FasParser Manual

[For version 2.4.0 | 2018-5-10]

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Contents

Introduction

Background

With the development of sequencing technology in recent times, a great number of molecular sequences (DNA and RNA) have been generated. Molecular analyses based on these sequences have become one of the most important measures for assessing their potential biological significance. The increase in the amount of available sequence data has made its manipulation tricky, especially for those without programming experience. Hence, it has now become necessary to develop one or more user-friendly software to perform such analyses in a batch mode.

Common manipulations may include extracting/removing subsequences or subalignments, estimating the sequence similarity and identifying the non-homologous sequence(s), converting between file formats, and/or alignment trimming to exclude poorly aligned regions from a multiple sequence alignment. While, with the increase in the amount of available sequence data, it made sequence manipulation tricky, especially for those without programming experience.

To achieve an objective of simplifying many common tasks in sequence manipulation for biologists, I provide a new program package named 'FasParser' for manipulating sequence files. It is designed with a user-friendly GUI and batch processing modes, which allows users to handle multiple sequence files in a simple way. Presently, the main functions of FasParser2 involve: (1) batch performing alignment construction with Muscle (Edgar, 2004), Mafft (Katoh, et al., 2002), or Prank (Loytynoja and Goldman, 2005), (2) alignment trimming on poorly-aligned regions, (3) alignment format conversion between FASTA and either PHYLIP, PAML, or NEXUS, (4) sorting & classifying sequences according to accession numbers or sequence length, which can also rename sequences, (5) concatenating & merging sequences for a particular set of samples from multiple sequence files, (6) translation and ORF prediction, (7) extracting and filtering sequences according to ID, sequence length, or sequence similarity, (8) detecting positive selection with CodeML (Yang, 2007), (9) designing PCR primers with Primer3 (Rozen and Skaletsky, 2000), and (10) extracting consensus-aligned regions between different aligner results (for a same gene). Moreover, there has been designed a specialized Editor for FasParser (v2.0+) to easily viewing and editing sequences.

Citations

If you use this package in your work, please cite these references:

1. *Yan-Bo Sun.* ***FasParser: a package for manipulating sequence data****, Zoological Research 38(2): 110-112, 2017*

2. *Yan-Bo Sun.* ***FasParser2: A Graphical Platform for Batch Manipulating Biological Sequence, Bioinformatics,*** *in press.*

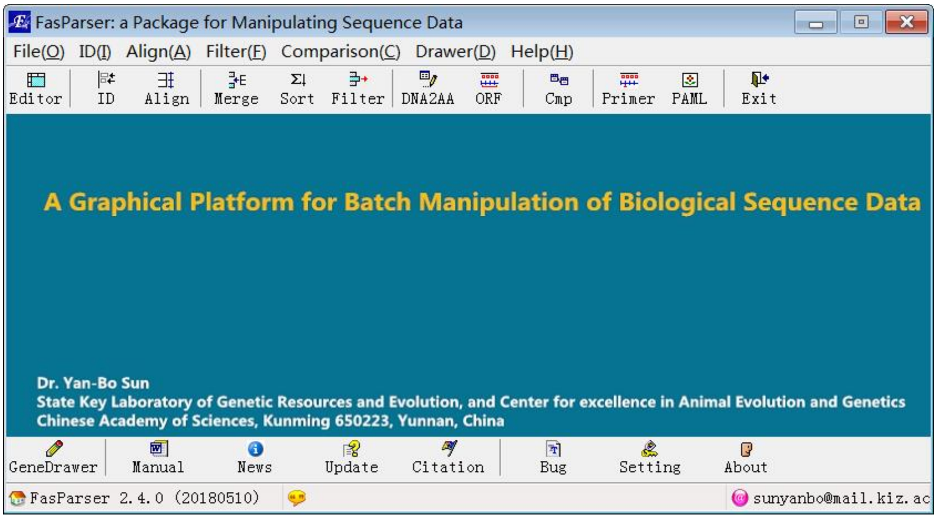
Below is a non-exhaustive list of publications related to the programs integrated:

1. *Edgar RC. 2004.* ***MUSCLE: a multiple sequence alignment method with reduced time and space complexity****. BMC Bioinformatics, 5: 113.*
2. *Katoh K, Misawa K, Kuma K, Miyata T. 2002.* ***MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform****. Nucleic Acids Research, 30: 3059-3066.*
3. *Loytynoja A & Goldman N. 2005.* ***An algorithm for progressive multiple alignment of sequences with insertions****. Proceedings of the National Academy of Sciences of the United States of America, 102: 10557-10562.*
4. *Nei M & Gojobori T. 1986.* ***Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions****. Molecular Biology and Evolution, 3: 418-426.*
5. *Untergasser A, et al. (2012)* ***Primer3--new capabilities and interfaces****. Nucleic Acids Res 40(15):e115.*
6. *Yang Z (2007)* ***PAML 4: phylogenetic analysis by maximum likelihood****. Molecular Biology and Evolution 24(8):1586-1591.*

Installation

The 'FasParser' has been developed into a standalone Windows System Application (compiled and tested on Windows 7/10). It can run on most Windows systems with no installation of other programs.

Download the newest setup program (i.e. 'FasParserX.X_setup.exe') from https://github.com/Sun-Yanbo/FasParser to your disk, and then double click it to install the whole package. Normally, it is well using the default installation parameters (by clicking the Next button to end). After successful installation, you would get a screen of the Home page of this package (Figure 1).


FasParser: a Package for Manipulating Sequence Data

A Graphical Platform for Batch Manipulation of Biological Sequence Data

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FasParser 2.4.0 (20180510)

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Figure 1. The Home page of the FasParser package.

Package Usage

[Select multiple files]

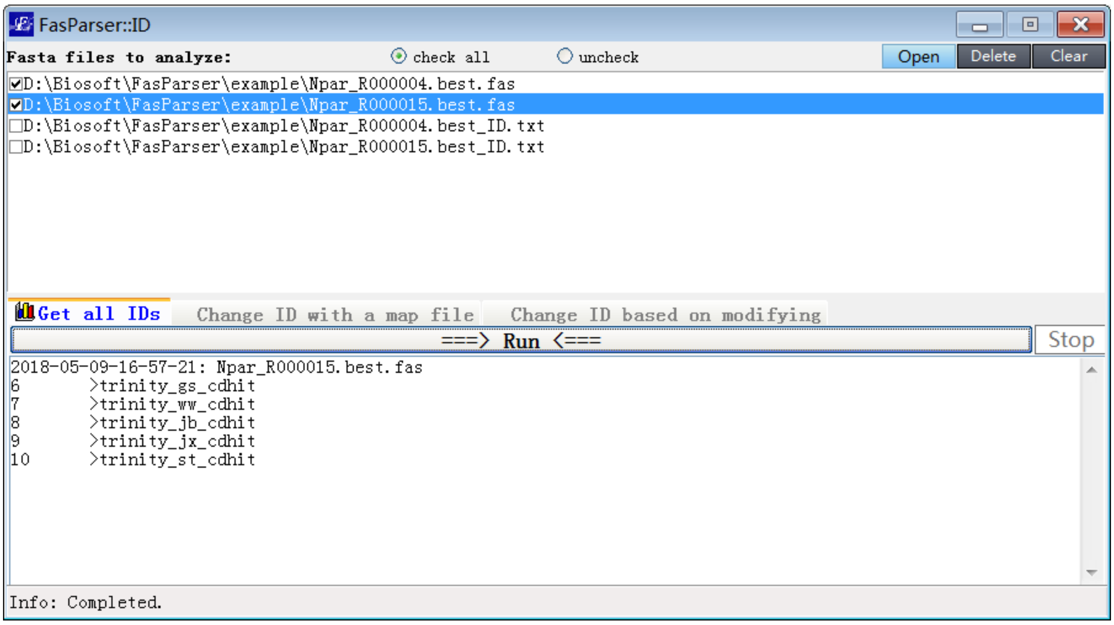
There are 3 different ways to select multiple files into the checking list box:

1. Using the button “upload”;
2. Drag the files into the checking list box;
3. Drag the folder which contains the sequence files into the checking list box;

[ID]

This program is designed to **extract IDs** of Fasta files, change raw IDs to another according to the user provided raw-new ID map, and change raw IDs based on modifying the raw IDs.

For extracting IDs:

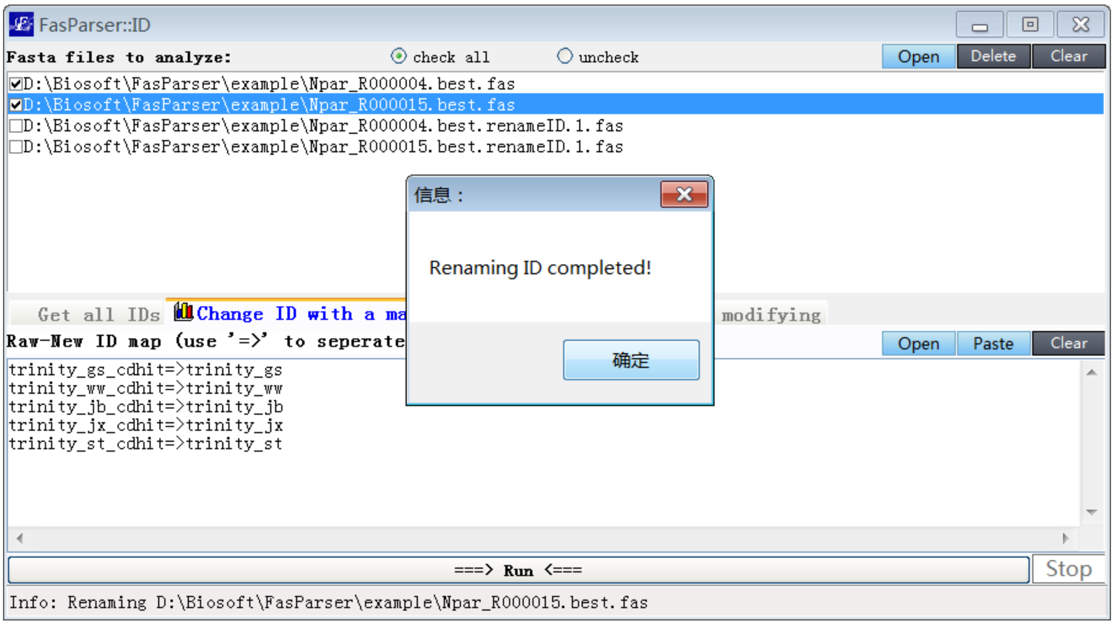


☑D:\Biosoft\FasParser\example\Npar_R000004.best.fas
☑D:\Biosoft\FasParser\example\Npar_R000015.best.fas
□D:\Biosoft\FasParser\example\Npar_R000004.best_ID.txt
□D:\Biosoft\FasParser\example\Npar_R000015.best_ID.txt

```
2018-05-09-16-57-21: Npar_R000015.best.fas
6       >trinity_gs_cdhit
7       >trinity_ww_cdhit
8       >trinity_jb_cdhit
9       >trinity_jx_cdhit
10      >trinity_st_cdhit
```

Info: Completed.

For changing raw IDs according to the user defined ID map file:



Fasta files to analyze: ● check all ○ uncheck

```
☑D:\Biosoft\FasParser\example\Npar_R000004.best.fas
☑D:\Biosoft\FasParser\example\Npar_R000015.best.fas
□D:\Biosoft\FasParser\example\Npar_R000004.best.renameID.1.fas
□D:\Biosoft\FasParser\example\Npar_R000015.best.renameID.1.fas
```

Renaming ID completed!

