BGI provides sequencing services for pre-made libraries from the 10x Genomics® Chromium™ system Single Cell 3’ Library Construction Kit v3 and Next GEM Single Cell 3’ Library Construction Kit v3.1. Once your pre-made library arrives at our sequencing facility, library QC will be performed to ensure the downstream service quality. For high quality, low duplication and index-hopping-free [1] results, your library will be converted into DNBs (DNA Nanoball) for sequencing with BGI’s proprietary DNBseq™ NGS technology. Upon request, sequencing can be performed with the Illumina HiSeq/NovaSeq platform.

We employ rigorous QC steps following each phase of the service workflow as described by the flow chart below. The qualified sequencing data will be offered for download and bioinformatics service is available upon request.

**Applications for Research and Drug Development**

- Identification of rare cell types
- Analysis of individual cellular heterogeneity
- Analysis of individual cellular signaling pathways
- Study of cellular ecosystems of tumors
- Analysis of individual cell differentiation
- Assessment of drug resistance

**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 flow cell. This densely patterned array technology provides optimal sequencing accuracy and increases flow cell utilization.

**The DNBseq Advantage**

- Superior performance of DNBseq technology when multiplexing samples for improved data integrity
- Hardly any index mis-assignment between samples.
- Sensitivity to detect low-abundance sequences in large sample numbers
- High system throughput without loss of data integrity
- Low duplication and error rate for optimal data yield
- Highest sample flexibility, service speed, and data accuracy
Performance Data

DNBseq and Illumina platforms provide comparable and often superior performance for sc-RNA Seq, as demonstrated in the summary of performance data below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Human iPSC</th>
<th>Human TMWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platform</td>
<td>NextSeq 500</td>
<td>DNBseq</td>
</tr>
<tr>
<td>Estimated cell number</td>
<td>12,859</td>
<td>12,916</td>
</tr>
<tr>
<td>Number of reads</td>
<td>159,010,774</td>
<td>159,715,620</td>
</tr>
<tr>
<td>Mean UMI counts/ cell</td>
<td>12,365</td>
<td>12,366</td>
</tr>
<tr>
<td>Number of detected genes</td>
<td>4,677</td>
<td>5,309</td>
</tr>
<tr>
<td>Median genes/cell</td>
<td>1,857</td>
<td>2,000</td>
</tr>
<tr>
<td>Valid barcodes (%)</td>
<td>97.2</td>
<td>96.4</td>
</tr>
<tr>
<td>Genome mapping rate (%)</td>
<td>80.6</td>
<td>97.8</td>
</tr>
<tr>
<td>Q30 in barcode (%)</td>
<td>93</td>
<td>87.9</td>
</tr>
<tr>
<td>Q30 in UMI (%)</td>
<td>92.2</td>
<td>87.3</td>
</tr>
<tr>
<td>Q30 in RNA (%)</td>
<td>55.9</td>
<td>86.6</td>
</tr>
<tr>
<td>Fraction of reads in cells (%)</td>
<td>79.2</td>
<td>80.1</td>
</tr>
</tbody>
</table>

BGI’s DNBseq outperforms Illumina NextSeq 500 by identifying more cells, genes, and UMI s. In another recent study[2], human iPSC (Induced Pluripotent Stem Cells) and Trabecular Meshwork Cells (TMWC) were used to compare sequencing performance for the widely used 10x Chromium platform against Illumina platforms. DNBseq showed better performance compared to the NextSeq 500 in sequencing quality, cell detection, UMI detection, and gene detection. The researchers were able to call an additional 1,065,659 SNPs from sequence data generated by the DNBseq platform, enabling an additional one in seven cells to be assigned to the correct donor from a multiplexed library.
Similar sensitivities and accuracies were demonstrated between DNBseq (yellow and light purple lines) and Illumina (red and blue lines) sequencers in a study published in 2019 [3], using mESC (yellow and red lines) and K562 (light purple and blue lines) cell lines. The grey dotted lines indicate downsampling at different read depths per cell, while the broken red line indicates saturation per cell.

DNBseq platform outperforms the competitor in Single Cell RNA Sequencing

In a pooled CRISPR single cell screen[4], DNBseq MGISEQ-2000 outperforms the NextSeq 500. With equalized read depths across DNBseq and Illumina platforms, both NextSeq 500 and DNBseq detected similar frequencies of gRNAs (A, left) and numbers of UMI per guide RNA (A, middle). The higher quality of DNBseq generated more qualified reads (A, right) which led to an additional 1,065,659 SNPs from the data (B). The additional SNPs allowed assignment of an additional 1,694 cells to the correct donor with the greater SNP detection.
Sequencing Service Specification

BGI transcriptome sequencing services are executed with the DNBseq sequencing technology, featuring cPAS and DNA Nanoballs (DNB™) technology for superior data quality.

**Sample preparation and services**
- Stranded and non-stranded sequencing is available
- 100bp and 150bp paired-end sequencing options available
- ≥30 Million reads per sample recommended
- Raw data and bioinformatics analysis are available in standard file formats
- Advanced and custom bioinformatics data analysis
- Cloud-based data storage and delivery system

**Sequencing quality standard**
- Guaranteed ≥80% of bases with quality score of ≥Q30

**Turnaround Time**
- Typically, 30 working days from library QC acceptance to filtered RAW data availability.
- Rapid service is available. Contact your local BGI specialist for details.

**Sample Requirements**

We can process libraries constructed with the 10x Genomics® Chromium™ system Single Cell 3’ Library Construction Kit v3 or with the Next GEM Single Cell 3’ Library Construction Kit v3.1, that meet the following requirements:

<table>
<thead>
<tr>
<th>Library parameter</th>
<th>Requirement</th>
<th>Preferred QC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragment size</td>
<td>400bp±100bp</td>
<td>Agilent 2100</td>
</tr>
<tr>
<td>Concentration</td>
<td>≥2ng/ul</td>
<td>Qubit</td>
</tr>
<tr>
<td>Total amount</td>
<td>≥30 ng</td>
<td>Qubit</td>
</tr>
</tbody>
</table>

Project Workflow

Highly experienced laboratory professionals follow strict quality procedures to ensure the integrity of your results.
Data Analysis

In addition to raw data output, BGI offers a range of standard and customized bioinformatics pipelines for your single cell sequencing project. Reports and output data files are delivered in industry standard FASTQ, BAM. Excel formats with publication-ready tables and figures.

### STANDARD ANALYSIS FOR SINGLE CELL RNA SEQUENCING

- Data filtering includes removing adaptors contamination low quality reads from raw reads
- Assessment of sequencing
- Gene expression and annotation (Gene coverage and coverage depth)
- Gene expression difference analysis
- Expression pattern analysis of DEGs
- Gene ontology analysis of DEGs
- Pathway enrichment analysis of DEGs
- Refinement of gene structures
- Identification of alternative spliced transcripts

### CUSTOMIZED ANALYSIS

Further customization of Bioinformatics analysis to suit your unique project is available:
Please contact your BGI technical representative

### References

1. Reliable Multiplex Sequencing with Rare Index Mis-Assignment on DNB-Based NGS Platform
5. Effects of Index Misassignment on Multiplexing and Downstream Analysis (Illumina white paper, 2017).
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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