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Comparative analysis of seven short-read sequencing platforms using the Korean Reference Genome: MGI and Illumina sequencing benchmark for whole-genome sequencing

--Manuscript Draft--

Manuscript Number:	GIGA-D-20-00072R1
Full Title:	Comparative analysis of seven short-read sequencing platforms using the Korean Reference Genome: MGI and Illumina sequencing benchmark for whole-genome sequencing
Article Type:	Data Note
Funding Information:	Ulsan National Institute of Science and Technology (1.200047.01) Dr. Jong Hwa Bhak
Abstract:	Background: MGISEQ-T7 is a new whole-genome sequencer developed by Complete Genomics and MGI utilizing DNA nanoball and combinatorial probe anchor synthesis technologies to generate short reads at a very large scale – up to 60 human genomes per day. However, it has not been objectively and systematically compared against Illumina short-read sequencers. Findings: By using the same KOREF sample, the Korean Reference Genome, we have compared seven sequencing platforms including BGISEQ-500, MGISEQ-T7, HiSeq2000, HiSeq2500, HiSeq4000, HiSeqX10, and NovaSeq6000. We measured sequencing quality by comparing sequencing statistics (base quality, duplication rate, and random error rate), mapping statistics (mapping rate, depth distribution, and %GC coverage), and variant statistics (transition/transversion ratio, dbSNP annotation rate, and concordance rate with SNP genotyping chip) across the seven sequencing platforms. We found that MGI platforms showed a higher concordance rate for SNP genotyping than HiSeq2000 and HiSeq4000. The similarity matrix of variant calls confirmed that the two MGI platforms have the most similar characteristics to the HiSeq2500 platform. Conclusions: Overall, MGI and Illumina sequencing platforms showed comparable levels of sequencing quality, uniformity of coverage, %GC coverage, and variant accuracy, thus we conclude that the MGI platforms can be used for a wide range of genomics research fields at a lower cost than the Illumina platforms.
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Response to Reviewers:

Reviewer reports:

Reviewer #1: In this manuscript, Kim et al. compared seven sequencing platforms, including 2 MGI platforms (BGISEQ-500 and MGISEQ-T7) and 5 Illumina platforms (HiSeq2000, HiSeq2500, HiSeq4000, HiSeqX10, and NovaSeq6000), by using one human genome. The sequencing quality of different sequencing platform was assessed by basic sequencing statistics, mapping statistic and variant statistic. Overall the manuscript is suitable to be published on Giga Science after a major revision. There are several major issues with the work presented in the manuscript, as listed below:
=> Thank you for precise and critical feedback. We have modified the text and added further analysis to accommodate the reviewer's suggestions. (See below for point-by-point responses).

1. This work only contains samples from one human individual. It's really hard to reach a confident conclusion based on such a small sample size.
=> It is a generally correct point. However, both platforms produce massive amounts of sequences and the sample number would not affect the conclusion much as our study rely on how the two sets of platforms are similar or dissimilar in terms of variant calling.

This work still needs more samples and even replicates (both Cross-platform replicates and intra-platform replicates) to do further analysis, and provide confident evidence.
=> We think this is a practically important point. Unfortunately, we have not generated replicates for each sequencer. First, this study is based on years of sequencing history with one reference sample and each sequencing batch can contain multiple replicates or not. It is because each platform has a different amount of sequence output per run, it is impossible to produce a controlled amount of sequences in a certain common replicate number. We stated these limitations in the discussion part of the manuscript. The purpose of this benchmarking work was to compare two major platforms (MGI and Illumina).

2. The samples for sequencing were extracted on different points of time from the individual, that we wonder if the differences between mutation sets of seven sequencing platforms were caused by different sampling time and the bias of sampling process.
=> There must be some problems caused by the different sampling time and the sampling process mentioned by the reviewer. We used a Korean male sample and the difference between the first and the last sampling time is about 7 years. It is known that the human germline mutation rate is approximately 0.5×10^{-9} per base pair per year (Scally A, 2016. [10.1016/j.gde.2016.07.008]), which means that 10.5 germline mutations can be accumulated in 7 years. In this respect, although the mutation rate of DNA of leukocyte, a somatic cell, is expected to be higher than that of a germline cell, the number of mutations accumulated over the 7 years would be much lower than the difference between platforms. Therefore, we think that the different sampling time had no significant effect on the results.
For the case of sampling process bias, we stated in the discussion part of the manuscript that there is a clear limitation in the sampling process. Although there are some limitations as the reviewer mentioned, we think our study is still meaningful in that it provides the data generated by the short read-based whole genome sequencing platform, which is the most used in the field. We compared the long existing common Illumina platforms with the relatively new MGISEQ-T7 platform using one human whole genome sequence (WGS) data which has not been done before.

3. This manuscript needs to show more detail about the sequencing process, such as the number of the flow cell and sequencing cycle, the run time of the sequencing process, the amount of DNA each sequencing platform needs.

=> We added the detailed methods for DNA extraction, library preparation, and sequencing process in the Materials and Methods section.

4. In order to compare, the sequencing data of seven sequencing platforms need to have the same genome coverage.
=> Very good point. As pointed out by the reviewer, we set the same genome coverage of the seven platforms and updated all subsequent analyses after analyzing the whole data. Please see Figure S5 and Table S4.

5. The results of the manuscript let me worry about the quality of the sequencing data generated from Hiseq2000 and Hiseq4000. More samples or replicates were needed to prove these results that the author found were normal.
=> HiSeq2000 and HiSeq4000 platforms are old, and their quality is not good compared to other platforms in our case. Currently it is not possible to have more replicates as these machines are often not available in sequencing centers and, also, it is quite expensive to run them now. Still, to compare with MGI platforms, we decided to add as many Illumina platforms as possible.

6. According to the official information, MGI platforms have low duplicate rate than any sequencing platform which needs PCR. But this work showed MGISEQ T7 had highest duplicate rate, I suggest the authors prove their finding by using other samples or individuals.
=> The official information showed a duplicate rate of less than 3% when using a PCR free library kit. However, we used the FS library kit that included the PCR process. Therefore, it seems that the duplicate rate is higher than the manufacturer's official information. We provide the table presenting the mapping rates and duplicate rates of other human samples produced simultaneously with the KOREF sample. We found that the duplicate rates of the other human samples that were sequenced simultaneously with the KOREF sample were also high (see link below).

https://github.com/howmany2/SequencingPlatformComparison/raw/master/Mapping%20and%20duplicate%20rate%20of%20samples%20using%20PE100%20protocol%20and%20MGISEQ-T7.xlsx

An FS library kit containing PCR steps was used for MGISEQ-T7 sequencing of the KOREF sample. Furthermore, according to the sequencing vendor, the PE100 (Paired-end 100 bp) protocol has a high duplication rate, and the new PE150 (Paired-end 150 bp) protocol has a duplication rate less than 3%. We used the PE100 protocol for the KOREF sample and it can be a reason for why relatively many duplicated reads were found from the reads generated by the MGISEQ-T7 platform. However, we think the duplicate rate does not affect variant results much because it was analyzed after removing duplicate reads and matching to the same genome coverage for the seven sequencing platforms.

