

# Pamir Manual

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# 1 Getting Started

## 1.1 Installation

Pamir can be obtained from <https://bitbucket.org/compbio/pamir>

### 1.1.1 Prerequisite

Pamir relies on specific version of the following tools:

- g++ 4.9.0 or higher  
(<https://gcc.gnu.org/releases.html>)
- Python 2.7 or higher (needed for the package `argparse` )
- boost library 1.57 or higher  
(<https://sourceforge.net/projects/boost/?source=directory>)
- You also need to define the boost library by typing on your shell  
`export BOOST_INCLUDE= the/BOOST/version/include/` (directory of BOOST in your machine).
- velvet 1.2.10 or higher
- BLAST 2.3.0+ or higher
- Latest BLAST nt database is also needed to be downloaded in `dir/to/blast/db` (needed for contamination detection).
  - `mkdir dir/to/blast/db`
  - `cd dir/to/blast/db`
  - `../bin/update_blastdb.pl nt`

Then

- `ln -s /dir/to/velvet/velveth /dir/to/pamir/velveth`
- `ln -s /dir/to/velvet/velvetg /dir/to/pamir/velvetg`
- `ln -s /dir/to/blast_folder /dir/to/pamir/blast`

### 1.1.2 Details and Troubleshooting

```
git clone --recursive git@bitbucket.org:compbio/pamir.git
cd pamir && make
```

## 1.2 Running Pamir

Pamir (Insertion Discovery tool for Whole Genome Sequencing Data) detects novel sequence insertions based on one-end anchors (OEA) and orphans from paired-end Whole Genome Sequencing (WGS) reads.

Note that reference genome is required for running Pamir in addition to sequencing or mapping data.

### 1.2.1 Project Name

To run Pamir you have to specify a project name such that Pamir will create a folder to store the results and intermediate files. You need to specify project name by `-p`.

### 1.2.2 Data Preparation

**Required Information.** Two information are required for running Pamir :

1. Reference Genome: You need to provide the reference genome in single fasta file by specifying the parameters `-r` or `--reference`.
2. Masking File: You can provide a file for masking reference genome. For example, you can ask Pamir to ignore events in repeat regions by giving `-m repeat.mask` . When you only want to consider events in genic regions, use `-m genic.region --invert-masker` and Pamir will mask those regions not in the given file.

**Read Length.** Now Pamir only accepts WGS datasets in which two mates of all reads are of equal length.

### 1.2.3 Sequencing Data

Pamir can take either FASTQ and SAM files as its input. It has three different options to accept inputs:

- **SAM/by mrsFAST-best-search:** A paired-end mapping result of your WGS data which satisfies the following conditions:
  - Two mates from a read are grouped together.
  - All mates are of equal length.

For example, a *best-mapping* SAM be a valid input file for Pamir . You can specify by `--files mrsfast-best-search=wgs.sam`. You can give multiple best-mapping files too, by comma separated or just the folder directory that includes the inputs. You can specify by

`--files mrsfast-best-search=sample1.sam,sample2.sam,sample3.sam` or  
`--files mrsfast-best-search=directory/to/sample_best_mapping_sam_files/`

- **FASTQ:** Pamir also accepts FASTQ format as the input data once it is a single gzipped file such that two (equal-length) mates of a read locate consecutively. You can specify by giving `--files fastq=wgs.fastq.gz`.
- **Alignment file SAM/BAM:** Pamir also accepts any other alignment output sorted by readname. Alignment output can be in SAM or BAM format. You can specify by `--files alignment=wgs.sam` or `--files alignment=wgs.bam`.

