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Cédric Cabau Corresp., [1] , **Frédéric Escudié** [2] , **Anis Djari** [3] , **Yann Guiguen** [4] , **Julien Bobe** [4] , **Christophe Klopp** [2]

[1] SIGENAE, GenPhySE, Université de Toulouse, INRA, INPT, ENV, Castanet Tolosan, France

[2] Plate-forme bio-informatique Genotoul, Mathématiques et Informatique Appliquées de Toulouse, INRA, Castanet Tolosan, France

[3] Laboratoire Génomique et Biotechnologie du Fruit, UMR990 INRA/INP-ENSAT, Auzeville, France

[4] UR1037 Fish Physiology and Genomics, INRA, Rennes, France

Corresponding Author: Cédric Cabau
Email address: Cedric.Cabau@toulouse.inra.fr

Background

De novo transcriptome assembly of short reads is now a common step in expression analysis of organisms lacking a reference genome sequence. Several software packages are available to perform this task. Even if their results are of good quality it is still possible to improve them in several ways including redundancy reduction or error correction. Trinity and Oases are two commonly used de novo transcriptome assemblers. The contig sets they produce are of good quality. Still, their compaction (number of contigs needed to represent the transcriptome) and their quality (chimera and nucleotide error rates) can be improved.

Results

We built a de novo RNA-Seq Assembly Pipeline (DRAP) which wraps these two assemblers (Trinity and Oases) in order to improve their results regarding the above-mentioned criteria. DRAP reduces from 1,3 to 15 fold the number of resulting contigs of the assemblies depending on the read set and the assembler used. This article presents seven assembly comparisons showing in some cases drastic improvements when using DRAP. DRAP does not significantly impair assembly quality metrics such are read realignment rate or protein reconstruction counts.

Conclusion

Transcriptome assembly is a challenging computational task even if good solutions are already available to end-users, these solutions can still be improved while conserving the overall representation and quality of the assembly. The de novo RNA-Seq Assembly Pipeline (DRAP) is an ease to use software package to produce compact and corrected transcript set. DRAP is free, open-source and available at http://www.sigenae.org/drap .

Title

Compacting and correcting Trinity and Oases RNA-Seq de novo assemblies

Authors

Cédric Cabau[1*], Frédéric Escudié[2*], Anis Djari[3], Yann Guiguen[4], Julien Bobe[4], Christophe Klopp[2]

* equal contribution

Affiliations

[1] SIGENAE, GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France

[2] Plate-forme bio-informatique Genotoul, Mathématiques et Informatique Appliquées de Toulouse, INRA, Castanet-Tolosan, France

[3] Laboratoire Génomique et Biotechnologie du Fruit, UMR990 INRA/INP-ENSAT, 24, Chemin de Borde Rouge - Auzeville, France

[4] INRA, UR1037 Fish Physiology and Genomics, F-35000 Rennes, France

Corresponding Author

Cédric Cabau

Cedric.Cabau@toulouse.inra.fr

Abstract

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Keywords: RNA-Seq, de novo assembly, compaction, correction, quality assessment

Background

Second generation sequencing platforms have enabled the production of large amounts of transcriptomic data permitting to analyze gene expression for a large variety of species and conditions. For species lacking a reference genome sequence, the now classical processing pipeline includes a de novo transcriptome assembly step. Assembling an accurate transcriptome reference is difficult because of the raw data variability. This variability comes from different factors: including 1. The variability of gene expression levels ranging usually between one and millions of copies 2. The biology of mRNA synthesis which goes through an early stage of pre-mRNA still containing introns and a late state in which mRNA can be decayed 3. The synthesis from pre-mRNA of numerous alternative transcripts 4. Potential sample contaminations 5. Sequencing quality biases 6. Most of the genome can be expressed in low abundance depending on the biological condition as presented by Djebali et al.[1] in the results of the ENCODE project.

Today there is no unique best solution to these RNA-Seq assembly problems but several software packages have proven to generate contig sets comprising most of the expressed transcripts correctly reconstructed. Trinity [2] and Oases [3] are good examples. The assembled contig sets produced by these packages often contain multiple copies of complete or partial transcripts but also chimeras. Chimeras are structural anomalies of a unique transcript (self-chimeras) or multiple transcripts (multi-transcripts chimeras). They are called “cis” if the

transcripts are in the same direction and "trans" if they are in opposite directions. Natural chimeric transcripts exist in some cancer tissues but are rare [4]. Yang and Smith [5] have shown the tendency of *de novo* transcriptome assemblers to produce self-chimeric contigs. The prevalence of the phenomenon depends on the assembly parameters. Multi-transcript chimeras distort contig annotation. The functions of the transcripts merged in the same contig can be very different and therefore the often-unique annotation given to such a chimeric contig does not reflect its content. Assemblies include also contigs corresponding to transcription or sequencing noise a phenomenon often referred as *illegitimate transcription* [6]. These contigs have often low coverage and are not found in the different replicates of the same condition.

Some contigs contain local biological variations or sequencing errors such as substitutions, insertions or deletions. These variations and errors can deeply impact the read back to contig mapping rate, create frameshifts which hinder annotation, limit the efficacy of primer design and generate false variations. Assemblies contain also polyA/T tails, which are posttranscriptional marks. They are usually removed before publication. For all these reasons contig sets usually need error correction.

Trinity and Oases have different algorithms, which give them advantages or disadvantages depending on gene expression levels. The main difference comes from their assembly strategy. Trinity chains a greedy algorithm with a de Bruijn graph one and Oases uses multiple de Bruijn graphs with different kmers. The first step of Trinity is very effective in assembling parts of highly expressed transcripts which will be connected at the second step. As shown by Yann Surget-Groba and Juan I. Montoya-Burgos [7], the Oases multi-kmer assembly approach is able

to build contigs corresponding to transcripts with very low to very high expression levels. However highly expressed genes with multiple transcripts will generate very complex graphs mainly because of the presence of variations or sequencing errors which will form new paths possibly considered as valid by the assembler and produce numerous erroneous contigs. No assembler is producing the best contig set in all situations. Bio-informaticians and biologists therefore use different strategies to maximize the reference contig set quality [8][9][10][11]. The simplest approach is to produce a reference set per software package or parameter set, to compare their metrics and choose the best one. It is also possible to merge different results and filter them.

Assemblies can be compared on different criteria. The usual ones are simple contig metrics such as total count, total length, N50, average length... Assembling equals summarizing (compressing the expression dimension) and therefore a good metric to check the summary quality is the proportion of reads mapped back to the contigs. As a large part of the transcripts correspond to mRNA, it is also possible to use as quality metric the number of correctly reconstructed proteins using a global reference as it is done by CEGMA [12] or BUSCO [13] or using a protein reference set from a phylogenetically closely related organism. Last, some software packages are also rating the contig set or the individual contigs using the above-mentioned criteria [14] or some other for example only related to the way reads map back to the contigs [15][16].

To try to overcome some of these assembly problems we built a *de novo* RNA-Seq Assembly Pipeline (DRAP) and ran it on seven different datasets. We will discuss the impact of the compaction and correction performed by this software package compared to raw assemblies.

Implementation

DRAP is written in Perl, Python, and shell. The software is a set of three command-line tools respectively called runDrap, runAssessment and runMeta. runDrap performs the assembly including compaction and correction (the corresponding workflows are presented in a graphical manner in Fig. S1). It produces a contig set but also a HTML log report presenting different assembly metrics. runAssessment compares different contig sets and gathers the results in a global report. runMeta merges and compacts different contigs sets and should be used for very large datasets for which memory or CPU requirements do not enable a unique global assembly or for highly complex datasets. The modules chained by each tool as well as the default parameters are presented in Fig. S2 and S3. Details on the compaction, correction and quality assessment steps of the tools are described hereafter. All software versions, parameters and corresponding default values are presented in Table S1.

