rna_cd Documentation

Release 0.3.0.dev0

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Contents:

Human DNA-seq experiments may be contaminated with RNA. This usually negatively influences downstream analysis. For example, it can introduce false positive variants around splice sites, suppress alternative alleles due to allele-specific expression, and alter coverage patterns which may then influence CNV calling. Moreover, it is typically hard to detect.

Modern Illumina sequencers unfortunately are more prone to such contamination, as the very large capacity of novaseq sequencers typically means multiple projects are sequenced on the same flowcells. Combined with the increased risk of index hopping in novaseq sequencers, this alltogether means that cross-contamination of samples is more likely - including contamination of RNA into DNA-seq experiments.

rna_cd is a python package and command line tool designed to detect such RNA contamination of DNA-seq experiments. It uses the altered coverage and softclip patterns in contaminated samples to train a Support Vector Machine that can classify BAM files into contaminated ("positive") and uncontaminated ("negative") groups.

rna_cd stands for *RNA contamination detector*.

CHAPTER 1

User documentation

1.1 Installation

`rna_cd` is now available on PyPI! Simply install the package in your favorite python environment with:

```
$ pip install rna-cd
```

This will install both the `rna_cd` python package, and install two command line tools:

1. `rna_cd-train`: For training a model using BAM files.
2. `rna_cd-classify`: For classifying new BAM files.

1.1.1 Supported python versions

We only support the following python versions:

- python 3.5
- python 3.6
- python 3.7

> **Note:** Python 2 is **not** supported in any way. Even attempting to install the package on python 2 will result in failures.

1.2 Training

To train the support vector machine, you need a set of contaminated ("positive") and a set of uncontaminated ("negative") BAM files. For each category, you can organize your BAM files in two distinct ways:

1. Place all BAM files of the category in the same directory.
2. Make a flat text file, where each line points to a path of a BAM file.

Note: Your BAM files must be indexed.

Once you have this in place, you need to choose the contig in your BAM file that you want to collect metrics for, and the chunksize. rna_cd will split your contig of interest in chunks with a maximum size `chunksize`, and collect a number of metrics for each chunk. When you later use the model for classifications you **must** use the same contig and chunksize as you used during the training step.

In our hands, the mitochondrial contig, at a chunksize of 100 bases, gives enough information to be trainable. When choosing the mitochondrial contig, one also benefits from its small size, which makes the training step fast.

Training can work in multicore mode. When using multiple cores, you will process multiple BAM files simultaneously. This can drastically speed up the metric collection for large numbers of BAM files.

Lastly, you have to set the amount of fold cross validations. By default this is 3, but you may set it to any positive integer.

The created model will saved to disk as a JSON file. The JSON file contains the pickled model.

Optionally, you can save a plot of the top two principal components of the training samples to disk.

1.2.1 Examples

Directory method, chrM, chunksize = 100, cores = 3

```
rna_cd-train -c chrM -pd positives_dir -nd negatives_dir -j 3 \
--chunksize 100 -o model.json
```

List method, chrM, chunksize = 100, cores = 3

```
rna_cd-train -c chrM -pl positives.list -nl negatives.list -j 3 \
--chunksize 100 -o model.json
```

List method, chrM, chunksize = 100, cores = 3, with plot

```
rna_cd-train -c chrM -pl positives.list -nl negatives.list -j 3 \
--chunksize 100 -o model.json --plot-out pca.png
```

1.2.2 Usage

rna_cd-train

```
rna_cd-train [OPTIONS]
```

Options

--chunksize <chunksize>
Chunksize in bases. Default = 100

-c, --contig <contig>
Name of mitochrondrial contig in your BAM files. Default = chrM

-pd, --positives-dir <positives_dir>
Path to directory containing positive BAM files. Mutually exclusive with –positives-list

-nd, --negatives-dir <negatives_dir>
Path to directory containing negative BAM files. Mutually exlusive with –negatives-list

-pl, --positives-list <positives_list>
Path to file containing a list of paths to positive BAM files. Mutually exclusive with –positives-dir

-nl, --negatives-list <negatives_list>
Path to file containing a list of paths to negative BAM files. Mutuallly exclusive with –negatives-dir

--cross-validations <cross_validations>
Number of folds for cross validation run. Default = 3

--verbosity <verbosity>
Verbosity value for cross validation step. Default = 1

-j, --cores <cores>
Number of cores to use for processing of BAM files and cross validations. Default = 1

--plot-out <plot_out>
Optional path to PCA plot.

