
pydamage

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Homepage: github.com/maxibor/pydamage



PYDAMAGE

Pydamage, is a Python software to automate the process of contig damage identification and estimation. After modelling the ancient DNA damage using the C to T transitions, Pydamage uses a likelihood ratio test to discriminate between truly ancient, and modern contigs originating from sample contamination.

1.1 Installation

1.1.1 With conda (recommended)

```
conda install -c bioconda pydamage
```

1.1.2 With pip

```
pip install pydamage
```

1.1.3 Install from source to use the development version

Using pip

```
pip install git+ssh://git@github.com:maxibor/pydamage.git@dev
```

By cloning in a dedicated conda environment

```
git clone git@github.com:maxibor/pydamage.git
cd pydamage
git checkout dev
conda env create -f environment.yml
conda activate pydamage
pip install -e .
```

1.2 Quick start

```
pydamage --outdir result_directory analyze aligned.bam
```

Note that if you specify `--outdir`, it has to be before the PyDamage subcommand, example: `pydamage --outdir test filter pydamage_results.csv`

1.3 CLI help

Command line interface help message

```
pydamage --help
```

1.4 Documentation

pydamage.readthedocs.io

1.5 Cite

PyDamage has been published in PeerJ: [10.7717/peerj.11845](https://doi.org/10.7717/peerj.11845)

```
@article{borry_pydamage_2021,
  author = {Borry, Maxime and Hübner, Alexander and Rohrlach, Adam B. and Warinner,
↵Christina},
  doi = {10.7717/peerj.11845},
  issn = {2167-8359},
  journal = {PeerJ},
  language = {en},
  month = {July},
  note = {Publisher: PeerJ Inc.},
  pages = {e11845},
  shorttitle = {PyDamage},
  title = {PyDamage: automated ancient damage identification and estimation for
↵contigs in ancient DNA de novo assembly},
  url = {https://peerj.com/articles/11845},
  urldate = {2021-07-27},
  volume = {9},
  year = {2021}
}
```


`pydamage.main.pydamage_analyze(bam, wlen=30, minlen=0, show_al=False, process=1, outdir="", plot=False, verbose=False, force=False, group=False)`

Runs the pydamage analysis for each reference separately

Parameters

- **bam** (*str*) – Path to alignment (sam/bam/cram) file
- **minlen** (*int*) – minimum reference sequence length threshold
- **wlen** (*int*) – window length
- **show_al** (*bool*) – print alignments representations
- **process** (*int*) – Number of processes for parallel computing
- **outdir** (*str*) – Path to output directory
- **verbose** (*bool*) – verbose mode
- **force** (*bool*) – force overwriting of results directory

Returns

pandas DataFrame containing pydamage results

Return type

pd.DataFrame

To access the help menu:

```
$ pydamage --help
```

The list of arguments of options is detailed below

3.1 pydamage

PyDamage: Damage parameter estimation for ancient DNA

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Contact: <borry[at]shh.mpg.de>

Homepage & Documentation: github.com/maxibor/pydamage

```
pydamage [OPTIONS] COMMAND [ARGS] ...
```

Options

--version

Show the version and exit.

-o, --outdir <outdir>

Output directory

Default

pydamage_results

3.1.1 analyze

Run the PyDamage analysis

BAM: path to BAM/SAM/CRAM alignment file. MD tags need to be set.

```
pydamage analyze [OPTIONS] BAM
```

Options

- w, --wlen <wlen>**
Window length (in bp) for damage modeling
Default
20
- m, --minlen <minlen>**
Minimum of length of reference sequence to consider
Default
0
- p, --process <process>**
Number of processes for parallel computing
Default
2
- s, --show_al**
Display alignments representations
- pl, --plot**
Write damage fitting plots to disk
- vv, --verbose**
Verbose mode
- f, --force**
Force overwriting of results directory
- g, --group**
Use entire BAM file as single reference for analysis (ignore reference headers)

Arguments

BAM

Required argument

3.1.2 cite

Get pydamage citation in bibtex format

pydamage cite [OPTIONS]

3.1.3 filter

Filter PyDamage results on predicted accuracy and qvalue thresholds.

CSV: path to PyDamage result file

`pydamage filter [OPTIONS] CSV`

Options

-t, --threshold <threshold>

Predicted accuracy filtering threshold. Set to 0 for finding threshold with kneed method

Default

0.5

Arguments

CSV

Required argument

OUTPUT

Pydamage generates both a tabular and a visual output.

