CIAlign - Clean and Interpret Alignments

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CIAlign is a command line tool which performs various functions to clean and analyse a multiple sequence alignment (MSA).

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The tool is designed to be highly customisable, allowing users to specify exactly which functions to run and which settings to use. It is also transparent, generating a clear log file and alignment markup showing exactly how the alignment has changed and what has been removed by which function.

This allows the user to:

- Remove sources of noise from their MSA
  - Remove insertions which are not present in the majority of sequences
  - Remove sequences below a threshold number of bases or amino acids
  - Crop poorly aligned sequence ends
  - Remove columns containing only gaps
  - Remove sequences above a threshold level percentage of divergence from the majority
  - Remove either end of an alignment where columns don't meet a minimum identity threshold and coverage level
- Generate consensus sequences
- Visualise alignments
  - Generate image files showing the alignment before and after using CIAlign cleaning functions and showing which columns and rows have been removed
  - Draw sequence logos
  - Visualise coverage and conservation at each position in the alignment
  - Generate position frequency, position probability and position weight matrices based on the alignment and produce output formated to be used as input for the BLAMM and MEME motif analysis tools.
- Analyse alignment statistics
  - Generate a similarity matrix showing the percentage identity between each sequence pair
- Make changes to the alignment
  - Extract a section of the alignment
  - Unalign the alignment
  - Replace U with T, or T with U in a nucleotide alignment

Citation

If you found CIAlign useful, please cite:

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Requirements

- python >= 3.6
- matplotlib >= 2.1.1
- numpy >= 1.16.3
- scipy >= 1.3.0

Installation

The easiest way to install CIAlign is using conda or pip3.

Conda

```
conda install -c bioconda cialign
```

link

pip3

```
pip3 install cialign
```

link

The current release of CIAlign can also be downloaded directly using this link,

If you download the package directly, you will also need to add the CIAlign directory to your PATH environment variable as described here

Usage

Basic Usage

```
CIAlign --infile INFILE --outfile_stem STEM --inifile my_config.ini
```

Parameters Parameters can be specified in the command line or in a config file using the naming system below.

A template config file is provided in `CIAlign/templates/ini_template.ini` - edit this file and provide the path to the –inifile argument. If this argument is not provided command line arguments and defaults will be used.

Parameters passed in the command line will take precedence over config file parameters, which take precedence over defaults.

Command help can be accessed by typing `CIAlign --help`

Parameter	Description	Default
`--infile`	Path to input alignment file in FASTA format	None
`--inifile`	Path to config file	None
`--outfile_stem`	Prefix for output files, including the path to the output directory	CIAlign
`--all`	Use all available functions with default parameters. Does not currently include crop_divergent	False
`--clean`	Use all available cleaning functions (except crop_divergent) with default parameters	False
`--visualise`	Use all available mini alignment visualisation functions with default parameters	False
`--interpret`	Use all available interpretation functions (except sequence logos) with default parameters	False
`--silent`	Do not print progress to the screen	False
`--help`	Show all available parameters with an explanation	None
`--version`	Show the version	None

Beside these main parameters, the use of every function and corresponding thresholds can be specified by the user by adding parameters to the command line or by setting them in the configuration file. Available functions and their parameters will be specified in the following section.

CIAlign always produces a log file, specifying which functions have been run with witch parameters and what has been removed. It also outputs a file that only specifies what has been removed with the original column positions and the sequence names.

Output files:

- `OUTFILE_STEM_log.txt` - general log file
- `OUTFILE_STEM_removed.txt` - removed columns positions and sequence names text file

Cleaning an MSA

Each of these steps (if specified) will be performed sequentially in the order specified in the table below.

The "cleaned" alignment after all steps have been performed will be saved as `OUTFILE_STEM_cleaned.fasta`

remove_divergent, remove_insertions, crop_ends and crop divergent require three or more sequences in the alignment, remove_short and remove_gap_only require two or more sequences.

The **retain** functions allow the user to specify sequences to keep regardless of the CIAlign results.