### 1.2.4 MrsFAST Parameters

Pamir uses mrsFAST for multi-mapping the orphan and OEA reads obtained from the best-mapping output. You can give your own mrsFAST parameters or Pamir will use the default values. Some of the parameters you may want to update are :

- **-mrsfast-n:** Maximum number of mapping loci of anchor of an OEA. Anchor with higher mapping location will be ignored. 0 for considering all mapping locations. (Default = 50)
- **-mrsfast-threads:** Number of the threads used by mrsFAST-Ultra for mapping. (Default = 1)
- **-mrsfast-errors:** Number of the errors allowed by mrsFAST-Ultra for mapping. In default mode Pamir does not give any error number to mrsFAST-Ultra, in which case it calculates the error value as  $0.06 \times \text{readlength}$ . (Default = -1)
- **-mrsfast-index-ws:** Window size used by mrsFAST-Ultra for indexing the reference genome. (Default = 12)

### 1.2.5 Other Parameters User can Define

- **–num-worker:** Number of independent prediction jobs to be created. You can define this parameter according to your core number. (Default = 1)
- **–resume:** Restart pipeline of an existing project from the stage that has not been completed yet.
- **–assembler:** The assembler to be used in orphan assembly stage (Options: velvet, minia, sga. Default = velvet).

## 1.3 Results

Pamir generates a VCF file for detected novel sequence insertions. You can run genotyping for each sample after obtaining the VCF file by:

```
python genotyping.py projectFolder/insertions.out_wodups.filtered_setcov_PASS.sorted.reference.fa.masked
sample1_FASTQ.1.fq sample1_FASTQ.2.fq readlength SAMPLENAME mrsfast-min mrsfast-max project-
FolderDirectory TEMP_LEN(1000)
```

## 1.4 Example Commands

- To start a new analysis from a mrsfast-best mapping result SAM file:

```
$ ./pamir.py -p my_project -r ref.fa --files mrsfast-best-search=sample.sam
```

- To make a pooled-run with multiple samples separated by comma:

```
$ ./pamir.py -p my_project -r ref.fa --files mrsfast-best-search=sample.sam,sample2.sam,sample3.sam
```

- To make a pooled-run with multiple samples which are in a folder called SAMPLEFOLDER:

```
$ ./pamir.py -p my_project -r ref.fa --files mrsfast-best-search=SAMPLEFOLDER
```

- To start from another mapping tool's alignment result SAM/BAM file:

```
$ ./pamir.py -p my_project -r ref.fa --files alignment=sample.bam
```

- To start from a gzipped fastq file,

```
$ ./pamir.py -p my_project -r ref.fa --files fastq=sample.fastq.gz
```

- To ignore regions in a mask file (e.g., repeat regions),

```
$ ./pamir.py -p my_project -r ref.fa -m repeat.txt --files mrsfast-best-search=sample.sam
```

- To analyze events only in some regions of the reference genome (e.g., genic regions),

```
$ ./pamir.py -p my_project -r ref.fa -m genic.region --invert-mask --files
mrsfast-best-search=sample.sam
```

- To make sure that mrsFAST will not report the mapping locations of an OEA read more after the 30th location:

```
$ ./mistrvar.py -p my_project -r ref.fa --mrsfast-n 30 --files mrsfast-best-search=sample.sam
```

- To specify the core number for mrsFAST during multi-mapping of OEAs:

```
$ ./pamir.py -p my_project -r ref.fa --mrsfast-threads 8 --files mrsfast-best-search=sample.sam
```

- To speed up the prediction process by defining the independent prediction jobs according to available core numbers:

```
$ ./pamir.py -p my_project -r ref.fa --num-worker 20 --files mrsfast-best-search=sample.sam
```

- To specify the assembler as sga for orphan assembly and also the number of prediction jobs will be 20:

```
$ ./pamir.py -p my_project -r ref.fa --num-worker 20 --assembler sga --files  
mrsfast-best-search=sample.sam
```

- To resume from the previously finished stage:

```
$ ./pamir.py -p my_project --resume
```

## 1.5 Example Invalid Commands

The following commands do not satisfy requirements of Pamir and will fail pamir.py:

- Project name is missing:

```
$ ./pamir.py -r ref.fa --files alignment=sample.sam
```

- Reference genome file is missing:

```
$ ./pamir.py -p my_project --files alignment=sample.sam
```

- Incorrect path of the mask file:

```
$ ./pamir.py -p my_project -m non-exist-mask-file --files alignment=sample.sam
```

- No input sequencing files:

```
$ ./pamir.py -p my_project -r ref.fa
```

- Multiple sequencing sources:

```
$ ./pamir.py -p my_project -r ref.fa --files mrsfast-best-search=sample.sam fastq=sample2.fastq.gz
```