Quality metrics for benchmarking sequences comparison tools

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METHODOLOGY

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Abstract

Background: Comparing sequences is a daily task in bioinformatics and many software try to fulfill this need by proposing fast execution times and accurate results. Introducing a new software in this field requires to compare it to recognized tools with the help of well defined metrics.

Results: A set of quality metrics is proposed that enables a systematic approach for comparing alignment tools. These metrics have been implemented in a dedicated software, allowing to produce textual and graphical benchmark artifacts.

Keywords: sequence; alignment; benchmark

1 Introduction

In Bioinformatics, the task of comparing genomic sequences is ubiquitous, leading to various software proposals over the years. With the NGS revolution, this task is becoming more and more time consuming as the size of NGS data truly explodes.

Software have tried to cope with this situation in different ways. First, by using known algorithms with optimized hardware usage. Second, by proposing new heuristics to reduce the searching space. The historically sequence comparison tools are:

- SSEARCH [1] that proposes an implementation of the Smith-Waterman algorithm with an efficient usage of multicore / SSE architecture; it provides an "exact" search alignment algorithm and is therefore slow, and consequently inadapted for large requests.
- BLAST [2] and FASTA [1] that both propose a seed based heuristics to speed-up the computation with a small loss of quality w.r.t. exact algorithms.

The trade-off between speed and quality is the key point. As a consequence, to evaluate new Alignement Search Tools (AST for short), benchmarking has to focus on time and quality metrics. Comparing execution times is straightforward. On the other hand, comparing quality is more challenging since it requires to define a precise quality metric.

The paper presents a methodology for comparing the results of AST. We explicitely target intensive sequence comparison for which there is an increasing demand due to high throuhput sequencing oportunities. A quality metric is defined and applied to a set of software and benchmarks.

2 Benchmarking AST

2.1 Configuration

To fairly benchmark AST, many configuration aspects must be taken into consideration:

1 For a given request, AST have to be configured for providing similars results. This is far from obvious since they generally propose a large set of parameters. In some cases, they can have hidden configuration.
2 Some AST implicitely modified the input banks. For instance, low complexity region [3], [4] can be systematically masked. These transformations may have significant influence on the results compared to other tools that are not applying these kinds of pre-processing.
3 AST often provide a statistical model telling whether an alignment has to be kept or not (for instance Karlin/Altschul model of Blast). Thus, AST with different models are likely to produce different alignments.

These points have a strong impact on the alignment list generated by the AST. Consequently, they need to be included in the quality metric itself. As a side effect, the AST command lines have to be explicitely described as part of the benchmark results.

In the following, we note $T^{(0)}$ (or T for short) an AST with its default configuration. Specific configurations of T will be noted as $T^{(i)}$ for $i \geq 1$.

2.2 Input request

To compare AST, a minimal set of common parameters needs to be specified. The following table lists the parameters we selected:

Table 1 Request parameters

r_1) query bank	r_6) reward cost
r_2) subject bank	r_7) penalty cost
r_3) e-value	r_8) substitution matrix
r_4) open gap cost	r_9) low complexity filtering
r_5) extend gap cost	r_{10}) number of cores

Parameters r_1 to r_9 have a direct impact on the output alignments. The r_{10} parameter has an impact on the execution time. The execution time can be used to analyze the scalability of AST as a function of the number of cores.

A request R can now be defined by a set of r_i. If not all r_i are specified, it is supposed that default values are used instead. A relation of equivalence can also be defined between two requests x and y:

$$x \sim y \iff \forall i \in [1..10];\ r_i(x) = r_i(y)$$

Here is an example of a request R_1 :

$$R_1 = \begin{cases} r_1 = \text{"}swissprot\text{"} \\ r_2 = \text{"}yeats\text{"} \\ r_3 = 1e-5 \end{cases}$$

2.3 Output data

Comparing AST qualities implies that AST generates the same nature of information. We choose the information provided by the Blast tabular output format; for each alignment, it provides 12 properties p_i as shown in table 2. Table 3 is an illustration of a Blast output.

