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TraPR Small RNA Isolation Kit

TraPR (Trans-kingdom, rapid, affordable Purification of RISCs) presents a gel- and bias-free, column-based method for isolation of functional small RNAs from RNA-induced silencing complexes (RISCs) of all organisms. Within 15 minutes, TraPR enables purification of RISC fractions even from challenging or inconsistent samples, cell types, tissues, and bio-fluids. The TraPR Small RNA Isolation Kit generates high-quality sRNA preparations suitable for Next Generation Sequencing (NGS) applications and thus provides a highly reproducible, time-saving method that outperforms all current gold-standard procedures for sRNA profiling.

Trans-kingdom, rapid, affordable Purification of RISCs (TraPR)

Regulatory small RNAs (sRNAs) play an essential role in mRNA turnover, translational regulation, and chromatin compaction and hence are important regulators of gene expression. These sRNAs associate with specific proteins of the Argonaute family (AGOs) to form RNA-induced silencing complexes (RISCs) and guide the AGO proteins to their respective targets in a process termed RNA interference.

Lexogen's TraPR Small RNA Isolation Kit enables the specific isolation of RISC-associated sRNAs via a simple 15 minute column purification. As a species-independent method, TraPR does not require any prior characterization of the sample. By purification of RISCs the TraPR Small RNA Isolation Kit enriches exclusively fully functional, physiologically relevant sRNAs including piRNAs, siRNAs, miRNAs, and scnRNAs. These sRNAs can be extracted even from degradation-prone material or from samples that are notoriously hard to work with. Contaminating RNAs such as degradation products of tRNA, rRNA, and mRNA are effectively excluded from the purified RISC fraction.

In summary, TraPR enables the universal, fast, and bias-free isolation of RISCs and provides an accurate representation of functional silencing sRNAs without requiring tedious and time-consuming gel extraction steps. As TraPR exploits the conserved biochemical properties of all AGO-family proteins it is a universally applicable method that outperforms procedures such as immunoprecipitation or affinity purification of labeled RISCs. After RISC elution, sRNAs can be isolated by phenol / chloroform extraction, and the resulting pure sRNAs are suitable for all molecular biology and Next Generation Sequencing (NGS) applications[1].

Workflow

Freshly harvested or flash frozen tissue or cells are lysed and loaded directly onto TraPR columns. While bulk RNA and DNA are retained on the column, only RISC-associated sRNAs are eluted. This workflow is completed in just 15 minutes (Fig. 1).
The RISC eluate can then serve as input for either biochemical analyses or for RNA extraction. The extracted sRNA is perfectly suited for direct NGS analysis, e.g., as input for Lexogen's Small RNA-Seq Library Prep Kit.

