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CRAST Leads to Homologous-ncRNA Search in Genomic Scale

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ABSTRACT

Motivation: Non-coding RNAs (ncRNAs) play important roles in various biological processes. In past, homologous-ncRNA search in genomic scale (e.g., search all house mouse ncRNAs for several human ones) is difficult since explicit consideration of secondary structure in alignment leads to impractical complexity on both of time and space.

Results: In this study, building the program *CRAST* (Context RNA Alignment Search Tool, available at "https://github.com/heartsh/crast" including the used validation/test set), we developed the CRAST algorithm, a *"seed-and-extend"* alignment one based on adaptive seed and RNA secondary structure context (motif probabilities) as in Fig. 1. The algorithm is $O(n : a\ sum\ of\ lengths\ of\ target\ sequences)$ on time through help of adaptive seed, implicitly considering both of sequence and secondary structure; it provides computation time comparable with other BLAST-like tools, significantly reduced from any variant of the Sankoff algorithm for alignment with the explicit consideration. It detects homologs as many as other BLAST-like tools and the lowest number of non-homologous ncRNAs.

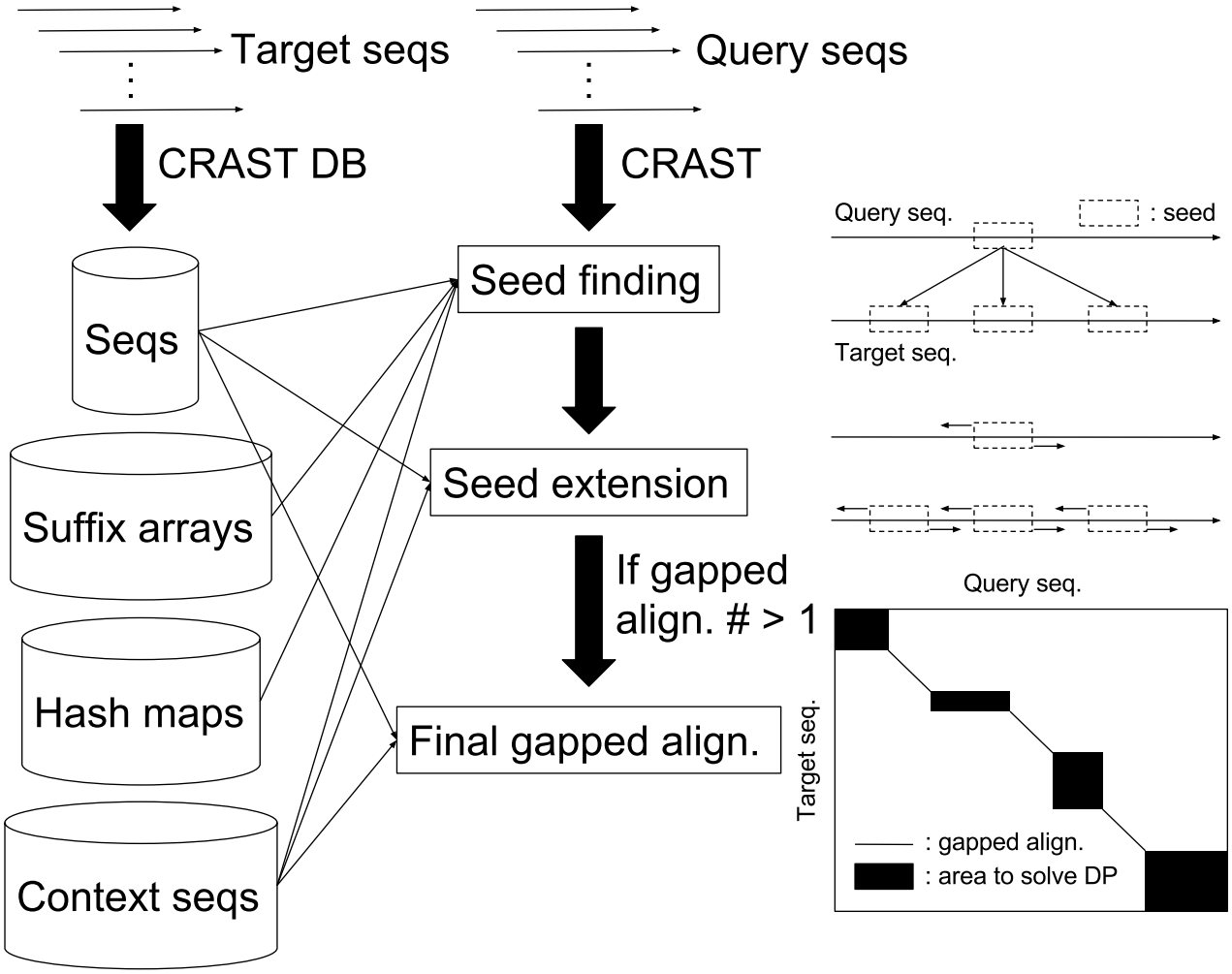

Figure 1: overview of CRAST

1 INTRODUCTION

NcRNAs are involved in diverse biological processes such as rRNA modification [1] and chromatin modification [2]. RNAs including ncRNAs prefer to form into 3D structures rather than DNAs due to contribution of 2' hydroxyl group to hydrogen bond and function based on it. Hence only sequence identity doesn't lead to ncRNA function. [3]

1.1 General genomic homolog-search

In genomic DNA/protein-homolog-search, tools based on "seed-and-extend" strategies such as BLAST [4] and LAST [5] are mostly used. In summary, these tools find *seeds* based on sequence identities of partial regions of 2 compared sequences, and extend the seeds from both sides of them til their scores based on sequence identities drop to some extent. They reduce the time complexity $O(mn)$ from the optimal search by the Smith-Waterman algorithm [6] with the suboptimal search.

1.2 Genomic ncRNA-homolog-search

On the other hand, secondary structure identity must be taken into account as well as sequence identity in genomic

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ncRNA homolog search from the aforementioned matter (especially for ncRNA with low sequence identity such as lncRNA). [7] Foldalign, an implementation of the Sankoff algorithm [8] finds ncRNA pairwise alignments by simultaneously folding and aligning sequences with pruning of dynamical programming matrix. [9] It is really time-consuming and not applicable to genomic data (e.g., all house mouse ncRNAs to search for several human ones) although its pruning discards any subalignment that doesn't have a score above a length-dependent threshold. The banded Sankoff algorithm, another variant of the Sankoff algorithm reduces the time/space complexity of the Sankoff algorithm $O(n^6)/O(n^4)$ to $O(n^4)/O(n^3)$, which again results in unapplicability to genomic data. [10] After all, explicitly considering RNA secondary structure and sequence results in the huge complexities on both time and space.

