

**A peer-reviewed version of this preprint was published in PeerJ on 16 February 2017.**

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Cabau C, Escudié F, Djari A, Guiguen Y, Bobe J, Klopp C. 2017. Compacting and correcting Trinity and Oases RNA-Seq *de novo* assemblies. PeerJ 5:e2988 <https://doi.org/10.7717/peerj.2988>

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

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## 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 as 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 easy to use software package to produce compact and corrected transcript set. DRAP is free, open-source and available at <http://www.sigene.org/drap>.

**Title**

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

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# **Abstract**

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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.

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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 as read realignment rate or protein reconstruction counts.

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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>.

**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. runDrapp, 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 over 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.

## Quality assessment

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

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

## Parallel processing and flow control

DRAP runs on Unix machines or clusters. Different steps of the assembly or assessment process 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

therefore limits the number of positions for which the correction can be made. As shown in Fig. 2 the Dr dataset is an outlier concerning this criteria. Regarding the five other datasets raw versus DRAP correction rates range from 1.7 to 18.6 for insertions, 3.1 to 27.1 for deletions and 2.7 to 14.1 for substitutions. DRAP correction steps lowers significantly the number of positions for which the consensus does not correspond to the major allele found in the alignment.

# Assembly quality assessment

The two previous parts have shown the beneficial impacts of DRAP on the assembly compactness and error rates but this should not impair quality metrics such as read and read pairs alignment rates, number of ORFs, complete ORFs found in the contigs, number of proteins of the known proteome mapped on the contigs or TransRate marks.

Read and read pair alignment rates differences between raw and DRAP assemblies are usually very low, between 1 and 2% and can sometimes be in favor of DRAP (Fig. 4) . In our test sets, the difference is significant (7.5%) for Dm when comparing Trinity to DRAP Trinity. This comes from the removal by DRAP of a highly expressed transcript (Ensembl: FBtr0100888 mitochondrial large ribosomal RNA) because that does not fulfill the criteria of having at least one 200 base pairs long ORF despite having over 11M reads aligned on the corresponding contig in the Trinity assembly. DRAP Oases assembly was not impacted because it builds a longer contig for this transcript with a long enough ORF to be selected in the additional part.

The reference proteome has been aligned on the contigs and matches with over 80% identity and 80% protein coverage have been counted. These figures give a good overview of the amount of 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.

Differences in compaction and correction are more important between Trinity and Oases than between pooled versus meta-assembly. Pooled assemblies collect significantly worse results for the number of reference proteins and number of read pairs aligned on the contigs. This comes from the filtering strategy which eliminates low-expressed contigs of a given condition when merging all the samples but will keep these contigs in a per sample assembly and meta-assembly strategy. Therefore we recommend using runMeta when the assembly input samples mix distinct conditions with specific and variable expression patterns.

# Assemblies fidelity check using simulated reads

The simulation process links each read with its transcript of origin. With this information it is possible to link contigs and transcripts. Here, the transcript-contig link was calculated using exon content and order in both sets (method explained in Data S1). The results presented in Table 5 first shows that the assembly process loses between 15.76 and 19.97% of the exons compared to the initial transcript set. This loss is close to 22% for all assemblies when the exon order is taken into account. As shown in Fig. 6, this is mainly the case for transcripts with low read coverage. The figures show once more that DRAP has a very limited negative impact on number of retrieved exons in correct order.

Table 5 shows the number of contigs linked to more than one gene. DRAP compaction and ORF splitting feature could have an antagonist impact for this criteria. But depending on the assembler, the figures are in favor or not of DRAP.