7. The methods for identifying the platform-specific covered region are unreasonable as different sequencing platforms had different coverage.
=> We agree with the reviewer's comment. We set the same genome coverage of the seven platforms and updated the result. As a result, the number of platform-specific covered regions of MGI platform decreased from 1,516 to 1,436, and in the case of Illumina, increased from 2,264 to 2,881. However, it was confirmed that the %GC ratio of the platform-specific covered region is the same as before meaning that the MGI platform covers a higher GC area (see Figure S10).

8. The Comparison of variants detected among seven platforms needs further analysis. Authors need a standard SNP and indel list of the Korean reference genome, which is verified by Sanger sequencing or other methods, to replace the dbSNP and SNP genotype chip as a compare object. What the relationship of FP, FN and the sequencing errors?
=> We agree with the reviewer's comment that it is a powerful tool to compare the variants to the gold standard variant set. However, to our knowledge, there is no gold standard variant set for the KOREF, which can give FP, FN, and sequencing error information, and, for this reason, we could not make a design for this study to conduct more precise and accurate comparison among the NGS platforms. As an alternative, we examined how much difference exists among the sequences generated by different

NGS platforms which are generally used methods for genome sequencing.

9. The introduction of this manuscript is too simple.
=> We added several sequencing platform comparative studies to the introduction section.

Minor revisions:
1. The coverages of BGISEQ-500 and HiseqX10 were not mentioned in the first section.
=> We added the coverages of BGISEQ-500 and HiSeqX10 in the first section.

2. Using the ratio of singletons may help you to bring out your findings more clearly.
=> We agree with the reviewer's comment. We examined the concordance rate of the singleton variants with SNP genotyping data to determine the accuracy of the singleton variants (see link below). However, it was difficult to obtain statistically significant results because there were very few overlapping positions between the singleton variants and the SNP chip data.

https://github.com/howmany2/SequencingPlatformComparison/raw/master/Comparison%20between%20singleton%20variant%20and%20SNP%20genotyping%20chip.xlsx

Reviewer #2: The submitted study has characterized sequencing quality, uniformity of coverage, %GC coverage, and variant accuracy of seven sequencing platforms. They found that MGI platforms showed a higher concordance rate of SNP genotyping than HiSeq series. The study is of interest to genomics and sequencing technologies areas. Two concerns must be addressed prior to acceptance.
=> Thank you for the feedback. We have modified the text and added further analysis to accommodate the reviewer’s suggestion. (See below point-by-point responses).

1)The author defined low-quality reads as those that had more than 30% of bases with a sequencing quality score lower than 20. I am wondering whether the results is stable once the definition changed?
=> As a supplementary analysis, we conducted an analysis without the filtering step to see how much the read filtering step affects in the result of this study. The supplementary analysis was conducted by matching the number of unfiltered reads with that of clean reads of prior analysis. The two tables below are the results of comparing the read mapping and variant statistics between the cases using clean (filtered) and unfiltered sequences (see link below).

https://github.com/howmany2/SequencingPlatformComparison/raw/master/Mapping%20rate%20and%20Variant%20statistics%20between%20clean%20reads%20and%20unfiltered%20reads.xlsx

As a result of using the unfiltered sequences, there was no notable difference in mapping and duplicate rates. The number of SNVs increased by 0.8% on average, and as the number of heterozygous SNVs increased, the hetero/homo ratio increased by 0.02 on average. Interestingly, the differences in total SNVs between clean and unfiltered reads in the two MGI platforms were less than that of the Illumina platforms. In the case of the Illumina platforms, on average, 44,000 additional SNVs were discovered when unfiltered reads were used compared to the case of the clean reads, while the increment in MGI platform was 800 SNVs on average when using unfiltered reads.

2) It looks the author ignored a highest duplicate ratio was found in MGISEQ-T7. More discussion and analysis should be performed to make this clear. The author claimed that duplicates and adapter contamination may be more affected by the process of sample preparation than by the sequencing instrument. However, again, no evidence was provided.
=> We agree with the reviewer’s concerns about the high duplicate ratio. We provide the table presenting the mapping rates and duplicate rates of other human samples produced simultaneously with the KOREF sample. We found that the duplicate rates of

other human samples that were sequenced simultaneously with the KOREF sample were also high (see Table below).
An FS library kit containing PCR steps was used for MGISEQ-T7 sequencing of the KOREF sample. Furthermore, according to the sequencing vendor, the PE100 (Paired-end 100 bp) protocol has a high duplication rate, and the new PE150 (Paired-end 150 bp) protocol reduces the duplication rate to less than 3%. We used the PE100 protocol for the KOREF sample sequencing and it can be a reason why relatively many duplicated reads were found from the reads generated by the MGISEQ-T7 platform. However, we think the duplicate rate does not affect variant calling results because it was analyzed after removing the duplicate reads and matching to the same genome coverage for the seven sequencing platforms (see link below).

https://github.com/howmany2/SequencingPlatformComparison/raw/master/Mapping%20and%20duplicate%20rate%20of%20samples%20using%20PE100%20protocol%20and%20MGISEQ-T7.xlsx

There are three main causes of duplicate reads generated by NGS technology.
1. Natural duplication
2. PCR duplicates (occur in library preparation step)
3. Optical duplicates (occur in sequencing step)
Natural duplications are not discussed in this section because it is difficult to distinguish them from PCR duplicates and optical duplicates. The following table showed the ratio of PCR duplication and optical duplication of the seven platforms (see link below).

https://github.com/howmany2/SequencingPlatformComparison/raw/master/Statistics%20of%20PCR%20duplicate%20and%20optical%20duplicate%20in%20seven%20sequencing%20platforms.xlsx

This result showed that PCR duplication occurs at least 2 times more than the optical duplication. (Unfortunately, the two MGI platforms were unable to calculate optical duplication.) This means that most duplication occurs during the library preparation rather than the sequencing steps.
The adapter contamination is caused by the sequencing of short DNA fragments that are shorter than the read length (Turner FS, 2014. 10.3389/fgene.2014.00005). For this reason, it can be expected that adapter contamination is mainly affected by the library preparation step, because size selection of DNA fragments is a part of the library preparation step; improper operation of size selection can introduce the shorter DNA fragments into the DNA library for sequencing.

Reviewer #3: The authors compare various short-insert, short-read whole-genome sequencing platforms used by academic researchers and clinical scientists.

My minor comments and suggestions are:

- As stated by the authors, Illumina platforms are indeed now considered 'historical.' However, many Illumina sequencers are still heavily used - in particular in pathology labs. This manuscript may prove very useful when arguing for an instrument upgrade in such a setting.

