

# Improving genome assemblies using multi-platform sequence data

Pınar Kavak<sup>1,2,\*</sup>, Bekir Ergüner<sup>1</sup> Duran Üstek<sup>3</sup>, Bayram Yüksel<sup>4</sup>, Mahmut Şamil Sağıroğlu<sup>1</sup>, Tunga Güngör<sup>2</sup>, and Can Alkan<sup>5,\*</sup>

- <sup>1</sup> Advanced Genomics and Bioinformatics Research Group (İGBAM), BİLGEM, The Scientific and Technological Research Council of Turkey (TÜBİTAK),  
41470 Gebze, Kocaeli, Turkey  
pınar.kavak@tubitak.gov.tr
- <sup>2</sup> Department of Computer Engineering, Boğaziçi University,  
34342 Bebek, İstanbul, Turkey
- <sup>3</sup> Department of Medical Genetics, İstanbul Medipol University,  
34810 Beykoz, İstanbul, Turkey
- <sup>4</sup> TÜBİTAK - MAM - GMBE (The Scientific and Technological Research Council of Turkey, Genetic Engineering and Biotechnology Institute),  
41470 Gebze, Kocaeli, Turkey
- <sup>5</sup> Department of Computer Engineering, Bilkent University,  
06800 Bilkent, Ankara, Turkey  
calkan@cs.bilkent.edu.tr

**Abstract.** Accurate De novo assembly using short reads generated by next generation sequencing technologies is still an open problem. Although there are several assembly algorithms developed for data generated with different sequencing technologies, and some that can make use of hybrid data, the assemblies are still far from being perfect. There is still a need for computational approaches to improve draft assemblies. Here we propose a new method to correct assembly mistakes when there are multiple types of data generated using different sequencing technologies that have different strengths and biases. We exploit the assembly of highly accurate short reads to correct the contigs obtained from less accurate long reads. We apply our method to Illumina, 454, and Ion Torrent data, and also compare our results with existing hybrid assemblers, Celera and Masurca.

**Keywords:** de novo assembly, assembly improvement, next generation multi-platform sequencing

## 1 Scientific Background

Since the introduction of high throughput sequencing (HTS) technologies, traditional Sanger sequencing is being abandoned especially for large-scale sequencing projects. Although cost effective for data production, HTS also imposes increased cost for data processing and computational burden. In addition, the data quality is in fact lower, with greater error rates, and short read lengths for most platforms. One of the main algorithmic problems to analyze HTS data is the de novo assembly: i.e. “stitching” billions of short DNA strings into a collection of larger sequences, ideally the size of chromosomes.

However, “perfect” assemblies with no gaps and no errors are still lacking due to many factors, including the short read and fragment (paired-end) lengths, sequencing errors in basepair level, and the complex and repetitive nature of most genomes. Some of these problems in de novo assembly can be ameliorated through using data generated by different sequencing platforms, where each technology has “strengths” that may be used to fix biases introduced by others.

There are three kinds of assemblers mainly used to do genome assembly: i) greedy assemblers [1–3], ii) overlap-layout-consensus (OLC) graph based assemblers [4–6] and de Bruijn graph based assemblers [7–11]. Greedy assemblers follow a greedy approach as follows: given one read or contig, at each step assembler adds one more read or contig with the largest overlap. The problem of greedy assemblers is that they can get stuck at local maxima. Therefore they are generally used for small genome assemblies. Since they also use more memory and are slower, it is not feasible to assemble large genomes with greedy assemblers. OLC graph based assemblers work well when the long reads are available for assembly. They generate all-against-all pairwise alignments and build the graph by representing reads as nodes and overlaps between reads as edges. They obtain the consensus assembly by following a Hamiltonian path on the graph. Assemblers that are based on de Bruijn graphs are designed primarily for short reads. They use a k-mer graph approach rather than calculating all-against-all pairwise alignments. They build the graph by using k-mers as edges and the overlaps between k-mers as nodes. They follow an Eulerian path through the k-mer graph to find a consensus assembly. Several assemblers use multiple read libraries [12, 13, 15, 16] for better assembly construction. CABOG [12] was initially designed for Sanger sequencing, and then was revised to use 454 data, but it also accepts Illumina data to generate a hybrid assembly. Masurca [13] is able to assemble Illumina reads together with longer 454 and Sanger reads. MIRAest [15] can use Sanger, 454, Illumina, Ion Torrent and corrected PacBio data for hybrid assembly. It works on small genomes. Cerulean [16] uses long PacBio reads and short Illumina reads to construct a hybrid assembly. It uses assembly graphs generated by ABySS [10] assembler with paired end Illumina reads and the mapping of long PacBio reads to the assembled contigs, as inputs.

