A reference human genome dataset of the BGISEQ-500 sequencer

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Abstract

Background: BGISEQ-500 is a new desktop sequencer developed by BGI. Using the DNA nanoball (DNB) and combinational probe anchor synthesis (cPAS) developed from Complete Genomics^{TM} sequencing technologies, it generates short reads in large scale.

Findings: Here, we present the first human whole genome sequencing dataset of BGISEQ-500. The dataset was generated by sequencing the widely used cell line, HG001 (NA12878) in two sequencing runs of pair-end 50 bp (PE50) and two sequencing runs of pair-end 100 bp (PE100). We also included examples of the raw images from the sequencer for reference. Finally, we identified variations using this dataset, estimated the accuracy of the variations and compared to that of the variations identified from similar amount of publicly available HiSeq2500 data.

Conclusions: We found similar SNP detection accuracy for the BGISEQ-500 PE100 data (false positive rate, FPR 0.00020%, and sensitivity 96.11%) comparing to the PE150 HiSeq2500 data (FPR 0.00017% and sensitivity 96.60%), better than the PE50 data (FPR 0.0006% and sensitivity 94.15%). But for insertions and deletions (indels), we found lower accuracy for BGISEQ-500 data (FPR 0.00069% and 0.00067% for PE100 and PE50 respectively, sensitivity 94.30% and 70.93%) than the HiSeq2500 data (FPR 0.00032% and sensitivity 96.28%). Our dataset can serve as the reference dataset providing basic information not just for future developing, but also for all the researches and applications based on the new sequencing platform.

Keywords: Genomics, sequencing, Next Generation Sequencing, BGISEQ-500

Data Description

Massively parallel sequencing technologies (also called as the second generation sequencing) generate large amount of data with lower cost, shorter reads and higher single base error rate comparing to Sanger sequencing technology[1]. With the large amount of data and well-developed analysis tools, second generation sequencing data can be used to effectively and accurately identify genomic variations[2]. Thus it has been widely applied in both researches and applications[3]. Currently there are several commercially available second generation sequencing platforms with different performances and data features[4, 5]. With more and more researches and applications to apply sequencing, new sequencing platforms are being developed. The BGISEQ-500 sequencer was first released by BGI in October, 2015. It was developed based on the Complete Genomics^{TM} sequencing technologies, and applied DNA NanoBalls (DNBs) technology[6] for sequencing library construction and combined primer anchor synthesis (cPAS) for sequencing. We present here a dataset generated from the BGISEQ-500 sequencer, including example of the raw images and the final sequences. We also conducted variation calling using this dataset and compared the variation calling result to that from other sequencers. This dataset can be served as a useful reference for community to develop bioinformatics methods and sequencing based applications on this new sequencing platform.

DNA preparation

NA12878 cell line genomic DNA was ordered from Coriell Institute, and it was 50 μg per tube. The genomic DNA was quantified by Qubit 3.0 fluorometer (Life Technologies, Paisley, UK) and the integrity was qualified on the 2% agarose gel to make sure the genomic DNA molecular was larger than 23kb and not substantially degraded.

Sequencing library preparation

For the sequencing library construction, the NA12878 genomic DNA was fragmented by ultrasonic on Covaris E220 (Covaris, Brighton, UK) to DNA fragments between 50 bp~800bp according to the manufacturer's instructions. The fragmented DNA was further selected to 100bp~300bp by AMPure XP beads (AGENCOURT). The selected DNA fragments were then repaired to obtain a blunt end and modified at 3'end to get a dATP as a stick end. The dTTP tailed adapter sequence was ligated to both ends of the DNA fragments. The ligation product was then amplified for 8cycles and subjected to the following single strand circularization process. The PCR product was heat-denatured together with a special molecular which was reverse complemented to one special strand of the PCR product, and the single strand molecular was ligated using DNA ligase. The remaining linear molecular was digested with the exonuclease, thus finally we obtained the single strand circle DNA library (Figure 1a).

Sequencing

We conducted sequencing according to the BGISEQ-500 protocol (Figure 1b). There were three steps including making DNBs, loading DNBs and sequencing. For making DNBs, 6 ng single strand circle DNA library was first PCR amplified for 10 minutes in an 80 µl reaction volume with pure water, buffer and DNB polymerase. After PCR reaction, 20 µl DNBs stopping buffer was added to stop the PCR reaction. Finally, we used the Qubit® ssDNA Assay Kit to quantify the DNBs on Qubit® Fluorometer (concentration ≥10 ng/µL).

For loading DNBs, we first added 33 µl DNBs loading buffer to DNBs product from the last step, and the mixture was placed on the BGIDL-50 (the sample preparation machine). Then we selected the DNBs loading process (Version: sample load 2.0) to load DNB onto the sequencing chip, which included 96 minutes' loading time and 30 minutes' incubation at room temperature.