Contig set compaction

Four different approaches are used to compact contig sets. The first is only implemented for Oases assemblies and corresponds to the sub-selection of only one contig per locus (NODE) produced by the assembler. Oases resolves the connected component of the de Bruijn graph and for complex sub-graphs generates several longest paths corresponding to different possible forms. These forms have shown (https://sites.google.com/a/brown.edu/bioinformatics-in-biomed/velvet-and-oases-transcriptome) to correspond to subpart of the same transcript, which

are usually included one in another. Oases provides the locus (connected component of the assembly graph) of origin of each contig as well as its length and depth. The Oasesv2.0.4BestTransChooser.py script sub-selects the longest and most covered contig of a locus. The second compaction method removes contigs included in longer ones. CD-HIT-EST [17] orders the contigs by length and removes all the included ones given identity and coverage thresholds. The third method elongates the contigs through a new assembly step. TGICL [18] performs this assembly in DRAP. The last approach either filters contigs using the individual TransRate quality score above the calculated threshold (--optimize parameter) or using read coverage according to the idea that lowly covered contigs often correspond to noise. By default, runDrap produces eight contigs sets, four include only protein coding transcripts and four others contain all transcripts. Each group comprises a contig set filtered for low coverage with respectively 1, 3, 5 and 10 fragments per kilobase per million (FPKM) thresholds. Compaction favors assemblies having contigs with multiple ORFs. Because a unique ORF is expected for contig annotation, DRAP splits multi-transcript chimera in mono-ORF contigs.

runMeta also performs a three step compaction of the contigs. The first is based on the contig nucleotide content and uses CD-HIT-EST. The second run CD-HIT on the protein translation of the longest ORF found by EMBOSS gertorf. The third, in the same way as runDrap, either filters contigs using their TransRate score (--optimize option) or using their expression producing the eight result files described in the previous paragraph.

Contig set corrections

DRAP corrects contigs in three ways. It first searches self-chimera and removes them by splitting contigs in parts or removing duplicated chimeric elements. An in house script aligns contigs on themselves using bl2seq and keeps only matches having an identity greater or equal to 96%. A contig is defined as a putative chimera if i) the longest self-match covers at least 60% of the contig length or ii) the sum of partial non-overlapping self-matches covers at least 80% of its length. In the first case, the putative chimera is split at the start position of the repeated block. In the second case, the contig is only a repetition of a short single block and is therefore discarded. For the second correction step, DRAP searches substitutions, insertions and deletions in the read realignment file. When found it corrects the consensus according to the most represented allele at a given position. Low read coverage alignment areas are usually not very informative therefore only positions having a minimum depth of 10 reads are corrected. The manual assessment made on DRAP assemblies has shown that a second path of this algorithm improves consensus correction. Part of the reads change alignment location after the first correction. runDrap, consequently, runs this step twice.

The last correction script eases the publication of the contig set in TSA (https://www.ncbi.nlm.nih.gov/genbank/tsa): NCBI transcript sequence assembly archive. TSA stores the de novo assembled contig sets of overs 1300 projects. In order to improve the data quality it performs several tests before accepting a new submission. These tests search for different elements such as sequencing adapters or vectors, polyA or polyT and stretches of unknown nucleotides (N). The thresholds used by TSA are presented at https://www.ncbi.nlm.nih.gov/genbank/tsaguide. DRAP performs the same searches on the contig set and corrects the contigs when needed.

179 Quality assessment

180

181 All three workflows create an HTML report. The report is a template including HighCharts
182 (http://www.highcharts.com) graphics and tables using JSON files as database. These files are
183 generated by the different processing steps. The report can therefore also be used to monitor
184 processing progression. Each graphic included in the report can be downloaded in PNG, GIF,
185 PDF or SVG. Some of the graphics can be zoomed in by mouse selecting the area to be enlarged.
186 The report tables can be sorted by clicking on the column headers and exported in CSV format.
187 For runDrap and runMeta, the reports present results of a single contig file.

188

189 runAssessment processes one or several contig files and one or several read files. It calculates
190 classical contig metrics, checks for chimeras, searches alignment discrepancies, produces read
191 and fragment alignment rates and assess completeness using an external global reference running
192 BUSCO. If provided, it aligns a set of proteins on the contigs to measure their overlap. Last, it
193 runs TransRate, a contig validation software using four alignment linked quality measures to
194 generate a global quality criteria for each contig and for the complete set. runAssessment does
195 not modify the contig set content but enables user to check and select the best candidate between
196 different assemblies.

197

198 Parallel processing and flow control

199

200 DRAP runs on Unix machines or clusters. Different steps of the assembly or assessment process
201 are run in parallel mode, if the needed computer infrastructure is available. All modules have

been implemented to take advantage of an SGE compliant HPC environment. They can be adapted to other schedulers through configuration file modification.

DRAP first creates a set of directories and shell command files and then launches these files in the predefined order. The '--write' command line parameter forces DRAP to stop after the first step. At this stage, the user can modify the command files for example to set parameters which are not directly accessible from runDRAP, runMeta or runAssessment and then launch the process with the '--run' command line option.

DRAP checks execution outputs at each processing step. If an error has occurred it adds an error file to the output directory indicating at which step of the processing it happened. After correction, DRAP can be launched again and it will scan the result directory and restart after the last error free step. The pipeline can easily be modified to accept other assemblers by rewriting the corresponding wrapper using the input files and producing correctly named output files.

Results and discussion

DRAP has been tested on seven different datasets corresponding to five species. These datasets are presented in Table 1 and include five real datasets (*Arabidopsis thaliana*: At, *Bos taurus*: Bt, *Drosophila melanogaster*: Dm, *Danio rerio*: Dr and *Homo sapiens*: Hs), one set comprising a large number of diverse samples (*Danio rerio* multi samples: Dd) and one simulated dataset (*Danio rerio* simulated: Ds). The simulated reads have been produced using rsem-simulate-reads (version rsem-1.2.18)[19]. The theta0 value was calculated with the rsem-calculate-expression program on the pineal gland sample (SRR1048059) Danio rerio read files. Table 1 also presents for each dataset: the number, length, type (paired or not) and strandedness of the reads, the public accession number, the tissue and experimental condition of origin. The results presented

hereafter compare the metrics collected from Trinity, Oases, DRAP Trinity and DRAP Oases assemblies of the six first datasets. The last dataset has been used to compare a strategy in which all reads of the different samples are gathered and processed as one dataset (pooled) to a strategy in which the assemblies are performed by sample and the resulting contigs joined afterwards (meta-assembly). The same assembly pipeline has been used in both strategies, except the contig set merging step, which is specific to the meta-assembly strategy.

Summary Table 2 and Table 3 present the metrics collected for the six first datasets. Table 2 provides metrics related to compaction and correction as Table 3 includes validation metrics and Table 4 collects all three metric types for pooled versus meta-assembly strategies.

Contig set compaction:

The improvement in compactness is measured by three criteria. The first is the number of assembled contigs presented in Fig.1. The differences between raw Oases and Trinity assemblies and DRAP assemblies are very significant ranging from 1.3 fold to 15 fold. The impact of DRAP on Oases assemblies (from 3,4 to 15 fold) is much more significant than on Trinity assemblies (from 1,3 to 2,2 fold). Oases multi-k-mers assembly strategy generates a lot of redundant contigs which are not removed at the internal Oases merge step. The second criterion is the percentage of inclusions i.e., contigs which are part of longer ones. Oases and Trinity inclusion rate range respectively from 55 to 75% and from 2.3 to 5.5% (Table 2). Because of its inclusion removal step this rate is null for DRAP assemblies. The last compaction criteria presented here is the total number of nucleotides in the contigs. The ratios between raw and DRAP assembly sizes for

Oases and Trinity range respectively from 3.4 to 14.8 fold and from 1.1 and 2.6 fold (Table 2). All these metrics show that DRAP produces less contigs with less redundancy resulting in an assembly with a much smaller total size.

Another metric that can be negatively correlated to compactness, but has to be taken into account, is the number of multi-ORF contigs found in the assemblies. The ratios of multi-ORF contigs found between raw and DRAP assemblies range from 11 and 116 folds (Table 2). DRAP multi-transcript chimera splitting procedure improves significantly this criterion.

In order to check if the compaction step only selects one isoform per gene, we compared the number of genes with several transcripts aligning on different contigs before and after DRAP. The test has been performed on the Danio rerio simulated dataset assembled with Trinity. A transcript is linked to a contig if its best blat hit has over 90 % query identity and 90 % query coverage. The results show that 82% (1470/1792) of these genes have still multiple isoforms in the resulting contig dataset.

