG009V0100).	

QuantSeq 3′ mRNA-Seq Library Prep Kit for Ion Torrent

Publication No. / Revision Date	Change	Page
012UG036V0130 Jun. 13, 2018	Added attention: Leave the reactions at 42 °C until step 4 (step 2).	11
	Added remark: Pre-warm mastermixes for first strand synthesis (step 3).	12
	Safe stopping point removed after step 4: Proceed immediately to RNA removal.	12
	Endpoint PCR test protocol reformatted in Appendix B and minor revisions of text in Appendices A, B, F.	24, 23-29
	Reduced Revision History table to show updates from 2016 onwards.	31
012UG036V0123 Jul. 5, 2017	Dry the beads only at room temperature.	13, 14
	Changed SIRVs text - Appendix A.	19
012UG036V0122 Mar. 28, 2017	Safe stopping point also added to finished library.	13, 16
	Fixed typo.	23
012UG036V0121 Aug. 3, 2016	Prevent internal priming by keeping RT at 42 °C (pipette on heating block, don't cool FS2/E1).	11
	Indication of safe stopping points.	11-14, 16
	Reseal Barcode Plate after usage to prevent crosscontamination.	12
	Possibility to select IonXpress barcodes from the machine.	23
012UG036V0120 Mar. 15, 2016	RT temperture raised from 37 °C to 42 °C.	11
	Protocol adjustments for or low input, FFPE, or low quality RNA in step 2, 6, 17, and 29.	11-13, 15-17
	Hyperlinks	14, 17, 20, 25
	Spike-in RNA Variant Control Mixes, Cat. No. 025.03.	19
012UG036V0111 Jul. 29, 2015	Changed Logo and Slogan.	1
	Keep temperature high to prevent internal oligodT priming.	11
	Barcode Set B (Barcodes 33-41, 43-48 extended) now compatible with IonXpress.	23
012UG036V0110 May 27, 2015	Introduction of Barcode Set B (barcodes 25-48) in Figure 2 and in Appendix D.	6, 23
	Explicit instructions to centrifuge at room temperature.	11, 12
	Recommendations to mix with pipettes set to larger volumes.	12
012UG036V0102 Jan. 15, 2015	PM renamed to PCR.	6, 14
	Reference to webpage: suggested PCR cycles for RNA from different organisms or tissues.	17
	PCR Add-on Kit for additional PCR reactions; Purification Module with Magnetic Beads.	17
012UG036V0101 Dec. 2, 2014	Corrected table footer; Consistency changes.	20
012UG036V0100 Sep. 4, 2014	Initial Release.	

QuantSeq-Flex Targeted RNA-Seq Library Prep Kit V2 for Illumina

Publication No. / Revision Date	Change	Page
015UG058V0230 Aug. 8, 2018	Pre-warm mastermixes for first-strand synthesis (step 3).	13-15
	After denaturing (step 2), leave the reactions on the thermocycler at 42 – 50 °C until step 4.	13-15
	Information on the handling of low quality, degraded, FFPE samples for first-strand synthesis added (steps 1-4).	13-15
	Safe stopping point removed after step 4: Proceed immediately to RNA removal	13,14
	Information on UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1, Cat. No. 081) added.	17
	Restructuring Appendix F (qPCR) and new Appendix G (Library Reamplification).	31, 32
	Addition of Appendix J (Modulating Insert Sizes) and Appendix K (UMIs).	36-38
	Shortened Revision History (only 2016 onwards).	43
015UG058V0220 Feb. 7, 2017	Included new QuantSeq FWD basic kit protocol (only RS, new SS1, less PB post SSS).	7, 16, 17
	Barcode plate (BC) was rearranged for improved balance and renamed to i7 Index Plate (7001-7096). Previous BC05: TAATCG replaced by 7025: TTTATG to avoid overlap with Illumina-specific indices.	7, 18, 32
	Barcode 00 (BC00) in PCR Add-on Kit renamed to P7 Primer 7000	28
	qPCR endpoint determination using only 1.7 µl template and set to 50 % FU (previously 33 %). Subtract 3 cycles from determined endpoint when using 10x as much template (17 µl in EP, 1.7 µl in qPCR)	28
	Evaluation tool to check the color balance of index subsets.	32
015UG058V0200 Oct. 19, 2016	New streamlined protocol for target-specific second strand synthesis.	4, 6, 15, 20, 31
	Restructuring of Kit Contents Figures.	6, 7
	Indication of safe stopping points.	13 - 20
	Increased insert size for 3' mRNA-Seq libraries with QuantSeq-Flex First Strand Synthesis Module.	13 - 14, 30
	Restructuring of Appendices (separate Appendices on qPCR and Typical Results).	24 - 35
	NCBI Primer Blast tool for Primer Pair Specificity Check.	25, 26
	qPCR endpoint determination now set to 50 % of the maximum fluorescence and using less template for qPCR.	28
	Typical Results for BRAF, ERBB2, and KRAS targeted primining in K562 RNA (Fig. 5).	30 - 31
	Input series for K562 RNA and BCR-ABL fusion transcript detection (Fig. 6).	31
015UG058V0110 Mar. 29, 2016	Format changes.	
	Changes in denaturation procedure for targeted priming during RT.	13, 20
	RT temperature set to at least 42 °C.	14, 20

