

# 2bRAD *de novo* scripts: a manual

October 14, 2014 – September 27, 2015

These are the printouts from scripts when run without arguments, in order of their appearance in the walkthrough.

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## **ngs\_concat.pl :**

concatenates files by matching pattern in their names

arg1: common pattern for files

arg2: perl-like pattern in the filename to recognize,  
use brackets to specify the unique part, as in  
"FilenameTextImmediatelyBeforeSampleID(.+)FilenameTextImmediatelyAfterSampleID"

Example (to concatenate files names like Sample\_Pop1\_L1.fastq, Sample\_Pop1\_L2.fastq):  
ngs\_concat.pl 'Sample' 'Sample\_(.+)\_L'

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## **2bRAD\_trim\_launch\_dedup.pl :**

Prints out list of calls to trim2bRAD\_2barcodes\_dedup.pl, one for each fastq file, to to trim and deduplicate raw 2bRAD reads.

See help for trim2bRAD\_2barcodes\_dedup.pl for more details.

prints to STDOUT

Arguments:

arg1, required: glob to fastq files

site=[pattern] perl-style pattern of the restriction site to recognise, in single quotes.

default is BcgI: \'.{12}CGA.{6}TGC.{12}I.{12}GCA.{6}TCG.{12}\'

if you need Alfi, here is how to define it: \'.{12}GCA.{6}TGC.{12}\'

adaptor=[dna sequence] adaptor sequence to look for on the far end of the read.

Default AGATC

sampleID=[integer] the position of name-deriving string in the file name

if separated by underscores, such as:

for input file Sample\_RNA\_2DVH\_L002\_R1.cat.fastq

specifying arg2 as \'3\' would create output

file with a name \'2DVH.trim'

barcode2=[integer] length of the in-line barcode immediately following  
the restriction fragment. Default 0.

Example:

2bRAD\_trim\_launch\_dedup.pl fastq sampleID=3 > trims

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**trim2bRAD\_2barcodes\_dedup.pl :**

This script does three things:

- Filters 2bRAD fastq reads to leave only those with a 100% matching restriction site, degenerate 5'-leader, secondary 3'-barcode, and adaptor on the far 3'end, trims away everything except the IIB-fragment itself;
- Deduplicates: removes all but one read sharing the same 64-fold degenerate leader, the first 34 bases of the insert sequence, and secondary barcode (this results in 128-fold dynamic range: 64-fold degeneracy x 2 strand orientations);
- Splits reads into separate files according to secondary barcode.

Writes trimmed fastq files named according to the secondary barcodes detected.

Arguments:

input=[fastq filename]

site=[perl-style pattern for restrictase site]

Default is BcgI: \'.{12}CGA.{6}TGC.{12}I.{12}GCA.{6}TCG.{12}\'

bc2=[perl-style barcode pattern] in-line barcode that immediately follows the RAD fragment, default \'[ATGC]{4}\'

adaptor=[far-end adaptor sequence] default AGATC

clip=[integer] number of bases to clip off the ends of the reads, default 0

minBCcount=[integer] minimum count per in-line barcode to output a separate file.

Default 100000.

sampleID=[integer] the position of name-deriving string in the file name

if separated by underscores, such as:

for input file Sample\_RNA\_2DVH\_L002\_R1.cat.fastq

specifying arg2 as \'3\' would create output

file with a name \'2DVH.trim\'

Example:

trim2bRAD\_2barcodes\_dedup.pl input=Myproject\_L8\_G3.fastq sampleID=2

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**uniquerOne.pl :**

Makes unquid 2bRAD read file for a single fastq file.

This is analogous to making 'stacks' in STACKS.

The script records unique tag sequences, the total

number of their appearances, and how many of those

were in reverse-complement orientation.