Finally, for sequencing, we referred to the BGISEQ-500 protocol. We selected sequence control software Version 1.1.0.10003, sequence process Version 1.0.06 and Zebracall process Version 0.5.0.13875 (the base calling software, and a detailed description can be found in the next section) for sequencing. Sequencing was initiated after the sequencing reagents pre-loaded and sequencing chip installed, and this process was finished in ~72 hours.

Base calling and raw images

During sequencing, four channels of 16-bit grey scale images were captured by high resolution sCMOS with ~5.5 million pixels per image. About ~570K DNBs were loaded onto the grid-patterned arrays of spots which were photolithographically etched and surface modified on the sequencing chip. The spots were illuminated by the lasers with different wavelengths. Intensity from neighbor channel would also be observed due to crosstalk effect. The sequences of DNBs were base-called by the software Zebra call (base calling software developed for BGISEQ-500). After background subtraction and registration of images from 4 channels, intensities of DNBs were extracted according to a template of grid-pattern. Correction within channels and neighbor cycles was applied to increase the quality and stabilization. The cross-talk between intensities from the four fluorophores is caused by the imperfect wavelength filtering of optical filters isolating the bands of wavelength from the four types of fluorophore molecules. A regression technique can identify correlations in our intensity data and correct for them. In order to correct the crosstalk between 2 channels (C and G), the correction of the C background intensity can be found by linear regression after eliminating DNBs that do have true signal in the C channel. Such DNBs can be identified by searching for DNBs that have the C intensity as the maximum of the four intensities and to keep just DNBs that are not too dim or noisy we take only DNBs that have all non-maximum intensities smaller than 80% of the C intensity. This leaves us with reasonably well performing DNBs that most likely don’t have C at the currently interrogated position. After the linear regression of these background G intensities as a function of the C intensities. All of the G intensities can in turn be corrected for this cross-talk from the C channel by subtracting from them the expected background intensity produced by a given C intensity. Such regression can be done for each channel and simultaneously correcting for all of the correlations outlined above using a multiple linear regression. After all correction steps, the base with highest probability will be called according to the scale of intensities. When the whole sequencing was finished, the binary file with bases and quality score were converted into FASTQ format with Phred+33 quality score. In order to mapping the base call quality to Phred+33 score, a prior probability model was constructed by the scale of intensities from channels. Bases were separated to 10404 groups according to different parameters which may affect the confidence level of base calling. The base-calling error probabilities(P) of each group was calculated by the mismatch distribution from repeated sequencing of the standard reference genome. Quality scores (Q) were calculated by the definition of Phred+33 quality scores:

$$Q = -10\ log_{10}P$$

A huge table was constructed and hard coded into the base call program to look up a corresponding quality score by different parameters.

An example dataset of the images was included and the base calling process was illustrated in Figure 2.

Results

Sequencing data summary

The sequencing data was consisted of four lanes, with two of PE100 and the other two of PE50 (Table 1). First, we analyzed the sequencing quality by identifying the low quality reads. We determined low quality reads as reads which had more than 10% bases with sequencing quality lower than 10, and reads which had more than 1% Ns (ambiguous bases). In this way, we identified 11.9% (9.2% low quality reads and 2.7% ambiguous reads) low quality reads in PE100 data and 12.3% low quality raw reads in PE50 data (5.4% low quality reads and 6.9% ambiguous reads). In order for comparison, we selected similar amount of data (8 sequencing libraries and 16 lanes, PE150 reads, ~98.5 G bp data) from a public Illumina HiSeq2500 dataset of this cell line generated by GIAB (Genome in a Bottle)[8]. Using the same criteria for low quality identification, we identified 7.95% low quality reads (7.7% low quality reads and 0.25% ambiguous reads). Excluding those low quality reads, we then further analyzed the reads quality by plotting the distributions of base quality scores and GC content against those of the HiSeq2500 data (Figure 3). Thus we found higher proportion of low quality reads, more stable base quality distribution along the reads (Figure 3 a-b) and lower overall single base quality scores (Figure 3 c). And we observed some secondary peak in the GC content distribution of BGISEQ-500 data, indicating higher GC bias (Figure 3 d).

Variation calling and false positive/negative ratios estimation

In order to further depict the data quality and test applications of the new sequencing platform, we carried out variation calling using this dataset. We adapted the widely used pipeline (BWA[9] and GATK[10-12], an illustration of the pipeline and key parameters can be found in Figure 4a) for variation calling. We observed higher mapping rate, similar sequencing coverage and similar sequencing uniformity of the two BGISEQ-500 datasets comparing to the HiSeq2500 dataset (Table 2). The lower unique mapping rate probably reflected the shorter read length of the dataset (2×50 bp and 2×100 bp comparing to 2×150 bp). We also observed relatively higher duplication rate, mismatch rate in the dataset comparing to the HiSeq2500 data (Table 2).