	SS1 dilutions for longer insert sizes. Only valid for random primed SSS.	16, 20
	Increased qPCR cycle number + NTC for low RNA input, low abundant targets.	22
	ERCC and SIRV Spike-in Mixes.	24
	Molecular barcodes in RT primer (requires PE sequencing run for read-out).	25
015UG058V0102 Jul. 28, 2015	SSS at temperatures of up to 50 °C.	15, 20, 25
	Remark to include PTOs in SSS oligos for increased specificity.	24
	Updated recommendation for primer concentrations.	24
	Example for targeted sequencing (BCR-ABL fusion transcript).	28
015UG058V0101 Jul. 8, 2015	Minor changes.	
015UG058V0100 Jul. 2, 2015	Initial Release.	

CORALL TOTAL RNA-Seq Library Prep Kit

Publication No. / Revision Date	Change	Page
095UG190V0110 Feb. 28, 2019	Initial Release.	
	Step 15 incubation time at 37 °C reduced to 30 minutes (from 1 hour).	14, 19
095UG190V0100 Jan. 3, 2019	Early Access Release.	

SENSE mRNA-Seq Library Prep Kit V2 for Illumina

Publication No. / Revision Date	Change	Page
001UG004V0322 Nov. 5, 2018	Temperature to hold the second strand synthesis is lowered to 4 °C.	14
	Restructuring of Appendix D (qPCR) and new Appendix E (Library Reamplification)	25, 26
	Shortened Revision History (only 2016 onwards)	33
001UG004V0321 Dec. 4, 2017	Consistency changes.	14, 15 ,17
	Edited remark text for step 37 regarding use of P7 Primer for qPCR.	16
	Safe stopping points added.	17, 18
	Updated text for Appendices C and D: PCR cycle numbers and qPCR.	24, 25
	Updated text for Appendix H: Data Analysis.	31
001UG004V0320 Feb. 7, 2017	Referral to i5 Dual Indexing Add-on Kit (Cat. No. 047) for up to 384 unique indexing options.	4-5, 27
	Update of Figures (Optional Dual Indexing). Kit Contents: i7 Index Plate, BC00 renamed to 7000.	6
	Barcode Plate (BC) was rearranged for improved balance and renamed to i7 Index Plate (7001-7096). Previous BC05: TAATCG replaced by 7025: TTTATG to avoid overlap with Illumina-specific indices.	6, 15, 18, 24, 27
	Rinse with RNAse-free water after RNaseZap usage!	8
	Restructuring of Appendices.	19-31
	qPCR endpoint determination using only 1.7 µl template and set to 50 % FU (previously 33 %). Subtract 3 cycles from determined endpoint (EP) when using 10x as much template (17 µl in EP, 1.7 µl in qPCR).	24
	Evaluation tool to check the color balance of index subsets.	27-28
001UG004V0313 Jun. 13, 2016	Indication of safe stopping points.	14, 15, 16, 18
	Fixed Typo in Figure 3 legend.	25
001UG004V0312 Feb. 23, 2016	Pierce or cut open new barcode sealing.	16
001UG004V0311 Feb. 2, 2016	Minor corrections.	18
001UG004V0310 Oct. 27, 2015	RNAse-free water removed from kit components. New Figure 2.	6
	Increased ST hybridization for lower RNA input at step 14.	13
	Increased RT/Lig time at step 16 for higher yield.	13
	New SSM formulation. Beads resuspension directly in SSM, one step less.	14
	Reduced PB amount in step 39 for RNA inputs lower than 50 ng.	16
	Minimum input RNA amount reduced to 1 ng.	19
	Table for lower UHRR RNA input amounts and required cycle numbers.	20
	Endpoint PCR set at 33 % of the maximum qPCR fluorescence.	20

