Tutoriel de MERLIN

1. MERLIN Input Files

MERLIN performs common pedigree analyses. Input files describe relationships between individuals in your dataset, store marker genotypes, disease status and quantitative traits and provide information on marker locations and allele frequencies.

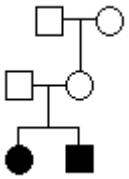
MERLIN supports input files in either QTDT or LINKAGE format. Although the two formats are very similar, in the discussion below we will focus on QTDT format.

a. Describing Relationships Between Individuals (.ped)

Although pedigrees can become quite complex, all the information that is necessary to reconstruct individual relationships in a pedigree file can be summarized in five items: a family identifier, an individual identifier, a link to each parent (if available) and finally an indicator of each individual's sex.

As an example of how family relationships are described, we will construct a *pedigree file* for a small pedigree with two siblings, their parents and maternal grand-parents.

For this simple pedigree, the five key items take the following values:



FAMILY	PERSON	FATHER	MOTHER	SEX
example	granpa	unknown	unknown	m
example	granny	unknown	unknown	f
example	father	unknown	unknown	m
example	mother	granpa	granny	f
example	sister	father	mother	f
example	brother	father	mother	m

These key values constitute the first five columns of any pedigree file. Because of restrictions in early genetic programs, text identifiers are usually replaced by unique numeric values. After replacing each identifier with unique integer and recoding sexes as 2 (female) and 1 (male), this is what a basic space-delimited pedigree file would look like:

<contents of basic.ped>

```
1 1 0 0 1
1 2 0 0 2
1 3 0 0 1
1 4 1 2 2
1 5 3 4 2
1 6 3 4 1
```

<end of basic.ped>

A pedigree file can include multiple families. Each family can have a unique structure, independent of other families in the dataset.

Describing Phenotypes and Genotypes (.ped)

Usually the five standard columns are followed by various types of genetic data, including phenotypes for discrete and quantitative traits and marker genotypes.

Disease status is usually encoded in a single column as

U or **1** for unaffecteds,
A or **2** for affecteds, and
X or **0** for missing phenotypes.

Quantitative traits are encoded as numeric values with **X** denoting missing values (it is also possible to use a peculiar numeric value to flag missing phenotypes, but the procedure is prone to error and not recommended).

Marker genotypes are encoded as two consecutive integers, one for each allele, optionally separated by a "/", or since version 1.1 using the letters "A", "C", "T" and "G". To denote missing alleles, either a 0, an X or an N can be used. The following are all valid genotype entries *1/1* (homozygote for allele 1), *0/0* (missing genotype), and *3 4* (heterozygote for alleles 3 and 4). In newer versions of Merlin *A/A*, *A/C*and *C/C* would also be valid genotypes. For the X chromosome, males should be encoded as if they had two identical alleles.

This is what the previous pedigree file might look like after adding a column for disease status, measurements for a quantitative trait and genotypes for two markers:

<**contents of basic2.ped**>

```
1  1  0 0 1  1    x  3 3  x x
1  2  0 0 2  1    x  4 4  x x
1  3  0 0 1  1    x  1 2  x x
1  4  1 2 2  1    x  4 3  x x
1  5  3 4 2  2 1.234  1 3  2 2
1  6  3 4 1  2 4.321  2 4  2 2
```

<**end of basic2.ped**>

Notice that the two siblings (individuals 5 and 6 in the last two rows) are marked as affected (value 2 in the sixth column), everyone else is marked as unaffected (value 1 in the sixth column). The quantitative trait (seventh column) takes values 1.234 and 4.321 for each sibling. Whereas everyone is genotyped at the first marker, for the second marker, only individuals 5 and 6 are genotyped.

Describing the pedigree file (.dat)

Pedigree files can include any number of marker genotype, disease status and quantitative trait variables, limited only by available memory. Since each pedigree file has a unique structure (apart from the first five columns), its contents must be described in a companion *data file*.

