# Supplementary Information

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# Contents



# Introduction

In this experiment, we used quaddRAD library prep to prepare the sample DNA. This means that there were both two unique outer barcodes (typical Illumina barcodes) *AND* two unique inner barcodes (random barcode bases inside the adapters) for each sample - over 1700 to be exact!

The sequencing facility demultiplexes samples based on the outer barcodes (typically called 5nn and i7nn). Once this is done, each file still contains a mix of the inner barcodes. We will refer to these as "sublibraries" because they are sort of halfway demultiplexed. We separate them out bioinformatically later.

## Raw Data File Naming - Sublibraries

Here's a bit of information on the file name convention. The typical raw file looks like this:

`AMH_macro_1_1_12px_S1_L001_R1_001.fastq.gz`

- These are author initials and "macro" stands for "Macrosystems". These are on every file.

  `AMH_macro`

- The first number is the *i5nn* barcode for the given sublibrary. We know all these samples have a i5nn barcode "1", so that narrows down what they can be. The second number is the *i7nn* barcode for the given sublibrary. We know all these samples have a i7nn barcode "1", so that further narrows down what they can be.

  `1_1`

- This refers to how many samples are in the sublibrary. "12px" means 12-plexed, or 12 samples. In other words, we will use the inner barcodes to further distinguish 12 unique samples in this sublibrary.

  `12px`

- This is a unique sublibrary name. S1 = 1 i5nn and 1 i7nn.

  `S1`

- This means this particular file came from lane 1 of the NovaSeq. There are four lanes. All samples should appear across all four lanes.

  `L001`

- This is the first (R1) of two paired-end reads (R1 and R2).

  `R1`

- The last part doesn't mean anything - it was just added automatically before the file suffix (`fastq.gz`)

  `001.fastq.gz`

### A Note on File Transfers

There are three main systems at play for file transfer: the local machine, the sequencing facility's (GRCF) Aspera server, and MARCC. The Aspera server is where the data were/are stored immediately after sequencing. MARCC is where we plan to do preprocessing and analysis. Scripts and text files are easy for me to edit on my local machine. We used Globus to transfer these small files from my local machine to MARCC.

Midway through this analyses, we transitioned to another cluster, JHU's Rockfish. Scripts below, with the exception of file transfer from the Aspera server, should reflect the new filesystem, though you will have to adjust the file paths accordingly.

## Preprocessing

### Step 1 - Transfer Files

Files can be found in the `01_transfer_files/` directory.

`01-aspera_transfer_n.txt`

These are text files containing the *names* of `fastq.gz` files that we wanted to transfer from the sequencing facility's Aspera server to the computing cluster (MARCC). This was to maximize ease of transferring only certain files over at once, since transferring could take a long time. We definitely did this piecemeal. Possible file names shown in Aspera Transfer File Names. There are multiple of these files so that we could parallelize (replace n with the correct number in the command used below). This text file will need to be uploaded to your scratch directory in MARCC.

Files were then transferred using the following commands. Before starting, make sure you are in a data transfer node. Then, load the aspera module. Alternatively, you can install the Aspera transfer software and use that.

```
module load aspera
```

Initiate the transfer from within your scratch directory:

```
ascp -T -l8G -i /software/apps/aspera/3.9.1/etc/asperaweb_id_dsa.openssh
--file-list=01-aspera_transfer_n.txt
--mode=recv --user=<aspera-user> --host=<aspera-IP> /scratch/users/<me>@jhu.edu
```

## Step 2 - Concatenate Files and Install Stacks

Files can be found in the `02_concatenate_and_check/` directory.

### Step 2a - Concatenate Files for each Sublibrary

We ran my samples across the whole flow cell of the NovaSeq, so results came in 8 files for each demultiplexed sublibrary (4 lanes * paired reads). For example, for sublibrary 1_1, we'd see the following 8 files:

```
AMH_macro_1_1_12px_S1_L001_R1_001.fastq.gz
AMH_macro_1_1_12px_S1_L001_R2_001.fastq.gz
AMH_macro_1_1_12px_S1_L002_R1_001.fastq.gz
AMH_macro_1_1_12px_S1_L002_R2_001.fastq.gz
AMH_macro_1_1_12px_S1_L003_R1_001.fastq.gz
AMH_macro_1_1_12px_S1_L003_R2_001.fastq.gz
AMH_macro_1_1_12px_S1_L004_R1_001.fastq.gz
AMH_macro_1_1_12px_S1_L004_R2_001.fastq.gz
```

The `02_concatendate_and_check/02-concat_files_across4lanes.sh` script finds all files in the working directory with the name pattern `*_L001_*.fastq.gz` and then concatenates across lanes 001, 002, 003, and 004 so they can be managed further. The "L001" part of the filename is then eliminated. For example the 8 files above would become:

```
AMH_macro_1_1_12px_S1_R1.fastq.gz
AMH_macro_1_1_12px_S1_R2.fastq.gz
```

Rockfish uses slurm to manage jobs. To run the script, use the `sbatch` command. For example:

```
sbatch ~/code/02-concat_files_across4lanes.sh
```

This command will run the script from within the current directory, but will look for and pull the script from the code directory. This will concatenate all files within the current directory that match the loop pattern.

### Step 2b – Download and Install Stacks

On Rockfish, Stacks will need to be downloaded to each user's code directory. Stacks, and software in general, should be compiled in an interactive mode or loaded via module. For more information on interactive mode, see `interact --usage`.

```
interact -p debug -g 1 -n 1 -c 1
module load gcc
```

Now download Stacks. We used version 2.60.

```
wget http://catchenlab.life.illinois.edu/stacks/source/stacks-2.60.tar.gz
tar xfvz stacks-2.60.tar.gz
```

Next, go into the stacks-2.60 directory and run the following commands:

```
./configure --prefix=/home/<your_username>/code4-<PI_username>
make
make install
export PATH=$PATH:/home/<your_username>/code4-<PI_username>/stacks-2.60
```

The filesystem patterns on your cluster might be different, and you should change these file paths accordingly.

## Step 3 - Remove PCR Clones

Files can be found in the `03_clone_filter/` directory.

### Step 3a - Run PCR Clone Removal Script

The `03-clone_filter.sh` script runs `clone_filter` from Stacks. The program was run with options `--inline_inline --oligo_len_1 4 --oligo_len_2 4`. The `--oligo_len_x 4` options indicate the 4-base pair degenerate sequence was included on the outside of the barcodes for detecting PCR duplicates. The script uses the file name prefixes listed for each single sub-pooled library in `03-clone_filter_file_names.txt` and loops to run `clone_filter` on all of them. Possible file names shown in `clone_filter` File Names.

### Step 3b - Parse PCR Clone Removal Results

If you want to extract descriptive statistics from the `clone_filter` output, you can use the `03.5-parse_clone_filter.py` script to do so. It can be run on your local terminal after transferring the `clone_filter.out` logs to your local computer.

```
source("03_clone_filter/examine_clones.R")
make_cloneplot()
```