	Reamplification primer in PCR Add-on Kit.	20, 25
	Spike-in RNA Variant Control Mixes, Cat. No. 025.03.	22
	Section on lower input RNA amounts included. New Figure 3.	25
001UG004V0302 Jan. 7, 2015	Changes in Figure 1 for easier understanding.	5
	SYBRGreen I recommendation.	7
001UG004V0301 Nov. 25, 2014	Consistency changes.	
	Increased ST hybridization for lower RNA input. PCR Add-on Kit for more qPCR assays.	19
001UG004V0300 Oct. 17, 2014	Initial Release SENSE mRNA-Seq V2.	
001UG004V0120 Jun. 1, 2014	Figure 1 with separate coloring for external barcode.	5
	Increased time on magnet for better beads separation.	11, 12, 13
	Added tube color codes to Short Protocol and Reference Card.	17
	Info: External barcode sequences for download under www.lexogen.com.	24
001UG004V0210 Feb. 1, 2014	Sentences reformulated in step 24 and 26 for a better understanding.	14
	Added details on choice of library size for gene expression and transcript assembly.	21
	Updated Figure 3 and Figure 4.	23
001UG004V0200 Jul. 31, 2013	Improved beads (MS150 oligodT beads; JSR Life Sciences).	11
	Less viscous storage solution for beads, same bead amount but reduced volume, and fewer pre-washes (2 now instead of 3).	11
	qPCR to determine the exact cycle number of your endpoint PCR (more E2).	18
	Fewer cycles recommended due to improved efficiency.	21
	Explanation of high molecular weight peak in bioanalyzer traces.	22
	Use Illumina Sequencing Primer for non-barcoded and externally barcoded SENSE libraries; CSP concentration for sequencing (0.5 µM final conc.).	28/29
001UG003V0110 Feb. 11, 2013	Adjust library size by RTS/RTL and by using different size cut-offs during purification.	12,13
	Table for chosing appropriate library size for the intended read length and updated Figures of library examples.	20, 22
	External barcoding during PCR reaction.	14, 23
	Introduction of customized sequencing primer (CSP).	27
001UG003V0100 Oct. 1, 2012	Initial Release SENSE mRNA-Seq.	

SENSE mRNA-Seq Library Prep Kit for Ion Torrent

Publication No. / Revision Date	Change	Page
006UG007V0107 Jul. 21, 2017	Indication of safe stopping points.	13-17
	Removal of the qPCR option.	1, 6-7, 14, 16, 18-19, 24
	SIRVs text updated.	0, 20
	Short procedure reformatted and page numbers for appendices were changed accordingly. Consistency changes.	16-17, all
006UG007V0106 Apr. 4, 2016	Updated Figure 1, Figure 2, and Kit Contents Table.	5, 6
	Prepare mastermixes in step 20 and step 32.	13, 14
	Extended qPCR description (example added, SYBR Green I usage).	17
006UG007V0105 Feb. 1, 2016	Consistency changes. Consistent labeling (ST01- ST24).	13, 23, 24
006UG007V0104 Nov. 26, 2015	Spike-in RNA Variant Control Mixes, Cat. No. 025.03.	0, 20
	Recommendation for Preparation of Mastermixes.	10
	Extended incubation time at steps 14 and 16 for higher yield.	13
	Endpoint PCR set at 33 % of the maximum qPCR fluorescence.	18
006UG007V0103 Jun. 1, 2015	Changes to front page - available kits and modules.	0
	Increased volumes for CW requiring more EtOH addition.	6
	Recommendation for SYBR Green I.	7
	Lowered SYBR Green I concentration in qPCR.	18
	Revision of Barcode Set B table.	24
006UG007V0102 Nov. 26, 2014	Consistency changes. Including Barcode Set B.	24
006UG007V0101 Feb. 1, 2014	Consistency changes.	
006UG007V0100 Aug. 30, 2013	Initial Release.	

