


TECHNICAL NOTE

Hi-C chromosome conformation capture sequencing of avian genomes using the BGISEQ-500 platform

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Abstract

Background: Hi-C experiments couple DNA-DNA proximity with next-generation sequencing to yield an unbiased description of genome-wide interactions. Previous methods describing Hi-C experiments have focused on the industry-standard Illumina sequencing. With new next-generation sequencing platforms such as BGISEQ-500 becoming more widely available, protocol adaptations to fit platform-specific requirements are useful to give increased choice to researchers who routinely generate sequencing data. **Results:** We describe an *in situ* Hi-C protocol adapted to be compatible with the BGISEQ-500 high-throughput sequencing platform. Using zebra finch (*Taeniopygia guttata*) as a biological sample, we demonstrate how Hi-C libraries can be constructed to generate informative data using the BGISEQ-500 platform, following circularization and DNA nanoball generation. Our protocol is a modification of an Illumina-compatible method, based around blunt-end ligations in library construction, using un-barcoded, distally overhanging double-stranded adapters, followed by amplification using indexed primers. The resulting libraries are ready for circularization and subsequent sequencing on the BGISEQ series of platforms and yield data similar to what can be expected using Illumina-compatible approaches. **Conclusions:** Our straightforward modification to an Illumina-compatible *in situ* Hi-C

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protocol enables data generation on the BGISEQ series of platforms, thus expanding the options available for researchers who wish to utilize the powerful Hi-C techniques in their research.

Keywords: Hi-C; BGISEQ-500; next-generation sequencing; chromosome conformation capture

Background

Determining the organization of chromatin and chromosomes within a cell nucleus carries great potential for describing genome function, regulation, interactions, and evolution. Advancements in molecular techniques since the beginning of the 21st century have allowed steady progress in uncovering these interactions using various forms of DNA-DNA proximity ligation [1] and chromosome conformation capture (3C) techniques [2]. These methods involve the covalent cross-linking of chromatin regions interacting in space, followed by restriction enzyme digestion and proximity ligation of fragments from interacting regions. This is then followed by DNA sequencing, yielding data that can be used to describe proximal interactions between distant genomic loci [3]. Earlier versions known as 3C and 4C generally focused on single or a few loci, using locus-specific primers and Sanger sequencing [4, 5]. Later, multiple parallel loci contacts could be quantified at once using either quantitative PCR approaches, microarray characterization, or early next-generation sequencing (NGS) platforms such as Roche 454, in a protocol known as 5C [6, 7].

More recently, however, NGS methods coupled with tailored bioinformatic tools have allowed a full range of interactions to be described at the genome scale using a method known as Hi-C [4]. These advances allow for insights not only into overall chromatin structure [8] but into the interactions between genes and their regulatory elements [9, 10] and their functions [11], revealing further layers of epigenomic activity to be decoded [12]. These genome-scale Hi-C analyses have used established NGS platforms as the preferred sequencing method, particularly those with short-read characteristics. Indeed, paired-end (PE) sequencing offered by the Illumina platforms [13] is widely used in Hi-C owing to their ubiquity and relatively universal final library constructs. In Hi-C, the mapping of both ends of a single read reveals a high sequential distance but at the same time implies a close physical proximity of the 2 ends, allowing for the identification of chromosomal contacts. While long-read platforms such as Pacific Biosciences are invaluable for *de novo* sequencing approaches owing to their ability to overcome nebulous genomic regions such as short tandem repeats or AT-rich regions, they cannot provide details of the structure of a genome. Therefore, for the time being, short, PE reads are the sequencing method of choice for identifying spatially close loci using 3C-based methods.

Although Illumina platforms have dominated the sequencing for these type of methods, alternative technologies are now appearing. One of these is the BGISEQ series of platforms, whose general workflow and stepwise sequencing procedures are similar to those of Illumina series, yet the sequencing templates have marked differences. Illumina uses hybridization clustering followed by bridge amplification [13], whereas the BGISEQ technology combines DNA nanoball nanoarrays [14] with polymerase-based stepwise sequencing. During this process, also called nanoball sequencing, the DNA undergoes an iterative ligation to circularize the DNA molecules, which are then replicated for the generation of DNA nanoballs. This iterative process generates billions of DNA nanoballs from each DNA molecule that are then loaded into a flow cell and sequenced [15]. The BGISEQ