Contig set corrections

DRAP corrects contigs in two ways: removing self-chimera and rectifying consensus substitutions, insertions and deletions when the consensus does not represent the major allele at the position in the read re-alignment file. Self-chimeras appear in Oases and Trinity contig sets at rate ranging respectively from 0.11 to 1.39 and from 0.09 to 0.56%. In DRAP, the corresponding figures drop to 0.01 to 0.16 and 0.00 to 0.01%. Concerning consensus correction only five datasets can be taken into account i.e. At, Bt, Dm, Ds and Hs. Dr Oases assembly generates such a large number of contigs and total length that it decreases significantly the average coverage and

271 therefore limits the number of positions for which the correction can be made. As shown in Fig.
272 2 the Dr dataset is an outlier concerning this criteria. Regarding the five other datasets raw versus
273 DRAP correction rates range from 1.7 to 18.6 for insertions, 3.1 to 27.1 for deletions and 2.7 to
274 14.1 for substitutions. DRAP correction steps lowers significantly the number of positions for
275 which the consensus does not correspond to the major allele found in the alignment.
276
277 Assembly quality assessment
278
279 The two previous parts have shown the beneficial impacts of DRAP on the assembly
280 compactness and error rates but this should not impair quality metrics such as read and read pairs
281 alignment rates, number of ORFs, complete ORFs found in the contigs, number of proteins of the
282 known proteome mapped on the contigs or TransRate marks.
283 Read and read pair alignment rates differences between raw and DRAP assemblies are usually
284 very low, between 1 and 2% and can sometimes be in favor of DRAP (Fig. 4) . In our test sets,
285 the difference is significant (7.5%) for Dm when comparing Trinity to DRAP Trinity. This
286 comes from the removal by DRAP of a highly expressed transcript (Ensembl: FBtr0100888
287 mitochondrial large ribosomal RNA) because that does not fulfill the criteria of having at least
288 one 200 base pairs long ORF despite having over 11M reads aligned on the corresponding contig
289 in the Trinity assembly. DRAP Oases assembly was not impacted because it builds a longer
290 contig for this transcript with a long enough ORF to be selected in the additional part.
291 The reference proteome has been aligned on the contigs and matches with over 80% identity and
292 80% protein coverage have been counted. These figures give a good overview of the amount of
293 well-reconstructed proteins in the contig sets. For all datasets except one (At) the number of

proteins are very close between raw and DRAP results. For this At dataset the difference is of 12.2% for Oases and 13.2% for Trinity. This is due to the FPKM filtering step performed by DRAP and the expression profile of this dataset that mixes different tissues (root, shoot and flower) and conditions (full nutrition and starvation). Contigs corresponding to low expression in one condition do not have sufficient overall expression to pass DRAP expression filter threshold and are therefore eliminated from the final set. Mixed libraries can benefit from the meta-assembly approach presented in the next section.

TransRate global scores (Fig. 5) are much higher for DRAP assemblies compared to raw ones. This comes from the compaction performed by DRAP and the limited impact it has on the read alignment rate.

DRAP has limited negative effect on the assembly quality metrics, and sometimes even improves some of them. Some cases in which multiple libraries are mixed with very distinct conditions can affect the results and it is good practice to systematically compare raw and DRAP assemblies. It is also to be noticed that Oases multi-k-mers strategy outperforms Trinity for all datasets regarding the number of well-reconstructed proteins.

Pooled versus meta-assembly strategies

In the previous sections we compared results from raw and DRAP assemblies. This section compares results from pooled versus meta-assembly strategies both using the DRAP assembly pipeline (Table 4). Because of the read re-alignment filtering thresholds used in DRAP, we expect different metrics between a pooled assembly and merged per sample assembly (meta-assembly). DRAP includes the runMeta workflow, which performs this task.

317 Differences in compaction and correction are more important between Trinity and Oases than
318 between pooled versus meta-assembly. Pooled assemblies collect significantly worse results for
319 the number of reference proteins and number of read pairs aligned on the contigs. This comes
320 from the filtering strategy which eliminates low-expressed contigs of a given condition when
321 merging all the samples but will keep these contigs in a per sample assembly and meta-assembly
322 strategy. Therefore we recommend using runMeta when the assembly input samples mix distinct
323 conditions with specific and variable expression patterns.
324
325 Assemblies fidelity check using simulated reads
326
327 The simulation process links each read with its transcript of origin. With this information it is
328 possible to link contigs and transcripts. Here, the transcript-contig link was calculated using exon
329 content and order in both sets (method explained in Data S1). The results presented in Table 5
330 first shows that the assembly process loses between 15.76 and 19.97% of the exons compared to
331 the initial transcript set. This loss is close to 22% for all assemblies when the exon order is taken
332 into account. As shown in Fig. 6, this is mainly the case for transcripts with low read coverage.
333 The figures show once more that DRAP has a very limited negative impact on number of
334 retrieved exons in correct order.
335 Table 5 shows the number of contigs linked to more than one gene. DRAP compaction and ORF
336 splitting feature could have an antagonist impact for this criteria. But depending on the
337 assembler, the figures are in favor or not of DRAP.
338 Table 5 also presents the maximum number of genes linked to a single contig. These clusters
339 correspond to zing finger gene family members which have been assembled a single contig.

Between 92.3 and 93.7% of the clustered transcripts belong to this family. De novo assembly tools are not able to distinguish transcript originating from different gene when the nucleotide content is highly similar.

Conclusion

Different software packages are available to assemble de novo transcriptomes from short reads. Trinity and Oases are commonly used packages which produce good quality references. DRAP assembly pipeline is able to compact and correct contig sets with usually very low quality loss. As no package out performs the others in all cases, producing different assemblies and comparing their metrics is a good general practice.

Abbreviations

RMP: reads per million

ORF: open reading frame

TSA: Transcript sequences archive

NCBI: National Center for Bio-Informatics

DRAP: De novo Rna-seq Assembly Pipeline

PNG, GIF, PDF or SVG: are image file formats.

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360

Figure 1(on next page)

Number of contigs

The figure shows for the different assemblers (Oases, DRAP Oases, Trinity, DRAP Trinity) the number of contigs produced for each dataset.

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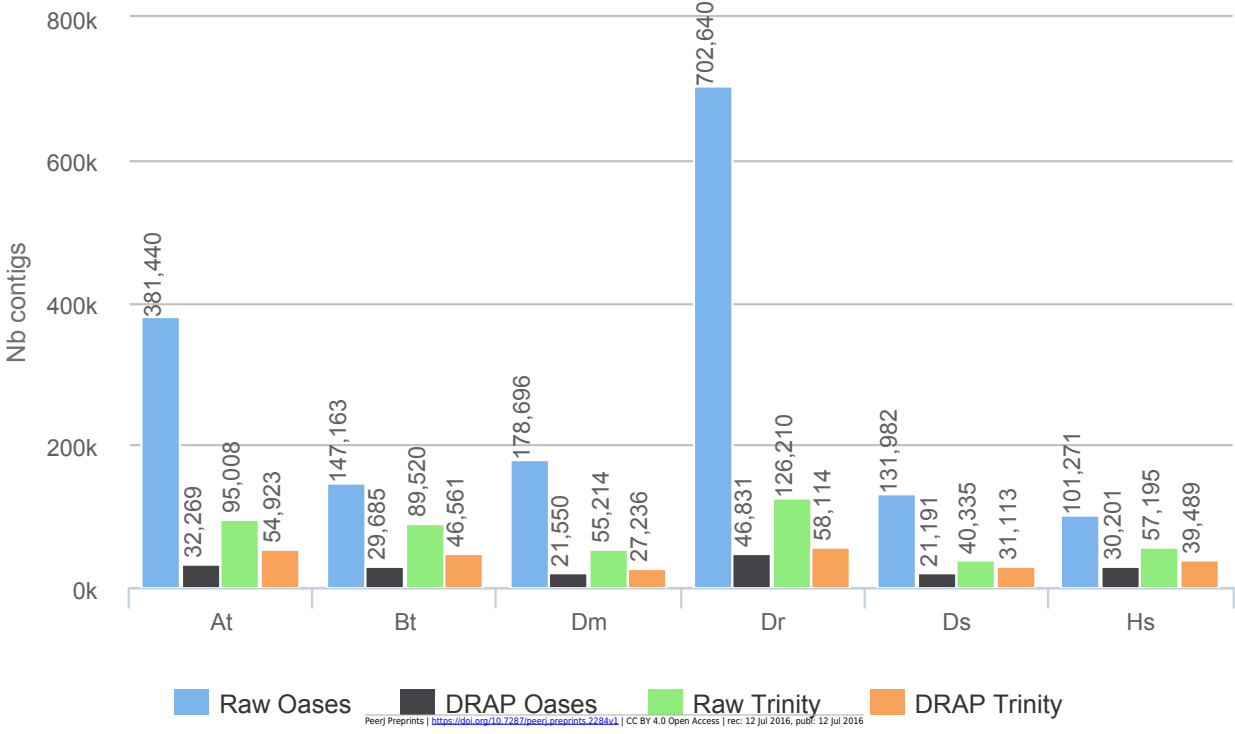

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Figure 2(on next page)

Consensus error rates

Figure A presents the global error rate of the four assemblers for each dataset. Figures B, C and D present the error rate respectively for substitution, insertions and deletions.