In total, we identified ~3.4 million SNPs using the BGISEQ-500 datasets (3.45 million for PE50 data and 3.48 million for PE100 data), less than 3.6 million SNPs identified using HiSeq2500 data (Table 3). While for indels (insertion and deletions), we identified 842,058 from PE100 BGISEQ-500 data, comparing to 553,842 identified from PE50 BGISEQ-500 data. Using the HiSeq2500 data, we identified 733,797 indels. The SNPs identified using BGISEQ-500 datasets were similar to those identified from HiSeq2500 data in different features including dbSNP rate, proportion of SNPs in different regions related to genes and Ti/Tv (transition/transversion) ratio, which indirectly reflected the SNP accuracy. We also observed similar situation for indels.

Further to assess the accuracy of the variations, we used the high confident variations previously identified in NA12878 provided by GIAB (Genome in A Bottle)[13]. Using the methods provided by GIAB, we estimated the false positive rates and sensitivity for BGISEQ-500 PE50 and PE100 data comparing to those of HiSeq2500 data (Table 4).

The SNP sensitivity was lower for the BGISEQ-500 datasets (96.11% for PE100 and 94.15% for PE50) than HiSeq2500 data (97.13%). And the SNP false positive rate (FPR) was similar for the BGISEQ-500 PE100 data (0.00020%) comparing to HiSeq2500 data (0.00024%), and lower than the BGISEQ-500 PE50 data (0.0006%). For indels, BGISEQ-500 PE100 data resulted in better performance with higher sensitivity (94.3%) than the HiSeq2500 PE150 data with sensitivity of 92.4%. In contrast, HiSeq2500 PE150 data shows lower FPR (0.00046%) than BGISEQ-500 PE100 data (0.00069%). The BGISEQ-500 PE50 data resulted in sensitivity of 70.9% and FPR of 0.00067%. The difference performances of indel calling might also be caused by read length difference (50 or 100 bp comparing to 150 bp), in addition to sequencing quality, mapping accuracy, etc..

Furthermore, to depict variation calling accuracy in different genomic regions, we compared the false negative rate (FNR), FPR and sensitivity in different genome context given by GIAB (Table S1). For the coding sequences, data from the two platforms have similar FNR, FPR and sensitivity (3.85% *vs.* 2.52%, 0.00012% *vs.* 0.00015% and 96.15% *vs.* 97.48% accordingly). For the regions which are difficult to sequence, including some of the promotors [http://genomebiology.com/2013/14/5/R51], substantially high GC content (>55%) regions, substantially low GC content (<30%) regions, regions with multiple variations (more than 1 variations within 50 bp), regions with compound variations, repeats and segmental duplications, BGISEQ-500 data have higher FNR, lower sensitivity and lower FPR (Figure S1).

Discussion

Using the new sequencer, BGISEQ-500, we obtained one run of PE50 data and the other run of PE100 data. The raw data were ~135.5 Gbp and ~153.6 Gbp respectively, and they were generated from two chips (~72h). Thus the sequencing throughput and turnaround time were comparable to HiSeq2500 sequencer Rapid mode v1 (~80 Gbp per single flow cell and ~40 hours). Both the single base quality and read quality (reflected by duplication rate, mapping rate and unique mapping rate) were basically comparable to those of the HiSeq2500 data. Furthermore, the variation calling result was similar to that identified using similar amount of the HiSeq2500 data, further reflecting that the sequencer can be used in different researches and applications. With Future improvements over data quality, sequencing length, different and optimized insert sizes of the paired reads, as well as specially modified or designed software/bioinformatics tools, the performance can be further improved. In the meantime, quality of the whole genome sequencing data also reflected feasibility of applying this sequencing platform for other sequencing purposes including transcriptome, epigenome, metagenome etc.. From this first reference dataset of sequencing data from BGISEQ-500 sequencer, we provided an overview and some basic information for the new sequencing platform. This dataset can serve as reference for all the researches using the BGISEQ-500 sequencing platform. And we anticipated it to help stimulating the further technical improvement and development of novel tools for accurately analyzing the data.

Availability of supporting data

The BGISEQ-500 dataset (sequences) described in this article is available in the GigaDB repository [http://gigadb.org/dataset/100252], and the European Nucleotide Archive under accession number ERP017158. This GigaDB repository for this article (http://dx.doi.org/10.5524/100252) also contains examples of the raw image data including images of all the sequencing cycles in a small region and images of the first and last 10 cycles of the whole flowcell. Future data which are to be generated will also be updated in this GigaDB repository with versions indicated.

