

# The PARA-suite – useful tools for NGS data analysis

## Overview

The PARA-suite provides tools for the analysis of NGS data, especially for (PAR-)CLIP sequencing reads. The most important tool is the mapping tool which embeds a modified version of BWA called the PARA-suite algorithm for read alignment and applies a best practice pipeline for PAR-CLIP read mapping. The following tools are available in the PARMA toolkit Version 0.5 alpha:

Table 1: Overview of tools accessible through the PARMA toolkit.

Tool	Description
map	Utilizes the PARA-suite algorithm to map a given sequencing read dataset against a reference sequence; optionally combines mapping against genomic and transcriptomic sequences
comb	Combines the results of genomic and transcriptomic read alignments; recalculates genomic mapping positions for transcriptomic hits
error	Calculates the error profile (mismatches and indels) for an aligned read dataset compared to the reference sequence
clust	Clusters an aligned PAR-CLIP read dataset to obtain RBP-bound genomic regions. Is able to filter T-C conversion sites that are annotated as SNPs in an appropriate database
simulate	Creates a simulated PAR-CLIP read dataset based on observations made for PAR-CLIP sequencing reads
benchmark	Calculates accuracy of an aligned simulated PAR-CLIP dataset
setup	Setup options for the PARA-suite, e.g. setting the path to the PARA-suite algorithm

## Getting started

The PARA-suite can be downloaded as a pre-compiled jar (java executable) including all dependent libraries (except CPAN Math::Random and samtools, see below).

```
git clone https://github.com/akloetgen/PARA-suite.git  
  
cd PARA-suite/bin/  
  
java -jar parasuite.jar
```

For optimal use of the PARA-suite, the following tools and libraries are required:

- Java (7 or 8 should work)
- Perl 5
- the PARA-suite algorithm ([https://github.com/akloetgen/PARA-suite\\_algorithm](https://github.com/akloetgen/PARA-suite_algorithm))
- samtools (Version 1.0 or newer, <https://github.com/samtools/samtools>)
- Perl CPAN Math::Random package (Version 0.71, <http://search.cpan.org/~grommel/Math-Random-0.71/>; you can use CPAN for easy installation of Perl modules)

The PARA-suite algorithm ([https://github.com/akloetgen/PARA-suite\\_algorithm](https://github.com/akloetgen/PARA-suite_algorithm)) should be included in the PATH environment, otherwise the PARA-suite is not able to access the algorithm. Alternatively, you can set the path to the PARA-suite algorithm installation using the following command:

```
java -jar parma.jar setup --parasuite myPATH_TO_THE_PARA-SUITE_ALIGNER
```

Alternatively, the source code of the PARA-suite can be downloaded and compiled, but additional libraries are required:

- HTSjdk-1.128.jar (<http://samtools.github.io/htsjdk/>)
- bzip2.jar (<http://www.kohsuke.org/bzip2/>)
- log4j-1.2.17.jar (<https://logging.apache.org/log4j/1.2/download.html>) (a newer version 2.1 is available but not yet supported)
- jmathplot.jar (<http://code.google.com/p/jmathplot/>)

## The PARA-suite

The basic command for executing the PARA-suite is as follows:

```
java -jar parasuite.jar MODE [options]
```

where *MODE* is one of the tools from Table 1. To print an overview of the available tools, just execute the jar-file without any further options. A more detailed description of every tool can be printed by executing the tool without further options, e.g. as follows for the mapping tool:

```
java -jar parasuite.jar map
```

which will print the instructions for the mapping tool.

We also provide example files in the subfolder “examples” for tools of the PARA-suite and an execution script (bin/examples.sh and bin/examples\_remove\_temp.sh) which applies every tool to those example files. This will also help to understand the file formats necessary for the individual tools. Please note, that the example files are too small to represent real results, but it gives a rough overview about the tools and their usage. You may have to make both scripts executable and run the following commands:

```
./examples.sh  
./examples_remove_temp.sh
```

The examples.sh script simulates a PAR-CLIP dataset, creates indices for genomic and transcriptomic reference sequences, executes the mapping pipeline including error profile estimation and mapping against multiple databases using the PARA-suite algorithm, calculates alignment accuracy, performs error profile estimation and combination of multiple database mappings as single executions, and in the

end piles up the aligned reads to clusters. The second script will delete all the temporary files created during the first script.

You may also have to set the path to the Math::Random library in the examples.sh script if you get an error during the PAR-CLIP read simulation (see “PAR-CLIP read simulation” paragraph for more information).

