An Improved Genome Assembly of *Azadirachta indica* A. Juss.

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Abstract

Neem (*Azadirachta indica* A. Juss.), an evergreen tree of the *Meliaceae* family, is known for its medicinal, cosmetic, pesticidal and insecticidal properties. We had previously sequenced and published the draft genome of the plant, using mainly short read sequencing data. In this report, we present an improved genome assembly generated using additional short reads from Illumina and long reads from Pacific Biosciences SMRT sequencer. We assembled short reads and error-corrected long reads using Platanus, an assembler designed to perform well for heterozygous genomes. The updated genome assembly (v2.0) yielded 3- and 3.5-fold increase in N50 and N75, respectively; 2.6-fold decrease in the total number of scaffolds; 1.25-fold increase in the number of valid transcriptome alignments; 13.4-fold less mis-assembly and 1.85-fold increase in the percentage repeat, over the earlier assembly (v1.0). The current assembly also maps better to the genes known to be involved in the terpenoid biosynthesis pathway. Together, the data represents an improved assembly of the *A. indica* genome.

The raw data described in this manuscript are submitted to the NCBI Short Read Archive under the accession numbers SRX1074131, SRX1074132, SRX1074133, and SRX1074134 (SRP013453).

Introduction

High-throughput sequencing platforms, especially those based on short-read technology, have enabled sequencing of many plant genomes (Michael and Jackson, 2013). This has substantially improved our understanding of genome organization, evolution and complexity in different plant species. However, most first generation genome assemblies are draft and incomplete assemblies. The correctness and accuracy of genome assembly depends on the length of the sequencing reads, errors generated during sequencing and the accuracy of the computational tools (assemblers and downstream annotation pipelines) used. Additionally, most genome assemblers are not suitable to assemble genomes of heterozygous plants, a characteristic feature of most plants in the wild

(Kajitani, Toshimoto, et al., 2014). Draft assemblies often bear significant gaps and,errors, yielding less accurate gene predictions and annotations. This is compounded by the usage of incomplete training-sets with gene prediction algorithms and absence of a representative transcriptome that can correctly anchor to the genome. Therefore, it is imperative to improve the quality of draft genome assemblies with the help of longer reads using genome assemblers tailored to handle heterozygosity, and make gene predictions using updated training-sets and gene annotations using combinatorial approaches not fully reliant on sequence similarity such as BLAST.

Neem (*Azadirachta indica* A. Juss.), belonging to the order *Rutales*, family *Meliaceae*, is an important woody angiosperm, given its many medicinal and agrochemical uses. We had previously sequenced and reported the draft genome and five organ-specific transcriptomes (Krishnan, Pattnaik, et al., 2011, Krishnan, Pattnaik, et al., 2012) of the neem tree. The neem genome was the 38th plant genome to be sequenced (Michael and Jackson, 2013). The genome assembly was generated using short paired-end reads (76 bases or shorter) from Illumina GAIIx with a first generation genome assembler, SOAPdenovo (Li, Zhu, et al., 2010). This was followed by genome annotation and gene prediction analysis, analysis of repeat elements, phylogenetic analysis and gene expression studies (Krishnan, Pattnaik, et al., 2012). In the current report, we have improved the quality of the neem genome assembly by using [a] additional long-insert libraries from Illumina Hiseq, [b] long reads from a third generation sequencer by Pacific Biosciences (PacBio), [c] LoRDEC (Salmela and Rivals, 2014), an algorithm that takes short reads from Illumina and uses those to correct errors in the PacBio reads, and [d] assembling the genome with short and error-corrected long reads using Platanus (Kajitani, Toshimoto, et al., 2014) which is better suited to assemble heterozygous genomes. We re-assembled all five organ-specific RNA libraries into a pooled representative transcriptome, using Trinity (Grabherr, Haas, et al., 2011, Haas, Papanicolaou, et al., 2013), and employed the Program to Assemble Spliced Alignments (PASA, Haas, Delcher, et al., 2003) to benchmark the completeness of previous (v1.0), intermediate, and current (v2.0) genome assemblies based on their mappability to this transcriptome. We also performed gene prediction analyses with GlimmerHMM (Majoros, Pertea, et al., 2004, v3.0.4) using updated training-sets from Citrus species, which were found to be evolutionarily closer to neem by our earlier phylogenetic analyses (Krishnan, Pattnaik, et al., 2012). Building on our draft assembly, here, we present data on different assembly parameters, accuracy, gaps, gene predictions and the total repeat content as evidence towards an improved neem genome assembly.

Materials and Methods

Assembly

In addition to the Illumina read libraries used for assembling the previously published draft neem genome (Krishnan, Pattnaik et al. 2012), four more libraries were used for updating the assembly. We included reads from three Illumina mate-pair (with insert sizes 4kb, 6kb and 10kb) and one PacBio (average read length >2kb, varying up to 17.64kb) libraries. Details of all libraries used are presented in Supplementary Table 1.

We pre-processed all the libraries as follows. In the case of Illumina libraries, exact read duplicates were removed using the '*in silico* normalization' utility from Trinity. For PacBio, reads were error-corrected using LoRDEC v0.4.1 based on the two paired-end Illumina libraries (Supplementary Table 1). K-mers ranging from 19 to 36 were tested for error-correction.

We made an effort to assemble intact PacBio reads following error-correction using the PacBioToCA (Koren, Schatz, et al., 2012) pipeline and Celera WGS assembler v7.1 (Myers, Sutton, et al., 2000). However, this process was CPU- and RAM-intensive, and also resulted in a sub-optimal assembly (data not shown). We, therefore, converted the PacBio reads, with and without error-correction, into Illumina-like paired-end reads (read lengths of 100 bases and average insert size of 350 bases) using SInC’s read generator (Pattnaik, Gupta, et al., 2014), which could be easily assembled using SOAPdenovo, SOAPdenovo2 (Luo, Liu, et al., 2012) and Platanus. Converting PacBio reads to Illumina-like reads did not nullify the advantage of the long reads, in terms of contiguity (Supplementary File 1).

We produced 13 intermediate assemblies (Supplementary Table 2) for quality comparison, as follows:

a) re-assembly of the published version using SOAPdenovo with Illumina short reads (R.S1/ v1.0)
b) assembly using additional Illumina libraries using SOAPdenovo2 (S2.DUP)
c) assembly of all Illumina duplicate-removed libraries using SOAPdenovo2 (S2)
d) assembly, using SOAPdenovo2, of all Illumina duplicate-removed libraries along with the error-corrected PacBio reads (S2.ecPB.21 and S2.ecPB.32, using kmers 21 and 32, respectively)
e) assembly using Platanus of all Illumina duplicate-removed libraries alone (P), or along with either the error-corrected PacBio reads using 19- (P.ecPB.19), 21- (P.ecPB.21), 32- (P.ecPB.32) and 36-mers (P.ecPB.36), or along with uncorrected PacBio reads (P.ucPB)
f) assembly and gap closing, using Platanus, of all Illumina duplicate-removed libraries and the PacBio library with (P.ecPB.32.gc/v2.0; kmer = 32) or without (P.ucPB.gc) error-correction.

All assembly QCs were performed using QUAST v2.3 (Gurevich, Saveliev, et al., 2013). The assembly NG50 was estimated assuming the neem genome size as 364Mb (Krishnan, Pattnaik, et al., 2012). We refer to the R.S1 assembly as v1.0 (previous) and the P.ecPB.32.gc assembly as the improved v2.0 (current), in our comparisons statistics below.

Assembly mapping to transcriptome using PASA

PASA r20140417 was used to compare and evaluate all the assemblies. The representative neem transcriptome was assembled *de novo* using Trinity v2.0.6 with five tissue-specific published RNA-seq libraries. This transcriptome was mapped to various genome assemblies using PASA and the numbers and lengths of valid alignments, failed alignments, and transcript assemblies were compared. In addition, the numbers and lengths of exon-only regions of the valid alignments were also extracted and compared across the assemblies.

Gene prediction using GlimmerHMM

GlimmerHMM was used for benchmarking the assemblies. We created training-sets based on *Citrus sinensis* and *Citrus clementina* (genes.gff3 files downloaded from http://phytozome.jgi.doe.gov/pz/portal.html), and used the inbuilt *Arabidopsis thaliana* training-set to predict genes and gene structures in the neem assemblies. Both citrus species were used here since they were found to be the evolutionarily closest to neem, among sequenced species (Krishnan, Pattnaik, et al., 2012).

Repeat analyses

RepeatModeler v1.0.8 (Smit and Hubley, 1989), employing Repeat Scout, Tandem Repeat Finder and Recon modules, was used to construct a library of novel repeats entirely based on the neem genome. Other tools such as LTR_finder v1.0.5 (Xu and Wang, 2007), TransposonPSI v08222010 (Haas, 2007-10) and MITE-hunter v11-2011 (Han and Wessler, 2010), were used to identify Long Terminal Repeats (LTRs), retro-transposons, and Miniature Inverted repeat Transposable Elements (MITEs), respectively. The neem genome assembly was masked using RepeatMasker v4.0.5 (Smit and Hubley, 1989) with all these repeats and the updated plant (*Viridiplantae*) libraries from Repbase (Kapitonov and Jurka, 2008), to estimate the non-redundant genomic repeat content. This was further classified using the RepeatClassifier module of RepeatModeler.