has several features that have proven attractive to researchers within different fields. Namely, it allows several sequencing read lengths (50, 100, 150 bp) either for single read or PE sequencing; it has a very high throughput where ≥ 2 billion PE reads per flow cell are generated in only a few days; and it allows an easy adaptation of library construction protocols. Comparisons between the Illumina HiSeq-2500 and BGISEQ-500 platforms have been made previously for the sequencing of shotgun DNA [16] and RNA [17], with both studies finding very little difference between platforms for standard metrics for each molecule type such as clonality, endogenous content, GC content, and sequence quality scores. Furthermore, the Illumina HiSeq-4000 and BGISEQ-500 have also been compared for sequencing of exomes [18] and transcriptomes [19], validating the capability of BGISEQ-500 to be established as a competitive and reliable platform for both exome and transcriptome analysis.

Given that the performance of the BGISEQ-500 as a sequencer is similar to Illumina, we explored whether the Hi-C method could be adapted to the BGISEQ-500 platform. Specifically we adapted the *in situ* Hi-C protocol published by Rao et al. [8] by changing the adapter ligation step to use blunt-end ligation, meaning omission of the A-tailing step in Illumina library construction. Additionally, we introduced a post-ligation fill-in step to remove overhangs present on the distal ends of the BGISEQ adapters. We also modified the sequences of the adapters and amplification primers to be compatible with the BGISEQ-500 platform. We then submitted amplified libraries to BGI-Europe for circularization, DNA nanoball construction, and sequencing, and analysed the data to assess the method's performance.

Results

We processed 3 different zebra finch (*Taeniopygia guttata*) tissue samples using the BGI-Hi-C protocol developed for this study and described in detail in the Supplementary Materials and Methods. The BGI-Hi-C libraries were quantified and visualized using a BioAnalyzer Instrument (Supplementary Fig. S1), pooled and sequenced on a partial lane of the BGISEQ-500 with PE100 sequencing mode. Sequencing reads were analysed using TADbit [20], a pipeline developed for Hi-C experiments to pre-process the reads, assess the quality of the Hi-C experiments (Supplementary Figs S2 and S3), map the reads to a reference genome, filter and normalize interaction data, analyse the resulting interaction matrices, and generate statistics and maps to model and explore 3C-based data.

The 3 zebra finch samples yielded ~29 million, ~3 million, and ~2 million PE sequence reads, respectively (Table 1). Using the TADbit pipeline we mapped and filtered all reads for the 3 samples (Table 2). Mapping was performed following a fragment-based method implemented in TADbit [20]. To map the sequence data we used the available reference genome on NCBI for the zebra finch (GCF.000151805.1.Taeniopygia.guttata-3.2.4).

Sample Oz13 yielded the largest number of reads (~29 million), with ~30% of the reads containing ≥ 1 ligation site, leading to a total of up to ~63% of uniquely mapped reads. The percentage of uniquely mapped reads was much higher for the other 2 samples (up to 78% and 75%, respectively) even though they were

Table 1: TADbit mapping and quality statistics of the Hi-C-BGI experimental results

Sample	Index	Read	Initial reads	Out of 100,000 reads		Uniquely mapped pairs (% initial)	Total No. interactions; both reads mapped (% initial)
				Digested sites (%)	Reads with ligation site (%)		
Oz13	10	1	29,436,352	83.6	31.6	18,670,087 (63.2%)	18,269,316 (62.1%)
		2	29,436,352	80.4	29.8	18,127,333 (61.6%)	
Mz13	18	1	3,069,136	68.5	14.8	2,396,713 (78.1%)	2,201,431 (71.7%)
		2	3,069,136	66.2	14.4	2,297,654 (74.9%)	
Mz17	19	1	2,274,286	44.8	7.8	1,711,896 (75.3%)	1,554,764 (68.4%)
		2	2,274,286	42.5	7.3	1,673,397 (73.6%)	

Table 2: For each of the 3 experiments, reads lost after each of the filters applied, approximate comparison to numbers expected for a standard Illumina experiment, and final number of valid pairs considered