Table 2 Alignment properties

p_1) query sequence id	p_7) start offset in query
p_2) subject sequence id	p_8) end offset in query
p_3) percentage of identity	p_9) start offset in subject
p_4) alignment length	p_{10}) end offset in subject
p_5) number of mismatches	p_{11}) e-value
p_6) number of gap openings	p_{12}) bitscore

Relying only on this set of properties means that only AST able to provide this information would be candidate. Fortunately, many AST can be parametrized to generate Blast-like tabular output format.

Table 3 Example of Blast tabular output

p_1	p_2	p_3	p_4	p_5	p_6	p_7	p_8	p_9	p_{10}	p_{11}	p_{12}
SPO13535	SPP02309	39.34	305	181	4	29	332	17	302	7.5e-62	231.6
SPO13535	SPQ91G55	34.93	209	134	2	42	250	46	252	1.5e-31	130.4
SPP02400	SPQ196T6	30.18	603	388	3	90	663	49	647	1.6e-76	282.4

3 Alignment Quality Metric

3.1 Definitions

Before specifying an alignment quality metric, we introduce the following definitions:

- I is a set of integers $i \in [1..12]$. I specifies a subset of properties p_i defined in table 2
- For a given set I, an alignment a is an object having properties p_i for $i \in I$
- Two alignments x and y are equivalent if their properties have the same values:

$$x \sim y \iff \forall i \in I;\ p_i(x) = p_i(y)$$

- an alignment set A contains alignments defined for a specific set I

An alignment set may contain equivalent items. In such a case, items equivalent to some alignment a are grouped into an equivalence class $\tilde{a}$.

$$\tilde{a} = \{x \in A; a \sim x\}$$

We define $\tilde{A}$ as the set built from A by keeping only one representant of each equivalence class $\tilde{a}$.

Taking the example of table 3, for $I = \{1\}$, we have the alignment set $A = \{a1, a2, a3\}$ and the set of equivalent alignments $\tilde{A} = \{\tilde{a_1}, \tilde{a_2}\}$ with:

$$\begin{cases} p_1(a1) = "SPO13535" \\ p_1(a2) = "SPO13535" \\ p_1(a3) = "SPP02400" \end{cases} \qquad \begin{cases} p_1(\tilde{a_1}) = "SPO13535" \\ p_1(\tilde{a_2}) = "SPP02400" \end{cases}$$

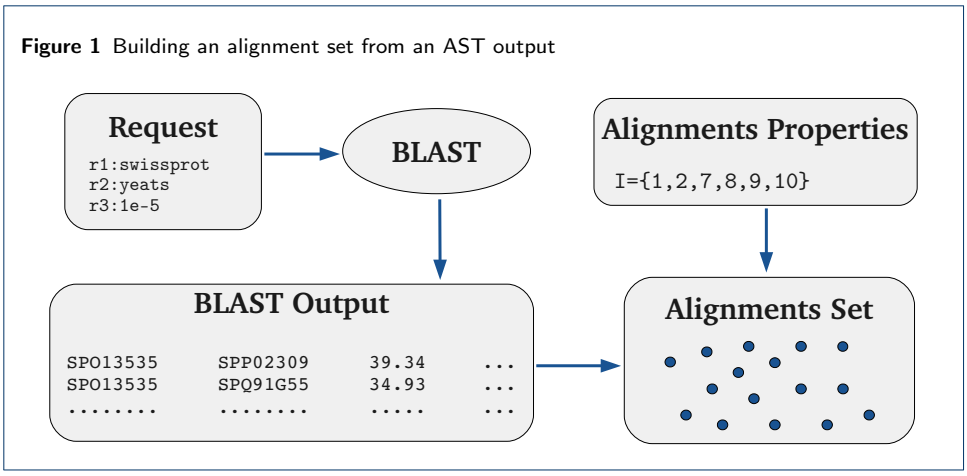


Figure 1 Building an alignment set from an AST output

3.2 From AST output to alignment set

With these definitions, an AST output can be formally transformed into an alignment set A where only some properties defined by the set I are kept. An alignment set can thus be defined as $A := \varphi(T, R, I)$ with T the AST, R the request and I the set of properties.

Figure 1 illustrates the transformation of a Blast output.