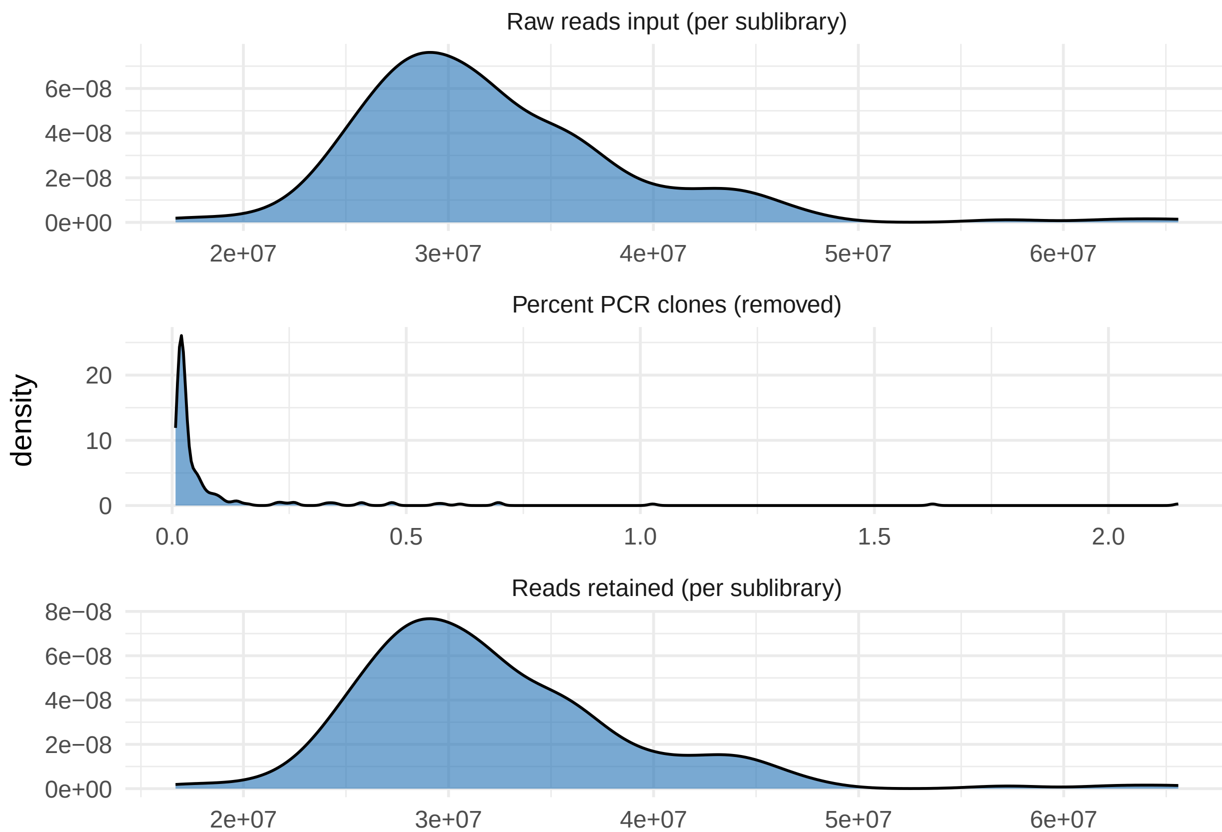


Figure 1: PCR clone removal statistics

## Step 4 - Demultiplexing and Sample Filtering

Files can be found in the `04_demux_filter/` directory.

### Step 4a - Demultiplex and Filter

The `04-process_radtags.sh` script runs `process_radtags` from Stacks. The program was run with options `-c -q --inline_inline --renz_1 pstI --renz_2 mspI --rescue --disable_rad_check`. The script uses the same file prefixes as Step 3 - `03-clone_filter.sh`. Each sub-pooled library has a forward and reverse read file that was filtered in the previous step. Like the above section, the script uses the file name prefixes listed for each single sub-pooled library in `04-process_radtags_file_names.txt` and loops to run `process_radtags` on all of them. Possible file names shown in `clone_filter` File Names.

Each sub-pooled library also has a demultiplexing file (`04-demux/` directory) that contains the sample names and inner(i5 and i7) barcodes. For example, the sublibrary 1_1, we'd see the following barcode file:

```
ATCACG  AGTCAA  DS.BA.PIK.U.1
CGATGT  AGTTCC  DS.BA.PIK.U.2
TTAGGC  ATGTCA  DS.BA.PIK.U.3
TGACCA  CCGTCC  DS.BA.PIK.U.4
ACAGTG  GTCCGC  DS.BA.PIK.U.5
GCCAAT  GTGAAA  DS.BA.DHI.U.1
CAGATC  GTGGCC  DS.BA.DHI.U.2
ACTTGA  GTTTCG  DS.BA.DHI.U.3
GATCAG  CGTACG  DS.BA.DHI.U.4
TAGCTT  GAGTGG  DS.BA.DHI.U.5
GGCTAC  ACTGAT  DS.BA.GA.U.1
CTTGTA  ATTCCT  DS.BA.GA.U.2
```

The 'process_radtags' command will demultiplex the data by separating out each sublibrary into the individual samples. It will then clean the data, and will remove low quality reads and discard reads where a barcode was not found.

### Step 4b - Organize files

In a new directory, make sure the files are organized by species. In the `process_radtags` script, we specified that files be sent to `~/scratch/demux/*sublibrary_name*` (reasoning for this is in Step 4c), but files should manually be organized into species folders (i.e., `~/scratch/demux/*SPP*`) after `process_radtags` is performed. For example, the file "DS.MN.L01-DS.M.1.1.fq.gz" should be sent to the `~/scratch/demux/DS` directory.

Note: this is not automated at this point but it would be nice to automate the file moving process so it's not forgotten at this point.

### Step 4c - Assess the raw, processed, and cleaned data

In the script for Step 4, we have specified that a new output folder be created for each sublibrary. The output folder is where all sample files and the log file will be dumped for each sublibrary. It is important to specify a different output folder if you have multiple sublibraries because we will be assessing the output log for each sublibrary individually (and otherwise, the log is overwritten when the script loops to a new sublibrary).

The utility `stacks-dist-extract` can be used to extract data from the log file. First, we examined the library-wide statistics to identify sublibraries where barcodes may have been misentered or where sequencing error may have occurred. We used:

```
stacks-dist-extract process_radtags.log total_raw_read_counts
```

to pull out data on the total number of sequences, the number of low-quality reads, whether barcodes were found or not, and the total number of retained reads per sublibary. Look over these to make sure there are no outliers or sublibraries that need to be checked and rerun.

Next, we used:

```
stacks-dist-extract process_radtags.log per_barcode_raw_read_counts
```

to analyze how well each sample performed. There are three important statistics to consider for each sample.

1. *The proportion of reads per sample for each sublibrary* indicates the proportion that each individual was processed and sequenced within the overall library. This is important to consider as cases where a single sample dominates the sublibrary may indicate contamination.
2. *The number of reads retained for each sample* can be an indicator of coverage. It is most likely a good idea to remove samples with a very low number of reads. Where you decide to place the cutoff for low coverage samples is dependent on your dataset. For example, a threshold of 1 million reads is often used but this is not universal.
3. *The proportion of reads retained for each sample* can also indicate low-quality samples and will give an idea of the variation in coverage across samples.

Output for sublibraries for this step are summarized in `process_radtags-library_output.csv`.

Output for individual samples for this step are summarized in `process_radtags-sample_output.csv`.

The script `04c-process_radtags_stats.R` was used to create many plots for easily assessing each statistic. Output from this step can be found in `figures/process_radtags/` where figures are organized by species.

```r
source("04_demux_filter/04c-radtags_filter_summary.R")
make_filterplot()
```

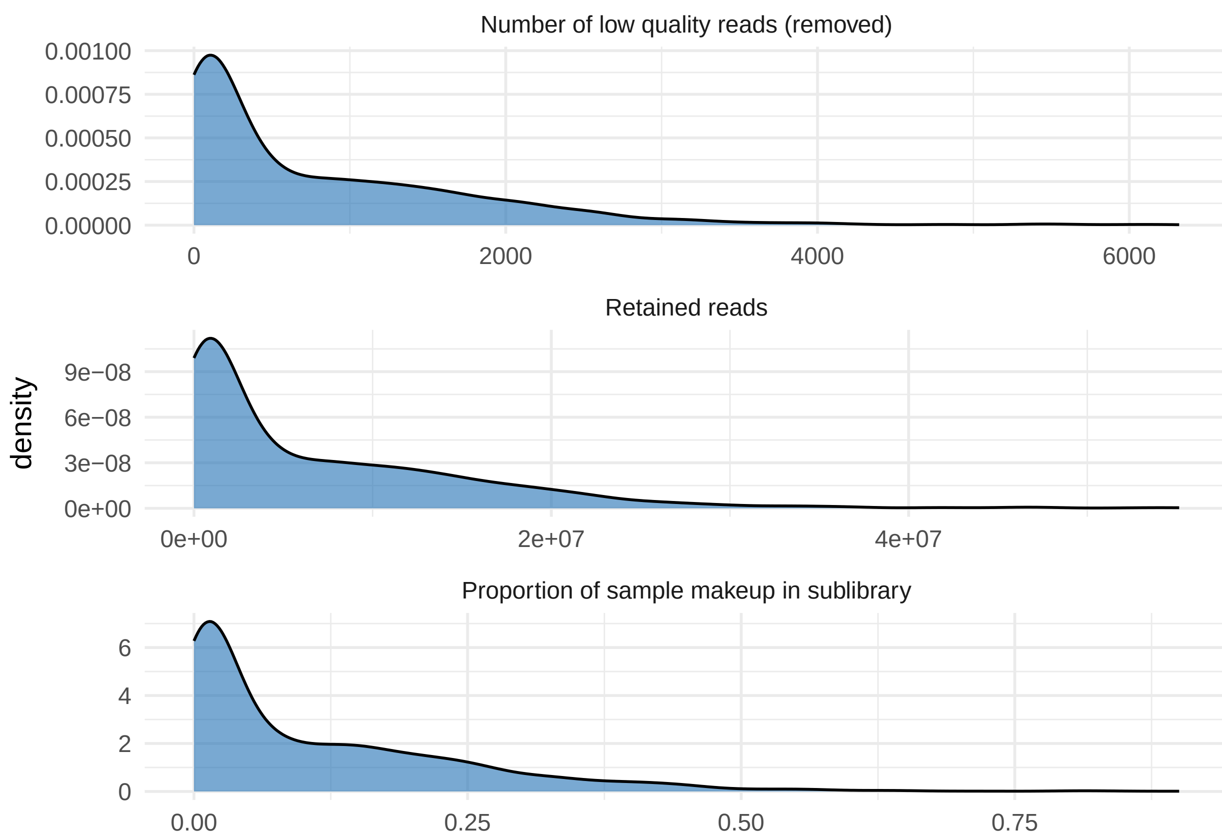


Figure 2: RAD tag processing statistics

#### Step 4d - Identify low-coverage and low-quality samples from `downstream analysis`

Using the same output log and the above statistics, we removed low-coverage and low-quality samples that may skew downstream analyses.