Identification of FDFT1 *and* SQLE *gene structures across assemblies*

We obtained the transcript sequences corresponding to *FDFT1* and *SQLE* genes in *C. clementina* from KEGG (Kanehisa and Goto, 2000; Kanehisa, Goto, et al. 2014), and created a database of these sequences using the makeblastdb utility in the BLAST package v2.2.29 (Altschul, Gish, et al. 1990). These genes belong to the sesqui- and tri-terpenoid biosynthesis pathways, involved in the synthesis of the commercially important compound, azadirachtin, and hence were chosen for comparative analyses here. The neem transcriptome was mapped against the database using BLAST with an Expect (E) value threshold of 0.001. The mapped neem transcripts were traced to their PASA alignments in various genome assemblies. In cases where the identified transcripts for the same reference gene aligned to multiple neem scaffolds, consensus exon-intron structures were inferred individually for each scaffold, and the one agreeing best with the *C. clementina* gene structure was considered. The gene structures for all assemblies were plotted along with the corresponding gene structure in *C. clementina* using 'Structure Draw' (http://www.bioinformatics.uni-muenster.de/tools/strdraw/index.hbi). Regions of gaps (N's) in the assembly were highlighted in red.

All scripts used in the assembly, QC, evaluation, genome-to-transcriptome mapping, gene prediction and repeat analyses pipeline are presented under Supplementary File 2.

Results

Quality comparison across all versions of neem genome assembly

We compared the correctness and completeness of all the assembly versions based on three measures:

1. Assembly statistics using QUAST
2. Metrics from transcriptome-to-assembly alignment using PASA
3. Gene and gene-structure prediction based on three different training-sets using

GlimmerHMM

The first measure strictly quantifies the completeness of the assembly, while the middle one mainly quantifies the correctness of the assembly, and its completeness to the extent that the draft transcriptome is complete, and the last measure quantifies the completeness of the assembly, but also its correctness, with the assumption that the genes and gene structures in the organisms used as a training-sets are present, as is, in the neem genome. Detailed metrics from all the benchmarking tools are provided in Supplementary Table 2.

Comparison of assembly statistics

Overall, assembly statistics improved with Platanus over SOAPdenovo or SOAPdenovo2 (Figure 1 and Supplementary Table 2), with the best assembly (v2.0) produced by Platanus using a combination of all duplicate-removed Illumina read libraries and error-corrected (*kmer* = 32) PacBio library in all the three stages - assembly, scaffolding and gap-closing. The scaffold numbers and the assembly size here were reduced by 2.6- and 3-fold, respectively, over those from the earlier draft assembly (v1.0; Figure 1). The assembly using uncorrected PacBio reads, in combination with Illumina libraries (P.ucPB), resulted in the longest scaffold (12,211,325 bases). However, other important quality metrics were compromised for this assembly. N50 and N75 were highest for Platanus assembly using all Illumina-only reads (P; 4,002,232 and 1,489,583 bases, respectively). The v2.0 assembly revealed a 13.4-fold reduction in gaps over the v1.0 assembly (an average of 5414.21 Ns per 100kb, Figure 1A) and a 2.26-fold lowered NG50. Incidentally, the NG50 for the Platanus assembly using Illumina-only reads (P; 1,587,838 bases) was comparable to that using SOAPdenovo (v1.0; 1,663,167 bases). Almost 60% of each assembly was covered at 5X when PacBio reads were assembled, along with Illumina read libraries, using SOAPdenovo2 or Platanus (Supplementary Table 2).

Comparison of transcriptome-to-genome alignment metrics

The numbers and cumulative lengths of all valid alignments and PASA assemblies were highest at 77,635 and 61,292, ~100Mb and ~99Mb, respectively, for the v2.0 assembly (Supplementary Table 2). The cumulative size of valid exonic alignments was also highest at ~48Mb for this assembly, and the corresponding numbers and lengths of all failed alignments were least at 6,584 and ~32Mb, respectively (Supplementary Table 2). The overall valid alignments increased 1.25-fold, and the ones in exons increased by 1.95-fold for the updated (v2.0) assembly over the old one (v1.0) (Figure 1B). Failed alignments went down by 3.5- and 5.9-fold in number and cumulative size, respectively (Figure 1B).

Comparison of predicted genes

We found the highest number of predicted genes and exons, using training-sets from any of the three organisms (*A. thaliana*, *C. sinensis*, *C. clementina),* with the v2.0 assembly (Supplementary Table 2 and Figure 2). The cumulative length of all predicted genes was highest for this assembly (68,723,917 bases) when *A. thaliana* was used as the training-set. When *Citrus* species were used as training-sets, however, the v1.0 assembly resulted in the highest cumulative predicted gene lengths (473,787,912 and 431,305,649 bases, respectively, with *C. sinensis and C. clementina*). The predicted gene lengths were comparable between both the assemblies after excluding gaps, suggesting this to be mostly a result of mis-assembly (Figure 2).

We found an abundance of smaller (< 100 bases) mRNAs and exons in gene predictions in the v1.0 assembly, especially with *Citrus* training-sets, which were substantially reduced in the v2.0 assembly (Figure 3). In contrast, the longer mRNAs were more abundant in the latter assembly, with Citrus training-sets, an indication of improvement in the assembly.

Comparison of gene structures of FDFT1 *and* SQLE *across various assemblies*

In order to demonstrate the biological significance of the improved assembly, we used *FDFT1* and *SQLE* genes, two important genes involved in the sesqui- and tri-terpenoid biosynthesis pathways. We observed that the gene structures of *FDFT1* and *SQLE* were more complete and accurate in the improved v2.0 assembly when compared to the v1.0 assembly (Figure 4 and Supplementary Figure 2). Using Platanus alone, and augmenting the libraries with additional short Illumina mate-pair libraries yielded a better *FDFT1* gene structure. Similarly, using Platanus as an assembler along with pre-and post- processing yielded a better assembly of the multi-isoform *SQLE* gene.

Estimation of repeat content

The non-redundant repeat content was estimated to be 54,375,206 bases (24.15% of v2.0), which is higher than the 47,427,034 bases reported earlier (13.03% of v1.0). We further classified the repeats into distinct classes, as shown in Supplementary Table 3.

Discussion

Here, we report an improved genome assembly of *A. indica* and provide quantitative evidence on various parameters in support of the improved assembly. The current assembly benefits from using additional Illumina mate-pair reads and long reads from PacBio, a third generation sequencing platform. Additionally, we have used Platanus, a tool designed to assemble heterozygous genomes, such as that of neem (Supplementary Figure 1), better, and an algorithm that uses short reads to correct the errors in long reads. Finally, we have used updated and near complete training-sets from closely related plant species to predict gene structures, and an equally enriched and updated repeat library to predict repeat sequences in the neem genome.

In our study, we employed PASA and GlimmerHMM to benchmark the assemblies, both of which have their limitations in the current context. PASA assumes that the transcriptome is free of mis-assembly errors. The caveat with GlimmerHMM, is that the gaps and errors in the genomic assembly extends to the predictions (Figure 2). We found that the number of gene predictions decreased across assemblies, post-redundancy removal using cd-HIT-EST (Li and Godzik, 2006). Additionally, the gene predictions are only as good as the training-sets used. Presence of a large number of very short, possibly spurious, exons in the *C. clementina* training-set manifested in a large number of similar predictions in the neem assembly (Figure 3). However, as expected, either these did not align to the neem transcriptome, or a large fraction of those that aligned did not meet the validity criteria set by PASA, suggesting incorrect predictions. This implied a larger number of gene predictions not to be an indicator of correct or complete assembly in neem. Instead, integration of results from multiple tools, preferably using additional information from orthogonal high-throughput platforms such as RNA-seq, and experimental validation, offered better benchmarking.

The presence of duplicate reads may give false assurance to the assembler in terms of artificially inflated read depth. Hence, removing exact read duplicates reduces mis-assemblies. Interestingly, we found that the assembly with SOAPdenovo2, after duplicate removal (S2), displayed worse statistics, but much improved transcriptome-genome mappings using PASA (Supplementary Table 2). SOAPdenovo, using fewer Illumina libraries, and without a duplicate removal step (v1.0), also displayed sub-optimal assembly statistics but a good NG50 number (Figure 1). This, most likely, is due to an abundance of gaps in the assembly, inflating the assembly size. Incidentally, the NG50 numbers for assemblies using libraries from the same platform were comparable (Supplementary Table 2). Such observations caution against deriving conclusions regarding best assembly based solely on assembly statistics tools, such as QUAST.

Exploring the finer details of individual genomic features, instead of macro-level statistics like NG50, may provide a better estimate of the improvement in the assembly quality, as exemplified by the improved assembly of *FDFT1* and *SQLE* genes in the improved neem assembly. Relying solely on sequence similarity-based approaches for gene identification can result in incomplete and/or inaccurate structural annotations. Using BLAST against *C. clementina* transcripts, with a stringent

E-value threshold of 0.001, identified only portions of the *FDFT1* and *SQLE* genes in our scaffolds, making us falsely deduce that we had assembled only certain exons from these genes. This would particularly be true for structurally conserved genes, which have few very important, and, therefore, conserved domains. In such genes, variable domains might not have significant sequence homology to the reference database(s) that include sequences from other species, causing the genes to not be annotated in their entirety. Therefore, our approach, of using the sequence similarity between *C. clementina* and neem transcripts to trace back the entire gene sequence, structure and combining both reference- and *de novo*-based identification techniques, is a better one (Figure 4).

In conclusion, genome assemblies need to be updated continuously by implementing accurate computational algorithms and supplementing with experimental evidence to obtain error-free and near complete assemblies. The process of obtaining accurate genome assembly is a dynamic and continuous process that needs to be undertaken, in our opinion, by groups or communities that have produced the first draft sequence of various genomes. This will facilitate research in genomics and create public resources to understand gene structure and function in plants better.

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Author Contributions

BP: Overall planning, conception and design of the study, data interpretation and manuscript writing; NMK: Conception, analysis and interpretation of data, manuscript writing; PJ and SG: Analysis and interpretation of data, manuscript writing; and AKH: Sequencing data production.