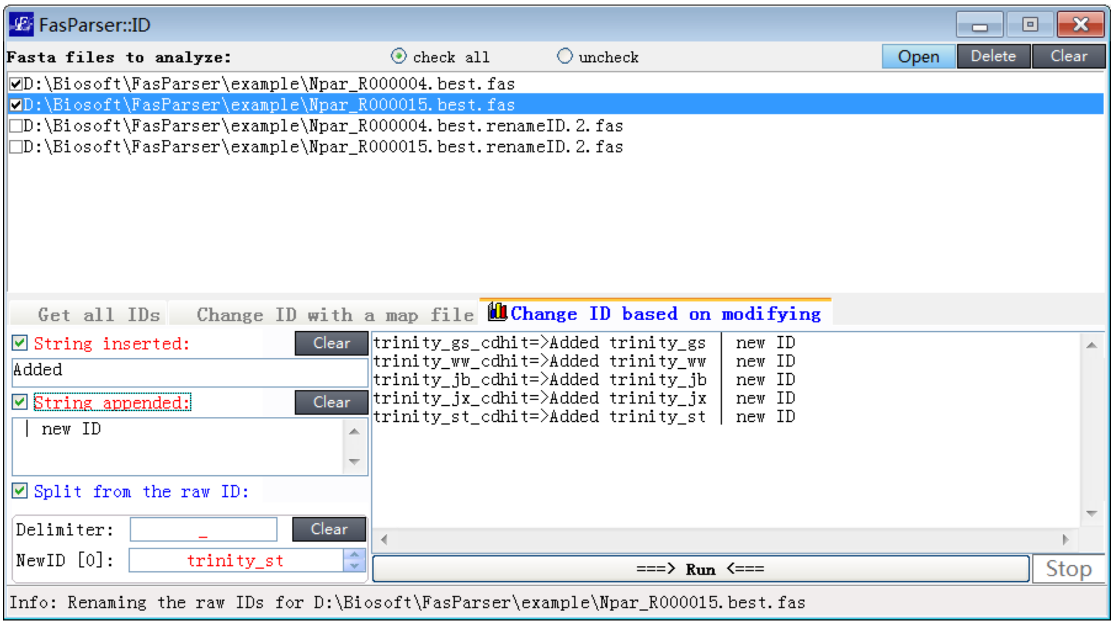
Raw-New ID map (use '=>' to seperate

```
trinity_gs_cdhit=>trinity_gs
trinity_ww_cdhit=>trinity_ww
trinity_jb_cdhit=>trinity_jb
trinity_jx_cdhit=>trinity_jx
trinity_st_cdhit=>trinity_st
```

Info: Renaming D:\Biosoft\FasParser\example\Npar_R000015.best.fas

Please note the symbol “=>” should be present between the old and new IDs.

For changing IDs based on modifying the raw IDs:



Fasta files to analyze: ● check all ○ uncheck

```
☑D:\Biosoft\FasParser\example\Npar_R000004.best.fas
☑D:\Biosoft\FasParser\example\Npar_R000015.best.fas
□D:\Biosoft\FasParser\example\Npar_R000004.best.renameID.2.fas
□D:\Biosoft\FasParser\example\Npar_R000015.best.renameID.2.fas
```

☑ String inserted: Added

☑ String appended: | new ID

☑ Split from the raw ID:

Delimiter: _

NewID [0]: trinity_st

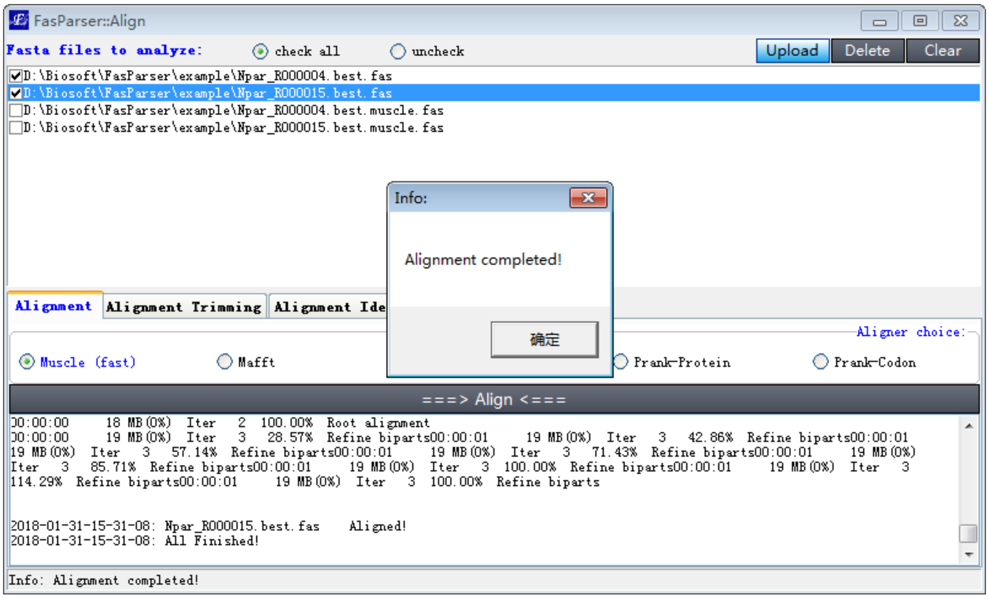
```
trinity_gs_cdhit=>Added trinity_gs | new ID
trinity_ww_cdhit=>Added trinity_ww | new ID
trinity_jb_cdhit=>Added trinity_jb | new ID
trinity_jx_cdhit=>Added trinity_jx | new ID
trinity_st_cdhit=>Added trinity_st | new ID
```

Info: Renaming the raw IDs for D:\Biosoft\FasParser\example\Npar_R000015.best.fas

The program can split the raw IDs based on defined delimiter, and add some other strings before the ID, and/or append some strings at the end of ID.

[Align]

This program is designed to construct alignments for multiple FASTA files. There are 3 aligners (MUSCLE, MAFFT, and PRANK, see below reference) have been integrated into FasParser. The first two aligners are always faster than Prank, but Prank could generate more accurate results. In addition, the first two aligners can auto recognize the input type (nuclear DNA or protein sequence), while, the Prank should be told the sequence type; user should select the corresponding aligner by yourself (including Prank-DNA, Prank-Codon, and Prank-AA, Figure 2). The running process information can be viewed in the bottom text box. User should use '*Scan'* button to select the Fasta files to analyze. The output files will be saved in the folder which contains the input files, with '*. <aligner>.fas'. Please note all the default parameters of the aligners are used by FasParser.


FasParser::Align

Fasta files to analyze: ● check all ○ uncheck

☑D:\Biosoft\FasParser\example\Npar_R000004.best.fas
☑D:\Biosoft\FasParser\example\Npar_R000015.best.fas
☐D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.fas
☐D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.fas

Info:

Alignment completed!

Aligner choice: ● Muscle (fast) ○ Mafft ○ Prank-Protein ○ Prank-Codon

```
00:00:00      18 MB(0%)  Iter   2  100.00%  Root alignment
00:00:00      19 MB(0%)  Iter   3   28.57%  Refine biparts00:00:01      19 MB(0%)  Iter   3   42.86%  Refine biparts00:00:01
19 MB(0%)  Iter   3   57.14%  Refine biparts00:00:01      19 MB(0%)  Iter   3   71.43%  Refine biparts00:00:01      19 MB(0%)
Iter   3   85.71%  Refine biparts00:00:01      19 MB(0%)  Iter   3  100.00%  Refine biparts00:00:01      19 MB(0%)  Iter   3
114.29%  Refine biparts00:00:01      19 MB(0%)  Iter   3  100.00%  Refine biparts


2018-01-31-15-31-08: Npar_R000015.best.fas    Aligned!
2018-01-31-15-31-08: All Finished!
```

Info: Alignment completed!

Figure 2. Overview of alignment construction

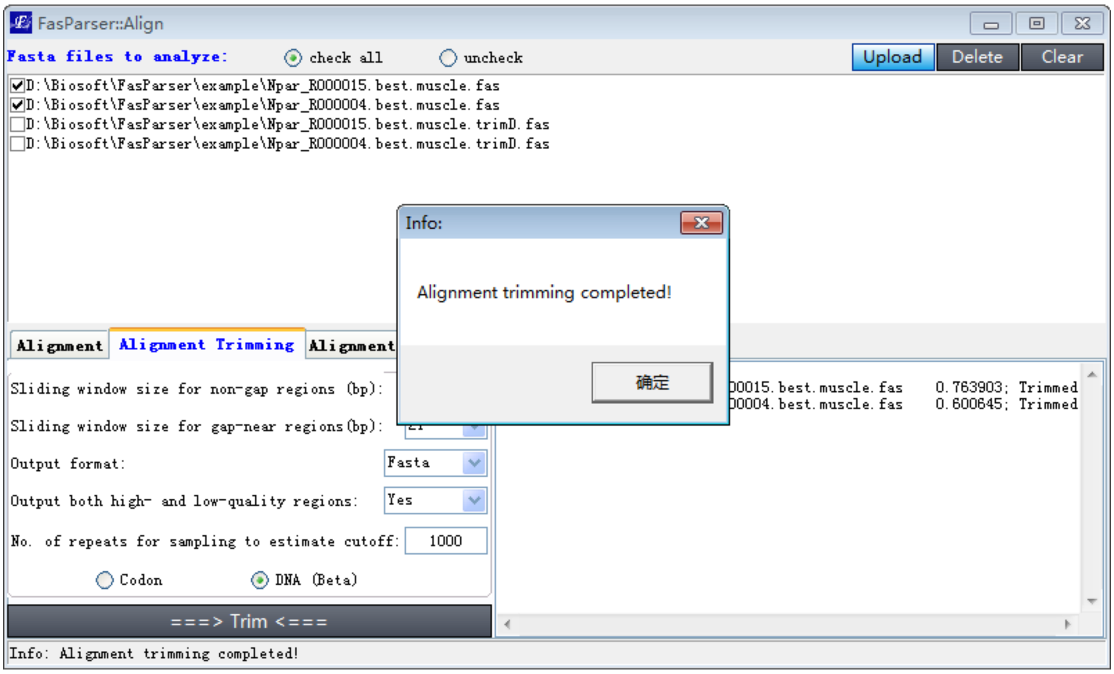
In versions >= 2.3.0, the aligned files can be displayed in the checking list box and can be viewed by double clicks. Moreover, the other aligning parameters can also be provided in FasParser with version > 2.3.1

[Trim]

Alignment quality is of great importance for downstream analyses, like phylogenetic inference and positive selection detection. The low-quality alignment always produces false positive results, like wrong phylogenetic positions of species. So, it is much better to strip the poorly-aligned regions in the raw alignments before conducting downstream evolutionary analyses. The 'Trim' function provides a simple and efficient trimming method to filter the low-quality regions in each alignment. [It is more suitable for preparing high-quality alignments for positive selection detection]

'Trim' will apply a sliding window method to identify which regions hold low-similarity sequences based on a dynamic cutoff that will be estimated from the total alignment (1000 times). There are two steps to trim an alignment. The 1st step is for the gap-near regions, and the window will be sliding from the gap "-" to both left and right ends. The 2nd step is for the block regions. After the 1st step trimming, the 2nd will trim again from the left end to right.

User should tell which type of seqs you provided: codon or DNA, and then you can click the "Start" button to start the trimming analyses for multiple files (Figure 3). The output files will be saved in the folder which contains the input files, with '*. trimC.fas' or '*. trimD.fas' for codon or DNA, respectively.



☑D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.fas
☑D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.fas
☐D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.trimD.fas
☐D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.trimD.fas

Alignment trimming completed!

Sliding window size for non-gap regions (bp):
Sliding window size for gap-near regions(bp):
Output format: Fasta
Output both high- and low-quality regions: Yes
No. of repeats for sampling to estimate cutoff: 1000
○ Codon ● DNA (Beta)

```
00015.best.muscle.fas     0.763903; Trimmed
00004.best.muscle.fas     0.600645; Trimmed
```

Info: Alignment trimming completed!

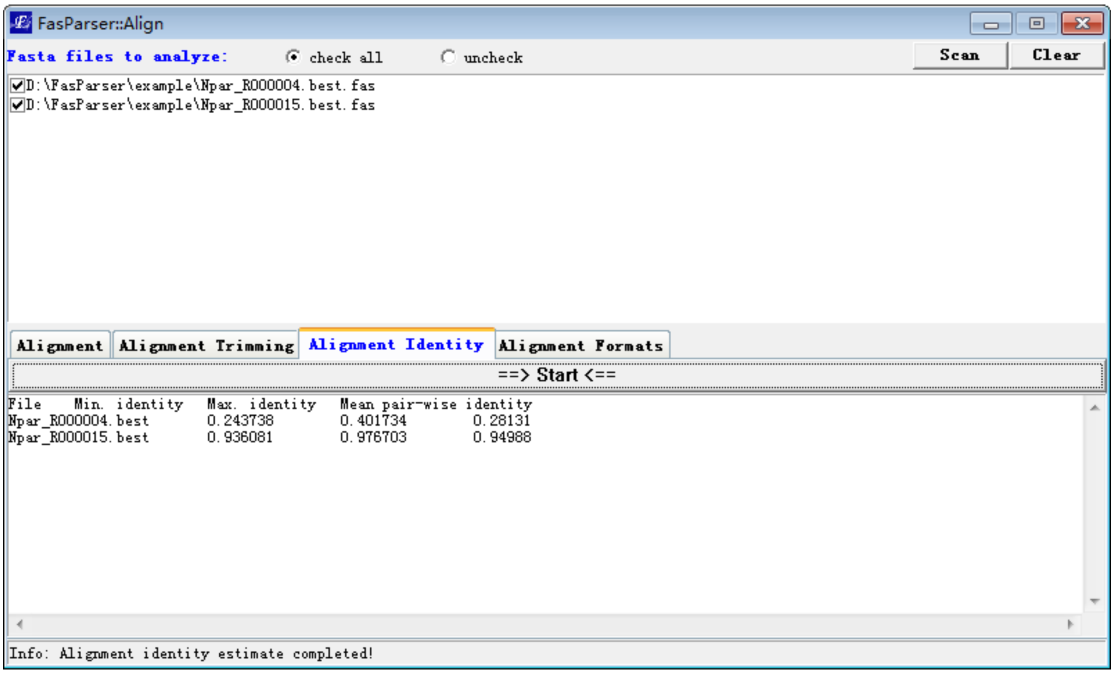
Figure 3. Overview of alignment trimming.