Additionally, strategies to merge different assemblies using different data sources into a single coherent assembly are described in the literature (e.g. [18]). Our method differs from that of [18], in data types. [18] works on Illumina, 454 and ABI SOLID data, where we work on Illumina, 454 and Ion Torrent data. Also pre- and post-processing steps of the two methods differ. [18] at first assembles 454, Illumina and SOLID data separately with different assemblers and then assembles the resulting contig collection again with another assembler.

In this work, we propose a method to improve draft assemblies (i.e. produced using a single data source, and/or single algorithm) by incorporating data generated by different HTS technologies, and applying novel correction methods. To achieve better improvements, we exploit the advantages of both short but low-error-rate reads and long but erroneous reads. We show that correcting the contigs built by assembling long reads through mapping short and high quality read contigs produce the best results, compared to the assemblies generated by algorithms that use hybrid data all at once. With this

study, we also have the opportunity to compare Ion Torrent and Roche/454 reads in terms of assembly performances.

## 2 Materials and Methods

We cloned a part of human chromosome 13 was into a bacterial artificial chromosome (BAC), and sequenced it separately using Illumina, Roche/454, and Ion Torrent platforms (Table 1). We also obtained a “gold standard” reference assembly for this BAC using GRCh37-guided assembly generated by Mira [14] using Roche/454 data, which we then corrected using the Illumina reads [17]. Since Roche/454 and Ion Torrent platforms have similar sequencing biases (i.e. problematic homopolymers), we separated this study into two different groups: Illumina & 454 and Illumina & Ion Torrent. We applied the same method on the two groups and evaluated them separately which gave us the opportunity to compare Roche/454 and Ion Torrent data. The flowchart of the pipeline is depicted in Figure 1.

Table 1. Properties of the data

Technology	Length range	Mean length	Mean base qual (phred s.)	Paired
Illumina	101bp (all reads have equal length)	101bp	38	paired
Roche/454	40bp-1027bp	650bp	28	single-end
Ion Torrent	5bp-201bp	127bp	24	single-end

Technology: The name of the sequencing technology used to produce the reads. Length range: Minimum and maximum lengths of the generated reads. Mean length: The mean length among all reads. Mean base qual: The average phred score sequence quality of all reads. Calculated by summing up all phred scores of the bases in a read and dividing it to sequence length of the read, over all reads. Paired: Represents whether the sequencing is performed as paired-end or single-end.

### 2.1 Pre-processing

Pre-processing steps consist of the following:

- First, we discard the reads that have low average quality value (phred score 17, i.e.  $\geq 2\%$  error rate).
- Then, we remove the reads with high N-density (with  $>10\%$  of the read consisting of Ns) from consideration.
- Third, groups of bases that seem to be non-uniform according to sequence base content (A,T,G,C) were trimmed (See Figure 2).
- Finally, we apply the pre-processing operations of each assembler we used.