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Figure Legends

Figure 1. Improvements (fold change between current, v2.0, over the previous, v1.0, assembly) in various A: assembly statistics and B: PASA mapping statistics. The Y-axis is plotted on a logarithmic scale and the minor grids conform to uniform intervals on positive and negative Y axis.

Figure 2. Improvements (fold change between current, v2.0, over the previous, v1.0, assembly) in the numbers (#s) and sizes (bases) of gene and exon predictions from GlimmerHMM. The Y-axis is plotted on a logarithmic scale and the minor grids conform to uniform intervals on positive and negative Y axis.

Figure 3. Proportion (%) of gene-bearing scaffolds/contigs with gene predictions of lengths <10 bases, 10-100 bases, and >100 bases, for *A. thaliana*, *C. sinensis* and *C. clementina* training sets.

Figure 4. Comparison of v1.0 and v2.0 assemblies for A: *FDFT1* and B: *SQLE* genes. The *FDFT1* and *SQLE* transcripts from *C. clementina* were mapped to the representative Trinity-assembled *A. indica* transcriptome using NCBI BLAST (*E*-value 0.001). The transcripts were traced to their neem genomic scaffold mappings from PASA, in order to extract the exon-intron structures of the corresponding genes. In the figures, boxes and lines denote exons and introns, respectively, and the red regions denote gaps in the assemblies. The scales are different for *FDFT1* and *SQLE* and are, therefore, indicated individually.

Supplementary Figure Legends

Supplementary Figure 1. kmer frequency curve. The frequency (%) of 17-mers is plotted as a function of the number of times they occur across paired-end libraries. The peaks for heterozygous, homozygous and repetitive kmers are highlighted by arrows.

Supplementary Figure 2. Comparison across assemblies for A: *FDFT1* and B: *SQLE* genes. The *FDFT1* and *SQLE* transcripts from *C. clementina* were mapped to the representative Trinity-assembled *A. indica* transcriptome using NCBI BLAST (*E*-value 0.001). The transcripts were traced to their neem genomic scaffold mappings from PASA, in order to extract the exon-intron structures of the corresponding genes. In the figures, boxes and lines denote exons and introns, respectively, and the red regions denote gaps in the assemblies. The scales are different for *FDFT1* and *SQLE* and are, therefore, indicated individually.

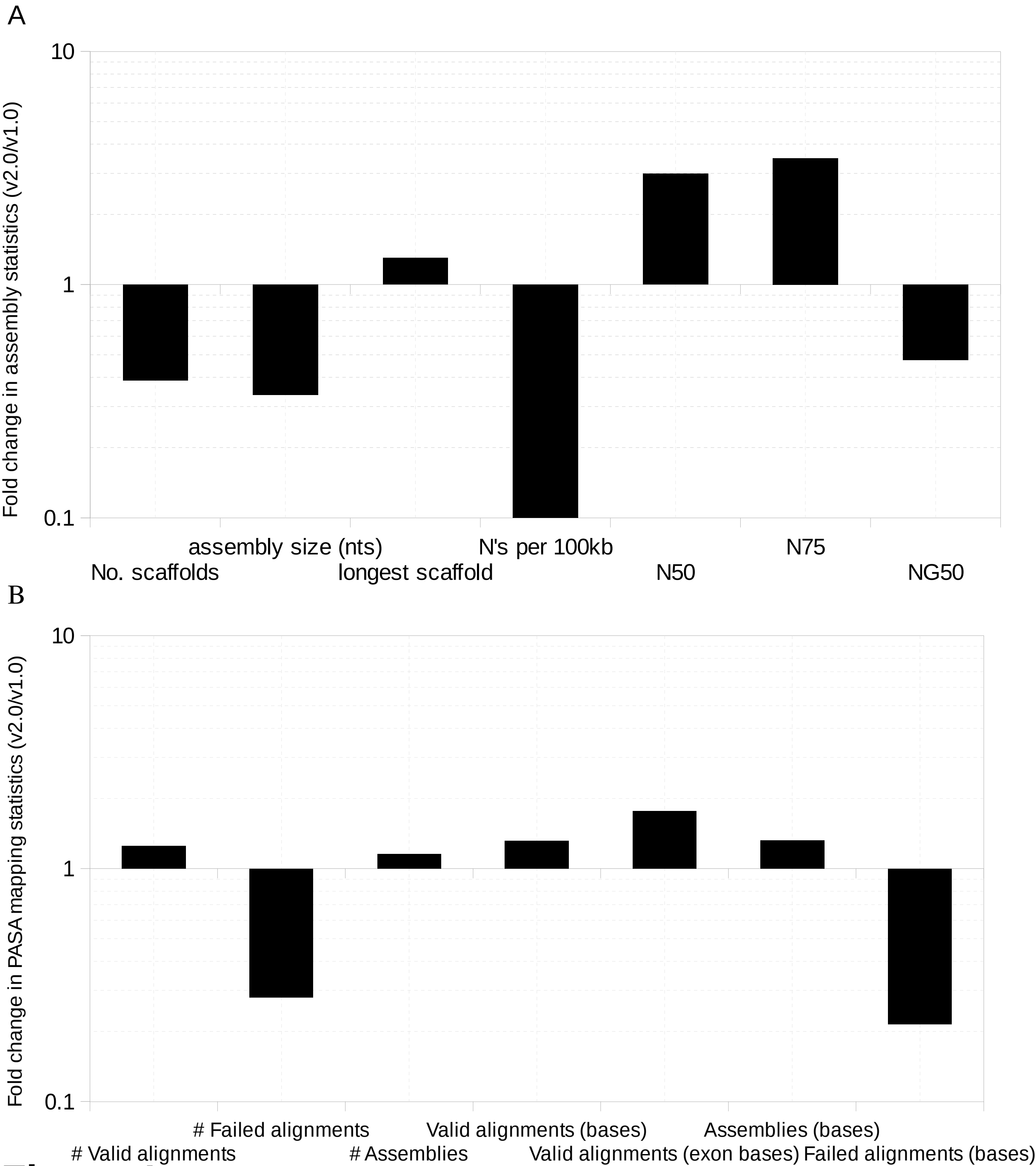

A
10
1
0.1
Fold change in assembly statistics (v2.0/v1.0)
No. scaffolds
assembly size (nts)
longest scaffold
N's per 100kb
N50
N75
NG50
B
10
1
0.1
Fold change in PASA mapping statistics (v2.0/v1.0)
# Valid alignments
# Failed alignments
# Assemblies
Valid alignments (bases)
Valid alignments (exon bases)
Assemblies (bases)
Failed alignments (bases)

Figure 1

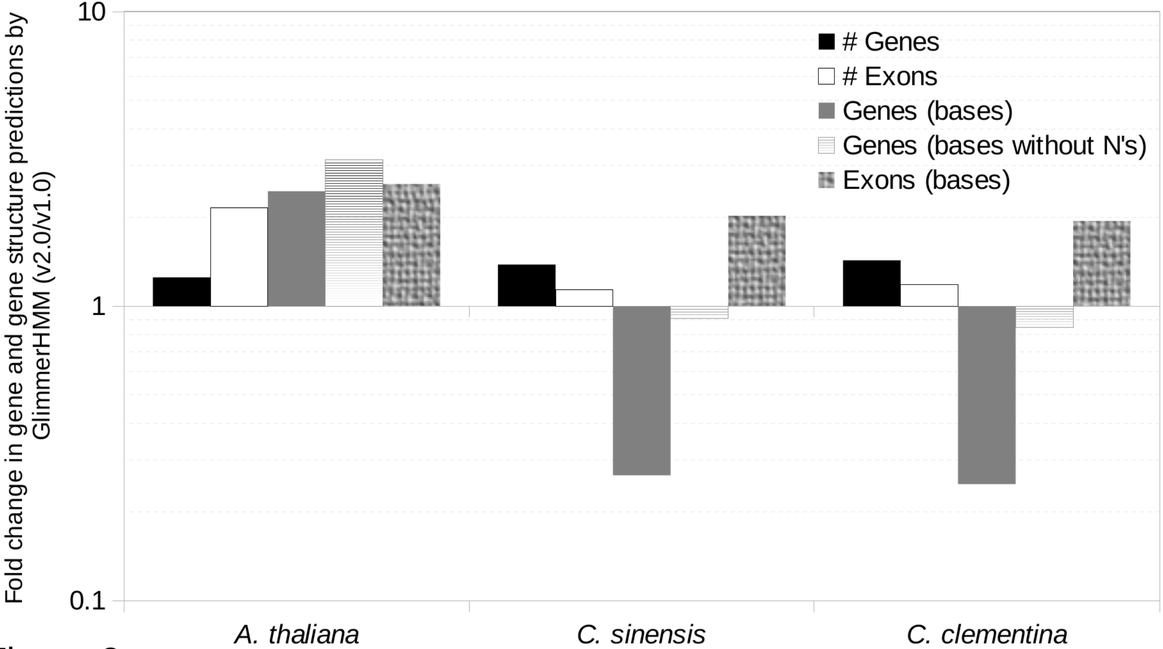

Fold change in gene and gene structure predictions by GlimmerHMM (v2.0/v1.0)
10
1
0.1
■ # Genes
□ # Exons
■ Genes (bases)
▤ Genes (bases without N's)
▨ Exons (bases)
A. thaliana
C. sinensis
C. clementina