Samples were identified and removed via the following procedure:

1. First, samples that represented less than **1% of the sequenced sublibrary** were identified and removed. These samples correlate to low-read and low-coverage samples.
2. Next, a threshold of **1 million retained reads per sample** was used to remove any remaining low-read samples. Low-read samples correlate to low coverage and will lack enough raw reads to contribute to downstream analyses.

Good/kept samples are summarized in `process_radtags-kept_samples.csv`.

Discarded samples are summarized in `process_radtags-discarded_samples.csv`.

```
source("04_demux_filter/04c-radtags_filter_summary.R")
make_manual_discard_plot()
```

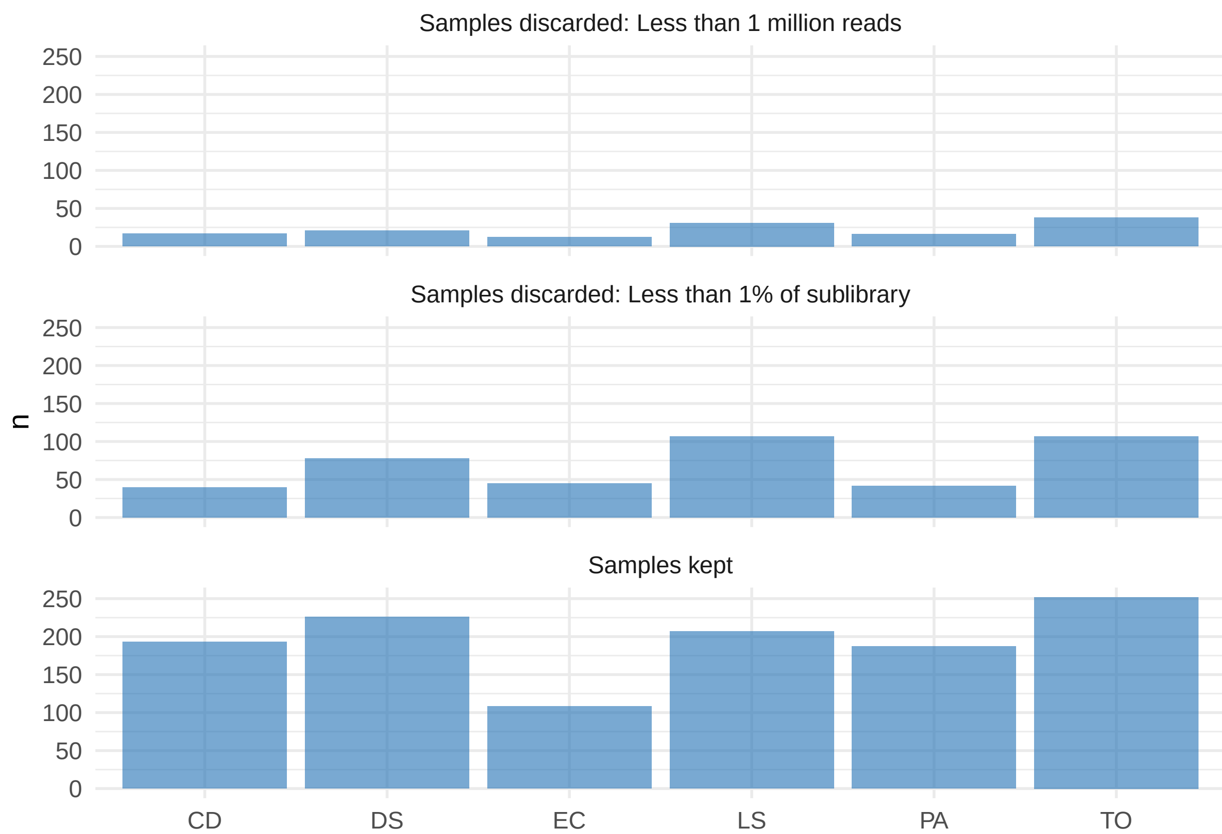


Figure 3: RAD tag manual filtering summary

Note: At this point, we started using Stacks 2.62 for its multi-threading capabilities. Functionality of the previous steps should be the same, however.

# Generating Stacks Catalogs and Calling SNPs

## Step 5 - Metapopulation Catalog Building and Parameter Search

Files can be found in the `05_ustacks_and_params/` directory.

Going forward, when we use the term **metapopulation**, we are referring to the collection of all samples within species among all cities where the species was present.

It is important to conduct preliminary analyses that will identify an optimal set of parameters for the dataset (see Step 5a). Following the parameter optimization, the program `ustacks` can be run to generate a catalog of loci.

### Step 5a - Run `denovo_map.sh`

Stack assembly will differ based on several different aspects of the dataset(such as the study species, the RAD-seq method used, and/or the quality and quantity of DNA used). So it is important to use parameters that will maximize the amount of biological data obtained from stacks.

There are three main parameters to consider when doing this:

1. $m$ = controls the minimum number of raw reads required to form a stack(implemented in `ustacks`)
2. $M$ = controls the number of mismatches between stacks to to merge them into a putative locus (implemented in `ustacks`)
3. $n$ = controls the number of mismatches allowed between stacks to merge into the catalog (implemented in `cstacks`)

There are two main ways to optimize parameterization:

1. an iterative method were you sequentially change each parameter while keeping the other parameters fixed (described in *Paris et al. 2017*), or
2. an iterative method were you sequentially change the values of $M$ and $n$(keeping $M = n$) while fixing $m = 3$, and then test $m = 2$, 4 once the optimal $M = n$ is determined(described in *Rochette and Catchen 2017*, *Catchen 2020*).

We performed the second method and used the `denovo_map.sh` script to run the `denovo_map.pl` command to perform iterations. This script requires that we first choose a subset of samples to run the iterations on. The samples should be representative of the overall dataset; meaning they should include all populations and have similar read coverage numbers. Read coverage numbers can be assessed by looking at the descriptive statistics produced from Step 4c.

Place these samples in a text file (`popmap_test_samples.txt`) with the name of the sample and specify that all samples belong to the same population. For example, `popmap_test_samples.txt` should look like...

```
DS.BA.GA.U.1    A
DS.PX.BUF.M.5   A
DS.BO.HC4.M.1   A
...
```

It is important to have all representative samples treated as one population because you will assess outputs found across 80% of the individuals. The script will read this text file from the `--popmap` argument.

The script also requires that you specify an output directory after `-o`. This should be unique to the parameter you are testing... for example, if you are testing $M = 3$, then you could make a subdirectory labeled `stacks.M3` where all outputs from `denovo_map.sh` will be placed. Otherwise, for each iteration, the outputs will be overwritten and you will lose the log from the previous iteration. The `denovo_map.sh` script also requires that you direct it toward where your samples are stored, which is your directory built in Step 4b. Make sure to run the `--min-samples-per-pop 0.80` argument.

To decide which parameters to use, examine the following from each iteration:

1. the average sample coverage: This is obtained from the summary log in the `ustacks` section of `denovo_map.log`. If samples have a coverage <10x, you will have to rethink the parameters you use here.
2. the number of assembled loci shared by 80% of samples: This can be found in the `haplotypes.tsv` by counting the number of loci: `cat populations.haplotypes.tsv | grep -v ^"#" | wc -l`
3. the number of polymorphic loci shared by 80% of samples: This can be found in `populations.sumstats.tsv` or by counting `populations.hapstats.tsv`: `cat populations.hapstats.tsv | grep -v "^#" | wc -l`
4. the number of SNPs per locus shared by 80% of samples: found in `denovo_map.log` or by counting the number of SNPs in `populations.sumstats.tsv`: `populations.sumstats.tsv | grep -v ^"#" | wc -l`

The script `05a-param_opt-figures_script.R` was used to create plots for assessing the change in shared loci across parameter iterations.