Table 5 also presents the maximum number of genes linked to a single contig. These clusters 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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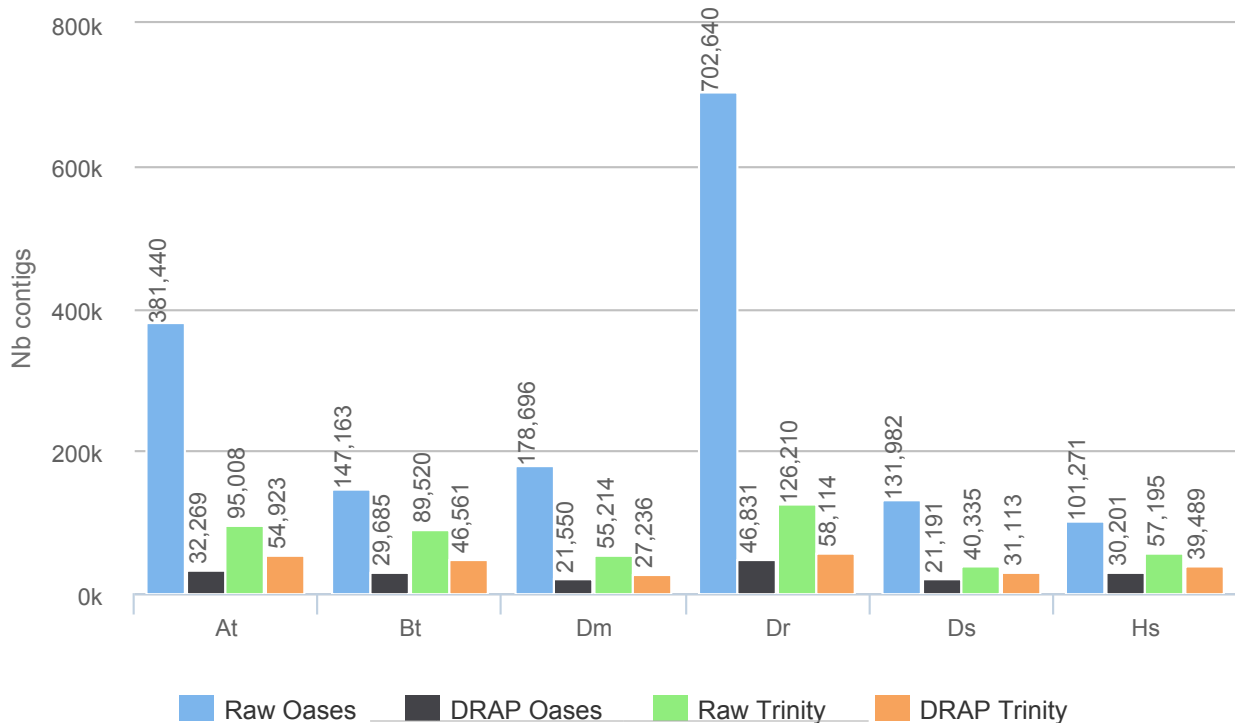
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# **Figure 1**(on next page)

Number of contigs

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



## 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.

(A)

Consensus error rate

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(B)