Remove Divergent

Removes divergent sequences from the alignment - sequences with <= `remove_divergent_minperc` positions at which the most common residue in the alignment is present

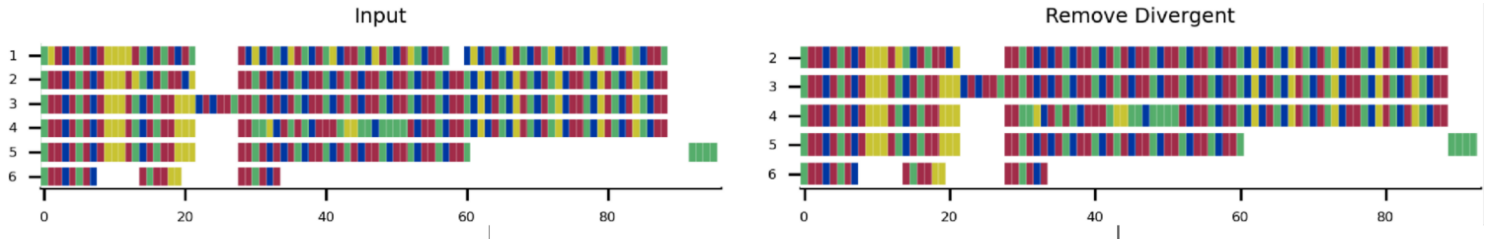


Figure 1: Remove Divergent

Parameter	Description	Default Value	Min	Max
`--remove_divergent`	Remove sequences with <= `remove_divergent_minperc` positions at which the most common base / amino acid in the alignment is present	False	NA	NA
`--remove_divergent_minperc`	Minimum proportion of positions which should be identical to the most common base / amino acid in order to be preserved	0.65	0	1
`--remove_divergent_retain`	Do not remove sequences with this name when running the remove divergent function	None	NA	NA
`--remove_divergent_retain_str`	Do not remove sequences with names containing this character string when running the remove divergent function	None	NA	NA
`--remove_divergent_retain_list`	Do not remove sequences with names listed in this file when running the remove divergent function	None	NA	NA

Remove Insertions

Removes insertions from the alignment which are found in <= `insertion_min_perc` of the sequences.

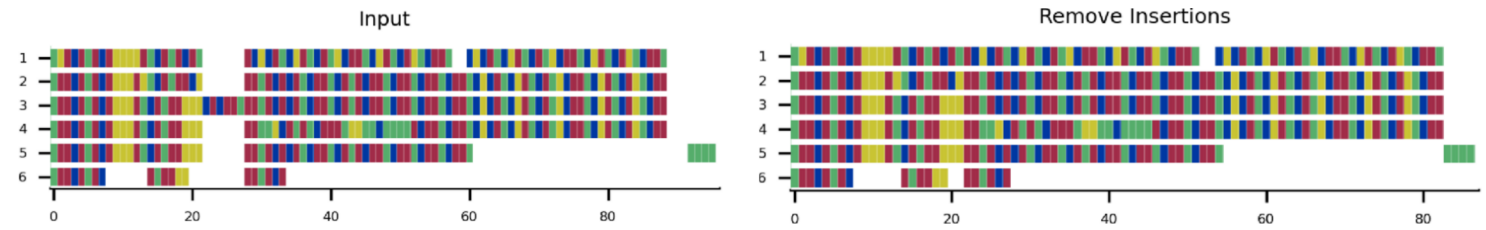


Figure 2: Remove Insertions

Parameter	Description	Default Value	Min	Max
`--remove_insertions`	Remove insertions found in <= `insertion_min_perc` of sequences from the alignment	False	NA	NA
`--insertion_min_size`	Only remove insertions >= this number of residues	3	1	n_col

Parameter	Description	Default Value	Min	Max
--insertion_max_size	Only remove insertions <= this number of residues	200	1	10000
--insertion_min_flank	Minimum number of bases on either side of an insertion to classify it as an insertion	5	0	n_col/2
--insertion_min_perc	Remove insertions which are present in less than this proportion of sequences	0.5	0	1

Crop Ends

Crops the ends of individual sequences if they contain a high proportion of gaps relative to the rest of the alignment.

