

# Improved *Gossypium raimondii* Genome Using a Hi-C-based Proximity-Guided Assembly

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## Research

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# Abstract

**Background:** Genome sequence plays an important role in both the basic and applied studies. *Gossypium raimondii*, the putative contributor of the D subgenome of Upland cotton (*Gossypium hirsutum*), highlights the need to improve the genome quality in a rapid and efficient way.

**Methods:** we performed Hi-C sequencing of *Gossypium raimondii* and reassembled its genome based on a set of new Hi-C data and previously published scaffolds. We identified and corrected errors of initial scaffolds before reassembled into chromosomes.

**Result:** A total of 98.42% of sequence was clustered successfully, among which 99.72% of the clustered sequence was ordered and 99.92% of the ordered sequence was oriented with high-quality. Further evaluation of results by heat-map and collinearity analysis revealed that the current reassembled genome is significantly improved than previous one.

**Conclusion:** This improvement in *Gossypium raimondii* genome not only provides a better reference genome to increase study efficiency, but also offers a new way to assemble cotton genomes. Furthermore, Hi-C data of *Gossypium raimondii* may be used for 3D structure research or regulating analysis.

## Introduction

Over the last decade, next generation sequencing (NGS) technologies has brought immense improvements in plant genome sequencing throughput and cost, and many plant genomes have been sequenced using this technology such as *Fragaria vesca* (Shulaev et al., 2011), *Cytisus cajan* (Varshney et al., 2012), *Gossypium raimondii* (Wang et al., 2012; Paterson et al., 2012, Udall et al., 2019), *Gossypium arboreum* (Li et al., 2014) and *Gossypium hirsutum* (Li et al., 2015; Zhang et al., 2015). However, *de novo* assembly of large eukaryotic genomes has remained a great challenge with the NGS platform due to significant amount of repeat contents (Rounsley et al., 2009). As a result, *de novo* assembly of chromosome-scale scaffolds has become a major constraint to the completion of high-quality genome sequence. Compared with the traditional method, high-throughput chromosome conformation capture (Hi-C) technology has assisted in the assembly of long scaffolds to produce chromosome-scale genome assemblies (Lightfoot et al., 2017). The Hi-C technique is based on existing scaffolds and restriction enzyme cutting sites, which are more evenly distribution and have much higher density.

The Hi-C-based proximity-guided assembly was initially developed to study the three-dimensional (3-D) conformation of chromosomes of yeast gene expression (van Berkum et al. 2010). There are two regular models with Hi-C data: the first one is that the rate of Hi-C interaction is inversely proportional to the genomic distance between the pairs of loci, the second one is that the rate of Hi-C interaction of pairs of loci within a chromosome is significantly higher than that in different chromosomes (Xie et al. 2015). Based on these two models, Hi-C-based proximity-guided assembly was applied for *de novo* assembling

of human, and subsequently for the assembling of mouse and *Drosophila* genomes which reported good results or improvement (Burton et al. 2013). With the success of testing and verifying this method in *Arabidopsis thaliana* (Xie et al. 2015), Hi-C based proximity-guide assembly has been reported as an effective and efficient method which subsequently has been used in many other plants.

Besides cotton's commercial value, it also serves as a perfect model system for studying cell wall biosynthesis (Zhang et al. 2018), cell elongation (Guo et al. 2017) and polyploidization (Yuan et al. 2015). The *Gossypium* genus comprises of more than 50 species including at least 5 tetraploid species and 45 diploid species. Diploid cottons are divided into 8 sub-genomes, denoted A-G and K based on chromosome pairing relationships (Wendel et al., 1992). Tetraploid cottons, such as cultivated *Gossypium hirsutum* (AD<sub>1</sub>) and *Gossypium barbadense* (AD<sub>2</sub>), had formed by an allopolyploidy event about 1-2 million years ago (Paterson et al. 2012). These tetraploid cotton species share common ancestors with the modern New World species *Gossypium raimondii* (D<sub>5</sub>) and the Old World A-genome species *Gossypium herbaceum* (A<sub>1</sub>) or *Gossypium arboreum* (A<sub>2</sub>). Previously, genomes of different cotton species sequenced and assembled including *Gossypium raimondii* (Wang et al. 2012), *Gossypium arboreum* (Li et al. 2014), and *Gossypium hirsutum* (Li et al., 2015).

Among these cotton species, the genome of *Gossypium raimondii* has the lowest complexity which has been sequenced and assembled using the next-generation Illumina paired-end sequencing strategy (Wang et al. 2012). Approximately 73% (281 scaffolds) of the assembled sequences were anchored to 13 chromosomes, covering 88.1% of the genome, while only 52.4% (228 scaffolds) of total sequence was both ordered and oriented. The completeness and accuracy of previous sequenced and assembled genome of *Gossypium raimondii* (Wang et al., 2012) was relatively low due to higher numbers of repeat elements and low numbers of genetic markers. In the present study, we conducted a *de novo* Hi-C sequencing of *Gossypium raimondii* genome, and incorporated the new Hi-C data with the existing *Gossypium raimondii* scaffolds (Wang et al., 2012) to improve the quality of the D-genome.

