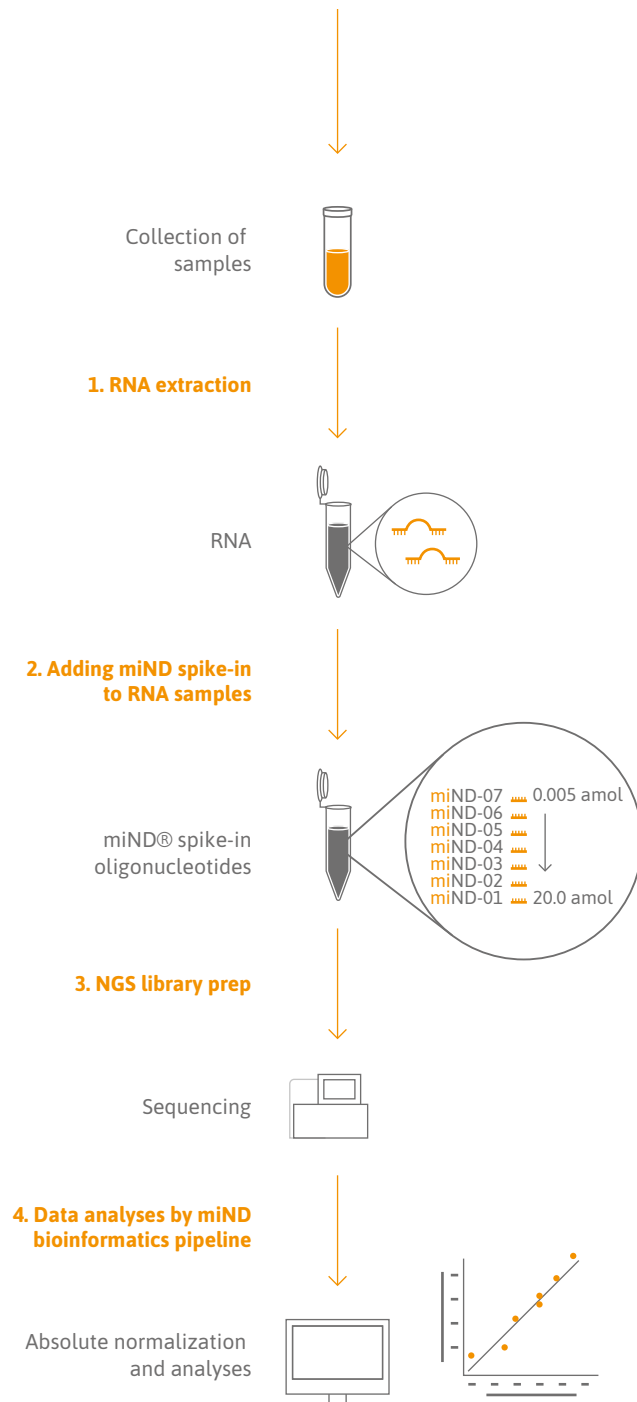# miND

miRNA NGS Data pipeline

A unique NGS workflow for absolute quantitation of microRNAs and other small RNAs in any biological sample and species

TAmiRNA

stability for life.

miND

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stability for life.

miND® (**mi**croRNA **N**GS **D**ata Analysis) is a combination of a novel **small RNA-seq workflow¹** and **bioinformatic pipeline²** for absolute quantitation of microRNAs in any biological matrix and species.

The miND® workflow uses proprietary miND spike-ins that are added to an RNA sample during the NGS library preparation.

miND® spike-ins consist of seven oligonucleotides with a unique design that reduces sequencing bias³. miND® spike-ins are provided in a specific ratio to cover the broad concentration range of endogenous small RNAs (see page 3).

The miND® data analysis pipeline processes NGS raw data and compiles all results in a simple but comprehensive report (see page 4).

The entire **miND® small RNA-seq workflow is provided as a service** by TAmiRNA. Get your quote via www.tamirna.com/small-rna-sequencing-services

miND® spike-ins can be purchased as a product to be included in any small RNA-sequencing project for QC and data normalization.