(STACKS would consider reverse-complements as separate loci)

prints to STDOUT

arg1: fastq filename

Example:

uniquerOne.pl G4\_TCAG.trim >G4\_TCAG.trim.uni

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### **mergeUniq.pl :**

Merges individual uniq files produced by uniquerOne.pl into one table.

arg1: common extension of uniq files

minDP=[integer] minimum sequencing depth to consider a unique tag. Default 5.

prints to STDOUT

Example:

```
mergeUniq.pl uni >mydataMerged.uniq
```

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### **uniq2loci.pl :**

assembles locus-annotated table from uniquer and cd-hit results

filters by read depth and strand bias (to avoid memory issues in genotype calling)

flips orientation of cluster members to match the most abundant tag in a cluster

saves cluster members seen just once and their references (singl.tab file) to use for error model development (requires previous running of mergeUniq.p with minDP=1)

arg1: uniquer file

arg2: cd-hit .clstr file

arg3: cd-hit .fasta file

minDP=[integer] minimum read depth (total counts for the tag in the whole dataset).

Default: 5

minSB=[integer] strand bias cutoff ( min(direct:reverse,reverse:direct)x100 ).

Default: 5

prints to STDOUT

Example:

```
uniq2loci.pl mydataMerged.uniq cdh_alltags.fas.clstr cdh_alltags.fas >cdh_alltags.ul
```

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### **haplocall\_denovo.pl :**

Calls genotypes (as population-wide RAD-tag haplotypes) in de novo 2bRAD data.

Outputs two files in VCF v.4 format:

"Vhap": whole RAD tags recoded as alternative alleles (for coalescent analysis and methods that take advantage of multi-allelic markers);

"Variants": RAD tags broken into individual SNPs, within-tag phase recorded (for methods requiring biallelic markers).

The QUAL field in VCF files is allele bias, the average fraction of minor allele counts in a heterozygote. Ideally this should be close to 50, more if the allele is found in homozygotes as well.

Recorded variant info fields:

SB : Strand bias (ratio x100)

AB : Minor allele bias in a heterozygote (ratio x100)

TP : SNP position in the tag

NS : Number of samples with data

DP : Combined read depth

AF : Allele frequency

Individual genotype fields:

GT : Genotype  
GQ : Phred-scaled genotype quality  
PS : Phase set (for multiple SNPs within a RAD tag)  
AD : Allele depth  
DP : Read depth

Homozygote quality is calculated as phred-scaled  
 $p(\text{error}) = 0.5^{(n-1)}$ , where  $n$  is the number of reads;  
Heterozygote quality is the same plus 20 on phred-scale  
(we are very sure about our heterozygotes, see allele filters below)

The ends of tags with more than one SNP in the terminal 3 bases  
are clipped off since such SNPs are most likely due to indel near the  
end of the tag.

Required arguments and defaults:

arg1                    input file (produced by uniq2loci.pl)

Optional arguments:

clip=0                number of bases to clip off the ends of reads

----- Allele filters -----

aobs=5                min number of times an allele must be observed across all samples.

strbias=10            strand bias cutoff: minimal ratio between reverse and  
                      direct reads, x100. 0 would mean no filtering.

abias=10              allele bias cutoff: minimal fraction of reads per locus corresponding  
                      to the candidate allele, averaged across individuals  
                      containing the allele, x100.

ind=2                 minimum number of individuals in which a candidate new allele  
                      has to be found.

----- Locus filters -----

found=0.25            fraction of individuals in which a locus has to be genotyped.  
                      Filter for this parameter later with vcftools.

hetero=0.5            maximum allowed fraction of heterozygotes. Guards against lumped  
paralogous loci.

mono=toss             whether to keep non-polymorphic loci. Specify mono=keep to keep them  
                      (if coalescent analysis is planned downstream).

----- Genotype filters -----

mindp=3               minimum depth to call a homozygote.

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Example: haplocalld\_denovo.pl cdh\_alltags.ul

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**replicatesMatch.pl** : (version 0.6, September 13 2015)

Selects polymorphic variants that have identical genotypes among replicates.