## Methods

### 1. Tissue collection and Hi-C sequencing

#### 1.1 Plant materials

The seeds of *Gossypium raimondii* D<sub>5-1</sub> were planted in an incubator at constant environmental condition having 27°C temperature, 60% relative humidity, 16/8-h light/dark photoperiod, and 100% fluorescent light. When sixth euphylla came out, these seedlings were transplanted into big pots. Approximately 3-gram young leaves from *Gossypium raimondii* plants were collected and immediately treated with formaldehyde.

#### 1.2 Hi-C pipeline

During this study, we have used the same Hi-C pipeline as in *Arabidopsis thaliana* (Xie, Zheng et al. 2015). Before start this experiment, we have tested the integrity of DNA from the formaldehyde-treated tissue, and then the DNA was isolated and digested by *MboI* instead of *HindIII* because of the shorter recognition site (only four bases of *MboI*). The resulting sticky ends were filled with nucleotides in which cytosine is biotinylated, and ligated the adjacent blunt ends to a chimeric circle under extremely dilute conditions. Subsequently, DNA was purified and broken into 300-500 base pairs using ultrasonic, pull-down the biotin labeled DNA and performed the PCR reaction (10 cycles). After DNA purification, the finished Hi-C library was sequenced with an Illumina Hiseq (PE150). A total of 570,412,361 read-pairs were obtained.

## 2. Genome assembly based on Hi-C data

Assembling of *Gossypium raimondii* genome involved three steps. First, valid Hi-C paired-end reads and contact matrix with a resolution of 100 kb were generated by HiC-Pro (Servant, Varoquaux et al. 2015). The raw sequence data with low quality, unmapped and invalid mapped paired reads were filtered out by HiC-Pro and contact matrix based on interaction frequency was created. At the second step, the *Gossypium raimondii* genome was assembled with the Hi-C data by Lachesis (Burton, Adey et al. 2013), which contained clustering, ordering and orienting. Lastly, the assembled *Gossypium raimondii* genome was assessed by Mummer and Python scripts, resulting heat-map and collinear analysis.

# Results

## 1. Hi-C data analysis by HiC-Pro

### 1.1 Mapping and filtering

Initially, the low-quality and invalid paired-reads were filtered out which was caused by sequencing errors from the raw Hi-C reads of *Gossypium raimondii* (Table 1). Results revealed that 95.6% of sequence is clean Q30 bases, showing a good quality of sequence data. Then, clean Hi-C reads were mapped to the previously sequenced genome of *Gossypium raimondii* (Wang et al., 2012) using Bowtie2 (Langmead and Salzberg 2012), and unique mapped paired-end reads were retained (Fig.1 and Table 2). Subsequent analysis was performed to remove the invalid paired-reads. The reference genome was broken into restriction fragments by cutting them at the *MboI* restriction enzyme site “GTAC”. Approximately 54.7% uniquely mapped paired-read was aligned to single restriction fragments. Among uniquely mapped reads, valid paired-end reads were present in different restriction fragments, but non-ligation, self-ligation and dangling end paired-end reads were recognized by mapping orientation information in the same restriction fragment (Belton, McCord et al. 2012) (Table 3, Fig. 2). After the Hi-C data were filtered out, results showed that 81.95% of uniquely mapped sequences are valid paired-end reads. Thus, the valid paired-end reads (223,304,666) were used for further analysis.

### 1.2 Creating contact matrix

The genome was into non-overlapping 100kb windows, and the number of valid paired-end reads in the 100kb windows was referred as the contact count. The Hi-C contact matrix was built and normalized by its restriction sites because the Hi-C signal was in linear proportion to the number of restriction sites.

### 1.3 Identification and correction of errors within scaffolds

Errors in scaffolds of the initial draft assembly were identified and corrected following the *Aedes aegypti's de novo* assembly procedure (Dudchenko, Batra et al. 2017). Briefly, the errors were corrected by identifying the bins where a scaffold's long-range contact pattern changes abruptly, which is unlikely for a correct scaffold. We cut out the error bins as a new scaffold. There are 259 errors within scaffolds. The list of error bins is presented in Supplemental.1.

## 2 Genome assembly by Lachesis

### 2.1 Clustering

Lachesis is a computational method that exploits Hi-C data sets for *de novo* genome assemblies (Burton, Adey et al. 2013). Hi-C data has two classical models, Hi-C interactions within one chromosome are distinctly more than it between two chromosomes, and Hi-C interactions between two loci are inversely proportional to their distance. Based on the first model, Lachesis clustered the scaffolds into 13 groups by agglomerative hierarchical clustering (Table 4). A total of 2,883 scaffolds were clustered successfully.

### 2.2 Ordering and orientation

In each clustered group, an acyclic spanning tree was built with vertexes corresponding to the scaffolds, while edge weights representing the normalized Hi-C interactions between pairs of scaffolds. A total of 1,328 scaffolds (744,578,885 bp, representing 99.72% of the total length) were ordered by Lachesis, among which 697 scaffolds (724,960,878 bp, representing 97.37% of the total length) are "trunk" (Table 5). For each ordered group, the acyclic spanning tree was built to represent all of the possible ways to orient the scaffolds. Lachesis has built a scoring function based on the difference between forward and backward interaction. Highest score represented the maximum likelihood through predicting orientations for each of the scaffolds. Among 1,328 ordered scaffolds, 1,129 scaffolds (743,948,690 bp, representing 99.92% of the ordered length) were of high scores (Table 6). All of the "trunk" scaffolds were of high score.