[Sim]

It is important to know the conservation level for a gene or a segment among different species (always refer to the species you studied). If the mean identity of an alignment is too low, it might be due to rapid evolution of this gene or non-homologous prediction for one or more species in this alignment. For each alignment, 'Sim' (Similarity) provides three identity calculations: the minimum pair-wise identity within the alignment (min.); the maximum pair-wise identity (max.); and the mean identity (mean).

Similarity, user should select files to analyze through clicking the '*Scan*' button, and then click the "*Start*" button to start the estimates (Figure 4).

Please note that during the similarity estimates, the gaps are also taken into account according to “Score = Score -1” if one base match a gap “-“.



☑D:\FasParser\example\Npar_R000004.best.fas
☑D:\FasParser\example\Npar_R000015.best.fas

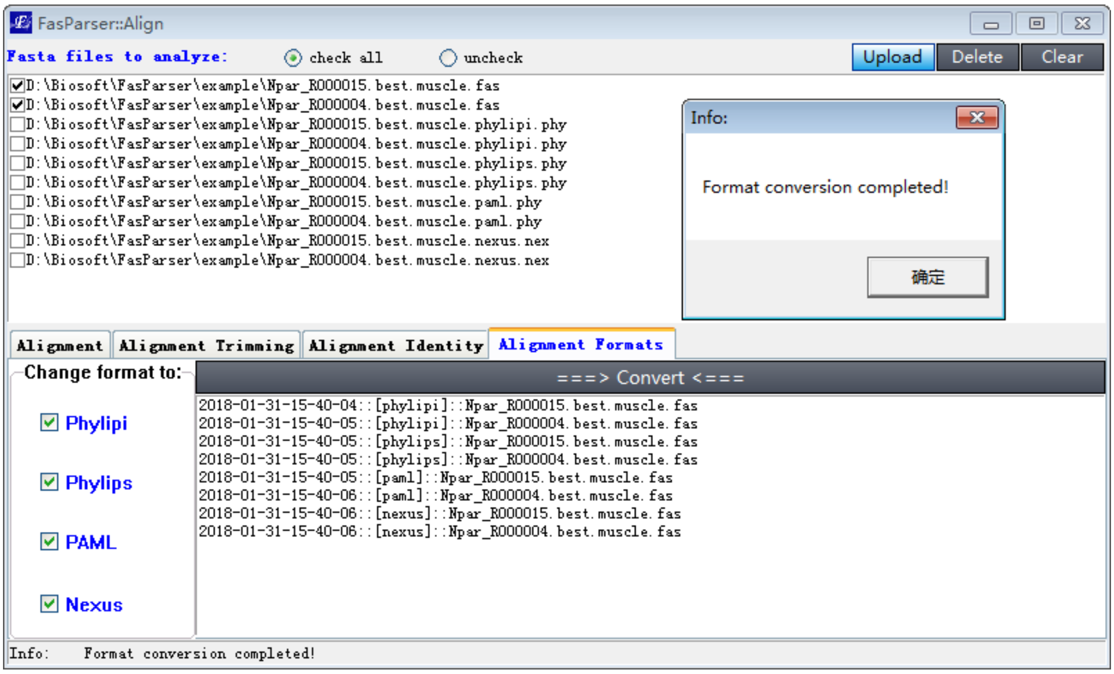
```
File    Min. identity    Max. identity    Mean pair-wise identity
Npar_R000004.best        0.243738         0.401734        0.28131
Npar_R000015.best        0.936081         0.976703        0.94988
```

Info: Alignment identity estimate completed!

Figure 4. Overview of alignment similarity estimates.

[Format]

This function is used to convert the FASTA files to other formatted ones. Presently, there are 4 different formats available: FASTA, PHYLIP (Phylipi and Phylips), PAML, and NEXUS (Figure 5). The output files will be saved in the folder which contains the input files, with ‘*. <format>.fas’. The result files are also displayed in the window and can be viewed by double clicks.



```
☑D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.fas
☑D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.fas
□D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.phylipi.phy
□D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.phylipi.phy
□D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.phylips.phy
□D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.phylips.phy
□D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.paml.phy
□D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.paml.phy
□D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.nexus.nex
□D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.nexus.nex
```

Format conversion completed!

☑ Phylipi

☑ Phylips

☑ PAML

☑ Nexus

```
2018-01-31-15-40-04::[phylipi]::Npar_R000015.best.muscle.fas
2018-01-31-15-40-05::[phylipi]::Npar_R000004.best.muscle.fas
2018-01-31-15-40-05::[phylips]::Npar_R000015.best.muscle.fas
2018-01-31-15-40-05::[phylips]::Npar_R000004.best.muscle.fas
2018-01-31-15-40-05::[paml]::Npar_R000015.best.muscle.fas
2018-01-31-15-40-06::[paml]::Npar_R000004.best.muscle.fas
2018-01-31-15-40-06::[nexus]::Npar_R000015.best.muscle.fas
2018-01-31-15-40-06::[nexus]::Npar_R000004.best.muscle.fas
```

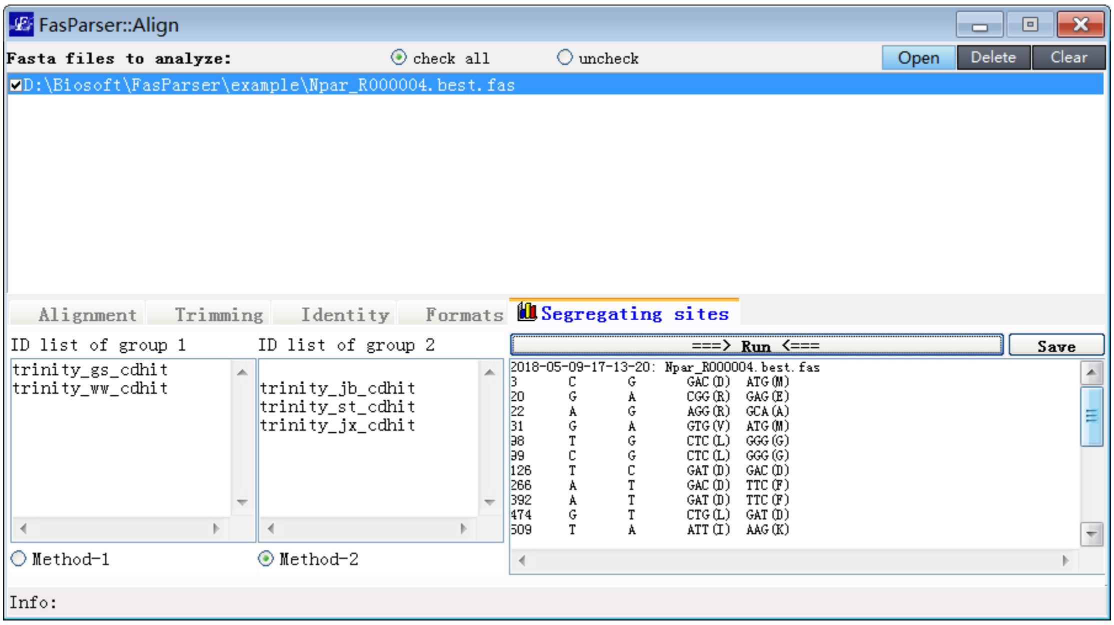
Info: Format conversion completed!

Figure 5. Overview of alignment format conversion.

[Segregating sites]

FasParser provides two methods to extract segregating sites that are same in a group of sequences, but different from the other group of sequences. Method-1 allowed the sites of a group of sequences are same, which can be not same in the other group of sequences. For example, one position in the 1st group of sequences are all "A", while the other sequences can be either G, C, or T.

Method-2 is more rigorous, which require the base of the same position are same in each group of sequences. For example, the 1st group is "A", and the others are all "G"



Fasta files to analyze: ● check all ○ uncheck

☑D:\Biosoft\FasParser\example\Npar_R000004.best.fas

ID list of group 1:

trinity_gs_cdhit
trinity_ww_cdhit

ID list of group 2:

trinity_jb_cdhit
trinity_st_cdhit
trinity_jx_cdhit

○ Method-1 ● Method-2

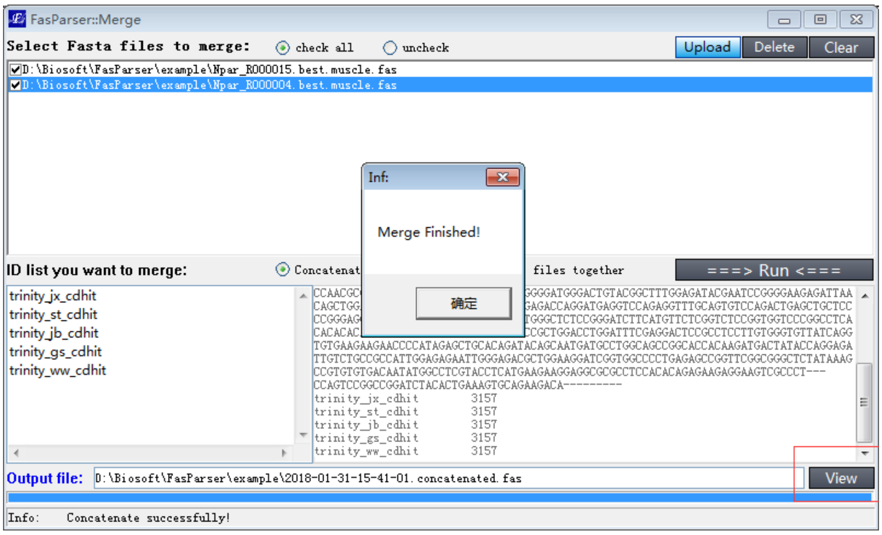
```
2018-05-09-17-13-20:  Npar_R000004.best.fas
3       C       G       GAC(D)  ATG(M)
20      G       A       CGG(R)  GAG(E)
22      A       G       AGG(R)  GCA(A)
31      G       A       GTG(V)  ATG(M)
38      T       G       CTC(L)  GGG(G)
39      C       G       CTC(L)  GGG(G)
126     T       C       GAT(D)  GAC(D)
266     A       T       GAC(D)  TTC(F)
392     A       T       GAT(D)  TTC(F)
474     G       T       CTG(L)  GAT(D)
509     T       A       ATT(I)  AAG(K)
```

Info:

[Merge]

Concatenate with same ID

This program is used to 'concatenate' sequences of the same IDs from multiple FASTA files. It is much useful in phylogenetic or other analyses, especially when users generated multiple loci sequences for a set of samples and want to generate a "*super*" sequence by concatenating all the loci sequences together for each sample.



Select Fasta files to merge: ● check all ○ uncheck

☑D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.fas
☑D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.fas

Merge Finished!

ID list you want to merge: ● Concatenat files together

trinity_jx_cdhit
trinity_st_cdhit
trinity_jb_cdhit
trinity_gs_cdhit
trinity_ww_cdhit

```
CCAACGC                          GGGGATGGGACTGTACGGCTTTGGAGATACGAATCCGGGGAAGAGATTAA
CAGCTGG                          GAGACCAGGATGAGGTCCAGAGGTTTGCAGTGTCCAGACTGAGCTGCTCC
CCGGGAG                          TGGGCTCTCCGGGATCTTCATGTTCTCGGTCTCCGGTGGTCCCGGCCTCA
CACACAC                          CCGCTGGACCTGGATTTCGAGGACTCCGCCTCCTTGTGGGTGTTATCAGG
TGTGAAGAAGAACCCCATAGAGCTGCACAGATACAGCAATGATGCCTGGCAGCCGGCACCACAAGATGACTATACCAGGAGA
TTGTCTGCCGCCATTGGAGAGAATTGGGAGACGCTGGAAGGATCGGTGGCCCCTGAGAGCCGGTTCGGCGGGCTCTATAAAG
CCGTGTGTGACAATATGGCCTCGTACCTCATGAAGAAGGAGGCGCGCCTCCACACAGAGAAGAGGAAGTCGCCCT---
CCAGTCCGGCCGGATCTACACTGAAAGTGCAGAAGACA---------
trinity_jx_cdhit        3157
trinity_st_cdhit        3157
trinity_jb_cdhit        3157
trinity_gs_cdhit        3157
trinity_ww_cdhit        3157
```

Output file: D:\Biosoft\FasParser\example\2018-01-31-15-41-01.concatenated.fas

Info: Concatenate successfully!

