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Reveal sRNA Potential

miRVEL Profiling PCR Add-on and Reamplification Kit

User Guide

Catalog Number:
243 (miRVEL Profiling Small RNA-Seq Library Preparation Kit)
246 (miRVEL Profiling PCR Add-on and Reamplification Kit)

246UG902V0100

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When describing a procedure using this product for publication, please refer to it as Lexogen's miRVEL Profiling PCR Add-on and Reamplification Kit for Illumina.

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1. Overview

This User Guide outlines the protocol for miRVEL Profiling PCR Add-on and Reamplification Kit (Cat. No. 246) and can only be used for libraries generated with the miRVEL Profiling Small RNA-Seq Library Preparation Kit.

The miRVEL Profiling PCR-Add on Kit can be used to perform a qPCR assay to determine the optimal PCR cycle number for endpoint PCR or re-amplification of undercycled libraries. The number of PCR cycles for library amplification must be adjusted according to RNA input amount, quality, and sample type.

The miRVEL Profiling PCR Add-on and Reamplification Kit (Cat. No. 246) contains PCR Mix (**PM** ○) and a PCR Enzyme (**PE** ○). The miRVEL Profiling qPCR Primer Mix (**qPCR** ●), which is required for the qPCR Assay. Primers bind to the synthesized cDNA. **ATTENTION:** Do not use **qPCR** ● primers for reampification of already indexed libraries as indices will be lost.

For already uniqely dual indexed miRVEL Profiling Small RNA-Seq libraries the Reamplification Primer (**RE** ○) must be used to reamplify undercycled libraries in order to obtain enough material for sequencing. The reamplification primers bind to the surface binding part of the Illumina libraries. **ATTENTION:** Do not use **RE** ○ primers in a qPCR assay, as the miRVEL cDNA lacks primer binding sites for **RE** ○ primers.

Please contact Lexogen at support@lexogen.com (see also p.8).

ATTENTION: Important notes for the qPCR Assay

- For the qPCR Assay, SYBR Green I nucleic acid dye (Sigma Aldrich, S9430; ThermoFisher S7563) is needed in addition to the PCR Add-on Kit and must be supplied by the user.
- The use of SYBR Green I-containing qPCR mastermixes for the qPCR Assay is explicitly not recommended.
- The instructions provided here relate to the use of SYBR Green I dye only for endpoint cycle calculation via qPCR Assay. The use of EvaGreen or other similar dyes at 0.1x or 1x final concentration in the qPCR reaction will also produce amplification curves. However, the calculation of endpoint PCR cycle numbers using the instructions provided for SYBR Green I may not apply. Therefore, cycle number calculations must be validated by the user.

2. Kit Components and Storage Conditions

Kit Component	Tube Label	Volume*	Storage
PCR Mix	**PM** ○	740 µl	-20 °C
Enzyme Mix	**PE** ○	106 µl	-20 °C
miRVEL Profiling qPCR Primer Mix	**qPCR** ●	1056 µl	-20 °C
Reamplification Primer Mix	**RE** ○	1056 µl	-20 °C

*including 10 % surplus

PCR Mix (**PM** ○) and Enzyme Mix (**PE** ○) provided in the miRVEL Profiling PCR Add-on and Reamplification Kit are the same as provided in the miRVEL Profiling Small RNA-Seq Library Preparation Kit and can therefore be used interchangeably.

3. User-Supplied Consumables and Equipment

Reagents

- SYBR Green I nucleic acid stain (10,000x in DMSO; e.g., Sigma-Aldrich S9430, or ThermoFisher S7563) has to be provided by the user.
- Purification Module with Magnetic Beads (Cat. No. 022) for purification of reamplified libraries.

Equipment

- Quantitative Real-Time PCR Machine, capable of SYBR Green I detection (emission maximum at 520 nm) - ROX passive reference detection should be turned off.
- Calibrated single- and / or multi-channel pipettes for handling 1 µl to 1,000 µl volumes.
- Ice bath or ice box, ice pellets, benchtop cooler (-20 °C for enzymes).

4. qPCR Assay

4.1 Detailed Protocol

The qPCR Assay is recommended to determine the exact number of cycles for the endpoint PCR in order to prevent under- or overcycling of the library. This assay uses cDNA from the miRVEL Profiling Small RNA-Seq Library Preparation Kit (step 13). Please refer to the User Guide for indication at which step the qPCR assay is recommended.