Figure 2

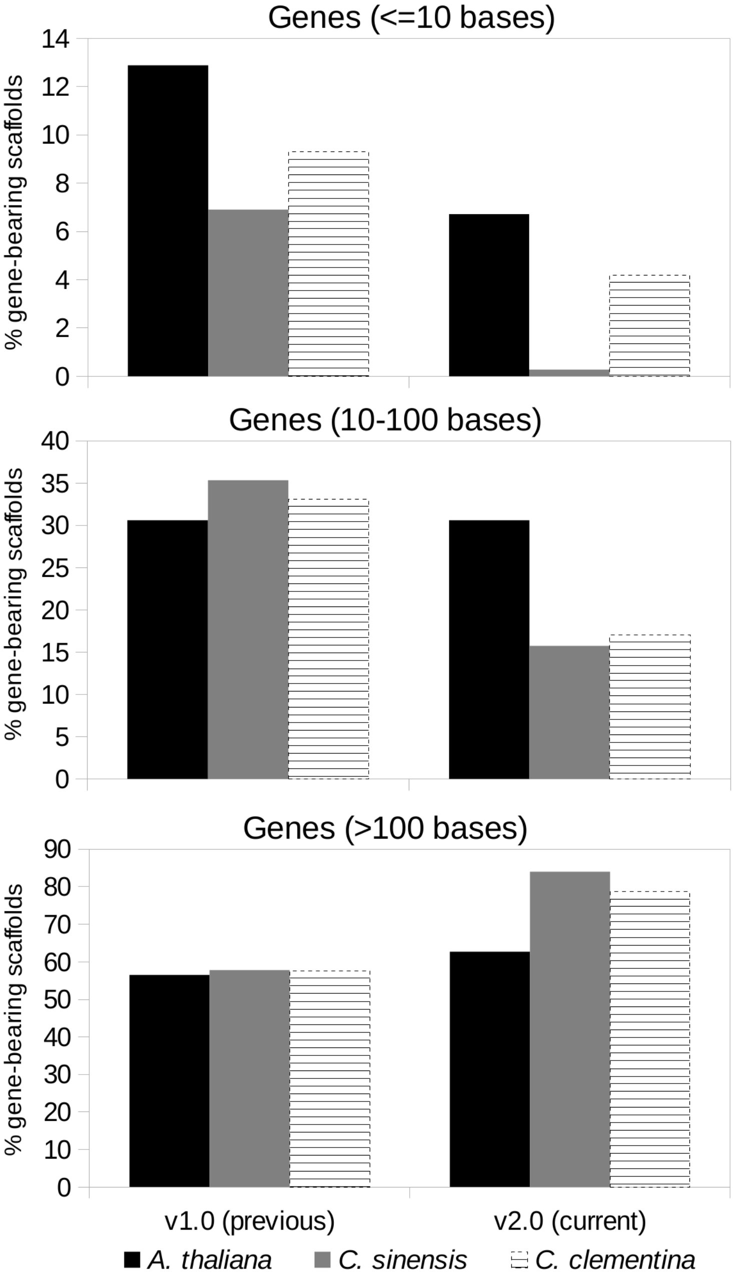

Genes (<=10 bases)
14
12
10
8
6
4
2
0
% gene-bearing scaffolds
Genes (10-100 bases)
40
35
30
25
20
15
10
5
0
% gene-bearing scaffolds
Genes (>100 bases)
90
80
70
60
50
40
30
20
10
0
% gene-bearing scaffolds
v1.0 (previous)
v2.0 (current)
■ *A. thaliana*
■ *C. sinensis*
▤ *C. clementina*

Figure 3

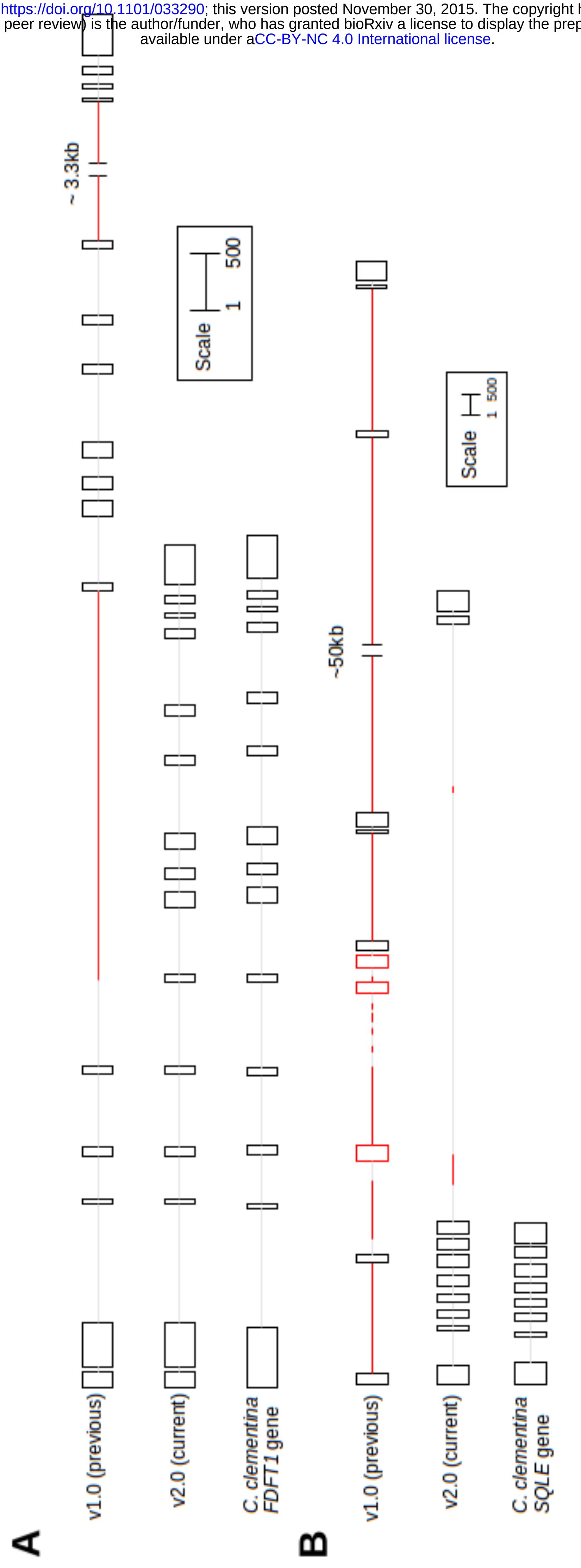
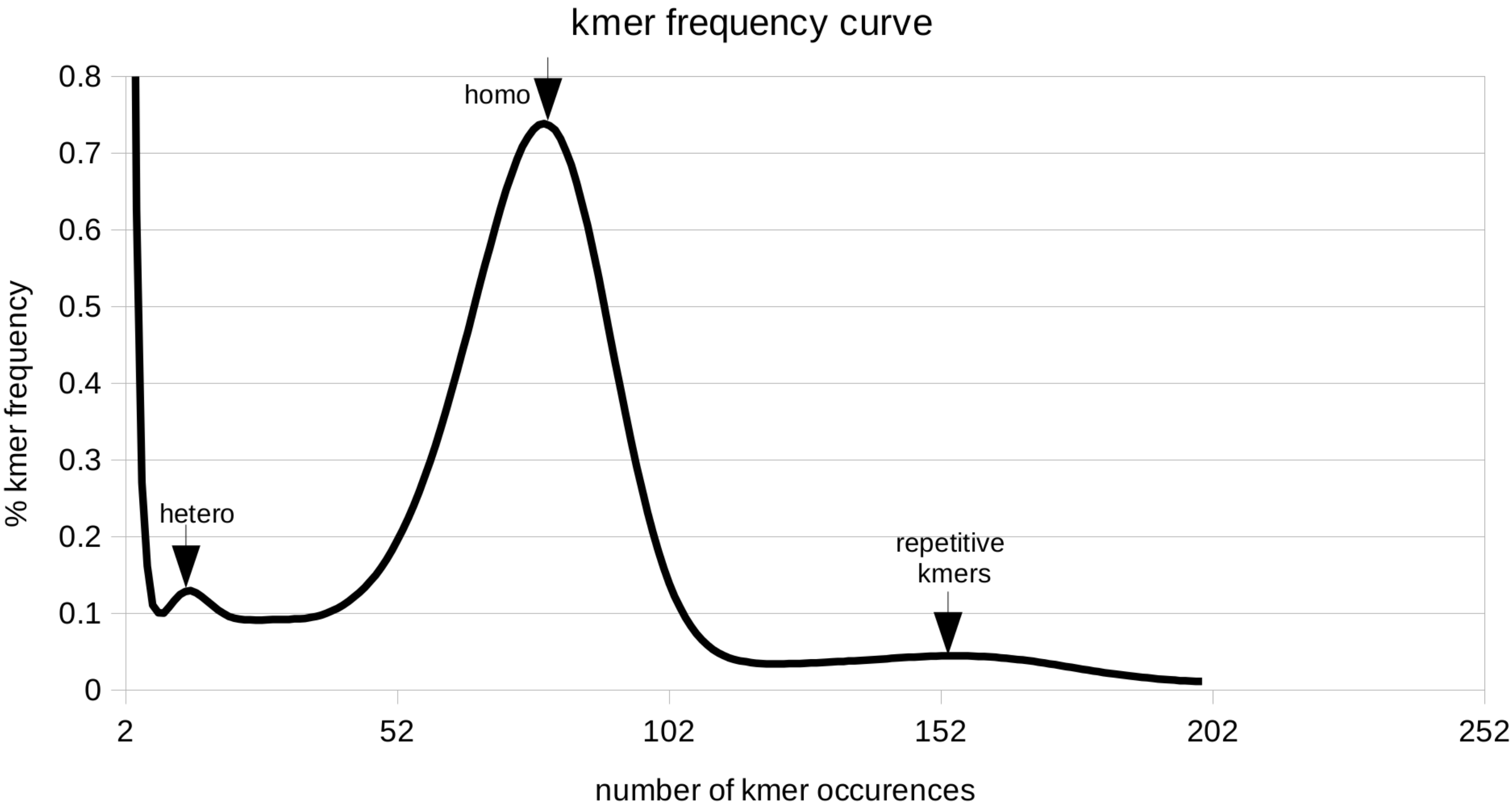
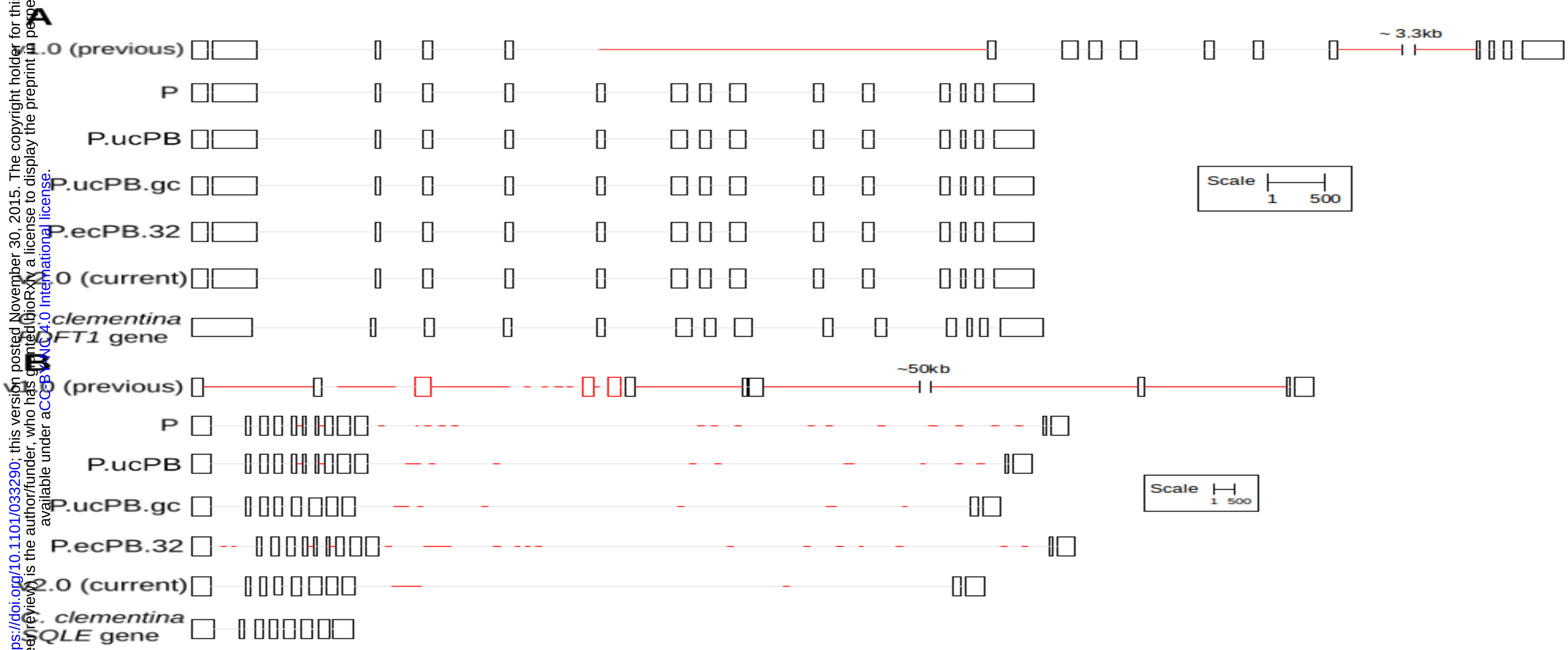