## 3 Assembling results

The genome of *Gossypium raimondii* were reassembled using Hi-C data (Supplemental.2). About 98.42% of total sequence length was clustered successfully, among which 99.72% and 97.37% of the clustered sequence were ordered and high-quality ordered, respectively. And approximately 99.92% of the ordered sequence was oriented with high-quality. The statistics of pseudo-chromosome length is shown in Table 7, while the indicator statistics of the initial draft genome and reassembly results are shown in Table 8 and 9, respectively. From the parameters like scaffolds number, N50 and N60 of previously draft genome

and reassemble genome, we found that the *Gossypium raimondii* genome using a Hi-C-based proximity-guided assembly is clearly much better than the reported draft genome (Wang et al., 2012).

Further, the results were also verified by heat-map (Fig. 3) and collinear-analysis (Fig.4). The heat-map directly proves the validity of the processing methods. Based on the two regular models as we mentioned above, the heat-map of the reassembled results revealed that the diagonal interaction is much higher. The boundaries of each pseudo-chromosome are relatively clear and it is under low background noise that shows good reassembling results (Fig. 3). In addition, the collinear relationship between the draft (Wang et al., 2012) and reassembled genomes (Fig. 4) showed that the current reassembled genome is quite different from the previous one. Further when we compared our results with another version of *Gossypium raimondii* genome (Paterson et al., 2012), results showed that current reassembled genome is improved with respect to both quality and integrity (Fig. 5).

## Conclusion And Discussion

With the development of sequencing techniques and bioinformatics tools, a high-quality genome sequence is the basis for cotton molecular breeding. In several previous studies, Illumina short sequencing reads are extensively used for *de novo* genome sequencing and assembling of different organisms (Ekblom and Wolf 2014) including cotton (Li et al., 2014; 2015). However, Illumina short sequencing reads are very short, which requires other types of sequencing data to assemble the genome such as BAC and fosmid libraries (Salzberg, Phillippy et al. 2012), jump libraries (Salzberg, Phillippy et al. 2012), optical mapping (Dong et al. 2013), genetic linkage maps (Fierst 2015), and single-molecule real-time sequencing (Bickhart et al. 2017). Previously, Wang et al. (2012) used the Illumina sequences and genetic linkage map to assemble the genome of *Gossypium raimondii* (Wang et al. 2012), but this technique is insufficient due to low density of markers available in genetic linkage map. Hi-C-based proximity-guided assembly was able to more accurately reassemble the *Arabidopsis thaliana* genome from a set of scaffolds into chromosomes (Xie et al. 2015). Nowadays, this method has been successfully used in many species such as *Aedes aegypt* (Dudchenko, Batra et al. 2017), *Amaranthus hypochondriacus* (Lightfoot et al. 2017), *Rubus occidentalis* (Jibran et al. 2018). In the present study, we applied *de novo* Hi-C sequencing of the *Gossypium raimondii* genome to improve the quality and accuracy of its previously reported draft genome from Wang et al. (2012).

Results from the comparative analysis of different parameters between the draft genome (Wang et al., 2012) and the current reassembled genome showed a significantly improved quality as compared to previous one. Such as we increased the rate of clustering from 73% of the previous draft assembly to 98.42% of current reassembly. Similarly, the rates of ordering and orienting were also from 52.4 % (previous draft assembly) to 98.07% (current reassembly), confirming the better quality of current reassembled *Gossypium raimondii* genome.

Previously, Paterson et al. (2012) also sequenced and assembled the *Gossypium raimondii* genome with good results and abundant markers. However, these markers are not evenly distributed across the

genome which might indicate some errors in its genome assembly. Thus in the current study, we also compare the reassembly genome with this version of *Gossypium raimondii* genome (Paterson et al., 2012) by the collinear analysis. Our results showed that despite of fewer differences, the current reassembled genome is improved with respect to both quality and integrity. However, based on the differences between the current reassembled genome and the genome reported by Paterson et al. (2012), further work may be necessary for integrating the two versions of *Gossypium raimondii* genome into a best version.

## Abbreviations

NGS: next generation sequencing

Hi-C: high-throughput chromosome conformation capture

CAAS: Chinese Academy of Agricultural Science

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

Not applicable.

### Funding

Not applicable.

### Authors' contributions

Yang QH and Song GL conceived and designed the experiments; all authors performed data analysis and interpretation; Yang QH, Javaria Ashraf and Song GL wrote the manuscript; All authors read and approved the final manuscript.