Abbreviations

DNBs: DNA nanoballs; SNPs: Single Nucleotide Polymorphisms; indels: insertions and deletions

Competing interests

JH, YX, SQ, XM, HC, TY, NS, ZY and SG are involved in the beta test of the BGISEQ-500 sequencer. X Liang, CG, YL, HL, HJ, X Liu and FM are involved in the BGISEQ-500 sequencer developing, library construction technology optimization, base calling software developing, or alpha and beta tests.

Authors' contributions

JH, ZY, FM and SG designed the project. YX, SQ and CG conducted sample preparation and sequencing library construction. HL, XM, HC, TY and NS conducted sequencing. X Liang, JR, JW, YL, X Liu, HJ, JR, JW, WZ, YC and SL conducted data analysis. X Liu, X Liang, YL, CG, HL, JH and HJ wrote the manuscript.

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Figure Legends

Figure 1. Flowchart of library construction and sequencing. The library construction includes fragmentation, size selection, end-repair and A-tailing, adaptor ligation and PCR amplification and splint circularization (**a**). The sequencing includes making DNBs, loading DNBs and sequencing (**b**).

Figure 2. Raw image data processing on the BGISEQ-500 platform. a. Registration of images from different channels. Relative coordinates will be calculated according to the pattern layout of DNBs. **b. Intensity correction between channels and cycles.** Correction of the optical and chemical interferences on different channels and the neighbor cycles was applied. **c. Connecting called bases to FASTQ.** Bases from all cycles will be collected and converted to FASTQ format. Phred score calculation and statistics will be applied during the conversion.

Figure 3. Quality control of the dataset after data filtering. Base-wise quality score distributions of the first read (**a**, from left to right, BGISEQ-500 PE50, BGISEQ-500 PE100 and HiSeq2500 PE150) and the second read (**b**, from left to right, BGISEQ-500 PE50, BGISEQ-500 PE100 and HiSeq2500 PE150)**.** For each position along the reads, the quality scores of all reads were used to calculate the mean, median and quantile values thus the box plot can be shown. The overall quality score distribution of BGISEQ-500 and HiSeq2500 data (**c**). GC content distribution of the BGISEQ-500 and HiSeq2500 data (**d**). FastQC [14] was used for the calculation.

Figure 4. Variation calling pipeline used for the assessment. The major steps included data filtering, alignment and variation calling, and the major parameters are also indicated.

Tables

Table 1. Summary of the dataset*.

Sequencing Type	Read ($\times 10^6$)	Bases (Gbp)	GC content	>Q20	>Q30
PE50	2,379	118.94	41.62%	96.00%	87.02%
PE100	1,159	115.88	41.28%	96.39%	87.13%

*This dataset was from two runs of the BGISEQ-500 sequencer (PE50 and PE100). '>Q20/Q30 percentage' indicates the percent of bases with quality score (-10×lg(error rate)) higher than 20 and 30 (indicating error rates of 1% and 1‰ respectively).

Table 2. Mapping statistics of the dataset*.

Metrics	BGISEQ-500 PE50	BGISEQ-500 PE100	HiSeq2500 PE150
Clean reads	2,378,725,921	1,136,008,901	708,941,148
Clean bases (bp)	118,936,296,050	113,600,890,100	104,923,289,904
Mapping rate	97.87%	99.22%	99.05%
Unique rate	93.17%	96.47%	97.06%
Duplicate rate	6.26%	2.47%	1.52%
Mismatch rate	0.34%	0.58%	0.56%
Average sequencing depth	37.57	37.44	34.52
Coverage	99.28%	99.12%	99.06%
Coverage at least 4×	98.90%	98.69%	98.60%
Coverage at least 10×	97.97%	97.81%	97.83%
Coverage at least 20×	95.78%	96.06%	94.81%

*The statistics shown here are calculated based on the clean reads (raw reads after filtering, the two platforms' data went through the same filtering process). Unique mapping rate indicates proportion of reads with unique alignment in the genome.

Table 3. Variation statistics of the dataset*.

	BGISEQ-500	**BGISEQ-500**	**HiSeq2500**
	PE50	**PE100**	**PE150**
SNPs	3,451,124	3,477,642	3,609,606
1000genome and dbSNP	3,242,083	3,288,653	3,347,441
1000genome specific	1,260	420	693
dbSNP specific	180,935	179,967	243,256
dbSNP rate	99.19%	99.74%	99.48%
Novel	26,846	8,602	18,216
Homozygous	1,426,328	1,433,490	1,472,063
Heterzygous	2,024,796	2,044,152	2,137,543
Synonymous	19,880	20,012	20,860
Ti/Tv	2.0462	2.065	2.0427
dbSNP Ti/Tv	2.0608	2.0693	2.0503
Novel Ti/Tv	0.8948	0.9775	1.0544
Indels	553,842	842,058	733,797
1000genome and dbSNP	260,157	320,741	314,161
1000genome specific	7,007	22,919	20,049
dbSNP specific	211,846	326,984	285,834
dbSNP rate	85.22%	76.92%	81.77%
Novel	74,832	171,414	113,753
Homozygous	206,163	295,492	300,013
Heterzygous	347,679	546,566	433,784