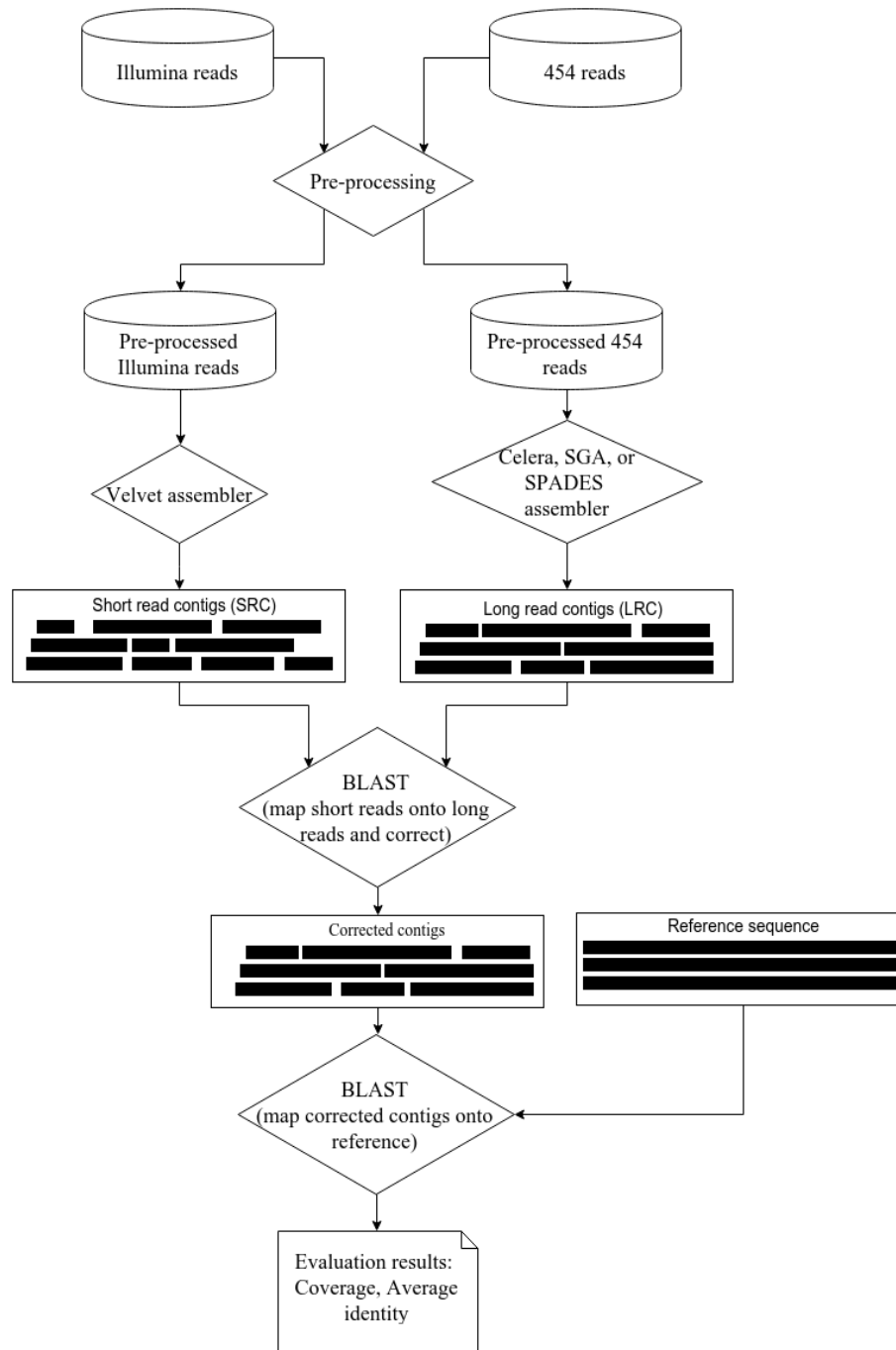


Fig. 1. Flow chart of the assembly improvement processes.