ATTENTION: Do not use the Reamplification Primer Mix (**RE** ○) for the qPCR Assay on the cDNA library, as non-amplified cDNA lacks binding sites for the Reamplification Primers. The Reamplification Primer can only be used on already amplified miRVEL Profiling Illumina libraries.

qPCR	
PM ○ – thawed at RT **PE** ○ – keep on ice or at -20 °C **qPCR** ● – thawed at RT **spin down before opening!**	**ATTENTION:** Spin down all solutions before opening tubes!
SYBR Green I nucleic acid dye (e.g., Sigma-Aldrich, Cat. No. S9430) - provided by user	
Thermocycler	95 °C, 60 sec 95 °C, 15 sec 60 °C, 15 sec 72 °C, 60 sec } 35 - 50x 72 °C, 6 min 10 °C, ∞

NOTE: SYBR Green I has an emission maximum at 520 nm, which for some qPCR machines has to be adjusted manually. ROX passive reference dye detection is not required for this qPCR Assay and should be turned off to ensure correct calculation of SYBR Green fluorescence intensity.

1. Prepare a 1:4,000 dilution of SYBR Green I dye in DMSO, for a 2.5x working stock concentration. **ATTENTION:** The final concentration in the reaction should be 0.1x. Higher concentrations of SYBR Green I will inhibit the amplification.
2. For each reaction, prepare a mastermix of 7 µl of PCR Mix (**PM** ○), 10 µl of qPCR Primer Mix (**qPCR** ●), 1 µl of Enzyme Mix (**PE** ○), and 1.4 µl of 2.5x SYBR Green I nucleic acid dye. Add 23.3 µl of Elution Buffer (**EB**) or molecular biology-grade water (**H_2O**) to bring the total volume to 32.7 µl per reaction. **ATTENTION:** Include a no template control!
3. Add 2.3 µl of miRVEL Profiling cDNA from step 13 to 32.7 µl of the qPCR mastermix. This equals 1/10th of the cDNA amount that will be used later for endpoint PCR.

4 Perform 35 cycles of PCR with the following program: Initial denaturation at 95 °C for 60 seconds, 35 cycles of 95 °C for 15 seconds, 60 °C for 15 seconds and 72 °C for 60 seconds, and a final extension at 72 °C for 6 minutes, hold at 10 °C.

5 Using the amplification curves in linear scale, determine the value at which the fluorescence reaches the plateau. Calculate 50 % of this maximum fluorescence (50 % MF) value and determine at which cycle this value is reached. As the endpoint PCR will contain 10x more cDNA than the qPCR, subtract three from this cycle number. This is then the final cycle number you should use for the Endpoint PCR with 23 µl of the template (step 13). **REMARK:** There is no need to purify or analyze the overcycled PCR product.

4.2 Example for Endpoint Calculation

100 ng total RNA input was used for generating libraries. Using 1.7 µl of cDNA for qPCR, the cycle number corresponding to 50 % MF was 15 cycles. The remaining 17 µl of the template should therefore be amplified with 12 cycles (15 - 3 cycles = 12 cycles).

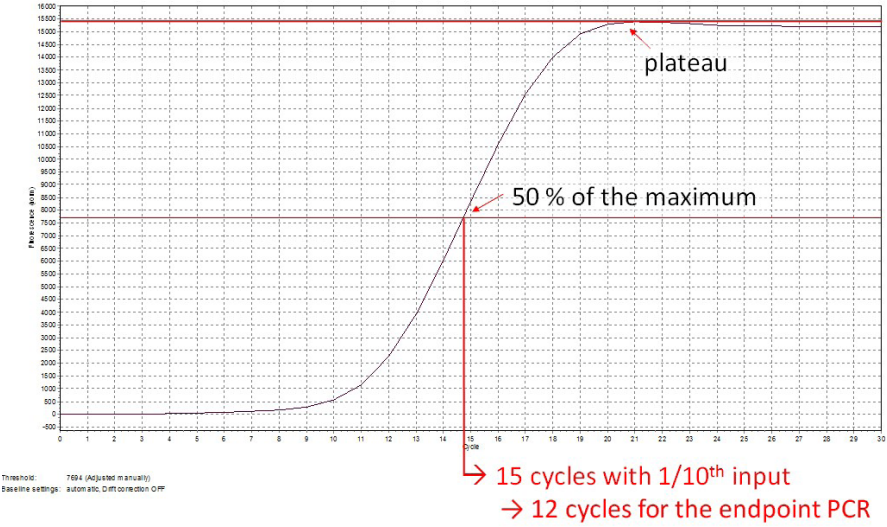


Figure 1. Calculation of the number of cycles for the endpoint PCR.