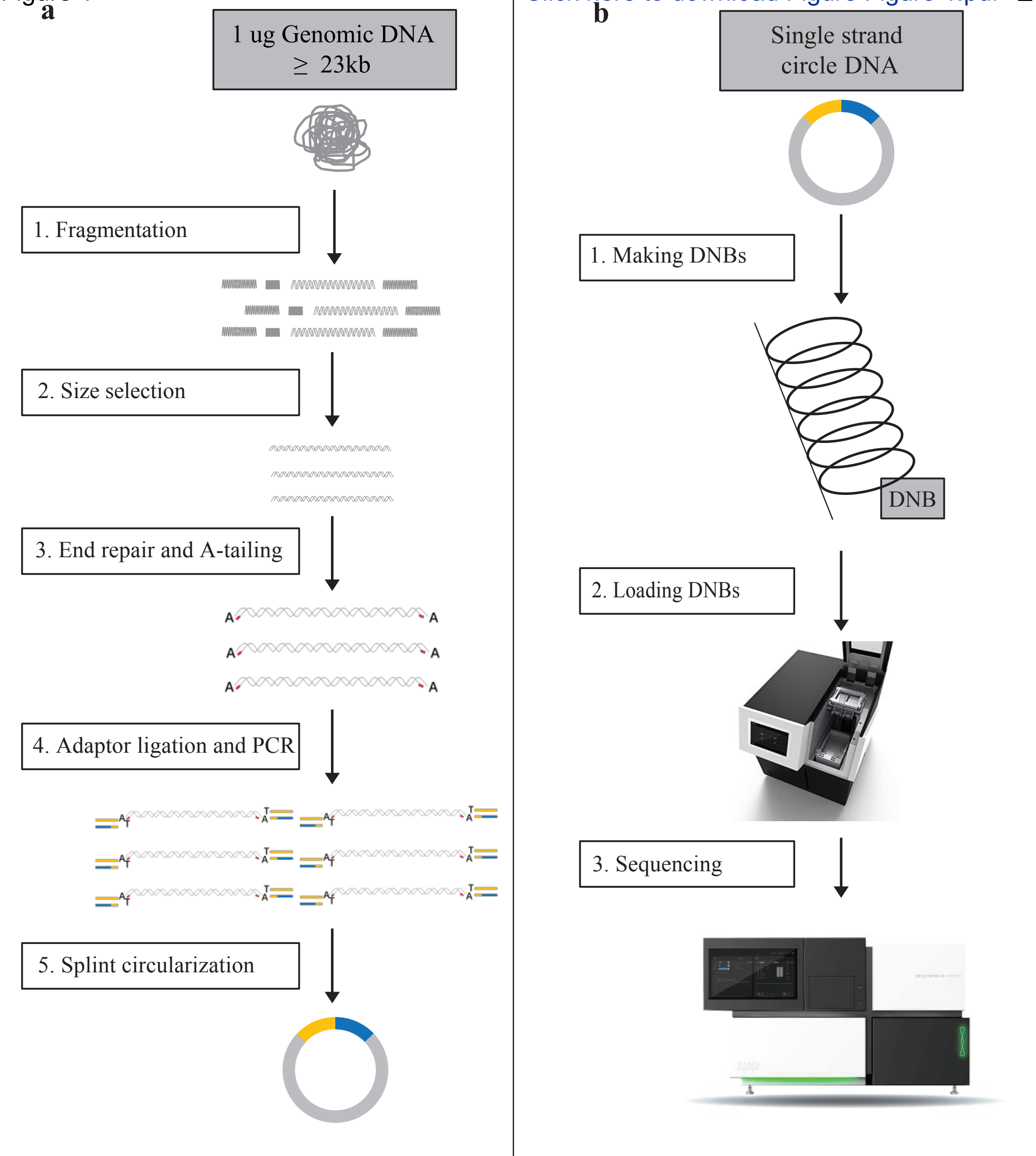
*1000genome and dbSNP equals the number of SNPs that are found in both 1000 genome and dbSNP databases (version 147 was used), 1000genome specific equals the number of SNPs that are only found in 1000 genomes database. dbSNP rate equals the number of SNPs found in dbSNP database/total detected SNPs. Novel SNP equals the number of SNPs that are not found in SNP database. Ti/Tv equals the ratio of SNP type are transition/SNP type are transversion.

Table 4. Performances of variation calling of dataset*.