Filter type	Illumina expected % (for Mbol)	Oz13	%	Mz13	%	Mz17	%
Self-circle	<1	11,385	0.06	540	0.02	440	0.03
Dangling end	2.3–58.4	6,072,260	33.2	934,066	42.4	781,281	50.3
Error	<1–3.4	8,941	0.05	586	0.02	587	0.03
Extra dangling end	9.5–70.1	5,275,752	28.9	744,126	33.8	517,306	33.3
Too close from RES	23–93.7	5,348,693	29.3	642,844	29.2	221,954	14.3
Too short	3.6–20	924,068	5	94,940	4.3	44,972	2.9
Too large	<1–0.14	21	0.0001	1	0.000045	0	0
Over-represented	3.16–13.2	3,362,668	18.4	446,571	20.3	64,224	4.1
Duplicated	1.94–64.2	13,637,482	74.6	1,186,193	53.9	259,406	16.7
Random breaks	<1–3.5	979,909	5.4	129,690	6.3	173,515	11.4
Valid pairs	7.5–98	4,043,904	22.1	848,181	38.5	1,117,826	71.7

Note that the number of reads does not necessarily add up to the total number of interactions because the same read can be categorized within >1 of the filter categories. Valid pairs represents the number of reads used for generating the Hi-C maps seen in Fig. 1. RES: restriction enzyme sites.

sequenced to a much lower depth (Table 1). Using a set of 316 *in situ* Hi-C experiments performed with Mbol/DpnII, available at the repository of the 4DGenome unit at the Centre for Genomic Regulation in Barcelona, we placed our 3 BGI processed samples within an Illumina context to compare the obtained values for 15 experimental parameters (Fig. 1).

The number of reads containing ≥ 1 ligation site is near optimal and in accordance with what is expected ($\sim 30\%$) for sample Oz13, albeit lower for the other 2 samples ($\sim 14\%$ for Mz13 and $\sim 7\%$ for Mz17), but still within the range observed in other Illumina-based Hi-C experiments (Fig. 1). For most of the analysed parameters, the 3 zebra finch samples fall within an expected range, except for duplicates in sample Oz13, which were larger than any of the 316 processed samples, which likely results in the final lower-than-expected number of valid pairs for this sample. We estimated the number of unique reads per total mapped reads (Fig. 2) and found that the high amount of duplicates in sample Oz13 is a consequence of a lack of library complexity as we reached sequencing saturation of the sample (Fig. 2).

We also note that all 3 samples exhibited a slightly (but not critically) higher than expected level of random breaks (Fig. 1), possibly reflecting over-sonication during the library construction process.

The final total number of interactions (the number of read pairs where both read 1 and 2 are mapped) and the uniquely mapped reads can be seen in Table 1 for each sample. Table 2 reports the values represented in Fig. 1, together with the percentage of expected numbers for the 316 samples.

Using TADbit, we then assembled the resulting valid pairs into a 500-kb resolution and Vanilla-normalized [8, 21] the interaction map for sample Oz13 (Fig. 3) and for samples Mz13 and Mz17 (Supplementary Fig. S3). The interaction matrix shows the chromosomal territories for the zebra finch genome, including identification of a translocation/inversion in chromosome NC.011465.1 in comparison to the reference genome (Fig. 3B).

Broadly speaking, standard sequencing experiments usually go beyond the coverage values we present here. Still, in light of our results, deeper sequencing experiments could be performed to obtain more detail in the genome structure. We are confident that the protocol presented here confirms that BGI sequencing protocol generates high-quality reads, potentially suitable for Hi-C pipeline analysis.

Methods

In situ BGI-Hi-C and sequencing

Samples were processed following the *in situ* BGI-Hi-C protocol, which is described in detail in the Supplementary Materials and Methods. Briefly, the 3 zebra finch tissue samples (50 mg) were coarsely crushed with the help of a scalpel and cross-linked with formaldehyde. Following cross-linking we proceeded to digest DNA with a restriction enzyme (Mbol), filling the 5' overhangs and biotin-tagging the ends of the fragments. We then ligated the resulting blunt-end fragments, sheared the DNA, and retrieved the biotinylated ligated fragments with streptavidin beads. To avoid the risk of DNA loss we skipped the size selection step described in the original protocol and contin-