Note also that the same output generated by a tool will be viewed differently for two distinct sets I_1 and I_2. Therefore several quality metrics based on different I sets can be defined to provide different benchmark points of view.

3.3 Comparing AST

For a request R (defined by a set of r_i from table 1) and a given set I, we note:

$$A_1 = \varphi(T_1, R, I),\ \textit{alignment set found by AST } T_1 \textit{ for request } R$$
$$A_2 = \varphi(T_2, R, I),\ \textit{alignment set found by AST } T_2 \textit{ for request } R$$

Comparing T_1 to T_2 consists in finding a set $M^{(I)}$ such as:

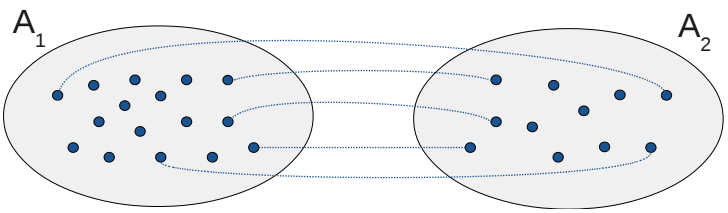
$$M^{(I)} = \begin{cases} (x,y) \in A_1 \times A_2; \quad x \sim y \\ \forall (x',y') \in A_1 \times A_2; \quad \begin{cases} x \sim y' \Rightarrow y' = y \\ x' \sim y \Rightarrow x' = x \end{cases} \end{cases}$$

The intuitive idea of this definition is to map an item from one set to at most one item of the other set. Note that some alignments in one set may not be connected to an alignment in the other set as shown figure 2.

The estimation of "how close two sets A_1 and A_2 are" can be done by considering the cardinal $|M^{(I)}|$ which represents the number of common alignments between A_1 and A_2. We define three numbers $N_c^{(I)}$, $N_1^{(I)}$ and $N_2^{(I)}$:

- $N_c^{(I)} := |M^{(I)}|$, number of common alignments between A_1 and A_2
- $N_1^{(I)} := |A_1| - |M^{(I)}|$, number of alignments specific to A_1
- $N_2^{(I)} := |A_2| - |M^{(I)}|$, number of alignments specific to A_2

Figure 2 Mapping equivalent alignments between two sets



Finally, the alignement quality metric for a given I by the $Q^{(I)}$ function is defined as:

$$\boxed{A_1 \times A_2 \xrightarrow{Q^{(I)}} \left(N_c^{(I)}, N_1^{(I)}, N_2^{(I)}\right)}$$

With the following properties:

1 $Q^{(I)}(A, A) = (|A|, 0, 0)$

2 $Q^{(I)}(A_1, A_2) = (x, y, z) \Longrightarrow Q^{(I)}(A_2, A_1) = (x, z, y)$

Note that this definition of the alignement quality metric is relative: it only gives information for a specific couple of alignment sets. It does not provide absolute assessment for one AST. The consequence is that one of the AST (T_1, T_2) needs to be chosen as a reference when comparing many AST together.

The percentage of alignments found by T_2 in the alignments of T_1 can also be defined as:

$$\alpha_2^{(I)} = \frac{N_c^{(I)}}{N_c^{(I)} + N_1^{(I)}}$$

Taking figure 2 as example, we have $Q^{(I)}(A_1, A_2) = \big(5, 11, 6\big)$. T_2 is able to find $\alpha_2^{(I)} = \frac{5}{5+11} = 31.25\%$ of the alignments found by T_1. Similary, T_1 finds $\alpha_1^{(I)} = \frac{5}{5+6} = 45.45\%$ of the alignments found by T_2.

Comparing N AST T_i is simply done by computing $Q^{(I)}(A_i, A_j)$ for all $(i, j) \in [1..N]^2$. Note that computation is only required for $i >= j$ because of the second property of the quality definition.

4 Results

4.1 List of benchmarked tools and quality metrics

The evaluation of our methodology has been done with the following tools :

T_1:=SSEARCH	T_2:=BLAST	T_3:=PLAST	T_4:=UBLAST

In this set of AST, SSEARCH is the only exact tool, the other ones are based on heuristics. Thus, SSEARCH is taken as the reference. In that way, it can be seen how close heuristics based tools are from an exact reference.