NOT PEER-REVIEWED

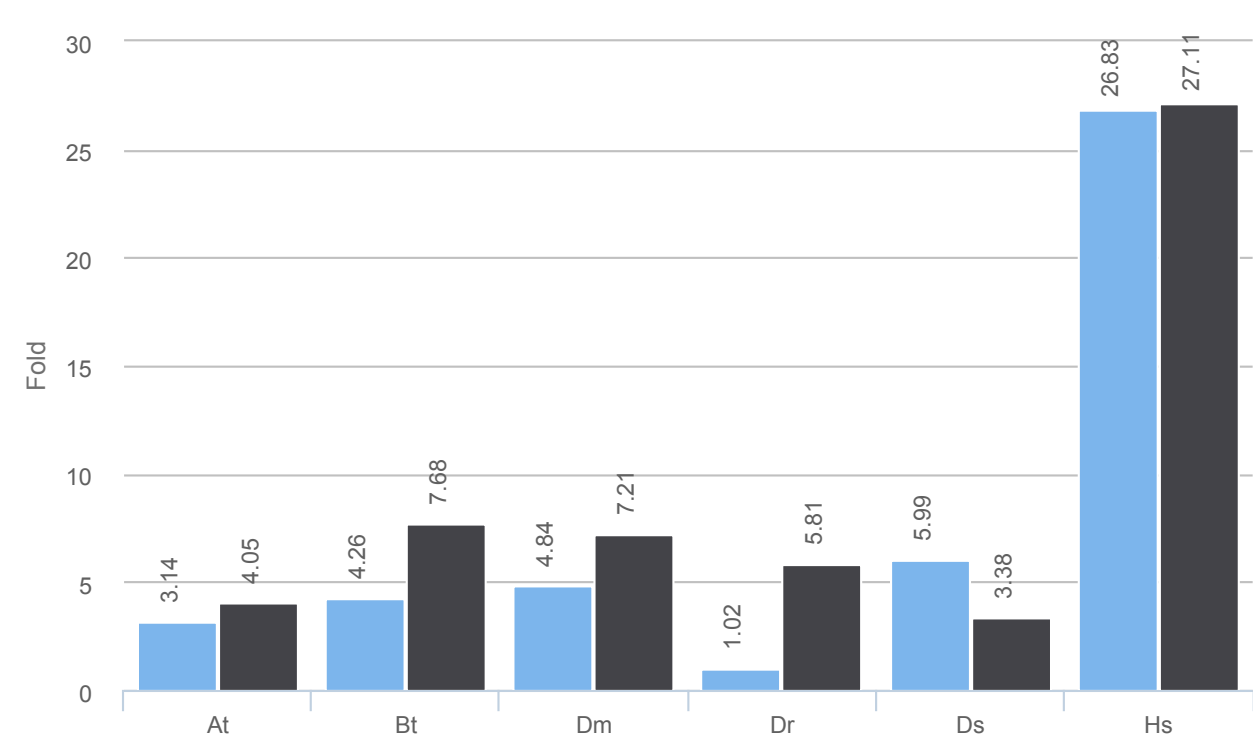
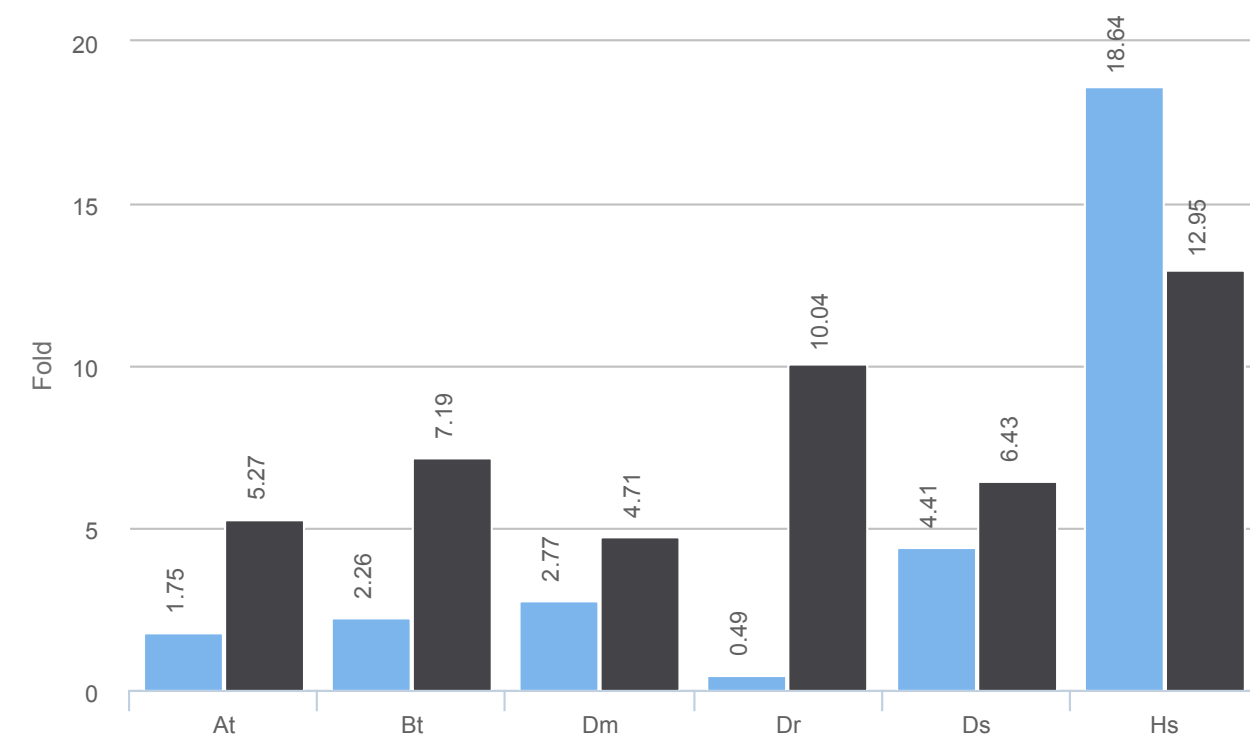
Consensus substitution rate

