DNBSEQ™ SERVICE OVERVIEW
Plant and Animal Whole Genome Re-Sequencing

Service Description

Plant and animal whole genome re-sequencing (WGRS) involves sequencing the entire genome of a plant or animal and comparing the sequence to that of a known reference genome. Re-sequencing of the plant and animal genome will identify genetic variations such as SNPs and Indels and discover other genetic changes of the sequenced species. It has been used for the identification of functional genes and markers of important traits to facilitate molecular breeding and to improve agricultural production and conservation.

Highlights of DNBSEQ™ Technology

·Even coverage of reads
·Much less duplication
·True PCR-Free
·Index hopping free

Sequencing Service Specification

BGI Plant and Animal Whole Genome Re-Sequencing services are executed with the DNBSEQ sequencing technology.

Sample Preparation and Services

· Library preparation
· 100bp and 150bp paired-end sequencing available
· Raw data, standard and customized data analysis
· Available data storage and bioinformatics applications

Sequencing Quality Standard

· Guaranteed ≥90% of clean bases with quality score of Q20
· Standard sequencing coverage of 10-30X is recommended for the study of individuals and 5-10X for population studies

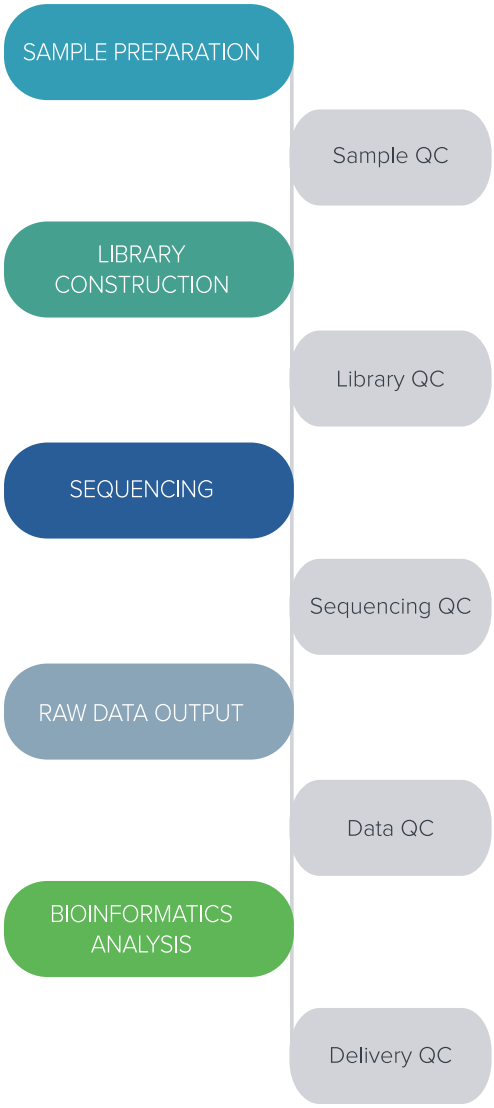
Turnaround Time

· Typical 30-40 working days from sample QC acceptance to filtered raw data availability
· Expedited services are available.Contact your local BGI specialist for details

Quality Data | Fast TAT | Cost Effective

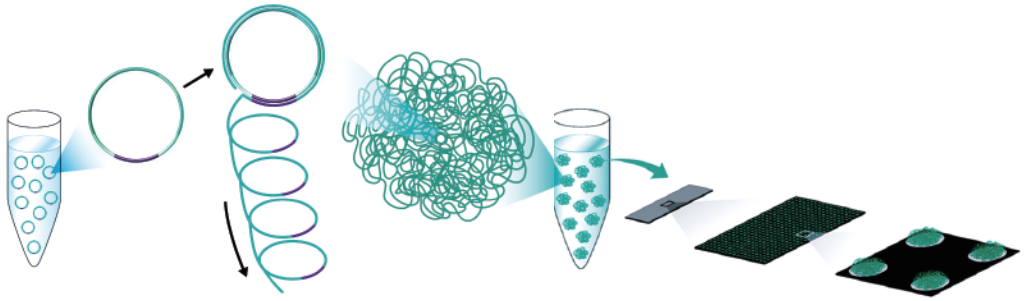
Project Workflow

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, developed by BGI's Complete Genomics subsidiary in Silicon Valley. 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

Besides clean data output, BGI offers a range of standard and customized bioinformatics pipelines for your whole genome re-sequencing project.