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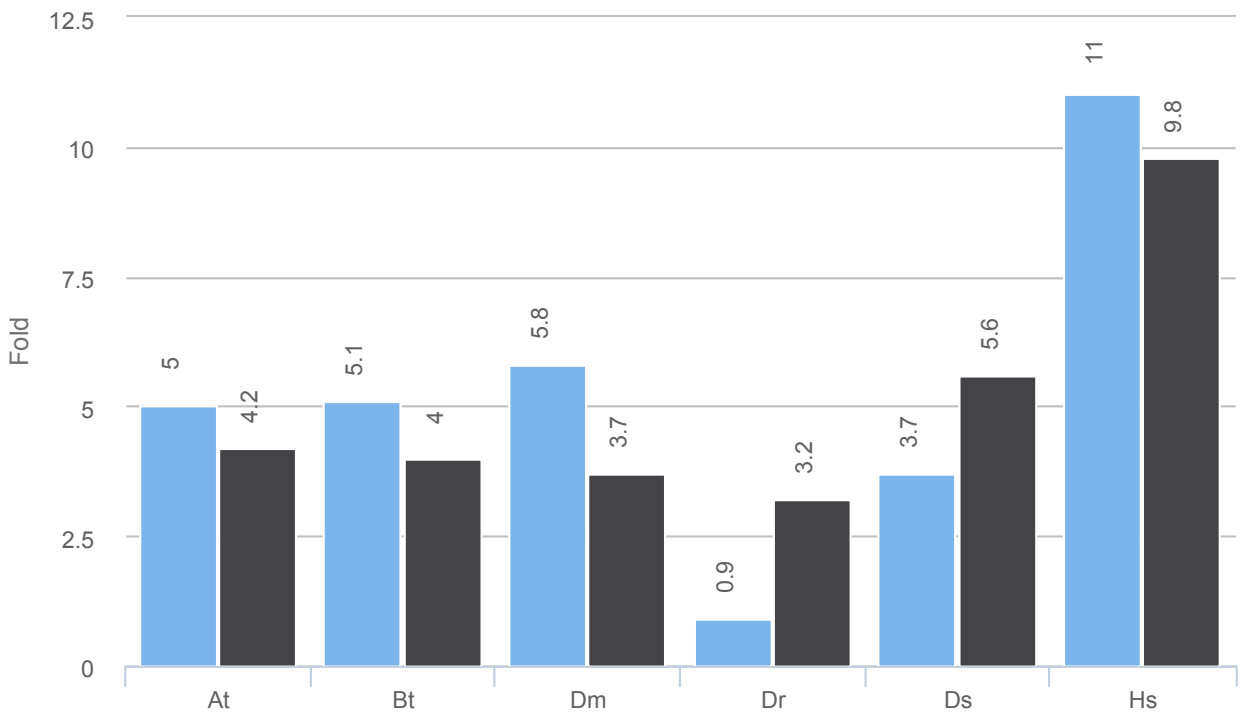
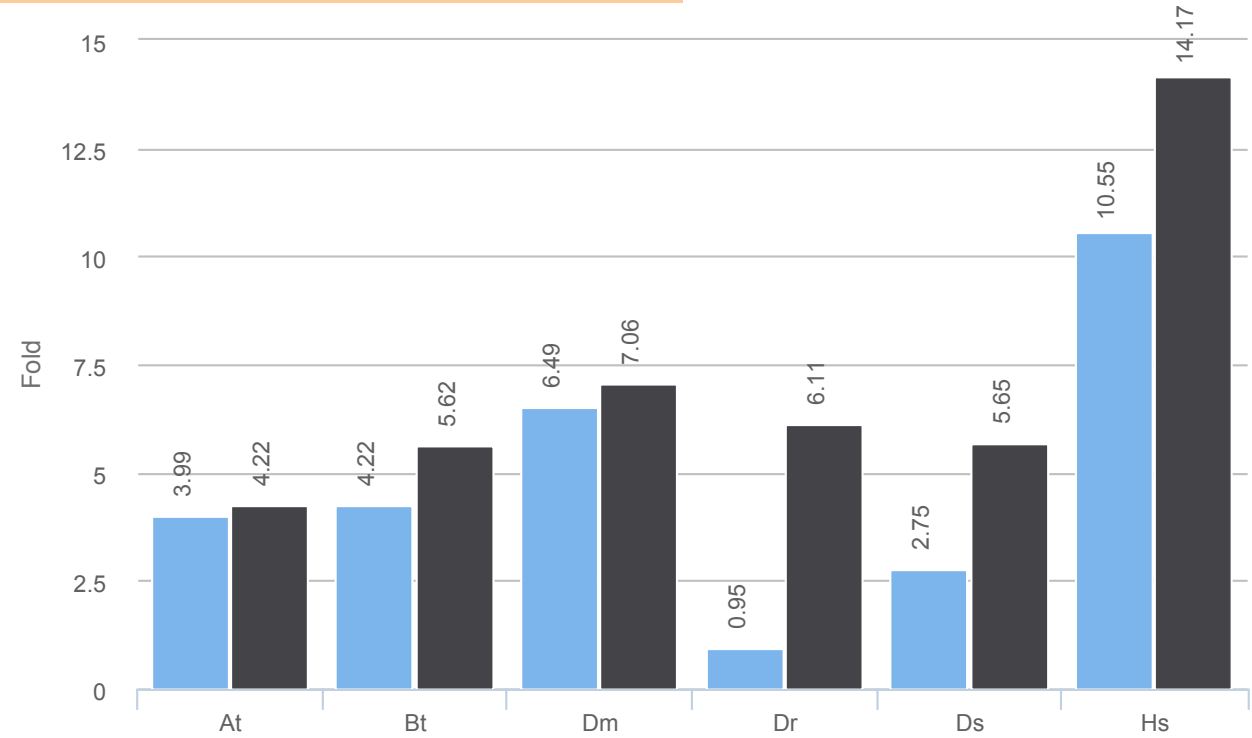
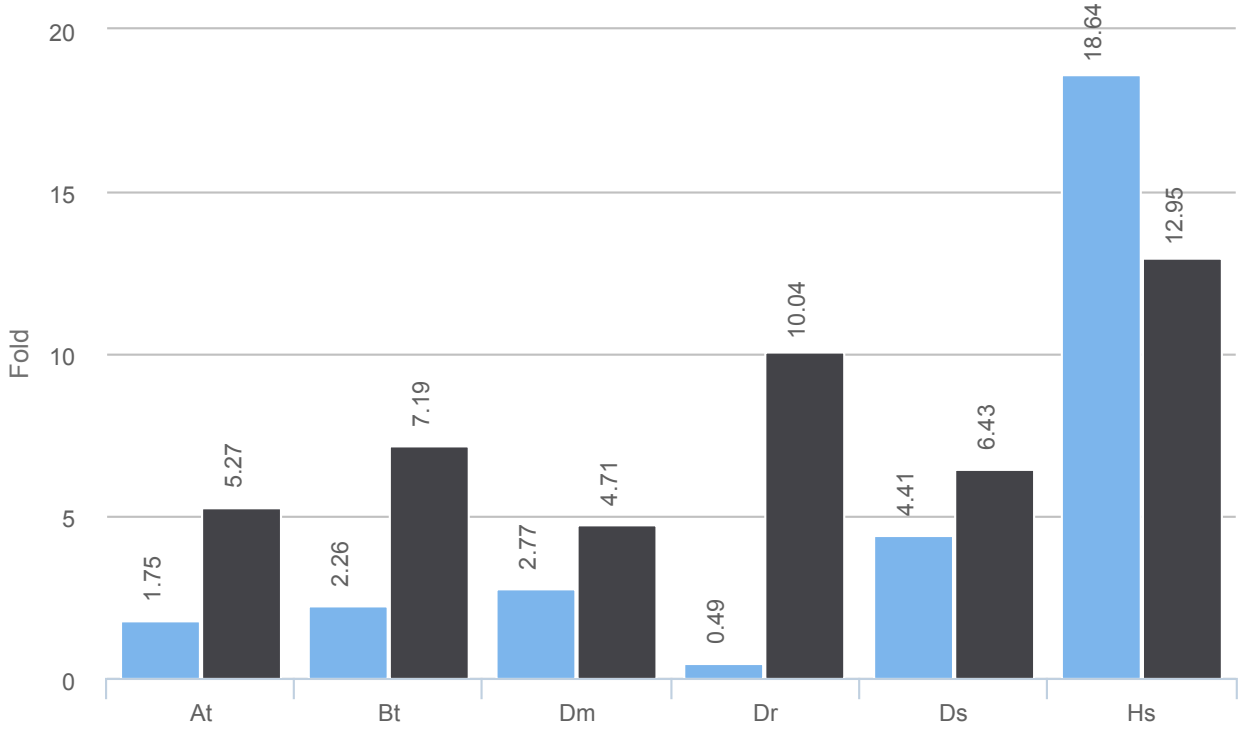
(A) Consensus error rate