(C)

Consensus insertion rate

(D)

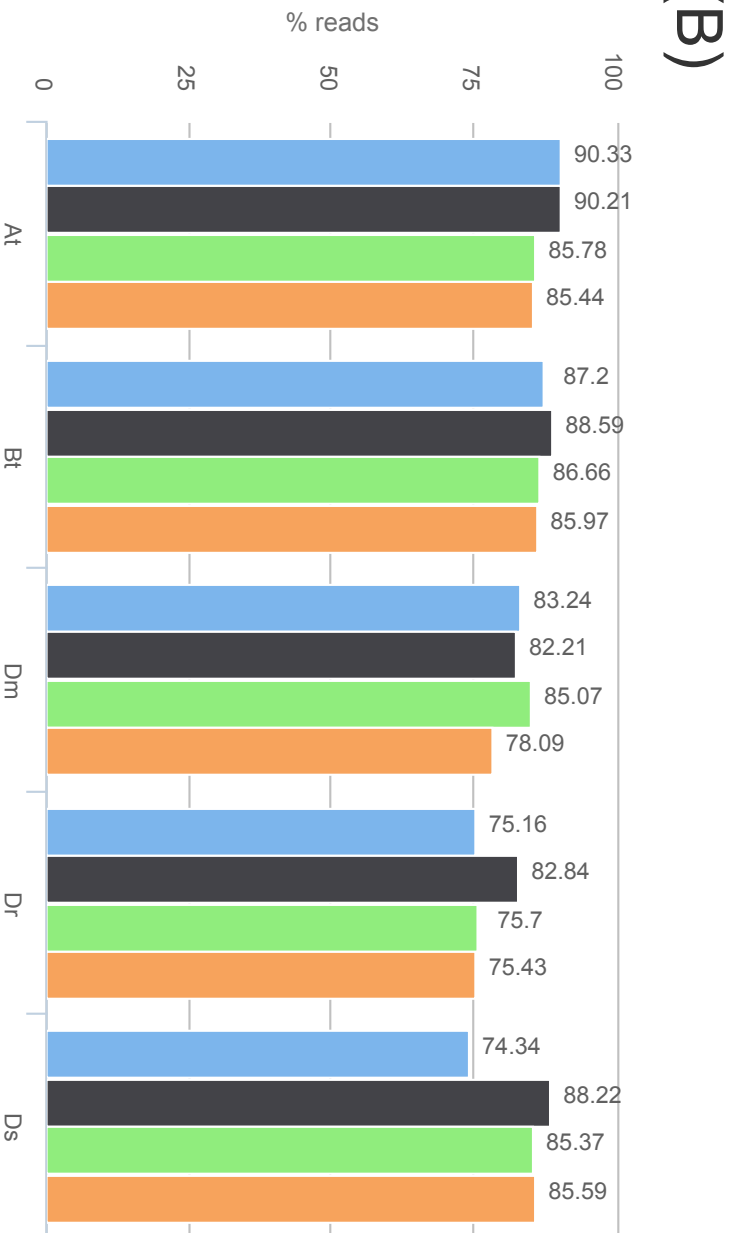
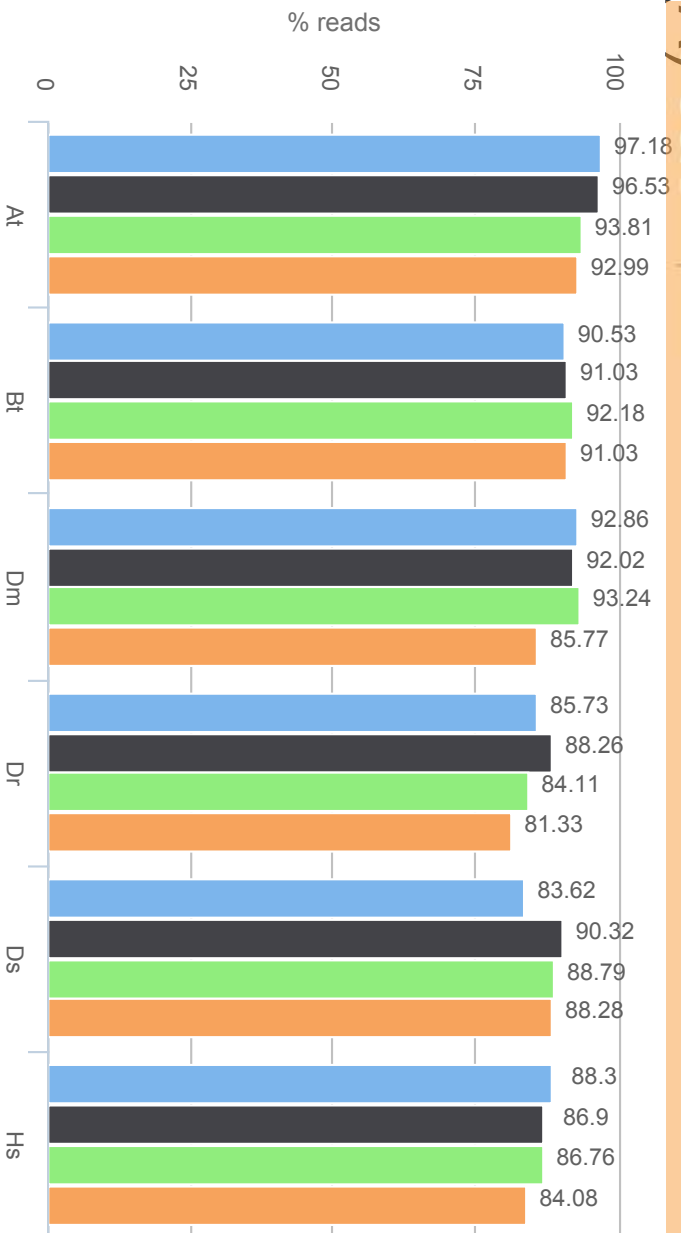
Consensus deletion rate