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## Tables

**Table 1: The statistics of Hi-C data filtering.**

Sample	<i>Gossypium raimondii</i>
Read Length /bp	100
Raw Paired-end Reads	570,412,361
Raw Bases /bp	114,082,472,200
Clean Paired-end Reads	503,917,093
Clean Paired-end Reads Rate /%	88.34
	10,734,587
Low-quality Paired-end Reads	1.88
Low-quality Paired-end Reads Rate /%	0.04
Ns Paired-end Reads	55,536,170
Ns Paired-end Reads Rate /%	9.74
Adapter Polluted Paired-end Reads	94.88
Adapter Polluted Paired-end Reads Rate /%	95.6
Raw Q30 Bases Rate /%	
Clean Q30 Bases Rate /%	
Clean paired-end reads are the high-quality reads after filtering. Ns paired-end reads having the more than 5% N's percentage. Q30 bases rate is the ratio of base's sequencing quality which is higher than 30, it means the base's sequencing error percentage is less than 0.1%.	

**Table 2: The statistics of mapped Hi-C data.**

Sample	<i>Gossypium raimondii</i>
Clean Paired-end Reads	503,917,093
Unmapped Paired-end Reads	20,995,471
Unmapped Paired-end Reads Rate /%	4.17
Paired-end Reads with Singleton	127,442,481
Paired-end Reads with Singleton Rate /%	25.29
Multi Mapped Paired-end Reads	82,998,864
Multi Mapped Ratio /%	16.47
Unique Mapped Paired-end Reads	272,480,277
Unique Mapped Ratio /%	54.07
Multi mapped paired-end read means Hi-C sequencing read mapped to more than one loci of reference sequence.	

**Table 3: The statistics of Hi-C data after filtering.**

Sample	<i>Gossypium raimondii</i>
Unique Mapped Paired-end Reads	272,480,277
Dangling End Paired-end Reads	1,151,621
Dangling End Rate /%	0.42
Self-circle Paired-end Reads	275,606
Self-circle Rate /%	0.10
Dumped Paired-end Reads	5,714,267
Dumped Rate /%	2.10
Interaction Paired-end Reads	265,338,783
Interaction Rate /%	97.38
Valid Paired-end Reads	223,304,666
Valid Rate /%	81.95
Dangling end paired-end read means the biotin labeled base is at the end of read. Dumped paired-end reads do not contain any biotin labeled base or it's inter size is out of range. Interaction paired-end reads were mapped to different restriction fragment. Valid paired-end reads are interaction paired-end which taken out repeat paired-end reads caused by PCR.	

**Table 4: The statistics of clustering results.**

Sample	<i>Gossypium raimondii</i>
Number of Sequence in Draft Genome	4,974
Length of Sequence in Draft Genome /bp	758,633,485
Number of Sequence in Clustering	2,883
Rate of Numbers in Clustering /%	57.96
Length of Sequence in Clustering /bp	746,659,745
Rate of Length in Clustering /%	98.42

**Table 5: The statistics of ordering results.**

Sample	Gossypium raimondii
Number of Sequence in Ordering	1,328
Rate of Number in Ordering /%	46.06
Length of Sequence in Ordering	744,578,885
Rate of Length in Ordering /%	99.72
Number of Sequence in Trunks	697
Rate of Number in Trunks /%	52.48
Length of Sequence in Trunks	724,960,878
Rate of Length in Trunks /%	97.37

**Table 6: The statistics of orienting results.**

Sample	Gossypium raimondii
Number of Sequence in Orienting	1,129
Rate of Numbers in Orienting /%	85.02
Length of Sequence in Orienting /bp	743,948,690
Rate of Length in Orienting /%	99.92

**Table 7: The statistics of pseudo-chromosome length.**

Pseudo-chromosome	Scaffolds number	Length /bp
chr1	171	73,966,406
chr2	98	65,258,067
chr3	93	63,950,047
chr4	94	62,276,720
chr5	117	60,231,642
chr6	76	60,016,944
chr7	88	59,096,273
chr8	107	58,208,991
chr9	69	56,972,541
chr10	125	53,706,612
chr11	90	51,080,163
chr12	99	49,533,500
chr13	101	30,412,479
Total anchored	1,328	744,710,385
Unanchored	3,646	14,054,600

We named the reassemble chromosome by its length order. The length of chromosome contained the 100 bp N between neighboring scaffolds.

**Table 8: The statistics of the draft genome.**

Parameters	Contigs length /bp	Scaffolds length /bp	Contigs number	Scaffolds number
Total	716,234,346	756,905,237	37,849	2,582
Max_length	333,622	10,920,000	-	-
Number>=2000bp	-	-	27,412	1,626
N50	44,885	1,600,000	4,810	139
N60	35,741	1,216,543	6,597	194
N70	27,678	947,408	8,872	264
N80	19,881	721,270	11,905	355
N90	11,331	439,650	16,558	485

All of the statistics got rid of the short scaffolds (scaffold length < 1,000bp).