We could implicitly and efficiently consider RNA secondary structure using CapR, a linear time/space complexity ($O(nw^2)/O(nw)$, but w could be considered as a constant) algorithm to estimate an RNA secondary structure context (motif probabilities) of each base in any RNA [11]. This probabilistic encoding of RNA secondary structure seems to enable to align ncRNA sequences in the same fashion as alignment for DNA/protein sequence.

So we built CRAST, a BLAST-like genomic ncRNA alignment search tool. We demonstrated this tool enables to align ncRNAs with consideration of secondary structure and sequence in a time complexity $O(n)$ where n is a sum of lengths of target sequences through helps of adaptive seed adopted in LAST [5], suffix array [12], and CapR.

2 METHODS

We implemented CRAST in Rust, a systems programming language [13]. It supports both of safe parallelism (multi-threading without data race) and safe zero-cost abstraction (e.g., runtime without garbage-collection and guarantee of memory safety when compiling), which results in both of more safety and computation efficiency almost the same as/more than C/C++. We implemented the bottlenecks (e.g., the calculation of the context sequences and alignment search) in a multi-threaded fashion.

2.1 NcRNA seed finding

"Seed-and-extend" using fixed-length seed such as one in BLAST leads to a quadratic number of seeds with target sequence length due to a non-uniform (oligo-)nucleotide composition of any real sequence. [5] Adaptive seed to find matches that occur at most f times in any target sequence guarantees a linear number of seeds and linear time complexity with a target sequence length. [5] We adopted this seed for extracting matches without need of repeat-masking which could hide potentially significant parts.

To find adaptive seeds, we initially build suffix arrays of target ncRNAs in a time/space complexity $O(n)$. Then we generate hash maps of short substrings of target sequences to corresponding index ranges in the suffix arrays for access in a time complexity $O(1)$. We store the suffix arrays, hash maps, target sequences, and context sequences of target sequences in compressed files (of the bzip2 format) as a database.

We find the shortest seeds for starting position pairs of any query sequence and target one with the hash maps for short matches; with binary-search using search ranges found in previous searches for long ones, as in [5]. These 2 techniques and search strategy reduce the time complexity $O(m \log n, m : a\ length\ of\ a\ match)$ of the binary-search for the substring search using suffix array into one less than $O(\log n)$. Steps til here are the same as in LAST, an adaptive seed implementation [5] except for the context sequences.

