Introduction

Single cell RNA-Seq enables transcriptome heterogeneity study at the resolution of a single cell. It also unlocks the spatial-temporal gene expression profile with cell-by-cell resolution, which is unarchivable by standard, bulk RNA sequencing. BGI’s Single Cell Sequencing Solution utilizes the 10x Genomics® Chromium™ system for sample prep and library construction, combined with BGI’s cutting edge DNBSEQ™ sequencing technology to ensure the downstream service quality. BGI also provides sequencing services for customer self-prepared libraries using 10x Genomics® Chromium™ system Single Cell 3’ Library Construction Kit v3 and Next GEM Single Cell 3’ Library Construction Kit v3.1, providing flexible tool set for the customers depending on their preference, equipment accessibility and schedule.

Sequencing using DNBSEQ™ (DNA nanoball technology) is accomplished through RCR (rolling circle replication), a linear amplification that each copy is amplified independently using the same single strand circle (DNA nanoball, DNB) as template. As a result, error reads will not accumulate, and most of the signal originate from correct indices [1]. This enables elimination of index mis-assignment which is the major cause for the index hopping problem on Illumina® sequencing platform[5]. With our extensive experience in single cell sequencing we offer the highest quality services in sample prep, library construction, DNB conversion, sequencing, and bioinformatics analysis to our customers. The high-quality results comparing with the competitors have been published in leading scientific journals[2][3][4].

Applications

Differentiation of different cell types and subtypes within a tissue
Analysis of cellular heterogeneity
Analysis of individual cellular signaling pathways
Study of cellular ecosystems of tumors
Analysis of individual cell differentiation
Case Studies

Accuracy and Sensitivity

DNBSEQ™ platforms provide comparable or often superior performance for sc-RNA Seq comparing to Illumina platforms, as demonstrated in the summary of performance data below.

Figure 1. 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.

Table 1. BGI's DNBSEQ™ outperforms Illumina NextSeq 500 by identifying more cells, genes, and UMIIs. In another recent study [4], single-cell RNA seq libraries derived from human iPSC (Induced Pluripotent Stem Cells) and Trabecular Meshwork Cells (TMWC) using 10x Chromium technology were sequenced on both DNBSEQ™ and Illumina platforms for a performance comparison. 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.

DNBSEQ™ outperforms NextSeq 500 in Single Cell RNA sequencing

<table>
<thead>
<tr>
<th>Sample platform</th>
<th>Human iPSC</th>
<th>Human TMWC</th>
</tr>
</thead>
<tbody>
<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>
Figure 2. In a pooled CRISPR single cell screen\[^4\], DNBSEQ™ MGISEQ-2000 outperformed 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.

### DNBSEQ™ has similar performance comparing with NovaSeq 6000

<table>
<thead>
<tr>
<th>Sample</th>
<th>Platform</th>
<th>Valid barcodes(%)</th>
<th>Genome mapping rate(%)</th>
<th>Q30 in barcode(%)</th>
<th>Q30 in UMI(%)</th>
<th>Q30 in RNA(%)</th>
<th>Fraction of reads in cells(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC1</td>
<td>NovaSeq 6000</td>
<td>98</td>
<td>95.3</td>
<td>96.1</td>
<td>95.9</td>
<td>92</td>
<td>93.7</td>
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<tr>
<td></td>
<td>MGISEQ-2000</td>
<td>97</td>
<td>98.1</td>
<td>91.8</td>
<td>91.8</td>
<td>89</td>
<td>94.8</td>
</tr>
<tr>
<td>PBMC2</td>
<td>NovaSeq 6000</td>
<td>98</td>
<td>95.2</td>
<td>96.1</td>
<td>95.9</td>
<td>92.2</td>
<td>94.1</td>
</tr>
<tr>
<td></td>
<td>MGISEQ-2000</td>
<td>97</td>
<td>97.9</td>
<td>90.5</td>
<td>90</td>
<td>88</td>
<td>95.2</td>
</tr>
</tbody>
</table>

Table 2. DNBSEQ™ and Illumina NovaSeq 6000 provide similar and comparable performance for sc-RNA Seq. BGI’s MGISEQ-2000 was compared with Illumina NovaSeq 6000 in identification of valid barcodes, genome mapping rates, quality of sequenced barcodes and UMIs, as well as quality and efficiency of RNA sequencing in single cell RNA sequencing. In a study two sets of human peripheral blood mononuclear cell assays (PBMC1 and PBMC2)\[^4\] have been used to compare sequence performance for 10x Chromium platform. MGISEQ-2000 showed similar performance versus NovaSeq 6000.
Conclusion

Transcriptome profiling at single-cell resolution is a demanding task requiring high sequencing quality. We employ rigorous QC steps following each phase of the service workflow using 10x Genomics® Chromium™ system and the linear amplification and DNB technology implemented sequencing platform. The combination of these two quality systems from sample prep to library sequencing generates the optimal outcomes which have been confirmed by the case studies.

References

1. Reliable Multiplex Sequencing with Rare Index Mis-Assignment on DNB-Based NGS Platform

   https://doi.org/10.1101/346106


   doi:10.1101/552588 https://www.biorxiv.org/content/10.1101/552588v2

5. Effects of Index Misassignment on Multiplexing and Downstream Analysis (Illumina white paper, 2017).

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