Based on this optimization step, we used the following parameters:

Table 1: Final parameter optimization values for the Stacks pipeline.

| Species | M (locus mismatches) | n (catalog mismatches) | m (minimum reads) |
|---|---|---|---|
| CD | 8 | 8 | 3 |
| DS | 10 | 10 | 3 |
| EC | 8 | 8 | 3 |
| LS | 7 | 7 | 3 |
| PA | 5 | 5 | 3 |
| TO | 6 | 6 | 3 |

#### Step 5b - Run `ustacks`

`ustacks` builds *de novo* loci in each individual sample. We have designed the `ustacks` script so that the process requires three files:

- `05-ustacks_n.sh` : the shell script that executes `ustacks`
- `05-ustacks_id_n.txt` : the sample ID number
- `05-ustacks_samples_n.txt` : the sample names that correspond to the sample IDs

The sample ID should be derived from the `order_id` column(first column) on the master spreadsheet. It is unique (1-1736) across all of the samples.

The sample name is the corresponding name for each sample ID in the spreadsheet. E.g., sample ID "9" corresponds to sample name "DS.BA.DHI.U.4". Sample naming convention is species.city.site.management_type.replicate_plant.

`05-ustacks_n.sh` should have an out_directory (`-o` option) that will be used for all samples (e.g., `stacks/ustacks`). Files can be processed piecemeal into this directory. There should be three files for every sample in the output directory:

- `<samplename>.alleles.tsv.gz`
- `<samplename>.snps.tsv.gz`
- `<samplename>.tags.tsv.gz`

Multiple versions of the `05-ustacks_n.sh` script can be run in parallel (simply replace n in the three files above with the correct number).

A small number of samples (13) were discarded at this stage as the `ustacks` tool was unable to form any primary stacks corresponding to loci. See output/ustacks-discarded_samples.csv.

#### Step 5c - Correct File Names

This step contains a script `05b-fix_filenames.sh` which uses some simple regex to fix filenames that are output in previous steps. Stacks adds an extra "1" at some point at the end of the sample name which is not meaningful. The following files:

- DS.MN.L02-DS.M.3.1.alleles.tsv.gz
- DS.MN.L03-DS.U.2.1.tags.tsv.gz
- DS.MN.L09-DS.U.1.1.snps.tsv.gz

become:

- DS.MN.L02-DS.M.3.alleles.tsv.gz
- DS.MN.L03-DS.U.2.tags.tsv.gz
- DS.MN.L09-DS.U.1.snps.tsv.gz

The script currently gives some strange log output, so it can probably be optimized/improved. The script should be run from the directory where the changes need to be made. Files that have already been fixed will not be changed.

#### Step 5d - Choose catalog samples/files

In the next step, we will choose the files we want to go into the catalog. This involves a few steps:

1. Create a meaningful directory name. This could be the date (e.g., `stacks_22_01_25`).
2. Copy the `ustacks` output for all of the files you want to use in the reference from Step 5b. Remember this includes three files per sample. So if you have 20 samples you want to include in the reference catalog, you will transfer 3 x 20 = 60 files into the meaningful directory name. The three files per sample should follow this convention:

- `<samplename>.alleles.tsv.gz`
- `<samplename>.snps.tsv.gz`
- `<samplename>.tags.tsv.gz`

3. Remember the meaningful directory name. You will need it in Step 6.

### Step 6 - Metapopulation catalog with `cstacks`

Files can be found in the `06_cstacks/` directory.

`cstacks` builds the locus catalog from all the samples specified. The accompanying script, `cstacks_SPECIES.sh` is relatively simple since it points to the directory containing all the sample files. It follows this format to point to that directory:

```
cstacks -P ~/directory ...
```

Make sure that you use the meaningful directory from Step 5c and that you have copied all the relevant files over. Otherwise this causes problems downstream. For example, you might edit the code to point to `~/scratch/stacks/stacks_22_01_25`.

```
cstacks -P ~/scratch/stacks/stacks_22_01_25 ...
```

The tricky thing is ensuring enough compute memory to run the entire process successfully. There is probably space to optimize this process.

The `cstacks` method uses a "population map" file, which in this project is `cstacks_popmap_SPECIES.txt`. This file specifies which samples to build the catalog from and categorizes them into your 'populations', or in this case, cities using two tab-delimited columns, e.g.:

```
DS.BA.GA.U.1    Baltimore
DS.BA.GA.U.2    Baltimore
```

```
DS.BA.GA.U.3    Baltimore
DS.BA.GA.U.4    Baltimore
DS.BA.GA.U.5    Baltimore
...
```

Make sure the samples in this file correspond to the input files located in e.g., `~/scratch/stacks/stacks_22_01_25`.

`cstacks` builds three files for use in all your samples (in this pipeline run), mirroring the sample files output by`ustacks`:

- `catalog.alleles.tsv.gz`
- `catalog.snps.tsv.gz`
- `catalog.tags.tsv.gz`

Table 2: Subset of samples used in SNP catalog creation.

| Sample | Species | City |
|---|---|---|
| DS.BA.PIK.U.1 | DS | BA |
| DS.BA.GA.U.4 | DS | BA |
| DS.BA.LH-1.M.4 | DS | BA |
| DS.BA.LH-3.M.1 | DS | BA |
| DS.BA.WB.U.2 | DS | BA |
| DS.BA.LL-4.M.5 | DS | BA |
| DS.BA.LH-2.M.5 | DS | BA |
| DS.BA.TRC.U.3 | DS | BA |
| DS.BA.W3.M.2 | DS | BA |
| DS.BA.RG-1.M.1 | DS | BA |
| DS.BA.LL-3.M.3 | DS | BA |
| DS.BA.RG-2.M.4 | DS | BA |
| DS.BO.HC1.M.3 | DS | BO |
| DS.BO.HC4.M.5 | DS | BO |
| DS.BO.LC1.M.3 | DS | BO |
| DS.BO.LC2.M.2 | DS | BO |
| DS.BO.LC3.M.5 | DS | BO |
| DS.BO.WL1.M.2 | DS | BO |
| DS.BO.WL2.M.1 | DS | BO |
| DS.BO.WL3.M.5 | DS | BO |
| DS.BO.I4.U.1 | DS | BO |
| DS.BO.R1.U.4 | DS | BO |
| DS.BO.R2.U.2 | DS | BO |
| DS.BO.R4.U.4 | DS | BO |
| DS.MN.L05-DS.M.3 | DS | MN |
| DS.MN.L09-DS.M.3 | DS | MN |
| DS.MN.L11-DS.M.1 | DS | MN |
| DS.MN.L02-DS.U.1 | DS | MN |
| DS.MN.L02-DS.M.4 | DS | MN |
| DS.MN.L03-DS.U.3 | DS | MN |
| DS.MN.L04-DS.U.5 | DS | MN |
| DS.MN.L06-DS.U.3 | DS | MN |
| DS.MN.L07-DS.U.3 | DS | MN |
| DS.MN.L09-DS.U.3 | DS | MN |
| DS.MN.L11-DS.U.1 | DS | MN |
| DS.MN.L11-DS.U.5 | DS | MN |
| DS.PX.BUF.M.1 | DS | PX |
| DS.PX.PIE.M.2 | DS | PX |

