SEQLinkage Documentation [Version 1.0 alpha]

Gao Wang and Di Zhang

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Contents

Chapter 1

SEQLinkage Reference Manual

1.1 Introduction

This program implements a *combined haplotype pattern* (CHP) method to generate markers from sequence data for linkage analysis. The core concept is that instead of treating each variant as a separate marker, we create regional markers for variants in specified genetic regions (e.g. genes) based on haplotype patterns within families, and perform linkage analysis on markers thus generated. CHP method outperforms traditional single marker based approach for compound heterozygosity and allelic heterogeneity in genes. We recommend the use of CHP in conjunction with filtering based variant prioritization method in the analyses of sequence data of human pedigrees.

For more details on the methodology, please refer to our paper: ...

Web resource: please visit http://bioinformatics.org/seqlink for more information including download & installation instructions, software updates and supports from SEQLinkage user forum.

1.2 SEQLinkage Program Command Options

To display the command interface

```
seqlink -h
```

SEQLinkage interface

```
usage: seqlink [--bin FLOAT] [-b FILE] [--single-markers] --fam FILE --vcf
               FILE [--freq INFO] [-c P] [--chrom-prefix STRING] [-o Name]
               [-f FORMAT [FORMAT ...]] [-K FLOAT] [--moi STRING] [-W FLOAT]
               [-M FLOAT] [--theta-max FLOAT] [--theta-inc FLOAT]
               [--run-linkage] [--output-entries N] [-h] [-j N]
               [--tempdir PATH] [--cache]
        SEQLinkage, linkage analysis using sequence data
        [1.0.alpha]
Collapsed haplotype pattern method arguments:
  --bin FLOAT           Defines theme to collapse variants. Set to 0 for
                        "complete collapsing", 1 for "no collapsing", r2 value
                        between 0 and 1 for "LD based collapsing" and other
                        integer values for customized collapsing bin sizes.
                        Default to 0.8 (variants having r2 >= 0.8 will be
                        collapsed).
  -b FILE, --blueprint FILE
                        Blueprint file that defines regional marker (format:
                        "chr startpos endpos name avg.distance male.distance
                        female.distance").
  --single-markers      Use single variant markers. This switch will overwrite
                        "--bin" and "--blueprint" arguments.
Input / output options:
  --fam FILE            Input pedigree and phenotype information in FAM
                        format.
  --vcf FILE            Input VCF file, bgzipped.
  --freq INFO           Info field name for allele frequency in VCF file.
  -c P, --maf-cutoff P  MAF cutoff to define "common" variants to be excluded
                        from analyses.
  --chrom-prefix STRING
                        Prefix to chromosome name in VCF file if applicable,
                        e.g. "chr".
  -o Name, --output Name
                        Output name prefix.
  -f FORMAT [FORMAT ...], --format FORMAT [FORMAT ...]
                        Output format. Default to LINKAGE.
LINKAGE options:
  -K FLOAT, --prevalence FLOAT
                        Disease prevalence.
  --moi STRING          Mode of inheritance, AD/AR: autosomal
                        dominant/recessive.
  -W FLOAT, --wt-pen FLOAT
                        Penetrance for wild type.
  -M FLOAT, --mut-pen FLOAT
                        Penetrance for mutation.
  --theta-max FLOAT     Theta upper bound. Default to 0.5.
  --theta-inc FLOAT     Theta increment. Default to 0.05.
  --run-linkage         Perform Linkage analysis using FASTLINK program.
  --output-entries N    Write the highest N LOD/HLOD scores to output tables.
                        Default to 10.
Runtime arguments:
  -h, --help            Show help message and exit.
  -j N, --jobs N        Number of CPUs to use.
  --tempdir PATH        Temporary directory to use.
  --cache               Load cache data for analysis instead of starting
                        afresh.
  -q, --quiet           Disable the display of runtime MESSAGE.
        Copyright (c) 2013 - 2014 Gao Wang <gaow@bcm.edu> and Di Zhang <di.zhang@bcm.edu>
        Distributed under GNU General Public License
        Home page: http://bioinformatics.org/seqlink
```

1.2.1 Input files

- `--vcf` **[required]**

Input genotype data must be bgzipped [1] VCF file indexed by tabix [2]. To create such files from plain VCF file, e.g. `data.vcf`:

```
bgzip data.vcf
tabix -p vcf -f data.vcf.gz
```

You should end up with two files `data.vcf.gz` and `data.vcf.gz.tbi`. In SEQLinkage command you can then use `--vcf data.vcf.gz` to load the genotype data.