SENSE Total RNA-Seq Library Prep Kit for Illumina

Publication No. / Revision Date	Change	Page
009UG013V0150 Oct. 10, 2018	Temperature to hold the second strand synthesis is lowered to 4 °C.	13
	Protocol notes regarding qPCR added in Library Amplification Section.	15
	Restructuring of Appendices B (RNA Input) and C (qPCR) and added new Appendix D (Library Reamplification).	22-26
	Information on dual indexing added.	30, 31
	Updated sequencing guidelines for Lexogen libraries.	32
	Shortened Revision History (2016 onwards).	33
009UG013V0141 Mar. 28, 2017	Safe stopping points added.	15-17
009UG013V0140 Feb. 7, 2017	Referral to i5 Dual Indexing Add-on Kit (Cat. No. 047) for up to 384 unique indexing options.	4, 27
	Update of Figures (Optional Dual Indexing). Kit Contents: i7 Index Plate, BC00 renamed to 7000.	5-7, 23
	Barcode plate (BC) was rearranged for improved balance and renamed to i7 Index Plate (7001-7096). Previous BC05: TAATCG replaced by 7025: TTTATG to avoid overlap with Illumina-specific indices.	6, 15, 21, 24, 27-28
	Rinse with RNase-free water after RNaseZap usage!	10
	qPCR endpoint determination using only 1.7 µl template and set to 50 % FU (previously 33 %). Subtract 3 cycles from determined endpoint (EP) when using 10x as much template (17 µl in EP, 1.7 µl in qPCR).	23
	Evaluation tool for color balance of index subsets at www.lexogen.com.	27-28
009UG013V0130 Jul. 26, 2016	Referral to SENSE FFPE Total RNA-Seq User Guide for degraded and FFPE RNA.	4, 12, 14, 17, 19
	Restructuring of Appendix Chapters (separate qPCR Appendix).	20 - 30
	Detailed instruction on preparation of lane mix for removal of side-products.	21
009UG013V0123 Jun. 27, 2016	FFPE RNA: Add 15 µl PB, 13 µl BD, and 27 µl PS.	13
009UG013V0122 May 24, 2016	Recommendations for low input and FFPE RNA (e.g, ST dilutions). New Appendix included for FFPE / low quality RNA. Renaming of subsequent Appendices. Page shifts.	12, 13, 16, 24
	Indication of safe stopping points.	13, 14, 17
	Reseal used barcode wells to prevent contamination!	15
009UG013V0121 Feb. 26, 2016	Pierce or cut open new barcode sealing. Consistency changes.	15
009UG013V0120 Dec. 18, 2015	RiboCop as recommended depletion kit.	4, 18
	Changes to protocol (3 min 94 °C denaturation of RNA / ST / RTL). Protocol recommendations for fragmented RNA input.	12

	Changes to E1 formulation.	12
	Reduced volumes of EB / PS in steps 13 and 14, respectively.	13, 14
	Changed cut-offs in step 10.	13, 22
	PB volume post PCR reduced to 27 µl.	15
	Endpoint PCR set at 33 % of the maximum fluorescence.	19
	Reamplification Primer in PCR Add-on Kit.	19
	Updated figures and tables in Appendix B and C with SENSE libraries synthesized from RiboCop rRNA depleted UHRR.	22, 23, 25
009UG013V0110 Jun. 1, 2015	Renaming of PS1 to BD.	4
	Figure 2 listing of Purification Modules 022.08., 022.24, and 022.96 (included in the kits).	5
	Extended recommendations regarding depletion kits.	11, 12
	ATTENTION: Reference values shown in Tables are for rRNA depleted UHRR.	21, 22
009UG013V0101 Dec. 16, 2014	Addition of PCR-Add-on Kit 020.96; 0.1x SYBR Green I recommendation.	18
009UG013V0100 Jun. 20, 2014	Initial Release	

