LEXOGEN
The RNA Experts

TraPR Small RNA Isolation Kit

- Unique, gel-free, fast, and easy single column workflow
- Save sequencing cost by focusing your reads on functional sRNAs only
- Generate consistent and reproducible results even from challenging and variable sample types

Introduction

Small RNAs are essential regulators of gene expression and involved in regulatory pathways, such as cancer, inflammation, and development. Furthermore, sRNAs serve as biomarkers for disease detection or monitoring and also as drug targets. 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.

Current methods for specific isolation of functional sRNAs involve co-immuno-precipitation (Co-IP) via their respective AGO proteins. Co-IP is a very specific and sensitive method to isolate functional sRNAs but it is limited to the one organism of interest and the availability of suitable, expensive antibodies. On the other hand, isolation with commercial sRNA extraction kits (using spin columns) is fast, cheap, and easy, but it does not confer any specificity since all RNAs below a common threshold of 200 nucleotides are purified. Thus, the majority of RNA-Seq reads will be wasted on non-functional RNA fragments derived from degradation.

Subsequent approaches to increase specificity use tedious gel extraction steps. But since selection is still purely based on the size, a distinction between functional sRNAs and degraded RNA fragments is not possible.

So far, there has been no method that combines the specificity of AGO Co-IP with the ease commercial sRNA extraction kits (Tab. 1).

Table 1 | TraPR combines the favorable features of sRNA-specific AGO Co-IP with fast and easy sRNA extraction kits.

	TraPR	Other sRNA Isolation Methods		
		AGO Co-IP	sRNA Extraction Kits	Gel Extraction
Specific	✓	✓	✗	✓*
Sensitive	✓	✓	✗	✗
Robust	✓	✓	✓	✗
Universal	✓	✗	✓	✓
Fast	✓	✗	✓	✗
Easy	✓	✗	✓	✗

* Specific for sRNA length, not functionality.

TraPR Combines Fast and Easy Handling with High Specificity for Functional sRNAs

TraPR (Trans-kingdom, rapid, affordable Purification of RISCs[1]) enables the specific isolation of functional sRNAs from RISCs using an easy 15 minutes column purification followed by standard RNA extraction of 1 hour (Fig. 1). 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 without the need for tedious gel extraction steps or prior knowledge of the sample's AGO composition.

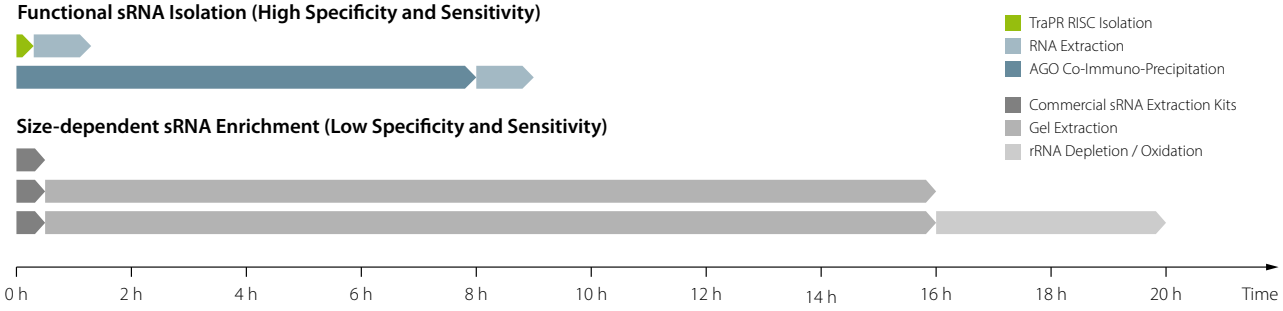

Figure 1 | TraPR delivers high sRNA specificity while saving up to 2 working days. Compared to AGO Co-IP, which delivers comparable specificity for functional sRNAs but takes 1-2 days, TraPR saves at least 8 hours of working time. While commercially available sRNA extraction kits (using spin columns) are fast and easy, they do not confer specificity for functional sRNAs. Subsequent size-dependent methods to increase sensitivity are tedious and extend to above 2 working days.

The TraPR Principle

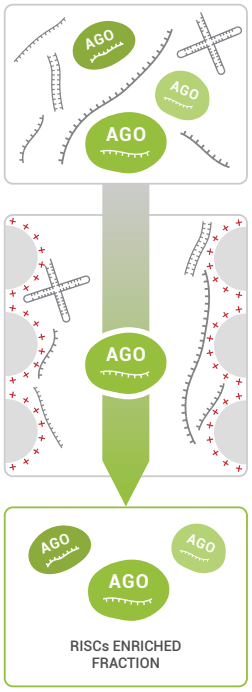

Figure 2 | Schematic depiction of the TraPR principle of RISC isolation.

