
pydamage

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Homepage: github.com/maxibor/pydamage



PyDamage

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ONE

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 conda-forge -c bioconda -c maxibor 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

```
@article{Borry2021_pydamage,
    author = {Borry, Maxime and Huebner, Alexander and Rohrlach, Adam B and Warinner,,
→ Christina G},
    doi = {10.1101/2021.03.24.436838},
    elocation-id = {2021.03.24.436838},
    eprint = {https://www.biorxiv.org/content/early/2021/03/24/2021.03.24.436838.full.
→pdf},
    journal = {bioRxiv},
    publisher = {Cold Spring Harbor Laboratory},
    title = {PyDamage: automated ancient damage identification and estimation for
→contigs in ancient DNA de novo assembly},
    url = {https://www.biorxiv.org/content/early/2021/03/24/2021.03.24.436838},
    year = {2021}
}
```

CHAPTER
TWO

API

```
pydamage.main.pydamage_analyze(bam, wlen=30, 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
- **wlen** (*int*) – window length
- **show_al** (*bool*) – print alignments representations
- **process** (*int*) – Number of processes for parellel computing
- **outdir** (*str*) – Path to output directory
- **verbose** (*bool*) – verbose mode
- **force** (*bool*) – force overwriting of results directory

Returns pandas DataFrame containg pydamage results

Return type pd.DataFrame

CLI

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

Author: Maxime Borry

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

-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

CHAPTER
FOUR

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 p₀ of the null model
- **null_model_p0_stdev**: standard error of the null model paramater p₀
- **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**: paramater p_min of the damage model. *This is the modelled damage baseline*
- **damage_model_pmin_stdev**: standard error of the paramater p_min of the damage model
- **damage_model_pmax**: paramater 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 paramater 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.

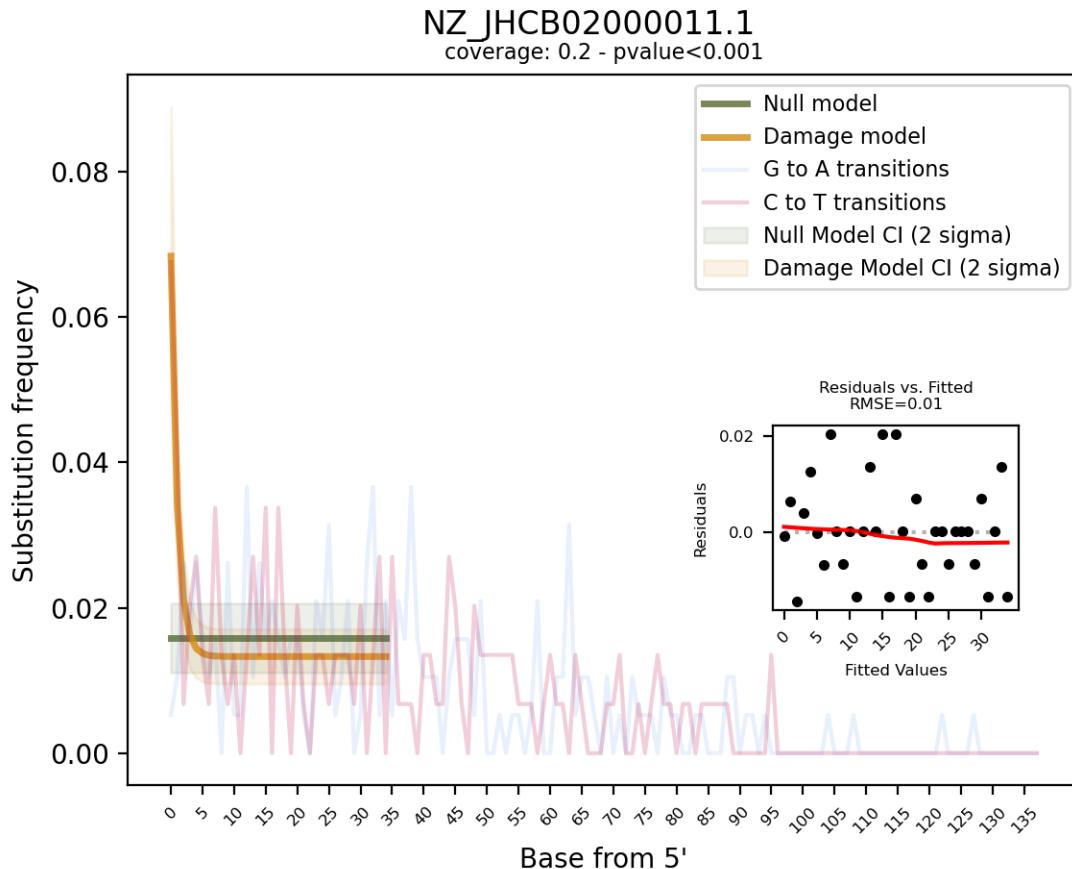
4.3 Plots

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**
- **Visual output**



CHAPTER
FIVE

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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