Reports and output data files are delivered in industry standard file formats: FASTQ, BAM, VCF, .xls, .png

Standard Analysis

- Data Filtering
- Alignment
- SNP/InDel/SV/CNV calling, annotation and statistics

Advanced Analysis

- Population evolution analysis
- Point mutation detection (wild vs. mutant)
- Linkage map construction and QTL mapping
- GWAS analysis
- BSA analysis

Customized Analysis

Further customization of bioinformatics analysis to suit your unique project is available.
Please contact your BGI technical representative for details.

Sample Requirements

We can process your gDNA samples from a variety of species, with the following general requirements:

	DNA Amount and Concentration	Minimum Sample Volume
Regular Samples	Intact genomic DNA ≥ 1μg, Concentration ≥ 12.5ng/μl	15μl
Low Input Samples	Intact genomic DNA ≥ 200ng, Concentration ≥ 2.5ng/μl	15μl
True PCR-Free	Intact genomic DNA ≥ 1.5 μg, Concentration ≥ 12.5 ng/μl	15μl

Data Performance

1 soybean sample and 1 mouse sample were used to validate the DNBSEQ sequencing technology. DNBSEQ sequencing datasets from 2 technical replicates of each species were generated and compared to datasets from the Illumina HiSeq 4000 sequencing system. Libraries were prepared according to the manufacturer's protocols.

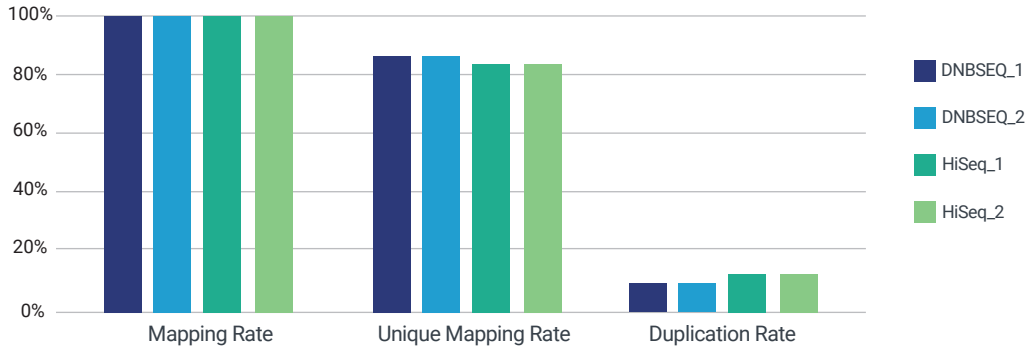
The DNBSEQ sequencing technology generated high quality PE100 data that is comparable to that from the HiSeq 4000 (Table 1). At similar sequencing output between different platforms of each species, over 95% of bases score above Q20 while the genome mapping rate and the unique mapping rate of DNBSEQ data and HiSeq data are at the comparative level. Notably, the clean data rates of all DNBSEQ replicates are higher than those of HiSeq replicates, generating more filtered bases for the following analysis. Moreover, the duplication rates of DNBSEQ reads of both species are constantly lower than those of HiSeq reads. Since the duplicated reads are usually skipped for downstream variant analysis, the DNBSEQ will generate more valid data for variant analysis than the HiSeq 4000 at the same data output.

▼ Table 1. Data quality for soybean and mouse samples (averages from 2 technical replicates).

