

About the STACKS Workflow

The STACKS analysis pipeline (<http://creskolab.uoregon.edu/stacks/>) is the *de facto* tool for SNP discovery in Genotyping By Sequencing (GBS) and Restriction-site Associated DNA sequencing (RAD) studies. This STACKS workflow aims at making the use of the STACKS pipeline easier and more structured so that people with GBS or RAD projects and limited UNIX/Linux experience can jump on the analysis wagon faster. It is being developed with the needs of our research group in mind and we make no claim about its use to other groups or other contexts.

The workflow has been tested with version 0.99999 of STACKS.

Licence

The STACKS workflow is licensed under the GPL3 license. See the LICENCE file for more details.

Overview of the steps

- Step 0 - Install and prepare the workflow
- Step 1 - Download raw datafiles (Illumina lanes)
- Step 2 - Extract individual information with process_radtags
- Step 3 - Rename samples and make links
- Step 4 - STACKS (ustack/pstacks, cstack, sstack, populations/genotypes)
- Step 5 - Filters
- Step 6 - Format for population genetics

The workflow

Step 0 - Install and prepare the workflow

- a) Download and install the most recent version of this workflow
 - From the terminal, run:

```
cd ~/Desktop
wget https://github.com/enormandeau/stacks_workflow/archive/master.zip
unzip master.zip
```

Use the extracted folder (**stacks_workflow-master**) as your working directory for the rest of the project. If you just updated the workflow, please use the MANUAL.pdf file that comes with that new version.

- b) Download and install STACKS
 - <http://creskolab.uoregon.edu/stacks/>
 - Unzip
 - From within the STACKS folder, run:

```
./configure
make
sudo make install
```

Step 1 - Download raw datafiles (Illumina lanes)

- a) Put them in the 'raw' folder of the gbs_workflow
 - NOTE: All file names MUST end with '.fastq.gz'

- b) Prepare the 'lane_info.txt' file automatically
- From the gbs_workflow folder, run:

```
./00-scripts/01-prepare_lane_info.sh
```

Step 2 - Extract individual information with process_radtags

- a) Prepare barcode information file
- barcodes.txt (1 barcode sequence per line)
- b) Launch process_radtags with:
TODO use discarded reads at each step rather than treating the whole file each time

```
./00-scripts/02-process_radtags.sh <trimLength> <enzyme>
```

Where:

trimLength = length to trim all the sequences

enzyme = name of enzyme (run 'process_radtags', without options, for list)

Step 3a - Rename samples and make links

- a) To rename and copy the samples, run:

```
./00-scripts/03_rename_samples.sh
```

- b) Join samples that should go together
TODO Implement neat way of doing this
- Go to 04-all_samples and join the .fq files that should go together
- Remove partial .fq files that have been joined
- Remove individuals with too few sequences (optional)

Step 3b - Align reads to a reference genome

- a) Install bwa
b) Download reference genome to the 01-info_files
c) Index reference genome, run:

```
bwa index -p genome -a bwtsv ./01-info_files/<genome reference>
```

- d) copy files

```
cp genome.* 01-info_files
```

- d) Aligned samples, run

```
for i in $(ls -1 04-all_samples/*.fq); do name=$(basename $i); bwa aln -n 5  
-k 3 -t 2 ./01-info_files/genome $i | bwa samse -r  
"@RG\tID: '$name'\tSM: '$name'\tPL: Illumina" ./01-info_files/genome -  
$i ./04ln-all_samples/$name.sam; done
```

Step 4 - STACKS (ustack/pstacks, cstack, sstack, populations/genotypes)

- a) Prepare population info file
- To prepare a template of that file, run:

```
./00-scripts/04-prepare_population_map_template.sh
```

- b) Rename the template file to 'population_map.txt' and remove '.fq' extensions in columns 1
- c) Open the stacks script in the 00-scripts folder and edit the options
- d) Run the STACKS programs, in order:
 - ustacks (or pstacks for reference assisted)

```
./00-scripts/stacks_1a_ustacks.sh
```

or

```
./00-scripts/stacks_1b_pstacks.sh
```

- cstacks

```
./00-scripts/stacks_2_cstacks.sh
```

- sstacks

```
./00-scripts/stacks_3_sstacks.sh
```

- populations or genotypes

```
./00-scripts/stacks_4_populations.sh
```

Step 5 - Filters

Use ./00-scripts/05_filterStacksSNPs.py to filter your SNPs. To print the documentation, type:

```
./00-scripts/05_filterStacksSNPs.py
```

Launch the script, example:

```
./00-scripts/05_filterStacksSNPs.py ./05-stacks/sumstats.tsv 8 2 1 0.6 0.05  
-0.3 0.3
```

Step 6 - Format for population genetics

To be done