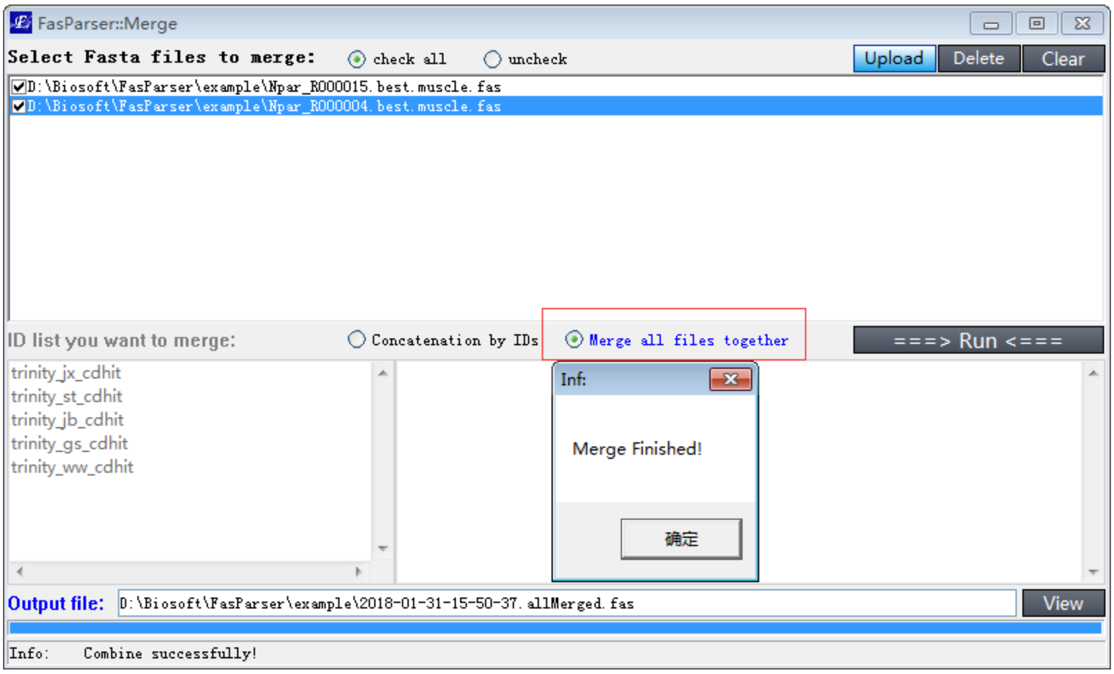
Figure 6.1. Overview of sequence concatenation manipulation.

Please note that for concatenate, if the raw FASTA files are not aligned before, the final concatenated FASTA file should be aligned first before conducting some other analyses. (We recommend user to construct alignment for each file before, and each file must have a same ID list).

To start this analysis, user should define an output file name and its location (can be automated created by FasParser). User can define which IDs will be concatenated by editing the ID list in the textbox (left-below, Figure 6.1). The View button can perform the user default execute program to open the result file.

Combine FASTA files together (append)

In addition to the concatenate, user may just want to 'combine' all the selected files together. It is easy to achieve this by using the Merge function in FasParser (Figure 6.2):



Select Fasta files to merge: ● check all ○ uncheck

☑ D:\Biosoft\FasParser\example\Npar_R000015.best.muscle.fas
☑ D:\Biosoft\FasParser\example\Npar_R000004.best.muscle.fas

ID list you want to merge: ○ Concatenation by IDs ● Merge all files together

trinity_jx_cdhit
trinity_st_cdhit
trinity_jb_cdhit
trinity_gs_cdhit
trinity_ww_cdhit

Merge Finished!

Output file: D:\Biosoft\FasParser\example\2018-01-31-15-50-37.allMerged.fas

Info: Combine successfully!

Figure 6.2. Overview of sequence combine manipulation

[Sort]

Sort Sequences

This program will allow users to sort their FASTA files according to either the ID names, sequence lengths, or a provided list of IDs. Part of this function (with the ID list provided by users) is much like the Extraction analysis in [Filter] module.

Please note that the ID is automatedly recognized from the raw ID, which is the first continuous string before symbols space, ".", and "|". For example, if the raw ID in a FASTA file is:

'>Uma_R000001.2 locus=scaffold79:384179:406202:-'

You can use the ID 'Uma_R000001.2' to search its sequence. Sometimes, the ID

'Uma_R000001' is also ok, if there is only one targeted ID. If the provided IDs cannot be recognized, there will be no sequence reported. Another example. You can use 'gi|947195581' to represent the sequence ID :

'>gi|947195581|ref|XP_006139827.2| PREDICTED…'

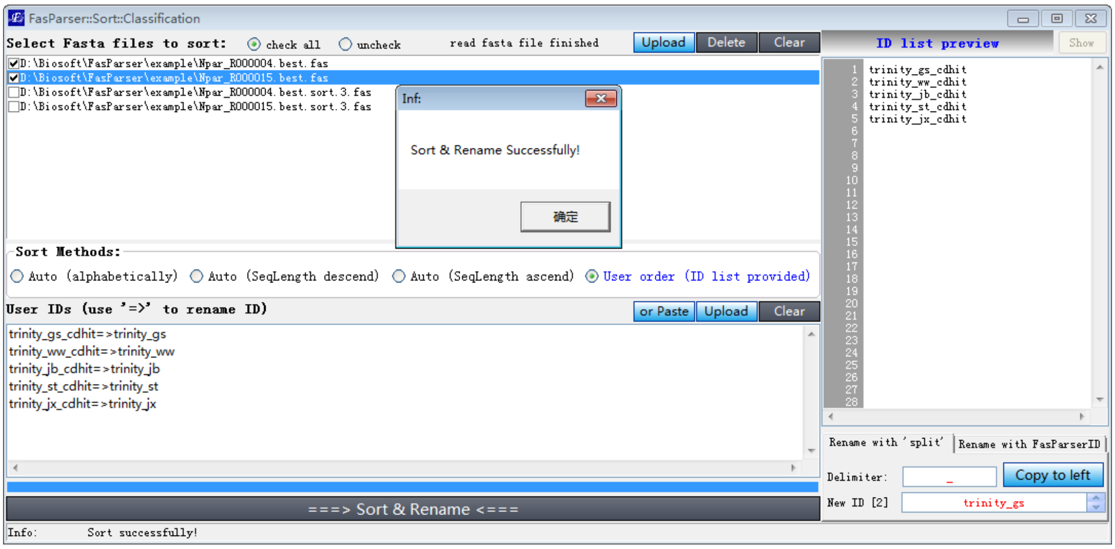
The output files will be saved in the folder which contains the input files, with '*. sort.1.fas' for alphabetically, '*. sort.2.1.fas' and '*. sort.2.2.fas' for length-descend and length-ascend, respectively.

Rename sequences (same with ID module)

With the provided ID list (**left-below textbox**), the program can also rename the raw sequence IDs. To achieve that, the ID list should have two columns, the first column refers to the raw ID, and the second column refers to the new ID, the separator between the two columns must be "=>", as below:

```
Uma_R000001.2=>R000001.2
Uma_R000003.2
Uma_R000002.2=>R000002
…
```

Under the above query IDs, program will use R000001.2 to replace Uma_R000001.2, and use R000002 to replace Uma_R000002.2. If there is only one column, the raw IDs will be retained (Figure 7). The output files will be saved in the folder which contains the input files, with '*. sort.3.fas'.



FasParser::Sort::Classification

Select Fasta files to sort: ● check all ○ uncheck   read fasta file finished

☑ D:\Biosoft\FasParser\example\Npar_R000004.best.fas
☑ D:\Biosoft\FasParser\example\Npar_R000015.best.fas
☐ D:\Biosoft\FasParser\example\Npar_R000004.best.sort.3.fas
☐ D:\Biosoft\FasParser\example\Npar_R000015.best.sort.3.fas

Inf:

Sort & Rename Successfully!

Sort Methods: ○ Auto (alphabetically) ○ Auto (SeqLength descend) ○ Auto (SeqLength ascend) ● User order (ID list provided)

User IDs (use '=>' to rename ID)

```
trinity_gs_cdhit=>trinity_gs
trinity_ww_cdhit=>trinity_ww
trinity_jb_cdhit=>trinity_jb
trinity_st_cdhit=>trinity_st
trinity_jx_cdhit=>trinity_jx
```

ID list preview

```
trinity_gs_cdhit
trinity_ww_cdhit
trinity_jb_cdhit
trinity_st_cdhit
trinity_jx_cdhit
```

Delimiter: _

New ID [2] trinity_gs

Info: Sort successfully!

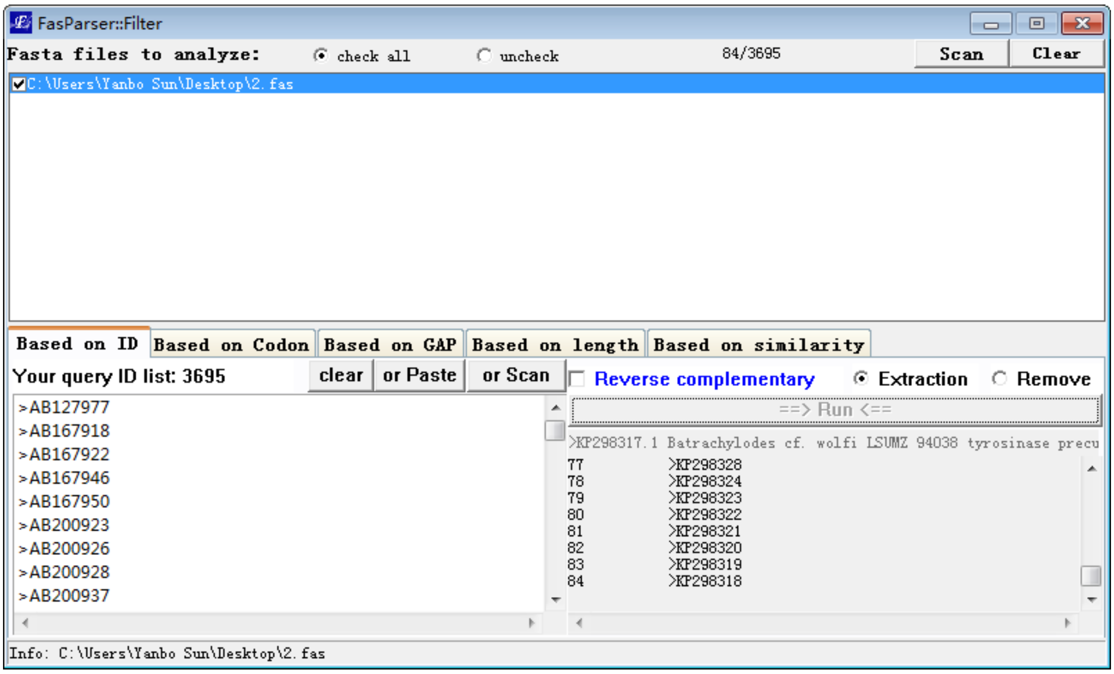
Figure 7. Overview of Sort & Rename sequences.

[Filter]

This program is designed to perform some extraction and filtration analyses within a particular FASTA file. It is a much common sequence manipulation. With this program, users can extract or remove a set of sequences from the raw FASTA file based on query IDs, the positions per codon, and can also filter the sequences based on the sequence length, or similarity with a provided reference sequence.

Extraction & Filtration based on IDs

Based on this function, user can extract or remove some sequences from one or more FASTA files. Just select the files you want to analyze, and then input the IDs (you want to query) in the left-below textbox. Select the appropriated operation: *extract* or *remove*, whether or not with reverse complement operation, and then click the "*Run*" button (Figure. 8.1). The output files will be saved in the folder which contains the input files, with '*.extract.fas' or '*.removed.fas'.