Genotypes of two replicates should be identical or missing; the maximal fraction of genotype pairs with missing genotypes is controlled by `missing=` argument.

Arguments:

`vcf=[file name]` input vcf file  
`replicates=[file name]` - a two column tab-delimited table listing pairs of samples that are replicates (at least 3 pairs)  
  
`matching=[float]` required fraction of matching genotypes (missing counts as match if there are other non-missing matching pairs), default 1 (all must match)  
  
`missing=[float]` allowed fraction of missing genotypes, default 0.25  
  
`altPairs=2` minimal number of matching genotypes involving non-reference alleles.  
  
`hetPairs=0` minimal number of matching heterozygotes.  
  
`max.het=0.5` maximum fraction of heterozygotes among non-missing genotypes (guards against lumped paralogous loci).  
  
`polyonly=[1|0]` extract only polymorphic sites. Default 0.

Example:

`replicatesMatch.pl vcf=round2.vcf replicates=clonepairs.tab > vqsr.vcf`

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**recalibrateSNPs.pl** :

Non-parametric variant quality recalibration based on INFO fields in the set of variants that are reproducibly genotyped among replicates (output of `replicatesMatch.pl`)

This script is for de novo pipeline. Use `recalibrateSNPs_gatk.pl` for reference-based pipeline.

Three parameters (INFO items) are assessed for each SNP:

- total coverage depth (DP)
- strand bias (SB)
- allele bias (AB)
- SNP position withn tag (TP)

For AB and SB, low values are deemed bad and high ones are good; for DP and TP, both low and high values are considered bad, the best values being around the median.

The script computes the product of quantiles for different INFO fields, determines the quantiles of the result within the 'true' set (JOINT quantiles), and then computes the new quality scores in the main vcf file.

These scores are supposed to correspond to the probability (x100) that the SNPs comes from the same distribution as the 'true' SNPs.

Output:

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- Recalibrated VCF (printed to STDOUT) with QUAL field replaced by the new score minus 0.1 (for easy filtering)
- a table (printed to STDERR) showing the \"gain\" at each recalibrated quality score setting, which is excess variants removed when filtering at this quality score. For example, if 40% of all variants are removed at the quality score 15, the gain is  $40 - 15 = 35\%$  (i.e., in addition to removing 15% of the 'true' variants, additional 35% of the dataset, likely corresponding to wrong variants, is removed). The optimal filtering score is the one giving maximum gain. Try different combinations of the four possible filters to find the one maximizing the gain.

Arguments:

vcf=[file name] : vcf file to be recalibrated

true=[file name] : vcf file that is subset of the above, with SNPs that are considered true because they show matching and polymorphic genotypes in

replicates  
(replicatesMatch.pl polyonly=1).

-nodp : do not use DP  
-noab : do not use AB  
-nosb : do not use SB  
-notp : do not use TP

Example:

recalibrateSNPs.pl vcf=cdh\_alltags.ul\_Vhap\_count10\_ab10\_sb10\_clip0.vcf \  
true=vqsr.vhap.vcf -nosb -notp >denovo.vhap.recal.vcf

#### **filterStats.pl :**

Calculates quantiles for three INFO fields in a VCF file:

- total coverage depth (DP)
- strand bias (SB)
- allele bias (AB)

For AB and SB, low values are bad and high values are good;  
for DP, both very low and very high values are bad, the best values  
should be around the median.

Arguments:

vcf=[file name] vcf file to analyze

Output:

Tables of quantiles (printed to STDOUT).

Example:

filter\_stats.pl vcf=alltags.ul\_Variants\_count10\_ab10\_sb10\_clip0.vcf

NOTE: only use this to select your filtering criteria if you don't have  
genotyping replicates.

### **repMatchStats.pl :**

Summarizes genotypic match between replicates in a vcf file.