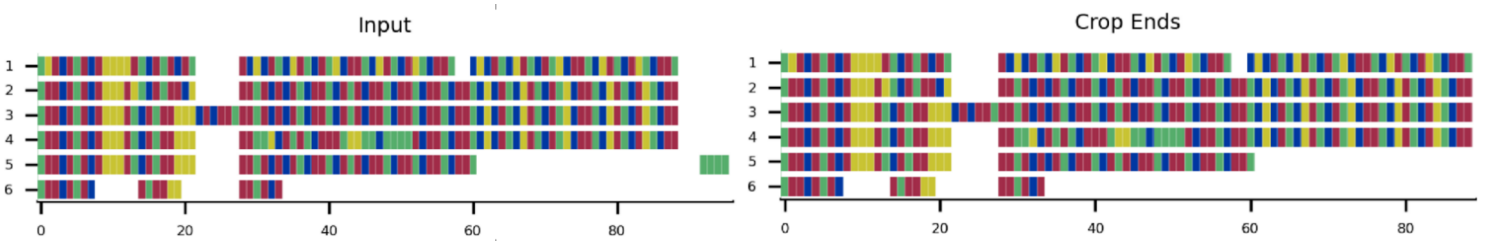


Figure 3: Crop Ends

Parameter	Description	Default Value	Min	Max
--crop_ends	Crop the ends of sequences if they are poorly aligned	False	NA	NA
--crop_ends_mingap_perc	Minimum proportion of the sequence length (excluding gaps) that is the threshold for change in gap numbers.	0.05	0	0.6
--crop_ends_redefine_perc	Proportion of the sequence length (excluding gaps) that is being checked for change in gap numbers to redefine start/end.	0.1	0	0.5
--crop_ends_retain	Do not crop sequences with this name when running the crop ends function	None	NA	NA
--crop_ends_retain_str	Do not crop sequences with names containing this character string when running the crop ends function	None	NA	NA
--crop_ends_retain_list	Do not crop sequences with names listed in this file when running the crop ends function	None	NA	NA

Note: if the sequences are short (e.g. < 100), a low crop_ends_mingap_perc (e.g. 0.01) will result in a change of gap numbers that is too low (e.g. 0). If this happens, the change in gap numbers will be set to 2 and a warning will be printed.

Remove Short

Removes sequences blow a threshold length.

Parameter	Description	Default Value	Min	Max
--remove_short	Remove sequences <= **remove_min_length** amino acids from the alignment	False	NA	NA
--remove_min_length	Sequences are removed if they are shorter than this minimum length, excluding gaps.	50	0	n_col
--remove_short_retain	Do not remove sequences with this name when running the remove short function	None	NA	NA
--remove_short_retain_str	Do not remove sequences with names containing this character string when running the remove short function	None	NA	NA
--remove_short_retain_list	Do not remove sequences with names listed in this file when running the remove short function	None	NA	NA

Keep Gap Only

Removes columns containing only gaps.

Parameter	Description	Default Value	Min	Max
`--keep_gaponly`	Keep gap only columns in the alignment	False	NA	NA

Crop Divergent

Crops columns from the sides of alignment to leave only a single conserved section, based on a threshold percentage of identical residues and percentage of gaps in each column.

Parameter	Description	Default Value	Min	Max
`--crop_divergent`	Crop either end of the alignment until > `crop_divergent_min_prop_ident` residues in a column are identical and > `crop_divergent_min_prop_nongap` residues are not gaps, over `buffer_size` consecutive columns	False	NA	NA
`--crop_divergent_min_prop_ident`	Minumum proportion of identical residues in a column to be retained by crop_divergent	0.5	0.01	1
`--crop_divergent_min_prop_nongap`	Minumum proportion of non gap residues in a column to be retained by crop_divergent	0.5	0.01	1
`--crop_divergent_buffer_size`	Minumum number of consecutive columns which must meet the criteria for crop_divergent to be retained	5	1	n_col

Retain

These parameters allow the user to specify sequences to not edit with any of the rowwise functions, regardless of the CIAlign results. The rowwise functions are currently remove_divergent, crop_ends and remove_short.