Fasta files to analyze: ● check all ○ uncheck 84/3695

☑ C:\Users\Yanbo Sun\Desktop\2.fas

Your query ID list: 3695

□ Reverse complementary ● Extraction ○ Remove

```
>AB127977
>AB167918
>AB167922
>AB167946
>AB167950
>AB200923
>AB200926
>AB200928
>AB200937
```

```
>KP298317.1 Batrachylodes cf. wolfi LSUMZ 94038 tyrosinase precu
77          >KP298328
78          >KP298324
79          >KP298323
80          >KP298322
81          >KP298321
82          >KP298320
83          >KP298319
84          >KP298318
```

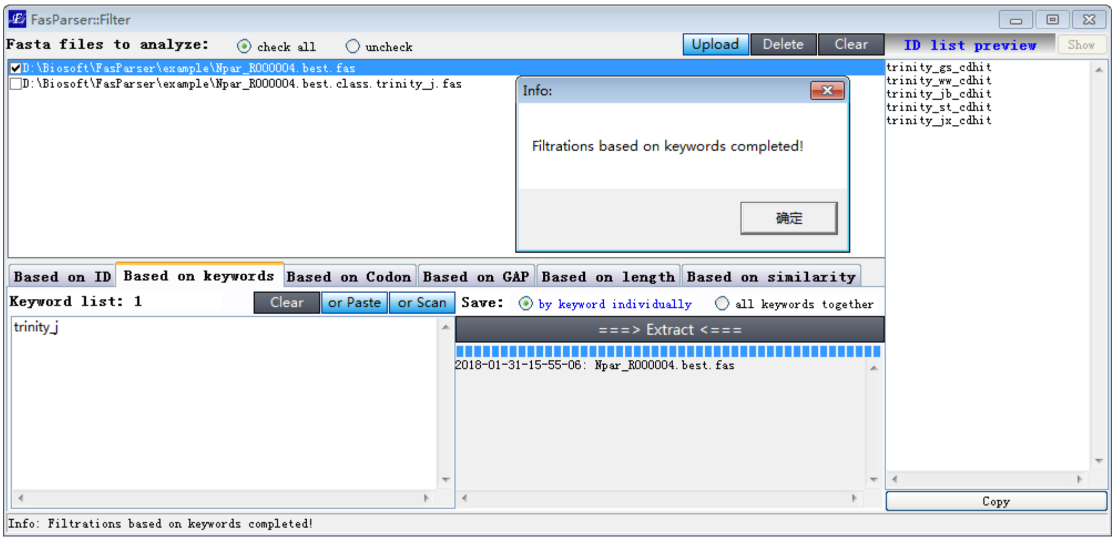
Info: C:\Users\Yanbo Sun\Desktop\2.fas

Figure 8.1. Overview of extraction & filtration based on query IDs.

Extraction based on keywords

In addition, this section also provides a Classification function, which means that you can use one or more keywords to extract sequences from a raw FASTA file and save them into separate FASTA files. The keywords can be the genus name, species name, or some other words, which should be part of the raw IDs (Figure 7.2).

The user should input the **keywords** in the right-below textbox, and then click the "*Classification*" button to run this analysis. The output files will be saved in the folder which contains the input files, with '*.class.[keyword].fas'.



☑ D:\Biosoft\FasParser\example\Npar_R000004.best.fas
□ D:\Biosoft\FasParser\example\Npar_R000004.best.class.trinity_j.fas

```
trinity_gs_cdhit
trinity_ww_cdhit
trinity_jb_cdhit
trinity_st_cdhit
trinity_jx_cdhit
```

Filtrations based on keywords completed!

Keyword list: 1

trinity_j

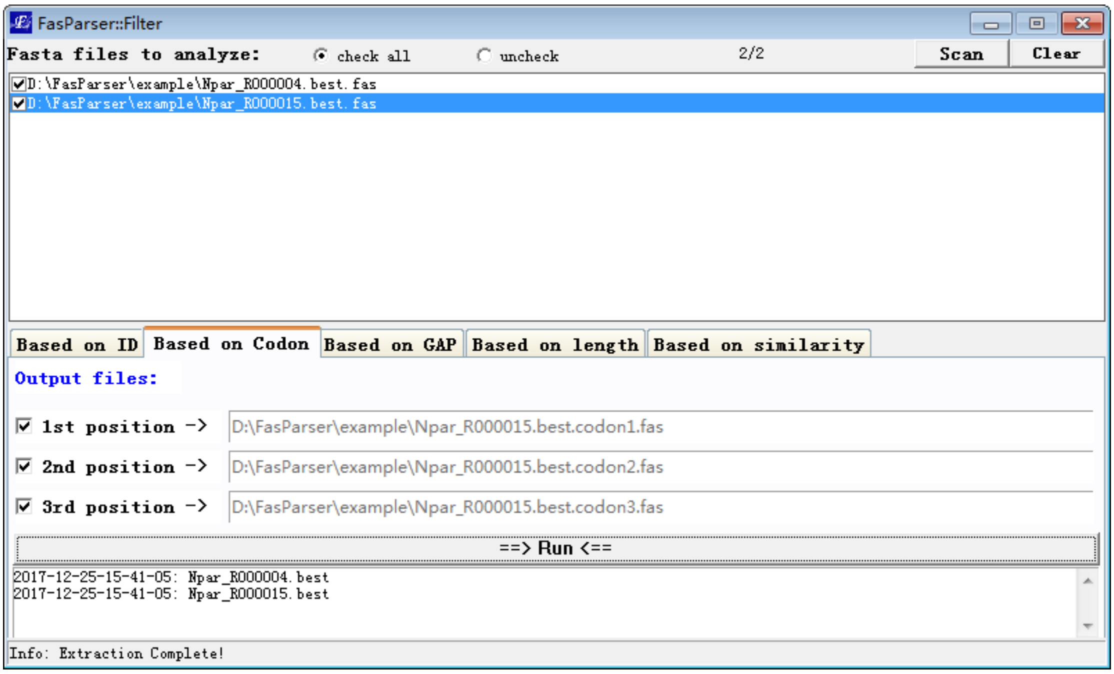
```
2018-01-31-15-55-06: Npar_R000004.best.fas
```

Info: Filtrations based on keywords completed!

Figure 7.2. Overview of extraction & filtration based on ID keywords.

Extract 3-site columns for Codon sequences

Based on this function, user can extract each-site columns of codon sequences for multiple FASTA files. Just select the files you want to analyze, and then click the "*Run*" button (Figure. 8.2). The output files will be saved in the folder which contains the input files, with '*.codon1.fas', '*.codon2.fas', and '*.codon3.fas'.



☑ D:\FasParser\example\Npar_R000004.best.fas
☑ D:\FasParser\example\Npar_R000015.best.fas

Output files:

☑ 1st position -> D:\FasParser\example\Npar_R000015.best.codon1.fas

☑ 2nd position -> D:\FasParser\example\Npar_R000015.best.codon2.fas

☑ 3rd position -> D:\FasParser\example\Npar_R000015.best.codon3.fas

```
2017-12-25-15-41-05: Npar_R000004.best
2017-12-25-15-41-05: Npar_R000015.best
```

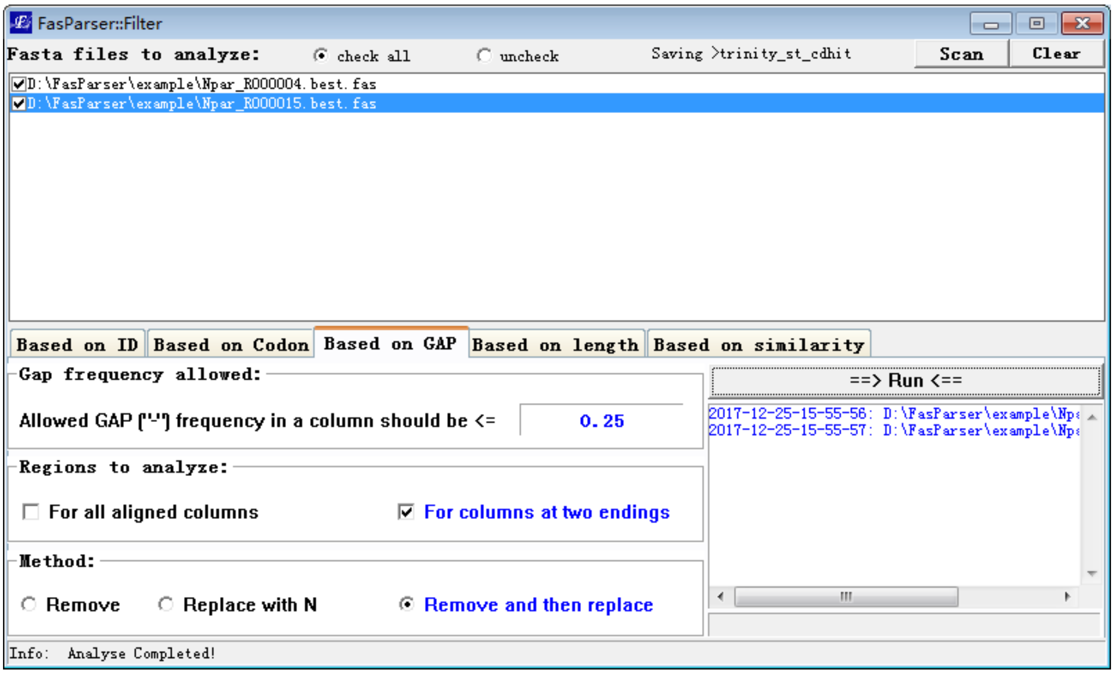
Info: Extraction Complete!

Figure 8.2. Overview of extraction of site columns for codon sequences.

Filtration of columns based on Gap frequency

This is a common function to cut the raw alignment by deleting columns with many gaps ('-'). This function can also fill up the missing data (with 'N') at the beginning and ending of an alignment, which is useful for some phylogenetic analyses. To perform this analysis, user should provide a gap-frequency cutoff value, and then select appropriated operation, like analyzing which region, and how to deal with the columns with a lower value (than the cutoff). Click the '*Run'* button to start this analysis (Figure 8.3).

The output files will be save in the folder which contains the raw input files, with a name format of "*.cut.end.fas" or "*.cut.all.fas".



Fasta files to analyze: ● check all ○ uncheck Saving >trinity_st_cdhit

☑ D:\FasParser\example\Npar_R000004.best.fas
☑ D:\FasParser\example\Npar_R000015.best.fas

Gap frequency allowed:

Allowed GAP ('-') frequency in a column should be <= 0.25

Regions to analyze:

□ For all aligned columns ☑ For columns at two endings

Method:

○ Remove ○ Replace with N ● Remove and then replace

```
2017-12-25-15-55-56: D:\FasParser\example\Np
2017-12-25-15-55-57: D:\FasParser\example\Np
```

Info: Analyse Completed!

Figure 8.3. Overview of the column-filtration based on Gap frequency.

Filtration of sequences based on sequence length

Maybe there are some too short sequences in a file, which would significantly influence the final available sequence length. So, it is usually useful to identify and remove the too short sequences. To do this, FasParser provides some different methods.

The 1st method is designed for alignment files (slower). The program will auto-estimate the block (no-gap regions) length under removal of each single sequence, and then identify the bad sequences whose removal can increase significantly the final block lengths.

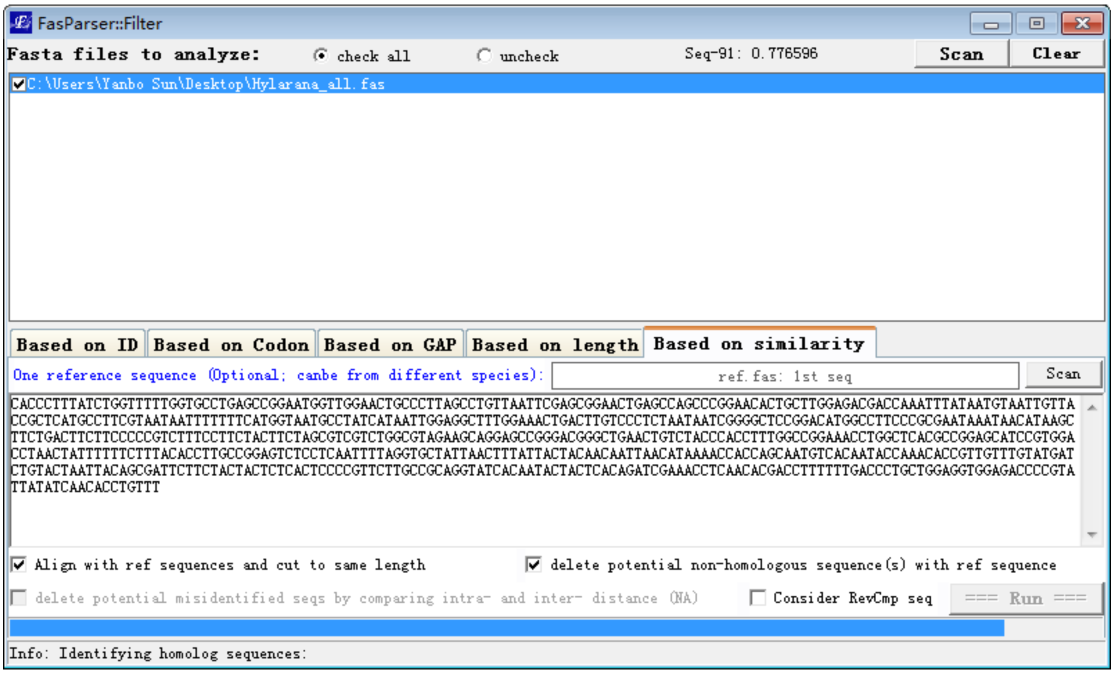
The 2nd method is designed for non-aligned files (faster). This method will identify directly the too short sequences according to their sequence lengths. The formula used is "mean - 3*std" if there are more than 100 sequences. If there are less than 100 sequences, Grubbs method will be used.