[1] bgzipped http://samtools.sourceforge.net/tabix.shtml
[2] tabix http://samtools.sourceforge.net/tabix.shtml

`--fam` [required]

This file contain information of pedigree structure, sample sex and disease status. It partially follows the LINKAGE format [3] convention: it has only 6 columns with each column being Family ID, Individual ID, Paternal ID, Maternal ID, Sex and Status.

`--blueprint` [default to RefSeq genes]

A "blueprint" file can be supplied to define regional marker units. SEQLinkage has a default built-in blueprint which is suitable for WES studies when it is desired to group variants to create regional markers by genes. Customized blueprint file can be provided by users for specific studies. Even for WES studies one can provide alternative blueprint based on exome sequencing capture targets rather than genes. The file should contain 7 columns:

- Chromosome name, without leading `chr` character, e.g. "5" not "chr5"
- Start position of the genetic region
- End position of the genetic region
- Region name, e.g. gene names
- Average genetic map distance of the region on average
- Female genetic map distance of the region on average
- Male genetic map distance of the region on average

Genetic map distance will be useful for performing multi-point linkage analysis. Users can output regional markers from SEQLinkage to, for example, Merlin format and perform linkage analysis using Merlin. In the built-in blueprint file we use the map distance of the variant at the median position of a genetic region as a substitute for the map distance of the genetic region. Such information can be interpolated using Rutgers Linkage-Physical Map [4] database. If multi-point linkage analysis is not the aim of your study you can leave these columns with a place holder symbol "." (a dot) for missing data in the blueprint file you provide to SEQLinkage. Example lines of a blue print file is shown below:

blueprint.txt

```
...
3        126111874        126113641        CCDC37-AS1        134.382        168.977        102.287
3        126113781        126155398        CCDC37        134.411        169.021        102.296
3        126156443        126194762        ZXDC        134.465        169.105        102.315
...
```

[3] LINKAGE format `http://www.jurgott.org/linkage/LinkagePC.html`
[4] Rutgers Linkage-Physical Map `http://compgen.rutgers.edu/maps`

1.2.2 Additional input options

- `--freq` **[default to sample MAF calculated from founders in data]**

Linkage analysis requires input of allele frequency for markers to control for type I error in the presence of missing genotypes. The `INFO` field name for population (minor) allele frequencies of variants in VCF file. For well defined populations we recommend using MAF for variants from publicly available data bases such as Exome Variant Server [5] or 1000 Genomes [6]. For variants not presented in these data bases it is safe to assign a very small proportion, e.g. 0.00015 which is roughly the MAF for a singleton variant in 3000 samples ($\frac{1}{3000\times 2} = 0.000167$). You may use other bioinformatics tools such as variant tools [7] to obtain and update such information to your VCF file. If this option is left unset, MAF estimated from founders in the sample will be used for linkage analysis.

- `--maf-cutoff` **[default to 1.0]**

When specified, variants having MAF (defined by `--freq` option) greater than this value will be excluded from analyses.

- `--chrom-prefix` **[default to empty]**

This option specifies the prefix to chromosome names in VCF file. For example for VCF files having chromosome names such as “1”, “5” and “X” there is no need to specify this option. For files having names such as “chr1”, “chr5” and “chrX” you need to use `--chrom-prefix chr` in SEQLinkage command.

1.2.3 Collapsed haplotype pattern method coding options

The CHP method has been described in the SEQLinkage paper (see “Introduction” section of this chapter). This section introduces the usage of parameters involved in implementing the CHP method.

- `--bin` **[default to $R^2 > 0.8$]**

This option defines the collapsing theme of variants in a genetic region, before computing haplotype patterns. Several collapsing themes are available via this option:

- “Linkage disequilibrium (LD) based collapsing”. The bin value takes a fraction number (between 0 and 1) as the R^2 cutoff to define LD blocks. Variant sites having LD greater than R^2 will be collapsed to binary codes.