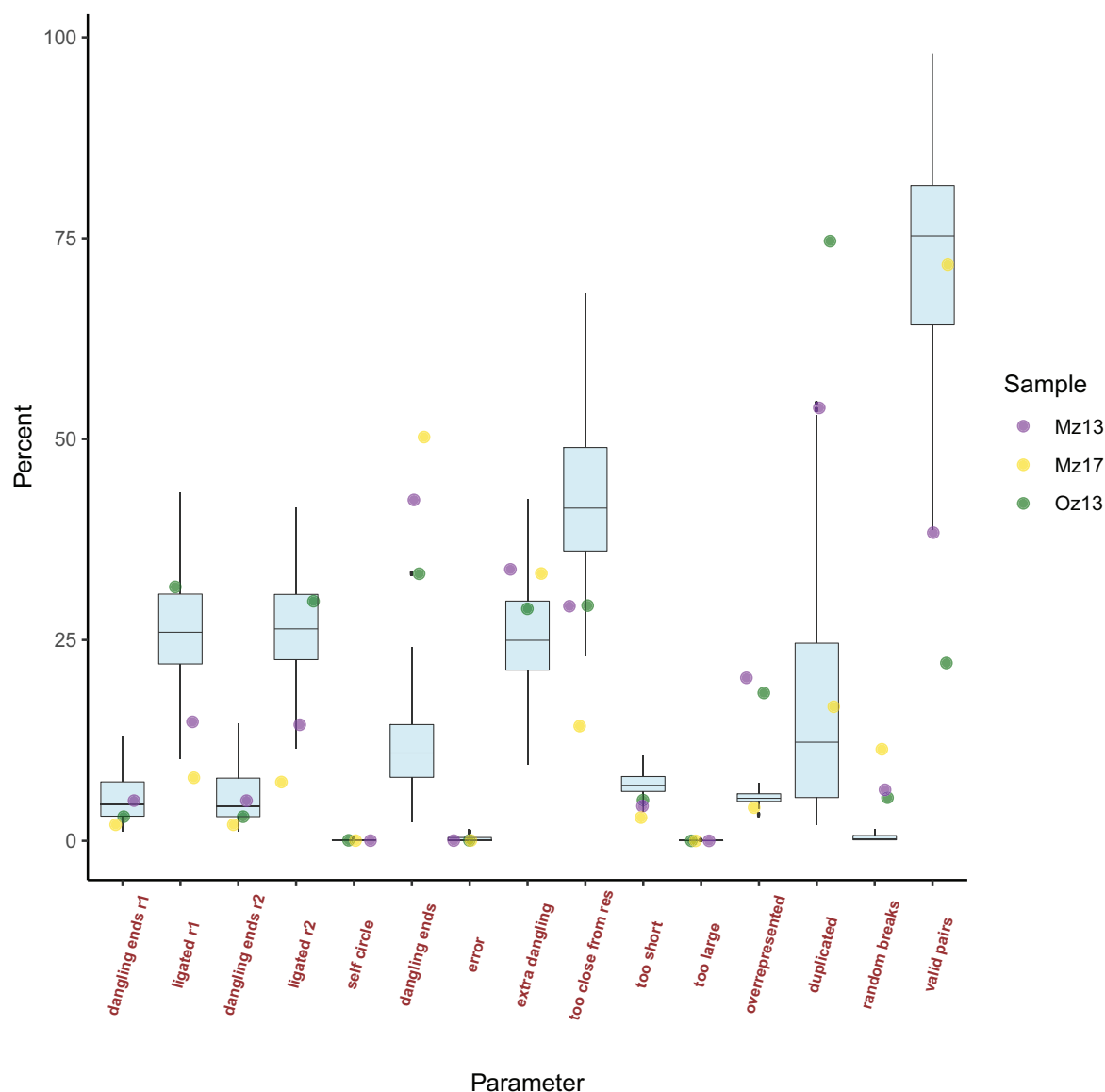


Figure 1: Comparison of values obtained for 15 parameters evaluated in our 3 samples within a context of 316 *in situ* Hi-C samples processed with the same restriction enzyme (RE). From the left, the first 4 parameters are intrinsic values for quality control of the experimental processing before mapping, where “dangling ends r1,2” for each read refers to the number of reads that have been digested but have not been mapped and “ligated” is the number of sites that have been re-ligated (contacting different fragments). The remaining 11 parameters are as follows: Self-circle: both read ends are mapped to the same RE fragment in opposed orientation. Dangling-ends: both read ends are mapped to the same RE fragment in facing orientation. Error: both read ends are mapped to the same RE fragment in the same orientation. Extra dangling end: the read ends are mapped to different RE fragments in facing orientation but are close enough (less than `max.molecule.length` bp) from the RE cut-site to be considered part of adjacent RE fragments that were not separated by digestion. The `max.molecule.length` parameter can be inferred from the `fragment.size` function previously detailed. Too close from RE sites (RES): the start position of 1 of the read ends is too close (5 bp by default) to the RE cutting site. Too short: 1 of the read ends is mapped to RE fragments of <75 bp. These are removed because there is ambiguity on where the read end is mapped because it could also belong to any of the 2 neighbouring RE fragments. Too large: the read ends are mapped to long RE fragments (default: 100 kb, $P < 10^{-5}$ to occur in a randomized genome) and they likely represent poorly assembled or repetitive regions. Over-represented: the read ends coming from the top 0.5% most frequently detected RE fragments; they may represent PCR