Filtration based on similarity with a reference sequence

This function is designed for manipulating multiple sequences, which are always collected from different sources without few sequence checking tasks. There may be some **non-homologous sequences** in the raw inputted sequences, or the sequences come from different parts of a same gene. Before downstream analyses, it is much better to do some filtration task on these sequences to improve their availability in other analyses.

To do this work, user should provide a reference sequence that is from your previous or other peoples' work to **identify the non-homologous sequences and cut the input sequences to a same length based on the alignment result with the reference sequence**.

After setting appropriated parameters, just click the "Run" button to run this analysis. There would generate 2 or 4 output files for each input file, which contain the final alignment (cut to a length based on the reference sequence), as well as the homologous identification results (Figure. 8.4).



Fasta files to analyze: ● check all ○ uncheck Seq-91: 0.776596 Scan Clear

☑ C:\Users\Yanbo Sun\Desktop\Hylarana_all.fas

One reference sequence (Optional; canbe from different species): ref.fas: 1st seq Scan

```
CACCCTTTATCTGGTTTTTGGTGCCTGAGCCGGAATGGTTGGAACTGCCCTTAGCCTGTTAATTCGAGCGGAACTGAGCCAGCCCGGAACACTGCTTGGAGACGACCAAATTTATAATGTAATTGTTA
CCGCTCATGCCTTCGTAATAATTTTTTTCATGGTAATGCCTATCATAATTGGAGGCTTTGGAAACTGACTTGTCCCTCTAATAATCGGGGCTCCGGACATGGCCTTCCCGCGAATAAATAACATAAGC
TTCTGACTTCTTCCCCCGTCTTTCCTTCTACTTCTAGCGTCGTCTGGCGTAGAAGCAGGAGCCGGGACGGGCTGAACTGTCTACCCACCTTTGGCCGGAAACCTGGCTCACGCCGGAGCATCCGTGGA
CCTAACTATTTTTTCTTTACACCTTGCCGGAGTCTCCTCAATTTTAGGTGCTATTAACTTTATTACTACAACAATTAACATAAAACCACCAGCAATGTCACAATACCAAACACCGTTGTTTGTATGAT
CTGTACTAATTACAGCGATTCTTCTACTACTCTCACTCCCCGTTCTTGCCGCAGGTATCACAATACTACTCACAGATCGAAACCTCAACACGACCTTTTTTGACCCTGCTGGAGGTGGAGACCCCGTA
TTATATCAACACCTGTTT
```

☑ Align with ref sequences and cut to same length ☑ delete potential non-homologous sequence(s) with ref sequence

□ delete potential misidentified seqs by comparing intra- and inter- distance (NA) □ Consider RevCmp seq === Run ===

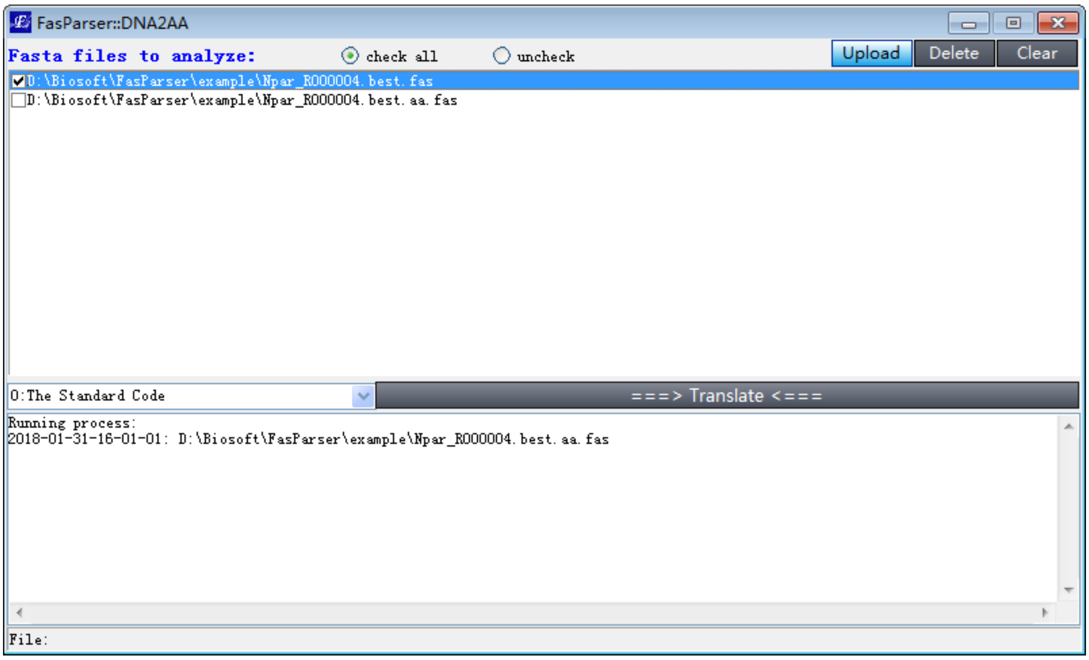
Info: Identifying homolog sequences:

Figure. 8.4. Overview of the sequence filtration based on similarity with a reference sequence.

[DNA2AA]

This function is used to translate the DNA sequences to protein sequences.

There are two modes available. If users have many FASTA files, you can use the batch mode to conduct such analyses, with genetic code selected. The output files will be saved into the folder which contains the raw input files, with a filename as "*.aa.fas". You can also input a pure DNA sequence or a FASTA file under the manual mode, the program can automate recognize them.



● check all ○ uncheck

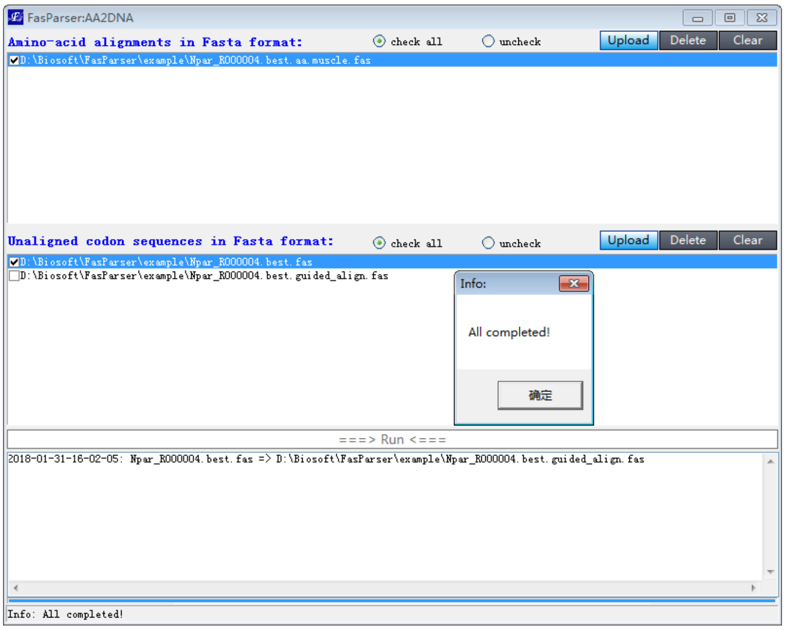
☑D:\Biosoft\FasParser\example\Npar_R000004.best.fas
□D:\Biosoft\FasParser\example\Npar_R000004.best.aa.fas

0:The Standard Code

```
Running process:
2018-01-31-16-01-01: D:\Biosoft\FasParser\example\Npar_R000004.best.aa.fas
```

[AA2DNA]

This function is used to generate codon alignments guided by the amino-acid alignments. The raw DNA sequence files (unaligned) and the amino-acid alignment files (aligned) are needed for this function. It is always used in transcriptome analyses. For example, you have 1000 genes with 5 species. Each gene has been translated to amino-acid sequences and aligned by Muscle or others. You can use this function to generate codon alignments based on the amino-acid sequences.



Amino-acid alignments in Fasta format: ● check all ○ uncheck

☑D:\Biosoft\FasParser\example\Npar_R000004.best.aa.muscle.fas

Unaligned codon sequences in Fasta format: ● check all ○ uncheck

☑D:\Biosoft\FasParser\example\Npar_R000004.best.fas
□D:\Biosoft\FasParser\example\Npar_R000004.best.guided_align.fas

Info:

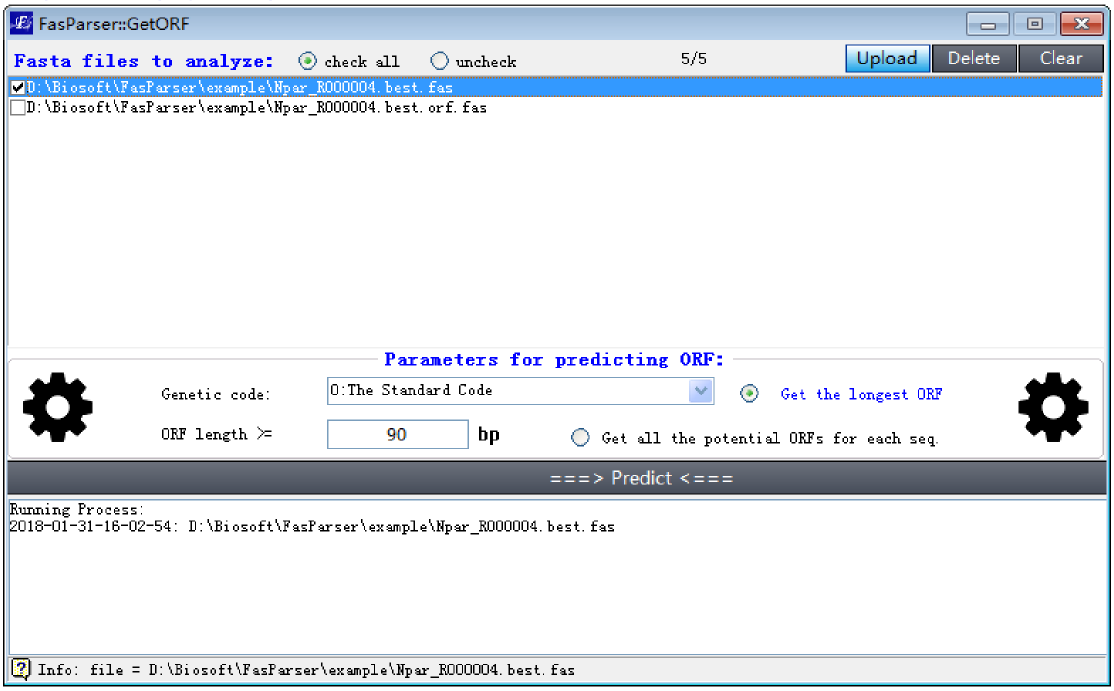
All completed!

```
2018-01-31-16-02-05: Npar_R000004.best.fas => D:\Biosoft\FasParser\example\Npar_R000004.best.guided_align.fas
```

Info: All completed!

[ORF]

This program is designed to identify the ORF for cDNA sequence(s). The cDNA sequences can be organized into a FASTA file or as single sequence. There are 2 choices to obtain the ORFs: a) get the longest one if there are present more than one potential ORF in the input cDNA sequence, and b) get all the potential ORF sequences (Figure 9).



Fasta files to analyze: ● check all ○ uncheck 5/5

☑D:\Biosoft\FasParser\example\Npar_R000004.best.fas
□D:\Biosoft\FasParser\example\Npar_R000004.best.orf.fas

Parameters for predicting ORF:

Genetic code: 0:The Standard Code

ORF length >= 90 bp

● Get the longest ORF
○ Get all the potential ORFs for each seq.