Heuristics based tools are configured by default to get the best trade-offs between speed and quality. Of course, these tools can also be configured by setting specific

parameters in order to get a better quality or a better speed. Table 4 gives several configurations used for the benchmark.

Table 4 Tools specific configurations

Name	Configuration	Purpose
BLASTN	blast -task blastn	nucl/nucl requests
MEGABLAST	blast -task megablast	nucl/nucl requests
BLASTQ	blast -max-target-seqs 25000	quality improvement
UBLASTQ	ublast -accel 1	quality improvement
PLASTS	plast -seeds-use-ratio 0.01	speed improvement

Three different quality metrics are considered:

- $I_{query} = \{1\}$ with $\tilde{A}$, the equivalent class alignment set; this metric compares the number of matched queries between tools
- $I_{hit} = \{1, 2\}$ with $\tilde{A}$, the equivalent class alignment set; this metric compares the number of matched couples [subject,query] between tools
- $I_{align} = \{1, 2, 7, 8, 9, 10\}$ with A, the alignment set; this metric compares the number of alignments sharing the same [subject,query] identifiers and the same [subject,query] boundaries.

These 3 metrics go from the worst to the best precision. As a matter of fact, the correponding sets I provide more and more selective relations of equivalence between alignments. As explained later, two AST may be close for I_{query} and even for I_{hit} but may have significant differences for I_{align}; this is shown by small value for $N_c^{(I_{align})}$ and big values for $N_1^{(I_{align})}$ and $N_2^{(I_{align})}$.

4.2 Results

In the following tables, we dump $\alpha_2^{(I)}$, the percentage of alignments found by T_2 in the alignment set of T_1.

Two ratios of execution time between T_1 and T_2 can be defined:

1 SU_{total} : speedup for the total execution time of a request, including bank preparation when needed (use of *makeblastdb* for instance)

2 SU_{AST} : speedup for the execution time of the sequences comparison only (exclude *makeblastdb* execution time for instance)

Table 5 [Escherichia Coli vs. uniprot_sprot, evalue=1e-3, nbcores=16]

T_1	T_2	$\alpha_2(I_{align})$	$\alpha_2(I_{hit})$	$\alpha_2(I_{query})$	SU_{total}	SU_{AST}
ssearch	*blast*	55.5	58.1	99.8	5.48	5.80
ssearch	*plast*	93.9	97.1	99.8	26.22	32.54
ssearch	*ublast*	29.0	64.4	99.8	64.41	146.60
ssearch	*blastQ*	94.2	97.4	99.8	4.90	5.08
ssearch	*plastS*	73.2	77.1	99.4	52.07	84.43
ssearch	*ublastQ*	30.4	69.1	99.8	40.61	62.63

Table 6 [Chitinophaga Pinensis vs. uniprot_sprot, evalue=1e-3, nbcores=16]

T_1	T_2	$\alpha_2(I_{align})$	$\alpha_2(I_{hit})$	$\alpha_2(I_{query})$	SU_{total}	SU_{AST}
ssearch	*blast*	61.4	64.2	98.7	5.33	5.45
ssearch	*plast*	92.0	95.9	97.9	29.23	33.26
ssearch	*ublast*	19.9	52.4	78.9	89.48	157.96

Tables 5 and 6 show a protein/protein comparison between swissprot [5] and two bacterias [6],[7] from which several comments can be done:

- Heuristics based tools have good speedups, especially UBLAST
- AST quality are very close to SSEARCH for $I^{(query)}$ (except UBLAST, Table 6)
- With default configuration, BLAST and UBLAST have poor results for $I^{(hit)}$ and $I^{(align)}$; only PLAST manages to recover most of the SSEARCH results
- For two similar requests, PLAST has the smallest discrenpencies for the 3 metrics, followed by BLAST and then by UBLAST

Table 7 [uniprot_sprot_40000b vs. uniprot_sprot_40000, evalue=1e-30, nbcores=16]

T_1	T_2	$\alpha_2(I_{align})$	$\alpha_2(I_{hit})$	$\alpha_2(I_{query})$	SU_{total}	SU_{AST}
ssearch	*blast*	86.5	89.9	97.1	8.40	8.46
ssearch	*plast*	88.9	92.4	96.3	44.62	46.48
ssearch	*ublast*	6.4	19.9	22.8	375.46	591.03

Tables 7 compares a subset of 40000 sequences from swissprot to another subset of 40000 sequences from swissprot. Here, BLAST and PLAST have similar values for the 3 metrics and are close to the reference; speedups are interesting, especially for PLAST. For this request, UBLAST is still very fast but has poor values for the 3 metrics. Other tools configurations give similar quality results.