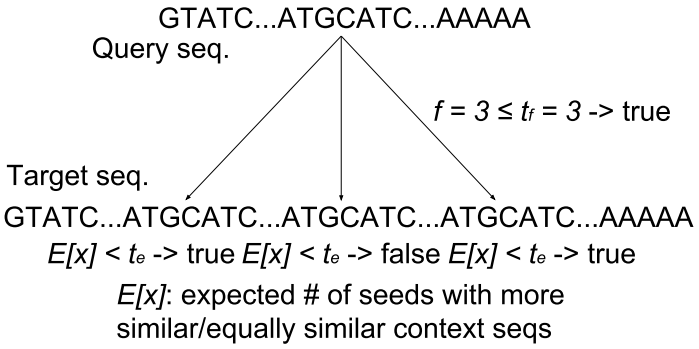

Figure 2: seed finding of CRAST
The seeds only satisfying both of the terms for sequence/context sequence match (the left/right one) are filtered in.

In CRAST, the seeds having similar context sequences are filtered in from the found seeds, using a threshold of an expected number of the seeds with more/equally similar context sequences as in Fig. 2.

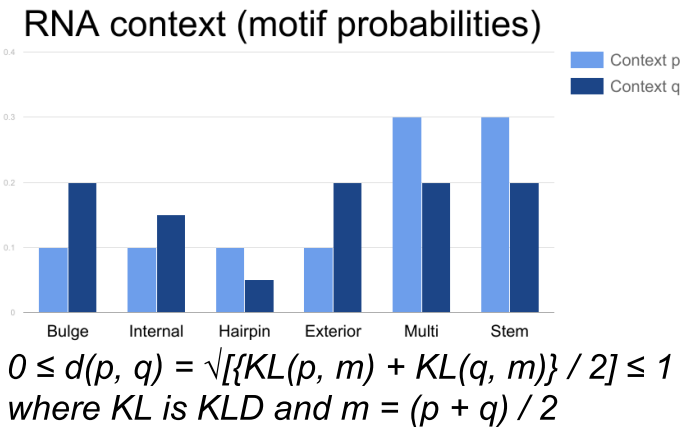

Figure 3: Jensen-Shannon distance of RNA context pair
Each term for the context is an RNA secondary structure motif.

We score any base pair having the Jensen-Shannon distance [14] of a pair of contexts as in Fig. 3 less than 0.25 as a match with a score +1; other than that as a mismatch with a score -1. The distance is a distance metric version of the Jensen-Shannon divergence [15] (not distance, often called the JSD) which is a symmetric finite measure of similarity between 2 probability distributions. The JSD doesn't satisfy triangle inequality required for any distance metric while the distance does. We considered a binomial distribution as a model of a probabilistic distribution of the series of the matches and mismatches such as a series of coin tosses: $B(n, p = 0.25)$.

2.2 Seed extension and scoring system

We score any base pair using both of match/mismatch of base/context: $s = rs_b + (1 - r)s_c$ where s is a fusion score, $0 \leq r \leq 1$ is a contribution ratio of base to the fusion score, s_b is a score of any base pair (+1/-1), and s_c is a score of any context pair (+1/-1) in the CRAST scoring system.

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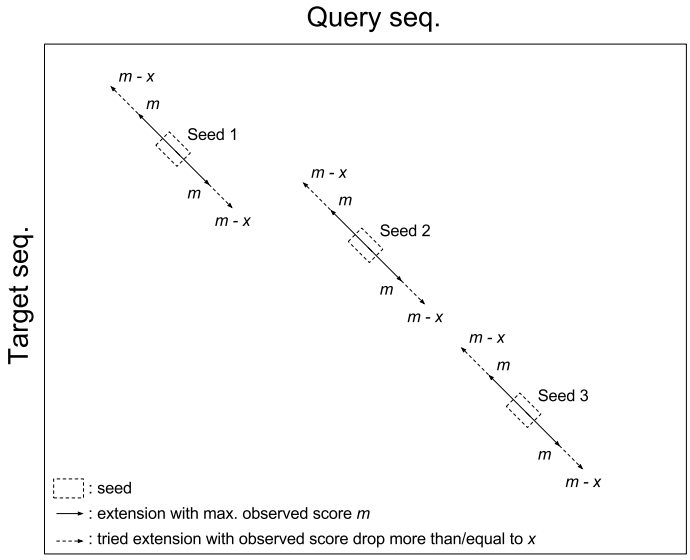

Figure 4: X-drop algorithm

We extend the seeds first without any gap using the X-drop algorithm [16]; then with gaps using the same algorithm. The algorithm greedily extends the seeds/ungapped alignments til their scores drop from maximum observed values m to less than/equal to $m - x$ where x is an aforehand determined value as in Fig. 4. [16]