artefacts, random breaks, or genome assembly errors. PCR artefacts or duplicated: the combination of the start positions, mapped length, and strands of both read ends are identical. In this case, only 1 copy is kept. Random breaks: the start position of 1 read end is too far (less than `minimum.distance.to.RE`) from the RE cut-site. These are produced most probably by non-canonical enzyme activity or by random physical breakage of the chromatin. Valid pairs: are those pairs of contacting reads that were kept as valid contacts, after removing all other 10 categories of read pairs. Additional details can be found in the filtering function of the TADbit method: https://3dgenomes.github.io/TADbit/tutorial/tutorial.6-Filtering_mapped_reads.html. In the plot, the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles) and the line in the middle is the median value. The upper whisker extends from the top hinge to the largest value no further than $1.5 \times \text{IQR}$ from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the hinge to the smallest value at most $1.5 \times \text{IQR}$ of the hinge. Data beyond the end of the whiskers are called “outlying” points and are plotted individually.

used with preparing our samples for BGI sequencing. We started by repairing the ends of the DNA fragments and removing the biotin from unligated ends. We then ligated the BGI adapters and

filled in the distal overhangs. Finally BGI-Hi-C libraries were indexed in 50 μL volume reactions and amplified for 25 cycles. PCR reaction consisted of 15 μL of BGI-Hi-C library template, 25 μL

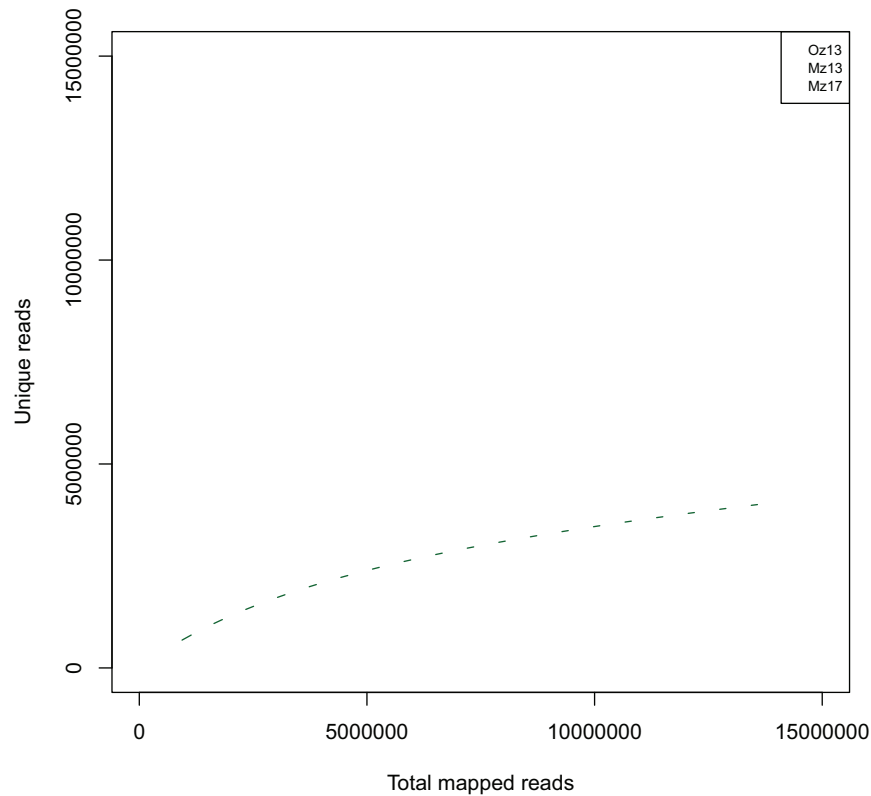


Figure 2: Unique reads per total mapped reads subsampling. Each point within the dotted lines indicates a 5% increase in the total number of the reads (X axis) for each sample. On the Y axis we show the proportion of unique reads mapped for each 5% increment.