[5] Exome Variant Server `http://evs.gs.washington.edu/EVS/`

[6] 1000 Genomes `http://www.1000genomes.org/`

[7] variant tools `http://varianttools.sourceforge.net`

- “No collapsing”. Set `--bin 1` which literally means collapsing variants by units of 1 variant site, i.e., no collapsing is applied to variants before computing haplotype patterns.
- “Complete collapsing”. Set `--bin 0` to collapse variant in the entire region to a single binary code.
- “Arbitrary collapsing”. Set `--bin` to any arbitrary positive integer value N to collapse N variants to a single binary code.

- `--single-markers` **[default to disabled]**

When this switch is turned on, single variant markers will be generated from data instead of regional markers, and both `--bin` and `--blueprint` options will be ignored.

1.2.4 Linkage analysis options

SEQLinkage has a built-in two-point linkage analysis routine to analyze data generated via the CHP method. Below are options for configuring linkage model parameters and producing graphic / HTML format analysis reports.

- `--prevalence` **[required]**

Disease prevalence.

- `--moi` **[required]**

Mode of inheritance, choose from “AD” (autosomal dominant) and “AR” (autosomal recessive).

- `--wt-pen` **[required]** / `--mut-pen` **[required]**

Penetrance of wild type / mutation.

- `--theta-max` **[default to 0.5]**

Recombination rate value upper bound (θ_{max}) up to which the linkage analysis will evaluate.

- `--theta-inc` **[default to 0.05]**

Increment steps from 0 to θ_{max}. At each step the θ value will be used to calculate a LOD score.

- `--run-linkage` **[default to disabled]**

When this switch is on, two-point linkage analysis will be performed.

- `--output-entries` **[default to 10]**

Output to HTML file the best N markers in terms of LOD and HLOD scores respectively. When $N = 0$, no heatmap graph or HTML file will be generated.

1.2.5 Format conversion options

SEQLinkage supports output in some population linkage software format including LINKAGE, Merlin and MEGA2. Many more linkage software format can be converted from MEGA2 format using the MEGA2 software. With the format conversion feature, CHP coding of sequence data can be written to these file formats for use in various linkage analysis software.

- `--format` **[default to LINKAGE]**

Output format for CHP coded data.

- `--output` **[default to LINKAGE]**

Output file / folder name prefix.

1.2.6 Runtime arguments

- `--jobs` **[default to 2]**

Number of CPUs to use for SEQLinkage. SEQLinkage supports analyzing many markers in parallel and the more CPUs it is assigned the shorter the computational time will be.

- `--tempdir` **[default to system temporary folder]**

The linkage analysis routine in SEQLinkage performs analysis per marker per family, thus involving frequent file I/O operations which can be a computational bottleneck. By default such I/O operations take place in one of the system temporary foldes, e.g. `/tmp`, `/var/tmp` in Linux system. To speed things up one can set the SEQLinkage temporary directory to some high speed hard drives, e.g. a solid state drive (SSD), or, if possible, a “RAM drive”. Below is an example to create a 5GB RAM drive in Linux:

```
sudo mkdir /tmp/ramdisk; sudo chmod 777 /tmp/ramdisk
sudo mount -t tmpfs -o size=5120M ramfs /tmp/ramdisk
```

With `--tempdir /tmp/ramdisk` option the newly created RAM drive will be used for the intensive file I/O in the analysis.

- `--cache` **[default to disabled]**

To speed up repeated runs of SEQLinkage on the same data set under similar parameter settings, data are archived to the `cache` folder under the work directory the first time SEQLinkage executes. With this switch on, SEQLinkage will used the archived data whenever appropriate to skip as many steps previously performed. For example in a repeated analysis under the same setting but only change `--output-entries` from 10 to 50, SEQLinkage will skip the CHP coding and linkage analysis step, only updating the result HTML table using archived analysis results.

Note that change of some input parameters will overwrite the effect of `--cache`. For example changing `--moi` will result in re-run of linkage analysis; changing `--vcf` or `--fam` input will result in re-run of CHP coding step.

- `--quiet` **[default to disabled]**

When this switch is on, the program will not display any log message during runtime. It will, however, display error message if an error occurs.