**Table 9: The statistics of the reassembled *Gossypium raimondii* genome.**

Parameters	Contigs length /bp	Scaffolds length /bp	Contigs number	Scaffolds number
Total	716,283,110	757,086,669	37,921	1,329
Max_length	333,622	73,966,406	-	-
Number>=2000bp	-	-	27,412	462
N50	44,885	60,016,944	4,810	6
N60	35,740	58,208,991	6,598	8
N70	27,675	56,972,541	8,874	9
N80	19,873	53,706,612	11,907	10
N90	11,327	49,533,500	16,562	12

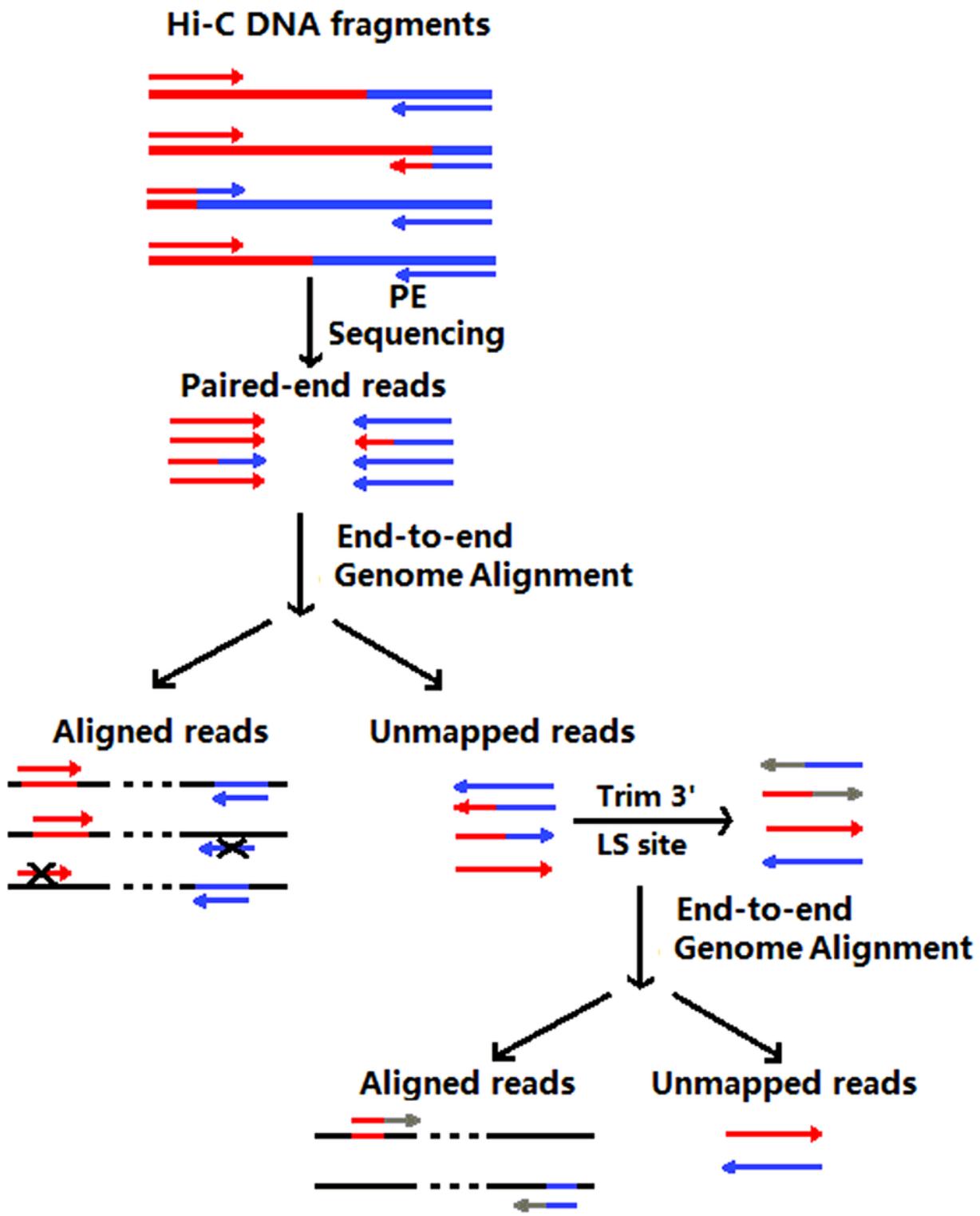
All of the statistics got rid of the short scaffolds (scaffold length < 1,000bp).

## Supplemental File 2

Supplemental 2: The reassemble genome.