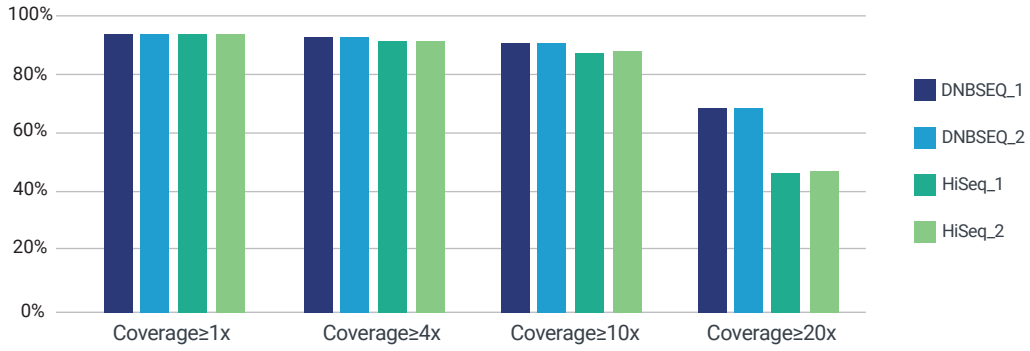
Species	Soybean		Mouse	
Sequencer	DNBSEQ	HiSeq	DNBSEQ	HiSeq
Raw data amount (Gb)	33.12	30.56	70.09	70.33
Clean data amount (Gb)	31.20	26.69	65.50	57.40
Clean rate (%)	94.19	87.34	93.45	81.62
Clean read Q20 (%)	96.59	97.81	96.16	97.66
Mapping rate (%)	99.53	99.77	99.76	99.92
Unique mapping rate (%)	82.90	82.61	86.57	83.97
Duplication rate (%)	3.64	6.42	7.85	11.21
Mismatch rate (%)	0.95	0.68	0.68	0.39
Average sequencing depth	29	26	22	18
Coverage (%)	95.48	94.59	93.51	93.26
Coverage at least 4X (%)	94.15	92.78	92.93	92.36
Coverage at least 10X (%)	92.05	89.32	91.00	87.72
Coverage at least 20X (%)	81.20	67.11	66.05	44.72

Mouse sample data performance

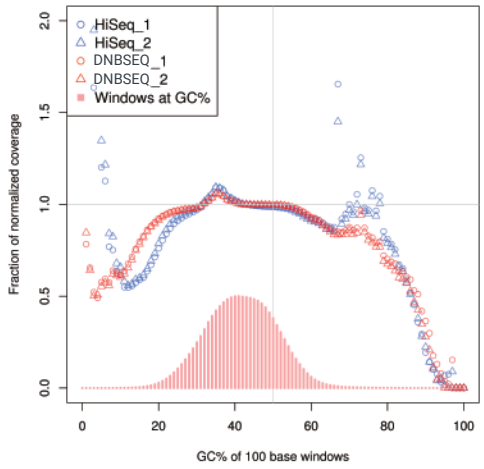
1 mouse liver DNA sample was sequenced by both DNBSEQ and HiSeq 4000 systems. 2 technical replicates for DNBSEQ and 2 for HiSeq are included in the experiment. Both DNBSEQ and HiSeq data show high mapping rates, while less bias is found in the read distribution of DNBSEQ datasets at low GC content (Fig. 1, 2 and 3). Moreover, DNBSEQ duplication rates are significantly lower (Fig. 1), due to less PCR bias from DNBSEQ's rolling circle replication, resulting in more uniform distribution of reads for variant analysis.



▲ Figure 1. Mapping rate and duplication rate of 2 DNBSEQ datasets and 2 HiSeq datasets with comparable data output of 70Gb.