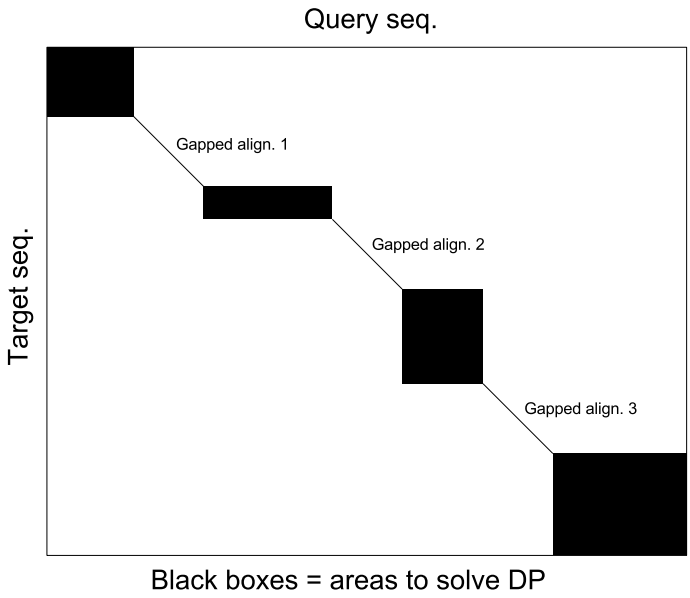

Figure 5: constrained DP matrix of CRAST
All the area of the DP matrix to solve the DP are reduced from mn.

Finally, we generate single gapped alignments derived from all the gapped ones, using DP matrices constrained by the gapped ones as in [17] and Fig. 5 when a number of the gapped ones is more than 1 for 1 strand. To constrain the matrices, we greedily merge the ungapped ones diagonally overlapped in the matrices; greedily remove one with a lower score of the ungapped ones non-diagonally (in parallel) overlapped. We greedily remove one with a lower score of the overlapped gapped ones for the same purpose. After each of the ungapped/gapped one, we discard some of the alignments based on an expected number of alignments with more/equally similar base/context sequences.

More specifically, we independently consider a binomial distribution of the series of the base/context matches and mismatches, which results in simple calculations of the E-values. The gapped one disables to model the distributions due to uncertainty of gap; we consider the gaps as given, which results in the same calculations of the E-values as the ungapped one.

3 RESULTS

3.1 Parameter-tuning

We set default CRAST parameters as in Table 3 to best perform with target/query sequences for validation. We used all 18,185 *Mus musculus* ncRNAs (derived from Ensembl [18]) as the target sequences; 34 *Homo sapiens* lncRNAs known as homologs to *M. musculus* corresponding ones including HOTAIR [19] and XIST [20] (derived from LncRNAdb [21]), as the query sequences. We fixed the parameters not referred to in each result.

3.2 Relation between seed E-value filtering and homolog detectability

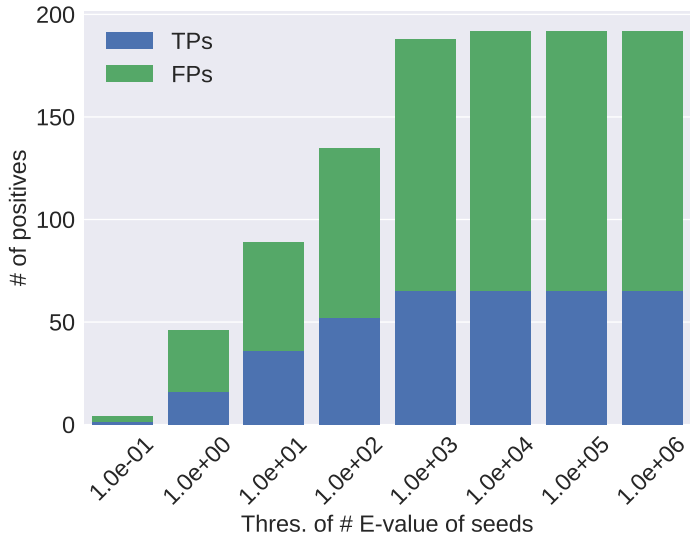

Figure 6: relation between seed E-value filtering and homolog detectability
We used the same target/query sequences for test as the target/query sequences for validation.
The "TP" is map of any *Homo sapiens* one to any corresponding *Mus musculus* one; the "FP" is any *Homo sapiens* one to any of the others.