# Figure 3(on next page)

Reads re-alignment rates

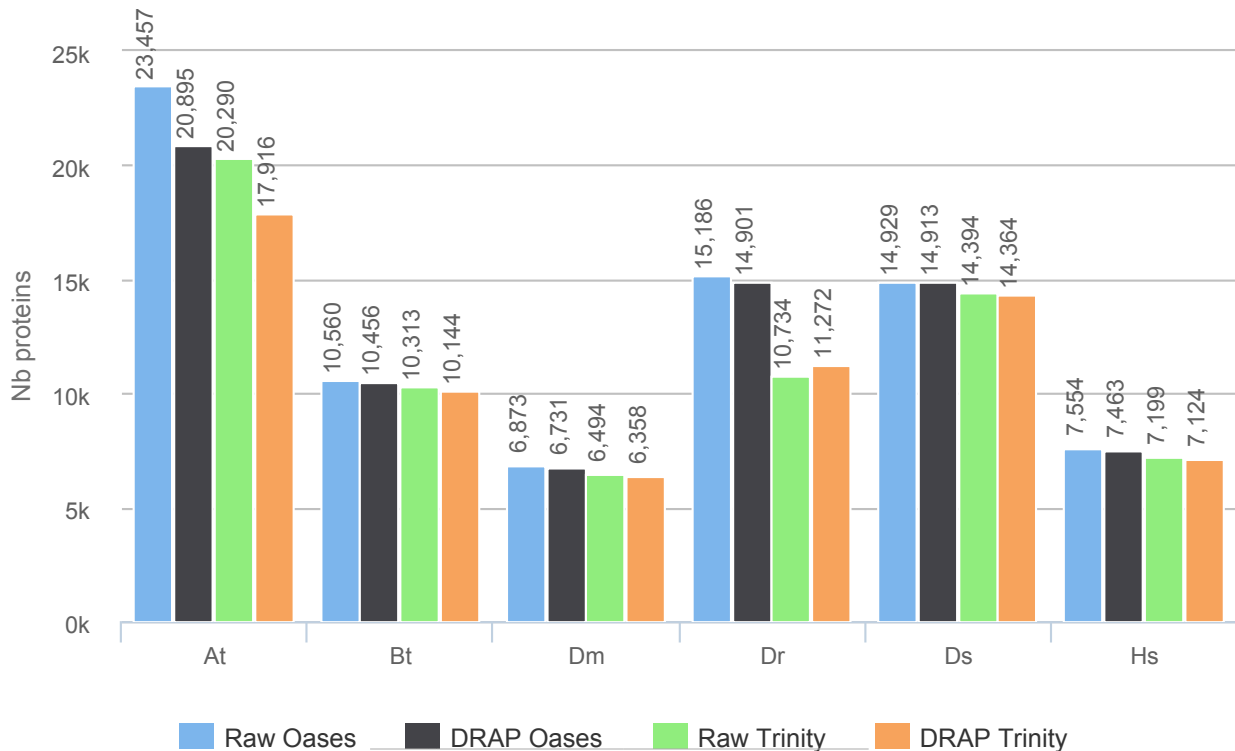
Figures A and B show respectively the alignment rates for reads and read pairs for the four assemblies of each dataset