Parameter	Description	Default Value	Min	Max
`--retain`	Do not edit or remove sequences with this name when running any rowwise function (currently remove divergent, crop ends and remove short)	None	NA	NA
`--retain_str`	Do not edit or remove sequences with names containing this character string when running any rowwise function	None	NA	NA
`--retain_list`	Do not edit or remove sequences with names listed in this file when running any rowwise function	None	NA	NA

Generating a Consensus Sequence

This step generates a consensus sequence based on the cleaned alignment. If no cleaning functions are performed, the consensus will be based on the input alignment. For the "majority" based consensus sequences, where the two most frequent characters are equally common a random character is selected.

Output files:

- `OUTFILE_STEM_consensus.fasta` - the consensus sequence only
- `OUTFILE_STEM_with_consensus.fasta` - the cleaned alignment plus the consensus

Parameter	Description	Default
`--make_consensus`	Make a consensus sequence based on the cleaned alignment	False

Parameter	Description	Default
--consensus_type	Type of consensus sequence to make - can be majority, to use the most common character at each position in the consensus, even if this is a gap, or majority_nongap, to use the most common non-gap character at each position	majority
--consensus_keep_gaps	If there are gaps in the consensus (if majority_nongap is used as consensus_type), should these be included in the consensus (True) or should this position in the consensus be deleted (False)	False
--consensus_name	Name to use for the consensus sequence in the output fasta file	consensus

Visualising Alignments

Each of these functions produces some kind of visualisation of your alignment.

Mini Alignments

These functions produce "mini alignments" - images showing a small representation of your whole alignment, so that gaps and poorly aligned regions are clearly visible.

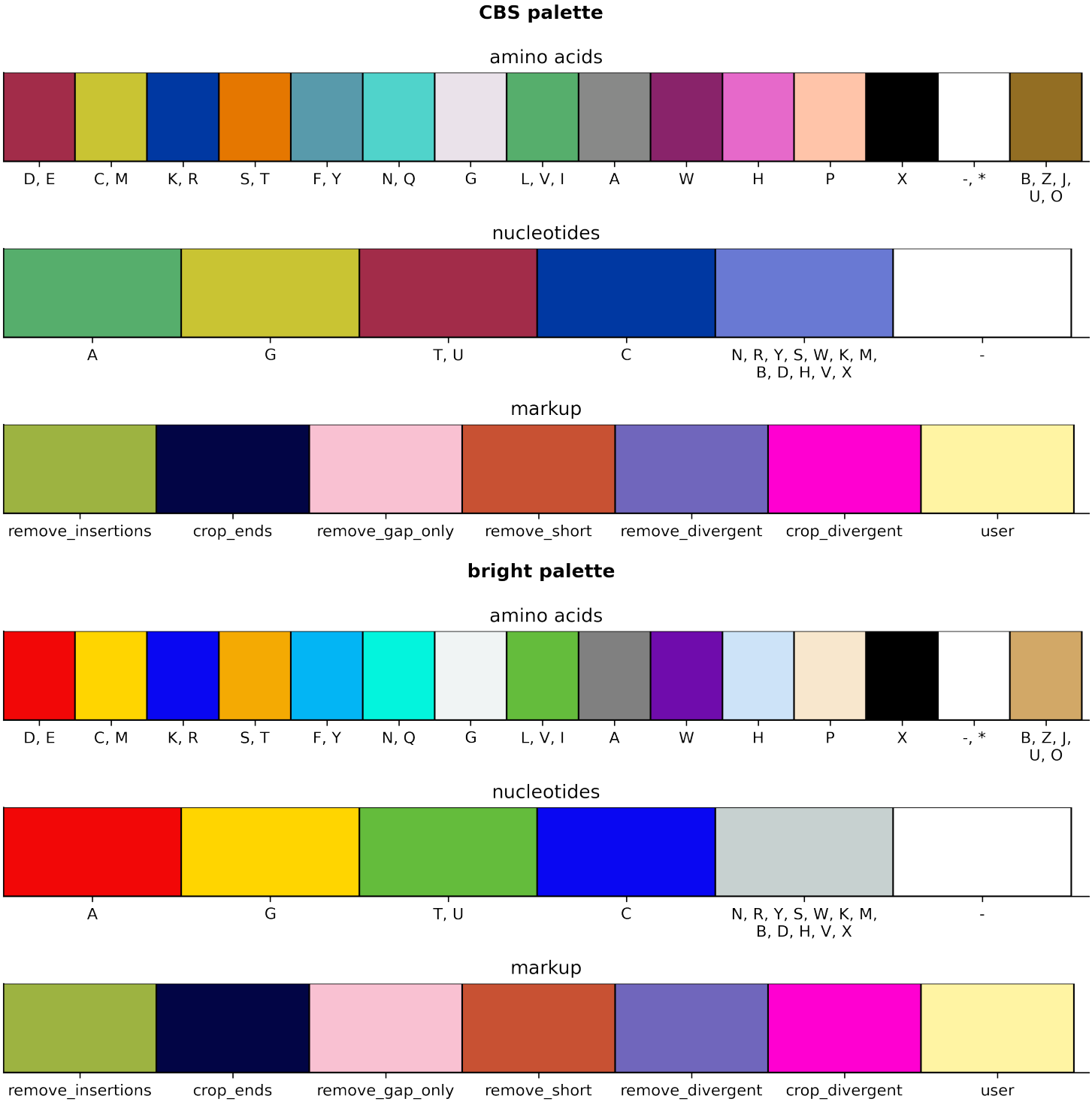
Output files:

- **`OUTFILE_STEM_input.png (or svg, tiff, jpg)`** - the input alignment
- **`OUTFILE_STEM_output.png (or svg, tiff, jpg)`** - the cleaned output alignment
- **`OUTFILE_STEM_markup.png (or svg, tiff, jpg)`** - the input alignment with deleted rows and columns marked

Parameter	Description	Default
`--plot_input`	Plot a mini alignment - an image representing the input alignment	False
`--plot_output`	Plot a mini alignment - an image representing the output alignment	False
`--plot_markup`	Draws the input alignment but with the columns and rows which have been removed by each function marked up in corresponding colours	False
--plot_dpi	DPI for mini alignments	300
--plot_format	Image format for mini alignments - can be png, svg, tiff or jpg	png
--plot_width	Mini alignment width in inches	5
--plot_height	Mini alignment height in inches	3
--plot_keep_numbers	Label rows in mini alignments based on input alignment, rather than renumbering	False
--plot_force_numbers	Force all rows in mini alignments to be numbered rather than labelling e.g. every 10th row for larger plots Will cause labels to overlap in larger plots	False

Palettes

This function sets the colour palette for the mini alignments. Currently available palettes are colour blind safe (CBS) and bright.



Parameter	Description	Default Value
`--palette`	Colour palette. Currently implemented CBS (colourblind safe) and bright	CBS