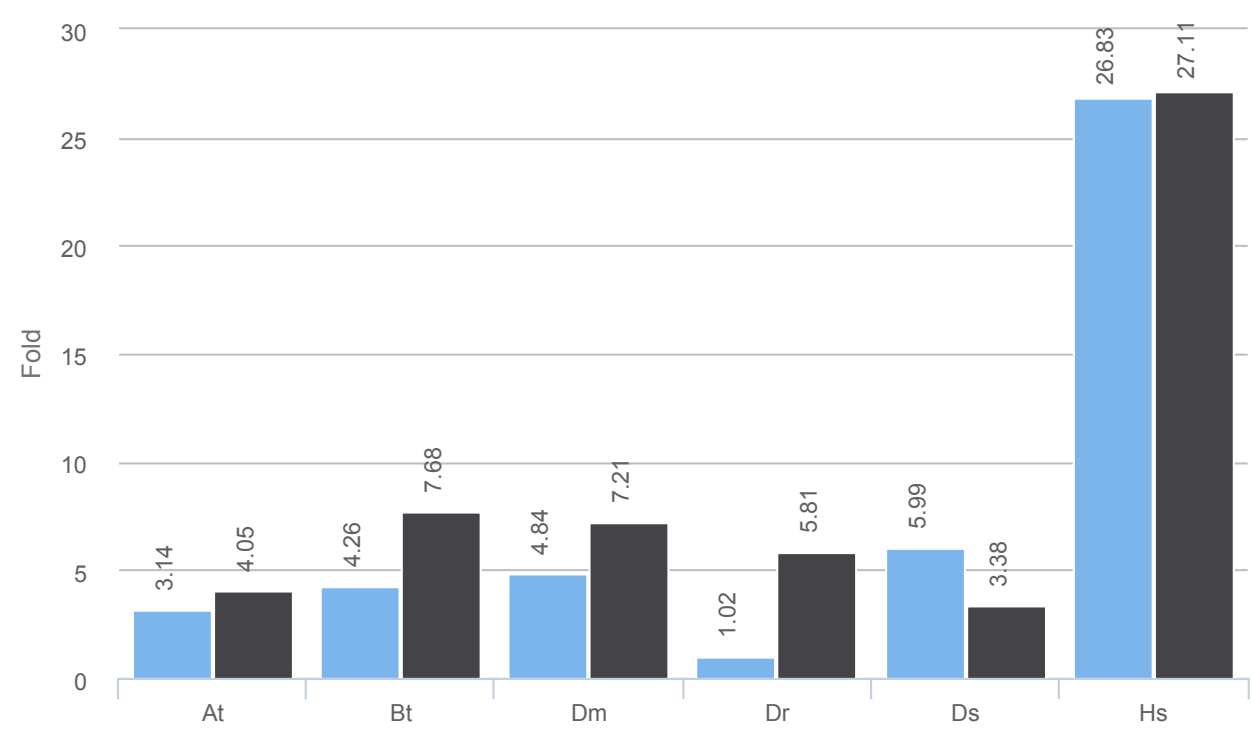
(B) Consensus substitution rate



(C) Consensus insertion rate



(D) Consensus deletion rate



Raw Oases+ DRAP Oases Raw Trinity+ DRAP Trinity

Figure 3(on next page)

Reads re-alignment rates

Figures A and B show respectively the alignment rates for reads and read pairs for the four assemblies of each dataset

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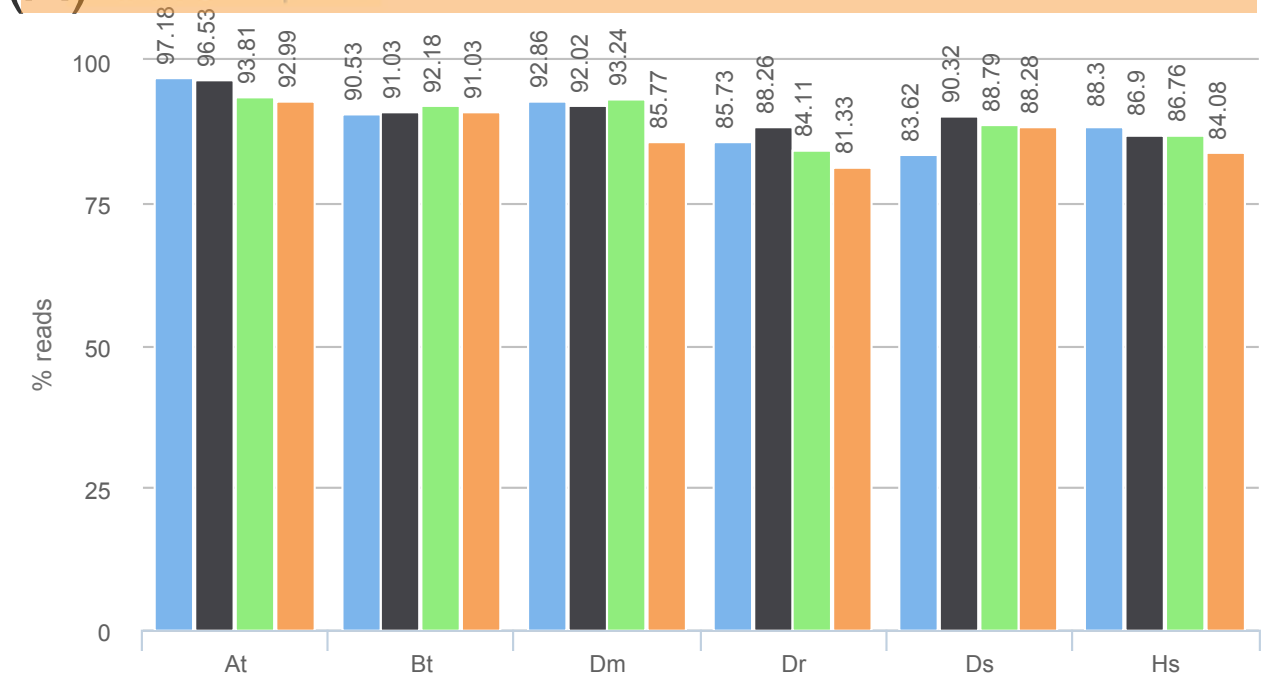