Fig. 6 shows the number of the FPs hits the ceiling at $t_e = 1.0 \cdot 10^4$ while the one of the TPs (homologs) hits the ceiling at $t_e = 1.0 \cdot 10^3$ where t_e is any threshold of an expected number of the seeds with more/equally similar context sequences. From that, we could think t_e as a parameter to discard the inappropriate seeds before the following extensions. It also implies only the seeds with highly similar context sequences would lead to incomplete capture of homologs.

3.3 Relation between contribution ratio of base to score r and homolog detectability

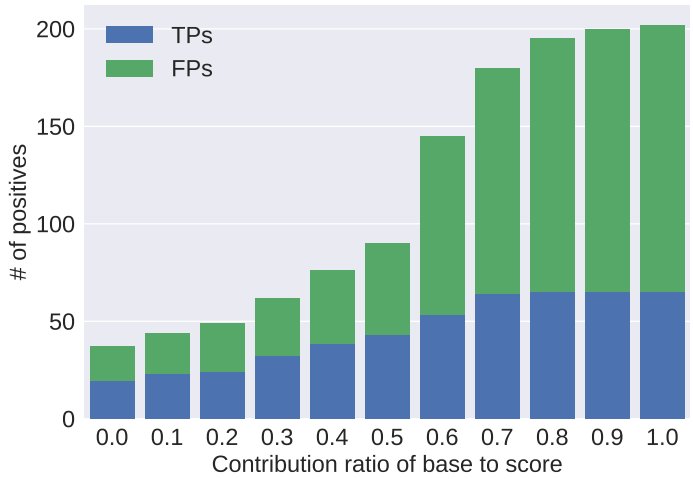

Figure 7: relation between contribution ratio of base to score r and homolog detectability
We used the same target/query ncRNAs as Fig. 6.

Fig. 7 represents the higher the contribution ratio of base to the fusion score r becomes, the more the FPs are obtained

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while the number of the TPs hits the ceiling at $r = 0.7$. From that, we could think r as a parameter to control a number of the FPs keeping the TPs as many as possible. It also implies consideration of only secondary structure would lead to incomplete capture of homologs.