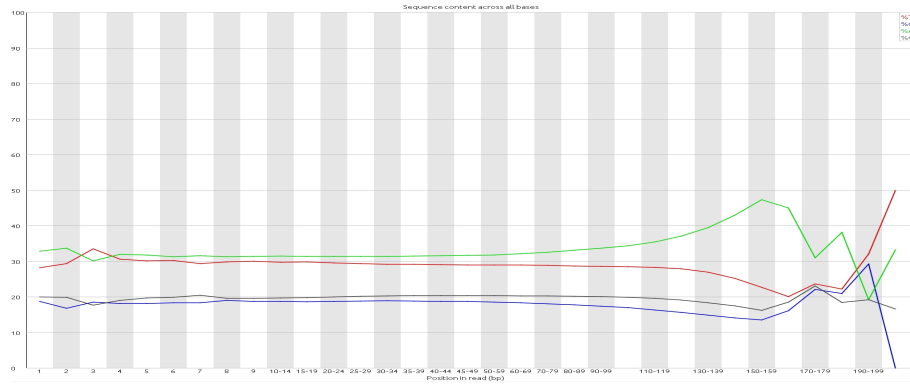


Fig. 2. Non-uniform A,T,G,C regions of Ion-torrent reads. First 8 bases and the bases after the 130<sup>th</sup> base are trimmed in pre-processing.

## 2.2 Assembly

After the pre-processing, we used several proper assembly tools to assemble different types of data: We used Velvet [7], a de Bruijn graph based assembler that is designed to assemble the short reads for assembling the Illumina reads. Considering the trimmed beginning and/or end parts of 101bp long paired-end reads from Illumina, and after testing kmers 21 and 31, we decided to use  $k=51$  for short read assembly. We ran Velvet with shortPaired mode with insert size 400bp, expected coverage 80, coverage cutoff 2, minimum contig length 100. N50 value of the resulting short read contigs was 8,865 bp. We used two different OLC assemblers: Celera [5], and SGA [6] to assemble the long read data sets (Roche/454 and Ion Torrent) separately. We ran Celera assembler in unmated mode and with default parameters to assemble 454 and Ion Torrent reads. N50 value of the assembly obtained with 454 and Ion Torrent reads with Celera was 1,308 bp and 1,284 bp respectively. We also used SGA assembler in unmated mode for the same data sets. We obtained N50 values of 505 bp and 117 bp for Roche/454 and Ion Torrent data respectively. In addition, we also used a de Bruijn graph based assembler, SPAdes [8], to assemble the long read data. Again, we applied default parameters. N50 values of the assemblies obtained with 454 and Ion Torrent reads with SPAdes were 212 bp and 259 bp, respectively.

We mapped all draft assemblies to the *E. coli* reference sequence using BLAST [19] to identify and discard *E. coli* contamination due to the cloning process. We discarded any contig that mapped to the *E. coli* reference sequence with sequence identity  $\geq 95\%$ . Finally, we obtained one short read, and three long read assemblies without contamination.

## 2.3 Correction

In the correction phase, we wanted to exploit the accuracy of the short read contigs (SRC) and the coverage of the long read contigs (LRC) to obtain a better assembly. Hence, we

mapped all SRCs onto all LRCs of each group and corrected the LRCs according to the mapping results. First, we used MegaBLAST [20] to map the SRC onto the LRC. We then used an in-house C++ program to process the MegaBLAST mapping results. Since MegaBLAST may report multiple mapping locations due to repeats, we only accepted the “best” mapping locations. Reasoning from the fact that the short reads show less sequencing errors, we preferred the sequence reported by the SRC over the LRC when there is a disagreement between the pair. By doing this, we patched the “less fragmented” long read assemblies. If there is an overlap between different SRC mappings at the same region on the LRC the latter overwrites the first. Figure 3 shows a visual representation of the strategy on correcting the LRCs.

Briefly, we apply our strategy as follows:

- If there is a mapping between a SRC and a LRC, and if the mapping does not start at the beginning of the LRC, add the unmapped prefix of the LRC.
- Also, if the mapping does not start at the beginning of the SRC (very rare situation), add the unmapped prefix of the SRC with lowercase (i.e. low confidence) letters.
- Over the mapping region between SRC and LRC, pick the SRC values.
- If the mapping does not end at the end of the SRC (rare), add the unmapped suffix of the SRC, again with lowercase letters. One may argue that it might disturb the continuity of the resulting contig, however, we observe such mapping properties very rarely. The reason for using lowercase letters is to keep track of the information that there is a disagreement between the SRC and LRC on these sections, so the basepair quality will be lower than other sections of the assembly.
- Finally, add the unmapped suffix of the LRC and obtain the corrected contig.