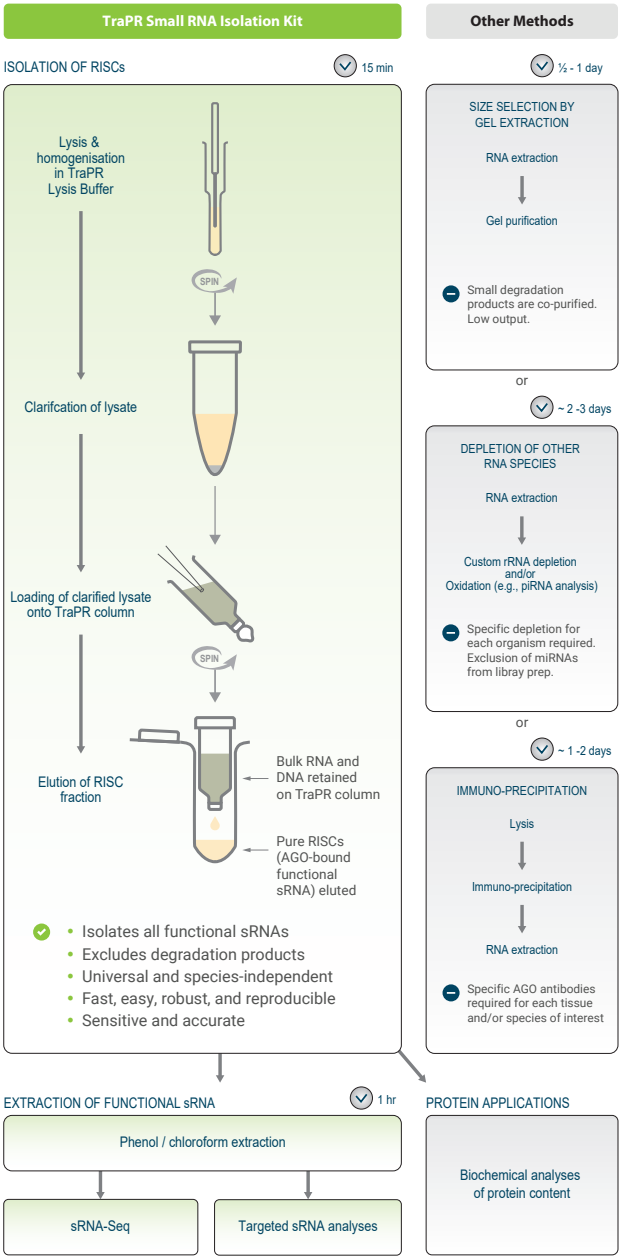

Figure 1 | Outline of the TraPR sRNA isolation method in comparison with alternatives. Using the TraPR column, the RISC fraction is isolated in only 15 minutes whereas other methods take days, need elaborate equipment, and are not as universal and sRNA-specific.

Species-Independent Isolation of Functional sRNAs

Lexogen's TraPR Small RNA Isolation Kit easily isolates all classes of RISC-associated sRNAs found in any organism, tissue, cell type, or bio-fluid. No previous characterization of the AGO/PIWI repertoire of the organism of interest is required (Fig. 2).

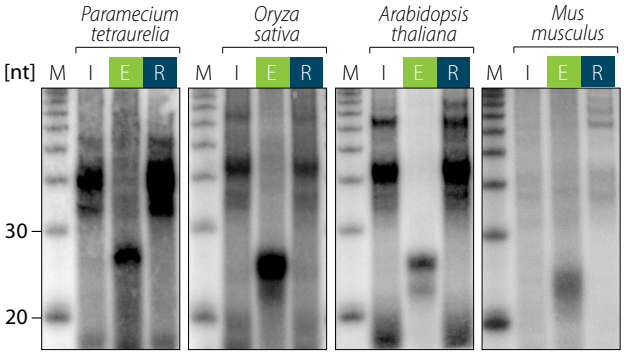

Figure 2 | TraPR is a universal, species-independent method for isolation of RISCs as exemplified for ciliate, plant, and mammalian samples. *RNA was extracted from input (I), TraPR eluate (E), and column-retained (R) fractions, radiolabeled and analyzed by gel electrophoresis and autoradiography[1].*

TraPR Eliminates the Need for Gel Extraction of sRNAs

Typically, sRNA-Seq libraries from total RNA contain only a minor fraction of reads corresponding to functional sRNAs (Fig. 3). Size selection methods such as gel extraction are commonly applied to increase the share of sRNA-mapping reads. However, these tedious processes often result in low yield and co-isolation of contaminating RNAs such as degradation products of tRNA, rRNA, and mRNA as selection is based purely on size. Such elaborate processing steps may also introduce additional bias that can distort the sRNA representation in sequencing data.

Lexogen's TraPR Small RNA Isolation Kit specifically isolates RISC-associated sRNAs via a single purification column, and subsequent RNA extraction channels only functional sRNAs into the library preparation. Thus, TraPR replaces lengthy and error-prone gel extractions with quick and easy column purifications and significantly decreases sequencing costs by re-directing reads from by-products towards functional sRNAs.