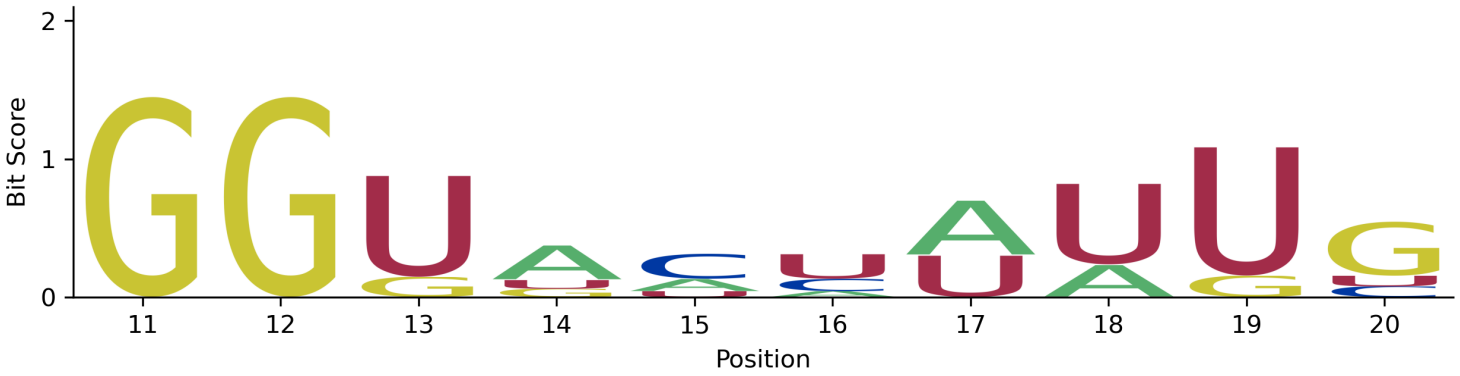
Sequence logos

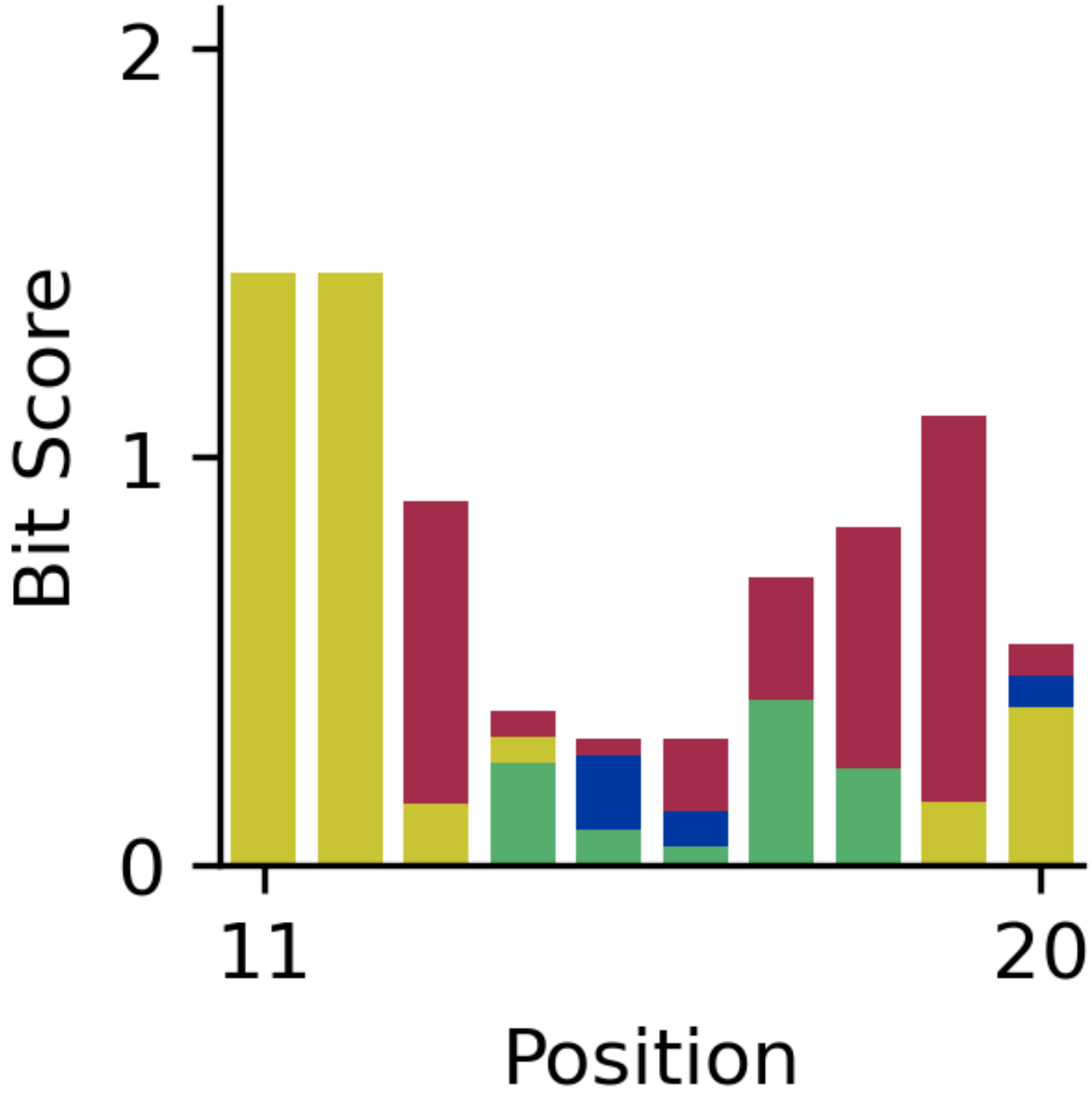
These functions draw sequence logos representing output (cleaned) alignment. You can also specify a subsection of the alignment using the `logo_start` and `logo_end` arguments, positions should be relative to the input alignment. If no cleaning functions are

