Two-point linkage analysis using the LINKAGE/FASTLINK programs

These exercises will introduce the LINKAGE file format which is the standard format for several linkage analysis computer programs (e.g. GENEHUNTER, ALLEGRO). Two datasets will be analyzed one for an autosomal dominant trait and the other for an autosomal recessive trait where the pedigree structures have consanguinity and marriage loops. Parametric two-point linkage analysis will be carried out using MLINK and ILINK of the LINKAGE/FASTLINK computer package.

Section I - Autosomal Dominant Disease

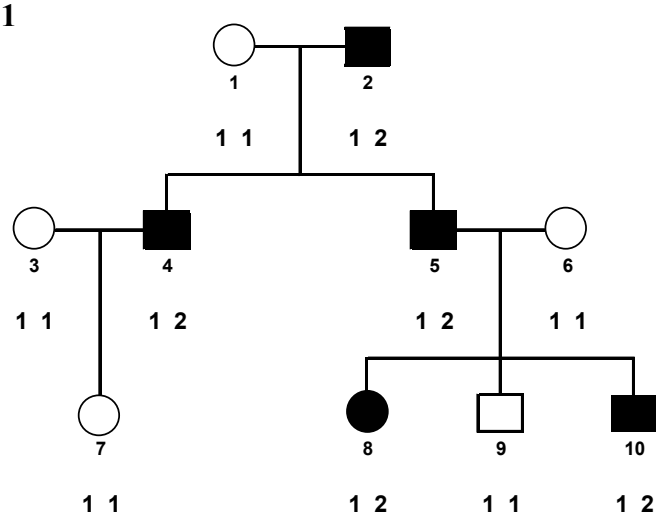
-Create a pedigree file for the following pedigrees below, using any text editor [e.g. pico, vi, emacs (UNIX, LINUX), edit, wordpad, textpad (WINDOWS)].

-In this file, each line represents one individual in the pedigree, and the columns contain the following information for each individual:

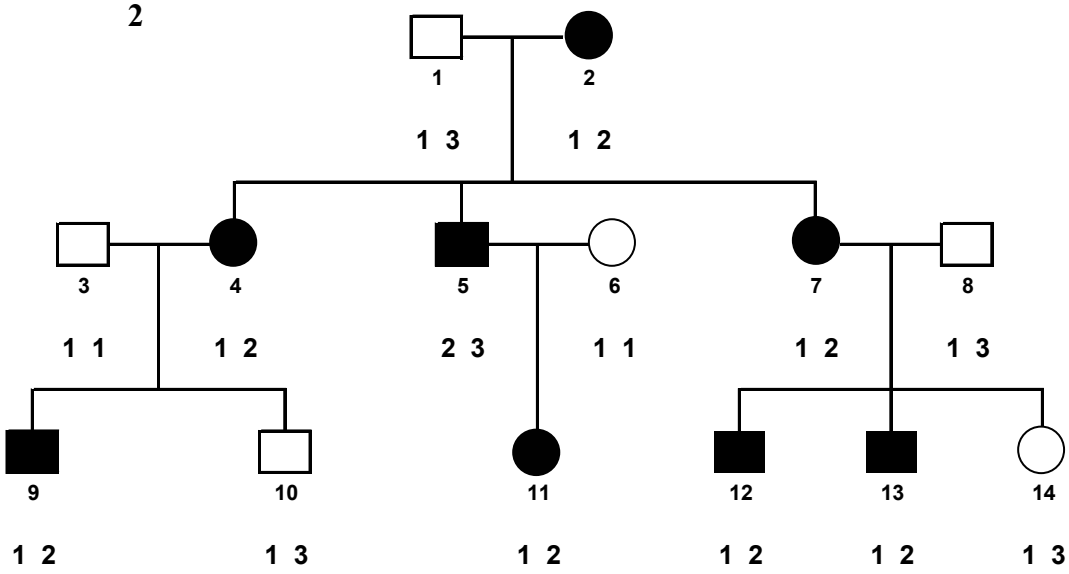
- Pedigree identifier
- Individual's identifier
- Father's identifier (0, if the father is unknown)
- Mother's identifier (0, if the mother is unknown)
- Sex (1 = male, 2 = female)
- Affection status (1 = unaffected, 2 = affected, 0 = unknown)
- 1st allele at marker #1 (alleles should be represented by integers)
- 2nd allele at marker #1
- 1st allele at marker #2
- 2nd allele at marker #2
- ……(for all markers)

-The information should be entered in the above order, separated by at least one space. Please note that you cannot have only one parent present in the pedigree file. For example if we only had information on individual 4 but not his wife, we would have to make a dummy individual for his wife making her phenotype and genotype information unknown. Unknown marker alleles are represented by 0 0. It is not possible to enter information only on one allele at a marker – for this situation both alleles must be made 0. There should be no spaces after the last character on the last line. The file should be saved as ending with a ".pre" extension (e.g. **pedsa.pre**). Note that for WINDOWS this file should be saved as an ASCII file, also known as a Text file (Tab delimited).

1



2



-Assume the disease is fully penetrant autosomal dominant and the individuals were genotyped at one marker locus. Designate the corresponding pedigree file **peds-a.pre**.

-Note: There should be no header line in the pedigree file (it is shown here and in the "Answers" section for demonstration purposes).

Pedigree	Individual	Father	Mother	Sex	Affection	First allele	Second allele
1	1	0	0	2	1	1	1
1	2	0	0	1	2	1	2
1	3	0	0	2	1	1	1
1	4	2	1	1	2	1	2
1	5	2	1	1	2	1	2
1	6	0	0	2	1	1	1
1	7	4	3	2	1	1	1
1	8	5	6	2	2	1	2
1	9	5	6	1	1	1	1
1	10	5	6	1	2	1	2
2	1	0	0	1	1	1	3
2	2	0	0	2	2	1	2
2	3	0	0	1	1	1	1
2	4	1	2	2	2	1	2
2	5	1	2	1	2	2	3
2	6	0	0	2	1	1	1
2	7	1	2	2	2	1	2
2	8	0	0	1	1	1	3
2	9	3	4	1	2	1	2
2	10	3	4	1	1	1	3
2	11	5	6	2	2	1	2
2	12	8	7	1	2	1	2
2	13	8	7	1	2	1	2
2	14	8	7	2	1	1	3

PREPLINK Program

-Run preplink to create the parameter file, **datafile.dat**, and to set the analysis parameters for MLINK.

> **preplink** **Enter**

Press ENTER to continue

> **Enter**

```
********** PRESENT STATUS **************
(a) Number of loci               :    2
(b) Sexlinked                    :    N
(c) Calculate Risk               :    N
(d) Mutation                     :    N
(e) Haplotype frequencies        :    N
(f) Locus Order                  :    1 2
(g) Interference                 :    N
(h) Recombination sex difference :    N
(i) Program used                 : MLINK
(j) Recombination values         :
      0.100
*********** OTHER OPTIONS ***************
(k) See or modify loci description
(l) See or modify recombination to vary
(m) Read datafile
(n) Write datafile
(o) Exit
*****************************************
Press letter to modify or see values
```

-For the first parameter, "(a) Number of loci", **2** is correct, since we only have two loci, the disease and the marker.

-Option "(b) Sexlinked" is set at its default **N**, therefore the disease is autosomal; this is also correct for this example since analysis is being carried out for an autosomal dominant locus.

-Option "(c) Calculate Risk" is used to specify the risk locus and allele when calculating genetic risks (for this example risks will not be calculated so the default no, **N** is correct).

-Option "(d) Mutation" is also set at **N**, since it is assumed that no mutations have occurred at the "disease" locus.

-Option "(e) Haplotype frequencies" is used to specify haplotype frequencies when incorporating linkage disequilibrium data into the analysis. In this example linkage equilibrium is assumed and this option is left at its default **N**.

-The "(f) Locus order" option (**1 2**) is also correct, since there are only two loci.

-Option "(g) Interference" should remain at its default **N** (no).

-Option "(h) Recombination sex difference" is used to set the different recombination rates in males and females; in this example it is assumed that there is no difference in male and female recombination rates, and this option is left at its default value **N** (no).

-Option (i) allows you to choose the program used for the analysis; for this example **MLINK** is used for the analysis.

-Option (j) sets the recombination fraction at which LOD scores will be calculated, to change this select option (j), and set the starting recombination fraction value at 0:
> **j** **Enter**

ENTER 1 NEW THETA(S)
> **0** **Enter**

```
********** PRESENT STATUS **************
(a) Number of loci               :    2
(b) Sexlinked                    :    N
(c) Calculate Risk               :    N
(d) Mutation                     :    N
(e) Haplotype frequencies        :    N
(f) Locus Order                  :    1 2
(g) Interference                 :    N
(h) Recombination sex difference :    N
(i) Program used                 : MLINK
(j) Recombination values         :
       0.000
*********** OTHER OPTIONS **************
(k) See or modify loci description
(l) See or modify recombination to vary
(m) Read datafile
(n) Write datafile
(o) Exit
****************************************
Press letter to modify or see values
```

-Next, select option (l), to set the increments of the recombination fraction at which the LOD scores will be calculated:
> **l** **Enter**

```
*******************************************
(a) RECOMBINATION TO VARY          :        1
(b) STARTING VALUE                 : 0.0000
(c) INCREMENT                      : 0.1000
(d) FINISHING VALUE                : 0.4500
(e) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
```

-We will calculate the LOD scores starting at a recombination fraction **0**, in increments of **0.01** and stopping at a value of **0.3**.