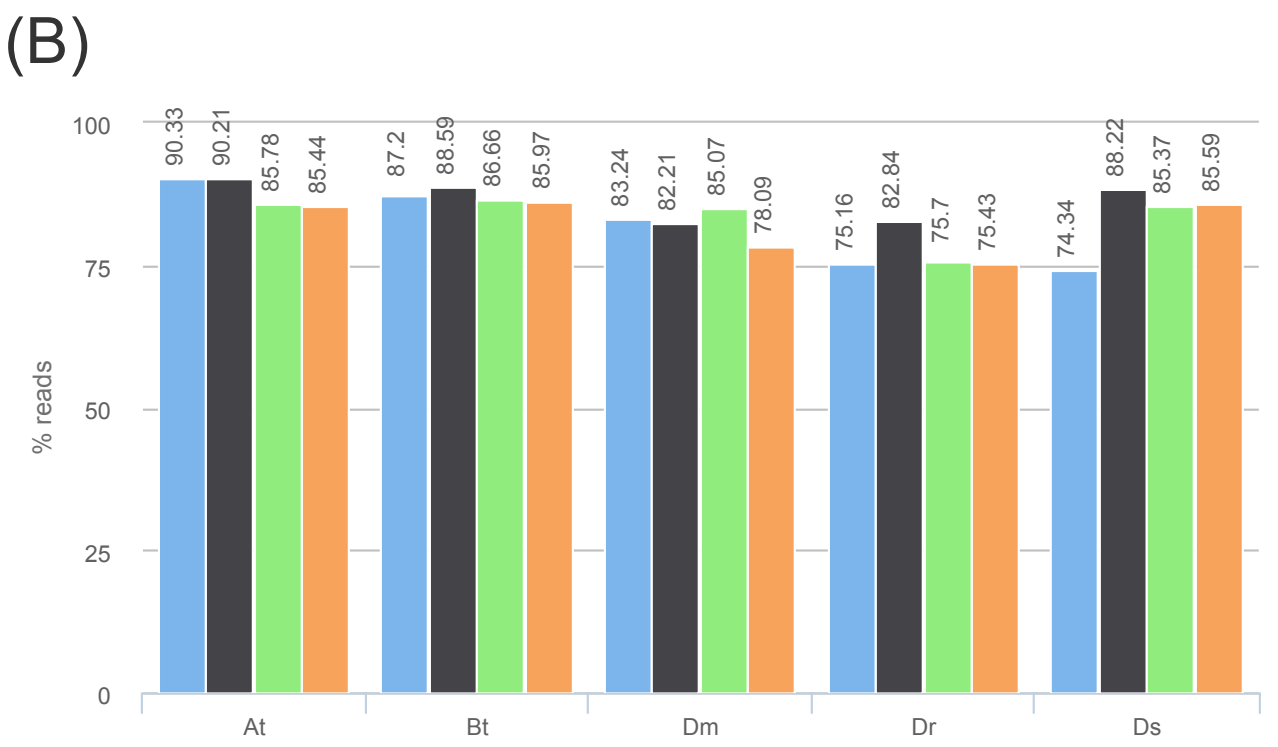

Figure 4(on next page)

Proteins realignment rates

The figure shows the number of proteins which have been aligned on the contig sets with more than 80% identity and 80% coverage for each assembler and dataset

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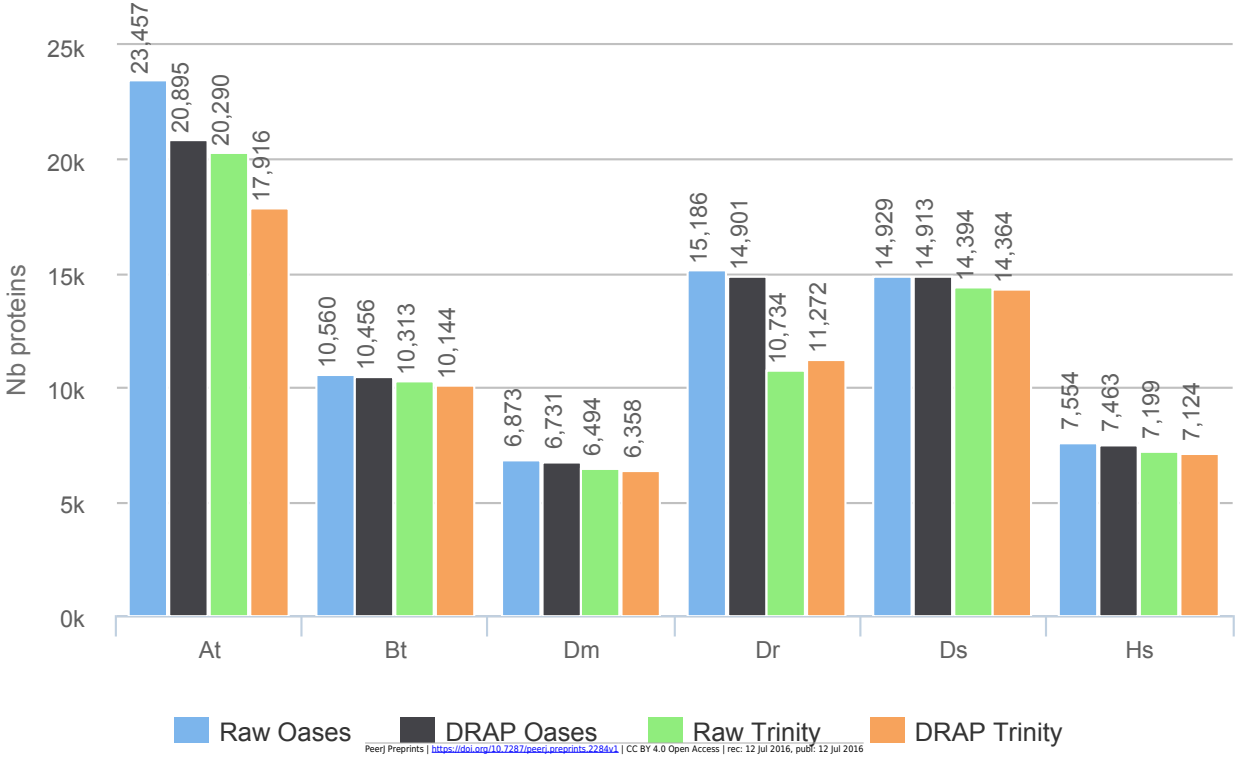

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Figure 5(on next page)

TransRate scores

Figure A presents TransRate scored of the four assemblers for each dataset

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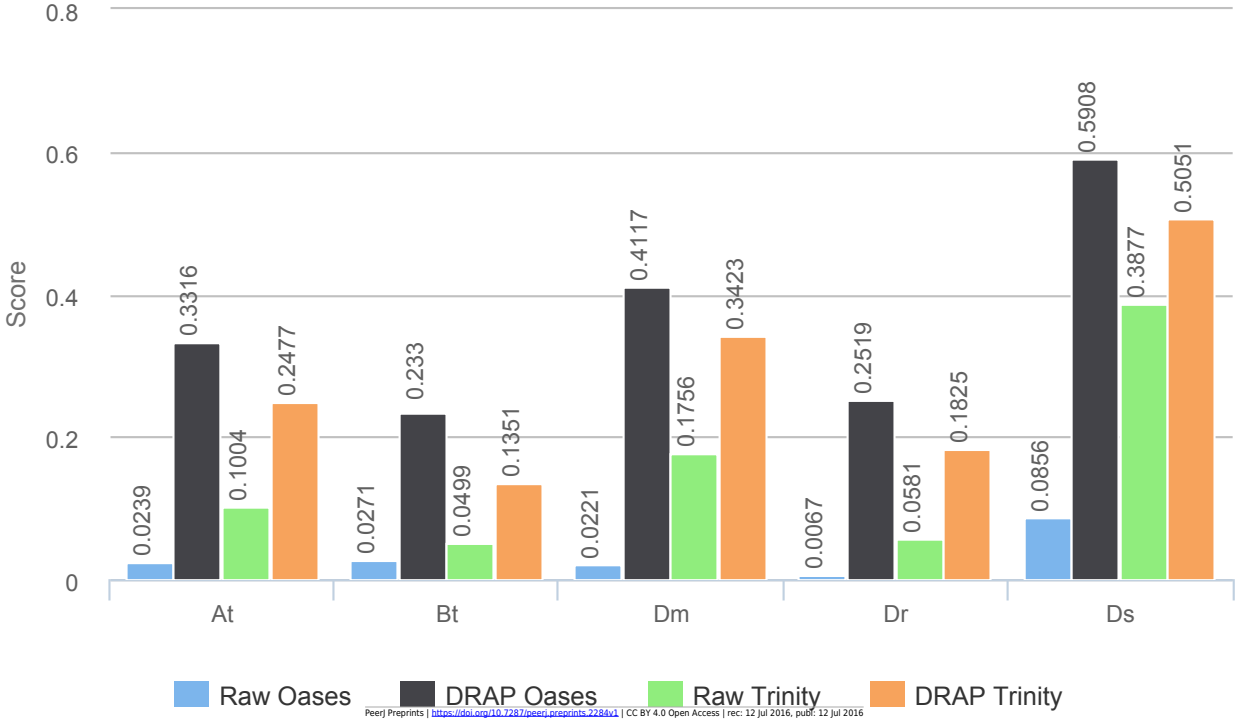