specified, the logo will be based on your input alignment.

Output_files:

- `OUTFILE_STEM_logo_bar.png (or svg, tiff, jpg)` - the alignment represented as a bar chart
- `OUTFILE_STEM_logo_text.png (or svg, tiff, jpg)` - the alignment represented as a standard sequence logo using text





Parameter	Description	Default
--make_sequence_logo	Draw a sequence logo	False
--sequence_logo_type	Type of sequence logo - bar/text/both	bar
--sequence_logo_dpi	DPI for sequence logo	300
--sequence_logo_font	Font (see NB below) for bases / amino acids in a text based sequence logo	monospace
--sequence_logo_nt_per_row	Number of bases / amino acids to show per row in the sequence logo, where the logo is too large to show on a single line	50
--sequence_logo_filetype	Image file type to use for the sequence logo - can be png, svg, tiff or jpg	png
--logo_start	Start sequence logo	0

Parameter	Description	Default
--logo_end	End of sequence logo	MSA length

NB: to see available fonts on your system, run CIAlign –list_fonts_only and view CIAlign_fonts.png

Position Frequency, Probability and Weight Matrices

These functions are used to create a position weight matrix, position frequency matrix or position probability matrix for your input or output (cleaned) alignment. These are numerical representations of the alignment which can be used as input for various other software, for example to find regions of another sequence resembling part of your alignment. PFMs, PPMs and PWMs are described well in the Wikipedia article here.

You can also specify a subsection of the alignment using the `pwm_start` and `pwm_end` arguments, positions should be relative to the input alignment.

Parameter	Description	Default
`--pwm_input`	Generate a position frequency matrix, position probability matrix and position weight matrix based on the input alignment	False
`--pwm_output`	Generate a position frequency matrix, position probability matrix and position weight matrix based on the cleaned output alignment	False
--pwm_start	Start the PWM and other matrices from this column of the input alignment	None
--pwm_end	Start the PWM and other matrices from this column of the input alignment	None
--pwm_freqtype	Type of background frequency matrix to use when generating the PWM. Should be 'equal', 'calc', 'calc2' or 'user'. 'equal', assume all residues are equally common, 'calc', frequency is calculated using the PFM, 'calc2', frequency is calculated using the full alignment (same as calc if pwm_start and pwm_end are not specified).	equal
--pwm_alphatype	Alpha value to use as a pseudocount to avoid zero values in the PPM. Should be 'calc' or 'user'. If alphatype is 'calc', alpha is calculated as frequency(base) * (square root(n rows in alignment)), as described in Dave Tang's blog here, which recreates the method used in Wasserman & Sandelin 2004. If alpha type is 'user' the user provides the value of alpha as `pwm_alphatype`. To run without pseudocounts set `pwm_alphatype` as user and `pwm_alphaval` as 0	calc
--pwm_alphaval	User defined value of the alpha parameter to use as a pseudocount in the PPM.	1
--pwm_output_blamm	Output PPM formatted for BLAMM software	False
--pwm_output_meme	Output PPM formatted for MEME software	False

Analysing Alignment Statistics

These functions provide additional analyses you may wish to perform on your alignment.