- You may like to comment on single tube long fragment read (stLFR), which enables the sequencing of long transcripts by sequencing bar-coded reads on the BGISEQ-500 platform [and, thus, probably also MGISEQ-T7) (10.1101/gr.245126.118). This technology is relatively cheap and is likely to decrease in cost - another argument for the adaption of MGI platforms in the laboratory.

- You may want to comment on Illumina library kits. It is possible that revisions [in the five-six years since the data in your study were generated] to these kits could improve the sequencing results (e.g., see 10.1371/journal.pone.0113501). I realize the effect may be minor, but it may nevertheless be useful to remind the reader about the potential for *slightly* better raw read statistics.

=> Thank you for your positive feedback and the suggestions. We added the idea suggested in your comments to the discussion part of the manuscript. (See Discussion

	section lines 209-210)
Additional Information:	
Question	Response
Are you submitting this manuscript to a special series or article collection?	No
Experimental design and statistics Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends. Have you included all the information requested in your manuscript?	Yes
Resources A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible. Have you included the information requested as detailed in our Minimum Standards Reporting Checklist?	Yes
Availability of data and materials All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the "Availability of Data and Materials" section of your manuscript.	Yes

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Comparative analysis of seven short-read sequencing platforms using the Korean Reference Genome: MGI and Illumina sequencing benchmark for whole-genome sequencing

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Abstract

Background: MGISEQ-T7 is a new whole-genome sequencer developed by Complete Genomics and MGI utilizing DNA nanoball and combinatorial probe anchor synthesis technologies to generate short reads at a very large scale – up to 60 human genomes per day. However, it has not been objectively and systematically compared against Illumina short-read sequencers. **Findings:** By using the same KOREF sample, the Korean Reference Genome, we have compared seven sequencing platforms including BGISEQ-500, MGISEQ-T7, HiSeq2000, HiSeq2500, HiSeq4000, HiSeqX10, and NovaSeq6000. We measured sequencing quality by comparing sequencing statistics (base quality, duplication rate, and random error rate), mapping statistics (mapping rate, depth distribution, and %GC coverage), and variant statistics (transition/transversion ratio, dbSNP annotation rate, and concordance rate with SNP genotyping chip) across the seven sequencing platforms. We found that MGI platforms showed a higher concordance rate for SNP genotyping than HiSeq2000 and HiSeq4000. The similarity matrix of variant calls confirmed that the two MGI platforms have the most similar characteristics to the HiSeq2500 platform. **Conclusions:** Overall, MGI and Illumina sequencing platforms showed comparable levels of sequencing quality, uniformity of coverage, %GC coverage, and variant accuracy, thus we conclude that the MGI platforms can be used for a wide range of genomics research fields at a lower cost than the Illumina platforms.

Keywords: MGISEQ-T7; whole-genome sequencing; sequencing platform comparison;

Introduction

Recently, due to the rapid technological advancement, the second- and third-generation sequencing platforms can produce a large amount of short- or long-read data at relatively low cost [1]. Depending on the application, these sequencers offer several distinct advantages. Short-read based second-generation sequencing can be used to efficiently and accurately identify genomic variations. Long-read based third-generation sequencing can be used to identify structural variations and build high quality *de novo* genome assemblies [2]. Short-read sequencing technologies are routinely used in large-scale population analyses and molecular diagnostic applications because of the low cost and high accuracy [3]. The recent platforms from Illumina are the HiSeqX10 and NovaSeq6000 short-read sequencers. A competing sequencer developed by Complete Genomics and MGI Tech is the MGISEQ-T7 (also known as DNBSEQ-T7). MGISEQ-T7 is a new sequencing platform after BGISEQ-500 that uses DNA nanoball and combinatorial probe anchor synthesis to generate short reads at a very large scale [4].

Recently, a paper was published showing similar accuracy of SNP detection for the BGISEQ-500 platform compared to the HiSeq2500 [5]. The quality of the data generated by BGISEQ-500 was shown to be of high quality. However, some of its characteristics showed lower quality compared to Illumina HiSeq2500. In addition, the comparison results for DNA, RNA, and metagenome sequencing of the Illumina and the MGI platforms have been reported [6-8]. Also, coronavirus analysis studies using an MGI platform have been reported in 2020 [9, 10]. Still, no study has compared Illumina platforms with MGISEQ-T7 for whole-genome sequencing (WGS). In the present study, we compared seven short-read based sequencers; two MGI platforms (BGISEQ-500 and MGISEQ-T7) and five Illumina platforms (HiSeq2000, HiSeq2500, HiSeq4000, HiSeqX10, and NovaSeq6000) (Table 1). We focused on how similar the two sets of platforms are rather than the accuracy of each sequencer, by comparing variants, platform-specific covered regions as well as the concordance rate to SNP genotyping chip.

Results

Sequencing data summary

We analyzed and benchmarked the whole-genome sequencing data quality generated by the seven sequencers using the KOREF (the Korean Reference Genome) [11] DNA. Due to the sequential release and distribution of the sequencers, KOREF sequencing has been carried out in nine years since 2010. Therefore, the blood samples, library construction, and sequencing conditions were not the same, although all the sample were from one individual. The Illumina platform data used here were from 2012 to 2019, while the MGI platform data were from 2017 and 2019. Also, the read length differs depending on the platform. The Illumina HiSeq2000 had the shortest read length of 90 bp paired-end (PE) and the HiSeq4000, HiSeqX10, and

NovaSeq6000 had 151 bp PE. The read length of the HiSeq2500 is 101 bp PE and that of the BGISEQ-500 and MGISEQ-T7 is 100 bp PE. Also, there is a difference in the amount of data as well. Thus, we randomly selected 35× coverage sequencing data for HiSeq2500 and NovaSeq6000 which have that much sequencing data matching to that of BGISEQ-500 and HiSeqX10. HiSeq2000, HiSeq4000, and MGISEQ-T7 had roughly 30× coverage.

Assessment of base quality and sequencing error in raw reads

Base quality is an important factor in evaluating the performance of sequencing platforms. We analyzed the sequencing quality by identifying low-quality reads. First, we investigated the base quality distribution of raw reads with the FastQC (FastQC, RRID:SCR_014583) [12]. All seven sequencing platforms showed that the quality of each nucleotide gradually decreased towards the end of a read (Fig. S1). The quality value of the HiSeq4000 and HiSeqX10 reads showed a tendency to decrease rapidly towards the end of the read. We defined low-quality reads as those that had more than 30% of bases with a sequencing quality score lower than 20. The fraction of low-quality reads ranged from 2.8% to 18.3% across the seven sequencing platforms (Fig. S2 and Table S1). Based on the filtering criteria, the newest platforms, NovaSeq6000 and MGISEQ-T7, showed the lowest percentage of low-quality reads (2.8% and 4.2%, respectively).