-Recombination varied should remain at **1** (for two-point analysis).

-The "starting value" is correct (**0.0000**). Next change the "increment" value to **0.01**
> **c** **Enter**

ENTER NEW INCREMENT
> **0.01** **Enter**

-Next set the "finishing value" at 0.3
> **d** **Enter**

ENTER NEW FINISHING VALUE
> **0.3** **Enter**

```
*******************************************
(a) RECOMBINATION TO VARY          :        1
(b) STARTING VALUE                 : 0.0000
(c) INCREMENT                      : 0.0100
(d) FINISHING VALUE                : 0.3000
(e) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
```

-In this example, LOD scores will be calculated starting at a recombination fraction of 0, then at 0.01, 0.02, 0.03, and so on until 0.3.

-Select option (e) to return to the main menu
> **e** **Enter**

-The main menu screen will reappear. This time select option (k):
> **k** **Enter**

```
*****************************************
(1) allele numbers   GENE FREQS :  0.50000  0.50000
(2) allele numbers   GENE FREQS :  0.50000  0.50000
*****************************************
(a) SEE OR MODIFY A LOCUS
(b) DELETE LOCUS
(c) ADD LOCUS
(d) CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE (NOT CHROMOSOME ORDER)
(e) CHANGE LOCUS TYPE
(f) RETURN TO MAIN MENU
*****************************************
Press letter to modify values
```

-Choose option (e) to change the locus type for locus one (the first locus in our analysis should correspond to the disease locus)
> **e** **Enter**

ENTER LOCUS TO CHANGE
> **1** **Enter**

```
ENTER NEW LOCUS TYPE:
(a) BINARY FACTORS
(b) QUANTITATIVE TRAIT
(c) AFFECTION STATUS
(d) ALLELE NUMBERS
```

> **c** **Enter**
(Choose (c) AFFECTION STATUS, to correspond to a disease locus)

-Then, the main menu screen will appear again.
-This time choose option "(a) SEE OR MODIFY A LOCUS", to change other parameters of the disease locus.
> **a** **Enter**
ENTER LOCUS NUMBER TO SEE OR MODIFY LOCUS (OR 0 TO EXIT)
> **1** **Enter**

```
*******************************
LOCUS NUMBER :          1
*******************************
(a) NUMBER OF ALLELES           :    2
(b) NUMBER OF LIABILITY CLASSES :    1
(c) PENETRANCES :
GENOTYPE  1 1  0.00000000
GENOTYPE  1 2  0.00000000
GENOTYPE  2 2  1.00000000
(d) GENE FREQUENCIES :
  0.50000  0.50000
(e) EXIT
*******************************
Press letter to modify values
```

-The first option, “number of alleles” is set at **2**, which is correct. The number of liability classes should be left at **1**. More than one liability class would be used for example for age specific penetrances. For this example, only one penetrance class will be used and it is assumed that the disease is fully penetrant with no phenocopies. The 2 allele is assigned as the causative variant at the disease locus. The penetrances need to be changed to **0 1 1**. The values 0 1 1 tell the program the probability of being affected given a certain genotype. Since 2 is the disease allele, an individual who is 1 1 at the disease locus (wild type) has a probability of 0 of being affected, since there are no phenocopies for this problem. If an individual has either a 1 2 or 2 2 genotype at the disease locus, their probability of being affected is 1, since the disease is fully penetrant.

> **c** **Enter**

ENTER NEW PENETRANCES
GENOTYPE 1 1 OLD PEN 0.00000000
?
> 0 **Enter**
GENOTYPE 1 2 OLD PEN 0.00000000
?
> 1 **Enter**
GENOTYPE 2 2 OLD PEN 1.00000000
?
> 1 **Enter**

-Next, choose option (d) in order to change the allele frequencies for the disease locus.
> **d** **Enter**

ENTER 2 NEW GENE FREQUENCIES
> **0.999** **0.001** **Enter**

-Note: The order of entering the allele frequencies is important. Since we defined allele 2 as the disease susceptibility allele, if the population disease allele frequency is 0.001, then the wild type allele frequency is 0.999, and **0.999** is entered first (for allele 1, the wild type allele), and then **0.001** (for allele 2, the disease-variant allele).

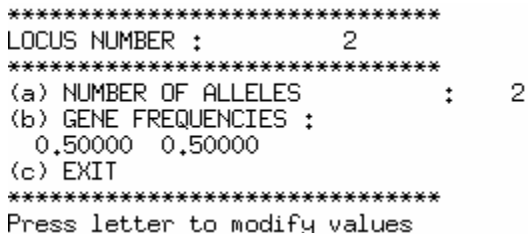
-Next, choose option “(e) EXIT” to go back to the main menu.
> **e** **Enter**

-This time we need to modify the parameters for the second locus, the marker locus.

-The locus type is set at “allele numbers” which is correct for our analysis.

-Choose option “(a) SEE OR MODIFY A LOCUS”.
> **a** **Enter**

ENTER LOCUS NUMBER TO SEE OR MODIFY LOCUS (OR 0 TO EXIT)
> **2** **Enter** (This time we choose 2, to modify locus 2)



-Since the marker locus has three alleles, choose the first option (a) to change the number of alleles to 3.
> **a** **Enter**

ENTER NUMBER OF ALLELES
> **3** **Enter**

-Assume the alleles at the marker locus have equal frequencies. Choose option (b) to give the alleles equal frequencies.
> **b** **Enter**

ENTER 3 NEW GENE FREQUENCIES
> 0.33330 0.33330 0.33330 Enter

-Select option (c) to go back to the main menu.
> **c** **Enter**

-Note: It is very important to enter the correct marker allele frequencies, and it is preferable to have the population allele frequencies for the marker studied, in the population studied. Incorrect allele frequencies can lead to false-positive results.

-Next choose option (f) to return to the uppermost menu.
> **f** **Enter**

-Now choose option "(n) Write datafile", to save the data file created.
> **n** **Enter**

Enter output file name - a file by the same name will be overwritten!
Press only Enter to skip
> **datafile.dat Enter**

-Finally choose "(o) Exit" to exit the program.
> **o** **Enter**

-The **datafile.dat** file should look like this:

```
 2 0 0 5  << NO. OF LOCI, RISK LOCUS, SEXLINKED (IF 1) PROGRAM
 0 0.0 0.0 0 << MUT LOCUS, MUT MALE, MUT FEM, HAP FREQ (IF 1)
  1  2
1   2  << AFFECTION, NO. OF ALLELES
  0.99900  0.00100 << GENE FREQUENCIES
 1 << NO. OF LIABILITY CLASSES
 0  1.0000  1.0000 << PENETRANCES
3   3  << ALLELE NUMBERS, NO. OF ALLELES
  0.33330  0.33330  0.33330 << GENE FREQUENCIES
 0 0  << SEX DIFFERENCE, INTERFERENCE (IF 1 OR 2)
  0.00000000 << RECOMBINATION VALUES
 1 0.01000 0.30000 << REC VARIED, INCREMENT, FINISHING VALUE
```

Using the PEDCHECK Program

-Pedcheck program detects genotype inconsistencies in pedigrees. There are four levels of error detection employed in pedcheck:

-*Level 1* checks the pedigree for Mendelian inconsistencies between parents and their offspring, and if there are any half-typed individuals.

-*Level 2* also detects Mendelian inconsistencies if they were not already reported after level 1 error detection. If no level 2 errors are detected, the pedigree does not contain any Mendelian inconsistencies.

-*Level 3* detects typed individuals that, when made unknown, remove the inconsistencies from the pedigree.

-*Level 4* determines the alternative genotypes that the individuals from level 3 can have, and assigns odds ratio statistics to help determine the most likely person with the error-causing genotype.

-Pedcheck requires the pedigree file (**peds-a.pre**) and the data file (**datafile.dat**) for input.

-Run pedcheck program to check for any Mendelian inconsistencies (Level 1 and 2 errors) in the pedigrees.

> **pedcheck -2 –p peds-a.pre –d datafile.dat Enter**

```
PedCheck has found 1 inconsistency in the pedigree data.

After fixing the pedigree file rerun PedCheck until
there are no more errors.

This screen output is also reproduced in the file 'pedcheck.err'.
```

-The errors detected will be outputted on the screen, and also reported in the file **pedcheck.err**.

-Note that pedcheck detected 1 inconsistency in the pedigree data. Open **pedcheck.err** to check what the error is.