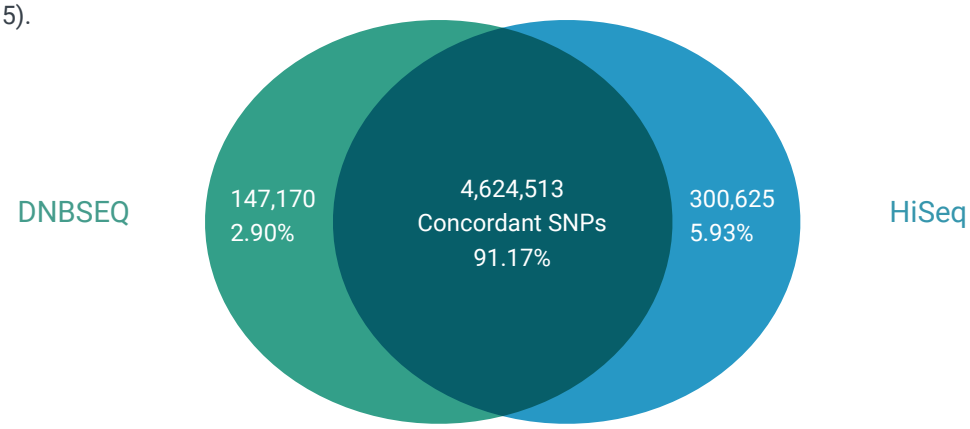


▲ Figure 2. Sequencing coverage of 2 DNBSEQ datasets and 2 HiSeq datasets with comparable data output of 70Gb.



▲ Figure 3. Normalized read coverage by GC content (duplication reads excluded). It shows a less biased distributionof DNBSEQ reads at low GC content when compared to HiSeq read distribution.

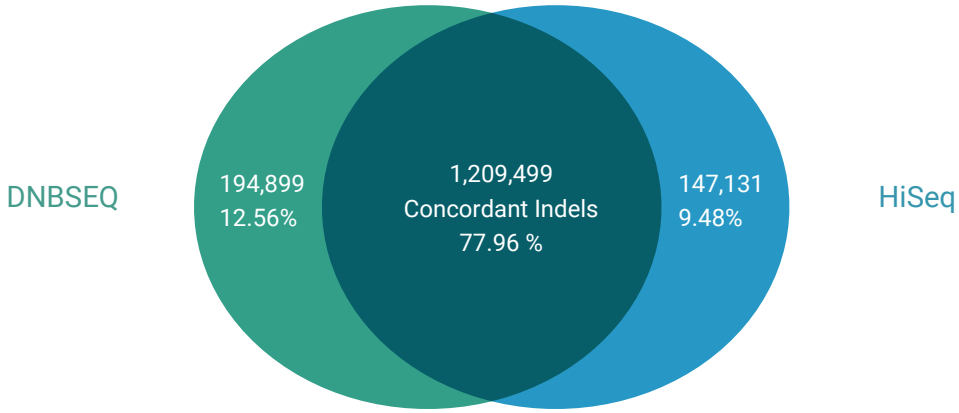
The DNBSEQ demonstrated equivalent variant calling reproducibility to HiSeq, with 93.63% and 76.64% for SNP and Indel calling, respectively. The concordant rates of SNP and Indel calling between platforms were 91.17% and 77.96%, respectively (Fig. 4 and Fig. 5).



DNBSEQ	
DNBSEQ_1	4,639,014
DNBSEQ_2	4,639,471
DNBSEQ total SNPs	4,791,741
Reproducibility	93.63%

HiSeq	
HiSeq_1	4,772,466
HiSeq_2	4,785,071
HiSeq total SNPs	4,946,249
Reproducibility	93.23%

▲ Figure 4. The SNP reproducibility and cross-platform consistency statistics from 2 DNBSEQ replicates and 2 HiSeq replicates.



DNBSEQ	
DNBSEQ_1	1,299,372
DNBSEQ_2	1,307,108
DNBSEQ total Indels	1,477,897
Reproducibility	76.64%

HiSeq	
HiSeq_1	1,250,891
HiSeq_2	1,269,891
HiSeq total Indels	1,414,150
Reproducibility	78.25%

▲ Figure 5. The Indel reproducibility and cross-platform consistency statistics from 2 DNBSEQ replicates and 2 HiSeq replicates.

Request for Information or Quotation

Contact your BGI account representative for the most affordable rates in the industry and to discuss how we can meet your specific project requirements or for expert advice on experiment design, from sample to bioinformatics.

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We Sequence, You Discover