We repeated this process to correct each of the three long read assembly contig sets. We applied our correction strategy on each data set multiple times until there is no improvement in the Coverage and Average Identity metrics.

## 2.4 Evaluation

To evaluate and compare the resulting and corrected assemblies all-against-all we mapped all of the assembly candidates, including primary assemblies and also final corrected assemblies to the “gold standard” BAC assembly. According to the alignment results, we calculated various statistics such as the number of mapped contigs, how many bases on the reference sequence were covered, how many gaps exist on the reference sequence, and total gap length. We calculated metrics such as “Coverage” and “Average Identity” and compared the resulting assemblies with these metrics.

To calculate these statistics, we kept an array of `arr_reference[0,0,0,...0]`, where, `length(arr_reference)= length(reference)`. We updated the contents of `arr_reference` according to the alignments. If there is a match at a location, we flipped it to as “1”, if there is a mismatch at a location, we set it as “-1”, and if that location is not included in any alignment, we left it as “0” (which means a gap). We assumed deletions in the contig (query) as mismatches. We also calculated the number of insertions in the contig. Going through the array and summing up the number of “1”s, “-1”s, “0”s and “insertionInQuery”, at the end, we calculated the number of matches, mismatches, gaps, and

insertions in contig. Using these numbers, we calculated the Coverage (Equation 1) and Average Identity values (Algorithm 1).

We also used two hybrid assemblers, Celera-CABOG [12] and Masurca [13], with Illumina & 454 and Illumina & Ion Torrent. These hybrid assemblers load all reads as input and assemble them with a hybrid method. We assembled the two data sets with these hybrid assemblers to compare our correction methodology with the results of them. The evaluation results are listed in Table 2.

$$\text{Coverage} = \left( \frac{\# \text{ of covered bases}}{\text{length of the reference}} \right) \quad (1)$$

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**Algorithm 1** Average identity

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while no contigs left do
  alignmentLength  $\leftarrow$  matches + mismatches + insertionInContig
  identity  $\leftarrow$   $\left( \frac{\text{matches}}{\text{alignmentLength}} \right)$ 
  avgIdentity  $\leftarrow$  avgIdentity + identity  $\times$  contigLength
end while
avgIdentity  $\leftarrow$   $\left( \frac{\text{avgIdentity}}{\sum_{i=1}^{\text{contigNum}} \text{contigLength}_i} \right)$ 

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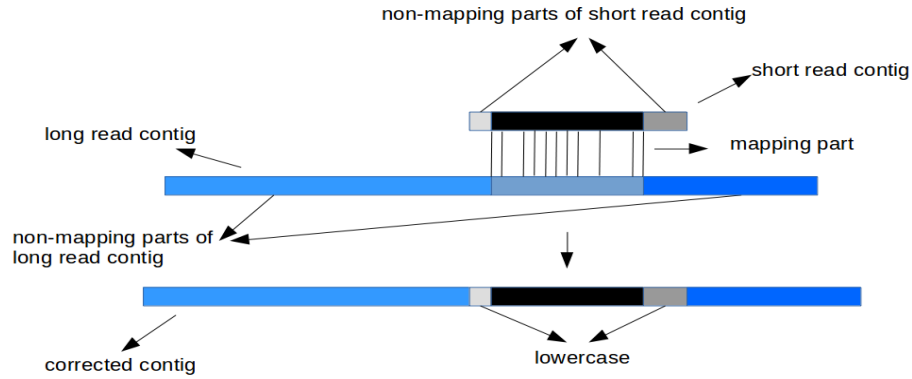


Fig. 3. Correction method: Correct the long read contig according to the mapping information of the short read contig.

### 3 Results and Conclusion

We present the results on Table 2, and interpret them in different categories.

