

# DNBSEQ™ Service Overview

## Small RNA Sequencing

### Service Description

Small RNAs are a type of non-coding RNA (ncRNA) molecule that are less than 200nt in length. They are often involved in gene silencing and post-transcriptional regulation of gene expression. Small RNA sequencing is used to discover novel small RNAs, examine the differential expression of all small RNAs and to characterize variations with single-base resolution.

### Unique Molecular Identifier (UMI) Service Option

Unique Molecular Identifiers (UMIs) can be utilized to eliminate undesirable PCR duplicates derived from a single molecule. After PCR, molecules sharing a UMI are assumed to be derived from the same input molecule. As such, UMI counts offer superior results to counting reads, leading to more accurate estimates of quantitative small RNA expression<sup>[1]</sup>. UMI technology is especially beneficial to customers doing research on rare and precious samples or samples containing less RNAs, such as exosomes.

DNBSEQ™ Small RNA sequencing service with optional UMI technology delivers accurate, affordable and high-quality sequencing data to support your academic and clinical research applications.

### Sequencing Service Specification

DNBSEQ™ Small RNA Sequencing Services are performed with the DNBSEQ™ technology, featuring cPAS and DNA Nanoballs (DNB™) technology for superior data quality<sup>[2]</sup>.



#### Sample preparation and services

- 50bp single-end sequencing reads
- Standard output 20 Million reads per sample
- UMI technology to enhance the quantification accuracy
- Sequencing data and bioinformatics analysis are available in standard file formats
- Advanced RNA data visualization and data mining with Dr.Tom system



#### Sequencing Quality Standard

- Guaranteed ≥80% of bases with quality score of ≥Q30

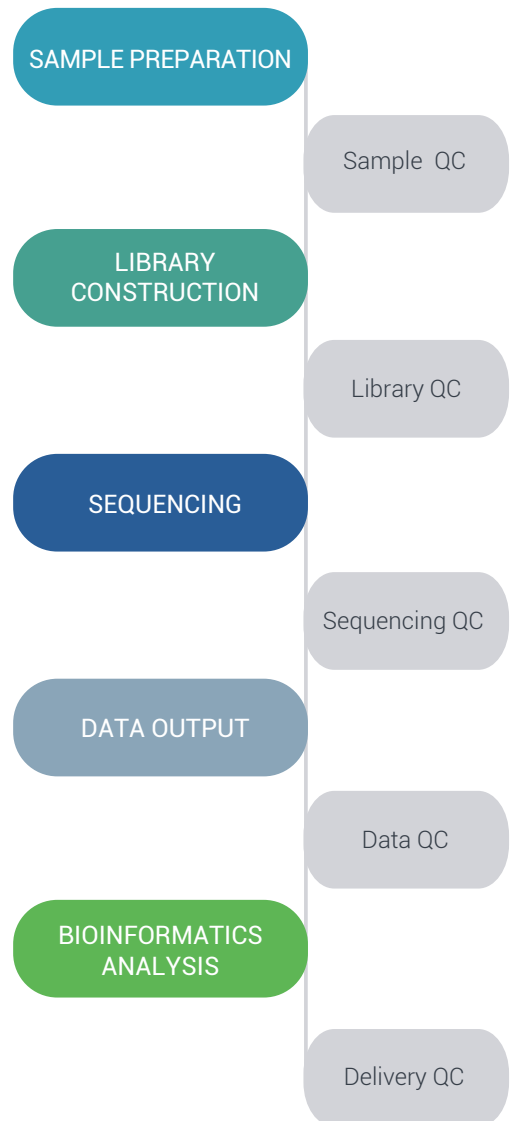


#### Turnaround Time

- Typical 27 working days from sample QC acceptance to filtered data availability
- Expedited services are available

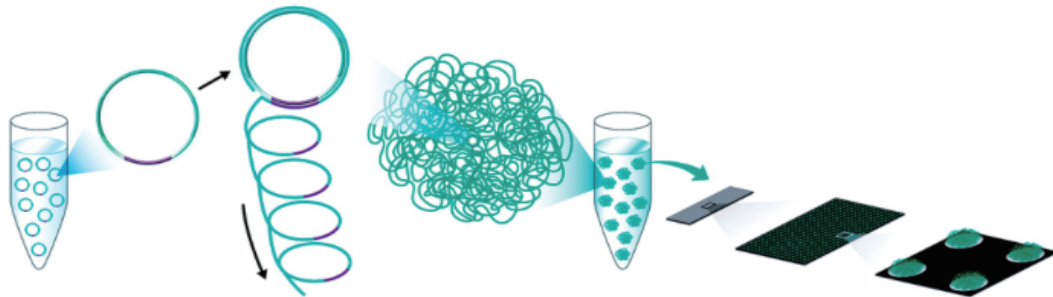
### Project workflow

We care for your samples from the start through to the result reporting. Highly experienced laboratory professionals follow strict quality procedures to ensure the integrity of your results.



## DNBSEQ™ Sequencing Technology

DNBSEQ™ is an innovative high-throughput sequencing solution. The system is powered by combinatorial Probe-Anchor Synthesis (cPAS), linear isothermal Rolling-Circle Replication and DNA Nanoballs (DNB™) technology, followed by high-resolution digital imaging.