The data file includes one row per data item in the pedigree file, indicating the data type (encoded as M - marker, A - affection status, T - Quantitative Trait and C - Covariate) and providing a one-word label for each item. A data file for the pedigree above, which has one affection status, followed by one quantitative trait and two marker genotypes might read:

<**contents of basic2.dat**>

```
A  some_disease
T  some_trait
M  some_marker
M  another_marker
```

<**end of basic2.dat**>

You can get a summary description of any pair of pedigree and data files using pedstats (included in the MERLIN distribution). To run pedstats you must provide the name of your data file (**-d** command line option) and pedigree file (**-p** command line option). In the MERLIN examples directory, try the following command:

```
prompt> pedstats -d basic2.dat -p basic2.ped
```

TIP: In newer versions of Merlin and Pedstats, it is possible to combine multiple pedigree and data files *on the fly*. This approach can be very convenient when analyzing multiple different phenotypic subsets or when you want to separate genotypes by chromosome or by region. For example, if your phenotypes are stored in files pheno.dat and pheno.ped and your genotypes are stored in files geno.dat and geno.ped, you could combine them using the command line:

```
prompt> pedstats -d pheno.dat,geno.dat -p pheno.ped,geno.ped
```

Genetic Maps

To analyse genetic markers, MERLIN requires information on their chromosomal location. This is usually provided in a *map file*. If you are using sex-average maps, this file has one line per marker with three columns, indicating chromosome, marker name and position (in centiMorgans). If you are using sex-specific maps, you will need two additional columns specifying the marker position along the female and male genetic maps, respectively.

The data file and map file can include different sets of markers, but markers that are absent from the map file will be ignored by MERLIN. Here is what a typical map file looks like:

<**contents of basic2.map**>

```
CHROMOSOME   MARKER         POSITION
24           some_marker    123.4
24           another_marker 136.2
```

<**end of basic2.map**>

And here is a refined version of the map file including sex-specific map positions for each marker:

<**contents of file with sex-specific map**>

```
CHROMOSOME   MARKER         POSITION   FEMALE_POSITION   MALE_POSITION
24           some_marker    123.4      146.8             100.0
24           another_marker 136.2      166.4             103.0
```

<**end of sex-specific map**>

Using separate data and map files makes for a very simple file structure and allows MERLIN to analyse multiple chromosomes in a single run.

Allele Frequency Files

LINKAGE format data files specify the number of alleles at each locus and their frequencies. When using QTDT format input files, MERLIN estimates allele frequencies by counting alleles across all individuals. If this is inappropriate for the analysis at hand you can request maximum likelihood allele frequency estimates (**-fm** command line option), specify equal allele frequencies (**-fe**), request estimates derived by counting among founders only (**-ff**) or provide a custom allele frequency file (**-f** *filename* option).

A custom allele frequency file indicates allele frequencies for all marker alleles at each marker. For each marker, a single header line naming the marker is followed by a list of allele frequencies, which can take multiple lines.

Each header line is labelled M and includes the marker name. This header is followed by a list of allele frequencies. There are two alternative formats for lines in the allele frequency list:

Classic format

Lines in the allele frequency list are labelled F and list frequencies for all alleles consecutively, starting with allele 1. This format is convenient for markers with a small number of alleles.

Extended format

Lines in the allele frequency list are labelled A and consist of a numeric allele label followed by an allele frequency. Alleles that are not specifically listed are assumed to have frequency zero.

Classic Allele Frequency Format

For example, if some_marker has four alleles with frequencies 0.1, 0.2, 0.3 and 0.4 respectively and another_marker has two alleles with frequencies 0.6 and 0.4 this is what the file might look like:

<**contents of basic2.freq**>
M some_marker
F 0.1 0.2 0.3 0.4
M another_marker
F 0.6 0.4
<**end of basic2.freq**>

An equivalent layout for the same information is:

<**contents of basic2.freq**>
M some_marker
F 0.1
F 0.2
F 0.3
F 0.4
M another_marker
F 0.6
F 0.4
<**end of basic2.freq**>

Extended allele frequency format

This format is recommended for microsatellites and other markers with large allele numbers. For example, if you are analysing a microsatellite marker with alleles of size 152, 154 and 156 base-pairs and their respective frequencies are 0.5, 0.4 and 0.1 your frequency file might read:

<**contents of allele frequency file**>
M some_microsatellite
A 152 0.5
A 154 0.4
A 156 0.1
<**end of allele frequency file**>

Parametric Linkage Analysis

Linkage analysis tests for co-segregation of a chromosomal region and a trait locus of interest. In parametric linkage analysis, a specific disease model is used to describe segregation of the trait locus. In this section, we will walk through a parametric linkage analysis using MERLIN.