| Sample | Species | City |
|---|---|---|
| DS.PX.ALA.M.1 | DS | PX |
| DS.PX.MTN.M.6 | DS | PX |
| DS.PX.LAP.M.3 | DS | PX |
| DS.PX.NUE.M.4 | DS | PX |
| DS.PX.WES.M.2 | DS | PX |
| DS.PX.DF1.M.1 | DS | PX |
| DS.PX.ENC.M.1 | DS | PX |
| DS.PX.DOW.M.1 | DS | PX |
| DS.PX.DOW.M.4 | DS | PX |
| DS.PX.DF2.M.3 | DS | PX |
| CD.BA.LA.U.2 | CD | BA |
| CD.BA.TRC.U.3 | CD | BA |
| CD.BA.WGP.M.2 | CD | BA |
| CD.BA.LH-2.M.2 | CD | BA |
| CD.BA.LL-4.M.1 | CD | BA |
| CD.BA.PIK.U.2 | CD | BA |
| CD.BA.WB.U.2 | CD | BA |
| CD.BA.CP.U.4 | CD | BA |
| CD.BA.FH.U.1 | CD | BA |
| CD.BA.PSP.M.4 | CD | BA |
| CD.BA.AA.U.4 | CD | BA |
| CD.BA.RG-1.M.2 | CD | BA |
| CD.BA.W3.M.3 | CD | BA |
| CD.BA.GA.U.3 | CD | BA |
| CD.BA.WBO.U.5 | CD | BA |
| CD.LA.WHI.M.3 | CD | LA |
| CD.LA.SEP.M.3 | CD | LA |
| CD.LA.SEP.M.4 | CD | LA |
| CD.LA.ROS.M.5 | CD | LA |
| CD.LA.MR2.M.2 | CD | LA |
| CD.LA.ALL.M.2 | CD | LA |
| CD.LA.ALL.M.5 | CD | LA |
| CD.LA.VAL.M.5 | CD | LA |
| CD.LA.HAR.M.4 | CD | LA |
| CD.LA.LUB.M.3 | CD | LA |
| CD.LA.GLO.M.4 | CD | LA |
| CD.LA.ZOO.M.3 | CD | LA |
| CD.LA.NWH.M.5 | CD | LA |
| CD.LA.KIN.M.3 | CD | LA |
| CD.LA.KIN.M.5 | CD | LA |
| CD.PX.CAM.U.5 | CD | PX |
| CD.PX.MON.U.5 | CD | PX |
| CD.PX.PKW.U.5 | CD | PX |
| CD.PX.LAP.M.4 | CD | PX |
| CD.PX.NES.U.4 | CD | PX |
| CD.PX.PAL.M.3 | CD | PX |
| CD.PX.ASU.M.1 | CD | PX |
| CD.PX.NUE.M.5 | CD | PX |
| CD.PX.WES.M.3 | CD | PX |
| CD.PX.MAN.M.4 | CD | PX |
| CD.PX.CLA.M.3 | CD | PX |
| CD.PX.DF1.M.5 | CD | PX |

| Sample | Species | City |
|---|---|---|
| CD.PX.COY.M.5 | CD | PX |
| CD.PX.RPC.M.3 | CD | PX |
| CD.PX.ENC.M.2 | CD | PX |
| EC.BA.LH-2.M.2 | EC | BA |
| EC.BA.WBO.U.4 | EC | BA |
| EC.BA.WB.U.5 | EC | BA |
| EC.BA.FH.U.3 | EC | BA |
| EC.BA.CP.U.2 | EC | BA |
| EC.BA.TRC.U.3 | EC | BA |
| EC.BA.LL-4.M.4 | EC | BA |
| EC.BA.WB.U.1 | EC | BA |
| EC.BA.PIK.U.5 | EC | BA |
| EC.BA.PSP.M.4 | EC | BA |
| EC.BA.GA.U.2 | EC | BA |
| EC.BA.LL-3.M.3 | EC | BA |
| EC.BA.ML.U.1 | EC | BA |
| EC.BA.TRC.U.5 | EC | BA |
| EC.BA.ML.U.3 | EC | BA |
| EC.LA.SGB.U.2 | EC | LA |
| EC.LA.SGB.U.5 | EC | LA |
| EC.LA.DUR.U.2 | EC | LA |
| EC.LA.HOW.U.2 | EC | LA |
| EC.LA.SAN.U.2 | EC | LA |
| EC.LA.VER.U.1 | EC | LA |
| EC.LA.VER.U.4 | EC | LA |
| EC.LA.VB2.U.4 | EC | LA |
| EC.LA.AC2.U.2 | EC | LA |
| EC.LA.AC1.U.1 | EC | LA |
| EC.LA.VB1.U.1 | EC | LA |
| EC.LA.VB1.U.3 | EC | LA |
| EC.LA.SGR.U.4 | EC | LA |
| EC.LA.SGR.U.5 | EC | LA |
| EC.LA.HOW.U.3 | EC | LA |
| EC.PX.BUF.M.1 | EC | PX |
| EC.PX.BUF.M.3 | EC | PX |
| EC.PX.ALA.M.3 | EC | PX |
| EC.PX.MTN.M.2 | EC | PX |
| EC.PX.WES.M.1 | EC | PX |
| EC.PX.WES.M.2 | EC | PX |
| EC.PX.MAN.M.1 | EC | PX |
| EC.PX.CLA.M.1 | EC | PX |
| EC.PX.PSC.M.1 | EC | PX |
| EC.PX.DF1.M.1 | EC | PX |
| EC.PX.DOW.M.1 | EC | PX |
| EC.PX.DOW.M.2 | EC | PX |
| EC.PX.COY.M.2 | EC | PX |
| EC.PX.COY.M.3 | EC | PX |
| EC.PX.ALA.M.5 | EC | PX |
| LS.BA.WB.U.1 | LS | BA |
| LS.BA.WB.U.2 | LS | BA |
| LS.BA.DHI.U.2 | LS | BA |
| LS.BA.GA.U.1 | LS | BA |

| Sample | Species | City |
|---|---|---|
| LS.BA.PIK.U.3 | LS | BA |
| LS.BA.PIK.U.5 | LS | BA |
| LS.BA.CP.U.2 | LS | BA |
| LS.BA.ML.U.2 | LS | BA |
| LS.BA.WBO.U.3 | LS | BA |
| LS.BO.WL3.M.4 | LS | BO |
| LS.BO.I1.U.1 | LS | BO |
| LS.BO.I2.U.1 | LS | BO |
| LS.BO.WL2.M.2 | LS | BO |
| LS.BO.R1.U.2 | LS | BO |
| LS.BO.R2.U.4 | LS | BO |
| LS.BO.R3.U.3 | LS | BO |
| LS.BO.HC4.M.3 | LS | BO |
| LS.BO.LC4.M.2 | LS | BO |
| LS.LA.VET.M.4 | LS | LA |
| LS.LA.SSV.M.1 | LS | LA |
| LS.LA.NAV.M.4 | LS | LA |
| LS.LA.SHO.M.2 | LS | LA |
| LS.LA.WES.M.3 | LS | LA |
| LS.LA.GLO.M.3 | LS | LA |
| LS.LA.HOW.U.5 | LS | LA |
| LS.LA.SAN.U.2 | LS | LA |
| LS.LA.ARR.U.2 | LS | LA |
| LS.MN.L06-LS.U.2 | LS | MN |
| LS.MN.L06-LS.U.5 | LS | MN |
| LS.MN.L07-LS.U.4 | LS | MN |
| LS.MN.L08-LS.U.5 | LS | MN |
| LS.MN.L09-LS.U.3 | LS | MN |
| LS.MN.L01-LS.M.4 | LS | MN |
| LS.MN.L01-LS.U.3 | LS | MN |
| LS.MN.L02-LS.U.1 | LS | MN |
| LS.MN.L05-LS.U.2 | LS | MN |
| LS.PX.MON.U.2 | LS | PX |
| LS.PX.PKW.U.5 | LS | PX |
| LS.PX.PIE.M.4 | LS | PX |
| LS.PX.ALA.M.3 | LS | PX |
| LS.PX.PAL.M.3 | LS | PX |
| LS.PX.MAN.M.2 | LS | PX |
| LS.PX.NUE.M.1 | LS | PX |
| LS.PX.ENC.M.4 | LS | PX |
| LS.PX.COY.M.3 | LS | PX |
| PA.BA.PIK.U.1 | PA | BA |
| PA.BA.LH-3.M.2 | PA | BA |
| PA.BA.LH-3.M.3 | PA | BA |
| PA.BA.WB.U.1 | PA | BA |
| PA.BA.AA.U.1 | PA | BA |
| PA.BA.WGP.M.3 | PA | BA |
| PA.BA.LL-4.M.3 | PA | BA |
| PA.BA.LA.U.2 | PA | BA |
| PA.BA.LH-2.M.2 | PA | BA |
| PA.BA.W3.M.3 | PA | BA |
| PA.BA.RG-1.M.2 | PA | BA |