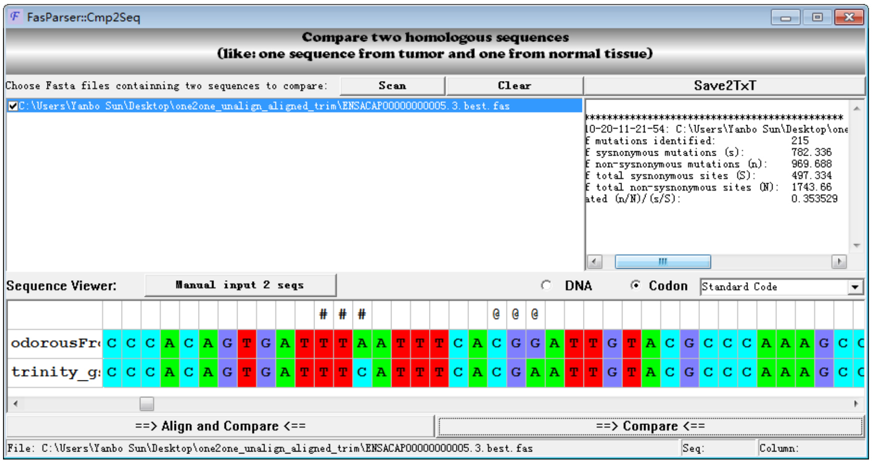
```
Running Process:
2018-01-31-16-02-54: D:\Biosoft\FasParser\example\Npar_R000004.best.fas
```

Info: file = D:\Biosoft\FasParser\example\Npar_R000004.best.fas

Figure 9. overview of identification of ORFs for sequences.

[2Seq]

The program "2Seq" is designed to easily count and view differences between two DNA sequences at both DNA and codon levels. Under the codon level, the program can also estimate the total number of synonymous (S) and non-synonymous (N) sites for the first sequence and then calculate the number of synonymous and non-synonymous substitutions between the two sequences. To do that, users just need to put two sequences into the two above textboxes and click the Run button. The program could then provide a view the differences between the two sequences in colors and the summary of identified mutations or substitutions (Figure 10).


FasParser::Cmp2Seq
Compare two homologous sequences
(like: one sequence from tumor and one from normal tissue)
Choose Fasta files containning two sequences to compare:
Scan
Clear
Save2TxT
Sequence Viewer:
Manual input 2 seqs
○ DNA
● Codon
Standard Code
==> Align and Compare <==
==> Compare <==

☑C:\Users\Yanbo Sun\Desktop\one2one_unalign_aligned_trim\ENSACAP00000000005.3.best.fas

```
*******************************************
10-20-11-21-54: C:\Users\Yanbo Sun\Desktop\one
f mutations identified:              215
f sysnonymous mutations (s):         782.336
f non-sysnonymous mutations (n):     969.688
f total sysnonymous sites (S):       497.334
f total non-sysnonymous sites (N):   1743.66
ated (n/N)/(s/S):                    0.353529
```

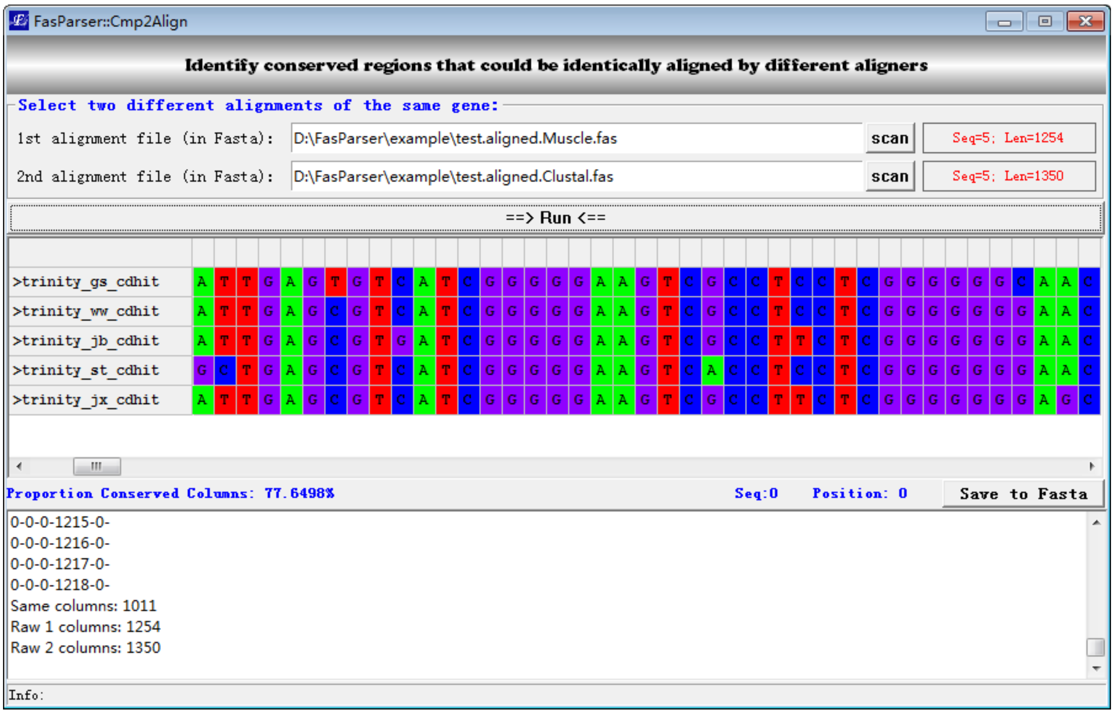
```
                                # # #                   @ @ @
odorousFr  C C C A C A G T G A T T T A A T T T C A C G G A T T G T A C G C C C A A A G C C
trinity_g  C C C A C A G T G A T T T C A T T T C A C G A A T T G T A C G C C C A A A G C C
```

File: C:\Users\Yanbo Sun\Desktop\one2one_unalign_aligned_trim\ENSACAP00000000005.3.best.fas | Seq: | Column:

Figure 10. Overview of comparison between two sequences.

[2Align]

This program "2Align" is designed to compare different alignments of a same gene that could be generated by different aligners. It is well known that there is almost no current method correctly aligns the entire sequence, and different aligners always correctly align different regions. One simple method is to identify the overlapped regions between different aligner-generated alignments, which might be useful for some other analyses, like phylogenetic reference and/or positive selection detection. This function needs users to input two alignments through the above scan buttons and click the "*Run*" button to view their overlaps (Figure 11). *This is the only function currently cannot run at the batch mode.



FasParser::Cmp2Align
Identify conserved regions that could be identically aligned by different aligners
Select two different alignments of the same gene:

1st alignment file (in Fasta): D:\FasParser\example\test.aligned.Muscle.fas scan Seq=5; Len=1254

2nd alignment file (in Fasta): D:\FasParser\example\test.aligned.Clustal.fas scan Seq=5; Len=1350

```
>trinity_gs_cdhit  A T T G A G T G T C A T C G G G G G A A G T C G C C T C C T C G G G G G G C A A C
>trinity_ww_cdhit  A T T G A G C G T C A T C G G G G G A A G T C G C C T C C T C G G G G G G G A A C
>trinity_jb_cdhit  A T T G A G C G T G A T C G G G G G A A G T C G C C T T C T C G G G G G G G A A C
>trinity_st_cdhit  G C T G A G C G T C A T C G G G G G A A G T C A C C T C C T C G G G G G G G A A C
>trinity_jx_cdhit  A T T G A G C G T C A T C G G G G G A A G T C G C C T T C T C G G G G G G G A G C
```

Proportion Conserved Columns: 77.6498% Seq:0 Position: 0

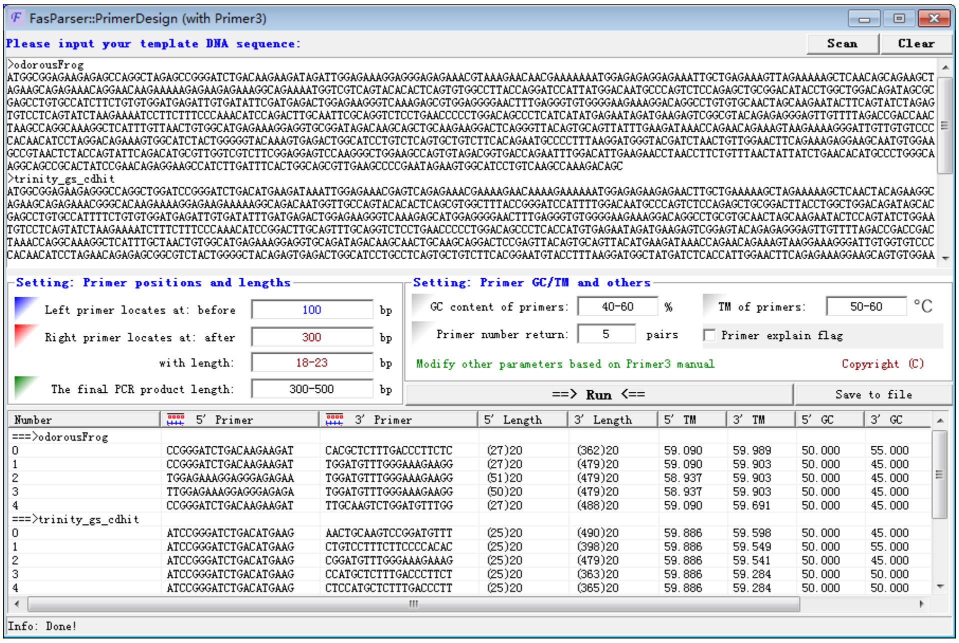
```
0-0-0-1215-0-
0-0-0-1216-0-
0-0-0-1217-0-
0-0-0-1218-0-
Same columns: 1011
Raw 1 columns: 1254
Raw 2 columns: 1350
```

Info:

Figure 11. Overview of the estimates of consensus alignment.

[Primer]

This is an interface to design PCR primers with an excellent Primer designing tool (Primer3). To do this, jst input the DNA template (either in Fasta or pure DNA sequence) and modify the parameters. Click the "Run" button will obtain the primer results if all run properly (Figure 12).



FasParser::PrimerDesign (with Primer3)
Please input your template DNA sequence:
Scan
Clear

```
>odorousFrog
ATGGCGGAGAAGAGAGCCAGGCTAGAGCCGGGATCTGACAAGAAGATAGATTGGAGAAAGGAGGGAGAGAAACGTAAAGAACAACGAAAAAAAATGGAGAGAGGAGAAATTGCTGAGAAAGTTAGAAAAAGCTCAACAGCAGAAGCT
AGAAGCAGAGAAACAGGAACAAGAAAAAGAGAAGAGAAAGGCAGAAAATGGTCGTCAGTACACACTCAGTGTGGCCTTACCAGGATCCATTATGGACAATGCCCAGTCTCCAGAGCTGCGGACATACCTGGCTGGACAGATAGCGC
GAGCCTGTGCCATCTTCTGTGTGGATGAGATTGTGATATTCGATGAGACTGGAGAAGGGTCAAAGAGCGTGGAGGGGAACTTTGAGGGTGTGGGGAAGAAAGGACAGGCCTGTGTGCAACTAGCAAGAATACTTCAGTATCTAGAG
TGTCCTCAGTATCTAAGAAAATCCTTCTTTCCCAAACATCCAGACTTGCAATTCGCAGGTCTCCTGAACCCCCTGGACAGCCCTCATCATATGAGAATAGATGAAGAGTCGGCGTACAGAGAGGGAGTTGTTTTAGACCGACCAAC
TAAGCCAGGCAAAGGCTCATTTGTTAACTGTGGCATGAGAAAGGAGGTGCGGATAGACAAGCAGCTGCAAGAAGGACTCAGGGTTACAGTGCAGTTATTTGAAGATAAACCAGAACAGAAAGTAAGAAAAGGGATTGTTGTGTCCC
CACAACATCCTAGGACAGAAAGTGGCATCTACTGGGGGTACAAAGTGAGACTGGCATCCTGTCTCAGTGCTGTCTTCACAGAATGCCCCTTTAAGGATGGGTACGATCTAACTGTTGGAACTTCAGAAAGAGGAAGCAATGTGGAA
GCCGTAACTCTACCAGTATTCAGACATGCGTTGGTCGTCTTCGGAGGAGTCCAAGGGCTGGAAGCCAGTGTAGACGGTGACCAGAATTTGGACATTGAAGAACCTAACCTTCTGTTTAACTATTATCTGAACACATGCCCTGGGCA
AGGCAGCCGCACTATCCGAACAGAGGAAGCCATCTTGATTTCACTGGCAGCGTTGAAGCCCCGAATAGAAGTGGCATCCTGTCAAGCCAAAGACAGC
>trinity_gs_cdhit
ATGGCGGAGAAGAGGGCCAGGCTGGATCCGGGATCTGACATGAAGATAAATTGGAGAAACGAGTCAGAGAAACGAAAAGAACAAAAGAAAAAATGGAGAGAAGAGAACTTGCTGAAAAAGCTAGAAAAAGCTCAACTACAGAAGGC
AGAAGCAGAGAAACGGGCACAAGAAAAGGAGAAGAAAAAGGCAGACAATGGTTGCCAGTACACACTCAGCGTGGCTTTACCGGGATCCATTTTGGACAATGCCCAGTCTCCAGAGCTGCGGACTTACCTGGCTGGACAGATAGCAC
GAGCCTGTGCCATTTTCTGTGTGGATGAGATTGTGATATTTGATGAGACTGGAGAAGGGTCAAAGAGCATGGAGGGGAACTTTGAGGGTGTGGGGAAGAAAGGACAGGCCTGCGTGCAACTAGCAAGAATACTCCAGTATCTGGAA
TGTCCTCAGTATCTAAGAAAATCTTTCTTTCCCAAACATCCGGACTTGCAGTTTGCAGGTCTCCTGAACCCCCTGGACAGCCCTCACCATGTGAGAATAGATGAAGAGTCGGAGTACAGAGAGGGAGTTGTTTTAGACCGACCGAC
TAAACCAGGCAAAGGCTCATTTGCTAACTGTGGCATGAGAAAGGAGGTGCAGATAGACAAGCAACTGCAAGCAGGACTCCGAGTTACAGTGCAGTTACATGAAGATAAACCAGAACAGAAAGTAAGGAAAGGGATTGTGGTGTCCC
CACAACATCCTAGAACAGAGAGCGGCGTCTACTGGGGCTACAGAGTGAGACTGGCATCCTGCCTCAGTGCTGTCTTCACGGAATGTACCTTTAAGGATGGCTATGATCTCACCATTGGAACTTCAGAGAAAGGAAGCAGTGTGGAA
```