3.4 Relation between alignment E-value filtering and homolog detectability

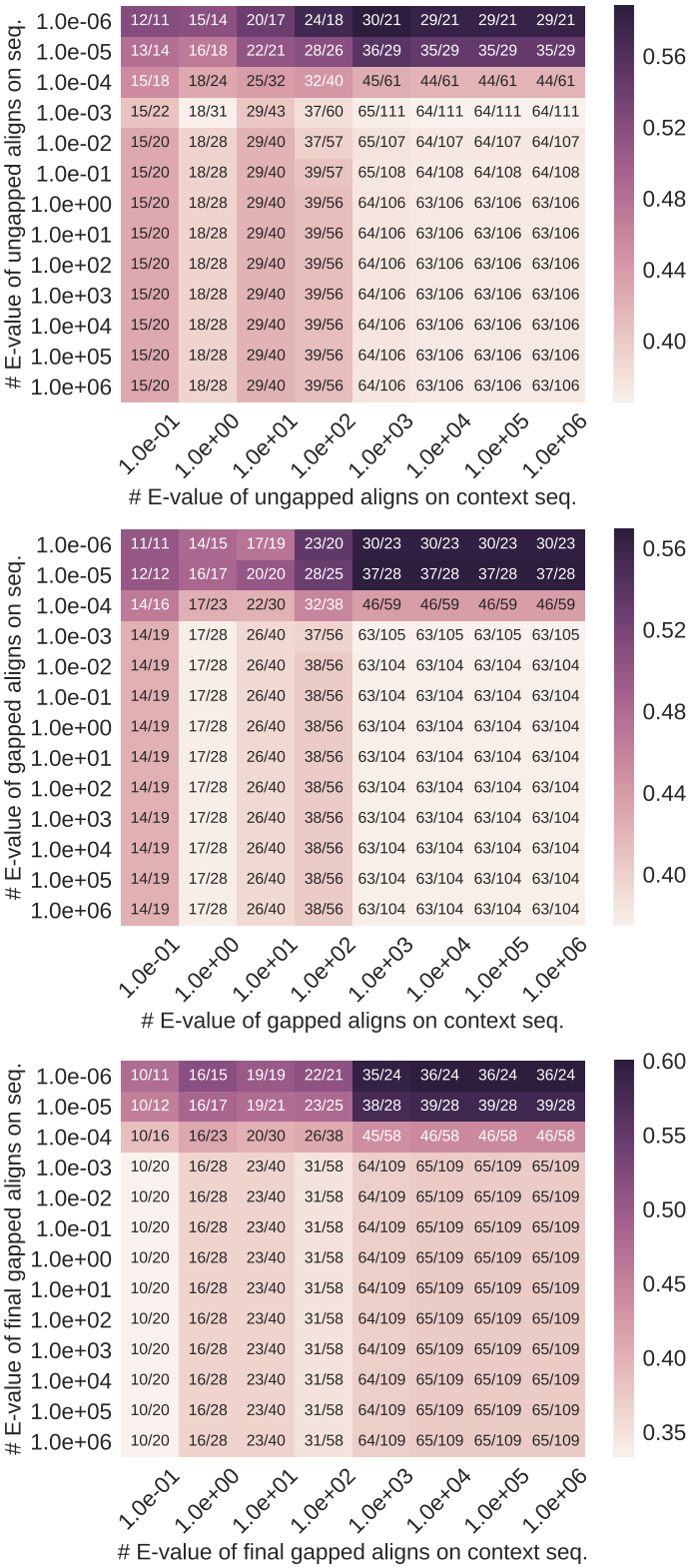

Figure 8: relation between alignment E-value filtering and homolog detectability
We used the same target/query ncRNAs as Fig. 6.
From the top, the relation for: ungapped one/gapped one/final gapped one. A color of each cell indicates precision $p = TP/(TP + FP)$; a number of each one does TP/FP number.

Fig. 8 shows the number pair of the TPs and FPs hits the ceiling around $t_{e1} = 1.0 \cdot 10^{-3}$ and $t_{e2} = 1.0 \cdot 10^{3}$ where t_{e1}/t_{e2} is any threshold of an expected number of the alignments with more/equally similar sequences/context sequences. From that, it is confirmed that each of the E-value thresholds could play a role as a parameter to discard the inappropriate alignments before the following alignment step/report. It also implies only the alignments with highly similar context sequences would lead to incomplete capture of homologs as well as the seeds.

3.5 Performance comparison with BLAST-like tools

Table 1: performance comparison among CRAST, LAST [5], BLASTN [4], and BLAT [22]

Tool/term	TPs/FPs/TNs/FNs	F-meas.	DB[s]	Align.[s]
CRAST	65/107/0/0	0.548	189.5[m]	148.0 (34.60)
LAST	63/585/1/0	0.177	7.246	0.193
BLASTN	63/1,834/445/0	0.064	1.646	1.152
BLAT	40/141/0/0	0.361	—	4.893

We used the same target/query ncRNAs as Fig. 6.
As a negative dataset, we made all the query ones di-nucleotide shuffled with UShuffle [23]. The "TN" is map of any shuffled query one to any of others than corresponding target ones; the "FN" is any shuffled query one to any of corresponding target ones.
The "F-meas." is the F-measure which is an weighted harmonic mean of precision $p = \frac{tp}{tp+fp}$ and recall $r = \frac{tp}{tp+fn}$: $f = \frac{2pr}{p+r}$. It p is high only when both of precision p and recall r are high.
The "align. time" of CRAST inside the parentheses is time except for the pre-processing. (The bzip2 decompression is time-consuming.) The pre-processing time excludes computation time of the CapR algorithm for the query sequences (807.8[s]) because the computation will be performed once per set of query sequences.
The computation environment is cpu Xeon E5 2680v2 with 20 cores and memory 64[GB].

We compared CRAST, LAST (ver. 719), BLASTN (BLAST for nucleotide, ver. 2.6.0+), and BLAT (ver. 35) for effect on ncRNA homolog detection of fixed-length/adaptive seed/seed-extension based on only substitution matrix/both of substitution matrix and RNA secondary structure context as in List. 1. We also measured running times of these programs on both database/alignment step. (In practice, database step is run only 1 time for any set of target sequences.)

Table 1 shows CRAST detects the homologs as many as the compared tools except for BLAT and the lowest number of the FPs. It also shows the database step of CRAST is significantly time-consuming due to domination by the CapR algorithm; the alignment step is relatively slow in spite of a lower number of the seeds.

There are 3 reasons why the alignment step is relatively slow: missing pre-computing of seed candidates (possible adaptive seeds) in the database step, the compute-intensive of the Jensen-Shannon distance, and no implemented repeat mask. First, we could reduce computation time of the alignment step by pre-computing the candidates in a certain database within a given range of seed frequencies (e.g., the frequencies around default one), which would lead to search of the candidates in query sequences, not target ones. But this pre-computation doesn't repesent the real performance of the CRAST algorithm because the speed-up is established only in the case when seed frequencies are within the range. Second, the Jensen-Shannon distance is computationally intensive compared with the L_p one such as the Euclidean one ($p = 2$) since it involves the intensive computation of logarithm compared with basic arithmetic operations. Finally, LAST implements repeat-masking in the database step whereas CRAST doesn't. We can mask repeats using repeat-masking programs such as RepeatMasker [24] in advance. However, we would like to demonstrate and compare the real performance of the CRAST algorithm. Considering these 3 reasons, the computation time of CRAST is reasonable.