1.3 Linkage Analysis Results

SEQLinkage summarizes two-point linkage analysis results to heatmap plots and tables in HTML format, which can be viewed with a web browser program. Below is screenshot of output from SEQLinkage analysis.

1.3.1 Tables of LOD and HLOD scores

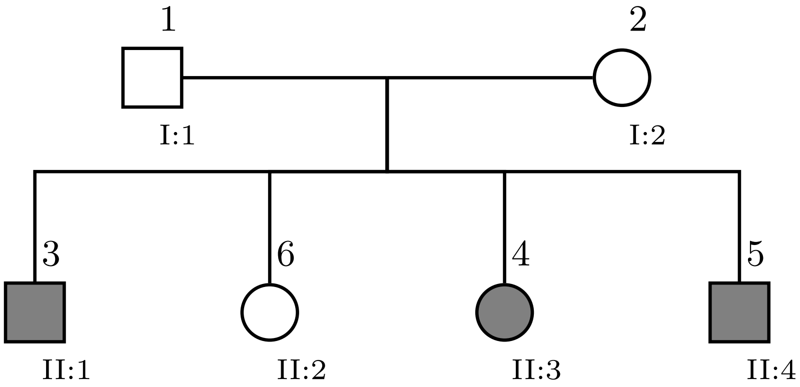
1.3.2 Heatmaps of LOD and HLOD scores

Chapter 2

Data Analysis Using SEQLinkage

2.1 Introduction

Here we demonstrate the use of SEQLinkage to generate regional markers from sequence data and perform linkage analysis. For demonstration purpose we will use a simulated example data set [1] of a nuclear family (see pedigree structure below) containing exome sequence data involving xx genes. From the phenotypic pattern it is reasonable to assume the disease follows a recessive mode of inheritance. We will first generate regional markers using CHP method with various collapsing themes, then perform two-point linkage analysis using regional markers. Finally we output markers to formats compatible with other linkage programs and perform non-parametric linkage analysis using Merlin.



2.2 Regional Markers from Sequence Data

2.2.1 Understanding terminal output and regional marker data

Here we perform a test run of SEQLinkage to generate regional marker data without performing linkage analysis. We (mostly) stick to default settings and examine the terminal output

[1] simulated example data set http://bioinformatics.org/seqlink/download/examples

generated by the program.

```
seqlink --fam seqlinkage-example.fam --vcf seqlinkage-example.vcf.gz -f MERLIN
```

▪ Terminal output

Command above generates the following output:

```
terminal info
MESSAGE: Binary trait detected in [/ramcache/slg/seqlinkage-example.fam]
MESSAGE: Checking local resources 5/5 ...
MESSAGE: 6 samples found in [/ramcache/slg/seqlinkage-example.vcf.gz]
MESSAGE: 1 families with a total of 6 samples will be scanned for 25,305 pre-defined units
MESSAGE: 15,657 units (from 145,908 variants) processed; 3,496 Mendelian inconsistencies and 6,482 recombination even
ts handled
MESSAGE: 994 tri-allelic loci were ignored
MESSAGE: 6,778 units ignored due to absence in VCF file
MESSAGE: 2,870 units ignored due to absence of variation in samples
MESSAGE: Archiving to directory [/ramcache/slg/cache]
MESSAGE: Generating MERLIN format output ...
MESSAGE: 0 units - family pairs skipped because of too many alleles
MESSAGE: Saving data to [/ramcache/slg/LINKAGE] ...
```

The 2^{nd} line of MESSAGE checks for some resource programs & files required for the execution of SEQLinkage. These files are stored in the folder $\sim$/`.SEQLinkage` on your computer. SEQLinkage will automatically download these files the first time it is executed on your computer so please make sure your computer is **connected to Internet when running SEQLinkage for the first time!** Out of the 5 resource files only one of them is relevant to the generation of regional markers: the *blueprint* file that defines genetic regions to be considered as one *marker*. This *blueprint* is based on UCSC RefSeq database. We use genomic coordinates of RefSeq genes to determine start and end positions for regional markers. The genomic coordinates are based on UCSC hg19 (or NCBI build 37) reference genome. To convert to previous builds for your data we recommend running the UCSC liftOver tool [2] to get updated *blueprint* and use the `--blueprint` option to load the file.