-Next, open the pedigree file (**peds-a.pre**) and correct the error by making individual 10 - 0 0 (unknown for the marker genotype). For this case we are assuming that you are sure about the other individuals' genotypes when you go back and examine their genotypes, but you are unsure about the genotype for individual 10. In most situations all of the genotypes that are involved in an inconsistency have to be removed; for this example this would involve removing the genotypes for individuals 3, 4 and 10 by making them 0 0 at the marker locus.

-Now re-run pedcheck, only this time use a higher level of error detection, level 4.
> **pedcheck -4 –p peds-a.pre –d datafile.dat Enter**

```
PedCheck has found NO inconsistencies in the pedigree data.
```

MAKEPED Program

-Run makeped program to modify the pedigree file for input into LINKAGE programs. The LINKAGE/FASTLINK programs require a post makeped format for the pedigree file. The output name for the file has to be **pedfile.dat**.

> **makeped peds-a.pre pedfile.dat Enter**

Does your pedigree file contain any loops? (y/n) -> **n (n = no loops; no consanguinity or marriage loops in the pedigree) Enter**

Do you want probands selected automatically? (y/n) -> **y Enter**

-You can also give the following command when you don't have any loops and you want the probands selected automatically by the program. If you are not carrying out a risk calculation you would want the program to select the probands automatically.

> **makeped peds-a.pre pedfile.dat n Enter**

-The pedfile.dat file should look like this:

```
1  1  0  0  4  0  0 2 0 1       1       1  Ped: 1  Per: 1
1  2  0  0  4  0  0 1 1 2       1       2  Ped: 1  Per: 2
1  3  0  0  7  0  0 2 0 1       1       1  Ped: 1  Per: 3
1  4  2  1  7  5  5 1 0 2       1       2  Ped: 1  Per: 4
1  5  2  1  8  0  0 1 0 2       1       2  Ped: 1  Per: 5
1  6  0  0  8  0  0 2 0 1       1       1  Ped: 1  Per: 6
1  7  4  3  0  0  0 2 0 1       1       1  Ped: 1  Per: 7
1  8  5  6  0  9  9 2 0 2       1       2  Ped: 1  Per: 8
1  9  5  6  0 10 10 1 0 1       1       1  Ped: 1  Per: 9
1 10  5  6  0  0  0 1 0 2       1       2  Ped: 1  Per: 10
2  1  0  0  4  0  0 1 1 1       1       3  Ped: 2  Per: 1
2  2  0  0  4  0  0 2 0 2       1       2  Ped: 2  Per: 2
2  3  0  0  9  0  0 1 0 1       1       1  Ped: 2  Per: 3
2  4  1  2  9  5  5 2 0 2       1       2  Ped: 2  Per: 4
2  5  1  2 11  7  7 1 0 2       2       3  Ped: 2  Per: 5
2  6  0  0 11  0  0 2 0 1       1       1  Ped: 2  Per: 6
2  7  1  2 12  0  0 2 0 2       1       2  Ped: 2  Per: 7
2  8  0  0 12  0  0 1 0 1       1       3  Ped: 2  Per: 8
2  9  3  4  0 10 10 1 0 2       1       2  Ped: 2  Per: 9
2 10  3  4  0  0  0 1 0 1       0       0  Ped: 2  Per: 10
2 11  5  6  0  0  0 2 0 2       1       2  Ped: 2  Per: 11
2 12  8  7  0 13 13 1 0 2       1       2  Ped: 2  Per: 12
2 13  8  7  0 14 14 1 0 2       1       2  Ped: 2  Per: 13
2 14  8  7  0  0  0 2 0 1       1       3  Ped: 2  Per: 14
```

-The columns correspond to the following:
Column 1: Pedigree identifier
Column 2: Individual's identifier
Column 3: Father's identifier
Column 4: Mother's identifier
Column 5: First offspring's identifier
Column 6: Next paternal sibling's identifier
Column 7: Next maternal sibling's identifier
Column 8: Sex (1 = male, 2 = female)
Column 9: Proband status (1 = proband, 0 = all others; higher than 1 indicates individuals duplicated in loop-breaking - see section III)
Column 10: Affection status (1 = unaffected, 2 = affected, 0 = unknown)
Column 11: 1st allele at marker #1
Column 12: 2nd allele at marker #1

UNKNOWN Program

-Run the unknown program

> **unknown Enter**

The files are ready for analysis by the LINKAGE/FASTLINK programs.

A) Two-point linkage analysis using the MLINK program

-MLINK requires the **datafile.dat** and **pedfile.dat** files for input.

-We already have these files, so run MLINK program for the analysis.
> **mlink Enter**

-The results are in **outfile.dat**.

B) LINKLODS program

-This program reads the outfile.dat from MLINK and summarizes the results.

-To run this program, copy outfile.dat to final.out.
> **cp outfile.dat final.out Enter**

-Run LINKLODS.

> **linklods Enter**

```
 ,-------------------------------------------------.
 | Program   LINKLODS   Copyright (C) 1989  J. Ott |
 `-------------------------------------------------'
   Version 1.80

   Reads output from LINKMAP or MLINK program and converts log likelihoods
to lod scores. An initial set of likelihoods, for a collection of families,
must be run for which the disease is off the map (theta=0.5).  Several such
initial 'baseline' sets of likelihoods may occur in one output file.
   Program will print current line numbers as it proceeds.  This may help
to localize an error in the input file.
   Input file to this program must be called FINAL.OUT.

Maximum values:
   400 families
   500 theta values

Opening "final.out" file for input

NOTE: The program will print to the screen when it finds header lines for
      the different programs whose output is in "final.out". If no such lines
      are printed, the program did not find any such lines.

Resulting lod scores will be written to the file FINAL.LOD.  Warning: an
existing file with that name will be overwritten!  To prevent this, enter
any character and press <Enter>. To continue, press only the <Enter> key.
```

> Enter

-The results are in **final.lod**.

PEDMANAGER Program

-An easier way to create the data file is through the **pedmanager** program.

-Run pedmanager.
> **pedmanager** **Enter**

```
*************************************************************************
*                                                                       *
*          PEDMANAGER - check, preprocess, and draw pedigrees           *
*                              (version 0.9)                            *
*                                                                       *
*************************************************************************

Type 'help' or '?' for help.
Can't find help file - detailed help information is not available.
See installation instructions for details.
```

pedmngr:1> **load peds-a.pre** **Enter**

```
no errors -> 2 pedigrees checked and loaded


            ==========PEDIGREE STATS==========

2 pedigrees loaded --> genotype data for 1 marker

Average pedigree size: 12.0 (24 individuals total)
        Smallest pedigree 10 individuals (1 pedigree)
        Largest pedigree 14 individuals (2 pedigree)

Number of individuals missing genotypes at all markers: 1 (4.2%)
   for the remaining individuals 0.0% of the genotypes are missing
            ==================================
```

pedmngr:2> **allele freq** **Enter**

==========

Write LINKAGE loci file? **y** **Enter**

1. Calculate allele frequencies from the genotype data
2. Give all alleles present the same frequency
   (use the second option if you have a small number of pedigrees or if you will be filling in allele frequencies from another source)

Enter the number of your choice, 1/2 [1]: **1** **Enter**
file to store results [linkage.loci]: **datain.dat** **Enter**

==========

Write a file with allele counts/frequency from the genotype data? y/n [y]: **n** **Enter**

==========

pedmngr:3> **quit** **Enter**

-Note that this time we gave the data file the name **datain.dat**.

-Also note that pedmanager checks for any formatting errors as well as Mendelian inconsistencies in the pedigree data.

-Edit the **datain.dat** file, using a text editor (see below in bold).

-The number at the beginning of the file (**2**) refers to the number of loci; in this example there are two, one disease locus and one marker locus.

-Complete the numbering of the markers on the third line (e.g. 3 4 5…) depending on the number of loci present; in this case there are 2 loci so do not edit this line.

-Correct the disease gene allele frequencies on the 5th line, with the wild-type allele frequency first, followed by the disease-causing allele frequency (**0.999 0.001**).

-Modify the disease penetrances on the 7th line; for a fully penetrant autosomal dominant disease, the penetrances are: 0 1 1. Here, the disease is fully penetrant autosomal dominant, so modify the penetrances to **0 1 1**.

-You can also enter the markers' names; delete everything underlined on line 8, and enter a "#" followed by the marker name (see the example in bold).

-Since we chose the option of calculating allele frequencies from the genotype data, the marker allele frequencies here (**0.7778 0.1111 0.1111**) differ from those in the initial data file created using preplink program where equal allele frequencies where used.

-Note: It is better to estimate marker allele frequencies from genotype data (rather than assigning equal frequencies for alleles at a marker). However, for accurate estimates, the pedigrees used should be large, or a large number of pedigrees (from the same population) should be used to estimate marker allele frequencies. The **pedmanager** program estimates the allele frequencies from the founders and the reconstructed genotypes from founders with missing genotype data.