Collection of samples

1. RNA extraction

RNA

2. Adding miND spike-in to RNA samples

miND® spike-in oligonucleotides

miND-07	0.005 amol
miND-06	
miND-05	
miND-04	
miND-03	
miND-02	
miND-01	20.0 amol

3. NGS library prep

Sequencing

4. Data analyses by miND bioinformatics pipeline

Absolute normalization and analyses

miND® spike-ins enable quality control and absolute quantitation of microRNAs in different sample types

Spike-in calibrator fit
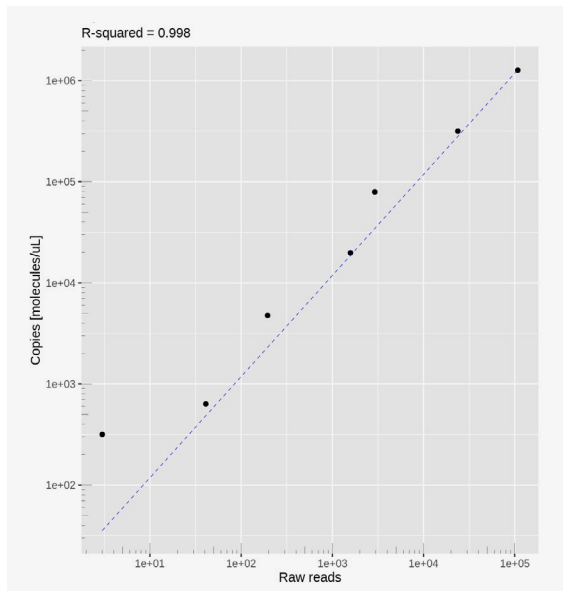
### miRNA and spike-in reads distribution
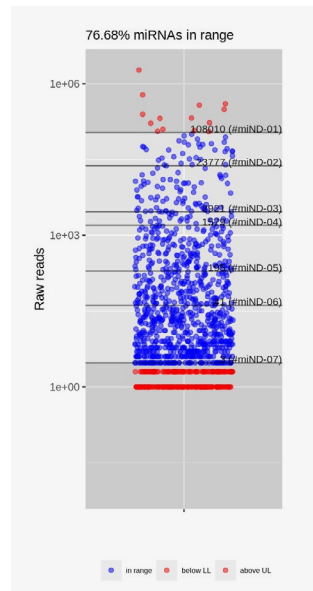
Spike-in calibrator applications:

- miND® spike-ins serve as a **quality control** for small RNA-sequencing experiments to confirm the dynamic range and sensitivity of the assay.

- miND® spike-ins are used to generate a linear regression model to **calculate absolute concentrations** of endogenous microRNAs

Fit-for-purpose analytical validation of the miND® pipeline has been completed[1]:

- Relative accuracy
- Precision
- Analytical measurement range
- Sequencing bias

The miND® pipeline was tested with plasma, serum, cerebrospinal fluid (CSF), synovial fluid (SF), and extracellular vesicles (EV)[1]
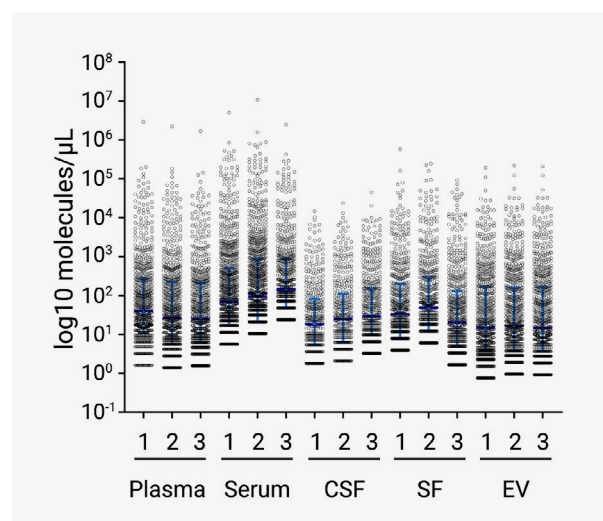
Contact us for a free consultation to discuss your project www.tamirna.com/services/contact-request
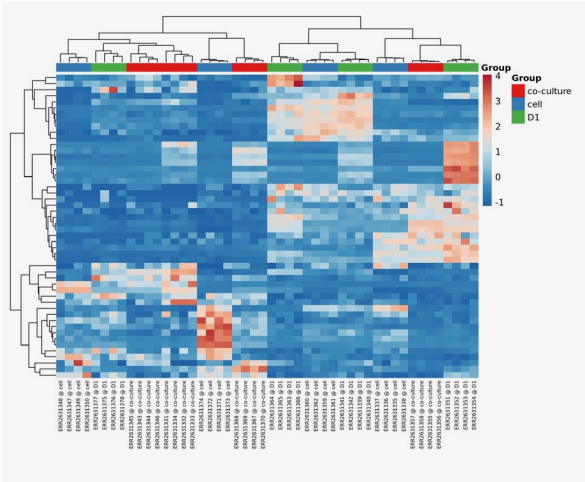
TAmiRNAs miND® pipeline provides user friendly and highly supportive data analysis reports