Setting: Primer positions and lengths

Left primer locates at: before 100 bp

Right primer locates at: after 300 bp

with length: 18-23 bp

The final PCR product length: 300-500 bp

Setting: Primer GC/TM and others

GC content of primers: 40-60 %

TM of primers: 50-60 °C

Primer number return: 5 pairs

□ Primer explain flag

Modify other parameters based on Primer3 manual

Copyright (C)

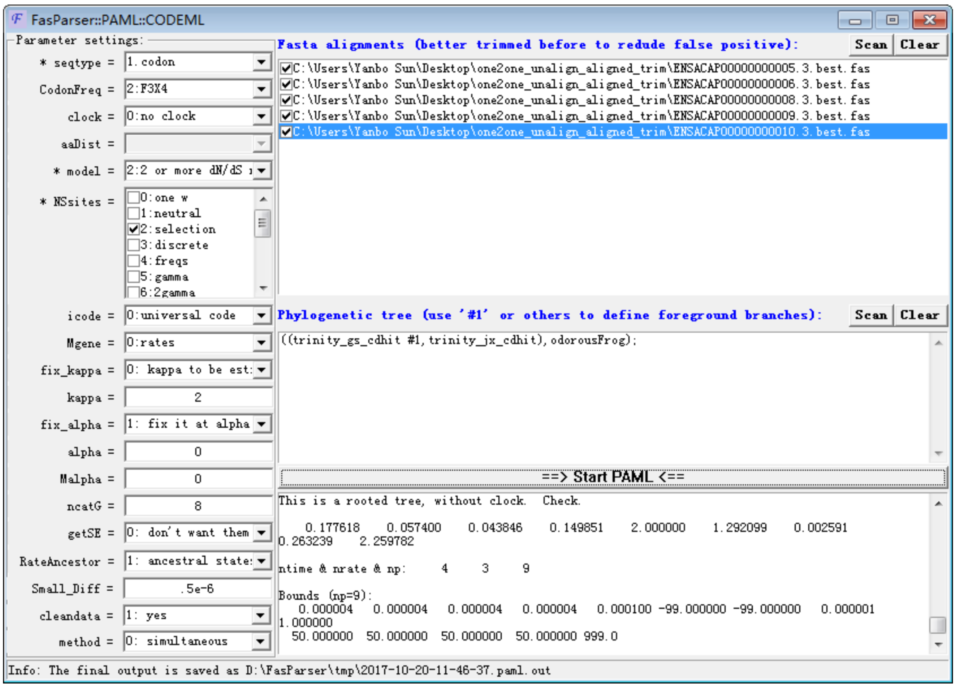
Number	5' Primer	3' Primer	5' Length	3' Length	5' TM	3' TM	5' GC	3' GC
===>odorousFrog								
0	CCGGGATCTGACAAGAAGAT	CACGCTCTTTGACCCTTCTC	(27)20	(362)20	59.090	59.989	50.000	55.000
1	CCGGGATCTGACAAGAAGAT	TGGATGTTTGGGAAAGAAGG	(27)20	(479)20	59.090	59.903	50.000	45.000
2	TGGAGAAAGGAGGGAGAGAA	TGGATGTTTGGGAAAGAAGG	(51)20	(479)20	58.937	59.903	50.000	45.000
3	TTGGAGAAAGGAGGGAGAGA	TGGATGTTTGGGAAAGAAGG	(50)20	(479)20	58.937	59.903	50.000	45.000
4	CCGGGATCTGACAAGAAGAT	TTGCAAGTCTGGATGTTTGG	(27)20	(488)20	59.090	59.691	50.000	45.000
===>trinity_gs_cdhit								
0	ATCCGGGATCTGACATGAAG	AACTGCAAGTCCGGATGTTT	(25)20	(490)20	59.886	59.598	50.000	45.000
1	ATCCGGGATCTGACATGAAG	CTGTCCTTTCTTCCCCACAC	(25)20	(398)20	59.886	59.549	50.000	55.000
2	ATCCGGGATCTGACATGAAG	CGGATGTTTGGGAAAGAAAG	(25)20	(479)20	59.886	59.541	50.000	45.000
3	ATCCGGGATCTGACATGAAG	CCATGCTCTTTGACCCTTCT	(25)20	(363)20	59.886	59.284	50.000	50.000
4	ATCCGGGATCTGACATGAAG	CTCCATGCTCTTTGACCCTT	(25)20	(365)20	59.886	59.284	50.000	50.000

Info: Done!

Figure 12. Overview of the primer designing function.

[PAML]

FasParser provided a batch mode to run codeml in PAML package (Yang et al. 2007). It is much better for user to read the manual of PAML to understand the parameter settings and add proper symbols to the phylogenetic tree (like below, Figure 13).



Parameter settings:

- * seqtype = 1. codon
- CodonFreq = 2:F3X4
- clock = 0:no clock
- aaDist =
- * model = 2:2 or more dN/dS r
- * NSsites = ☐0:one w ☐1:neutral ☑2:selection ☐3:discrete ☐4:freqs ☐5:gamma ☐6:2gamma
- icode = 0:universal code
- Mgene = 0:rates
- fix_kappa = 0: kappa to be est:
- kappa = 2
- fix_alpha = 1: fix it at alpha
- alpha = 0
- Malpha = 0
- ncatG = 8
- getSE = 0: don't want them
- RateAncestor = 1: ancestral state:
- Small_Diff = .5e-6
- cleandata = 1: yes
- method = 0: simultaneous

Fasta alignments (better trimmed before to redude false positive):

- ☑C:\Users\Yanbo Sun\Desktop\one2one_unalign_aligned_trim\ENSACAP00000000005.3.best.fas
- ☑C:\Users\Yanbo Sun\Desktop\one2one_unalign_aligned_trim\ENSACAP00000000006.3.best.fas
- ☑C:\Users\Yanbo Sun\Desktop\one2one_unalign_aligned_trim\ENSACAP00000000008.3.best.fas
- ☑C:\Users\Yanbo Sun\Desktop\one2one_unalign_aligned_trim\ENSACAP00000000009.3.best.fas
- ☑C:\Users\Yanbo Sun\Desktop\one2one_unalign_aligned_trim\ENSACAP00000000010.3.best.fas

Phylogenetic tree (use '#1' or others to define foreground branches):

```
((trinity_gs_cdhit #1,trinity_jx_cdhit),odorousFrog);
```

```
This is a rooted tree, without clock.  Check.

    0.177618    0.057400    0.043846    0.149851    2.000000    1.292099    0.002591
0.263239    2.259782

ntime & nrate & np:     4     3     9

Bounds (np=9):
   0.000004    0.000004    0.000004    0.000004    0.000100 -99.000000 -99.000000    0.000001
1.000000
  50.000000   50.000000   50.000000   50.000000 999.0
```

Info: The final output is saved as D:\FasParser\tmp\2017-10-20-11-46-37.paml.out

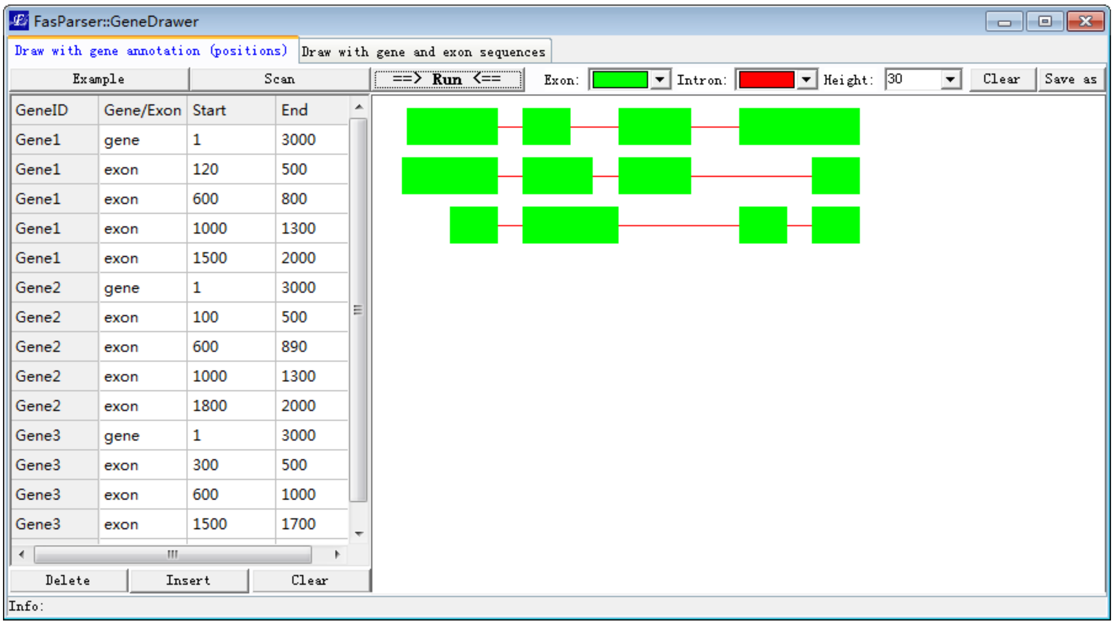
Figure 13. Positive selection detection.

Below is an example of running output:

```
GeneID  Ln Alternative  Ln Null Deta    Sig.
ENSACAP00000000005.3.best.fas   -4271.375567    -4271.375567    0
ENSACAP00000000006.3.best.fas   -6538.150389    -6538.150389    0
ENSACAP00000000008.3.best.fas   -1092.144468    -1092.144468    0
ENSACAP00000000009.3.best.fas   -2101.001357    -2094.010118    13.9829 P < 0.05
ENSACAP00000000010.3.best.fas   -1165.844923    -1165.844923    0
```

[GeneDrawer]

FasParser (since version 2) provides a gene structure drawing fiction. It is very easy to use. It can be run in two different mode, based on the data type of inputs. It now can read either gene annotation or gene sequences (Figure 14). Presently, the drawer function has litter photo-editing interfaces, which will be modified in the future versions.



Exon: 🟩 Intron: 🟥 Height: 30

GeneID	Gene/Exon	Start	End
Gene1	gene	1	3000
Gene1	exon	120	500
Gene1	exon	600	800
Gene1	exon	1000	1300
Gene1	exon	1500	2000
Gene2	gene	1	3000
Gene2	exon	100	500
Gene2	exon	600	890
Gene2	exon	1000	1300
Gene2	exon	1800	2000
Gene3	gene	1	3000
Gene3	exon	300	500
Gene3	exon	600	1000
Gene3	exon	1500	1700

Figure 14. Overview of gene structure drawing function.

Contact

If you have any question, or program bug, please feel free to contact the me (Yan-Bo Sun). My email address is sunyanbo@mail.kiz.ac.cn

FasParser also has a bug report interface ([Bug]), through which you can also contact me and report the errors of this program. I believe FasParser will grow better under your suggestions.

[TODO list]

1. Genbank file parser;
2. Phylogenetic tree parser;
3. Primer blast analysis
4. Improve the drawer function
5. …