STACKS Workflow

The STACKS analysis pipeline () 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 can jump in the analysis wagon faster. It is being developped with the needs of our research group in mind and we make no claim about its use to other groups or other contextes.

Licence

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 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/01b-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 discarted reads at each step rather than treating the whole file each time
   > ./00-scripts/02b-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 bwtsw ./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 - $i > ./04aln-all_samples/$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/04a-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 and use the printed help

Step 6 - Format for population genetics

To be done