-The edited **datain.dat** file for **peds-a.pre** should look like this:

2 0 0 5 << **NO. OF LOCI**, RISK LOCUS, SEXLINKED (IF 1), PROGRAM
0 0.0 0.0 0 << MUT LOCUS, MUT RATE, HAPLOTYPE FREQUENCIES (IF 1)
1 2 #### insert rest of map order here ####
1 2 << AFFECTION, NO. OF ALLELES
0.999 0.001 << GENE FREQUENCIES ##### **correct as necessary**####
1 << NO. OF LIABILITY CLASSES
0 1 1 << PENETRANCES ##### **correct as necessary**####
3 3 << ALLELE NUMBERS, NO. OF ALLELES (Marker #2) (**e.g. # D1S200)**
0.7778 0.1111 0.1111
0 0 << SEX DIFFERENCE, INTERFERENCE (IF 1 OR 2)
0.10 #### insert map distances here ####
1 0.1000 0.4500 << REC VARIED, INCREMENT, FINISHING VALUE

-Note: The next to last line contains the recombination value at which LOD scores will be calculated (**0.10**), and the last line states that **1** recombination fraction will be varied, in increments of **0.1000**, and stopping at **0.4500**.

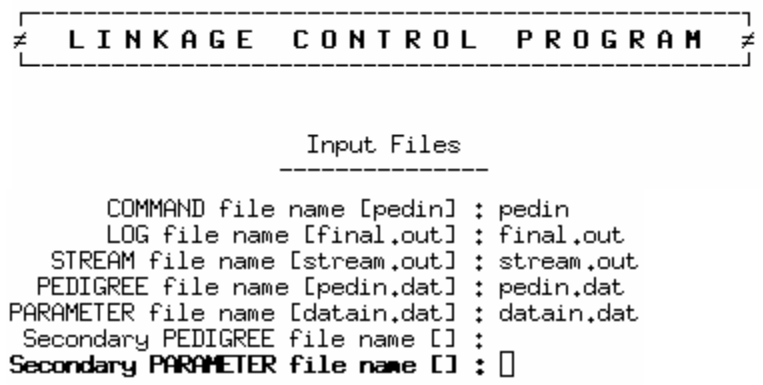
C) Two-point linkage analysis using the linkage control program (LCP)

-The input files for LCP are the pedigree file (pedfile.dat) and the parameter file (**datain.dat**). These files can have any file name except for pedfile.dat and datafile.dat, respectively. So, copy pedfile.dat to **pedin.dat**, the default name for LCP input (see below); or any other file name but make sure to change the "PEDIGREE file name" entry on the "Input Files" screen.

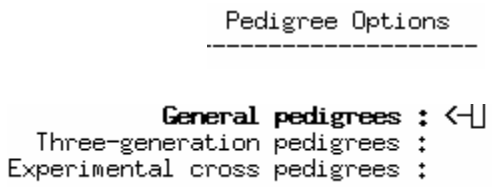
> cp **pedfile.dat pedin.dat** **Enter**

-Run the LCP program.

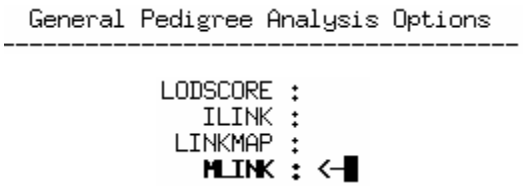
> **lcp** **Enter**



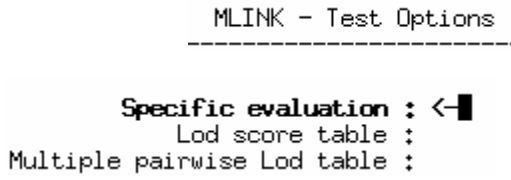
> ^ **N** (to advance to the next screen)



-Choose "**General pedigrees**"
> ^ **N**

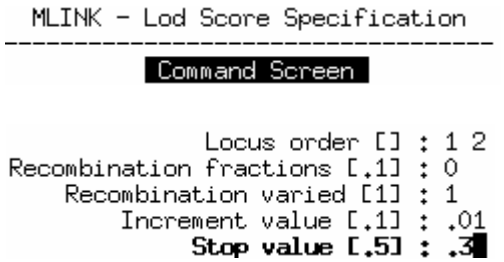


-Choose "**MLINK**", or "**ILINK**" (depending on the analysis required)
> ^ **N**



-Choose "**Specific evaluation**"
> ^ **N**

-Choose "**No sex difference**" (the only choice for MLINK analysis)
> ^ **N**



-On the Command Screen, enter the desired analysis parameters.

-For Locus order, start with 1 2 (this calculates LOD scores at the first marker), then 1 3 (for the second marker), and so on; here since there is only one marker, enter **1 2** and stop.

-Recombination fractions should be set at the desired starting value of the recombination fraction (here, **0**).

-Recombination varied should remain at **1** (for two-point analysis).

-The increment value should be set at the desired value of increments of the recombination fraction at which the LOD scores will be calculated (here, **0.01**).

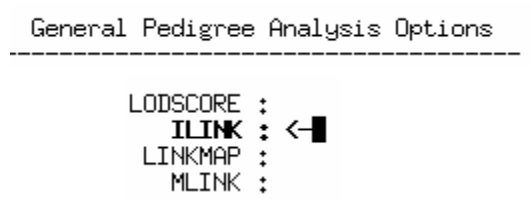
-And the stop value (**0.3**) tells the program at which value of the recombination fraction to stop.

-In this example, LOD scores will be calculated starting at a recombination fraction of 0, then at 0.01, 0.02, 0.03, and so on until 0.3.

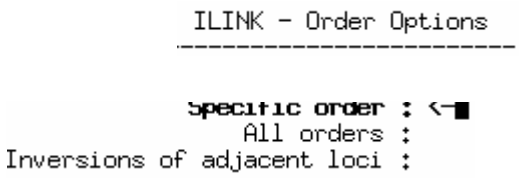
-After making all the changes to this screen hit ^**N** before going on to the next step.

-<u>WARNING</u>: If you do not hit ^**N** before either exiting LCP (^**Z**) or going back to set up another analysis as shown below (^**P**) your changes on this final screen will not be recorded!

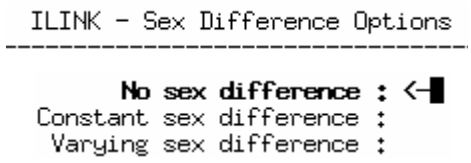
-Next, set up the analysis using ILINK; hit ^**P** (move to the previous screen) until you get the "**General Pedigree Analysis Options**" screen. Repeat the above steps, this time choose **ILINK**.



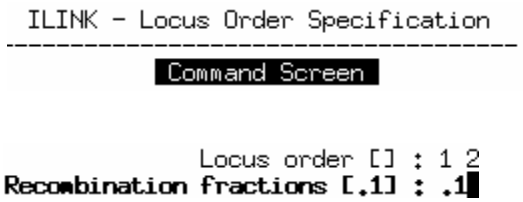
> ^ **N** (to advance to the next screen)



-Choose "**Specific order**" (for two-point analysis)
> ^ **N**



-Choose "**No sex difference**"
> ^ **N**



-On the command screen, enter the locus order. Since there are two loci, enter **1 2**. For the recombination fractions, enter **0.1**.

> ^**N**

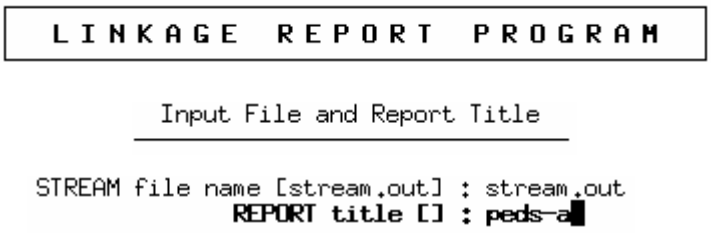
-When finished, exit the program (^**Z**). And type **pedin** to start the analysis.
> ^ **Z**
>**pedin** **Enter**

-The results will be contained in the **final.out** file (the MLINK results, followed by the ILINK results).

LRP Program

-To generate a report of the results in table format, run the linkage report program (LRP).

> **lrp** **Enter**



-Enter the desired report title (ex: **peds-a**)
> ^ **N**

-Choose the "**General pedigree reports**"
> ^ **N**

-Choose "**Lod table report (MLINK)**" (This will generate a report for the MLINK analysis results)
> ^ **N**

-Choose "**Table format**"
> ^ **N**

-Choose "**Yes**" for the "**Include Pedigrees**" option
> ^ **N**

-Choose "**Output report to a file**" option
> ^ **N**

-Enter the desired report file name (e.g. **report-a.txt**), and report page width (usually **500** will be enough)
> ^ **N**

-When finished, exit the program (**^Z**). The report file you created and saved (**report-a.txt**) should be in the current directory.

Questions:

1.) What is the maximum LOD score for pedigree 1_____? At what value of theta did it occur_____?