1. Quality control
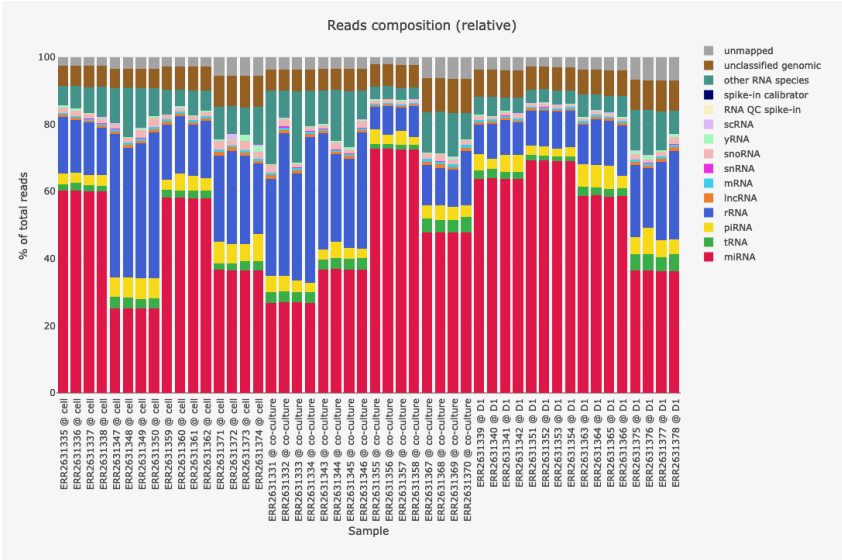
Reads classification plots (shown here) provide insights into the RNA composition of each sample. This is complemented by information on mapping statistics, microRNA numbers, and read length.

2. Unsupervised analysis (heatmap, PCA, t-SNE)

Heatmaps visualize RPM normalized reads and information on the clustering of samples and microRNAs.

3. Supervised analysis (differential expression)
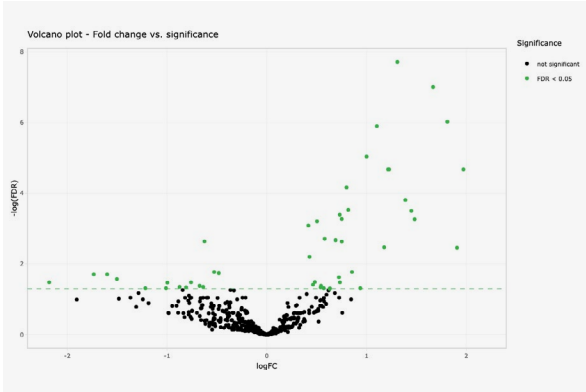
Volcano plots (shown here) and MA plots visualize the relation of the logFC (how much did a specific miRNA change between the groups) and the statistical significance.

4. Full access: all raw and normalized data, tables, plots, and differential expression results can be downloaded in CSV or XLX Format

Download an exemplary report here:

The miND® pipeline was tested in a variety of samples types and species:

	cells	tissue	media	biofluid
Required Input	> 50 cells	> 10,000 µm²	> 50 µL	> 50 µL
EV / exosome purification			SEC, ExoQuick, Ultracentrifugation	SEC, ExoQuick, Ultracentrifugation
EV analysis (NTA, Flow Cytometry)			⊘	⊘
Total RNA isolation and QC	⊘	⊘	⊘	⊘
miND® small RNA-seq	⊘	⊘	⊘	⊘

Additional options:

- **Species compatibility**: our bioinformatic pipeline has been tested with human, mouse, rat, pig, cow, and horse samples. Any species with known miRNAs can be analyzed.

- **Sample types:** besides cells and tissues we have tested conditioned media, plasma (various anti-coagulants), serum, urine, CSF, brain microdialysate, and synovial fluid. This includes enrichment of EV/exosomes from all biofluids.

- **Laser microdissection:** the miND® service can be used to analyze dissected tissue compartments for increased precision. Learn more here: https://www.tamirna.com/space-resolved-rna-profiling-in-complex-tissues/

- **Other RNAs:** mRNA, tRNA, rRNA, piRNA, lncRNA, and sn/snoRNAs are also picked up by our data and can be used for exploratory analyses.

- **RNA-seq:** we offer mRNA-seq (polyA and total RNA) alongside our small RNA-seq workflow to generate high quality microRNA/mRNA datasets.

References:

1 Khamina, K et al. A MicroRNA Next-Generation-Sequencing Discovery Assay (miND) for Genome-Scale Analysis and Absolute Quantitation of Circulating MicroRNA Biomarkers. Int. J. Mol. Sci.2022,23,1226
2 Diendorfer A, et al. miND (miRNA NGS Discovery pipeline): a small RNA-seq analysis pipeline and report generator for microRNA biomarker discovery studies. F1000Research 2022, 11:233
3 Nodine et al. Novel spike-in oligonucleotides for normalization of sequence data. EP3354746B1. Granted in EP and JPN. Pending in US, CN, and CA

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