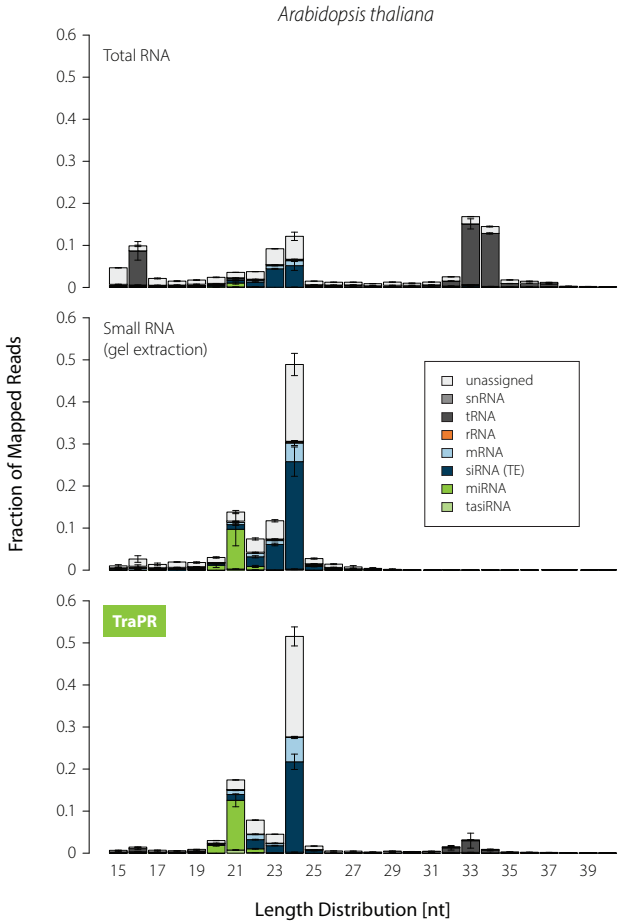

Figure 3 | TraPR enriches functional sRNA without the need for gel extraction. *Size distribution and biotypes of mapped reads from NGS libraries prepared from Total RNA (TRIzol extraction), gel-purified, or TraPR-isolated sRNA from Arabidopsis thaliana. All samples were prepared in triplicates[1].*