2.) What is the maximum LOD score for pedigrees 1 and 2_____? At what value of theta did it occur_____?

3.) What is the difference between the MLINK and ILINK results for both pedigrees_____?

4.) Is the disease locus linked to this marker_____?

5.) Since everybody is genotyped for pedigrees 1 and 2, would using equal allele frequencies (incorrect) affect your results_____?

Section II - Reduced Penetrance

-Consider pedigrees 1 and 2 from "Section I". This time, assume the disease is autosomal dominant with age-specific penetrance. Assume that for this disease no one is affected before the age of 15 and if an individual carries a copy of the disease gene they will be affected by age 30. Assume the ages of individuals in pedigrees 1 and 2 are as follows: for pedigree 1, individuals 1 through 6 are above 30 years old, individual 9 is 12 years old, and individuals 7, 8, and 10 are 17 years old. For pedigree 2, individuals 1 through 8 are older than 30, and individuals 10 and 14 are younger than 15, and individuals 9, 11, 12 and 13 are18, 22, 24 and 27 years old, respectively.

-Redo the analysis for these pedigrees. First, create the pedigree file, and edit it, making all individuals below the age of 15 unknown for affection status. Also, you have to add an additional column (after the affection status column), to assign each individual to their corresponding liability class. Assign individuals above 30 years of age to liability class 1, and individuals between the ages of 15 and 30 to liability class 2. For individuals below the age of 15, it does not matter which liability class they are assigned, since their affection status was made unknown.

- Note: It does not matter whether you assign individuals older than 30 to liability class 1 or 2, and individuals between 15 and 30 to class 1 or 2, as long as it is consistent with the data file and throughout. For this example all individuals older than age 30 are assigned to liability class 1, while all individuals between the ages of 15-30 are assigned to liability class 2. Individuals less than 15 years of age can be assigned to either liability class but their affection status must be made unknown.

-Designate the pedigree file **peds-b.pre**.

-Then run pedcheck to check for errors.

-Next, create the **datain.dat** file using preplink program as before, but this time for the disease locus, change the number of liability classes to 2. For the first liability class, enter the penetrances: **0 1 1** (the first liability class will represent individuals above 30 years of age). For the second class, enter the penetrances: **0 0.6 0.6** (this represents individuals between the ages of 15 and 30).

-Redo the rest of the analysis steps as before: run makeped, copy datain.dat to datafile.dat, run unknown, copy pedfile.dat to pedin.dat, run LCP using the same batch file that we created previously, by typing **pedin**. Finally, run LRP to create the new report file (**report-b.txt**).

Questions:

1.) What is the maximum LOD score for pedigree 1_____? At what value of theta did it occur_____?

2.) What is the maximum LOD score for pedigrees 1 and 2_____? At what value of theta did it occur_____?

3.) Do individuals under age 15 provide linkage information_____?

4.) If an individual is between the ages of 15-30 and is affected, do they provide as much linkage information as an affected individual who is older than 30 years of age_____?

5.) Why_____?

6.) If an individual is between the ages of 15-30 and is unaffected, do they provide as much linkage information as an unaffected individual who is older than 30 years of age_____?

7.) Why_____?

Section III - Autosomal Recessive Disease and Pedigrees with Loops

-There are two types of pedigree loops:

a) *Consanguinity loops*: There is inbreeding; the parents of an individual must be related (e.g. pedigree 3 below, individuals 7 and 8 are first cousins).

b) *Marriage loops*: There is no inbreeding. For example, two brothers marry two sisters. (e.g. pedigree 4 below, individuals 6 and 7 are not related, and individuals 5 and 8 are not related, but there is a loop since two brothers had children with two sisters).

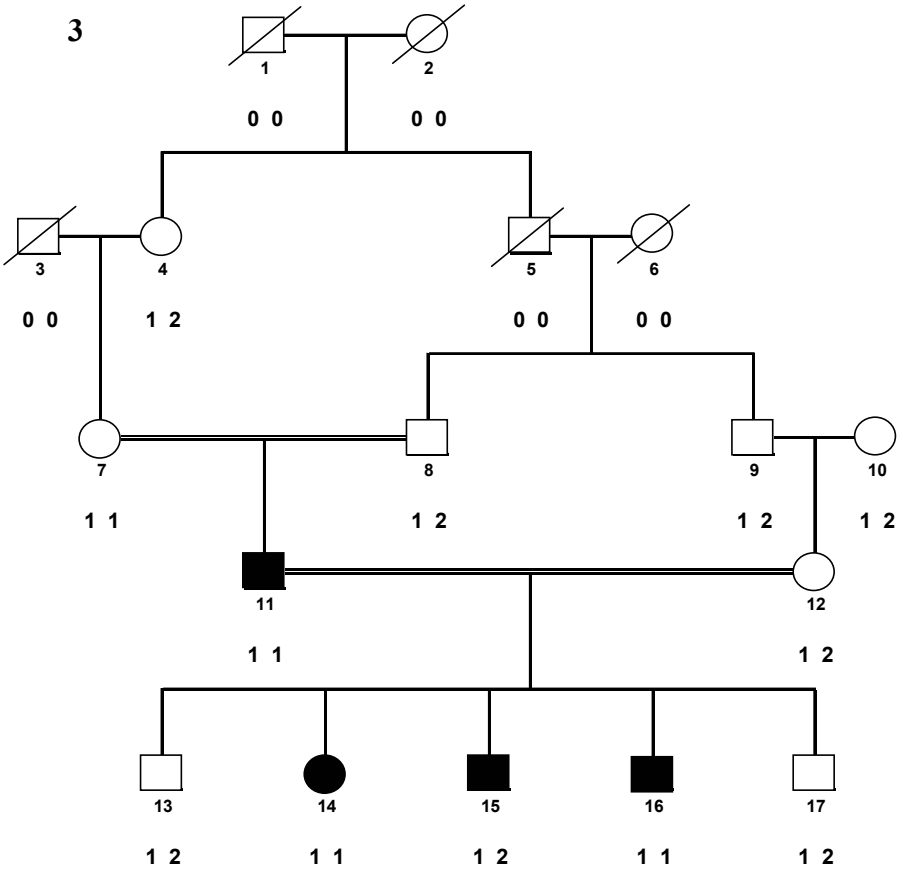
-Note: LINKAGE programs can not distinguish the two types of loops.

-Loop: starting at an individual in a pedigree and drawing a connected sequence of lines ending up back at the same individual without retracing the line.

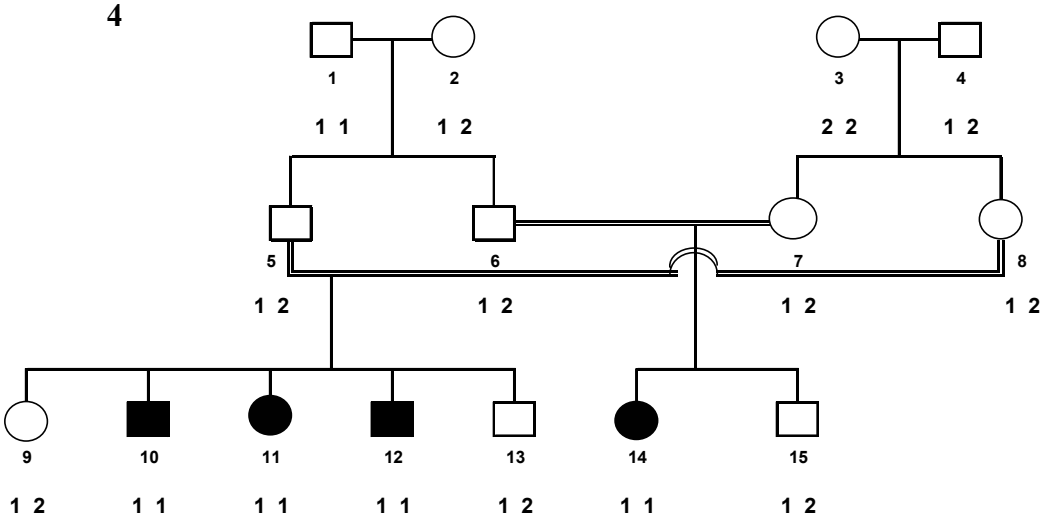
-LINKAGE programs require that the loops be "broken"; for each loop, one individual who is both a parent and an offspring must be duplicated.

-For pedigrees with loops, use the unknown program to automatically break the loops. This is done by running unknown with the "-l" option (**unknown –l**). Note that it is also possible to manually break the pedigree loops as is done in the example below.

-As an example consider the following pedigrees:



4



-Assume the disease is fully penetrant autosomal recessive and the individuals were genotyped at one marker locus. Create a pedigree file for the pedigrees using any text editor, and designate the file **peds-c.pre**.

-Run pedmanager program to check for errors in the pedigree structure, and to create the **datain.dat** file.

-Edit the **datain.dat** file, using a text editor.