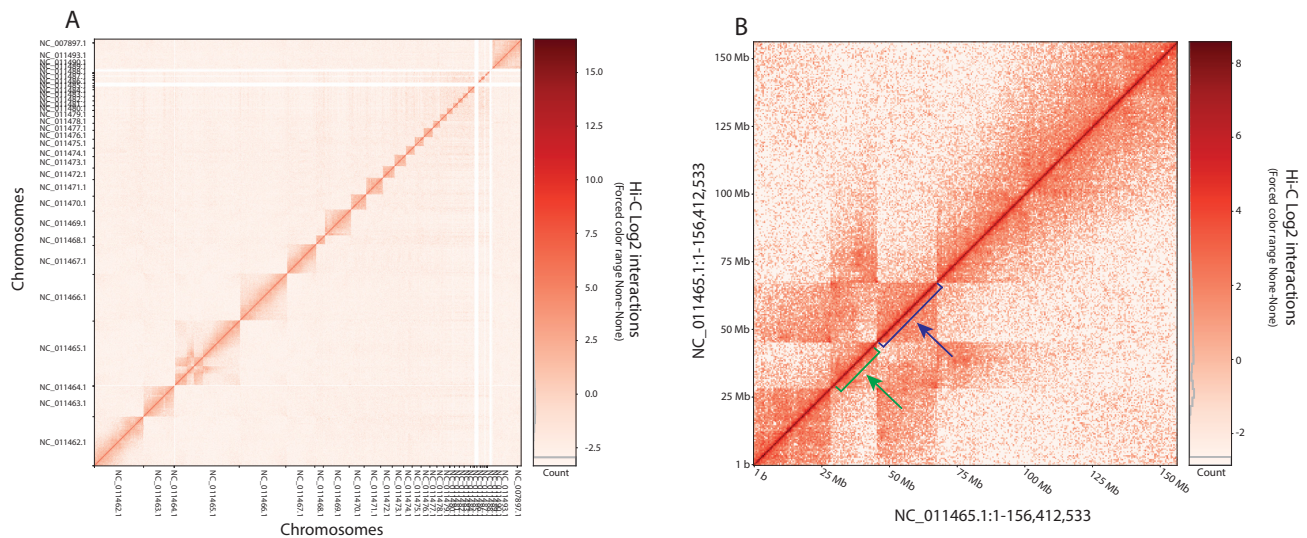


Figure 3: (A) Hi-C Vanilla-normalized contact matrix representation of the genome of sample Oz13. Note the presence of the rearrangement/translocations in chromosome NC.011465.1, as mentioned in the text. (B) Close-up detail of the chromosome NC.011465, Vanilla normalized at a 500-kb resolution. Marked with blue and green arrows are the intrachromosomal rearrangements with relation to the reference genome.

of 2× Phusion Hi-Fi PCR Master Mix, 0.8 μ L bovine serum albumin (20 mg/mL), 2 μ L of each primer (10 μ M BGI forward primer and indexed reverse primer), 1 μ L of dimethyl sulfoxide, and water. Thermocycling conditions were set as follows: 30 s at 98°C, followed by 25 cycles of 30 s at 98°C, 30 s at 60°C, and 30 s at 72°C, and a final 7-min elongation step at 72°C. The number of cycles was estimated using quantitative PCR. Following amplifi-

cation, PCR products were cleaned using 1× of AmpureXP beads, washing twice with 200 μ L of 80% ethanol, followed by a 5-min drying incubation at room temperature. Amplified BGI-Hi-C libraries were eluted in 32 μ L of EB buffer after a 5-min incubation at 37°C. BGI-Hi-C libraries were visualized and quantified using a BioAnalyzer instrument and pooled along with other samples. Pooled BGI-Hi-C libraries were circularized, and sequenced as

100PE on the BGISEQ-500 platform at BGI Europe, Copenhagen. Demultiplexing was performed in house, and resulting FastQ files were delivered electronically.