SENSE Total FFPE RNA-Seq Library Prep Kit for Illumina

Publication No. / Revision Date	Change	Page
09UG102V120 Oct. 10, 2018	Temperature to hold the second strand synthesis is lowered to 4 °C.	13
	Protocol notes regarding qPCR added in Library Amplification Section.	15
	Restructuring of Appendices B (Input) and C (qPCR) and new Appendix D (Library Reamplification).	23-26
	Updated sequencing guidelines for Lexogen libraries.	32
	Information on dual indexing added.	30, 31
	Shortened Revision History (only 2016 onwards)	34
009UG102V0111 Mar. 28, 2017	Safe stopping points added.	15-17
009UG102V0110 Feb. 7, 2017	Referral to i5 Dual Indexing Add-on Kit (Cat. No. 047) for up to 384 unique indexing options.	4, 27
	Update of Figures (optional dual indexing). Kit Contents: i7 Index Plate, BC00 renamed to 7000.	5-7, 23
	Barcode plate (BC) was rearranged for improved balance and renamed to i7 Index Plate (7001-7096). Previous BC05: TAATCG replaced by 7025: TTTATG to avoid overlap with Illumina-specific indices.	6, 15, 21, 27-28
	Rinse with RNAse-free water after RNaseZap usage!	10
	qPCR endpoint determination using only 1.7 µl template and set to 50 % FU (previously 33 %). Subtract 3 cycles from determined endpoint (EP) when using 10x as much template (17 µl in EP, 1.7 µl in qPCR).	23
	Evaluation tool for color balance of index subsets at www.lexogen.com.	27-28
009UG102V0100 Jul. 7, 2016	Initial Release.	

Small RNA-Seq Library Prep Kit for Illumina

Publication No. / Revision Date	Change	Page
052UG128V0102 Jun. 19, 2018	Updated table in Appendix B.	20
	Added details for agarose gel extraction and example gel images to Appendix H.	28-31
052UG128V0101 Nov. 7, 2017	Recommendations to save beads and supernatant in Appendix G as a precaution. Corrected volumes for CB, CW, EB in table, Appendix H.	25-26, 28
052UG128V0100 Aug. 8, 2017	Initial Release.	

SLAMseq Explorer and Kinetics Kits

Publication No. / Revision Date	Change	Page
059UG142V0103 Jul. 11, 2018	Kit Component figures updated.	6-9
	Added table for Cell Viability Assay culture volumes.	10
	Corrected S4US concentrations in HPLC standards table.	17
	Added new table for S4U dilution series in Appendix A.	28
	Added new figure to Appendix B.	31
	Updated ATTENTION notes for each module.	11-25
	Added *Pseudomonas aeruginosa* $IC_{10,\ 12\ hr}$ to table in Appendix E.	34
059UG142V0102 Nov. 22, 2017	Fixed error in RNA extraction procedure and removed incorrect references.	14, 15, 20, 24
	Added information for SLAMdunk data analysis software access.	31
059UG142V0101 Oct. 3, 2017	Kit component layout modified.	6, 8, 9
059UG142V0100 Oct. 2, 2017	Initial Release.	

SIRV-Set 1 (Iso Mix E0, E1, E2)

Publication No. / Revision Date	Change	Page
025UG063V0111 Mar. 28, 2017	Kit content updated.	5
	Spike-in Data Evaluation using the SIRV Suite added.	22
025UG063V0110 Sep. 10, 2015	Product Release 2015-09-04.	
025UI063V0100 Jun. 3, 2015	Initial Release 2015-06-03, first release of the documentation together with the FASTA and GTF sequence file package, *name*_150601a.*extension*.	

SIRV-Set 2 (Iso Mix E0) and SIRV-Set 3 (Iso Mix E0/ERCC)

Publication No. / Revision Date	Change	Page
050UG134V0100 Jul. 14, 2017	Initial Release.	