-Note: For this example, it is assumed that the disease is fully penetrant autosomal recessive with no phenocopies, and the 2 allele is assigned as the causative variant. Thus, the penetrances need to be changed to **0 0 1**. The values 0 0 1 tell the program the probability of being affected given a certain genotype. Since 2 is the disease allele, an individual who is either 1 1 or 1 2 at the disease locus (wild type or carrier, respectively) has a probability of 0 of being affected, since there are no phenocopies. If an individual has a 2 2 genotype at the disease locus, their probability of being affected is 1, since the disease is fully penetrant.

-Run pedcheck program to check for errors.

Manually Breaking Loops using the Makeped Program

-Run makeped program to modify the pedigree file for input into LINKAGE programs.

-Note: The makeped program has an option that allows the user to manually break any pedigree loops:

> **makeped peds-c.pre pedfile.dat Enter**

Does your pedigree file contain any loops? (y/n) -> **y** (*answer yes, to proceed to break loops manually*)
Enter

Do you have a file of loop assignments? (y/n) -> **n** **Enter**
Enter identifiers for each pedigree and person...
enter pedigree 0 when finished.

Pedigree -> **3** (*the first pedigree with a loop*) **Enter**
Person -> **8** (*an individual from pedigree 3 who is in the first loop and is both a parent and an offspring*) **Enter**

Pedigree -> **3** **Enter**
Person -> **4** (*an individual from pedigree 3 who is in the second loop and is both a parent and an offspring*) **Enter**

Pedigree -> **4** (*the second pedigree with a loop*) **Enter**
Person -> **6** (*an individual from pedigree 4 who is in the loop and is both a parent and an offspring*) **Enter**

Pedigree -> **0** **Enter**

Do you want these selections saved for later use? (y/n) -> **n** **Enter**
Do you want probands selected automatically? (y/n) -> **y** **Enter**

-The pedfile.dat file should look like this:

```
3  1  0  0  4  0  0 1 0 1      0      0  Ped: 3  Per: 1
3  2  0  0  4  0  0 2 0 1      0      0  Ped: 3  Per: 2
3  3  0  0  7  0  0 1 1 1      0      0  Ped: 3  Per: 3
3  4  1  2  0  5  5 2 3 1      1      2  Ped: 3  Per: 4
3 19  0  0  7  0  0 2 3 1      1      2  Ped: 3  Per: 4  <--
3  5  1  2  8  0  0 1 0 1      0      0  Ped: 3  Per: 5
3  6  0  0  8  0  0 2 0 1      0      0  Ped: 3  Per: 6
3  7  3 19 11  0  0 2 0 1      1      1  Ped: 3  Per: 7
3  8  5  6  0  9  9 1 2 1      1      2  Ped: 3  Per: 8
3 18  0  0 11  0  0 1 2 1      1      2  Ped: 3  Per: 8  <--
3  9  5  6 12  0  0 1 0 1      1      2  Ped: 3  Per: 9
3 10  0  0 12  0  0 2 0 1      1      2  Ped: 3  Per: 10
3 11 18  7 13  0  0 1 0 2      1      1  Ped: 3  Per: 11
3 12  9 10 13  0  0 2 0 1      1      2  Ped: 3  Per: 12
3 13 11 12  0 14 14 1 0 1      1      2  Ped: 3  Per: 13
3 14 11 12  0 15 15 2 0 2      1      1  Ped: 3  Per: 14
3 15 11 12  0 16 16 1 0 2      1      2  Ped: 3  Per: 15
3 16 11 12  0 17 17 1 0 2      1      1  Ped: 3  Per: 16
3 17 11 12  0  0  0 1 0 1      1      2  Ped: 3  Per: 17
4  1  0  0  5  0  0 1 1 1      1      1  Ped: 4  Per: 1
4  2  0  0  5  0  0 2 0 1      1      2  Ped: 4  Per: 2
4  3  0  0  7  0  0 2 0 1      2      2  Ped: 4  Per: 3
4  4  0  0  7  0  0 1 0 1      1      2  Ped: 4  Per: 4
4  5  1  2  9  6  6 1 0 1      1      2  Ped: 4  Per: 5
4  6  1  2  0  0  0 1 2 1      1      2  Ped: 4  Per: 6
4 16  0  0 14  0  0 1 2 1      1      2  Ped: 4  Per: 6  <--
4  7  4  3 14  8  8 2 0 1      1      2  Ped: 4  Per: 7
4  8  4  3  9  0  0 2 0 1      1      2  Ped: 4  Per: 8
4  9  5  8  0 10 10 2 0 1      1      2  Ped: 4  Per: 9
4 10  5  8  0 11 11 1 0 2      1      1  Ped: 4  Per: 10
4 11  5  8  0 12 12 2 0 2      1      1  Ped: 4  Per: 11
4 12  5  8  0 13 13 1 0 2      1      1  Ped: 4  Per: 12
4 13  5  8  0  0  0 1 0 1      1      2  Ped: 4  Per: 13
4 14 16  7  0 15 15 2 0 2      1      1  Ped: 4  Per: 14
4 15 16  7  0  0  0 1 0 1      1      2  Ped: 4  Per: 15
```

-Note that for each loop, one individual who is both a parent and an offspring was duplicated; these individuals are indicated by arrows for demonstration purposes. For example, individual 19 is the duplicate of individual 4. Also note that in the proband status column for those individuals for which a loop has been broken a number greater than 1 is assigned to the original and its duplicate. For example individuals 4 and 19 are both assigned the number 3 in the proband status column.

-Copy **datain.dat** into **datafile.dat** (for input into unknown program).

-Run the unknown program.
> **unknown Enter**

-Run LCP program, by first copying **pedfile.dat** to **pedin.dat**, then typing pedin (like before).
> **pedin Enter**

-Repeat the steps for running LRP (to generate the new report and designate a different file name for the report, e.g. **report-c.txt**).

-An alternate method to break pedigree loops is using the unknown program. This method is faster and less tedious than using makeped. Repeat the above steps, only this time, when running makeped, place the "n" option in the command line:

> **makeped peds-c.pre pedfile.dat n Enter**

-Copy **datain.dat** into **datafile.dat**.

Using Unknown Program to Break Loops

-Run the unknown program with the loop breaking option (-l). The unknown will generate **lpedfile.dat**, the pedigree file with no loops.
> **unknown –l Enter**

-Repeat the steps for running LCP, only this time start by copying lpedfile.dat (instead of pedfile.dat) to pedin.dat.
> **cp lpedfile.dat pedin.dat Enter**
-Then run LCP by typing pedin (you do not need to repeat all the steps; since we are not changing any analysis parameters, we can use the same batch file that we created previously).
> **pedin Enter**

-Repeat the all steps for running LRP.

Questions:

1.) How many loops does pedigree 3 have_____?

2.) How many loops does pedigree 4 have_____?

3.) What is the LOD score for pedigree 3 at theta equal zero_____?

4.) What is the maximum LOD score for pedigree 4_____? At what value of theta did it occur_____?

5.) What is the maximum LOD score for pedigrees 3 and 4_____? At what value of theta did it occur_____?

6.) Were you able to establish linkage_____?

Results: Section I

A) MLINK: **outfile.dat** (Edited)

```
------------------------------------
THETAS  0.500
------------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
------------------------------------
      1   -22.633929    -9.829769 LOD=     0.000000
      2   -31.069683   -13.493363 LOD=     0.000000
------------------------------------
TOTALS      -53.703612   -23.323133
-2 LN(LIKE) =  1.07407e+02 LOD SCORE =     0.000000
------------------------------------
------------------------------------
THETAS  0.000
------------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
------------------------------------
      1   -19.169193    -8.325057 LOD=     1.504713
      2   -26.220648   -11.387459 LOD=     2.105905
------------------------------------
TOTALS      -45.389841   -19.712515
-2 LN(LIKE) =  9.07797e+01 LOD SCORE =     3.610617
------------------------------------
```

-*summary:*

θ	Pedigree 1	Pedigree 2	Total LOD
0	1.504713	2.105905	3.610617
0.1	1.235592	1.740601	2.976194
0.2	0.949787	1.337760	2.287547
0.3	0.648849	0.900338	1.549187
0.5	0	0	0

B) linklods: **final.lod** (Edited)

```
THETAS  0.000
Male map position:    0.0000 (Haldane)    0.0000 (Kosambi)
    PED      LOD
      1    1.505
      2    2.106
  TOTALS    3.611
```

-*summary:*

θ	Pedigree 1	Pedigree 2	Total LOD
0	1.505	2.106	3.611
0.1	1.236	1.741	2.976
0.2	0.950	1.338	2.288
0.3	0.649	0.900	1.549