A
v1.0 (previous)
v2.0 (current)
C. clementina
FDFT1 gene
~ 3.3kb
Scale
1
500
B
v1.0 (previous)
v2.0 (current)
C. clementina
SQLE gene
~50kb
Scale
1
500

Figure 4



Supplementary Figure 1



Supplementary Figure 2

Supplementary Table 1. Deatils of sequencing libraries. PE: short-insert paired end, MP: long-insert mate pair libraries.

Lib no.	Read length	Insert size	Type	Used for earlier published assembly (v1.0)* using SOAPdenovo	Used for v2.0 and intermediate assemblies
1	76	350	Illumina PE	✔	✔
2	76	350	Illumina PE	✔	✔
3	36	1500	Illumina MP	✔	✔
4	36	3000	Illumina MP	✔	✔
5	36	10000	Illumina MP	✔	✔
6	100	4000	Illumina MP		✔
7	100	6000	Illumina MP		✔
8	100	10000	Illumina MP		✔
9	Variable (mean >2Kbp, longest 17.64Kbp)	NA	PacBio		✔

* The original assembly contained both Ion Torrent and Sanger libraries with very low coverage (0.5X and 0.001X, respectively) which were excluded from our current assembly.

Supplementary File 1.
NUCMER based mapping of smaller Illumina reads coming from a single long PacBio read, to the assembly.

NUCMER (Read 1)

```
   [S1]     [E1]  |     [S2]     [E2]  |  [LEN 1]  [LEN 2]  |  [% IDY]  | [TAGS]
================================================================================
==============
      22      100  |      726      648  |       79       79  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_190_681_12
_1_0    scaffold24417_len726_cov2219_single
       1      100  |      712      613  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_225_666_14
_1_0    scaffold24417_len726_cov2219_single
       1      100  |      693      594  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_244_671_22
_1_0    scaffold24417_len726_cov2219_single
      10       87  |       78        1  |       78       78  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_55_488_4_1
_0       scaffold23831_len619_cov1962_single
```

NUCMER (Read 2)

```
   [S1]     [E1]  |     [S2]     [E2]  |  [LEN 1]  [LEN 2]  |  [% IDY]  | [TAGS]
================================================================================
==============
       1      100  |      306      405  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_106_532_11
_1_1    scaffold24417_len726_cov2219_single
       1      100  |      276      375  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_111_562_6_
1_1      scaffold24417_len726_cov2219_single
       1      100  |      260      359  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_134_578_2_
1_1      scaffold24417_len726_cov2219_single
       1      100  |      235      334  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_140_603_15
_1_1    scaffold24417_len726_cov2219_single
       1      100  |      232      331  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_146_606_19
_1_1    scaffold24417_len726_cov2219_single
       1      100  |      157      256  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_190_681_12
_1_1    scaffold24417_len726_cov2219_single
       1      100  |      172      271  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_225_666_14
_1_1    scaffold24417_len726_cov2219_single
       1      100  |      349      448  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_22_489_25_
1_1      scaffold24417_len726_cov2219_single
       1      100  |      167      266  |      100      100  |   100.00  |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_244_671_22
_1_1    scaffold24417_len726_cov2219_single
     1      100 |      356      455 |      100      100 |   100.00 |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_2_482_17_1
_1      scaffold24417_len726_cov2219_single
     1      100 |      350      449 |      100      100 |   100.00 |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_55_488_4_1
_1      scaffold24417_len726_cov2219_single
     1      100 |      311      410 |      100      100 |   100.00 |
m121022_124828_42181_c100388272550000001523034010251242_s1_p0/7/0_806_65_527_8_1
_1      scaffold24417_len726_cov2219_single
```

abbreviation	assembler	library type	QUAST				
			# of scaffolds	assembly size (nts)	longest scaffold	N's per 100kb	N50
R.S1 (v1.0)	soapdenovo	illumina (wo dupRem) reassembled	65735	669685450	6070869	72683.84	879363
P.ecPB.32.gc (v2.0)	platanus	illumina (dupRem) + ec (32) pacbio (5X) + gc	25560	225115251	7894471	5414.21	2629187
S2.DUP	soapdenovo2	illumina (wo dupRem)	107964	449656122	7814495	31319.29	557365
S2	soapdenovo2	illumina (dupRem)	110581	344150202	6186666	32999.82	580956
S2.ecPB.21	soapdenovo2	illumina (dupRem) + ec (21) pacbio (5X)	578405	241236331	20326	19079.17	1388
S2.ecPB.32	soapdenovo2	illumina (dupRem) + ec (32) pacbio (5X)	567634	236057722	20326	15290.74	1325
P	platanus	illumina (dupRem)	35075	250268532	10980712	15531.55	4002232
P.ecPB.19	platanus	illumina (dupRem) + ec (19) pacbio (5X)	24458	247175992	7115459	14799.02	2264224
P.ecPB.21	platanus	illumina (dupRem) + ec (21) pacbio (5X)	24750	250209332	8603192	16201	2902226
P.ecPB.32	platanus	illumina (dupRem) + ec (32) pacbio (5X)	25560	243319034	8307765	13435.44	2889126
P.ecPB.36	platanus	illumina (dupRem) + ec (36) pacbio (5X)	21743	246909418	8609061	15312.29	2044474
P.ucPB	platanus	illumina (dupRem) + pacbio (5X)	68309	246188646	12211325	12200.65	3137410
P.ucPB.gc	platanus	illumina (dupRem) + pacbio (5X) + gc	68309	239373060	11935676	9428.23	3044020