We analyzed the frequency of random sequencing errors (ambiguous base, N), which is also an important factor to evaluate the quality of the sequencing platform. We found that the HiSeq2000, HiSeq4000, and HiSeqX10 showed a high random error ratio in certain sequencing cycles (Fig. S3 and Table S2). Furthermore, in the case of HiSeq2000, the random error tended to increase gradually after each sequencing cycle. We also investigated the sequencing error using *K*-mer analysis. Most erroneous *K*-mers caused by sequencing error appeared at very low frequency and form a sharp left-side peak [13, 14]. Distribution of *K*-mer frequencies showed similar distributions between the platforms (Fig. 1). However, there was a difference in the proportion of low-frequency *K*-mer (≤ 3 *K*-mer depth), which was considered as putative sequencing errors (Table S3). The NovaSeq6000 showed the lowest amount of erroneous *K*-mer (3.91%), while the HiSeq4000 contained the highest amount of erroneous K-mer (13.91%) among the seven sequencing platforms. The BGISEQ-500 and MGISEQ-T7 showed a moderate level of erroneous *K*-mer (7.72% and 6.39%, respectively).

We examined the duplication rate and adapter contamination in the seven sequencing platforms (Table S2). We examined the exact duplicates, which are identical sequence copies, from raw sequence data. The HiSeq2000 and MGISEQ-T7 showed the highest duplicate ratio (8.71% in HiSeq2000 and 3.04% in MGISEQ-T7). The HiSeq4000, HiSeqX10 and NovaSeq6000 showed higher adapter contamination rates than other platforms, probably due to longer sequence length (151 bp). However, duplicates and adapter contamination may be more affected by the process of sample preparation than by the sequencing instrument.

Genome coverage and sequencing uniformity

In order to assess genomic coverage and sequencing uniformity, we aligned quality-filtered reads to the human reference genome (GRCh38). All seven sequencing platforms showed a mapping rate of more than 99.98% and genome coverage of more than 99.6% (≥ 1×; Table 2). We observed a higher duplicate mapping rate in the HiSeq2000 (15.35%) and MGISEQ-T7 (8.77%) than the other platforms and the same pattern as the duplication rates of raw reads (see Table S2). The insert-size for paired-end libraries corresponds to the targeted fragment size for each platform (Fig. S4). It has been reported that the depth of coverage is often far from evenly distributed across the sequenced genome [15]. To assess the sequencing uniformity, we analyzed the distribution of mapping depth for all chromosomes (Fig. S5). All seven platforms showed a similar pattern of depth distribution, but interestingly, we found that the depth near the centromere regions was lower exclusively in the HiSeq4000 (Figs. S6-S9). We speculate that this may have been due to a bias in the library preparation step on the HiSeq4000 platform.

In order to examine the platform-specific covered region of MGI and Illumina platforms, we defined a platform-specific covered region that had significantly different depths (five times difference with an average depth between MGI and Illumina platforms) based on the 100 bp non-overlapping windows. Prior to examining the platform-specific covered regions, mapped reads were down-sampled for all platforms to 24x coverage, which is the minimum coverage among the platforms, for a fair comparison. (Table S4). We found 144 Kb and 288 Kb of the platform-specific covered regions from MGI and Illumina platforms, respectively (Table S5). A total of 172 and 854 genes were overlapped in MGI and Illumina specific covered regions, respectively, and most of them were intronic. Interestingly, however, the platform-specific covered regions showed a significantly different distribution of GC ratios between the MGI and Illumina platforms (Fig. S10). The MGI platforms tend to cover regions relatively high in GC content (Wilcoxon rank-sum test, P = 7.92 × 10^{-187}). Nevertheless, it is obvious that platform-specific covered regions for Illumina platforms are slightly longer than those of the MGI platforms, and these regions were not sufficiently covered by the MGI platforms.

Biases in PCR amplification create uneven genomic representation in classical Illumina libraries [16, 17] as PCR is sensitive to extreme GC-content variation [18]. Thus, we analyzed the GC biases for seven sequencing platforms. We examined the distribution of GC content in sequencing reads and found that raw reads of all the seven sequencing platforms showed a similar GC content distribution to the human reference genome (Fig. S11). To better understand what parts of the genome were not covered properly, we generated GC-bias plots, showing relative coverage at each GC level. Unbiased sequencing would not be affected by GC composition, resulting in a flat line along with relative coverage = 1. We found that all the seven sequencing platforms provided nearly even coverage in the moderate-GC range 20% to 60%, which represents approximately 95% of the human genome (Fig. 2). On the other hand, the relative coverage of the HiSeq2000 platform dropped fast above 60% GC than other platforms, while the NovaSeq6000 covered well above 60% GC, unlike the other platforms.

Comparison of variants detected among seven sequencing platforms

To investigate the performance of variant calling for the seven sequencing sequencers, we adopted the widely used pipeline BWA-MEM (BWA, RRID:SCR_010910) [19] and GATK (GATK, RRID:SCR_001876) [20-22]. We identified an average of 4.14 million single nucleotide variants (SNVs), and 0.61 million indels (insertion and deletion) on each of the seven sequencing platforms (Table 3). The statistics of SNVs were similar across all the seven in terms of the dbSNP annotation rate (dbSNP153) and the transition/transversion (Ti/Tv) ratio, which indirectly reflects SNV calling accuracy. About 3.7 million SNV loci were found on all the seven sequencing platforms, and this accounts for 87% to 91% of the discovered SNVs on each platform (Table S6). We found 13,999 and 9,691 platform-specific SNVs on the MGI and Illumina platforms, respectively. Interestingly, the number of singletons, variations found only in one platform, was higher for the Illumina (~0.10 million SNVs on average) than MGI (~0.05 million SNVs on average; Table S7) sequencers. This means that the difference within the Illumina platforms is greater than the difference between the MGI platforms. We also analyzed the number of SNVs found in any six of the seven sequencing platforms, which we considered false negatives. The HiSeq2000 had the largest number of false negatives (64,856 SNVs) among the seven sequencing platforms. The two MGI platforms (MGISEQ-T7 and BGISEQ-500) had 18,826 and 15,657 false negatives, respectively, and those of the NovaSeq6000 showed the smallest number of false negatives (6,999 SNVs). To investigate the relationship between the sequencing platforms, an unrooted tree was constructed using a total of 1,036,417 loci where the genotypes of one or more platforms differ from the rest of the platforms (Fig. 3 and Table S8). We found that the two MGI platforms grouped together, and they are the closest to the Illumina HiSeq2500 platform. The Illumina platforms were divided into two subgroups in the tree: a long read length (151 bp) group, containing the HiSeq4000, HiSeqX10, and NovaSeq6000 platforms and a short read length (≤101 bp) group, containing the HiSeq2000 and HiSeq2500 platforms. Read length primarily affects the detection of variants through alignment bias and alignment errors, which are higher for short reads because there is less chance of a unique alignment to the reference sequence than with longer reads [23].