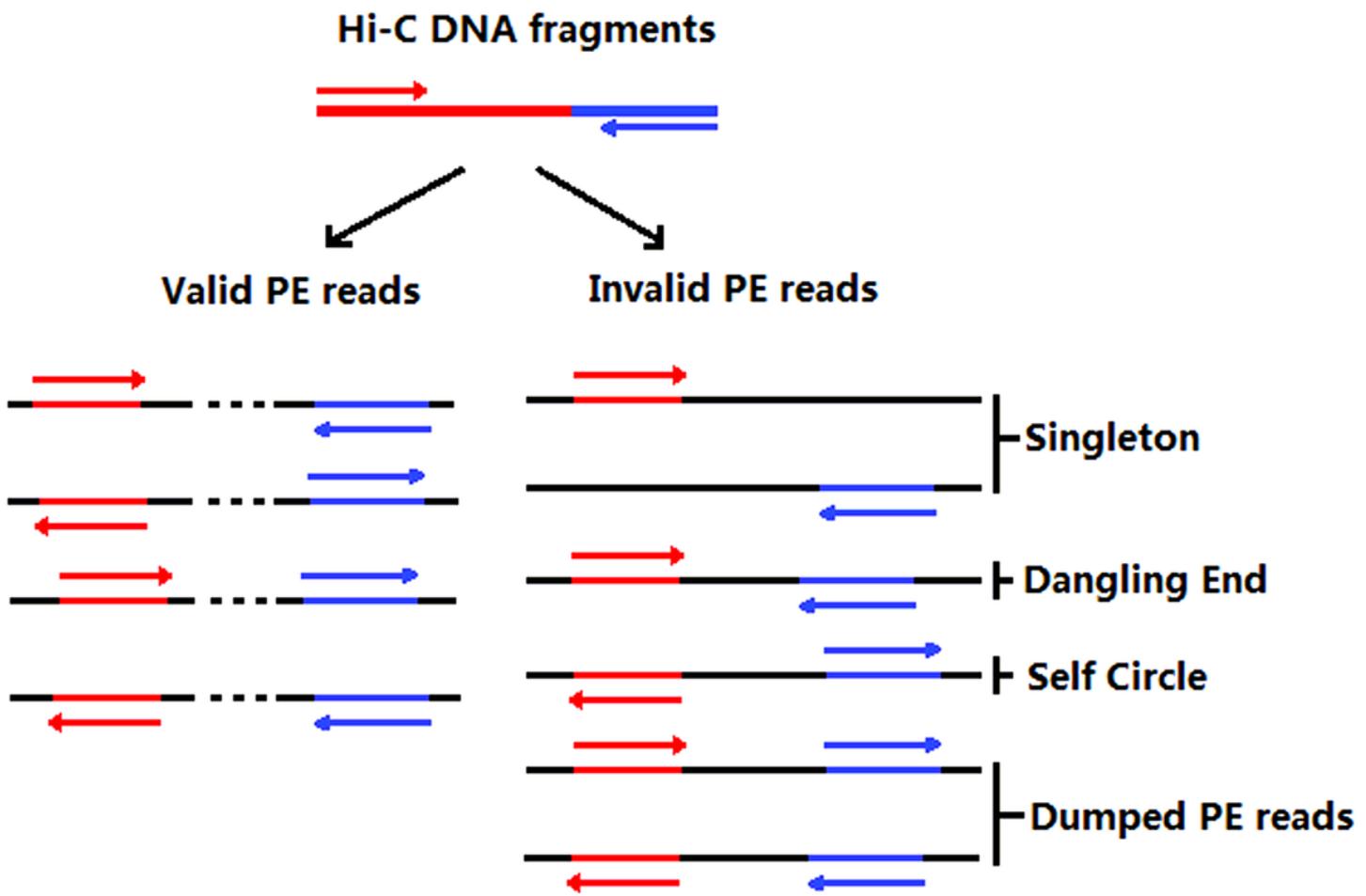
Supplemental file 2 was not provided with this version of the manuscript.

## Figures



**Figure 1**

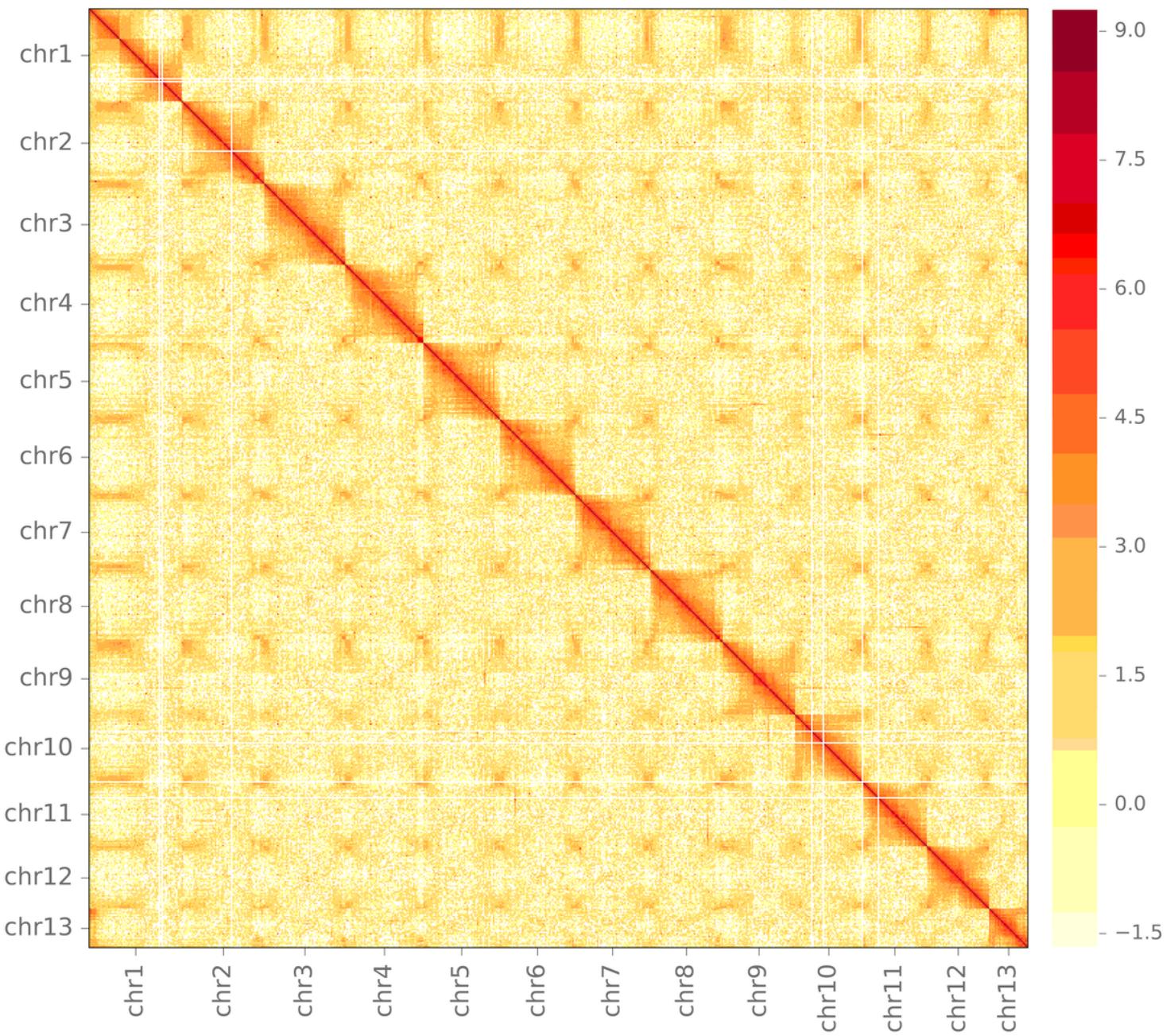
The pipeline of bowtie2. First, Reads1 and Reads2 are aligned to reference genome and then we cut the tails from 3' to the ligation-site and realigned it to reference genome. At last we merged the two results as the final mapped results.