		NG50 (364 mb)	% covered at 5X	% covered at 10X	PASA (#s)			PASA (Lengths)			PASA (Exon Lengths)
N75	assembly size (no gaps)				Valid alignments	Failed alignments	Assemblies	Valid alignments	Failed alignments	Assemblies	Valid alignments
329406	182932349	1663167	13.66	6.83	61991	23523	52924	75719343	190033044	74739553	24479791
1149129	213036786	734691	65.50	32.75	77635	6584	61292	99453593	32030331	98694338	47829727
181445	308827017.1	836066	37.41	18.71	40416	43883	35880	21118152	214585399	20954880	9219905
194116	230581254.8	681660	32.88	16.44	72582	10851	58508	53795060	120907710	53154195	22605627
837	195210441.3	290	60.95	30.47	40082	26780	34723	11708648	13883902	11566814	9821552
821	199962749.5	270	62.38	31.19	40226	25494	34769	11794632	13941208	11647110	9899586
1489583	211397949.8	1587838	45.22	22.61	73800	11324	59057	85780130	53260936	85251475	41362113
876306	210596367.5	958049	59.64	29.82	70309	14030	56274	80711062	60735939	80164716	38639958
986022	209672918.1	1175333	58.76	29.38	69116	15189	55456	77277179	68307801	76849819	37102672
1243543	210628051.2	1112734	60.52	30.26	74576	8833	59412	91112744	40801767	90400020	43230588
973439	209101931.9	1030861	59.72	29.86	65304	19054	52778	66319838	80933035	65982106	31721203
1274166	216152031	1270061	60.16	30.08	73763	9728	58895	86665009	44526788	85994624	41389567
1252388	216804417.3	646875	61.87	30.94	73855	10533	58699	88744677	50211652	88084027	42629241

abbreviation					predicted length = 1 nucleotide						
	GlimmerHMM (#s)		GlimmerHMM (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (I
	Genes	Exons	Genes	Exons	Genes	Exons	Genes	Exons	Valid	Failed	Valid
R.S1 (v1.0)	22816	59432	27884404	11837318	0	3	0	3	0	0	0
P.ecPB.32.gc (v2.0)	28553	128053	68723917	30727025	0	13	0	13	2	1	2
S2.DUP	34484	102919	54852164	20474483	0	12	0	12	1	9	1
S2	32304	108656	60771387	23656610	0	5	0	5	1	2	1
S2.ecPB.21	59707	90723	25691472	18635045	0	2	0	2	0	0	0
S2.ecPB.32	60610	92091	26159705	19002432	0	1	0	1	0	0	0
P	25707	112438	60757663	25795802	0	9	0	9	2	2	2
P.ecPB.19	26159	114069	62047674	26457349	0	5	0	5	2	1	2
P.ecPB.21	25694	112341	60941486	25691804	0	13	0	13	2	6	2
P.ecPB.32	25863	115978	62504333	26766180	0	13	0	13	4	1	4
P.ecPB.36	24954	109392	58493707	23706845	0	7	0	7	1	2	1
P.ucPB	25911	115639	62033977	26174038	0	14	0	14	4	3	4
P.ucPB.gc	27356	121406	65264827	28187804	0	10	0	10	4	2	4

	predicted length <= 10 nucleotide										
engths)	GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (Lengths)		GlimmerHMM (#s)		GlimmerHM
Failed	**Genes**	**Exons**	**Genes**	**Exons**	**Valid**	**Failed**	**Valid**	**Failed**	**Genes**	**Exons**	**Genes**
0	553	2012	3269	12424	451	724	2872	4524	1308	9296	22679
1	98	1599	673	9985	534	151	3351	965	286	11559	6071
9	526	2280	3066	14448	180	885	1188	5771	1349	13925	23681
2	522	2145	3051	13350	345	526	2169	3390	1311	12662	23602
0	1745	2232	10644	13682	90	128	588	801	7585	12001	181203
0	1721	2214	10485	13566	97	115	632	732	7635	12062	183275
2	211	1682	1251	10462	497	234	3176	1543	501	10644	8613
1	132	1612	760	9953	430	333	2711	2106	334	10414	6347
6	116	1584	682	9828	450	344	2782	2152	313	10314	5886
1	136	1572	812	9792	480	186	2993	1235	343	10715	6130
2	156	1667	890	10488	430	425	2708	2787	351	10949	5802
3	127	1646	778	10306	493	257	3070	1656	317	10986	5550
2	67	1602	381	9969	486	247	3026	1538	245	11165	5284

predicted length <= 50 nucleotide					predicted length <= 100 nucleotide							
IM (Lengths)	PASA (#s)		PASA (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (Lengths)	
Exons	Valid	Failed	Valid	Failed	Genes	Exons	Genes	Exons	Valid	Failed	Valid	Failed
233878	3019	3875	84914	102181	1875	23993	65004	1352381	9540	11079	582283	651332
346117	5825	1210	195579	36596	478	45725	20609	2959090	31051	5248	2133009	345098
384755	1529	7424	46934	223183	1925	40351	66008	2386791	5753	26156	369403	1646989
351635	3358	4682	106802	142899	1957	40903	71841	2497568	14455	18716	954945	1214213
319161	962	1143	30716	36185	15636	34432	793299	2036255	4417	5570	297024	379502
321267	950	1156	30010	37114	15752	34772	800883	2060085	4389	5650	295320	385927
311165	5117	1906	169515	57635	676	40990	21659	2632658	26056	8056	1776258	528900
306500	4761	2398	159521	72508	486	40778	18170	2631236	24833	10141	1701956	664971
303668	4646	2621	153767	79546	449	40713	16357	2630525	24304	10920	1663611	714191
318217	5286	1603	177301	48875	503	42146	18052	2723607	27911	6710	1914064	440339
321218	4368	3379	145263	102697	486	41038	15720	2620577	21680	14105	1473488	924155
323189	5267	1913	175573	56968	453	42407	15753	2727163	26973	7970	1842651	520660
332259	5305	1937	178157	58931	407	43702	17516	2822003	27776	8299	1904545	545248

abbreviation					predicted length = 1 nucleotide								predicted length <= 10 nucl				
	GlimmerHMM (#s)		GlimmerHMM (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PAS
	Genes	Exons	Genes	Exons	Genes	Exons	Genes	Exons	Valid	Failed	Valid	Failed	Genes	Exons	Genes	Exons	Valid
R.S1 (v1.0)	30871	74109	473787912	14430572	0	3	0	3	0	0	0	0	343	2311	2288	14293	178
P.ecPB.32.gc (v2.0)	42683	84499	126158109	29180337	0	0	0	0	0	0	0	0	5	283	49	1770	55
S2.DUP	56689	96506	268082598	24620354	0	0	0	0	0	0	0	0	133	934	889	6033	19
S2	49104	89693	228939681	25339719	0	0	0	0	0	0	0	0	153	886	992	5671	48
S2.ecPB.21	48805	55174	19800195	17469270	0	0	0	0	0	0	0	0	38	41	228	243	1
S2.ecPB.32	49794	56236	20235698	17885517	0	0	0	0	0	0	0	0	30	33	189	201	1
P	37941	77774	149393202	24654768	0	0	0	0	0	0	0	0	19	448	125	2815	56
P.ecPB.19	39096	78862	145710378	25404608	0	0	0	0	0	0	0	0	21	464	130	2958	55
P.ecPB.21	38248	77893	150652290	24668484	0	0	0	0	0	0	0	0	11	517	75	3172	57
P.ecPB.32	38334	78786	144880464	25392144	0	0	0	0	0	0	0	0	15	440	98	2760	64
P.ecPB.36	36783	75516	144880464	25392144	0	0	0	0	0	0	0	0	22	487	169	3116	53
P.ucPB	38717	78794	144328106	25049627	0	0	0	0	0	0	0	0	18	387	118	2444	49
P.ucPB.gc	40838	81839	136545510	26929248	0	0	0	0	0	0	0	0	8	314	56	1947	39

leotide			predicted length <= 50 nucleotide								predicted length <= 100 nucleotide							
A (#s)	PASA (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (Lengths)	
Failed	Valid	Failed	Genes	Exons	Genes	Exons	Valid	Failed	Valid	Failed	Genes	Exons	Genes	Exons	Valid	Failed	Valid	Failed
277	1097	1717	1376	12425	31257	329489	1442	2119	43073	62276	2110	29995	85898	1657523	5783	8204	379544	532693
21	335	141	60	2548	1891	79566	742	200	25626	6291	173	13087	10581	905953	7016	1343	525975	97014
91	136	592	907	6820	24287	198018	261	1347	9045	46361	1772	21978	88487	1358458	1833	10476	133098	766607
107	314	719	826	5953	21003	169956	572	978	18573	30250	1642	19627	82445	1224231	3991	5785	289538	409182
1	6	6	441	510	14865	17589	51	43	2047	1716	3740	5168	281733	395076	721	890	56566	70851
0	6	0	367	437	12667	15461	48	44	1930	1790	3698	5131	282342	396039	705	899	55610	71659
57	324	332	103	3466	2692	103096	746	489	25324	14611	267	14628	15193	973591	6225	2610	462138	181108
73	335	462	139	3374	3883	99509	647	532	21809	15722	316	14272	17479	950027	5817	3039	433022	212963
92	351	571	132	3641	3653	106017	663	652	22281	19328	301	14615	16653	961438	5748	3375	427637	232851
50	397	331	113	3367	3048	100048	743	386	25420	11681	269	14391	14759	960162	6556	2051	487815	143491
76	334	486	120	3754	3129	111711	653	765	22356	23737	273	14995	14645	984574	5107	4223	376203	295495
48	293	316	117	3279	3192	99246	713	422	24670	12806	245	14454	13250	972087	6272	2474	468186	174500
33	247	198	81	2932	2355	90908	707	399	25092	12669	202	13818	12107	942511	6419	2377	481125	167951