Since it was not possible to conduct standard benchmarking procedures and determine error values for each platform in this study, we compared the variations called by the seven whole-genome sequences with an SNP genotyping chip as an independent platform. Of the total 950,585 comparable positions, more than 99.3% of the genotypes matched the WGS-based genotypes from the seven sequencing platforms (Table S9). We found that 4,356 loci in the SNP genotyping were inconsistent across all seven WGS-based genotyping results, suggesting that these loci are probably errors in the SNP genotyping chip. With the exception of HiSeq2000 and HiSeq4000, all the other platforms showed a similar concordance rate.

Discussion

Our benchmark can provide a useful but rough estimation of the quality of short-read based whole-genome sequencers. We used the same individual's samples for all seven sequencing platforms but collected at different time points in the past nine years. Just one human sample cannot justify the variation that may occur among different individuals, extracted DNA molecules, and overall sequencing qualities. Furthermore, the sequencing quality may vary much depending on the version of the library preparation kit even, on the same platform [24]. These are clear limitations in our benchmarking, however, as our purpose was to compare two major platforms, namely Illumina and MGI, still, just one person sample can function as an intuitive index for researchers who consider purchasing large sequencers to generate a very large amount of data. Our method of statistical analysis does not allow us to conclude which of the seven sequencing instruments is the most accurate and precise as there is much variation in the sample preparation and sequencer specifications. Nevertheless, overall, the data generated by the Illumina and MGI sequencing platforms showed comparable levels of quality, sequencing uniformity, %GC coverage, and concordance rate with SNP genotyping, thus it can be broadly concluded that the MGI platforms can be used for a wide range of research tasks on a par with Illumina platforms at a lower cost [7].

Materials and Methods

Genomic DNA extraction and SNP genotyping

Genomic DNA used for genotyping and sequencing were extracted from the peripheral blood of a Korean male sample donor (KOREF). The genomic DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. DNA quality was assessed by running 1 μl on the Bioanalyzer system (Agilent) to ensure size and analysis of DNA fragments. The concentration of DNA was assessed using the dsDNA BR assay on a Qubit fluorometer (Thermo Fisher). We conducted a genotyping experiment using the Illumina Infinium Omni1 quad chip according to the manufacturer's protocols. The Institutional Review Board (IRB) at Ulsan National Institute of Science and Technology approved the study (UNISTIRB-15-19-A).

Illumina paired-end library construction and sequencing

High-molecular weight genomic DNA was sheared using a Covaris S2 ultra sonicator system, in order to get appropriate sizes. Libraries with short inserts of 500 bp for HiSeq2000, 400 bp for HiSeq2500 and HiSeq4000, and 450 bp for HiSeqX10 and NovaSeq6000 for paired-end reads were prepared using TruSeq DNA sample prep kit following the manufacturer's protocol. Products were quantified using the Bioanalyzer (Agilent, Santa Clara, CA, USA) and the raw data were generated by each Illumina platform. Further image analysis and base calling were conducted with the Illumina pipeline using default settings.

MGI paired-end library construction and sequencing

The KOREF genomic DNA was fragmented by Frag enzyme (MGI) to DNA fragments between 100 bp and ~1,000 bp suitable for PE100 sequencing according to the manufacturer's instructions (MGI FS DNA library prep set, cat no; 1000005256). The fragmented DNA was further selected to be between 300 bp and ~500 bp by DNA clean beads (MGI). The selected DNA fragments were then repaired to obtain a blunt end and modified at the 3'end to get a dATP as a sticky end. The dTTP tailed adapter sequence was ligated to both ends of the DNA fragments. The ligation product was then amplified for seven cycles and subjected to the following single-strand circularization process. The PCR product was heat-denatured together with a special molecule that was reverse-complemented to one special strand of the PCR product, and the single-strand molecule was ligated using DNA ligase. The remaining linear molecule was digested with the exonuclease, finally obtaining a single-strand circular DNA library. We sequenced the DNA library using BGISEQ-500 and MGISEQ-T7 with a pair-end read length of 100bp.

Raw data preprocessing

We used the FastQC v0.11.8 [12] to assess overall sequencing quality for MGI and Illumina sequencing platforms. PCR duplications (reads were considered duplicates when forward read and reverse read of the two paired-end reads were identical) were detected by the PRINSEQ v0.20.4 (PRINSEQ, RRID:SCR_005454) [25]. The random sequencing error rate was calculated by measuring the occurrence of 'N' bases at each read position in raw reads. Reads with sequencing adapter contamination were examined according to the manufacturer's adapter sequences (Illumina sequencing adapter left = "*GATCGGAAGAGCACACGTCTGAACTCCAGTCAC*", Illumina sequencing adapter right =

"*GATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT*", MGI sequencing adapter left = “*AAGTCGGAGGCCAAGCGGTCTTAGGAAGACAA*”, and MGI sequencing adapter right = “*AAGTCGGATCGTAGCCATGTCGTTCTGTGAGCCAAGGAGTTG*”). We conducted base quality filtration of raw reads using the NGS QC Toolkit v2.3.3 (cutoff read length for high quality 70; cutoff quality score, 20) (NGS QC Toolkit, RRID:SCR_005461) [26]. We used clean reads after removing low-quality reads and adapter containing reads for the mapping step.

Mapping, variant calling, and coverage calculation

After the filtering step, clean reads were aligned to the human reference genome (GRCh38) using BWA-MEM v0.7.12, and duplicate reads were removed using Picard v2.6.0 (Picard, RRID:SCR_006525) [27]. After removing duplicate reads, we down-sampled the deduplicated clean reads of all the sequencing platforms to 24× coverage according to the amount of the deduplicated clean reads of HiSeq2000 for a fair comparison. Realignment and base score recalibration of the bam file was processed by GATK v3.3. Single nucleotide variants, short insertions, and deletions were called with the GATK (Unifiedgenotyper, options --output_mode EMIT_ALL_SITES --genotype_likelihoods_model BOTH). The resulting variants were annotated with the dbSNP (v153) database [28]. Coverage was calculated for each nucleotide using SAMtools v1.9 (SAMTOOLS, RRID:SCR_002105) [29]. We defined a specific covered region based on the 100 bp non-overlapping windows by calculating the average depth of the windows. We used more than five times the difference with an average depth in each window between MGI and Illumina platforms. GC coverage for raw reads and the genome was calculated by the average %GC of the 100bp non-overlapping windows.

Variant comparison and concordance rate with SNP genotyping

The chromosome position and genotype of each variant called from each sequencing platform was used to identify the relationship between seven sequencing platforms. We compared 1,036,417 loci found on one or more platforms for locations where genotypes were determined on all the seven platforms. An unrooted tree was generated using FastTree v2.1.10 (FastTree, RRID:SCR_015501) [30] with the generalized time-reversible (GTR) model. For calculating the concordance rate between SNP genotyping and WGS-based genotype, the coordinates of SNP genotyping data were converted to GRCh38 assembly using the UCSC LiftOver tool [31]. We removed unmapped positions and indel markers and used only markers that were present on the autosomal chromosomes.