### 3.1 454 vs. Ion Torrent

As we see in Table 1, Ion Torrent reads are shorter than 454 reads and they have less mean base quality. So, we did not expect to have better assembly with Ion Torrent reads than 454 reads. The results in Table 2 agree with our expectations. In Table 2, we see that the assembly of 454 reads perform better on evaluation metrics than Ion Torrent with all kind of assemblers. The assembly of Ion Torrent reads with Celera assembler has very low coverage value: 26.94%. The reason for the low coverage might be because Celera assembler is not designed for Ion Torrent read type (shorter reads with lower quality), even 454 and Ion Torrent reads have similar error types at the homopolymer regions. SGA assembly with Ion Torrent reads perform better on Coverage (86.57%) but it cannot reach to the Coverage of SGA assembly with 454 reads (99.83%). The assembly of Ion Torrent reads has the highest coverage with SPAdes assembler (94.94%). Correction of the Ion Torrent contigs improves the assembly quality but even after correction phase Ion Torrent corrected assembly cannot reach the results of 454 corrected assembly.

### 3.2 Assemblers

On Table 2, the assembly obtained by Velvet with only short Illumina reads showed good coverage (99%) and average identity rates (97.5%). The number of contigs obtain with Velvet assembly is 455, of which 437 maps to the reference. There are 39 gaps and the total size of the gaps is 1,671 bp. Our aim was to increase the coverage, improve the average identity and decrease the number of contigs and gaps, and shrink the lengths of the gaps.

Since we observed that 454 reads resulted better assembly than Ion Torrent reads in Section 3.1, we compared different assemblers through 454 contigs. The assembly of Celera with the 454 long reads has 97.5% coverage and 92.6% average identity, which are lower than Illumina-Velvet values. Number of contigs (735) is reasonable but number of gaps and total gap length are high (18 and 4,280 bp, respectively). SGA assembly using 454 reads has very high coverage (99.83%) and identity (97.4%). It has just one gap with size 297 bp, but the number of contigs is also very high (101,514), which is an unwelcome situation. SPAdes-454 assembly also had a large number of contigs (49,824) which completely cover the reference sequence with 98% average identity. SPAdes assembly resulted in lower number of contigs and had higher coverage and average identity than SGA. If we evaluate the results according to the number of contigs, Celera-454 results seem more reasonable than SGA or SPAdes results, since it returned a reasonable number of contigs even with low coverage and average identity.

### 3.3 Correction

We observed that the correction method improved both 454 and Ion Torrent based assemblies generated with all assemblers we tested (Table 2). In the remainder of the paper, we only mention the 454-based assemblies for simplicity.

When we applied our correction method on Celera-454 assembly using the Velvet-Illumina assembly, we achieved better coverage and average identity rates: the coverage of 454 assembly increases up to 99.7% and the average identity rate increases up to



94.4% on the first correction cycle. The second correction cycle increases the coverage and average identity rates up to 99.7% and 94.5%, the correction converges. The number of contigs decrease to 245 from 735, and the number of gaps decrease down to 4 (500 bp) from 18 (4,280 bp). Since the third correction cycle does not give better results it is not shown on Table 2.

Our correction method increased the coverage of SGA-454 assembly up to 99.9% from 99.8% but with less average identity and with more gaps although the total length of the gaps is decreased. Correction using the short read assembly decreased the number of contigs down to a reasonable number (335). Corrected SGA assembly has the largest coverage rate among all, and also with more identity than Velvet-Illumina assembly.

The number of contigs in SPAdes assembly also decreased to 298 from 49,691 using our correction method. With the decrease in number of contigs, the coverage also decreased (99.7%) as well as the average identity (96.5%). The number of gaps increased to 5 from 0 with total size 389.

In summary, we obtained substantial assembly correction in draft assemblies by using advantages of different technologies.

### 3.4 Hybrid Assemblers

We also compared the results of two hybrid assemblers on our multiple type of data. We ran Masurca and Celera-CABOG with default parameters given two groups of hybrid data as input: Illumina & 454 and Illumina & Ion Torrent. Hybrid assemblers Masurca and CABOG did not show good assembly rates. We obtained zero coverage with 454 and Illumina reads using Masurca. The only contig left after the contamination removal did not map to the reference sequence. We also observed very low coverage (1.1%) with 98.2% average identity with Ion Torrent & Illumina reads. Therefore, we conclude that Masurca did not work very well in our case with our data types.