**Figure 2**

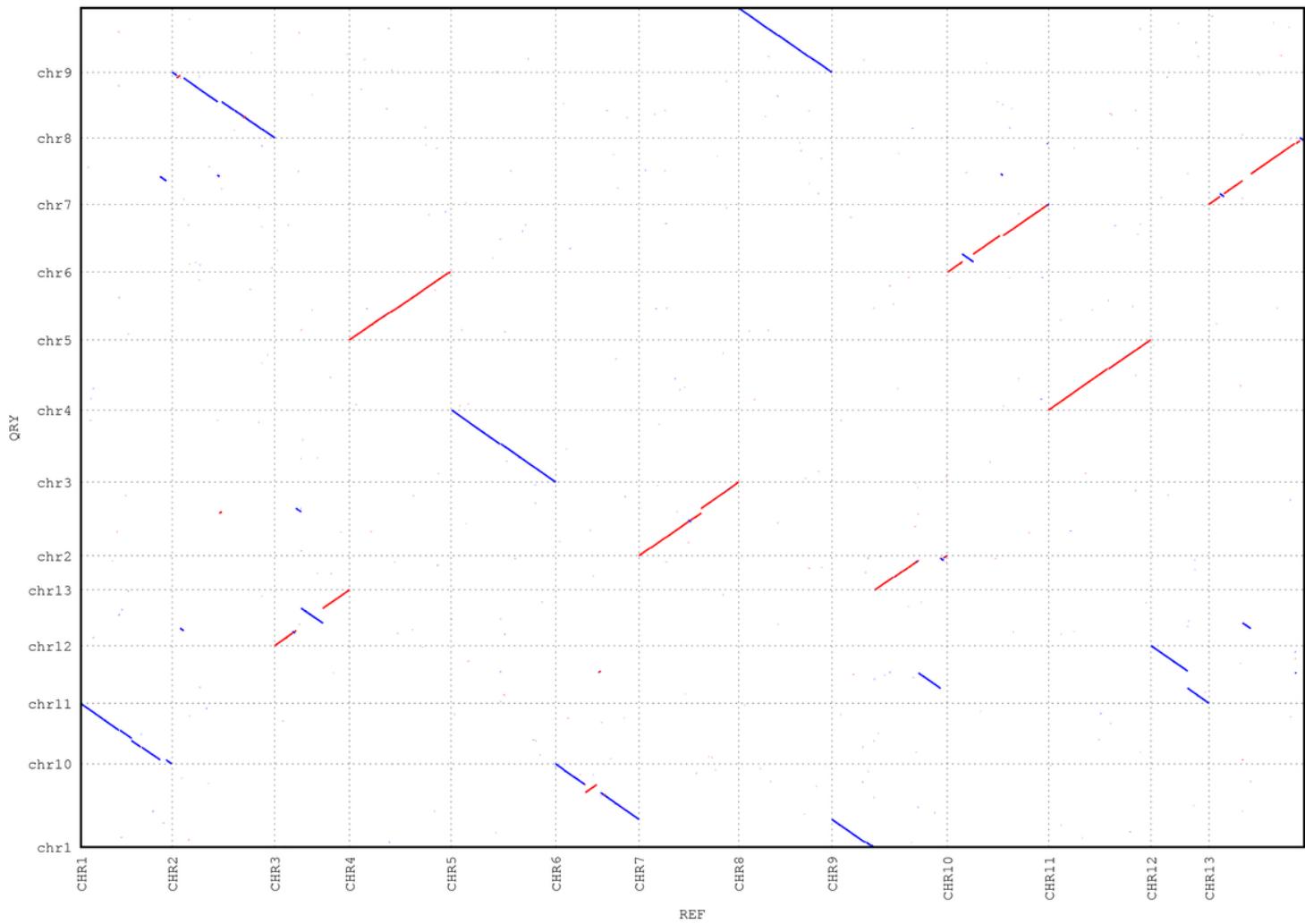
The corresponding situations of different alignment orientations.