Availability of Supporting Data and Materials

All sequences generated in this study, including the HiSeq2000, HiSeq2500, HiSeq4000, HiSeqX10, NovaSeq6000, BGISEQ-500, and MGISEQ-T7 sequencing reads, were deposited in the NCBI Sequence Read Archive database under BioProject PRJNA600063. All data will be hosted and distributed from http://biosequencer.org.

Additional Files

Additional file 1: **Figure S1**. Distribution of nucleotide quality across seven sequencing platforms. **Figure S2**. Base quality filtration statistics for seven sequencing platforms. **Figure S3**. Random error ratio for seven sequencing platforms. **Figure S4**. Insert-size distributions for seven sequencing platforms. **Figure S5**. The coverage distribution of two MGI and five Illumina platforms. **Figure S6**. Depth distribution of chromosome 8. **Figure S7**. Depth distribution of chromosome 12. **Figure S8**. Depth distribution of chromosome 18. **Figure S9**. Depth distribution of chromosome 20. **Figure S10**. GC distribution of platform-specific covered regions. **Figure S11**. The GC composition distribution of the human genome and sequencing reads. **Table S1**. Base quality summary. **Table S2**. Duplicate reads, random error base, and adapter read rate. **Table S3**. The putatively erroneous *K*-mers (≤ 3 *K*-mer depth) for seven sequencing platforms. **Table S4**. Statistics of clean reads for seven sequencing platforms. **Table S5**. Statistics for platform-specific covered regions. **Table S6**. The number of shared SNVs for seven sequencing platforms. **Table S7**. The number of SNVs that were singleton or not found in a specific platform. **Table S8**. Genotype concordance rate among seven sequencing platforms. **Table S9**. Genotype comparison between SNP genotyping and WGS.

List of abbreviations

PE: paired-end;

WGS: whole-genome sequencing;

BWA: burrows-wheeler aligner;

SNVs: single nucleotide variants;

indels: insertions and deletions;

Ti/Tv: transition/transversion;

GATK: Genome Analysis ToolKit;

Competing Interests

H.M.K., O.C., Y.S.C., J.H.J., H.Y.L., and Y.Y. are employees, J.B. is the chief executive officer of Clinomics Inc. H.M.K., Y.S.C., and J.B. have an equity interest in the company. All other co-authors declare that they have no competing interests.

Funding

This work was supported by the Research Project Funded by the Ulsan City Research Fund (1.200047.01) of the Ulsan National Institute of Science & Technology (UNIST) and Clinomics and Geromics Ltd internal funding.

Authors' contributions

J.B. supervised and coordinated the project. J.B. and Y.S.C. conceived and designed the experiments. H.M.K., S.J., O.C., J.H.J., H.Y.L., and Y.Y. conducted the bioinformatics data processing and analyses. H.M.K., S.J., D.M.B., and J.B. wrote and revised the manuscript. A.B. and H.S.K. reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

We thank the Korea Institute of Science and Technology Information (KISTI) for providing us with the Korea Research Environment Open NETwork (KREONET). We thank Jaesu Bhak for editing.

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Figures

Figure 1. Distribution of *K*-mer frequency for 21-mers using raw reads from seven sequencing platforms. The x-axis represents *K*-mer depth, and the y-axis represents the proportion of *K*-mer, as calculated by the frequency at that depth divided by the total frequency at all depths.

Figure 2. GC-bias plots for seven sequencing platforms. Unbiased coverage is represented by a horizontal dashed line at relative coverage = 1. A relative coverage below 1 indicates lower than expected coverage and above 1 indicates higher than expected coverage.

Figure 3. An unrooted tree for seven sequencing platforms showing the similarity of the variant calling. Numbers of nodes denote bootstrap values based on 1,000 replicates.

Tables

Table 1. Raw read statistics for seven sequencing platforms

	Illumina platforms					MGI platforms	
Metrics	HiSeq2000	HiSeq2500	HiSeq4000	HiSeqX10	NovaSeq6000	BGISEQ-500	MGISEQ-T7
Production date	2012	2015.03	2015.10	2015.12	2019.04	2017.04	2019.09
Quality range	Illumina 1.5+	Illumina 1.8+	Illumina 1.8+	Illumina 1.8+	Illumina 1.8+	Illumina 1.8+	Illumina 1.8+
# of Total read	1,044M	1,500M	629M	833M	833M	1,171M	1,035M
Read length (bp)	90 PE	101 PE	151 PE	151 PE	151 PE	100 PE	100 PE
Total bases	94 Gb	151.5 Gb	95 Gb	125.8 Gb	125.8 Gb	117.1 Gb	103.4 Gb
Sequencing depth (×, based on 3 Gb)	31.31	50.52	31.65	41.94	41.94	39.04	34.49

Table 2. Mapping and coverage statistics

Metrics	HiSeq2000	HiSeq2500	HiSeq4000	HiSeqX10	NovaSeq6000	BGISEQ-500	MGISEQ-T7
# of clean reads	935,951,974	1,050,028,628	512,891,970	705,987,420	706,000,000	1,060,837,856	991,021,996
Read length	90	101	151	151	151	100	100
Clean bases (Gb)	84.23	106.05	77.45	106.60	106.6	106.08	99.1
Clean read depth (based on 3 Gb, ×)	28.08	35.35	25.82	35.53	35.54	35.36	33.03
Mapping rate	99.986%	99.999%	99.990%	99.999%	99.9996%	99.983%	99.999%
Properly mapped rate*	96.67%	98.30%	97.24%	96.91%	97.15%	97.44%	98.17%
Duplicate rate	15.35%	3.01%	3.19%	5.08%	3.39%	2.56%	8.77%
Duplicate clean read depth (×)	23.90	34.29	24.99	33.73	34.33	34.46	30.14
Down-sampled depth (×)	23.90	23.90	23.90	23.90	23.90	23.90	23.90
Coverage	99.68%	99.82%	99.71%	99.81%	99.76%	99.83%	99.83%
Coverage at least 5×	98.62%	99.30%	98.37%	99.30%	99.19%	99.34%	99.24%
Coverage at least 10×	94.63%	96.65%	93.98%	97.05%	96.89%	97.05%	96.61%
Coverage at least 15×	85.10%	88.54%	85.08%	90.23%	90.36%	90.11%	89.36%

* Both of the read mates are in the correct orientation.

Table 3. Variant statistics of Illumina and MGI sequencing platforms.