The combination of linear amplification and DNB™ technology reduces the error rate while enhancing the signal. The size of the DNB is controlled in such a way that only one DNB is bound per active site on the DNBSEQ™ flow cell. This densely patterned array technology provides optimal sequencing accuracy and increases flow cell utilization.

## Data Analysis

In addition to data output, we offer a range of standard and customized bioinformatics pipelines for your small RNA sequencing project.

Reports and output data files are delivered in industry standard FASTQ, and Excel file formats with publication-ready tables and figures.

### STANDARD BIOINFORMATICS ANALYSIS

- Data filtering;
- Length distribution of small RNAs;
- Small RNAs distribution across selected genome;
- Identification of rRNAs, tRNAs, snRNAs, snoRNAs, etc.;
- Identify homology small non-coding RNAs by aligning to Rfam database;
- Identification of small RNA sequences which could align to exon/intron;
- Identify repeat associated small RNAs;
- Identification of known miRNAs by aligning to designated part of miRbase;
- Identify known non-coding RNAs by aligning to RNAcentral database;
- Novel miRNAs prediction; phasiRNA prediction in plants; Novel piRNAs prediction in animals;
- Analysis of the expression pattern of known and novel miRNAs;
- Target genes prediction of miRNA;
- GO annotation and KEGG pathway analysis of different miRNA Target genes;
- Time Clustering analysis of known and novel small RNA.

### Dr. Tom SYSTEM ANALYSIS

- miRNA quantification;
- Differentially expressed miRNA detection;
- miRNA target gene analysis;
- GO and Pathway annotation fortarget genes;
- miRNA-mRNA interaction, lncRNA-mRNA interaction analysis;
- Protein-Protein Interaction (PPI) analysis;
- Co-expression Interaction Network Analysis.

## Sample Requirements

We can process your sample of human, plant, or animal samples with the following general requirements:

	RNA Amount and Concentration	Minimum Sample Volume	Quantitative Result
Regular sample	Mass $\geq 1\mu\text{g}$ Concentration $\geq 50\text{ng}/\mu\text{l}$	15 $\mu\text{l}$	RIN $\geq 6.5$ (plant) RIN $\geq 8.0$ (human/animal)
FFPE RNA	Mass $\geq 1\mu\text{g}$ Concentration $\geq 50\text{ng}/\mu\text{l}$	15 $\mu\text{l}$	RIN $\geq 2.0$ DV <sub>200</sub> $\geq 30\%$
Small RNA of Plasma/serum/exosome	Mass $\geq 5\text{ng}$ Concentration $\geq 0.5\text{ng}/\mu\text{l}$	10 $\mu\text{l}$	N/A

## Stable and High-Quality Data Performance

### Technical reproducibility <sup>[3]</sup>

To demonstrate the high technical reproducibility of the DNBSEQ™ technology platform, six human brain samples, two heart samples and two blood samples were sequenced. Reproducibility was assessed by using six technical replicates of human brain sample (see Fig. 1). The median correlation between the six replicates was 0.98, and the 25% and 75% quantile were 0.98 and 0.99, respectively.



Fig. 1 Correlation matrix of brain (six technical replicates), heart (two technical replicates), and blood (two biological replicates) samples, sequenced by the DNBSEQ system.

## References

- [1] Fu Y, Wu PH, Beane T, Zamore PD, Weng Z: Elimination of PCR duplicates in RNA-seq and small RNA-seq using unique molecular identifiers. *BMC Genomics* 2018;19:531.
- [2] Drmanac R, Sparks AB, Callow MJ, Halpern AL, Burns NL, Kermani BG, Carnevali P, Nazarenko I, Nilsen GB, Yeung G, Dahl F, Fernandez A, Staker B, Pant KP, Baccash J, Borcharding AP, Brownley A, Cedeno R, Chen L, Chernikoff D, Cheung A, Chirita R, Curson B, Ebert JC, Hacker CR, Hartlage R, Hauser B, Huang S, Jiang Y, Karpinchyk V, Koenig M, Kong C, Landers T, Le C, Liu J, McBride CE, Morenzoni M, Morey RE, Mutch K, Perazich H, Perry K, Peters BA, Peterson J, Pethiyagoda CL, Pothuraju K, Richter C, Rosenbaum AM, Roy S, Shafto J, Sharanhovich U, Shannon KW, Sheppy CG, Sun M, Thakuria JV, Tran A, Vu D, Zaranek AW, Wu X, Drmanac S, Oliphant AR, Banyai WC, Martin B, Ballinger DG, Church GM, Reid CA: Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science* 2010;327:78-81.
- [3] Fehlmann T, Reinheimer S, Geng C, Su X, Drmanac S, Alexeev A, Zhang C, Backes C, Ludwig N, Hart M, An D, Zhu Z, Xu C, Chen A, Ni M, Liu J, Li Y, Poulter M, Li Y, Stahler C, Drmanac R, Xu X, Meese E, Keller A: cPAS-based sequencing on the BGISEQ-500 to explore small non-coding RNAs. *Clin Epigenetics* 2016;8:123.

## To Learn more

If you have any questions or would like to discuss how our services can help you with your research, please don't hesitate to contact us at [P\\_contact@innomics.com](mailto:P_contact@innomics.com). We look forward to hearing from you!

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