SPLIT RNA Extraction Kit

Publication No. / Revision Date	Change	Page
008UG005V0220 Mar. 23, 2016	**CHANGES TO USER GUIDE -** The kit content was not changed.	
	New workflow overview graphic.	5
	Incorporation of homogenization protocols for plant tissue and fluid samples.	11, 13
	Column-based purification now one section for total and large/small RNA.	15 - 18
	Added tested RNA sources.	21
	Consistency changes.	
008UG005V0211 May 11, 2015	**CHANGES TO USER GUIDE -** The kit content was not changed.	
	General text changes to account for consistency.	
	Note added on RNasin 230 nm absorption to be considered for OD blanking.	14, 17
	Optional RNasin addition added to Short Procedures.	18, 19
008UG005V0210 Aug. 27, 2014	**CHANGES TO USER GUIDE -** The kit content was not changed.	
	Figure 3 updated to include miRNA spike-in experiment.	21
008UG005V0206 Jul. 8, 2014	**CHANGES TO USER GUIDE -** The kit content was not changed.	
	The extra chloroform extraction was removed. Workflow, preparation table, number of PLG-tubes, volumes of user-supplied reagents and the phenol-chloroform extraction protocol were adapted accordingly.	5, 6, 7, 12
	Storage of all kit components can now be at +2 to +8 °C (+4 °C).	6
	Incubation and centrifugation times were shortened.	12 - 17
	Isopropanol volume increased to 1.75 x to maximize miRNA recovery.	13
	Max. loading volume of purification column increased from 600 µl to 800 µl.	13, 15, 16
	No re-elution but optional second elution into new micro-tube.	14, 17
	Short Procedures were adapted accordingly.	19 - 20
008UG005V0100 Aug. 19, 2013	Initial Release	

RiboCop rRNA Depletion Kit V1.2 (Human/Mouse/Rat)

Publication No. / Revision Date	Change	Page
037UG073V0202 Aug. 29, 2018	Use 150 µl of 80 % EtOH in step 24 when using 1.5 ml tubes.	11
	Reduced Revision History table to show updates from 2016 onwards.	14
037UG073V0201 Jul. 26, 2017	Consistency changes.	
	Added Attention note to step 16.	10
037UG073V0200 Aug. 19, 2016	Released RiboCop V1.2.	
	Lowered input amount to 1 ng.	5
	Introduction of Hybridization Solution (HS) in step 2.	5
	Adjusted rpm-values.	9
	Adjusted reaction volumes in step 16, 17, 27.	10, 11
037UG073V0104 Mar. 29, 2016	Lowered input amount to 10 ng.	4, 9, 12
037UG073V0103 Dec. 15, 2015	Update of Figure 1.	4
037UG073V0102 Dec. 15, 2015	Added section "Typical Results".	13
	Increased input to 1 µg.	4, 9
	Increased time on magnet in step 18.	11
	Protocol available for human/mouse/rat.	
037UG073V0101 Nov. 18, 2015	Consistency changes.	
037UG073V0100 Nov. 10, 2015	Initial Release.	

Poly(A) RNA Selection Kit

Publication No. / Revision Date	Change	Page
039UG069V103 Dec. 14, 2017	Consistency changes.	0, 2, 4, 6-7, 9-17
	Added note at step 7.	11
	Added safe stopping points.	11-12
	Added adjusted volumes for Bead Wash Buffer.	15
039UG069V102 Dec. 12, 2016	Consistency changes.	
	Restructuring of Appendices.	13 - 19
	Figure 4: Comparison of total RNA and poly(A) selected RNA.	17
039UG069V101 Nov. 26, 2015	Initial Release.	

TeloPrime Full-Length cDNA Amplification Kit

Publication No. / Revision Date	Change	Page
013UG022V0200 (V2) Nov. 22, 2018	**Version 2 Kit Update:** The following kit components were updated: Second Strand Mix (SS), DNA Buffer, TeloPrime PCR Mix (Telo PCR), Enzyme Mix 3 (E3), PCR Forward Primer (FP), and PCR Reverse Primer (RP).	
	Detailed protocol was updated for: second strand synthesis, qPCR and endpoint PCR (steps 19 - 42). Elution volume reduced to 12 µl (steps 19 - 20), Second Strand Mix (SS) and Mastermix 4 volumes increased (steps 21 - 22), denaturing temperature for step 23 changed to 95.8 °C, PCR Primer volumes reduced to 1 µl, each PCR program, temperatures, and timing was changed. Notes added before purification sections.	11-17
	Kit workflow timing, kit components figures, and volume table updated.	5, 6
	Store Column Binding Buffers 1 (CB1) and 2 (CB2) at -20 °C until first use, then afterwards at room temperature.	6
	Short protocol steps and volumes updated.	18-19
	Safe stopping points indicated at steps 20, 29, 36, and 42.	13, 15
	Updated text and figures in Appendices A-D.	20-26
013UG022V0110 (V1) Mar. 23, 2018	Trial kit reagent volumes included in kit components table.	6
	Equilibrate samples to room temperature after storage at -20 °C before continuing with the protocol.	9, 15
	Safe stopping points indicated at steps 20, 29, 36, and 42.	13, 15
	Appendix D: Added recommendations for preparing TeloPrime cDNA for PacBio Iso-Seq™ library preparation.	22
013UG022V0106 (V1) Mar. 13, 2017	Added note that additional reagents are included for qPCR.	6
	Adjustment of reagent volumes.	6
	Addition of ethanol to CW is indicated on the tube label only.	6
013UG022V0105 (V1) Apr. 1, 2016	Table formatting.	
013UG022V0104 (V1) Jan. 12, 2016	Consistency changes. Label color of PCR, RP, FP, SS, E3 changed to white.	
013UG022V0103 (V1) Oct. 6, 2015	Remark to discard flow-through in step 16.	13
	Increased elution volume in step 28 from 19 µl to 20 µl DNA Buffer.	13