Similarly, we obtained zero coverage with Ion Torrent & Illumina using CABOG. All of the resulting contigs obtained from the assembly were removed as contamination. However, CABOG performed substantially better with Illumina & 454, and generated assembly with 98.58% coverage and 92.5% average identity. The assembly composed of 891 contigs and 12 gaps with total gap length of 2,513 bp. Still, the performance of CABOG was not better than the corrected assembly results described above.

## 4 Conclusion

Here we presented a new method to improve draft assemblies by correcting high contiguity assemblies using the relatively more fragmented contigs obtained using high quality short reads. Assembling short and long reads separately using both de Bruijn and OLC graph based assemblers according to data types and then using correction methods gives better results than using only hybrid assemblers.

However, the need to develop new methods that exploit different data properties of different HTS technologies, such as short/long reads or high/low quality of reads, remains. In this manner, as future work, our correction algorithm can be improved by exploiting the paired end information of the short, high quality reads after the correction phase to close the gaps between corrected contigs.

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Table 2. Results of assembly correction method on BAC data.

Name	Length	# of Contigs	# of Mapped Contigs	# of Covered bases	Coverage	Avg. Identity	# of Gaps	Size of Gaps
Reference	176,843							
Velvet								
III-Velvet	197,040	455	437	175,172	0.99055	0.97523	39	1,671
Celera								
454 Celera	908,008	735	735	172,563	0.97580	0.92599	18	4,280
Ion Celera	39,347	27	27	47,638	0.26938	0.96932	47	129,205
Corrected Celera								
III-454 Celera	371,065	250	250	176,071	0.995635	0.944558	5	772
III-454 Celera <sup>2*</sup>	365,802	245	245	176,343	0.9971	0.9455	4	500
III-Ion Celera	93,909	30	28	81,819	0.46267	0.96327	36	95,024
III-Ion Celera <sup>2</sup>	145,262	30	28	91,962	0.52002	0.97412	33	84,881
III-Ion Celera <sup>3</sup>	216,167	30	28	99,645	0.56347	0.98066	34	77,198
SGA								
454 SGA	62,909,254	108,095	101,514	176,546	0.99832	0.97439	1	297
Ion SGA	842,997	6,417	6,122	153,092	0.86569	0.99124	197	23,751
Corrected SGA								
III-454 SGA	295,009	335	335	176,757	0.99951	0.96823	5	86
III-Ion SGA	197,509	291	291	175,052	0.98987	0.97501	45	1,791
III-Ion SGA <sup>2</sup>	203,064	291	291	175,676	0.99340	0.97413	34	1,167
SPADES								
454 SPADES	12,307,761	49,824	49,691	176,843	1.0	0.98053	0	0
Ion SPADES	176,561	110	107	167,890	0.94937	0.92909	9	8,953
Corrected SPADES								
III-454 SPADES	290,702	298	298	176,454	0.99780	0.96538	5	389
III-Ion SPADES	198,665	52	52	171,977	0.97248	0.94215	4	4,866
III-Ion SPADES <sup>2</sup>	200,307	52	52	172,101	0.97319	0.94230	2	4,742
Masurca								
III-454 Masurca	380	1	0	0	0	0	0	0
III-Ion Masurca	2,640	8	8	1,952	0.01104	0.98223	9	174,891
Celera-CABOG								
III-454 Celera	1,101,716	891	891	174,330	0.98579	0.92452	12	2,513
III-Ion Celera	0	0	0	0	0.0	0.0	0	0.0

Name: the name of the data group that constitute the assembly; # of contigs: the number of contigs that belong to the resulting assembly; # of Mapped Contigs: the number of contigs that successfully mapped onto the reference sequence; # of Covered bases: the number of bases on the reference sequence that are covered by the assembly; Coverage: percentage of covered reference; Avg. identity: percentage of the correctly predicted reference bases; # of Gaps: The number of gaps that cannot be covered on the reference genome; Size of Gaps: total number of bases on the gaps.

\* “2” represents the results of the second cycle of correction, “3” represents the third cycle.