# Figure 4(on next page)

## Proteins realignment rates

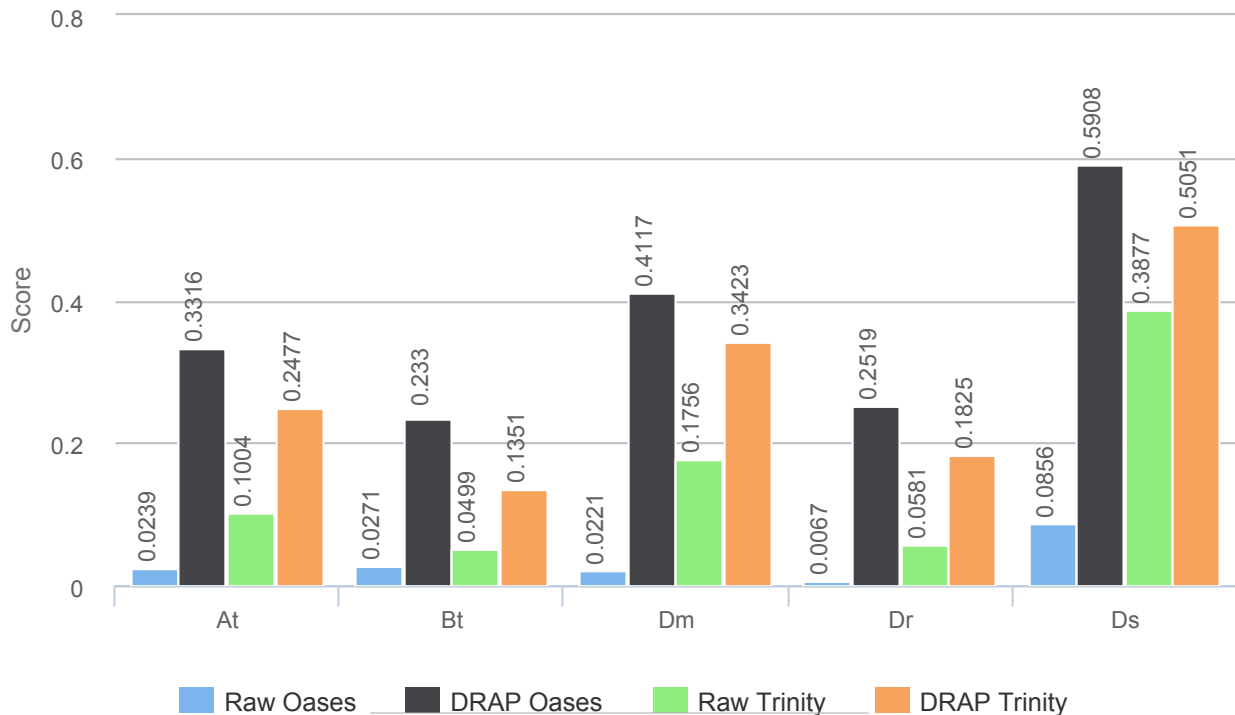
The figure shows the number of proteins which have been aligned on the contig sets with more than 80% identity and 80% coverage for each assembler and dataset