The tabular outputs are comma-separated file (.csv) with the following columns, for each analysed reference:

4.1 pydamage_results.csv

- **reference**: name of the reference genome/contig
- **predicted_accuracy**: Predicted accuracy of Pydamage prediction, from the GLM modelling
- **null_model_p0**: parameter p_0 of the null model
- **null_model_p0_stdev**: standard error of the null model parameter p_0
- **damage_model_p**: parameter p of the damage model
- **damage_model_p_stdev**: standard error of the parameter p of the damage model
- **damage_model_pmin**: parameter p_{\min} of the damage model. *This is the modelled damage baseline*
- **damage_model_pmin_stdev**: standard error of the parameter p_{\min} of the damage model
- **damage_model_pmax**: parameter p_{\max} of the damage model. *This is the modelled amount of damage on the 5' end.*
- **damage_model_pmax_stdev**: standard error of the parameter p_{\max} of the damage model
- **pvalue**: p-value calculated from the likelihood-ratio test-statistic using a chi-squared distribution
- **qvalue**: p-value corrected for multiple testing using Benjamini-Hochberg procedure. *Only computed when multiple references are used*
- **RMSE**: residual mean standard error of the model fit of the damage model
- **nb_reads_aligned**: number of aligned reads
- **coverage**: average coverage along the reference genome
- **CtoT-N**: Proportion of CtoT substitutions observed at position N from 5' end
- **GtoA-N**: Proportion of GtoA substitutions observed at position N from 5'

4.2 pydamage_filtered_results.csv

Same file as above, but with contigs filtered with `qvalue <= 0.05` and `predicted_accuracy >= threshold` with a user defined filtering threshold (default = 0.5), or determined with the [kneedle](#) method.

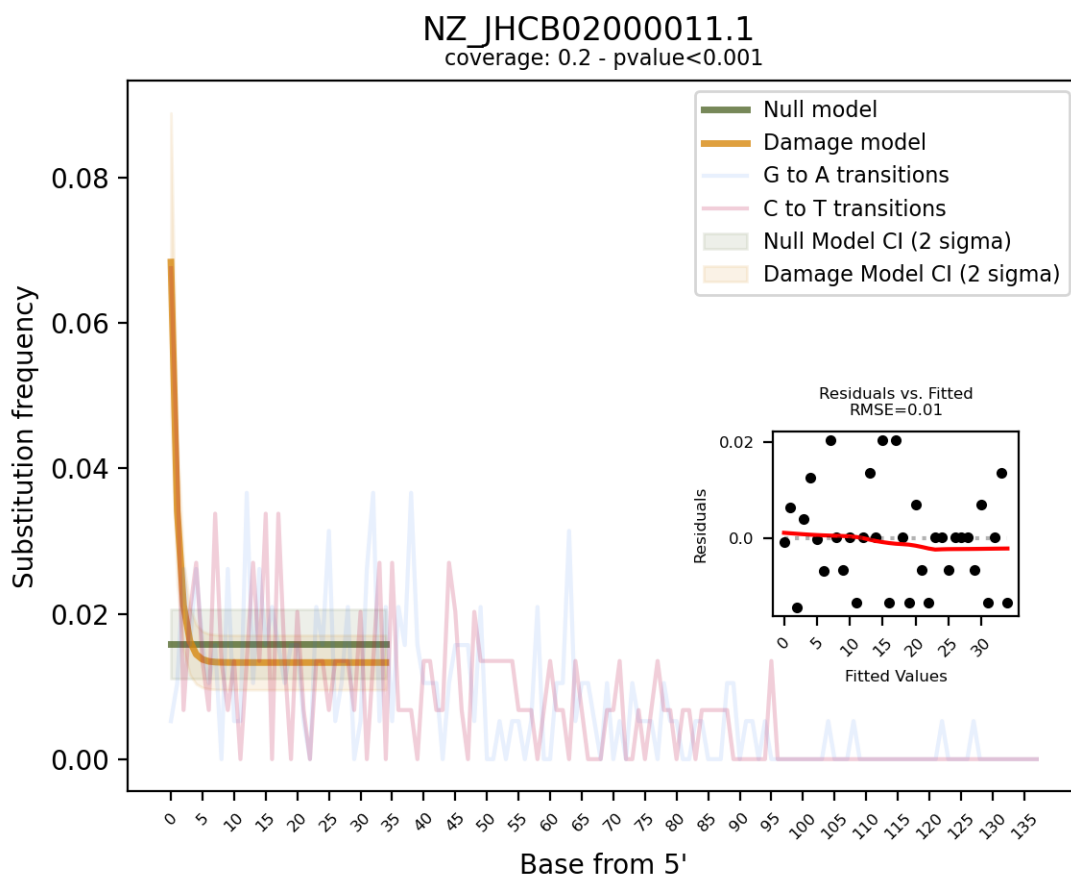
4.3 Plots

The visual output are PNG files, one per reference contig. They show the frequency of observed C to T, and G to A transition at the 5' end of the sequencing data and overlay it with the fitted models for both the null and the damage model, including 95% confidence intervals. Furthermore, it provides a “residuals versus fitted” plot to help evaluate the fit of the pydamage damage model. Finally, the plot contains information on the average coverage along the reference and the p-value calculated from the likelihood-ratio test-statistic using a chi-squared distribution.

The visual output is only produced when using the `--plot` flag

4.4 Example

- **Tabular output**
 - `pydamage_results.csv`
 - `pydamage_filtered_results.csv`
- **Visual output**



TROUBLESHOOTING

5.1 My alignment files don't have a MD tag

You can use `samtools calmd` to set the MD tag

Example:

```
samtools calmd -b alignment.bam reference.fasta > aln.bam
```


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