For this example, we will use a simulated data set that you will find in the examples subdirectory of the MERLIN distribution or in the download page.

The dataset consists of a 10-cM scan of candidate chromosome in a single pedigree where a rare dominant disorder is segregating (the pedigree is picture above). Ten microsatellite markers, each with 4 equally frequent alleles, were genotyped in all pedigree members. The genotypes and phenotypes are described in 3 files, a data file (*parametric.dat*), a pedigree file (*parametric.ped*) and a map file (*parametric.map*). An overview of MERLIN input files is available elsewhere.

The recommended first step in any analysis is to verify that input files are being interpreted correctly. So let's start by running pedstats... Pedstats requires an input data file (**-d** parameter) and pedigree file (**-p**parameter):

```
prompt> pedstats -d parametric.dat -p parametric.ped
```

By examining the abbreviated pedstats output below, you should be able to confirm that there is a single pedigree, with a total of 16 individuals (8 of these individuals are affected), and that there is no missing phenotype or genotype data.

```
Pedigree Statistics - 0.5.4
(c) 1999-2005 Goncalo Abecasis, 2002-2005 Jan Wigginton

The following parameters are in effect:
            Pedigree File :  parametric.ped (-pname)
               Data File :  parametric.dat (-dname)

PEDIGREE STRUCTURE
==================

     Individuals: 16
       Founders: 5 founders, 11 nonfounders
         Gender: 6 females, 10 males
       Families: 1

     Generations
        Average: 3.00 (3 to 3)
   Distribution: 3 (100.0%), 0 (0.0%) and 1 (0.0%)

AFFECTION STATISTICS
====================

              [Diagnostics]     [Founders] Prevalence
VERY_RARE_DISEASE      16 100.0%       5 100.0%     50.0%
        Total      16 100.0%       5 100.0%

MARKER GENOTYPE STATISTICS
```

```
============================

          [Genotypes]     [Founders]     Hetero
  MRK1      16 100.0%       5 100.0%      87.5%
  MRK2      16 100.0%       5 100.0%      75.0%
(...statistics for other markers would appear here...)
  MRK10     16 100.0%       5 100.0%      75.0%
  Total    160 100.0%      50 100.0%      78.1%
```

The pedigree and data file seem to be okay. In addition to the standard Merlin input files, parametric linkage analyses require disease locus parameters to be specified in a separate text file. This text file has one row for each of the disease models to be evaluated, and can include as many different models as available memory allows. For this analysis, the file *parametric.model* specifies a single rare dominant disease model. Here are its contents:

Affection	Disease Allele Frequency	Penetrances	Model Name
VERY_RARE_DISEASE	0.0001	0.0001,1.0,1.0	Rare_Dominant

In general, the file should be tab or space delimited, with 4 fields: affection status label (matching the data file), disease allele frequency, probability of being affected for individuals with 0, 1 and 2 copies of the disease allele (penetrances), and finally a label for the analysis model. A header line is included in the table above, for readability, but is not required. This file can also specify penetrance functions thatdepend on a covariate, such as age.

Okay ... let's run merlin! We will need to specify an input data file (**-d** parameter), pedigree file (**-p** parameter) and map file (**-m** parameter) as well as the file with trait model parameters (**--model** command line option). Since parametric linkage LOD scores tend to dip at marker locations, we will request an analyses at three equally spaced locations between each consecutive pair of markers with the **--step 3**option. With all these options, the command line will look like this:

```
prompt> merlin -d parametric.dat -p parametric.ped -m parametric.map --model parametric.model --step 3
```