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Figure 6(on next page)

Gene reconstruction versus expression depth using simulated reads

The figure presents the proportion of correctly build transcripts (method presented in Data S1) versus the read count per transcript

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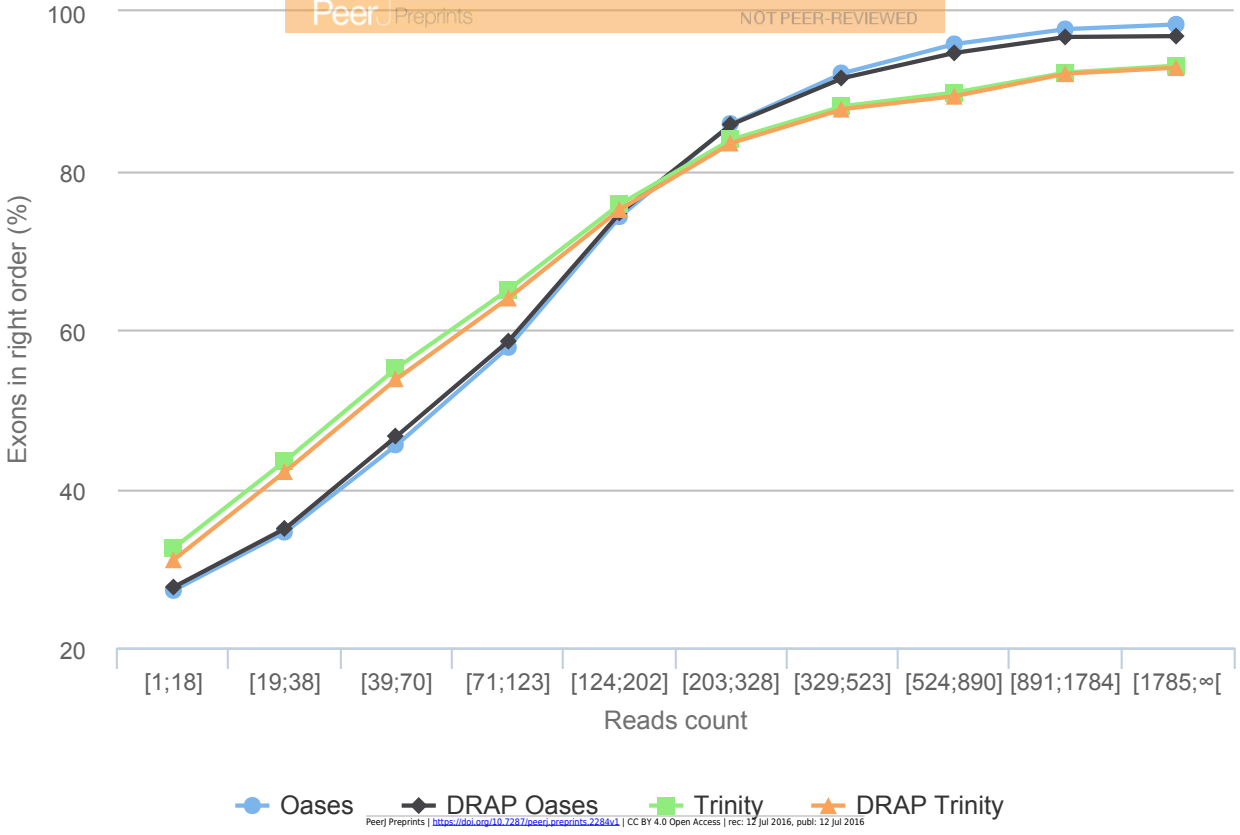

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Table 1(on next page)

Datasets

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Name	Species	Layout			Library		Protocol	
		Paired	Stranded	Length (nt)	Nb R1	SRA ID	Tissue	Condition
At	Arabidopsis thaliana	Yes	-	100	32 041 730	SRR1773557	Root	Full nutrition
		Yes	-	100	30 990 531	SRR1773560	Shoot	Full nutrition
		Yes	-	100	24 898 527	SRR1773563	Root	N starvation
		Yes	-	100	54 344 171	SRR1773569	Flower	Full nutrition
		Yes	-	150	31 467 967	SRR1773580	Shoot	N starvation
Bt	Bos taurus	Yes	No	100	30 140 101	SRR2635009	Milk	Day 70 with low milk production
		Yes	No	75	15 339 206	SRR2659964	Endometrium	-
		Yes	Yes	50	13 542 516	SRR2891058	Oviduct	-
Dd	Danio rerio	Yes	No	100	35 368 936	SRR1524238	Brain	5 months female
					54 472 116	SRR1524239	Gills	5 months female
					85 672 616	SRR1524240	Heart	5 months male and female
					34 032 976	SRR1524241	Muscle	5 months female
					59 248 034	SRR1524242	Liver	5 months female
					46 371 614	SRR1524243	Kidney	5 months male and female
					96 715 965	SRR1524244	Bones	5 months female
					43 187 341	SRR1524245	Intestine	5 months female
					55 185 501	SRR1524246	Embryo	2 days embryo
					24 878 233	SRR1524247	Unfertilized eggs	5 months female
					22 026 486	SRR1524248	Ovary	5 months female
					59 897 686	SRR1524249	Testis	5 months male
Dm	Drosophila melanogaster	Yes	Yes	75	21 849 652	SRR2496909	Cell line R4	Time P17
					21 864 887	SRR2496910	Cell line R4	Time P19
					20 194 362	SRR2496918	Cell line R5	Time P17
					22 596 303	SRR2496919	Cell line R5	Time P19
Dr	Danio rerio	Yes	No	100	5 072 822	SRR1048059	Pineal gland	Light
					8 451 113	SRR1048060	Pineal gland	Light
					8 753 789	SRR1048061	Pineal gland	Dark
					7 420 748	SRR1048062	Pineal gland	Dark
					9 737 614	SRR1048063	Pineal gland	Dark
Ds	Danio rerio	Yes	No	100	30 000 000	Simulated	-	-
Hs	Homo sapiens	No	No	25-50	15 885 224	SRR2569874	TK6 cells	pretreated with the protein kinase C activating tumor
					15 133 619	SRR2569875	TK6 cells	pretreated with the protein kinase C activating tumor
					19 312 543	SRR2569877	TK6 cells	pretreated with the protein kinase C activating tumor
					21 956 840	SRR2569878	TK6 cells	pretreated with the protein kinase C activating tumor

1

Table 2 (on next page)