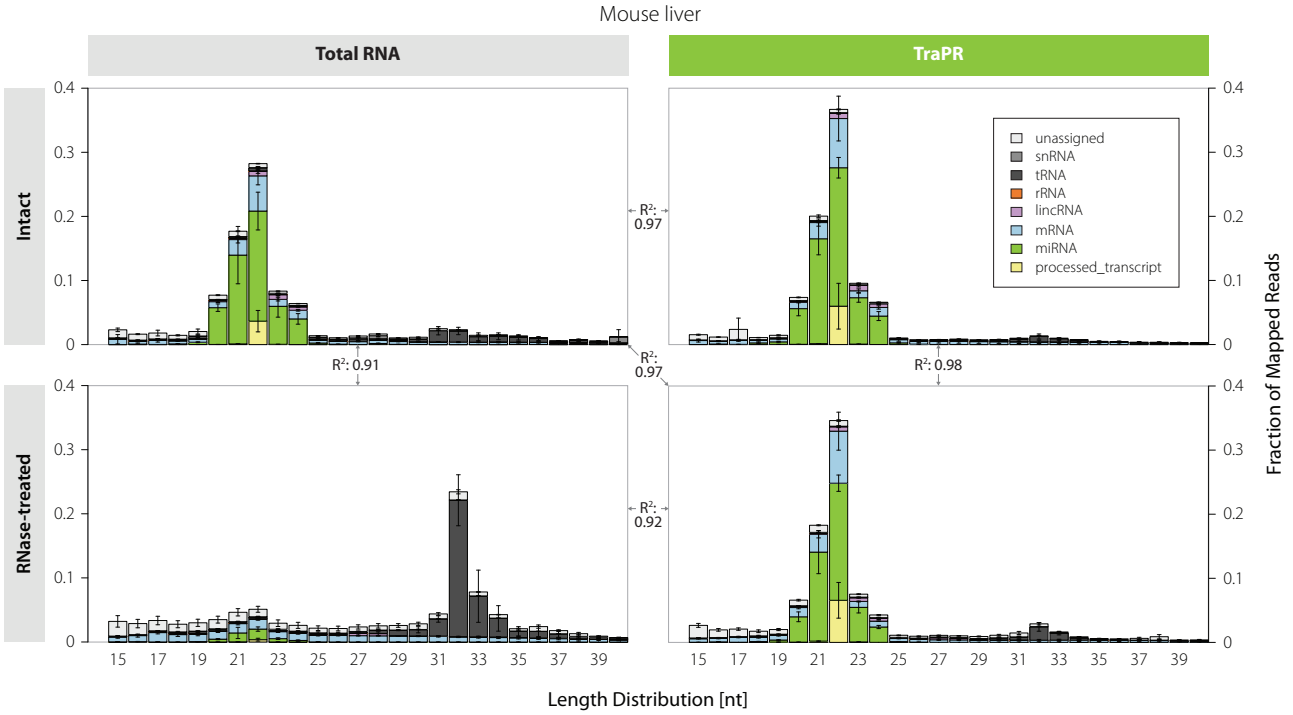

Figure 4 | TraPR enables clean isolation of high-quality sRNA from RNA-degraded samples. *Total RNA (TRIzol extraction) or TraPR-isolated sRNA from intact or RNase-treated samples were converted to NGS libraries using Lexogen's Small RNA-Seq Library Prep Kit and sequenced. Mus musculus liver lysates were treated for 30 minutes with RNase T1 to simulate RNA degradation. Size distribution and biotypes of mapped reads are shown. The R^2 values between the panels refer to the respective miRNA read count correlations. All samples were prepared in triplicates[1].*

TraPR Isolates High-Quality sRNA from Challenging Samples

Size-based selection does not distinguish between functional sRNAs and short RNA fragments originating from degradation. This is particularly problematic for low-quality samples or such that are prone to RNA degradation during handling or storage, such as biopsy samples. TraPR enables isolation of pure high-quality sRNA even from highly degraded input as functional sRNAs are protected within stable RISCs.

To demonstrate this quality, mouse liver lysate was treated with RNase to mimic RNA degradation (Fig. 4). Total RNA libraries from the RNase-treated lysate are heavily contaminated with tRNA-derived fragments leading to a strong reduction in the level of regulatory sRNAs. In contrast, TraPR isolation preserves the sRNA size distribution for degraded samples which is represented by the dominant ~22-nt miRNA peak. The high miRNA NGS read count correlation (R^2: 0.97) between intact Total RNA and TraPR preparation of degraded samples further demonstrates the extraordinary robustness of the procedure. This is also reflected by a high correlation of miRNA read counts between intact and degraded samples for TraPR-isolated sRNAs (R^2: 0.98). TraPR therefore allows comparison of miRNA compositions regardless of sample quality.

TraPR Increases Sensitivity for Low-Abundant miRNAs

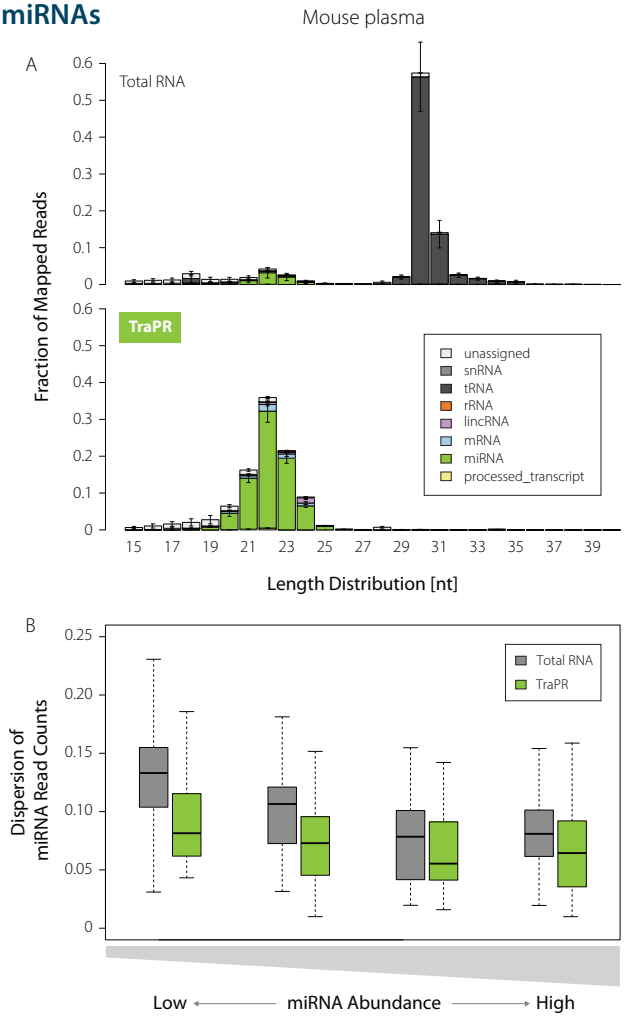

Figure 5 | TraPR robustly detects low-abundant sRNA from plasma samples. *Total RNA (TRIzol) or TraPR-isolated RNA from 150 µl Mus musculus plasma were converted to NGS libraries and sequenced (n = 4).* ***A)*** *Size distribution and biotypes of mapped reads illustrate the superior specificity of TraPR for functional sRNAs.* ***B)*** *Box plot representation of miRNA NGS read count dispersion, grouped by miRNA abundance. TraPR consistently shows lower dispersion levels due to a higher reproducibility and accuracy of TraPR-derived sRNA preparations*[1].