After running the command, you should first see the MERLIN banner and a summary of currently selected options:

```
MERLIN DEMO-VERSION - (c) 2000-2005 Goncalo Abecasis

The following parameters are in effect:
                Data File :  parametric.dat (-dname)
           Pedigree File :  parametric.ped (-pname)
              Map File :  parametric.map (-mname)
       Allele Frequencies : ALL INDIVIDUALS (-f[a|e|f|file])

Data Analysis Options
        General : --information, --likelihood, --model [parametric.model]
      Positions : --steps [3], --maxStep, --minStep, --grid, --start,
                  --stop
```

Notice that allele frequencies were estimated by counting among all individuals (the default). In this

case, this does not matter because all founders are genotyped. In practice, when analysing small datasets such as this one, it might be a good idea to genotype additional unrelated individuals to obtain better estimates of allele frequencies or to use an allele frequency file with custom frequencies.

After a minute or two, you should see analysis results at each location:

Parametric Analysis, Model Dominant_Model

==========

POSITION	LOD	ALPHA	HLOD
(... some results edited to save space ...)			
35.000	-1.291	0.000	0.000
37.500	2.037	1.000	2.037
40.000	2.263	1.000	2.263
42.500	2.358	1.000	2.358
45.000	2.388	1.000	2.388
47.500	2.201	1.000	2.201
50.000	1.959	1.000	1.959
52.500	1.585	1.000	1.585
55.000	-9.291	0.000	0.000
(... results continue at other locations...)			

Each row indicates the estimated multipoint LOD score at a particular location. This is followed by the estimate proportion of linked families (since there is only one informative family in this sample, the proportion will always be 0.000 or 1.000), and the corresponding maximum heterogeneity LOD score. In this case the maximum LOD score of 2.407 is observed at position 45.000, the position of marker MRK5 in the map file.

Useful options for parametric linkage analyses options include requesting output with marker names, instead of cM positions (**--markerNames** option), requesting analysis along a grid of equally spaced locations (**--grid** *n* for an n-cM grid) rather than at a fixed number of steps between markers (**--steps** *n* for n-steps between consecutive markers), or requesting a graph summarizing results (**--pdf**). Try them out! For example...

```
prompt> merlin -d parametric.dat -p parametric.ped -m parametric.map --model parametric.model --grid 1 --markerNames --pdf
```

... would calculate the parametric LOD scores for a 1-cM grid along the chromosome and generate a PDF file with the resulting statistics.

That is it! That is all you need to get started with parametric linkage analysis in Merlin. Remember to set your disease model carefully, as an appropriate and careful choice of disease model is essential for parametric linkage analyses.

To learn about other analyses options, you might want to check the non-parametric linkage analysis section to find out how to conduct affecteds only linkage analyses. Or you could proceed to the error detection (improves power!), haplotyping, simulation or ibd estimation sections.

Options de MERLIN :

Les options sont précédées de « - » ou « -- ». On les rédige sous la forme :

merlin -p fichier.ped -d fichier.dat -m fichier.map –npl –prefix exemple

Input Files and Basic Parameters

-d *datafile*

Selects input data file, in linkage or QTDT format.

-p *pedfile*

Selects pedigree file, with genotype, phenotype and family structure information

Newer versions of Merlin (>1.1) can combine multiple data and pedigree files on the fly. To do this, list multiple data files separated by commas after the -d option, for example, -d pheno.dat,geno.dat, and also list the corresponding pedigree files separated by commas after the -p option, for example, -p pheno.ped,geno.ped.

Linkage Analyses

--npl
Use the Whittemore and Halpern NPL all statistic to test for allele sharing among affected individuals. Also calcu

--model *parametric_models.tbl*
Calculate parametric LOD scores, using the models specified in *parametric_models.tbl*.

--singlepoint
Consider each marker individually.

Output Formatting

--markerNames
Use marker names, rather than cM positions, to label results

--pdf
Output LOD score plots to pdf file *merlin.pdf*.

--tabulate

Generate tables summarizing key analysis results in tab-delimited format. These tables can be convenient for subsequent analysis.

--prefix *label*

Requests that output file names should be derived from *label*. For example,
e
s
t
i
m
a
t
e
d

h
a
p
l
o
t
y
p
e
s

s
h
o
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d

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d

i
n

a

f
i
l
e

c