G\_raimondii resolution=100000  
Genome-wide all-by-all Hi-C interaction



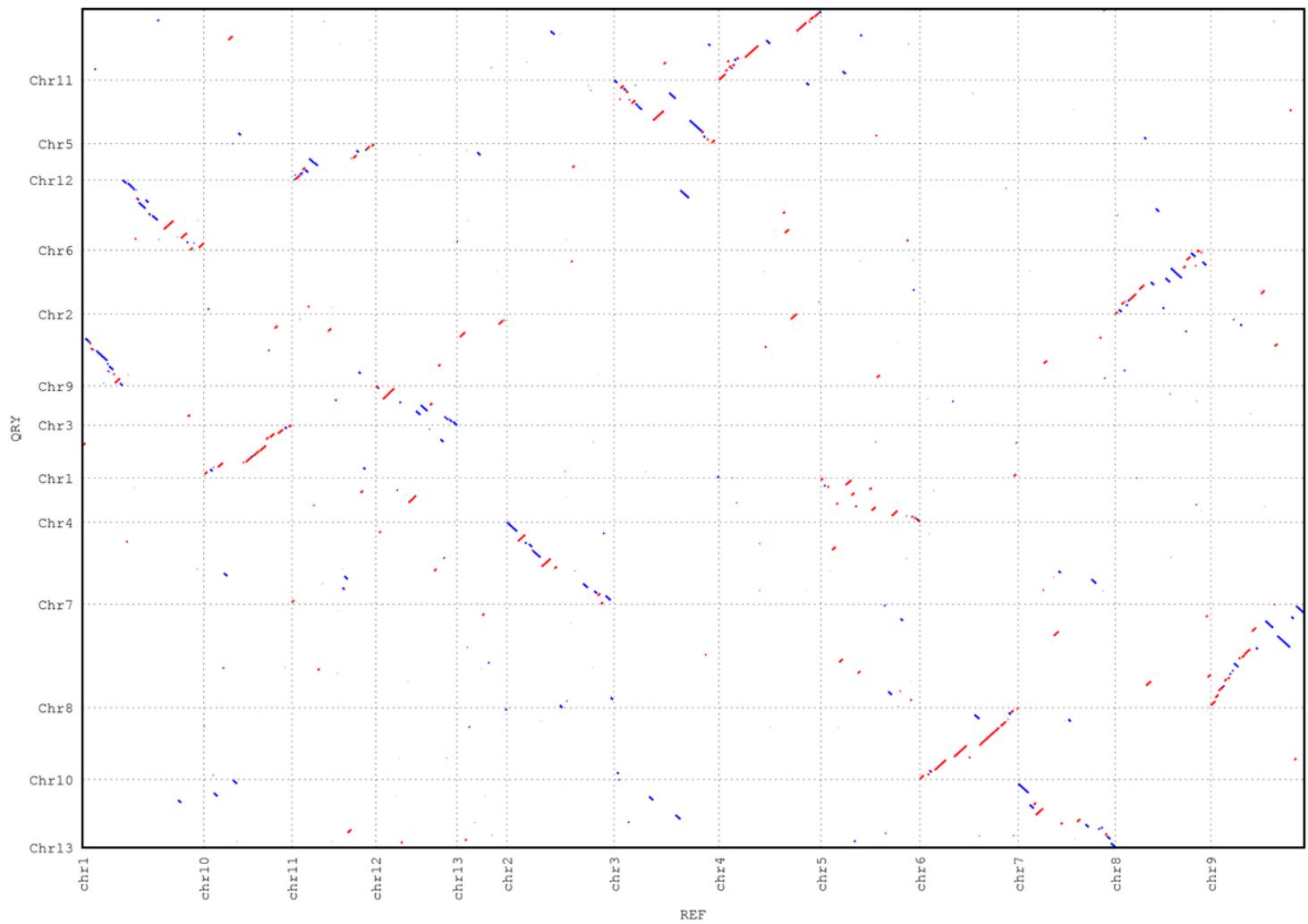
**Figure 3**

The heat-map of the reassembled result at a 100kb resolution. The darker red dot is, representing the higher interaction between bins



**Figure 4**

The collinearity analysis between reassemble (ORY) and draft genome (REF).



**Figure 5**

The collinearity analysis between the reassemble genome (ORY) and draft genome from Paterson et al. (2012) (REF).

## Supplementary Files

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