Data analysis

As a first step of the processing of the sequencing reads, BGI adapters were removed from each sample's FastQ files using cutadapt (v.1.11) [22], with default parameters for PE reads and allowing for 10% mismatch. Trimmed reads were analysed using and following the TADbit [20] pipeline. The TADbit pipeline starts by performing a quality control on the raw data (FastQ files) to assess the quality of the sequencing reads and the efficiency of the digestion and ligation steps of the Hi-C experiments. Next, the PE reads were aligned in TADbit to the available reference genome for the zebra finch (GCF_000151805.1.Taeniopygia_guttata-3.2.4) using the GEM mapper (v2) [23]. Once the reads had been mapped we proceeded to find the intersection of both reads and extract the interacting pairs, followed by a fragment-based filtering step to correct experimental biases/errors. Finally, we binned the valid pairs into a 500-kb resolution and Vanilla-normalized [8, 21] the interaction map.

The detailed bioinformatic pipeline used to analyse the data is available as a Jupyter Notebook and can be found at the project home page.

Conclusions

As the number of available high-throughput sequencing platforms available increases, each with their own specific profiles of cost, input requirement, and data return, developing the ability to easily tailor experiments to different platforms based on only minor changes to existing protocols is becoming increasingly important.

In this regard, this study represents the first exploration of the applicability of the BGISEQ-500 as an alternative sequencing platform to the Illumina series for the generation of Hi-C sequencing data. With some simple modifications and adaptations to the existing Hi-C protocol, we were able to sequence Hi-C libraries on the BGISEQ-500 platform, proving that there are other options available for researchers who wish to utilize the powerful Hi-C techniques in their research. We find that the modified protocol shows performance similar to that of Illumina experiments. Although we acknowledge that our analyses are limited to a small sample size, our observations suggest that the BGISEQ-500 holds the potential to become a valid and valuable alternative platform for Hi-C data generation that is worthy of future exploration. It is important and it will be interesting for future studies to perform direct comparisons to investigate possible sources of sequencing platform biases, although other studies comparing the BGISEQ and Illumina platforms in other contexts (e.g., exomes, ancient DNA, RNA) show no evidence of such.

Availability of Supporting Data and Materials

The trimmed sequencing read data supporting the results of this article are available at the University of Copenhagen Electronic Research Data Archive (ERDA repository) [24] with the ID 0af6e87de023ee3508e59a7a868c256b and can be accessed through the archive [24]. Other supporting data are available in the GigaScience repository, GigaDB [25].

Availability of Source Code and Requirements

Project name: BGI-Hi-C Computational Analysis TADbit Pipeline
Project home page: <https://github.com/pollicipes/BGI-Hi-C-Computational-Analysis>

Operating system(s): Tested for Ubuntu/Linux and MacOSX

Programming language: Python and Bash

Other requirements: Conda and Jupyter Notebook (optional), TADbit and GEM mapper (mandatory)

License: GNU General Public License v3.0

Additional Files

Supplementary Materials and Methods. BGI-Hi-C protocol

Supplementary Figure S1. BioAnalyzer fragment length distribution images

Supplementary Figure S2. Quality assessment plots of the Hi-C experiment

Supplementary Figure S3. Hi-C contact matrix for samples Mz13 and Mz17

Abbreviations

bp: base pairs; GC: guanine-cytosine; Hi-C: high-throughput chromosome conformation capture; kb: kilobase pairs; NCBI: National Center for Biotechnology Information; NGS: next-generation sequencing; PE: paired end; IQR: inter-quartile range.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

M.T.P.G. conceived of the study. M.S.-V., O.S., and C.P.E. designed the Hi-C experiments and performed the laboratory work. M.S.-V. and O.S. optimized the Hi-C protocol adapted for BGI sequencing. O.S. designed the BGI-specific aspects of the library construction. G.Z. produced the sequencing data. J.A.R. and M.A.M.-R. analysed the data. M.S.-V., O.S., and J.A.R. wrote the manuscript with input from all authors. M.T.P.G., M.A.M.-R., and E.L.A. supervised the work.

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