# Figure 5(on next page)

TransRate scores

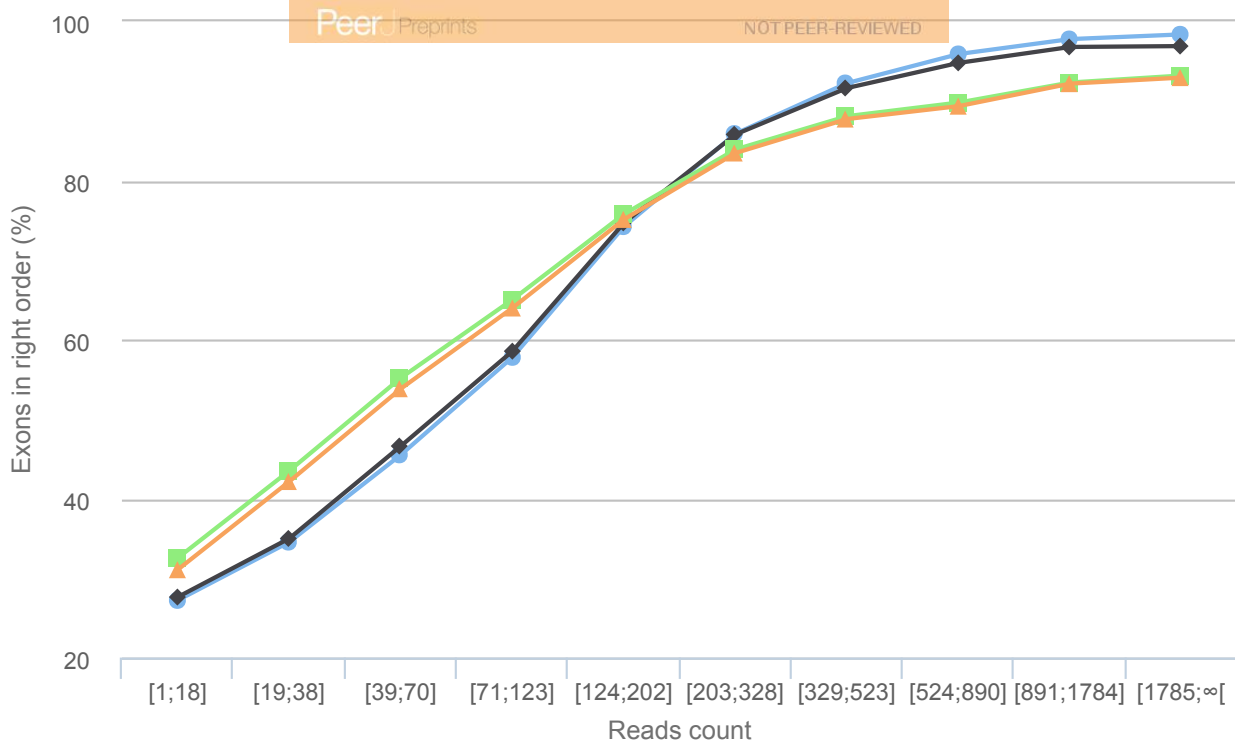
Figure A presents TransRate scored of the four assemblers for each dataset



# **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



● Oases

◆ DRAP Oases

■ Trinity

▲ DRAP Trinity

# **Table 1** (on next page)

Datasets

| 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

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

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

# **Table 3**(on next page)

Validation DRAP against standard assembler

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

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

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

1  
2

# **Table 5**(on next page)

Structure validation on Ds dataset

| 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 %         | <b>77.83 %</b>        | <b>77.61 %</b>       | <b>2.77 % (537)</b>           | 221                           |
| DRAP_oases    | <b>80.21 %</b>  | 77.54 %               | 77.29 %              | 4.13 % (671)                  | <b>203</b>                    |
| Raw Trinity   | <b>84.24 %</b>  | <b>77.30 %</b>        | <b>77.10 %</b>       | 3.65 % (717)                  | 339                           |
| DRAP_trinity  | 83.30 %         | 76.65 %               | 76.47 %              | <b>3.17 % (602)</b>           | <b>327</b>                    |

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

1  
2