Compaction and correction in DRAP and standard assembler

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Dataset	Assembler	Nb seq	N50 (nt)	L50 (nt)	Sum(nt)	Median Length (nt)	Included Contigs (%)	Contigs with Multi-ORF (%)	Contigs with Multi-prot (%)	Chimeric Contigs (%)	Contigs with Bias (%)
At	Oases	381 440	**2 971**	**92 020**	834 329 264	**1 816**	72.75	27.89	**0.26**	0.80	13.88
	DRAP_oases	**32 269**	2 014	9 563	**56 122 047**	1 547	**0.00**	**0.24**	1.40	**0.04**	**2.78**
	Trinity	95 008	**2 198**	**19 140**	130 969 737	991	4.05	15.63	1.22	0.20	11.29
	DRAP_trinity	**54 923**	1 761	15 857	**80 258 659**	**1 287**	**0.00**	**0.20**	**0.52**	**0.00**	**2.68**
Bt	Oases	147 163	**2 739**	**31 441**	269 085 141	**1 359**	71.19	7.45	**0.06**	0.66	6.29
	DRAP_oases	**29 685**	2 441	6 029	**47 727 730**	1 111	**0.00**	**0.28**	0.32	**0.03**	**1.23**
	Trinity	89 520	**2 184**	**12 080**	90 989 611	431	4.12	3.69	0.17	0.12	5.98
	DRAP_trinity	**46 561**	2 129	9 183	**64 809 448**	**927**	**0.00**	**0.23**	**0.14**	**0.00**	**1.50**
Dm	Oases	178 696	2 220	**29 086**	232 776 717	756	75.48	5.14	**0.18**	0.35	13.11
	DRAP_oases	**21 550**	**2 309**	3 674	**29 372 261**	**804**	**0.00**	**0.09**	0.45	**0.06**	**2.27**
	Trinity	55 214	**2 266**	**7 126**	57 209 890	438	5.19	4.58	0.95	0.22	13.33
	DRAP_trinity	**27 236**	2 146	5 240	**37 249 612**	**914**	**0.00**	**0.07**	**0.31**	**0.00**	**3.59**
Dr	Oases	702 640	2 715	**114 042**	1 059 904 844	857	70.99	2.80	**0.01**	1.39	**11.52**
	DRAP_oases	**46 831**	**2 757**	9 046	**82 268 872**	**1 173**	**0.00**	**0.15**	0.27	**0.16**	13.05
	Trinity	126 210	1 279	**21 003**	96 279 046	418	5.56	0.81	**0.08**	0.56	23.63
	DRAP_trinity	**58 114**	**1 644**	13 022	**68 900 396**	**866**	**0.00**	**0.07**	0.12	**0.00**	**7.41**
Ds	Oases	131 982	2 975	**28 618**	280 469 694	1 619	75.05	3.05	**0.06**	0.14	4.07
	DRAP_oases	**21 191**	**3 000**	4 872	**46 994 928**	**1 744**	**0.00**	**0.08**	0.25	**0.02**	**1.10**
	Trinity	40 335	**2 398**	**7 159**	58 571 859	910	3.12	1.82	0.37	0.09	6.47
	DRAP_trinity	**31 113**	2 381	6 492	**51 580 407**	**1 205**	**0.00**	**0.04**	**0.14**	**0.00**	**1.15**
Hs	Oases	101 271	**2 048**	**20 131**	132 681 065	**895**	55.73	5.55	**0.03**	0.11	7.51
	DRAP_oases	**30 201**	1 880	5 542	**34 670 862**	540	**0.00**	**0.15**	0.08	**0.00**	**0.68**
	Trinity	57 195	1 687	**7 843**	47 639 190	384	2.63	2.85	0.12	0.09	5.79
	DRAP_trinity	**39 489**	**1 705**	6 621	**38 557 758**	**540**	**0.00**	**0.11**	**0.06**	**0.00**	**0.59**

1 Bold values are “best in class” values between raw and DRAP assemblies

Table 3(on next page)

Validation DRAP against standard assembler

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Dataset	Assembler	% contigs by ORF count		Contigs with Complete ORF (%)	% contigs by Proteins count		Nb reference Proteins aligned	Reads mapping (%)		Score * 100
		0	1		0	1		Mapped	Properly paired	
At	Oases	18.96	53.15	65.72	94.27	5.57	**23 457**	**97.18**	**90.33**	2.39
	DRAP_oases	**9.90**	**89.86**	**72.38**	**39.38**	**59.22**	20 895	96.53	90.21	33.16
	Trinity	38.97	45.40	40.32	81.09	17.69	**20 290**	**93.81**	**85.78**	10.04
	DRAP_trinity	**13.89**	**85.91**	**55.51**	**69.85**	**29.64**	17 916	92.99	85.44	24.77
Bt	Oases	36.07	56.48	**28.29**	93.33	6.61	**10 560**	90.53	87.20	2.71
	DRAP_oases	**32.59**	**67.13**	25.70	**67.63**	**32.05**	10 456	**91.03**	**88.59**	23.30
	Trinity	64.13	32.18	15.33	89.48	10.35	**10 313**	**92.18**	**86.66**	4.99
	DRAP_trinity	**38.55**	**61.23**	**24.86**	**79.95**	**19.91**	10 144	91.03	85.97	13.51
Dm	Oases	**46.19**	48.67	20.27	96.43	3.39	**6 873**	**92.86**	**83.24**	2.21
	DRAP_oases	48.80	**51.11**	**31.45**	**70.30**	**29.25**	6 731	92.02	82.21	41.17
	Trinity	67.53	27.89	18.49	89.63	9.42	**6 494**	**93.24**	**85.07**	17.56
	DRAP_trinity	**45.94**	**53.99**	**32.23**	**77.76**	**21.93**	6 358	85.77	78.09	34.23
Dr	Oases	56.81	40.39	23.37	97.98	2.01	**15 186**	85.73	75.16	0.67
	DRAP_oases	**40.20**	**59.65**	**33.43**	**70.89**	**28.84**	14 901	**88.26**	**82.84**	25.19
	Trinity	66.76	32.43	9.79	92.34	7.58	**10 734**	**84.11**	**75.70**	5.81
	DRAP_trinity	**39.74**	**60.19**	**20.16**	**82.44**	**17.44**	11 272	81.33	75.43	18.25
Ds	Oases	24.52	72.43	41.60	89.47	10.47	**14 929**	83.62	74.34	8.56
	DRAP_oases	**12.80**	**87.11**	**53.73**	**35.56**	**64.19**	14 913	**90.32**	**88.22**	59.08
	Trinity	37.72	60.46	30.29	67.37	32.26	**14 394**	**88.79**	85.37	38.77
	DRAP_trinity	**22.85**	**77.11**	**37.65**	**57.53**	**42.33**	14 364	88.28	**85.59**	50.51
Hs	Oases	**44.51**	49.94	**21.18**	93.04	6.93	**7 554**	**88.30**	NA	NA
	DRAP_oases	46.95	**52.91**	20.06	**77.28**	**22.64**	7 463	86.90	NA	NA
	Trinity	69.02	28.13	11.70	88.53	11.35	**7 199**	**86.76**	NA	NA
	DRAP_trinity	**55.48**	**44.41**	**16.07**	**83.46**	**16.48**	7 124	84.08	NA	NA

1 Bold values are “best in class” values between raw and DRAP assemblies

Table 4(on next page)

Pooled samples vs meta-assembly strategie

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Assembly strategy		Pooled_oases	Meta_oases	Pooled_trinity	Meta_trinity
Compaction					
Nb seq		**42 726**	43 049	**62 327**	65 271
N50 (nt)		**3 565**	3 379	2 027	**2 237**
L50 (nt)		**10 409**	9 259	**14 956**	13 106
Sum (nt)		114 371 598	**99 928 206**	**94 993 910**	98 421 439
Median length (nt)		**2 182**	1 766	**1 217**	1 052
Contigs with multi-ORF (%)		**0.33**	0.50	**0.13**	0.17
Contigs with multi-prot (%)		**1.39**	1.73	**0.64**	0.95
Correction					
Chimeric contigs (%)		**0.11**	0.21	0.00	0.00
Contigs with bias (%)		75.19	**68.00**	**58.79**	61.88
Validation					
% contigs by ORF count	0	**24.79**	38.77	**37.24**	50.63
	1	**74.88**	60.72	**62.63**	49.20
Contigs with complete ORF (%)		**61.84**	46.36	**38.80**	31.55
% contigs by proteins count	0	58.52	**57.15**	75.23	**72.02**
	1	40.09	**41.13**	24.13	**27.03**
Nb reference proteins aligned		32 367	**35 432**	26 041	**33 385**
Reads mapping (%)	Mapped	87.38	**87.57**	77.82	**85.19**
	Properly paired	78.88	**80.13**	70.13	**77.30**
Score * 100		28.66	**29.49**	17.97	**23.36**

1 Bold values are “best in class” values between raw and DRAP assemblies
2

Table 5(on next page)

Structure validation on Ds dataset

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Assembly	Retrieved Exons	Exons in Right contig	Exons in Right order	Contigs with More than 1 gene	Max number Of genes by contig
Real assembly	99.81 %	99.81 %	99.50 %	0.16 % (46)	5
Raw Oases	80.03 %	**77.83 %**	**77.61 %**	**2.77 % (537)**	221
DRAP_oases	**80.21 %**	77.54 %	77.29 %	4.13 % (671)	**203**
Raw Trinity	**84.24 %**	**77.30 %**	**77.10 %**	3.65 % (717)	339
DRAP_trinity	83.30 %	76.65 %	76.47 %	**3.17 % (602)**	**327**

1 Bold values are “best in class” values between raw and DRAP assemblies
2