| Sample | Species | City |
|---|---|---|
| PA.BA.LL-3.M.5 | PA | BA |
| PA.BO.I2.U.3 | PA | BO |
| PA.BO.HC1.M.4 | PA | BO |
| PA.BO.R3.U.2 | PA | BO |
| PA.BO.HC4.M.5 | PA | BO |
| PA.BO.R4.U.2 | PA | BO |
| PA.BO.WL2.M.5 | PA | BO |
| PA.BO.WL4.M.4 | PA | BO |
| PA.BO.LC4.M.4 | PA | BO |
| PA.BO.HC2.M.1 | PA | BO |
| PA.BO.R1.U.2 | PA | BO |
| PA.BO.WL1.M.1 | PA | BO |
| PA.BO.I1.U.5 | PA | BO |
| PA.LA.ALL.M.5 | PA | LA |
| PA.LA.SEP.M.1 | PA | LA |
| PA.LA.SEP.M.5 | PA | LA |
| PA.LA.WHI.M.2 | PA | LA |
| PA.LA.ROS.M.5 | PA | LA |
| PA.LA.LUB.M.2 | PA | LA |
| PA.LA.GLO.M.2 | PA | LA |
| PA.LA.ZOO.M.4 | PA | LA |
| PA.LA.ZOO.M.5 | PA | LA |
| PA.LA.NWH.M.2 | PA | LA |
| PA.LA.KIN.M.4 | PA | LA |
| PA.LA.POP.M.4 | PA | LA |
| PA.PX.BUF.M.3 | PA | PX |
| PA.PX.PIE.M.4 | PA | PX |
| PA.PX.LAP.M.5 | PA | PX |
| PA.PX.ALA.M.1 | PA | PX |
| PA.PX.PAP.M.2 | PA | PX |
| PA.PX.PAP.M.5 | PA | PX |
| PA.PX.DF1.M.2 | PA | PX |
| PA.PX.RPP.U.3 | PA | PX |
| PA.PX.ENC.M.4 | PA | PX |
| PA.PX.ENC.M.5 | PA | PX |
| PA.PX.COY.M.1 | PA | PX |
| PA.PX.BUF.M.2 | PA | PX |
| TO.BA.WBO.U.4 | TO | BA |
| TO.BA.CP.U.1 | TO | BA |
| TO.BA.FH.U.1 | TO | BA |
| TO.BA.LH-3.M.4 | TO | BA |
| TO.BA.WGP.M.3 | TO | BA |
| TO.BA.GA.U.4 | TO | BA |
| TO.BA.PIK.U.4 | TO | BA |
| TO.BA.PSP.M.1 | TO | BA |
| TO.BA.RG-2.M.2 | TO | BA |
| TO.BO.HC1.M.4 | TO | BO |
| TO.BO.HC2.M.5 | TO | BO |
| TO.BO.HC3.M.1 | TO | BO |
| TO.BO.HC4.M.5 | TO | BO |
| TO.BO.LC1.M.1 | TO | BO |
| TO.BO.LC2.M.5 | TO | BO |

| Sample | Species | City |
|---|---|---|
| TO.BO.LC3.M.1 | TO | BO |
| TO.BO.WL2.M.1 | TO | BO |
| TO.BO.I2.U.3 | TO | BO |
| TO.LA.WHI.M.5 | TO | LA |
| TO.LA.HAR.M.4 | TO | LA |
| TO.LA.MR1.M.1 | TO | LA |
| TO.LA.GLO.M.5 | TO | LA |
| TO.LA.ZOO.M.1 | TO | LA |
| TO.LA.NWH.M.4 | TO | LA |
| TO.LA.VNS.M.2 | TO | LA |
| TO.LA.PEP.M.5 | TO | LA |
| TO.LA.COM.M.4 | TO | LA |
| TO.MN.L11-TO.M.3 | TO | MN |
| TO.MN.L02-TO.U.1 | TO | MN |
| TO.MN.L04-TO.U.1 | TO | MN |
| TO.MN.L06-TO.U.2 | TO | MN |
| TO.MN.L08-TO.U.5 | TO | MN |
| TO.MN.L09-TO.U.2 | TO | MN |
| TO.MN.L11-TO.U.3 | TO | MN |
| TO.MN.L05-TO.M.5 | TO | MN |
| TO.MN.L08-TO.M.5 | TO | MN |
| TO.PX.BUF.M.1 | TO | PX |
| TO.PX.ALA.M.2 | TO | PX |
| TO.PX.LAP.M.4 | TO | PX |
| TO.PX.WES.M.1 | TO | PX |
| TO.PX.CLA.M.1 | TO | PX |
| TO.PX.DF1.M.1 | TO | PX |
| TO.PX.DF2.M.1 | TO | PX |
| TO.PX.COY.M.1 | TO | PX |
| TO.PX.COY.M.6 | TO | PX |

## Step 7 - Metapopulation locus matching with `sstacks`

Files can be found in the `07_sstacks/` directory.

All samples in the population (or all samples you want to include in the analysis) are matched against the catalog produced in[`cstacks`] (#step-6—cstacks) with `sstacks`, run in script `stacks_SPECIES.sh` and `stacks_SPECIES_additional.sh`. It runs off of the samples based in the output directory *and* the listed samples in `sstacks_samples_SPECIES.txt` and `sstacks_samples_SPECIES_additional.txt` (respectively), so make sure all your files (sample and catalog, etc.) are there and match. `sstacks_samples_SPECIES.txt` takes the form:

```
DS.BA.GA.U.1
DS.BA.GA.U.2
DS.BA.GA.U.3
DS.BA.GA.U.4
DS.BA.GA.U.5
...
```

There should be a new file produced at this step for every sample in the output directory:

- `<samplename>.matches.tsv.gz`

## Step 8 - Metapopulation oriented by locus tsv2bam

Files can be found in the `08_tsv2bam/` directory.

`tsv2bam` and the proceeding programs in the pipeline use a populations map text file to specify which samples to include in the analysis. As such, a new population map (that differs from `06-cstacks_popmap.txt`) should be created that includes all samples (including those used to create your catalog) that you want to include in the analysis. This file will include the same samples specified in `sstacks_samples_SPECIES.txt` with a colomn specifying population. Here, this file is `popmap_SPECIES.txt`.

We run `tsv2bam` using the script `tsv2bam_SPECIES.sh`.

This is the step at which it's usually discovered that some samples are bad(don't have any useable matches to the catalog). These samples were excluded from `popmap_SPECIES.txt`. For example we might simply cut out the following rows:

```
DS.MN.L10-DS.M.4    Minneapolis
DS.MN.L01-DS.M.4    Minneapolis
DS.BO.WL2.M.4   Boston
```

The following samples were discarded because they contained less than 300 sample loci matched to catalog loci:

Table 3: Samples discarded at the `tsv2bam` stage of the Stacks pipeline.

| Sample | City |
|---|---|
| CD.BA.RG-1.M.4 | Baltimore |
| CD.BA.RG-1.M.5 | Baltimore |
| CD.BA.DHI.U.2 | Baltimore |
| CD.BA.DHI.U.3 | Baltimore |
| DS.MN.L04-DS.U.2 | Minneapolis |
| DS.BO.WL3.M.4 | Boston |
| DS.BO.WL1.M.4 | Boston |
| DS.BO.LC1.M.2 | Boston |
| DS.BO.HC4.M.4 | Boston |
| EC.BO.R4.U.1 | Boston |
| LS.PX.PAL.M.5 | Phoenix |
| LS.PX.PAL.M.2 | Phoenix |
| LS.PX.PAL.M.1 | Phoenix |
| LS.MN.L01-LS.M.1 | Minneapolis |
| LS.PX.PKW.U.3 | Phoenix |
| LS.PX.ENC.M.2 | Phoenix |
| LS.MN.L01-LS.U.1 | Minneapolis |
| LS.LA.ARR.U.4 | Los Angeles |
| LS.BO.WL2.M.3 | Boston |
| LS.BO.WL2.M.1 | Boston |
| LS.BO.R4.U.4 | Boston |
| LS.BO.R4.U.2 | Boston |
| LS.BO.R4.U.1 | Boston |
| LS.BO.R3.U.2 | Boston |
| LS.BO.R2.U.1 | Boston |
| LS.BO.LC4.M.5 | Boston |
| LS.BO.LC2.M.1 | Boston |
| LS.BO.I4.U.1 | Boston |
| LS.BO.HC4.M.4 | Boston |
| LS.BO.HC4.M.2 | Boston |

| Sample | City |
|---|---|
| LS.BA.WB.U.5 | Baltimore |
| PA.BA.AA.U.3 | Baltimore |
| PA.BA.AA.U.4 | Baltimore |
| PA.BA.LH-3.M.1 | Baltimore |
| PA.BA.LH-3.M.4 | Baltimore |
| PA.BA.LL-4.M.1 | Baltimore |
| PA.BA.RG-1.M.1 | Baltimore |
| PA.BO.HC2.M.4 | Boston |
| PA.BO.LC2.M.3 | Boston |
| PA.BO.LC2.M.4 | Boston |
| PA.BO.R3.U.3 | Boston |
| PA.PX.ALA.M.2 | Phoenix |
| PA.PX.ALA.M.3 | Phoenix |
| PA.PX.RPP.U.1 | Phoenix |
| PA.PX.RPP.U.2 | Phoenix |
| TO.BA.DHI.U.5 | Baltimore |
| TO.BA.TRC.U.1 | Baltimore |
| TO.BA.TRC.U.2 | Baltimore |
| TO.BA.TRC.U.3 | Baltimore |
| TO.BO.R2.U.2 | Boston |
| TO.BO.R4.U.1 | Boston |
| TO.BO.R4.U.2 | Boston |

## Step 9 - Metapopulation SNP calling with `gstacks`

Files can be found in the `09_gstacks/` directory.