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3.6 Performance comparison with Foldalign

Table 2: performance comparison with Foldalign [9]

Tool/term	TPs/FPs/TNs/FNs	F-meas.	DB[s]	Align.[s]
CRAST	65/156/219/0	0.454	728.2	7.582
Foldalign	32/1,923/1,923/32	0.031	—	> 3[d]

We used only all 219 homologs to the query ncRNAs from the same target ncRNAs as Fig. 6 as other target sequences for test due to huge computation of Foldalign.
We define the "FP" as any target one not mapped to the corresponding query one; the "TN" as any target one not mapped to the corresponding shuffled query one.
Everything except for them is the same as Table 1.

We compared CRAST and Foldalign (ver. 2.5) for effect on ncRNA homolog detection of alignment with context sequence/simultaneous folding as in List. 2.

Table 2 represents there are numerous incorrect maps of the target ones to others than the corresponding query ones for Foldalign ($32 + 1,923 - 219 = 1,768$) compared with CRAST ($65 + 156 - 219 = 2$); a half of correct maps of the target ones to the corresponding ones for Foldalign compared with CRAST; and several incorrect maps of the target ones to the corresponding shuffled query ones for Foldalign (32) compared with CRAST (0). It also implies alignment with simultaneous folding could detect numerous non-homologous ones rather than sufficient homologs despite the huge computation compared with CRAST.

4 DISCUSSION

4.1 Principal findings

We developed the CRAST algorithm (the time complexity $O(n : a\ sum\ of\ lengths\ of\ target\ sequences)$) to pairwise-align numerous ncRNAs with consideration of both of sequence/secondary structure identity. Instead of explicit consideration of secondary structure like the Sankoff algorithm, we utilized RNA context (motif probabilities) from the CapR algorithm and fused the score from sequence/secondary structure into the fusion score for the implicit consideration.

We demonstrated it could successfully reduce detections of non-homologous ones keeping detections of homologs as many as other BLAST-like tools with reasonable computation-time in case of lncRNA, in other words, low sequence identity requiring viewpoint of secondary structure identity. This reduction of the false detections leads to improvement of product quality/computation time from subsequent process in genome comparative analysis such as binary classification of ncRNA by RNAz [25, 26] and ncRNA clustering by GraphClust [27].

Surprisingly, Foldalign, a variant of the Sankoff algorithm could detect numerous non-homologous ones rather than sufficient homologs despite the huge computation compared with CRAST in case of lncRNA. It may be because of a fact which only highly similar secondary structure would lead to incomplete capture of homologs as in Fig. 6 and Fig. 8, and even excess capture of non-homologous ones.

To verify it, we set the seed E-value parameter to 0.75; other E-value ones to 1; and contribution ratio of base to score r to 0.5 for relaxing/restricting sequence/secondary structure identity and equally taking both of the identities into account in the scoring system. Then we got 31/1,088/1,062/28 as TPs/FPs/TNs/FNs in the same condition as Table 2; the number of the TPs is comparable with the Foldalign result in Table 2. The lower number of the FPs/TNs could be due to adaptive seed requiring rare exact match on sequence.

4.2 Method appraisal

We independently modelled a distribution of series of matches and mismatches on sequence/context sequence as a binomial distribution $B(n, p = 0.25)$ due to uncertainty of the score distribution/gap. In general, Modelling a distribution of scores as the Gumbel distribution instead of matches is frequently used to handle the uncertainties. But the fusion score depends on not only the match/mismatch score and gap opening/extension cost but also the contribution ratio of base to the fusion score r and estimated RNA context sequence controlled by the parameter w from the CapR algorithm. We could model it as the distribution by fitting random data for roughly possible combinations of the parameters.

We demonstrated the algorithm between only human and house mouse due to limited availability of sufficient annotation of lncRNA homolog. However, the more evolutional distance on ncRNA between 2 compared species gets diverse, the more homolog detectability of CRAST/other BLAST-like tools/the Sankoff algorithm may increase/decrease. If we got the sufficient availability, we would robustly parameter-tune the algorithm.