Metrics		HiSeq2000	HiSeq2500	HiSeq4000	HiSeqX10	NovaSeq6000	BGISEQ-500	MGISEQ-T7
Reference homozygous		2,839,358,003	2,855,619,759	2,855,062,233	2,864,272,103	2,861,198,782	2,851,898,568	2,853,066,635
# of no call positions		80,241,142	63,980,549	64,532,078	55,244,498	58,311,103	67,747,107	66,584,361
No call rate		2.74%	2.19%	2.21%	1.89%	1.99%	2.32%	2.28%
SNVs	Total SNVs	4,133,925	4,132,468	4,138,296	4,216,589	4,223,612	4,088,645	4,082,103
	Total SNVs in dbSNP	4,094,212	4,114,993	4,112,253	4,198,005	4,184,100	4,070,101	4,064,986
	dbSNP rate	99.04%	99.58%	99.37%	99.56%	99.06%	99.55%	99.58%
	Singleton	159,429	78,109	98,574	100,158	104,052	52,127	51,978
	Singleton in dbSNP	126,762	68,673	78,361	89,094	73,177	41,092	41,743
	dbSNP rate for Singleton	79.51%	87.92%	79.49%	88.95%	70.33%	78.83%	80.31%
	Homozygous	1,703,616	1,690,878	1,704,813	1,708,639	1,714,752	1,688,328	1,689,834
	Heterozygous	2,430,309	2,441,590	2,433,483	2,507,950	2,508,860	2,400,317	2,392,269
	Het/Hom ratio	1.43	1.44	1.43	1.47	1.46	1.42	1.42
	Ti/Tv ratio	1.91	1.92	1.9	1.88	1.85	1.92	1.92
Indels	Total Indels	526,504	546,918	491,899	689,357	708,062	703,873	631,163
	Total Indels in dbSNP	524,738	544,866	489,777	686,916	705,553	701,802	629,314
	dbSNP rate	99.66%	99.62%	99.57%	99.65%	99.65%	99.71%	99.71%
	Singleton	7,864	7,444	8,094	17,036	23,596	41,384	12,092
	Singleton in dbSNP	7,612	7,259	7,915	16,784	23,303	41,183	11,964
	dbSNP rate for Singleton	96.80%	97.51%	97.79%	98.52%	98.76%	99.51%	98.94%

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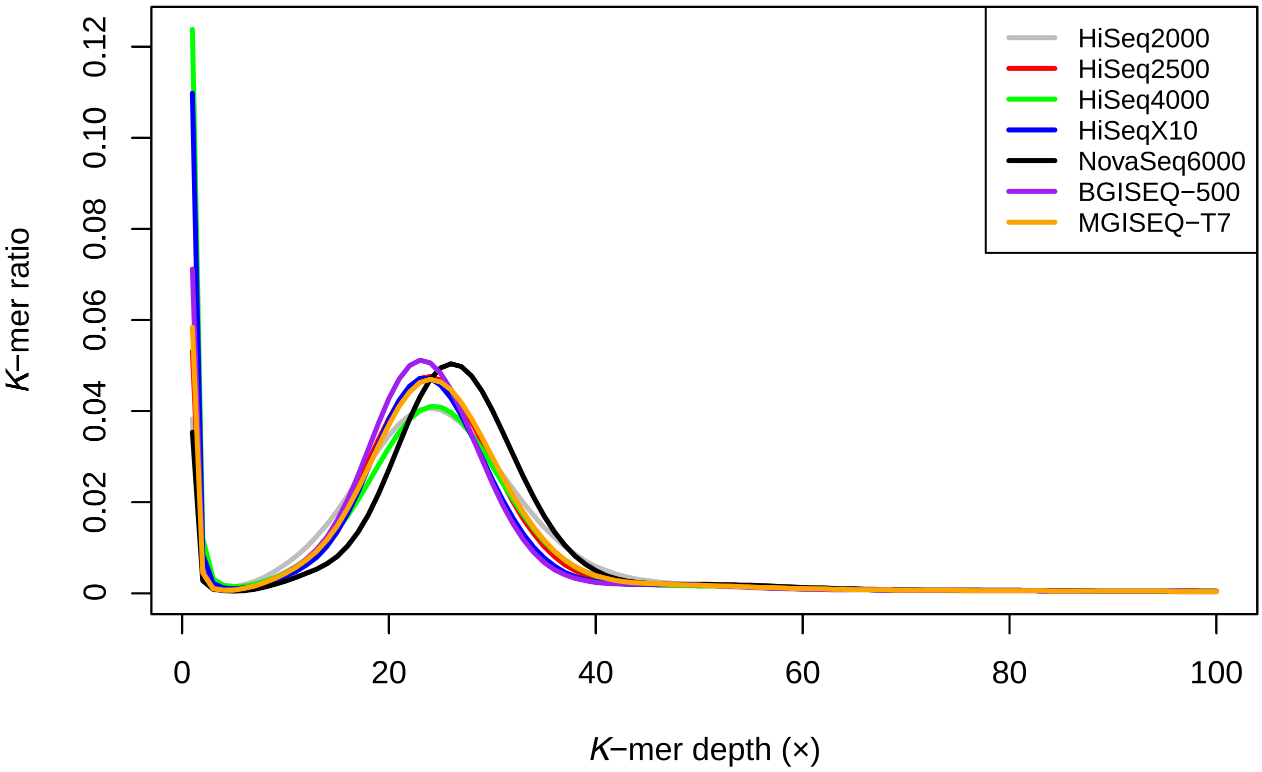