		BGISEQ-500 PE50	**BGISEQ-500 PE100**	**HiSeq2500 PE150**
SNPs	True Positive	3,006,132	3,071,579	3,084,449
	False Positive	15,203	6,907	4,318
	False Negative	186,825	121,379	108,508
	Precision	99.50%	99.78%	99.86%
	Sensitivity	94.15%	96.20%	96.60%
	FPR	0.00060%	0.00020%	0.00017%
	FNR	5.85%	3.80%	3.40%
indels	True Positive	261,867	326,810	355,728
	False Positive	16,931	22,246	7,981
	False Negative	107,311	42,391	13,751
	Precision	93.93%	93.63%	97.81%
	Sensitivity	70.93%	88.52%	96.28%
	FPR	0.00067%	0.00069%	0.00032%
	FNR	29.7%	11.48%	3.72%

*True Positive (TP), False Positive (FP), False Negative (FN), precision and sensitivity were calculated using the software rtg-tools. TP is the number of SNPs that are found in high-confidence reference dataset, FP is the number of SNPs that are not found in reference dataset, FN is the number of SNPs that are found in high-confidence reference dataset but are not found in reference dataset. FPR is calculated using the formula of FP/(all high-confident region length-TP-FN), where all high-confident region length equals 252,9164,928bp that comes from GIAB released high confidence variants datasets (ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/NA12878_HG001/NISTv3.3/NA12878_GIAB_highconf_CG-IllFB-IllGATKHC-Ion-Solid-10X_CHROM1-X_v3.3_highconf.bed). FNR is calculated using the formula of FN/(FN+TP).

Figure 1


a
1 ug Genomic DNA
≥ 23kb
1. Fragmentation
2. Size selection
3. End repair and A-tailing
A
A
A
A
A
A
4. Adaptor ligation and PCR
T
A
5. Splint circularization
b
Single strand
circle DNA
1. Making DNBs
DNB
2. Loading DNBs
3. Sequencing