We reasoned why the alignment step of CRAST was relatively slow: missing pre-computing of seed candidates (possible adaptive seeds) in the database step, the compute-intensive of the Jensen-Shannon distance, and no implemented repeat mask. Except for the second factor, we would implement them in CRAST, and let it faster and more accurate.

4.3 Scientific implications

We discovered the Sankoff algorithm, a traditional ncRNA alignment could lead to numerous non-homologous ones rather than sufficient homologs and BLAST-like tools work well in case of lncRNA contrary to our expectations. The result and performance of CRAST lead to comparing a large amount of ncRNA pairs in the efficient and accurate fashion. We could adapt the algorithm to ncRNA multiple alignment with consideration of secondary structure such as [28] and ncRNA clustering with the consideration such as [27].

5 ACKNOWLEDGEMENTS

The computations were performed on the NIG supercomputer at the ROIS National Institute of Genetics.

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6 APPENDICES

6.1 Default CRAST parameters

Table 3: default CRAST parameters from parameter-tuning

Term	Description	Value
ms	Maximum span between paired bases for CapR algorithm	200
ts	Threshold of frequency of adaptive seeds	3
x1	Threshold of drop of score when ungapped alignment	5
x2	Threshold of drop of score when gapped alignment	15
b	Contribution ratio of base to score	0.65
e1	Threshold of # E-value of seeds with better scores on secondary structure	$6.5 \cdot 10^2$
e2	Threshold of # E-value of ungapped alignments with better scores on sequence	$5 \cdot 10^{-3}$
e3	Threshold of # E-value of ungapped alignments with better scores on secondary structure	$9 \cdot 10^2$
e4	Threshold of # E-value of gapped alignments with better scores on sequence	$5 \cdot 10^{-4}$
e5	Threshold of # E-value of gapped alignments with better scores on secondary structure	$9 \cdot 10^2$
e6	Threshold of # E-value of final gapped alignments with better scores on sequence	$5 \cdot 10^{-4}$
e7	Threshold of # E-value of final gapped alignments with better scores on secondary structure	$6.5 \cdot 10^3$
go	Gap opening penalty	-7
ge	Gap extension penalty	-1

E-values $E[M]$ are per target sequence: $E[M] = s(1 - B(M \leq m; n, p = 0.25))$ where s is a possible number of the seed occurrence and M/m is a random variable/observed value for a number of matches of bases/secondary structures.
We set ms equal to the default one of the CapR algorithm and go/ge equal to the default one of LAST.

6.2 Zsh commands for BLAST-like tools/Foldalign

Listing 1: Zsh commands for BLAST-like tools

```
$ # DB step for LAST (command ''time'' is for
    measuring elapsed time during given one)
$ time lastdb asts/last_m_mscls_ncrna_db/
    m_mscls_ncrna_db asts/m_mscls_ncrnas.fa
$ # Align. step for LAST
$ time lastal -P $(nproc) -s 1 -D 1e+6 asts/
    last_m_mscls_ncrna_db/m_mscls_ncrna_db asts/
    h_spns_m_mscls_hmlg_lncrnas.fa > asts/
    last_h_spns_m_mscls_hmlg_lncrnas_2_m_mscls_ncrnas
    .maf
$ # DB step for BLASTN
$ time makeblastdb -in ../m_mscls_ncrnas.fa -
    parse_seqids -dbtype nucl
$ # Align. step for BLASTN
$ time blastn -strand plus -query asts/
    h_spns_m_mscls_hmlg_lncrnas.fa -db asts/
    blast_m_mscls_ncrna_db/m_mscls_ncrnas.fa -out
    asts/
    blastn_h_spns_m_mscls_hmlg_lncrnas_2_m_mscls_ncrnas
    .dat -num_threads $(nproc) -outfmt 6 -task
    blastn-short
$ # Align. step for BLAT
$ time blat asts/m_mscls_ncrnas.fa asts/
    h_spns_m_mscls_hmlg_lncrnas.fa -out=maf asts/
    blat_h_spns_m_mscls_hmlg_lncrnas_2_m_mscls_ncrnas
    .maf
```

Listing 2: Zsh commands for Foldalign

```
$ # Align. step for Foldalign
$ time foldalign -number_of_processors $(nproc)
    asts/h_spns_m_mscls_hmlg_lncrnas.fa asts/
    hmlg_m_mscls_ncrnas.fa > asts/
    fldlgn_h_spns_m_mscls_hmlg_lncrnas_2_m_mscls_ncrnas
    .dat
```

For the shuffled query sequences, just replace the query file with the one for them, prepending "shfl_" to the name of the query file when issuing a command of an alignment step.