C) LCP: **final.out** (Edited)

```
*******************************************************************************
                                        MLINK
*******************************************************************************

-----------------------------------
THETAS  0.500
-----------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-----------------------------------
      1   -17.800556    -7.730667 LOD=     0.000000
      2   -28.433535   -12.348501 LOD=     0.000000
-----------------------------------
TOTALS      -46.234091   -20.079168
-2 LN(LIKE) =  9.24682e+01 LOD SCORE =     0.000000
-----------------------------------
-----------------------------------
THETAS  0.000
-----------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-----------------------------------
      1   -14.335820    -6.225954 LOD=     1.504713
      2   -23.584500   -10.242596 LOD=     2.105905
-----------------------------------
TOTALS      -37.920320   -16.468551
-2 LN(LIKE) =  7.58406e+01 LOD SCORE =     3.610617
-----------------------------------
-----------------------------------
THETAS  0.010
-----------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-----------------------------------
      1   -14.396000    -6.252090 LOD=     1.478577
      2   -23.664871   -10.277501 LOD=     2.071000
-----------------------------------
TOTALS      -38.060871   -16.529591
-2 LN(LIKE) =  7.61217e+01 LOD SCORE =     3.549577
-----------------------------------
-----------------------------------
THETAS  0.300
-----------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-----------------------------------
      1   -16.306523    -7.081818 LOD=     0.648849
      2   -26.360426   -11.448163 LOD=     0.900338
-----------------------------------
TOTALS      -42.666949   -18.529981
-2 LN(LIKE) =  8.53339e+01 LOD SCORE =     1.549187
*******************************************************************************
                                        ILINK
*******************************************************************************
CHROMOSOME ORDER OF LOCI :
  1  2
*********************************************************
THETAS:
 0.001
*********************************************************
-2 LN(LIKE) =  7.58406e+01
LOD SCORE = 3.61062e+00
NUMBER OF ITERATIONS =     3
NUMBER OF FUNCTION EVALUATIONS =     9
PTG = -3.26864e+01
```

-*summary:*

MLINK results are summarized in **report-a.txt**

	Max LOD	θ
Pedigree 1	1.5	0
Pedigree 2	2.11	0
Total	3.61	0

ILINK result (gives the maximum LOD score for both pedigrees combined and the θ at which it occurred)

Max LOD	θ
3.6106200	0.001

Results: Section II

A) LCP: **final.out** (Edited)

```
*************************************************************************
                                    MLINK
*************************************************************************
LINKAGE (V5.1) WITH  2-POINT AUTOSOMAL DATA
 ORDER OF LOCI:   1  2
-----------------------------------
-----------------------------------
THETAS  0.500
-----------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-----------------------------------
       1   -22.625961    -9.826309 LOD=     0.000000
       2   -31.726691   -13.778698 LOD=     0.000000
-----------------------------------
TOTALS      -54.352652   -23.605007
-2 LN(LIKE) =  1.08705e+02 LOD SCORE =     0.000000
-----------------------------------
-----------------------------------
THETAS  0.000
-----------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-----------------------------------
       1   -20.190844    -8.768753 LOD=     1.057556
       2   -27.570804   -11.973822 LOD=     1.804875
-----------------------------------
TOTALS      -47.761647   -20.742576
-2 LN(LIKE) =  9.55233e+01 LOD SCORE =     2.862431
-----------------------------------
```

```
*************************************************************************
                                    ILINK
*************************************************************************
CHROMOSOME ORDER OF LOCI :
 1  2
******************************************************
THETAS:
 0.001
******************************************************
-2 LN(LIKE) =  9.55233e+01
LOD SCORE = 2.86243e+00
NUMBER OF ITERATIONS =     3
NUMBER OF FUNCTION EVALUATIONS =     9
PTG = -2.87766e+01
******************************************************
```

-*summary:*

MLINK results are summarized in **report-b.txt**.

	Max LOD	θ
Pedigree 1	1.06	0
Pedigree 2	1.80	0
Total	2.86	0

ILINK result (gives the maximum LOD score for both pedigrees combined and the θ at which it occurred)

Max LOD	θ
2.86243	0.001

Results: Section III

A) LCP: **final.out** (Edited)

```
*************************************************************************
                                    MLINK
*************************************************************************
LINKAGE (V5.1) WITH  2-POINT AUTOSOMAL DATA
 ORDER OF LOCI:   1  2
-------------------------------------
-------------------------------------
THETAS  0.500
-------------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-------------------------------------
       3   -23.363497   -10.146616 LOD=     0.000000
       4   -51.419359   -22.331096 LOD=     0.000000
-------------------------------------
TOTALS      -74.782856   -32.477713
-2 LN(LIKE) =  1.49566e+02 LOD SCORE =     0.000000
-------------------------------------
-------------------------------------
THETAS  0.000
-------------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
```

```
-----------------------------------
      3 -10000000000000000000.000000 -43429355638650388480.000000 LOD=  -999.999999
      4   -45.704783   -19.849293 LOD=     2.481804
-----------------------------------
TOTALS    -10000000000000000000.000000 -43429355638650388480.000000
-2 LN(LIKE) =  2.00000e+20 LOD SCORE = -43429355638650388480.000000
-----------------------------------
-----------------------------------
THETAS  0.040
-----------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-----------------------------------
      3   -22.578976    -9.805904 LOD=     0.340713
      4   -46.228811   -20.076875 LOD=     2.254222
-----------------------------------
TOTALS      -68.807787   -29.882778
-2 LN(LIKE) =  1.37616e+02 LOD SCORE =     2.594934
-----------------------------------
-----------------------------------
THETAS  0.300
-----------------------------------
PEDIGREE |  LN LIKE  | LOG 10 LIKE
-----------------------------------
      3   -22.931208    -9.958876 LOD=     0.187740
      4   -49.760736   -21.610767 LOD=     0.720330
-----------------------------------
TOTALS      -72.691944   -31.569643
-2 LN(LIKE) =  1.45384e+02 LOD SCORE =     0.908070
*******************************************************************************
                                          ILINK
*******************************************************************************
CHROMOSOME ORDER OF LOCI :
  1  2
*****************************************************
THETAS:
 0.043
*****************************************************
-2 LN(LIKE) =  1.44095e+02
LOD SCORE = 2.59660e+00
NUMBER OF ITERATIONS =     3
NUMBER OF FUNCTION EVALUATIONS =     8
PTG = -8.16421e-04
*****************************************************
```

-*summary:*

MLINK results are summarized in **report-c.txt**.

	Max LOD	**θ**
Pedigree 3	0.49	0.1
Pedigree 4	2.48	0
Total	2.59	0.04

ILINK result (gives the maximum LOD score for both pedigrees combined and the θ at which it occurred)

Max LOD	**θ**
2.5966	0.043

Answers:

Section I

1.) What is the maximum LOD score for pedigree 1___**1.5**_____? At what value of theta did it occur_____**0**_____?

2.) What is the maximum LOD score for pedigrees 1 and 2____**3.6**_____? At what value of theta did it occur_____**0**_____?

3.) What is the difference between the MLINK and ILINK results for both pedigrees___**The same; ILINK only gives the maximum LOD score and the theta at which it occurred**_?

4.) Is the disease locus linked to this marker_____**Yes**_____?

5.) Since everybody is genotyped for pedigrees 1 and 2, would using equal allele frequencies (incorrect) affect your results___**No**_____?

Section II

1.) What is the maximum LOD score for pedigree 1____**1.06**_____? At what value of theta did it occur_____**0**_____?

2.) What is the maximum LOD score for pedigrees 1 and 2____**2.86**_____? At what value of theta did it occur_____**0**_____?

3.) Do individuals under age 15 provide linkage information__**No**____?

4.) If an individual is between the ages of 15-30 and is affected, do they provide as much linkage information as an affected individual who is older than 30 years of age_**Yes**___?

5.) Why__? **Once an individual is affected and since there are no phenocopies in our model, the probability that they are disease gene carrier is 1**.

6.) If an individual is between the ages of 15-30 and is unaffected, do they provide as much linkage information as an unaffected individual who is older than 30 years of age___**No**_____?

7.) Why____? **Once an individual is above the age of onset, and is unaffected, for the penetrance model used, the probability that they carry a copy of the disease allele is 0. For individuals who are unaffected and are between the ages of 15-30, they can either be homozygous wild type or carry a copy of the disease allele. The amount of linkage information these individuals provide is based on the ratio of them being unaffected and wild type to being unaffected and a disease carrier. In this example, the ratio is 1:0.4 which is 2.5, while for the unaffected individual who is over the age of 30 the ratio is 1:0 which is infinity.**

Section III

1.) How many loops does pedigree 3 have____**2**_____?

2.) How many loops does pedigree 4 have____**1**____?

3.) What is the LOD score for pedigree 3 at theta equal zero___ **-infinity**_____?

4.) What is the maximum LOD score for pedigree 4___**2.48**_____? At what value of theta did it occur_____**0**_____?

5.) What is the maximum LOD score for pedigrees 3 and 4___**2.59**_____? At what value of theta did it occur____**0.04**_____?

6.) Were you able to establish linkage___**No**_____?