abbreviation					predicted length = 1 nucleotide								predicted length <= 10 nucleotide						
	GlimmerHMM (#s)		GlimmerHMM (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (I
	Genes	Exons	Genes	Exons	Genes	Exons	Genes	Exons	Valid	Failed	Valid	Failed	Genes	Exons	Genes	Exons	Valid	Failed	Valid
R.S1 (v1.0)	54500	97841	4.3E+08	1.8E+07	148	185	148	185	0	0	0	0	603	3464	3177	20146	243	459	1555
P.ecPB.32.gc (v2.0)	78021	115562	1.1E+08	3.6E+07	0	66	0	66	1	0	1	0	66	556	130	3057	120	47	762
S2.DUP	114527	153380	2.3E+08	3.7E+07	139	179	139	179	0	0	0	0	303	1623	1244	9141	27	200	174
S2	93737	132158	2E+08	3.5E+07	169	204	169	204	0	0	0	0	340	1608	1329	8958	117	232	751
S2.ecPB.21	77294	84692	2.5E+07	2.3E+07	559	568	559	568	1	3	1	3	661	689	1274	1403	10	9	61
S2.ecPB.32	78991	86476	2.6E+07	2.3E+07	544	555	544	555	1	2	1	2	627	657	1119	1250	11	10	71
P	69929	106381	1.3E+08	3.1E+07	134	140	134	140	0	0	0	0	163	956	328	5125	109	124	682
P.ecPB.19	71427	107756	1.2E+08	3.1E+07	45	51	45	51	0	0	0	0	67	869	207	4990	118	130	728
P.ecPB.21	70084	106364	1.3E+08	3.1E+07	50	64	50	64	0	0	0	0	79	1000	257	5841	121	150	764
P.ecPB.32	70608	107431	1.2E+08	3.1E+07	76	85	76	85	1	0	1	0	100	868	234	5001	134	82	864
P.ecPB.36	67855	103858	1.3E+08	2.9E+07	45	52	45	52	0	1	0	1	71	937	231	5596	100	172	613
P.ucPB	72000	108702	1.2E+08	3.1E+07	67	76	67	76	0	1	0	1	92	833	247	4734	129	90	798
P.ucPB.gc	75114	112381	1.2E+08	3.3E+07	33	44	33	44	0	1	0	1	43	677	107	3948	119	77	736

	predicted length <= 50 nucleotide								predicted length <= 100 nucleotide							
engths)	GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (Lengths)		GlimmerHMM (#s)		GlimmerHMM (Lengths)		PASA (#s)		PASA (Lengths)	
Failed	Genes	Exons	Genes	Exons	Valid	Failed	Valid	Failed	Genes	Exons	Genes	Exons	Valid	Failed	Valid	Failed
2862	1862	16063	37773	407084	1901	2914	57170	83268	2788	37081	107490	2005806	7108	9993	460903	630127
302	141	3857	2544	115861	1237	325	41353	9807	340	17382	18369	1170784	9021	1705	658050	118323
1238	1163	9224	27025	255843	366	2124	12203	70975	2338	28647	115491	1749508	2286	12847	164734	915748
1467	1212	8279	27355	225200	946	1589	29541	48212	2319	25696	111480	1567929	5220	7549	366574	517553
40	1985	2207	48461	56113	142	152	5194	5416	9307	11896	629479	829282	1306	1604	99113	123559
48	1917	2148	47265	55149	137	159	4880	5706	9263	11877	630691	831311	1301	1618	98816	124031
770	317	5252	4694	146697	1205	848	40134	24675	567	19164	23802	1227977	7908	3455	571511	228602
797	213	5058	4752	143087	1132	861	37029	24857	457	18710	23691	1206458	7470	3916	540496	265242
977	193	5252	3544	146627	1043	975	34482	28504	429	19104	21680	1223274	7262	4338	527553	291587
510	232	4992	4315	142129	1222	577	39916	17371	462	18903	22056	1223364	8409	2604	608198	176624
1048	213	5463	4430	154569	1005	1256	33439	37757	435	19666	21462	1259239	6552	5532	473329	372864
575	233	4982	4334	141844	1191	647	39052	19491	470	19017	23024	1233823	8027	3135	581825	213970
461	132	4421	2984	129634	1156	604	38194	17946	370	18274	22125	1209139	8195	3081	596091	212526

Supplementary Table 3. Repeat element classification

Repeat category	**Sub-category**	**Number**	**Content (bases)**	**Content (% of genome assembly size)**
Retrotransposons	*LTR retrotransposon*			
	Gypsy	16983	9699390	4.31
	Copia	14183	9031714	4.01
	Caulimovirus	1298	919770	0.41
	Unclassified	19	8004	0.004
	Non-LTR retrotransposon			
	LINEs	2428	845972	0.38
	SINEs	0	0	0
DNA Transposons	*Terminal Inverted Repeats(TIRs)*			
	MuLE-MuDR	6261	2797241	1.24
	hAT	1776	833096	0.37
	PIF/Harbinger	407	152389	0.07
	En-Spm	2143	969277	0.43
	others	80	4328	0.002
MITEs		3782	707942	0.31
RC/Helitron		173	11906	0.005
Unclassified Sequences		98680	30568122	13.58
Simple repeats		1297	284765	0.13
Non redundant Repeat Content		N.A.	**54375206**	**24.15**

Supplementary File 2.

Scripts used in the study

1. In silico normalization using Trinity utils

```
perl /Apps/Trinity/trinityrnaseq_r20140717/util/insilico_read_normalization.pl –seqType fq ---JM 10G –max_cov 5 –left s_1234_1.fastq –right s_1234_2.fastq –output s_1234 –CPU 12
```

2. Assemble using SOAPdenovo 2.0

```
/Apps/SOAPdenovo2/SOAPdenovo2-src-r223/SOAPdenovo-63mer all -s config -K 31 -d 9 -F -R -o 64 -p 12
```

```
config:
[LIB]
avg_ins=350
reverse_seq=0
asm_flags=3
rd_len_cutoff=76
rank=1
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/s_1234_1.fastq.normalized_K25_C50_pctSD200.fq
q2=/storage/fastqs/s_1234_2.fastq.normalized_K25_C50_pctSD200.fq

[LIB]
avg_ins=350
reverse_seq=0
asm_flags=3
rd_len_cutoff=76
rank=2
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/s_567_1.fastq.normalized_K25_C50_pctSD200.fq
q2=/storage/fastqs/s_567_2.fastq.normalized_K25_C50_pctSD200.fq

[LIB]
avg_ins=1500
reverse_seq=1
asm_flags=3
rd_len_cutoff=36
rank=3
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/s34_1.fastq.normalized_K25_C50_pctSD200.fq
q2=/storage/fastqs/s34_2.fastq.normalized_K25_C50_pctSD200.fq

[LIB]
avg_ins=3000
reverse_seq=1
asm_flags=3
```

```
rd_len_cutoff=36
rank=4
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/s56_1.fastq.normalized_K25_C50_pctSD200.fq
q2=/storage/fastqs/s56_2.fastq.normalized_K25_C50_pctSD200.fq

[LIB]
avg_ins=4000
reverse_seq=1
asm_flags=3
rd_len_cutoff=100
rank=5
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/Az_3.5_4.5KB_R1.fastq.normalized_K25_C50_pctSD200.fq
q2=/storage/fastqs/Az_3.5_4.5KB_R2.fastq.normalized_K25_C50_pctSD200.fq

[LIB]
avg_ins=6000
reverse_seq=1
asm_flags=3
rd_len_cutoff=100
rank=6
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/Az_5_7KB_R1.fastq.normalized_K25_C50_pctSD200.fq
q2=/storage/fastqs/Az_5_7KB_R2.fastq.normalized_K25_C50_pctSD200.fq

[LIB]
avg_ins=10000
reverse_seq=1
asm_flags=3
rd_len_cutoff=100
rank=7
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/Az_8_11KB_R1.fastq.normalized_K25_C50_pctSD200.fq
q2=/storage/fastqs/Az_8_11KB_R2.fastq.normalized_K25_C50_pctSD200.fq

[LIB]
avg_ins=10000
reverse_seq=1
asm_flags=3
rd_len_cutoff=36
rank=8
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/10kb_R1.fastq_30012014_QC_passed.fastq.normalized_K25_C50_pctSD200.fq
q2=/storage/fastqs/10kb_R2.fastq_30012014_QC_passed.fastq.normalized_K25_C50_pctSD200.fq

[LIB]
```

```
avg_ins=350
reverse_seq=0
asm_flags=3
rd_len_cutoff=76
rank=9
pair_num_cutoff=3
map_len=32
q1=/storage/fastqs/hybrid.fasta_1_350_35_5.0_100.fq
q2=/storage/fastqs/hybrid.fasta_2_350_35_5.0_100.fq
```

3. Assemble using Platanus:

assemble:

```
/Apps/platanus assemble -o P.ecPB.32 -f
/storage/fastqs/s_1234_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s_1234_2.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s_567_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s_567_2.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/hybrid_1_350_35_5.0_100.fq /storage/fastqs/hybrid_2_350_35_5.0_100.fq -k 32 -s
5 -t 12 -m 100
```

scaffold:

```
/Apps/platanus scaffold -o P.ecPB.32 -c P.ecPB.32_contig.fa -b P.ecPB.32_contigBubble.fa -IP1
/storage/fastqs/s_1234_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s_1234_2.fastq.normalized_K25_C50_pctSD200.fq -IP2
/storage/fastqs/s_567_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s_567_2.fastq.normalized_K25_C50_pctSD200.fq -IP3
/storage/fastqs/hybrid_1_350_35_5.0_100.fq /storage/fastqs/hybrid_2_350_35_5.0_100.fq -OP4
/storage/fastqs/s34_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s34_2.fastq.normalized_K25_C50_pctSD200.fq -OP5
/storage/fastqs/s56_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s56_2.fastq.normalized_K25_C50_pctSD200.fq -OP6
/storage/fastqs/10kb_R1.fastq_30012014_QC_passed.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/10kb_R2.fastq_30012014_QC_passed.fastq.normalized_K25_C50_pctSD200.fq -
OP7 /storage/fastqs/Az_3.5_4.5KB_R1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/Az_3.5_4.5KB_R2.fastq.normalized_K25_C50_pctSD200.fq -OP8
/storage/fastqs/Az_5_7KB_R1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/Az_5_7KB_R2.fastq.normalized_K25_C50_pctSD200.fq -OP9
/storage/fastqs/Az_8_11KB_R1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/Az_8_11KB_R2.fastq.normalized_K25_C50_pctSD200.fq -n1 315 -n2 315 -n3 315
-n4 1350 -n5 2700 -n6 9000 -n7 3500 -n8 5000 -n9 8000 -a1 350 -a2 350 -a3 350 -a4 1500 -a5 3500
-a6 10000 -a7 4000 -a8 6000 -a9 9500 -d1 35 -d2 35 -d3 35 -d4 150 -d5 350 -d6 1000 -d7 400 -d8
600 -d9 950
```