The script `gstacks_SPECIES.sh` also uses the population map specified in Step 8, `popmap_SPECIES.txt`. The `gstacks` program aligns the paired-end reads and assembles the paired-end contigs and then calls SNPs.

Produces the following:

- `catalog.fa.gz` : consensus catalog loci, contains the consensus sequence for each locus as produced by `gstacks` in a standard FASTA file
- `catalog.calls` : per-nucleotide genotypes, contains the output of

## Step 10 - Metapopulation summaries with `populations`

Files can be found in the `10_populations/` directory.

The populations program will use the script `species_populations.sh` and the population maps specified in Step 9 (`popmap_SPECIES.txt`) to calculate population-level summary statistics. Specifically, the script iterates through all species and several levels of the parameter `--min-samples-overall`, the minimum percentage of individuals across populations required to process a locus

You will most likely run the populations program multiple times if you are looking at different 'sub-populations'. A new directory should be created and the population program should run out of that directory for each iteration of the population program. Alternativley, you can specify a new directory as the output folder in the script using the command `-O`.

Ultimately, the key parameters we used for running `populations` were as follows:

- `--write-random-snp`: restrict data analysis to one random SNP per locus
- `--min-samples-overall`: 20 - locus must be in 20% of individuals

- `--min-maf`: 0.05 - minimum minor allele frequency required to process a nucleotide site at a locus

## Step 11 - Examine Within-city Catalogs and Populations

Files can be found in the `11_city_catalogs/` directory.

For any given species within city, there is likely to be a slightly different set of SNPs compared to the whole metapopulation of five cities. We examined 24 sets of species-city combinations. Within the folder `11_city_catalogs` there are 24 folders:

```
CD_BA_22_09_14
CD_LA_22_09_14
CD_PX_22_09_14
DS_BA_22_09_14
DS_BO_22_09_14
DS_MN_22_09_14
DS_PX_22_09_14
EC_BA_22_09_14
EC_LA_22_09_14
EC_PX_22_09_14
LS_BA_22_09_14
LS_BO_22_09_14
LS_LA_22_09_14
LS_MN_22_09_14
LS_PX_22_09_14
PA_BA_22_09_14
PA_BO_22_09_14
PA_LA_22_09_14
PA_PX_22_09_14
TO_BA_22_09_14
TO_BO_22_09_14
TO_LA_22_09_14
TO_MN_22_09_14
TO_PX_22_09_14
```

All folders contain a script and set of samples used to build the species-city catalog. Because parameter search had already been performed at the metapopulation level, we ran all species-city combinations using `denovo_map.pl`. Input samples came from step 4. For example, input files should look like this:

```
CD.BA.AA.U.1.1.fq.gz
CD.BA.AA.U.1.2.fq.gz
CD.BA.AA.U.1.rem.1.fq.gz
CD.BA.AA.U.1.rem.2.fq.gz
```

A population map is also included within each directory to ensure only appropriate samples (i.e., ok coverage) are included in the catalog.

### Step 11b - City Level Population Analysis

The city-level populations program will use the script `11-city_catalogs/11-city_populations.sh`. The goal of this script is to run the Stacks function `populations` on each city-species combination while toggling the `--min-samples-overall` option. Toggling this option allows us to hone in on a reasonable number of SNPs while also getting rid of SNPs with a lot of missing data, therefore creating a more robust population structure snapshot. As described in step 11, each city-species combination with adequate data is represented by a folder. The `11-city_populations.sh` script iterates through folders, within which it runs several `--min-samples-overall` levels and outputs each parameter permutation to its own new directory. This script uses the `11-city_catalogs/11-city_catalog_names.txt` to iterate rather than doing all folders by default. This is convenient if you only want to iterate through a subset of the folders (city-species combinations).

# File Organization

All data files for the Macrosystems project are permanently stored under Meghan Avolio's group resources in the Johns Hopkins University Rockfish computing cluster. Files are stored under the 'data' directory under the following subdirectories:

- **`01-raw_data`**: This folder contains the raw, unprocessed data files that were obtained directly from the sequencing server. There are eight `fastq.gz` files per sublibrary that correspond to the four sequencing lanes for each read direction.
- **`02-concatenated_data`**: This folder contains the concatenated, unprocessed files for each sublibrary (i.e., the files containing the sequences for each lane were combined to create one file per read direction).
- **`03-pcr_filtered_data`**: Here, you will find the resulting data files from the `clone_filter` program, where pcr replicates/clones have been removed from the raw sequences. There are two `fq.gz` files per sublibrary.
- **`04-process_radtags`**: This folder contains various subdirectories that correspond to the `process_radtags` program that demultiplexes and cleans the data. The `demux_txt_files` folder contains the .txt files used to identify barcodes and separate out the individual samples from each sublibrary. The resulting data files from the `process-radtags` program are separated by individual and can be found in the relevant species folder(i.e., CD, DS, EC, LS, PA, TE, TO). Each individual sample has four data files; `sampleID.1.fq.gz` and `sampleID.2.fq.gz` correspond to the forward and reverse reads for each sample and `sampleID.rem,1/2.fq.gz` contain the remainder reads that were cleaned and removed from the data sequence.
- **`05-ustacks-denovo_data`**: This folder contains species subdirectories that store the resulting data files from the `ustacks` program for each individual. There are three files per individual; `sampleID.allelles.tsv.gz`, `sampleID.snps.tsv.gz`, and, `sampleID.tags.tsv.gz`. These files should be permanently stored here and copied to a new directory for any new catalogs and/or when a group of samples are being aligned to a new catalog.
- **`catalogs_by_city`**: For any given species within city, there is likely to be a slightly different set of SNPs compared to the whole metapopulation of five cities. We examined 24 sets of species-city combinations. These catalogs are permanently stored here.
- **`catalogs_by_species`**: Metapopulation catalogs are stored within this folder for each species. The metapopulation catalog was created using samples from all populations to create a national catalog.

  Some notes about catalog directories:

  - Catalogs contain three files; `catalog.alleles.tsv.gz`, `catalog.snps.tsv.gz`, and `catalog.tafs.tsv.gz`. If you would like to use the catalog on a new project, you will need to copy all three files to a new project folder.
  - You can determine which individuals were used to create the catalog by looking at the `cstacks_popmap.txt` found within each folder. Specifically for the metapopulation catalogs, this information is also found in the cstacks-metapop-catalog_samples-included.csv
  - You can determine which individuals were subsequently aligned to the catalog and used in the subsequent stacks analysis by looking at the `popmap*.txt` found within each folder.
  - Each folder also contains the relevant ustacks and stacks pipeline scripts and output files (i.e., from `cstacks`, `gstacks`, `stacks`, `tsv2bam`, and `populations`),

# Appendix

## Aspera Transfer File Names

```
/Hoffman_macrosystems/AMH_macro_1_1_12px_S1_L001_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_1_12px_S1_L001_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_1_12px_S1_L002_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_1_12px_S1_L002_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_1_12px_S1_L003_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_1_12px_S1_L003_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_1_12px_S1_L004_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_1_12px_S1_L004_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_10_8px_S10_L001_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_10_8px_S10_L001_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_10_8px_S10_L002_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_10_8px_S10_L002_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_10_8px_S10_L003_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_10_8px_S10_L003_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_10_8px_S10_L004_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_10_8px_S10_L004_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_1_11_8px_S11_L001_R1_001.fastq.gz
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/Hoffman_macrosystems/AMH_macro_9_5_8px_S117_L001_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_5_8px_S117_L002_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_5_8px_S117_L002_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_5_8px_S117_L003_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_5_8px_S117_L003_R2_001.fastq.gz
```

```
/Hoffman_macrosystems/AMH_macro_9_5_8px_S117_L004_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_5_8px_S117_L004_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_6_8px_S118_L001_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_6_8px_S118_L001_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_6_8px_S118_L002_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_6_8px_S118_L002_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_6_8px_S118_L003_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_6_8px_S118_L003_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_6_8px_S118_L004_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_6_8px_S118_L004_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_7_8px_S119_L001_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_7_8px_S119_L001_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_7_8px_S119_L002_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_7_8px_S119_L002_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_7_8px_S119_L003_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_7_8px_S119_L003_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_7_8px_S119_L004_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_7_8px_S119_L004_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_8_8px_S120_L001_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_8_8px_S120_L001_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_8_8px_S120_L002_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_8_8px_S120_L002_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_8_8px_S120_L003_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_8_8px_S120_L003_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_8_8px_S120_L004_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_8_8px_S120_L004_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_9_12px_S121_L001_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_9_12px_S121_L001_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_9_12px_S121_L002_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_9_12px_S121_L002_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_9_12px_S121_L003_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_9_12px_S121_L003_R2_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_9_12px_S121_L004_R1_001.fastq.gz
/Hoffman_macrosystems/AMH_macro_9_9_12px_S121_L004_R2_001.fastq.gz
```