-o, --model-out <model_out>
Path where model will be stored. [required]

1.3 Classification

As with the training step, you can organize your BAM files in two distinct ways:

1. Place all BAM files of the category in the same directory.
2. Make a flat text file, where each line points to a path of a BAM file.

This time, there are no separate categories, as all BAM files are a-priori unknown.

Note: Your BAM files must be indexed.

Warning: As mentioned before, you **must** use the **exact** same contig and chunksize settings in this step as were used during the training step.

As with the training step, metric collection can run in multicore mode during classifcation as well.

Once you have prepared your BAM files, and chosen your parameters, you will use the model you generated during the training step to classify your BAM files into contaminated (“positive”) and uncontamined (“negative”) groups.

Additionally, there is an optional third category with label “unknown”. This represents samples for which we are unsure to which category they belong to. By default, samples are categorized as “unknown” when the class probability

of the most likely category is below 0.75. You can override this parameter, but it must be higher than 0.5 and lower than 1.0.

The classifications will be stored to disk in a three-column tab-delimited text file, with the following columns:

1. Name of the BAM file that was classified.
2. Assigned category ("pos" or "neg" for positive and negative classifications, respectively, and "unknown" for the unknown category).
3. The probability of the assigned category.

E.g. an example output file could look like

```
filename    predicted_class class_probability
a.bam   neg 0.95
b.bam   neg 0.88
c.bam   pos 0.75
d.bam   unknown 0.55
```

1.3.1 Examples

Directory method, chrM, chunksize = 100, cores = 3

```
rna_cd-classify -m model.json -d bams_dir -j 3 -c chrM \
--chunksize 100 -o classifications.out
```

List method, chrM, chunksize = 100, cores = 3

```
rna_cd-classify -m model.json -l bams.list -j 3 -c chrM \
--chunksize 100 -o classifications.out
```

1.3.2 Usage

rna_cd-classify

```
rna_cd-classify [OPTIONS]
```

Options

--chunksize <chunksize>
Chunksize in bases. Default = 100

-c, --contig <contig>
Name of mitochrondrial contig in your BAM files. Default = chrM

-j, --cores <cores>
Number of cores to use for processing of BAM files. Default = 1

-d, --directory <directory>
Path to directory with BAM files to be tested. Mutually exclusive with –list-items

-l, --list-items <list_items>
Path to file containing list of paths to BAM files to be tested. Mutually exclusive with –directory

-m, --model <model>
Path to model. [required]

-o, --output <output>
Path to output file containing classifications. [required]

-t, --unknown-threshold <unknown_threshold>
Threshold of most likely probability below which sampleswll be assinged as 'unknown'. Default = 0.75

1.4 Changelog

1.4.1 0.2.0-dev

- Add some more columns to the classification output

1.4.2 0.1.0

- Make tool
- Add tests
- Add sphinx documentation
- Add optional 'unknown' category.

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get its source.  For example, if your program is a web application, its
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of the code.  There are many ways you could offer source, and different
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CHAPTER 2

API documentation

2.1 API documentation

2.1.1 bam_process

rna_cd.bam_process.**chop_contig**(*size: int*, *chunksize: int*) → Iterator[Tuple[int, int]]
For a contig of given size, generate regions maximally chunksize long. We use _0_ based indexing

rna_cd.bam_process.**softclip_bases**(*reader: pysam.libcalignmentfile.AlignmentFile, contig: str, region: Tuple[int, int]*) → int
Calculate amount of softclip bases for a region

rna_cd.bam_process.**coverage**(*reader: pysam.libcalignmentfile.AlignmentFile, contig: str, region: Tuple[int, int], method: Callable = <function mean>*) → float
Calculate average/median/etc coverage for a region

rna_cd.bam_process.**process_bam**(*path: pathlib.Path*, *chunksize: int = 100*, *contig: str = 'chrM'*) → numpy.ndarray
Process bam file to an ndarray

Returns numpy ndarray of shape (n_features,)

rna_cd.bam_process.**make_array_set**(*bam_files: List[pathlib.Path], labels: List[Any], chunksize: int = 100, contig: str = 'chrM', cores: int = 1*) → Tuple[numpy.ndarray, numpy.ndarray]
Make set of numpy arrays corresponding to data and labels. I.e. train/testX and train/testY in scikit-learn parlance.