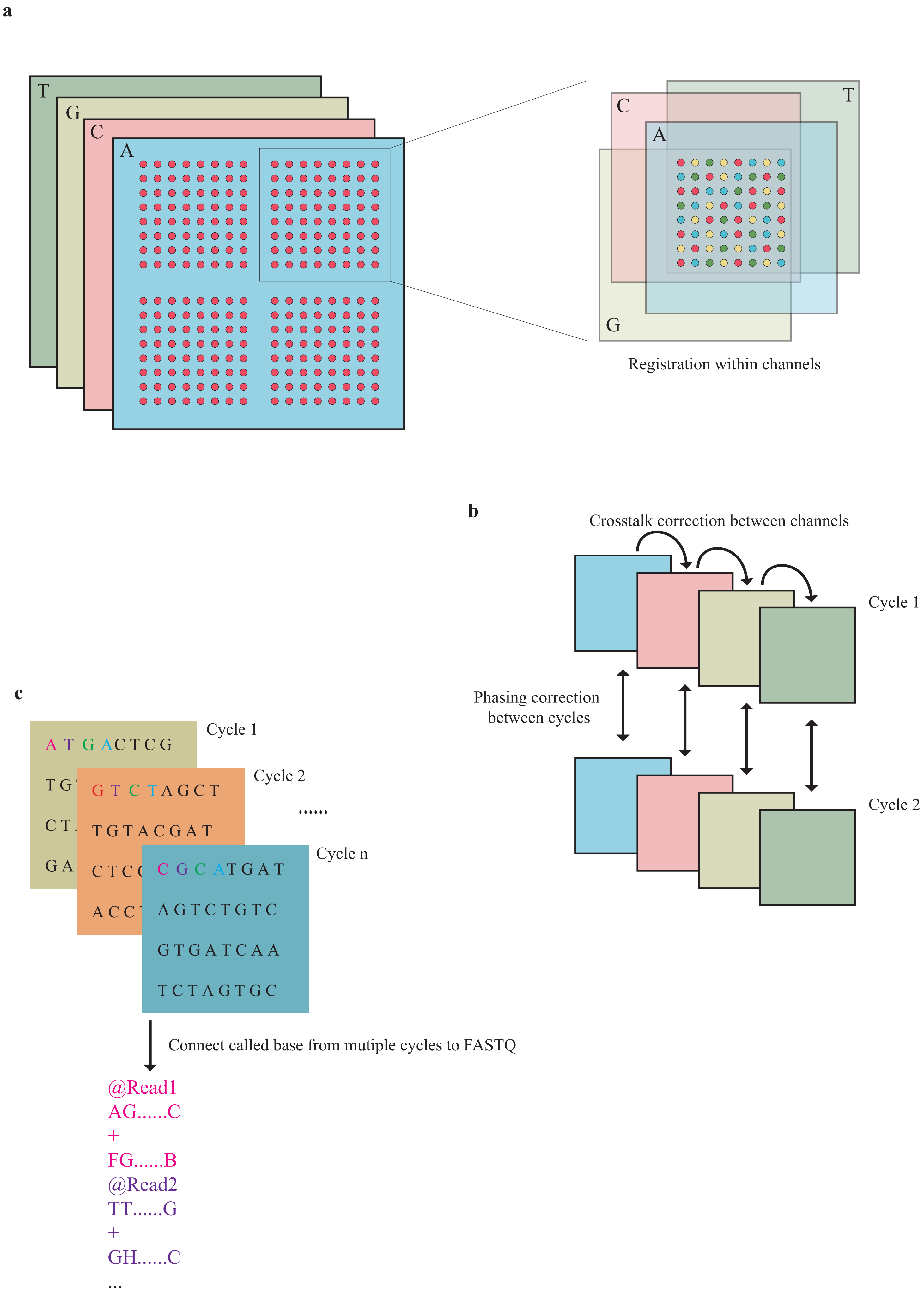

a
T
G
C
A
C
T
A
G
Registration within channels
b
Crosstalk correction between channels
Cycle 1
Phasing correction
between cycles
Cycle 2
c
Cycle 1
A T G A C T C G
T G
C T
G A
Cycle 2
G T C T A G C T
T G T A C G A T
C T C
A C C
......
Cycle n
C G C A T G A T
A G T C T G T C
G T G A T C A A
T C T A G T G C
Connect called base from mutiple cycles to FASTQ
@Read1
AG......C
+
FG......B
@Read2
TT......G
+
GH......C
...

Figure 3

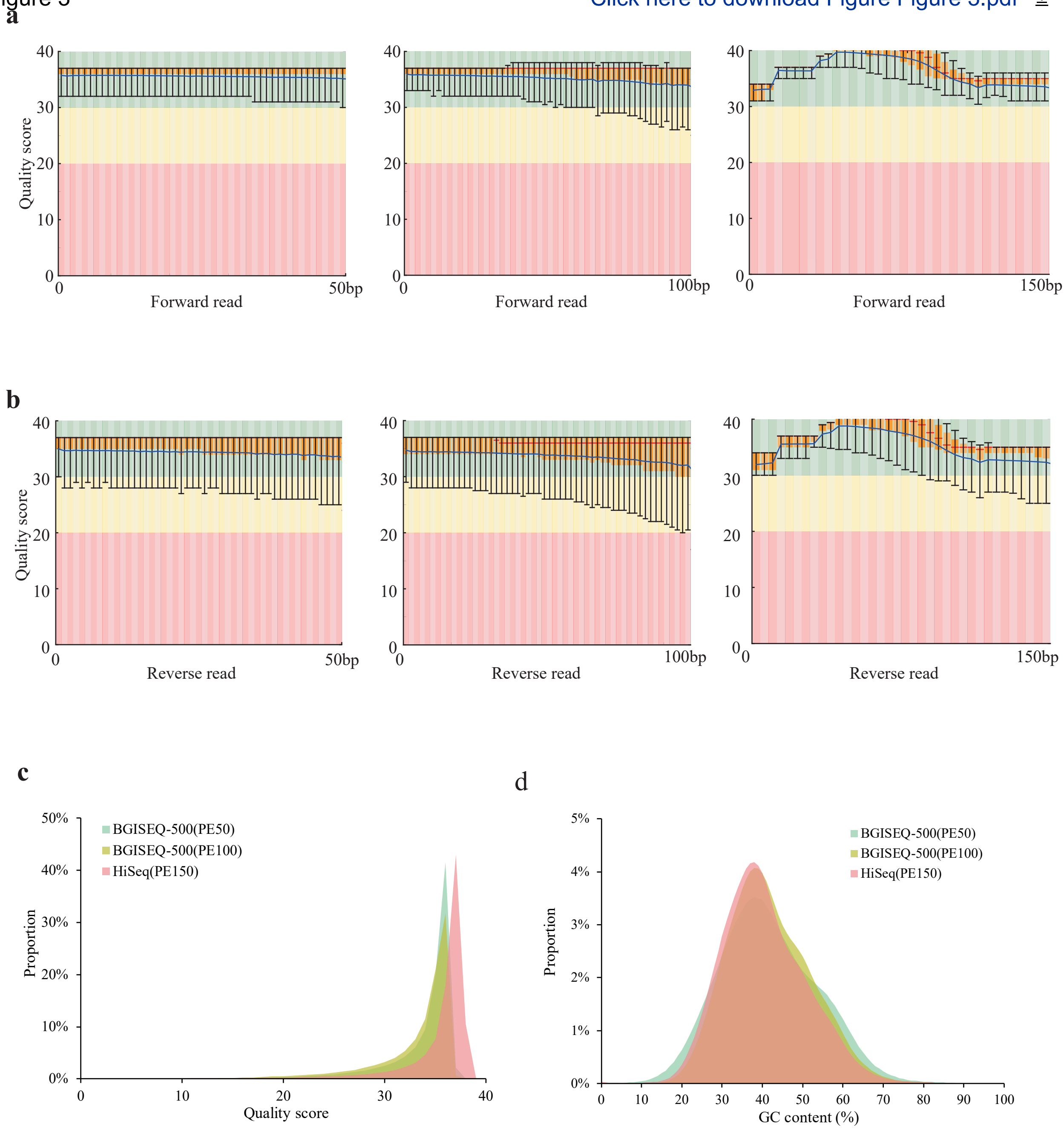
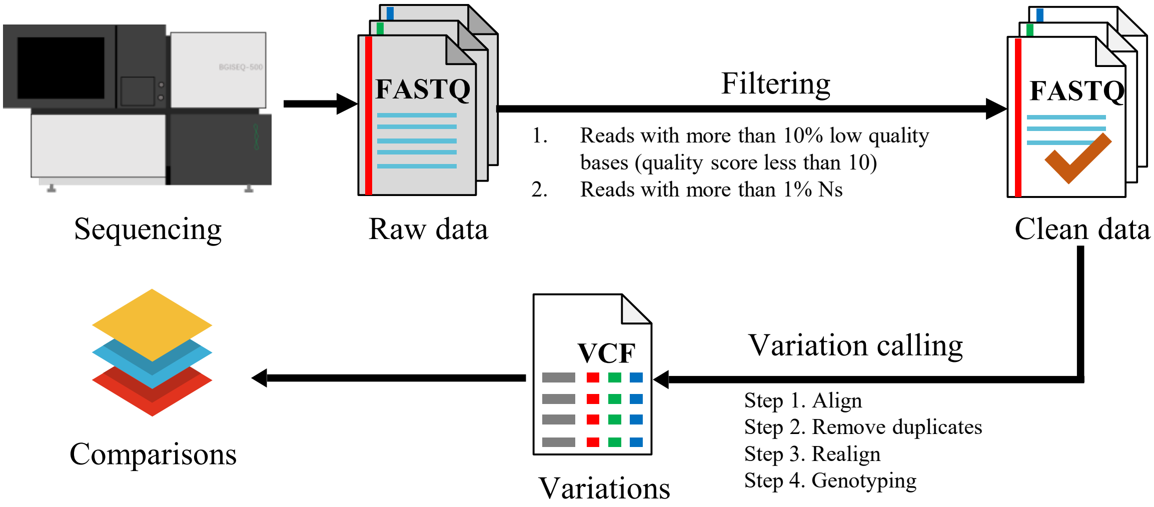