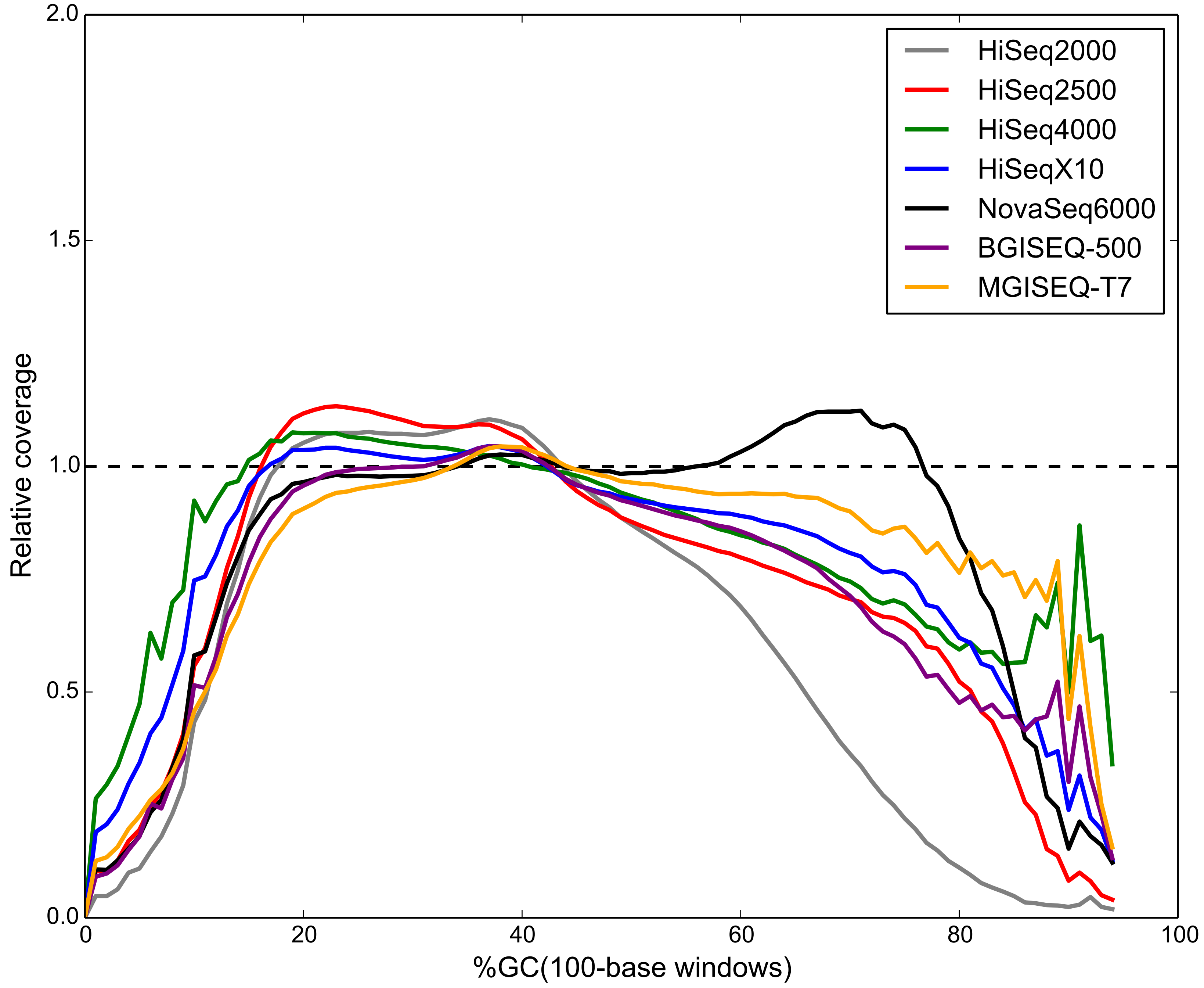
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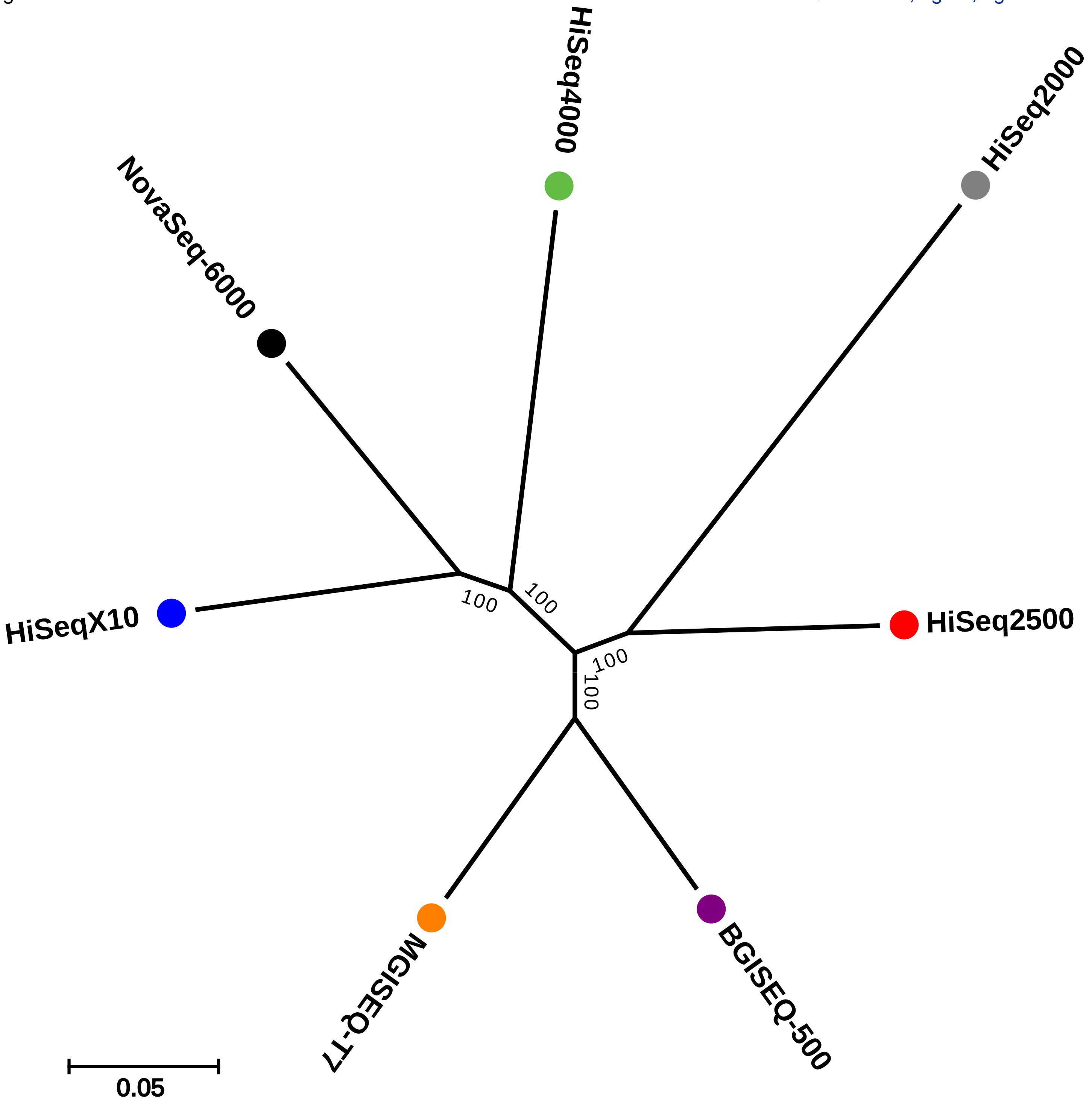

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GIGA-D-20-00072

Dear ***GigaScience*** editors,

Thank you for considering our manuscript for publication in *GigaScience*.

The reviewer #1' criticisms on "the sequencing data of seven sequencing platforms need to have the same genome coverage" was useful to improve the quality of our analyses and manuscripts.

To accommodate the reviewer #1' criticisms, we matched the seven sequencing platforms to the same genome coverage and re-analyzed the downstream analyses, including variant comparison, platform-specific covered regions, and concordance rate with SNP genotyping, to remove the bias due to the different genome coverage. As a result, we could compare the seven sequencing platforms more objectively.

We have also edited the manuscript to accommodate the reviewers' concerns on clarity and better presentation of the results. Please see the detailed point-by-point revision notes that are submitted on-line.

We hope that our revision will be suitable for your journal's standards.

Sincerely yours,

Dan M. Bolser & Jong Bhak
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