Arguments:

vcf=[file name] : input vcf file  
replicates=[file name] : a two column tab-delimited table listing  
pairs of samples that are replicates

Output:

A table (printed to STDOUT) of the following form:

pair	gtyped	match	[ 00 01 11 ]	HetMatch	HomoHetMism	HetNoCall	HetsDiscRate
K210:K212	7328	7169(97.8%)	[ 78% 17% 5% ]	1200	139	5	0.94
K212:K213	7369	7117(96.6%)	[ 78% 17% 5% ]	1202	179	4	0.93

The first four columns are self-evident;  
the last four columns show how good is the match between heterozygote calls  
(HetNoCall being a heterozygote in one and missing data in another replicate).  
The last column is the most important: it is the heterozygote discovery rate,  
the fraction of all heterozygotes discovered in each replicate.

Example: repMatchStats.pl vcf=denovo.filt.recode.vcf replicates=clonepairs.tab

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### **thinner.pl (v.4.2) :**

Leaves only one SNP per specified nucleotide distance interval, chosen by its  
allele frequency, sequencing depth, combination of those, or randomly.

The minimal distance between selected SNPs is half the specified interval.

The thinning process uses random starts for interval specification, potentially  
resulting in different SNPs selected.

Arguments:

vcf=[file name] : vcf file name  
  
interval=[integer] : interval length, default 80 (for 2bRAD tags)  
  
criterion=[maxAF|maxDP-random|maxDP-maxAF|random] : SNP choosing criterion.  
maxAF - maximum minor allele frequency;  
maxDP-random - randomly selected from those with maximum  
sequencing depth (for dadi);  
maxDP-maxAF - maximum allele frequency among those with  
maximum sequencing depth (default);  
random - random selection.

Output: thinned VCF, printed to STDOUT

Example: thinner.pl vcf=denovo.recal.vcf > thinDenov.vcf

NOTES: - Do not thin variants if you want to calculate pi or Tajima's D.

### **hetfilter.pl :**

Filters out loci with too many heterozygotes, to guard against lumped paralogous loci. Also filters out loci with too many missing genotypes.

Arguments:

vcf=[file name] input vcf file  
maxmiss=[float] allowed fraction of missing genotypes, default 0.5.  
maxhet=[float] maximum fraction of heterozygotes among non-missing genotypes.  
Default 0.75

Prints to STDOUT

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### **vcf2genepop.pl :**

converts VCF to multiallelic GENEPOP, preserves chromosome and position info

Arguments:

vcf=[filename] : vcf file to convert  
pops=[list] : comma-separated perl patterns to determine population  
affiliation of samples in the VCF file based on sample names

Output:

GENEPOP formatted genotypes, printed to STDOUT

Example:

vcf2genepop.pl vcf=filtered.vcf pops=O,K,M,S > filtered.gen

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### **vhap2migrate.pl :**

converts \"Vhap\" vcf file (produced by haplocalldenoovo) to MIGRATE-n sequence input (make sure you keep monomorphic loci when running haplocalldenoovo: mono=keep)

Arguments:

arg1 : input vhap vcf file  
arg2 (optional) : comma-delimited list of files listing samples from specific  
populations

Output:

MIGRATE-n formatted genotypes (printed to STDOUT)

Example:

vhap2migrate.pl denovo.vhap.filt.recode.vcf keppel.pop,orpheus.pop,maggi.pop

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### **vhap2fasta.pl :**

extracts reference 2bRAD tags from a vcf \"Vhap\" file (produced by haplocalldenoovo.pl) in fasta format

Arguments:

arg1: vhap.vcf file name  
altalleles=[0|1] whether to output alternative alleles. Default 0.

prints to STDOUT



**vcf2dadi.pl :**

Converts VCF format to dadi data format  
(requires bioperl)

Usage: perl vcf2dadi.pl <genome file> <vcf file> <list file>

Genome file is in fasta format;  
List file gives population designations, like this:

```
BEGIN
sample1    population1
sample2    population1
sample3    population2
END
```