The optimal cycle number can also be determined using the amplification data from the qPCR software to calculate the 50 % MF value. Take the cycle number that is the next closest fluorescence value above the 50 % MF and subtract 3 to get the endpoint cycle number.

NOTE: Once the number of cycles for the Endpoint PCR is established for one type of sample (same input amount, tissue, and RNA quality), there is no need for further qPCRs. 23 µl of miRVEL Profiling cDNA can be used directly for the Endpoint PCR.

5. Library Reamplification

Unique dual indexed miRVEL Profiling Small RNA-Seq Illumina libraries can be reamplified using this kit. Even if only a small peak is visible on a quality control trace (Bioanalyzer or similar), the quantity is often sufficient for sequencing. The Library Quantification Calculation Sheet available from www.lexogen.com/support-tools/lane-mix-calculation can be used to check whether you have sufficient library for pooling.

ATTENTION: Do not use the qPCR Primer Mix (**qPCR** ●) for reamplification of already indexed libraries! This will lead to a loss of indices, and failure to amplify properly in an NGS run.

NOTE: Additional purification reagents (Cat. No. 022) will be required for purification of reamplified libraries.

Reamplification PCR	Purification
PM ○ – thawed at RT **PE** ○ – keep on ice or at -20 °C **RE** ○ – thawed at RT **spin down before opening!**	**PB** – stored at +4 °C **PS** – stored at +4 °C **80 % EtOH** – provided by user; **prepare fresh!** **EB** – stored at +4 °C
Thermocycler 95 °C, 60 sec 95 °C, 15 sec 60 °C, 15 sec 72 °C, 60 sec } 3 - 10x 72 °C, 6 min 10 °C, ∞	Equilibrate all reagents to room temperature for 30 minutes prior to use!

1 For each reaction, combine: 7 µl of PCR Mix (**PM** ○), 10 µl of Reamplification Primer Mix (**RE** ○), 1 µl of Enzyme Mix (**PE** ○), and up to 17 µl of PCR amplified dual indexed miRVEL Profiling Small RNA-Seq libraries. If required, add Elution Buffer (**EB**) or molecular biology-grade water (**H_2O**) to bring the total volume to 35 µl.

4 Conduct 3 - 10 additional PCR cycles with the following program: initial denaturation for 60 seconds at 95 °C, 3 - 10 cycles of 15 seconds at 95 °C, 15 seconds at 60 °C, 60 seconds at 72 °C, and a final extension for 6 minute at 72 °C, hold at 10 °C.

5 At this point, the libraries are finished and should be purified according to the miRVEL Profiling User Guide (from step 18 onwards) using the Purification Module with Magnetic Beads (Cat. No. 022).

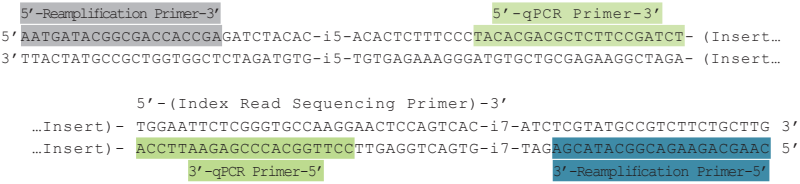
5.1 PCR Cycle Number for Library Reamplification

If your libraries are undercycled but have measurable yields, perform 3 - 6 cycles of reamplification PCR, calculating approximate doubling of the original yield per cycle.

EXAMPLE: If your library concentration is 0.05 ng/µl, perform 3 - 5 cycles to obtain a concentration in the range of 0.4 - 1.5 ng/µl.

If you do not see a library at all e.g., on a Bioanalyzer trace (Agilent Technologies), and / or have no measurable library concentration, use 6 - 10 cycles for reamplification. For further PCR cycle recommendations for reamplification, please contact: support@lexogen.com.

6. Appendix: Primer Sequences



ATTENTION: The Reamplification Primer can only be used on already amplified PCR libraries. Do not use it on non amplified cDNA as it lack primer binding sited for the Reamplification Primer Mix (**RE** ○)!

7. Appendix: Revision History

Publication No. / Revision Date	Change	Page
246UG902V0100 Oct. 1, 2025	Initial release.	

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Associated Products:

008 (SPLIT RNA Extraction Kit)
022 (Purification Module with Magnetic Beads)
128 (TraPR Small RNA Isolation Kit)
243 (miRVEL Profiling Small RNA-Seq Library Prep Kit for Illumina)

PCR Add-on and Reamplification Kit V2 for Illumina · User Guide

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