Small RNA profiling from easily accessible material such as plasma is of growing interest for biomarker discovery. However, widespread degradation, low sRNA content, and diversity of sRNA carriers such as exosomes, micro-vesicles, or apoptotic bodies pose a great challenge. Furthermore, even slightest changes in extraction procedures are known to affect reproducibility dramatically. TraPR overcomes these limitations by enriching sRNAs from minute amounts present in samples such as mammalian plasma and increases the number of usable reads by at least one order of magnitude (Fig. 5A).

TraPR further reduces the read count dispersion particularly for low-abundant miRNAs, resulting in more precise quantification of this elusive fraction (Fig. 5B). Small RNA isolation from liquid samples thereby becomes easily accessible and TraPR is perfectly suited for biomarker applications that require high accuracy and consistency.

TraPR Works Consistently Over a Large Input Range and Eliminates the Need for rRNA Depletion

Small RNA profiling of certain difficult tissue types requires extensive and laborious sample preparation due to the vast abundance of contaminating RNA species of similar length. *Drosophila* ovaries, for instance, harbor 21-nt siRNAs, ~22-nt miRNAs and 23- to 30-nt piRNAs. These sRNA subtypes are bound by Ago2, Ago1, and PIWI proteins. A severe complication to sRNA profiling in flies is caused by the high abundance of the 30-nt long 2S rRNA. Therefore, a typical piRNA library generation from fly gonads is a two- to three-day process that entails gel-based size selection and removal of 2S rRNA and other abundant RNA fragments by antisense-oligo-based depletion and oxidation. While oxidation effectively removes contaminants, it also eliminates the majority of miRNAs. In contrast, sRNA isolation using TraPR fully preserves miRNAs, siRNAs, and piRNAs without requiring depletion of contaminating non-regulatory RNAs (Fig. 6A).

Furthermore, TraPR can be used over a broad input range starting from as little as two ovary pairs. Small RNA profiles are maintained, and robustness is demonstrated by perfect correlation of read counts for piRNAs (R^2: 0.999) and miRNAs (R^2: 0.997) from two and 50 ovary pairs (Fig. 6B).

TraPR Applications

- Detection and quantification of miRNAs, siRNAs, piRNAs, and scnRNAs.
- Analysis of differential sRNA expression between tissues, developmental stages, or environmental stresses.
- Investigation of unannotated sRNAs and screening for novel sRNAs.
- Discovery of novel biomarkers for the detection of diseases or monitoring of the response to drug treatments.
- Characterization of miRNA isoforms and modifications to study their effect on gene expression.
- *Bona fide* identification and characterization of regulatory tRNA fragments active in RNA interference pathways.
- Isolation of RISCs for biochemical analyses.