013UG022V0102 (V1) Jul. 29, 2015	Consistency changes.	
	Discontinuation of 013.04.	1, 6
	Option to ligate over night.	12
	Indication of safe stopping points at steps 20 and 29.	12, 13
013UG022V0101 (V1) Sep. 4, 2014	Consistency changes.	
013UG022V0100 (V1) Aug. 27, 2014	Initial Release.	

PCR Add-on Kit for Illumina

Publication No. / Revision Date	Change	Page
020IM064V0122 Aug. 8, 2018	Reference to Reamplification Add-on Kit for the reamplification of dual-indexed libraries.	2
	RE renamed to i7-RE.	2, 3, 6, 7
	Restructuring of Section 4 and 5.	4-6
020IM064V0121 Feb. 24, 2017	Fixed typo in step 2 and 3.	4
020IM064V0120 Feb. 7, 2017	BC00 renamed to 7000.	3-5
	qPCR endpoint determination using only 1.7 µl template and set to 50 % FU (previously 33 %). Subtract 3 cycles from determined endpoint (EP) when using 10x as much template (17 µl in EP, 1.7 µl in qPCR). Figure 2.	4-5
020IM064V0113 Oct. 12, 2016	PCR volume in Kit Contents Table corrected.	3
	Updates to schematic representation of libraries in Appendix.	7
020IM064V0112 May 17, 2016	For reamplification no SYBR Green I is needed.	6
020IM064V0111 Apr. 27, 2016	Corrected step numbers for SENSE mRNA-Seq.	4, 6
	Endpoint PCR set at 33 % of the maximum qPCR fluorescence.	4, 6
020IM064V0110 Aug. 8, 2015	Addition of Reamplification Primer (RE).	1, 3, 6
	Addition of primer sequences.	7
020IM064V0100 Jun. 19, 2015	Initial Release PCR Add-on Kit for Illumina.	

Lexogen i5 6 nt Dual Indexing Add-on Kits (5001 - 5096)

Publication No. / Revision Date	Change	Page
047IM109V0210 Mar. 12, 2019	Kit naming changed to "Lexogen i5 6 nt Dual Indexing Add-on Kits (5001-5096)", and kit component naming changed to "Lexogen i5 6 nt Index Sets (5001 - 5004, and 5001 - 5096)".	
	Custom Sequencing Primer (CSP) changed to Version 5, sequence and schematics updated.	10-13
	Notes for dual indexing of CORALL Total RNA-Seq libraries added.	2, 4-7, 9, 11-13
	Updated sequencing guidelines for Lexogen libraries.	14
047IM109V0200 May 24, 2018	Addition of 047.96 i5 Index Plate, 96 indices (5001-5096), Kit Components.	2, 3
	Updated Appendix A text and sample multiplexing information.	7-9
	Updated Appendix B text for dual indexing sequencing workflows.	10-13
	Updated Appendix C text for reamplifiaction of dual-indexed libraries.	14
047IM109V0101 Feb. 7, 2017	Renaming of i5 Kit Components. Rearreanged i7 Index Plate 7001-7096 replaces Barcode Plate.	2-11
	Inclusion of QuantSeq-Flex.	1-11
047IM109V0100 Dec. 5, 2016	Initial Release.	

LEXOGEN

Enabling complete transcriptome sequencing

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