Pedigree Files:

peds-a.pre

Pedigree	Individual	Father	Mother	Sex	Affection	First allele	Second allele
1	1	0	0	2	1	1	1
1	2	0	0	1	2	1	2
1	3	0	0	2	1	1	1
1	4	2	1	1	2	1	2
1	5	2	1	1	2	1	2
1	6	0	0	2	1	1	1
1	7	4	3	2	1	1	1
1	8	5	6	2	2	1	2
1	9	5	6	1	1	1	1
1	10	5	6	1	2	1	2
2	1	0	0	1	1	1	3
2	2	0	0	2	2	1	2
2	3	0	0	1	1	1	1
2	4	1	2	2	2	1	2
2	5	1	2	1	2	2	3
2	6	0	0	2	1	1	1
2	7	1	2	2	2	1	2
2	8	0	0	1	1	1	3
2	9	3	4	1	2	1	2
2	10	3	4	1	1	1	3
2	11	5	6	2	2	1	2
2	12	8	7	1	2	1	2
2	13	8	7	1	2	1	2
2	14	8	7	2	1	1	3

peds-a.pre -- *corrected*

Pedigree	Individual	Father	Mother	Sex	Affection	First allele	Second allele
1	1	0	0	2	1	1	1
1	2	0	0	1	2	1	2
1	3	0	0	2	1	1	1
1	4	2	1	1	2	1	2
1	5	2	1	1	2	1	2
1	6	0	0	2	1	1	1
1	7	4	3	2	1	1	1
1	8	5	6	2	2	1	2
1	9	5	6	1	1	1	1
1	10	5	6	1	2	1	2
2	1	0	0	1	1	1	3
2	2	0	0	2	2	1	2
2	3	0	0	1	1	1	1
2	4	1	2	2	2	1	2
2	5	1	2	1	2	2	3
2	6	0	0	2	1	1	1
2	7	1	2	2	2	1	2
2	8	0	0	1	1	1	3
2	9	3	4	1	2	1	2
2	10	3	4	1	1	0	0
2	11	5	6	2	2	1	2
2	12	8	7	1	2	1	2
2	13	8	7	1	2	1	2
2	14	8	7	2	1	1	3

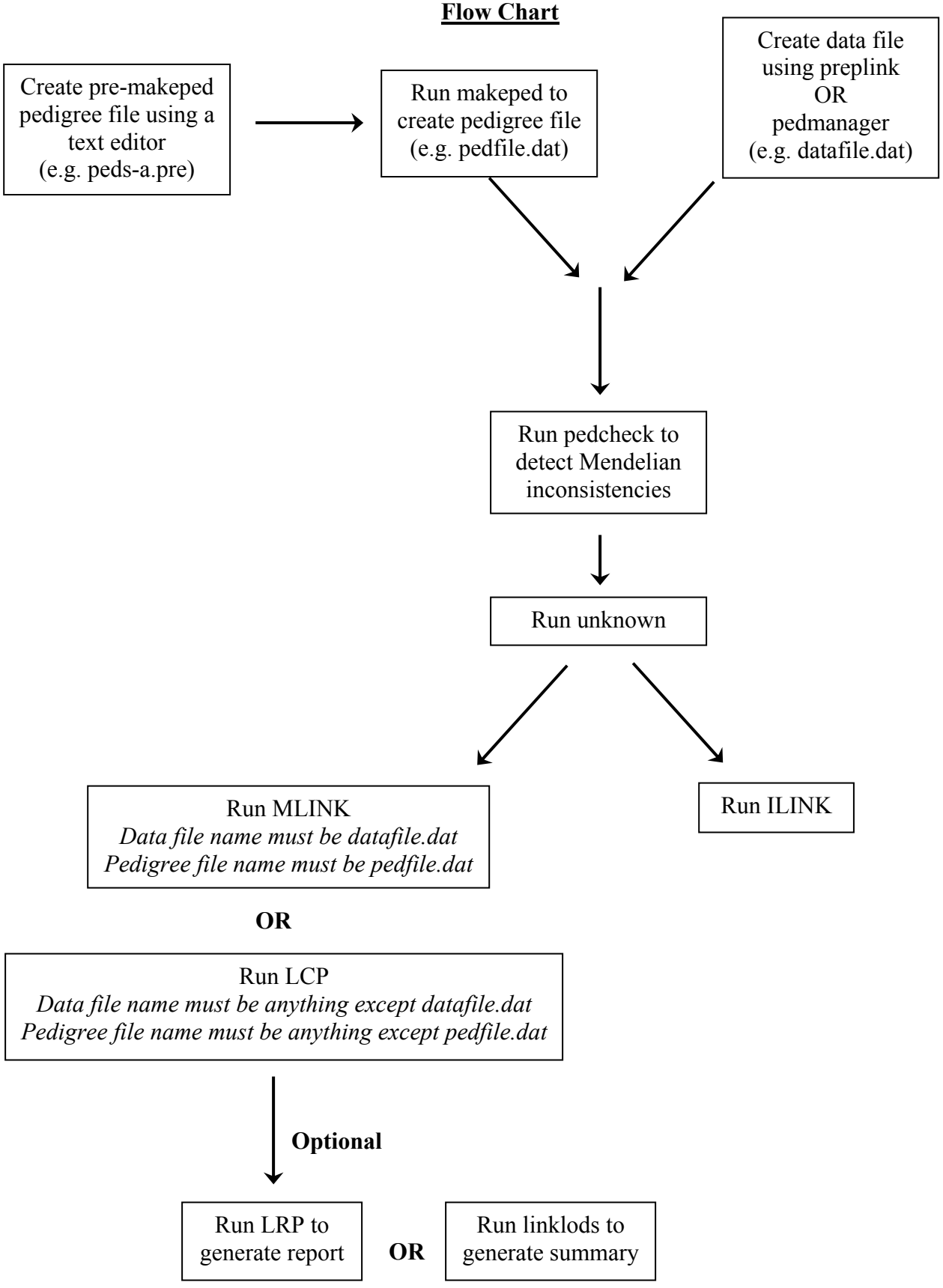
peds-b.pre

Pedigree	Individual	Father	Mother	Sex	Affection	Liability	First allele	Second allele
1	1	0	0	2	1	1	1	1
1	2	0	0	1	2	1	1	2
1	3	0	0	2	1	1	1	1
1	4	2	1	1	2	1	1	2
1	5	2	1	1	2	1	1	2
1	6	0	0	2	1	1	1	1
1	7	4	3	2	1	2	1	1
1	8	5	6	2	2	2	1	2
1	9	5	6	1	0	2	1	1
1	10	5	6	1	2	2	1	2
2	1	0	0	1	1	1	1	3
2	2	0	0	2	2	1	1	2
2	3	0	0	1	1	1	1	1
2	4	1	2	2	2	1	1	2
2	5	1	2	1	2	1	2	3
2	6	0	0	2	1	1	1	1
2	7	1	2	2	2	1	1	2
2	8	0	0	1	1	1	1	3
2	9	3	4	1	2	2	1	2
2	10	3	4	1	0	2	0	0
2	11	5	6	2	2	2	1	2
2	12	8	7	1	2	2	1	2
2	13	8	7	1	2	2	1	2
2	14	8	7	2	0	2	1	3

peds-c.pre

Pedigree	Individual	Father	Mother	Sex	Affection	First allele	Second allele
3	1	0	0	1	1	0	0
3	2	0	0	2	1	0	0
3	3	0	0	1	1	0	0
3	4	1	2	2	1	1	2
3	5	1	2	1	1	0	0
3	6	0	0	2	1	0	0
3	7	3	4	2	1	1	1
3	8	5	6	1	1	1	2
3	9	5	6	1	1	1	2
3	10	0	0	2	1	1	2
3	11	8	7	1	2	1	1
3	12	9	10	2	1	1	2
3	13	11	12	1	1	1	2
3	14	11	12	2	2	1	1
3	15	11	12	1	2	1	2
3	16	11	12	1	2	1	1
3	17	11	12	1	1	1	2
4	1	0	0	1	1	1	1
4	2	0	0	2	1	1	2
4	3	0	0	2	1	2	2
4	4	0	0	1	1	1	2
4	5	1	2	1	1	1	2
4	6	1	2	1	1	1	2
4	7	4	3	2	1	1	2
4	8	4	3	2	1	1	2
4	9	5	8	2	1	1	2
4	10	5	8	1	2	1	1
4	11	5	8	2	2	1	1
4	12	5	8	1	2	1	1
4	13	5	8	1	1	1	2
4	14	6	7	2	2	1	1
4	15	6	7	1	1	1	2

Flow Chart


Create pre-makeped pedigree file using a text editor (e.g. peds-a.pre)
Run makeped to create pedigree file (e.g. pedfile.dat)
Create data file using preplink OR pedmanager (e.g. datafile.dat)
Run pedcheck to detect Mendelian inconsistencies
Run unknown
Run MLINK
Data file name must be datafile.dat
Pedigree file name must be pedfile.dat
Run ILINK
OR
Run LCP
Data file name must be anything except datafile.dat
Pedigree file name must be anything except pedfile.dat
Optional
Run LRP to generate report
OR
Run linklods to generate summary