Table 8 [SRR142736 vs. uniprot_sprot, evalue=1e-3, nbcores=16]

T_1	T_2	$\alpha_2(I_{align})$	$\alpha_2(I_{hit})$	$\alpha_2(I_{query})$	SU_{total}	SU_{AST}
blastQ	*blast*	73.8	72.3	100.0	1.00	1.00
blastQ	*plast*	77.7	95.4	98.1	6.70	7.10
blastQ	*ublast*	19.8	64.9	82.0	40.30	68.91

Table 9 [TARA sample vs. uniprot_sprot, evalue=1e-3, nbcores=16]

T_1	T_2	$\alpha_2(I_{align})$	$\alpha_2(I_{hit})$	$\alpha_2(I_{query})$	SU_{total}	SU_{AST}
blastQ	*blast*	82.1	80.8	100.0	1.00	1.00
blastQ	*plast*	80.8	94.7	83.1	6.50	6.68
blastQ	*ublast*	17.2	59.2	29.9	71.10	105.50

Tables 8 shows a genomic query and table 9 a meta-genomic query [8], with swissprot as subjet bank. SSEARCH can't be used as reference here so BLASTQ is used instead. BLAST and PLAST are similar for $I^{(align)}$, with better results for PLAST on $I^{(hit)}$ and better results for BLAST on $I^{(query)}$. UBLAST is still the fastest tool with poor quality, in particular in the meta-genomic case.

Table 10 [SRR027344 vs. nt 2000000 seqs, evalue=1e-30, nbcores=16]

T_1	T_2	$\alpha_2(I_{align})$	$\alpha_2(I_{hit})$	$\alpha_2(I_{query})$	SU_{total}	SU_{AST}
blastQ	*blast*	64.8	64.6	100.0	1.03	1.03
blastQ	*megablast*	61.1	61.0	96.6	5.17	12.48
blastQ	*plast*	94.2	94.2	96.8	5.38	14.07
blastQ	*ublast*	90.1	94.9	95.6	0.63	0.65

Tables 10 is a nucleotide/nucleotide request, comparing a set of reads to $2,000,000$ sequences from the nt [9] database. Here, PLAST and UBLAST provide the best quality metrics w.r.t. BLASTQ, with good speedups for PLAST.

In brief, we summarize this benchmark with the following observations:

- As expected, heuristics based tools are fast at the expense of quality; for instance, UBLAST is very fast but has often poor quality for the chosen metrics

- Surprisingly, BLAST with default configuration has sometimes poor quality for some metrics; a special configuration like BLASTQ is needed for recovering full quality
- PLAST is a good trade-off between speed and quality; it is faster than BLAST with similar quality values.

5 Conclusion

In this paper, we have defined a set of quality metrics for comparing alignment search tools (AST). Comparing AST is important to understand how the AST behave and what should be expected from the alignments they produce. In particular, these metrics allow the distance between exact and heuristics based tool to be precisely evaluated.

These metrics have been tested on standard AST. The benchmark results provides some interesting hints that can be used to match a specific tool with user needs; for instance, PLAST is a good trade-off in terms of speed/quality, and UBLAST is interesting if speed is crucial and if quality loss is acceptable (particularly for alignment and hit metrics).

Other quality metrics are currently under investigation to better capture user needs according to the application domains where AST are involved. A next step is also to gather in a public database many experimentatons and offer to the scientific community a way to select the best AST according to their needs. Other AST will also be added to extend the scope of this methodology. Finally, a web interface [10] is under development and already provides a graphical representation of the metrics.

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