a
40
30
20
10
0
Quality score
0
50bp
Forward read
100bp
Forward read
150bp
Forward read
b
Quality score
50bp
Reverse read
100bp
Reverse read
150bp
Reverse read
c
50%
40%
30%
20%
10%
0%
Proportion
BGISEQ-500(PE50)
BGISEQ-500(PE100)
HiSeq(PE150)
0
10
20
30
40
Quality score
d
5%
4%
3%
2%
1%
0%
Proportion
BGISEQ-500(PE50)
BGISEQ-500(PE100)
HiSeq(PE150)
0
10
20
30
40
50
60
70
80
90
100
GC content (%)

Figure 4


Filtering
FASTQ
FASTQ
1. Reads with more than 10% low quality bases (quality score less than 10)
2. Reads with more than 1% Ns
Sequencing
Raw data
Clean data
VCF
Variation calling
Step 1. Align
Step 2. Remove duplicates
Step 3. Realign
Step 4. Genotyping
Comparisons
Variations

Rebuttal Tables and Figures

Point-to-point response letter with tables and figures

Dear Editor,

Thanks for considering our manuscript entitled *A reference human genome dataset of the BGISEQ-500 sequencer* for possible publication as a datanote in *GigaScience*. We have revised our manuscript according to the suggestions by the two reviewers, and we have addressed all the questions raised by the reviewers thus we are including a rebuttal letter with point-to-point response letter. Furthermore, as we have obtained the pair-end 100 bp (PE100) sequencing data from BGISEQ-500, we have also included the description and analysis of PE100 data here which would make it a unique and comprehensive reference dataset for BGISEQ-500. And sorry for the delay caused by adding the PE100 data. We have also uploaded the PE100 data to ENA as well as GigaDB to make it available for the public.

During the revision, and also for the data generation and analysis of the PE100 data, several people have made substantial contribution thus we would like to include them as authors for this manuscript, with author contributions indicated in the revised manuscript (in the Authors' Contributions section).

The revised manuscript has ~2,300 words in the main text (including the abstract), with four figures and four tables, and one supplementary file (with detailed parameters for the analysis as well as the accuracy assessment in different genome content).

Please let us know if further revision required, and we are looking forward to feedbacks from you and the reviewers. Thanks!

Xin Liu,
BGI Research