Statistics Plots

For each position in the alignment, these functions plot: * Coverage (the number of non-gap residues) * Information content * Shannon entropy

Output files:

- `OUTFILE_STEM_input_coverage.png (or svg, tiff, jpg)` - image showing the input alignment coverage
- `OUTFILE_STEM_output_coverage.png (or svg, tiff, jpg)` - image showing the output alignment coverage
- `OUTFILE_STEM_input_information_content.png (or svg, tiff, jpg)` - image showing the input alignment information content
- `OUTFILE_STEM_output_information_content.png (or svg, tiff, jpg)` - image showing the output alignment information content

- **OUTFILE_STEM_input_shannon_entropy.png (or svg, tiff, jpg)** - image showing the input alignment Shannon entropy
- **OUTFILE_STEM_output_shannon_entropy.png (or svg, tiff, jpg)** - image showing the output alignment Shannon entropy

Parameter	Description	Default
--plot_stats_input	Plot the statistics for the input MSA	False
--plot_stats_output	Plot the statistics for the output MSA	False
--plot_stats_dpi	DPI for coverage plot	300
--plot_stats_height	Height for coverage plot (inches)	3
--plot_stats_width	Width for coverage plot (inches)	5
--plot_stats_colour	Colour for coverage plot (hex code or name)	#007bf5
--plot_stats_filetype	File type for coverage plot (png, svg, tiff, jpg)	png

Similarity Matrices

Generates a matrix showing the proportion of identical bases / amino acids between each pair of sequences in the MSA.

Output file:

- **OUTFILE_STEM_input_similarity.tsv** - similarity matrix for the input file
- **OUTFILE_STEM_output_similarity.tsv** - similarity matrix for the output file

Parameter	Description	Default
--make_similarity_matrix_input	Make a similarity matrix for the input alignment	False
--make_similarity_matrix_output	Make a similarity matrix for the output alignment	False
--make_simmatrix_keepgaps	0 - exclude positions which are gaps in either or both sequences from similarity calculations, 1 - exclude positions which are gaps in both sequences, 2 - include all positions	0
--make_simmatrix_dp	Number of decimal places to display in the similarity matrix output file	4
--make_simmatrix_minoverlap	Minimum overlap between two sequences to have non-zero similarity in the similarity matrix	1

Replacing U or T

This function replaces the U nucleotides with T nucleotides or vice versa without otherwise changing the alignment.

Output files:

- **OUTFILE_STEM_T_input.fasta** - input alignment with T's instead of U's
- **OUTFILE_STEM_T_output.fasta** - output alignment with T's instead of U's

or

- **OUTFILE_STEM_U_input.fasta** - input alignment with U's instead of T's
- **OUTFILE_STEM_U_output.fasta** - output alignment with U's instead of T's

Parameter	Description	Default
--replace_input_tu	Generates a copy of the input alignment with T's instead of U's	False
--replace_output_tu	Generates a copy of the output alignment with T's instead of U's	False
--replace_input_ut	Generates a copy of the input alignment with U's instead of T's	False
--replace_output_ut	Generates a copy of the output alignment with U's instead of T's	False

Unaligning the Alignment

This function simply removes the gaps from the input or output alignment and creates and unaligned file of the sequences.

Output files:

- `OUTFILE_STEM_unaligned_input.fasta` - unaligned sequences of input alignment
- `OUTFILE_STEM_unaligned_output.fasta` - unaligned sequences of output alignment

Parameter	Description	Default
`--unalign_input`	Generates a copy of the input alignment with no gaps	False
`--unalign_output`	Generates a copy of the output alignment with no gaps	False