TraPR is an easy and robust column purification method: the sample is lysed, and the clarified lysate is loaded onto the TraPR column. TraPR exploits the conserved properties of RISCs to elute them while bulk RNA and DNA are retained on the column (Fig 2). 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. Thus, sRNAs can be isolated even from degradation-prone material or from samples that are notoriously hard to work with (e.g. plasma). Contaminating RNAs such as degradation products of tRNA, rRNA, and mRNA are effectively excluded from the purified RISC fraction.

Isolation of Functional sRNAs Saves Sequencing Costs

Lexogen's TraPR Small RNA Isolation Kit specifically isolates RISC-associated sRNAs (Fig. 3) via a single purification column, and subsequent RNA extraction channels only functional sRNAs into the library preparation. Thus, TraPR decreases sequencing costs by re-directing reads from by-products towards functional sRNAs, dramatically lowering the overall cost per sample for sRNA-Seq (Fig. 4).

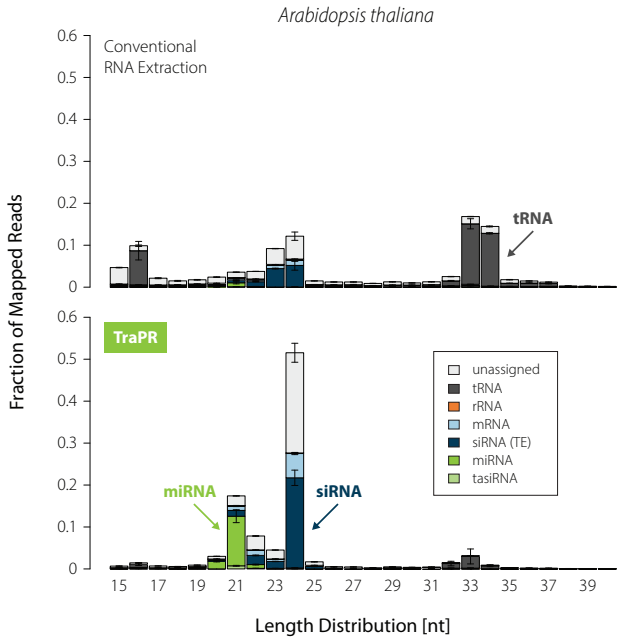

Figure 3 | TraPR enriches functional sRNA. Size distribution and biotypes of mapped reads from NGS libraries prepared from total RNA (conventional RNA extraction) or TraPR-isolated sRNA from *Arabidopsis thaliana*[1].

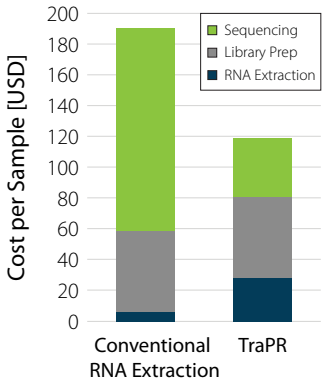

Figure 4 | TraPR significantly reduces overall sRNA analysis cost. Almost all reads obtained from TraPR-isolated RNA map to functional sRNAs whereas using conventional RNA extraction, only ~40 % of reads map to functional sRNAs. Hence, TraPR allows pooling of more libraries per lane resulting in decreased sequencing and overall cost per sample.

Summary

TraPR presents a gel- and bias-free, column-based method for isolation of functional small RNAs from RISCs of all eukaryotic 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 NGS applications and thus provides a highly reproducible, time-saving method that outperforms all current gold-standard procedures for sRNA profiling.

Benefits

- **Specific:** Isolate only fully functional, physiologically relevant silencing sRNAs.
- **Sensitive:** Detect even low-abundant sRNAs with high confidence and accuracy.
- **Robust:** Generate consistent and reproducible results effortlessly and extract high-quality sRNA from degradation-prone material, challenging, and variable sample types.
- **Universal:** Apply TraPR to all eukaryotic organisms: no need for characterization of the sample's AGO-protein composition prior to functional sRNA analysis.
- **Fast:** Save time with the fast protocol (15 min + 1 hour).
- **Easy:** Skip all pre-processing steps, e.g. gel extraction and perform the full procedure with only a benchtop centrifuge.
- **Cost efficient:** Save sequencing costs and simplify downstream analysis.

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

128 (TraPR Small RNA Isolation Kit)
135 (Small RNA-Seq Library Prep Kit for Illumina with TraPR)

For more information and additional resources on TraPR visit our website.

Find more about TraPR at www.lexogen.com.
Contact us at info@lexogen.com, +43 1 345 1212-41, or find your local contact at www.lexogen.com/distributors.