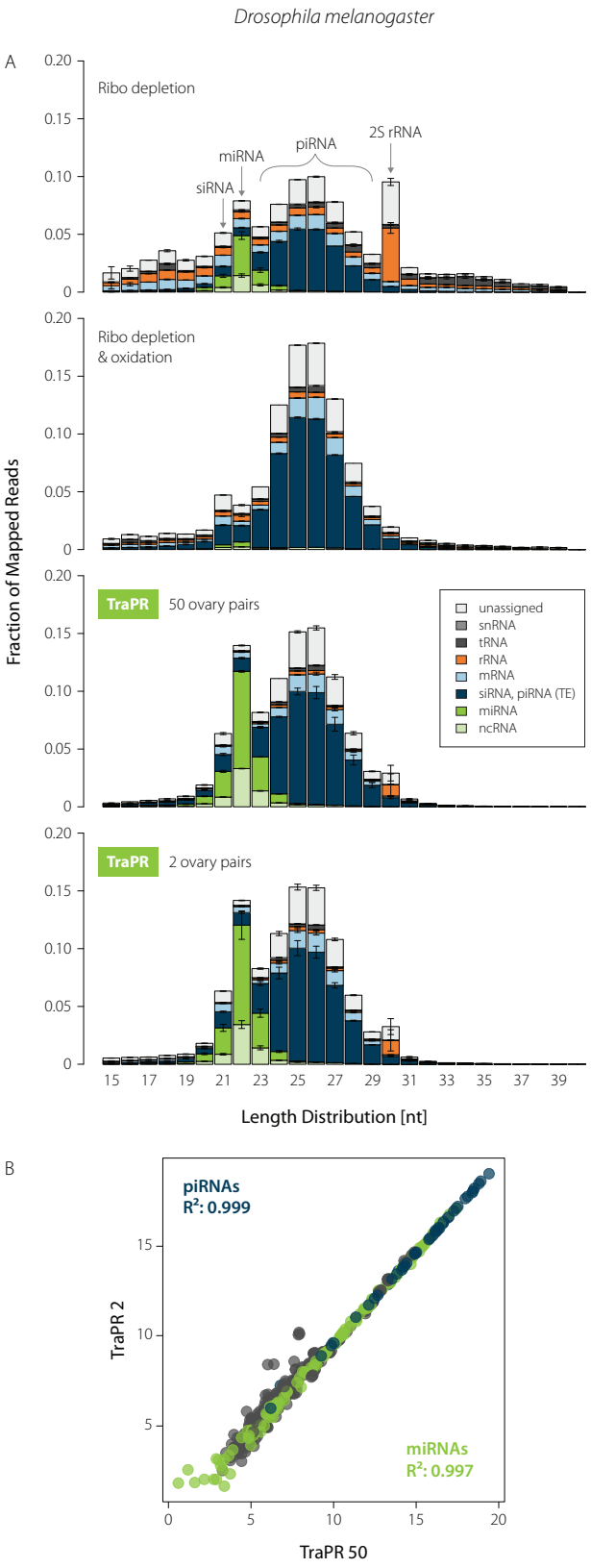

Figure 6 | TraPR robustly enriches all classes of sRNAs while eliminating 2S rRNA from Drosophila ovaries. A) Size distribution and biotypes of mapped reads from NGS libraries. 10 µg of total RNA were used for gel selection followed by ribo-depletion (+/- oxidation). For TraPR-based isolation only two or 50 ovary pairs were used as input. Averages of two independent experiments are shown. B) Correlation plot comparing read counts corresponding to miRNAs (green), piRNAs (blue), and rRNA (gray) between TraPR-derived sRNA library preparations using either two or 50 ovary pairs[1].

Summary

Lexogen's TraPR Small RNA Isolation Kit allows isolation of all RISC-associated sRNAs found in any organism, tissue, cell type, or bio-fluid in a matter of minutes. TraPR enables the consistent isolation of *bona fide* sRNAs, leading to highly reproducible sequencing results even from RNA-degradation-prone material or from samples with inferior quality. TraPR offers substantially higher reliability for detection of low abundant miRNAs rendering the method exceedingly suitable for biomarker discovery from samples containing minute amounts of sRNA.

Benefits

- Isolate only fully functional, physiologically relevant silencing sRNAs including piRNAs, siRNAs, miRNAs, scnRNAs, and AGO-bound tRNA fragments.
- Benefit from an easy, robust, and fast (15 min) protocol.
- Generate consistent and reproducible results effortlessly.
- Work with standard laboratory equipment, perform the complete procedure with only a benchtop centrifuge.
- Remove the need for characterization of the sample's AGO-protein composition prior to functional sRNA analysis.
- Extract high-quality sRNA from degradation-prone material, challenging, and variable sample types.
- Work confidently with samples containing low amounts of sRNA, such as plasma.
- Skip all pre-processing steps, such as size selection, rRNA depletion, oxidation, or immuno-precipitation.
- Reduce time, input amounts, effort, and inconsistency.
- Simplify downstream analyses and save sequencing costs.
- Preserve the RISC composition for downstream biochemical analyses.

Reference

[1] Grentzinger T., Oberlin, S., Schott, G., et. al. (2020) A universal method for the rapid isolation of all known classes of functional small RNAs. Nucleic Acids Res, DOI: 10.1093/nar/gkaa472.

Ordering Information

Cat. №	Product Name
128	TraPR Small RNA Isolation Kit
242	miRVEL Discovery Small RNA-Seq Library Prep Kit
243	miRVEL Profiling Small RNA-Seq Library Prep Kit

TraPR
Functional sRNA Isolation

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