Parameters

- **bam_files** – List of paths to bam files
- **labels** – list of labels.
- **cores** – number of cores to use for processing

Returns tuple of X and Y numpy arrays. X has shape (n_files, n_features). Y has shape (n_files,).

2.1.2 cli

rna_cd.cli.**directory_callback**(*ctx*, *param*, *value*)
Click callback function for getting bam/cram files from a directory.

rna_cd.cli.**list_callback**(*ctx*, *param*, *value*)
Click callback function for getting bam/cram files from a list file.

rna_cd.cli.**path_callback**(*ctx*, *param*, *value*)
Generic str to path callback. To be used for click.Path types that ought to return pathlib.Path

rna_cd.cli.**unknown_threshold_callback**(*ctx*, *param*, *value*)
Click callback function for threshold that has to be between 0.5 and 1.0

2.1.3 models

class rna_cd.models.**PredClass**
An enumeration.

rna_cd.models.**plot_pca**(*searcher: sklearn.model_selection._search.GridSearchCV*, *arr_X: numpy.ndarray*, *arr_Y: numpy.ndarray*, *img_out: pathlib.Path*) → None
Plot PCA with training samples of pipeline.

rna_cd.models.**predict_labels_and_prob**(*model*, *bam_files: List[pathlib.Path]*, *chunksize: int = 100*, *contig: str = 'chrM'*, *cores: int = 1*, *unknown_threshold: float = 0.75*) → List[rna_cd.models.Prediction]
Predict labels and probabilities for a list of bam files.

Parameters **unknown_threshold** – The probability threshold below which samples are considered to be 'unknown'. Must be between 0.5 and 1.0

Returns list of Prediction classes

rna_cd.models.**train_svm_model**(*positive_bams: List[pathlib.Path]*, *negative_bams: List[pathlib.Path]*, *chunksize: int = 100*, *contig: str = 'chrM'*, *cross_validations: int = 3*, *verbosity: int = 1*, *cores: int = 1*, *plot_out: Optional[pathlib.Path] = None*) → sklearn.model_selection._search.GridSearchCV
Run SVM training on a list of positive BAM files (i.e. _with_ contamination) and a list of negative BAM files (i.e. _without_ contamination).

For all bam files features are collected over one contig. This contig is binned, and for each bin two different metrics of coverage are collected, in addition to the softclip rate.

These features are then fed to a sklearn pipeline with three steps:

1. A scaling step using StandardScaler
2. A dimensional reduction step using PCA.
3. A classification step using an SVM.

Hyperparameters are tuned using a grid search with cross validations.

Optionally saves a plot of the top two PCA components with the training samples.

Parameters

- **positive_bams** – List of BAM files with contaminations
- **negative_bams** – List of BAM files without contaminations.

- **chunksize** – The size in bases for each chunk (bin)
- **contig** – The name of the contig.
- **cross_validations** – The amount of cross validations
- **verbosity** – Verbosity parameter of sklearn. Increase to see more messages.
- **cores** – Amount of cores to use for both metric collection and training.
- **plot_out** – Optional path for PCA plot.

Returns GridSearchCV object containing tuned pipeline.

2.1.4 utils

rna_cd.utils.**dir_to_bam_list**(*path: pathlib.Path*) → List[pathlib.Path]
Load a directory containing bam or cram files

rna_cd.utils.**echo**(*msg: str*)
Wrapper around click.secho to include datetime

rna_cd.utils.**load_list_file**(*path: pathlib.Path*) → List[pathlib.Path]
Load a file containing containing a list of files

rna_cd.utils.**load_sklearn_object_from_disk**(*path: pathlib.Path*) → Any
Load a JSON-serialized object from disk

rna_cd.utils.**save_sklearn_object_to_disk**(*obj: Any*, *path: pathlib.Path*)
Save an object with some metadata to disk as serialized JSON

CHAPTER 3

Indices and tables

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- modindex
- search

Python Module Index

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