gap-close:

```
/Apps/platanus gap_close -o P.ecPB.32.gc -c P.ecPB.32.scaffold.fa -IP1
/storage/fastqs/s_1234_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s_1234_2.fastq.normalized_K25_C50_pctSD200.fq -IP2
/storage/fastqs/s_567_1.fastq.normalized_K25_C50_pctSD200.fq
```

```
/storage/fastqs/s_567_2.fastq.normalized_K25_C50_pctSD200.fq -IP3
/storage/fastqs/hybrid.fasta_1_350_35_5.0_100.fq
/storage/fastqs/hybrid.fasta_2_350_35_5.0_100.fq -OP4
/storage/fastqs/s34_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s34_2.fastq.normalized_K25_C50_pctSD200.fq -OP5
/storage/fastqs/s56_1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/s56_2.fastq.normalized_K25_C50_pctSD200.fq -OP6
/storage/fastqs/10kb_R1.fastq_30012014_QC_passed.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/10kb_R2.fastq_30012014_QC_passed.fastq.normalized_K25_C50_pctSD200.fq -
OP7 /storage/fastqs/Az_3.5_4.5KB_R1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/Az_3.5_4.5KB_R2.fastq.normalized_K25_C50_pctSD200.fq -OP8
/storage/fastqs/Az_5_7KB_R1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/Az_5_7KB_R2.fastq.normalized_K25_C50_pctSD200.fq -OP9
/storage/fastqs/Az_8_11KB_R1.fastq.normalized_K25_C50_pctSD200.fq
/storage/fastqs/Az_8_11KB_R2.fastq.normalized_K25_C50_pctSD200.fq -t 1 &
```

4. SINC to generate Illumina-like 100bp reads with 350±35 bp insert size from PacBio reads

```
/Apps/SInC/SInC_readGen -D 350 -S 35 -C 5 -T 10 -R 100 hybrid.fasta
/Apps/SInC/100_bp_read_1_profile.txt /Apps/SInC/100_bp_read_2_profile.txt 1> sinc.log 2>&1
```

5. LoRDEC to error-correct PacBio reads using Illumina libraries

```
/Apps/LoRDEC-0.4.1/lordec-correct -T 4 -i /storage/fastqs/filtered_subreads.fastq -2
s_1234_1.fastq s_1234_2.fastq s_567_1.fastq s_567_2.fastq -k 19 -o hybrid -s 3
```

6. Assembly QC using QUAST

```
python quast.py -t 4 --scaffolds P.ecPB.32.gc.fa
```

7. Transcriptome Assembly using Trinity

```
/Apps/Trinity/trinityrnaseq_r20140717/Trinity --seqType fq --JM 5G --CPU 10 --
min_contig_length 72 --trimmomatic --quality_trimming_params "LEADING:20 TRAILING:20
MINLEN:36" --left ../fastqs/flower_index5_ACAGTG_L007_R1_001.fastq
../fastqs/fruit_index2_CGATGT_L007_R1_001.fastq
../fastqs/leaf_index4_TGACCA_L007_R1_001.fastq
../fastqs/root_index6_GCCAAT_L007_R1_001.fastq
../fastqs/stem_index7_CAGATC_L007_R1_001.fastq --right
../fastqs/flower_index5_ACAGTG_L007_R2_001.fastq
../fastqs/fruit_index2_CGATGT_L007_R2_001.fastq
../fastqs/leaf_index4_TGACCA_L007_R2_001.fastq
../fastqs/root_index6_GCCAAT_L007_R2_001.fastq
../fastqs/stem_index7_CAGATC_L007_R2_001.fastq --output 5organs_combined_forDE
```

8. Mapping genome to transcriptome using PASA

```
sed 's/ path=\[.*\]$//g' Trinity.fasta | sed 's/[ =]/_/g' > Trinity_headerMod.fasta
/Apps/PASA_r20140417/seqclean/seqclean/seqclean Trinity_headerMod.fasta
/Apps/PASA_r20140417/scripts/Launch_PASA_pipeline.pl -c alignAssembly.config -C -R -g
P.ecPB.32.gc.fa-t Trinity_headerMod.fasta.clean -T -u Trinity_headerMod.fasta --ALIGNERS
gmap --CPU 12 1>pasa.out 2>pasa.err &
```

9. Training set creation and Gene prediction using GlimmerHMM-Train and GlimmerHMM

```
# training with C. sinsensis

# formatting exon file for input to trainGlimmerHMM
egrep "exon|mRNA" Csinensis_154_gene_exons.gff3 |cut -f 1,3,4,5,7 | sed 's/.*\tmRNA\t.*//'|awk -
F"\t" '{if($5=="-") {print $1"\t"$4"\t"$3;} else {print $1"\t"$3"\t"$4}}' | sed 1d >
Csinensis_154_gene_exons_forGlimmerHMM.tsv
/Apps/GlimmerHMM/GlimmerHMM3.0.4/train/trainGlimmerHMM Csinensis_154.fa
Csinensis_154_gene_exons_forGlimmerHMM.tsv -d Csinensis.glimmerTraining


# training with C. clementina
# formatting exon file for input to trainGlimmerHMM
egrep "exon|mRNA" Cclementina_182_v1.0.gene_exons.gff3 |cut -f 1,3,4,5,7 | sed
's/.*\tmRNA\t.*//'|awk -F"\t" '{if($5=="-") {print $1"\t"$4"\t"$3;} else {print $1"\t"$3"\t"$4}}' | sed
1d > Cclementina_182_v1.0.gene_exons_forGlimmerHMM.tsv
/Apps/GlimmerHMM/GlimmerHMM3.0.4/train/trainGlimmerHMM Cclementina_182_v1.fa
Cclementina_182_v1.0.gene_exons_forGlimmerHMM.tsv -d Cclementina.glimmerTraining


# running GlimmerHMM with Arabidopsis
/Apps/GlimmerHMM/GlimmerHMM3.0.4_mod/bin/glimmhmm.pl
/Apps/GlimmerHMM/GlimmerHMM3.0.4_mod/bin/glimmerhmm_linux_x86_64 P.ecPB.32.gc.fa
/Apps/GlimmerHMM/GlimmerHMM3.0.4_mod/trained_dir/arabidopsis -g >
P.ecPB.32.gc.arabidopsis.glimmerhmm.txt 2>glimmer.arabidopsis.err
# running GlimmerHMM with C. sinensis
/Apps/GlimmerHMM/GlimmerHMM3.0.4_mod/bin/glimmhmm.pl
/Apps/GlimmerHMM/GlimmerHMM3.0.4_mod/bin/glimmerhmm_linux_x86_64 P.ecPB.32.gc.fa
Csinensis.glimmerTraining -g > P.ecPB.32.gc.csinensis.glimmerhmm.txt 2>glimmer.csinensis.err


# running GlimmerHMM with C. clementina
/Apps/GlimmerHMM/GlimmerHMM3.0.4_mod/bin/glimmhmm.pl
/Apps/GlimmerHMM/GlimmerHMM3.0.4_mod/bin/glimmerhmm_linux_x86_64 P.ecPB.32.gc.fa
Cclementina.glimmerTraining -g > P.ecPB.32.gc.cclementina.glimmerhmm.txt
2>glimmer.cclementina.err
```

10. Repeat analyses pipeline

```
# Mite-hunter

perl /Apps/MITE_Hunter/MITE_Hunter_manager.pl -i P.ecPB.32.gc.fa -g AZ -S 12345678 -c 12

# transposon-PSI
nohup /Apps/TransposonPSI_08222010/transposonPSI.pl P.ecPB.32.gc.fa nuc
```

```
# LTR_finder
nohup /Apps/ltrFinder_1.0.5/ltr_finder -w 0 P.ecPB.32.gc.fa > P.ecPB.32.gc.ltrFinder1.log 2>
P.ecPB.32.gc.err

egrep "^\[|^[35]'-LTR|^TSR" P.ecPB.32.gc.ltrFinder.log | sed 's/ Len:.*//;s/.*: //;s/^\[[0-9][0-9]*\]
//;s/ - .* , .* - \([0-9][0-9]*\) \[.*/\t\1/;s/ - /\t/' | awk '{if(FNR%4==1) {scaf=$1} else if(FNR%4==2)
{start1=$1-1;end1=$2} else if(FNR%4==3) {start2=$1-1;end2=$2} else {if($0!~/NOT FOUND/)
{start1=$1-1; end2=$2;} print scaf"\t"start1"\t"end1"\n"scaf"\t"start2"\t"end2}}' >
P.ecPB.32.gc.ltrFinder.bed
fastaFromBed -fi P.ecPB.32.gc.fa -bed P.ecPB.32.gc.ltrFinder.bed -fo P.ecPB.32.gc.ltrOut.fa

# RepeatModeler
perl /Apps/RepeatModeler/BuildDatabase -name P.ecPB.32.gc P.ecPB.32.gc.fa
perl /Apps/RepeatModeler/RepeatModeler -engine ncbi -database P.ecPB.32.gc 1>> run.log 2>&1

# RepeatMasker
nohup /Apps/RepeatMasker/RepeatMasker -s -nolow -gff -no_is -norna -pa 16 -lib allRepeats.fa
P.ecPB.32.gc.fa 1>rm.P.ecPB.32.gc.log 2>&1


# RepeatClassifier
/Apps/RepeatModeler/RepeatClassifier -consensi allRepeats.fa
```