## Workflow for mapping

First, a fasta-index for the reference genome sequence has to be created using the index function of samtools as follows (note, that you can also create BWT-indices but in case none exist, the PARMA toolkit will recognize this and create the respective indices on its own):

```
samtools faidx REFERENCE
```

Afterwards, the PARMA mapping tool can be executed as follows:

```
java -jar parasuite.jar map -q INPUT -r REFERENCE -p THREADS -o OUTPUT --refine
```

To allow mapping against multiple databases, the command just needs the indexed transcript reference filename as additional input:

```
java -jar parasuite.jar map -q INPUT -r REFERENCE -p THREADS -o OUTPUT -t  
TRANSCRIPT_REFERENCE --refine
```

where *TRANSCRIPT\_REFERENCE* is a multiple fasta file containing sequences of known transcripts for a given organism. It is important that the fasta header of the *TRANSCRIPT\_REFERENCE* looks as follows (which could be downloaded e.g. from Ensembl BioMart):

```
>Gene_ID|Transcript_ID|Chr|Exon_start_site1;Exon_start_site2;...|Exon_end_site1;Exon_end_site  
2;...|Strand
```

## Combine tool

The combination of results of a genomic reference mapping and the results of a transcriptomic reference mapping is possible using the combine tool. Therefore, the two alignment files must be stored in a BAM-format and are used as input for the tool, as follows:

```
java -jar parasuite.jar comb GENOMIC_MAPPING TRANSCRIPT_MAPPING OUTPUT
```

The result is saved in the *OUTPUT* file in a BAM-format. Note, that the *TRANSCRIPT\_MAPPING* needs a specific format for the fasta-header for each transcript sequence as described above.

### Error profile tool

The calculation of the error profile for a given sequence read dataset is possible using the error profile tool of the PARMA toolkit. Therefore, a reference-based read alignment has to be calculated (and stored in a BAM-file) and can be used as input for the error profile tool:

```
java -jar parasuite.jar error MAPPING REFERENCE MAX_READ_LENGTH
```

### Clustering tool

A first postprocessing analysis for (PAR-)CLIP data is the pile-up of aligned reads into clusters representing the RBP-bound regions in the genome. This can be done using the clustering tool which also excludes T-C conversion sites that are annotated as SNP loci in an appropriate SNP database (SNP database must be in a VCF-format and indexed e.g. using <http://samtools.sourceforge.net/tabix.shtml>). The additional parameter *MIN\_COVERAGE* is necessary to already pre-filter the list of clusters for those that contain at least *MIN\_COVERAGE* sequencing reads.

```
java -jar parasuite.jar clust MAPPING REFERENCE OUTPUT SNP_DB MIN_COVERAGE
```

### PAR-CLIP read simulation

As common sequencing read simulators are not applicable to simulate realistic PAR-CLIP reads, we provide a PAR-CLIP read simulator based on PAR-CLIP read specific properties. The results are saved to *OUTPUT\_PREFIX.fastq* in the common fastq-format. To achieve sequencing reads for which the genomic positions can be tracked, the header line of the transcript-fasta file should have the following format:  
>*TRANSCRIPT\_ID|CHR|TRANSCRIPT\_START|TRANSCRIPT\_END*

The following command executes the PAR-CLIP read simulator:

```
java -jar parasuite.jar simulate TRANSCRIPTS OUTPUT_PREFIX ERROR_PROFILE T2C_PROFILE  
T2C_POSITION_PROFILE QUALITIES INDEL_PROFILE BOUND_PROB
```

If you get an error such as the following:

```
java -jar parasuite.jar simulate TRANSCRIPTS OUTPUT_PREFIX ERROR_PROFILE T2C_PROFILE  
T2C_POSITION_PROFILE QUALITIES INDEL_PROFILE BOUND_PROB -I  
PATH_TO_MATH_RANDOM
```

Note, that the header of the transcriptome fasta is slightly different to the one used for the transcript mapping (will be updated soon, so that 1 file is enough):

```
> TRANSCRIPT_ID|CHR|TRANSCRIPT_START|TRANSCRIPT_END
```

## Benchmarking read alignments of a simulated PAR-CLIP dataset

After a simulated PAR-CLIP read set was aligned against a reference sequence, this tool can assess the alignment accuracy of the respective aligner on PAR-CLIP reads:

```
java -jar parasuite.jar benchmark MAPPING OUTPUT SIM_READS_FILE
```

## Setup

To set up some properties, the setup mode can be executed. So far, the paths to different aligners can be set unless they are already in the environment path:

```
java -jar parasuite.jar setup --parma PATH_TO PARA-SUITE_ALGORITHM
```

If any further questions arise or if you note a bug, please contact us: [andreas.kloetgen@hhu.de](mailto:andreas.kloetgen@hhu.de)