## `clone_filter` File Names

```
AMH_macro_1_1_12px_S1
AMH_macro_1_10_8px_S10
AMH_macro_1_11_8px_S11
AMH_macro_1_12_8px_S12
AMH_macro_1_13_8px_S13
AMH_macro_1_14_8px_S14
AMH_macro_1_2_12px_S2
AMH_macro_1_3_12px_S3
AMH_macro_1_4_12px_S4
AMH_macro_1_5_8px_S5
AMH_macro_1_6_8px_S6
AMH_macro_1_7_8px_S7
AMH_macro_1_8_8px_S8
AMH_macro_1_9_8px_S9
AMH_macro_10_1_8px_S127
AMH_macro_10_10_12px_S136
AMH_macro_10_11_12px_S137
```

```
AMH_macro_10_12_12px_S138
AMH_macro_10_13_8px_S139
AMH_macro_10_14_8px_S140
AMH_macro_10_2_8px_S128
AMH_macro_10_3_8px_S129
AMH_macro_10_4_8px_S130
AMH_macro_10_5_8px_S131
AMH_macro_10_6_8px_S132
AMH_macro_10_7_8px_S133
AMH_macro_10_8_8px_S134
AMH_macro_10_9_12px_S135
AMH_macro_11_1_8px_S141
AMH_macro_11_10_12px_S150
AMH_macro_11_11_12px_S151
AMH_macro_11_12_12px_S152
AMH_macro_11_13_8px_S153
AMH_macro_11_14_8px_S154
AMH_macro_11_2_8px_S142
AMH_macro_11_3_8px_S143
AMH_macro_11_4_8px_S144
AMH_macro_11_5_8px_S145
AMH_macro_11_6_8px_S146
AMH_macro_11_7_8px_S147
AMH_macro_11_8_8px_S148
AMH_macro_11_9_12px_S149
AMH_macro_12_1_8px_S155
AMH_macro_12_10_12px_S164
AMH_macro_12_11_12px_S165
AMH_macro_12_12_12px_S166
AMH_macro_12_13_8px_S167
AMH_macro_12_14_8px_S168
AMH_macro_12_2_8px_S156
AMH_macro_12_3_8px_S157
AMH_macro_12_4_8px_S158
AMH_macro_12_5_8px_S159
AMH_macro_12_6_8px_S160
AMH_macro_12_7_8px_S161
AMH_macro_12_8_8px_S162
AMH_macro_12_9_12px_S163
AMH_macro_13_1_8px_S169
AMH_macro_13_10_8px_S178
AMH_macro_13_11_8px_S179
AMH_macro_13_12_8px_S180
AMH_macro_13_2_8px_S170
AMH_macro_13_3_8px_S171
AMH_macro_13_4_8px_S172
AMH_macro_13_5_8px_S173
AMH_macro_13_6_8px_S174
AMH_macro_13_7_8px_S175
AMH_macro_13_8_8px_S176
AMH_macro_13_9_8px_S177
AMH_macro_14_1_8px_S181
AMH_macro_14_10_8px_S190
AMH_macro_14_11_8px_S191
```

AMH_macro_14_12_8px_S192
AMH_macro_14_13_4px_S193
AMH_macro_14_2_8px_S182
AMH_macro_14_3_8px_S183
AMH_macro_14_4_8px_S184
AMH_macro_14_5_8px_S185
AMH_macro_14_6_8px_S186
AMH_macro_14_7_8px_S187
AMH_macro_14_8_8px_S188
AMH_macro_14_9_8px_S189
AMH_macro_2_1_12px_S15
AMH_macro_2_10_8px_S24
AMH_macro_2_11_8px_S25
AMH_macro_2_12_8px_S26
AMH_macro_2_13_8px_S27
AMH_macro_2_14_8px_S28
AMH_macro_2_2_12px_S16
AMH_macro_2_3_12px_S17
AMH_macro_2_4_12px_S18
AMH_macro_2_5_8px_S19
AMH_macro_2_6_8px_S20
AMH_macro_2_7_8px_S21
AMH_macro_2_8_8px_S22
AMH_macro_2_9_8px_S23
AMH_macro_3_1_12px_S29
AMH_macro_3_10_8px_S38
AMH_macro_3_11_8px_S39
AMH_macro_3_12_8px_S40
AMH_macro_3_13_8px_S41
AMH_macro_3_14_8px_S42
AMH_macro_3_2_12px_S30
AMH_macro_3_3_12px_S31
AMH_macro_3_4_12px_S32
AMH_macro_3_5_8px_S33
AMH_macro_3_6_8px_S34
AMH_macro_3_7_8px_S35
AMH_macro_3_8_8px_S36
AMH_macro_3_9_8px_S37
AMH_macro_4_1_12px_S43
AMH_macro_4_10_8px_S52
AMH_macro_4_11_8px_S53
AMH_macro_4_12_8px_S54
AMH_macro_4_13_8px_S55
AMH_macro_4_14_8px_S56
AMH_macro_4_2_12px_S44
AMH_macro_4_3_12px_S45
AMH_macro_4_4_12px_S46
AMH_macro_4_5_8px_S47
AMH_macro_4_6_8px_S48
AMH_macro_4_7_8px_S49
AMH_macro_4_8_8px_S50
AMH_macro_4_9_8px_S51
AMH_macro_5_1_8px_S57
AMH_macro_5_10_8px_S66

AMH_macro_5_11_8px_S67
AMH_macro_5_12_8px_S68
AMH_macro_5_13_8px_S69
AMH_macro_5_14_8px_S70
AMH_macro_5_2_8px_S58
AMH_macro_5_3_8px_S59
AMH_macro_5_4_8px_S60
AMH_macro_5_5_12px_S61
AMH_macro_5_6_12px_S62
AMH_macro_5_7_12px_S63
AMH_macro_5_8_12px_S64
AMH_macro_5_9_8px_S65
AMH_macro_6_1_8px_S71
AMH_macro_6_10_8px_S80
AMH_macro_6_11_8px_S81
AMH_macro_6_12_8px_S82
AMH_macro_6_13_8px_S83
AMH_macro_6_14_8px_S84
AMH_macro_6_2_8px_S72
AMH_macro_6_3_8px_S73
AMH_macro_6_4_8px_S74
AMH_macro_6_5_12px_S75
AMH_macro_6_6_12px_S76
AMH_macro_6_7_12px_S77
AMH_macro_6_8_12px_S78
AMH_macro_6_9_8px_S79
AMH_macro_7_1_8px_S85
AMH_macro_7_10_8px_S94
AMH_macro_7_11_8px_S95
AMH_macro_7_12_8px_S96
AMH_macro_7_13_8px_S97
AMH_macro_7_14_8px_S98
AMH_macro_7_2_8px_S86
AMH_macro_7_3_8px_S87
AMH_macro_7_4_8px_S88
AMH_macro_7_5_12px_S89
AMH_macro_7_6_12px_S90
AMH_macro_7_7_12px_S91
AMH_macro_7_8_12px_S92
AMH_macro_7_9_8px_S93
AMH_macro_8_1_8px_S99
AMH_macro_8_10_8px_S108
AMH_macro_8_11_8px_S109
AMH_macro_8_12_8px_S110
AMH_macro_8_13_8px_S111
AMH_macro_8_14_8px_S112
AMH_macro_8_2_8px_S100
AMH_macro_8_3_8px_S101
AMH_macro_8_4_8px_S102
AMH_macro_8_5_12px_S103
AMH_macro_8_6_12px_S104
AMH_macro_8_7_12px_S105
AMH_macro_8_8_12px_S106
AMH_macro_8_9_8px_S107

AMH_macro_9_1_8px_S113
AMH_macro_9_10_12px_S122
AMH_macro_9_11_12px_S123
AMH_macro_9_12_12px_S124
AMH_macro_9_13_8px_S125
AMH_macro_9_14_8px_S126
AMH_macro_9_2_8px_S114
AMH_macro_9_3_8px_S115
AMH_macro_9_4_8px_S116
AMH_macro_9_5_8px_S117
AMH_macro_9_6_8px_S118
AMH_macro_9_7_8px_S119
AMH_macro_9_8_8px_S120
AMH_macro_9_9_12px_S121