The 3^{rd} line checks samples from VCF file against FAM file. For our test data samples in VCF file matches those in FAM file. SEQLinkage allows for samples in VCF file but not in FAM file, or otherwise. For such cases only samples in both files will be analyzed and a warning message will be given if samples are found in FAM but not VCF file.

The 4^{th} line summarizes data information, mostly from FAM file, VCF header and the blueprint file. In the example one family with six members are found in both VCF and FAM input; also there are 25,305 pre-defined genetic regions in the blueprint file.

The 5^{th} line is dynamic: it was a progress meter during runtime, and becomes a summary of runtime statistics after the CHP algorithm is complete for all regional marker units. "xxx units (from xxx variants) were processed" is based on those variants in both VCF file and covered

[2] UCSC liftOver tool `http://genome.ucsc.edu/cgi-bin/hgLiftOver`

by the blueprint definition. **You should comparing the number of variants processed with the total number of variants in the VCF file to evaluate how much data was covered by the pre-defined regional marker positions in blueprint file**, and decide whether or not a customized blueprint should be provided. SEQLinkage performs Mendelian error check on the fly, ignoring genotype calls due to Mendelian inconsistency when there is not enough information to infer them correctly. It also deals with recombination events during haplotype construction and CHP coding process, and for those regions with recombination events the regional markers are divided into sub-units. This will be discussed in details later.

The $6^{th} \sim 8^{th}$ lines provides additional information on variants and units ignored in the analysis. Note that values on lines 7 and 8 plus the number of units on line 5 equals the total number of pre-defined units in the blueprint (line 4).

The last line of MESSAGE displays the path of output data, which in our case is in Merlin format. We will examine next the content of the output.

▪ Regional marker data

[marker name is gene name. allele numbering based on per family per unit]

2.2.2 Collapsing themes

▪ LD based collapsing

The default collapsing theme is LD based with the $R^2 > 0.8$ rule; variants within LD blocks thus defined will be collapsed to binary codes before haplotype patterns are computed. You may set `--bin` to other values $R^2 \in (0, 1)$ for different LD block definition. The output result will be written to MERLIN format (via the `--format MERLIN` argument) for use later.

```
seqlink --fam seqlinkage-example.fam --vcf seqlinkage-example.vcf.gz --format MERLIN --output RMBPt8 --jobs 8
```

terminal info

```
FIXME
```

▪ Complete collapsing

Setting `--bin 0` will collapse all variants in the region to generate one marker per region. Haplotype patterns are thus simply either “1” for all wild type or “2” for any mutation in the region.

```
seqlink --fam seqlinkage-example.fam --vcf seqlinkage-example.vcf.gz --format MERLIN --output RMB0 --jobs 8 --bin 0
```

terminal info

```
FIXME
```

No collapsing

Setting `--bin 1` will compute the haplotype pattern for the region as is, without collapsing.

```
seqlink --fam seqlinkage-example.fam --vcf seqlinkage-example.vcf.gz --format MERLIN --output RMB1 --jobs 8 --bin 1
```

terminal info

```
FIXME
```

Comparison between different themes

[FIXME: visualize the output]

2.2.3 Population vs. founder allele frequencies

In the examples above we left `--freq` option unspecified, thus frequency from samples are used. The example VCF file contains an INFO field `EVSEAAF`, the allele frequency for European Americans in Exome Variant Server (EVS). We assume the simulated variant data is from European American samples, and we incorporated the information to the VCF file as long as the variant is found in EVS; for variants not found in EVS we use `EVSEAAF=0.00015`. This input alters the resulting estimate of regional marker frequency, shown in the comparison below:

2.2.4 Recombination events

For regional markers sub-divided into smaller units by recombination events, we use `[1]`, `[2]`, `...`, `[i]` convention to mark different units (see below). They can be treated different regional markers. In the linkage analysis pipeline incorporated in SEQLinkage, we choose the one sub-unit that gives strongest evidence of linkage to represent the entire region under consideration.

OUTPUT

```
FIXME
```

2.3 Two-point Linkage Analysis

[Examples, particularly choice of parameters ...]

2.4 SEQLinkage with Other Linkage Programs

[give one example output to MEGA2 with –bin 0; and another example output to Merlin and run some analysis in Merlin]