

# Linkage Analysis Package

## User's Guide to Support Programs

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## Chapter 1: Linkage Analysis Package

### *1.0 Introduction*

The Linkage Analysis Package (LINKAGE) is an integrated system of programs designed to perform linkage analysis and genetic risk calculation for an arbitrary number of loci.

LINKAGE is composed of two groups of programs. The first group consists of a “core” of linkage analysis programs, which perform such tasks as maximum likelihood estimation of recombination rates, calculation of lod score tables, and analysis of genetic risk. Activity at the “core” level requires an understanding of how these programs interact, and a knowledge of how to create and modify the appropriate data files. These “core” programs are referred to as the “Linkage Analysis Programs.”

The second group consists of a series of programs that form an interactive “shell” or “front end” around the “core” programs. This “shell” provides a friendly, easy-to-use interface to the “core” programs. The “shell” enables a user to run the linkage analysis programs without specific knowledge of the programs or the data files that support them. In addition to providing an interactive shell around the Linkage Analysis Programs, the Support Programs provide the user with linkage file preparation, data checking facilities and report generation from the linkage results. These “shell” programs are collectively referred to as the “Linkage Support Programs.”

This document describes the use of the Support Programs. For information on the use of the Linkage Analysis Programs, see *LINKAGE ANALYSIS PACKAGE II - User’s Guide to Analysis Programs*. The relationship of these groups is illustrated in Figure 1-1. This document is an updated version of document version 4.8 and describes the programs made available at Columbia University, New York.

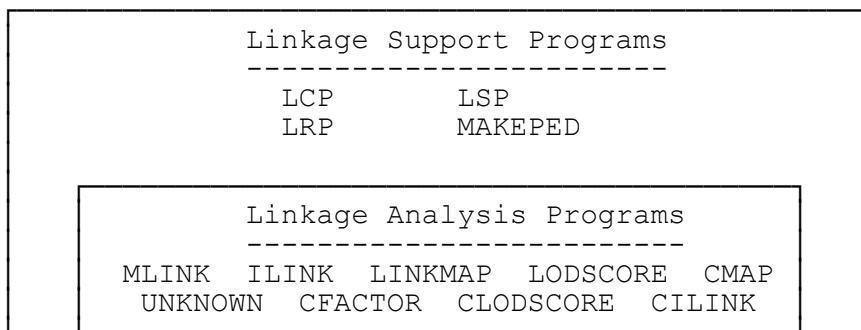


Figure 1-1

The linkage analysis process can be broken into three phases: the data preparation and management phase, the data analysis phase, and the linkage results report generation phase. The functions of the data preparation phase are creation, modification and checking of the linkage input files. These files are then analyzed by the analysis phase. This phase gives the user the ability to perform a wide variety of analysis on the same data input files. The final phase provides the user with ability to preview and generate reports from the results of the analysis phase. Figure 1-2 is an overview of the phases of linkage analysis and the files they require.

For a practical introduction on how to perform analyses with the LINKAGE and other programs, please consult Terwilliger and Ott (1994). A general introduction to human linkage analysis and its theoretical basis is Ott (1999). In publications, the LINKAGE programs may be referred to by quoting Lathrop et al. (1984). Additional information is available in the document, <http://www.genemapping.cn/linkhelp.htm>. Windows versions of the programs may be downloaded from <http://www.genemapping.cn/LinkageWin.zip>.

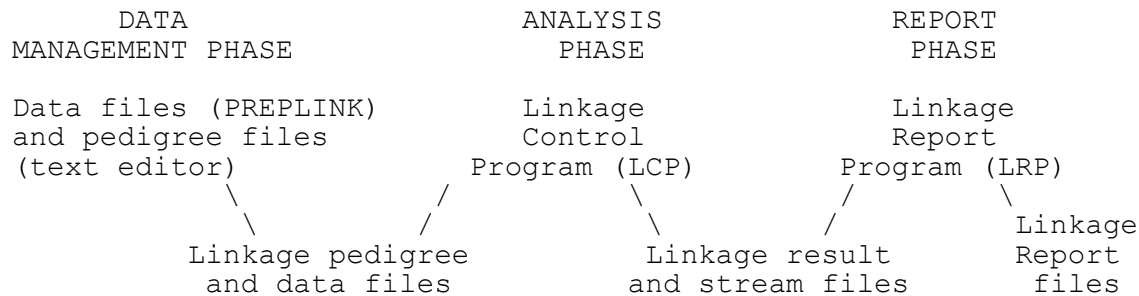


Figure 1-2

### 1.1 LINKAGE Programs

The Linkage Analysis Package contains the following programs (for additional information on LCP and MAKEPED see the **LINKHELP** document):

#### Support Programs:

- LCP**            The Linkage Control Program (LCP) is an interactive menu program for building command files to perform linkage analysis. *Note:* Risk calculation and general parameter estimation are not supported by LCP. Risk calculation and other nonsupported procedures may be carried out by manipulating datafiles created by LCP. See LINKHELP file and book by Terwilliger and Ott (1994).
- LRP**            The Linkage Report Program (LRP) is an interactive menu program for displaying and formatting the stream output from the linkage analysis programs.
- LSP**            The Linkage Setup Program (LSP) selects specified loci from the primary (master) pedigree and data files and builds new pedigree and data files.
- MAKEPED**        MAKEPED converts pedigree files of the form: pedigree name (or number), ID name (or number), father ID, mother ID, etc. to a form that includes offspring and sib-pointers; it also interactively breaks loops. MAKEPED currently is a batch program that first calls the MAKEPED1 program to carry out the tasks described above and then calls the LOOPS program to verify that no pedigree loops remain after MAKEPED1 has run.

#### Analysis Programs:

- MLINK**        The MLINK program performs n-point linkage and risk calculations in general pedigrees. This program can be used to construct lod score tables.
- LODScore**     The LODSCORE program performs iterative maximum likelihood estimation of recombination fractions from two-locus data in general pedigrees.
- ILINK**        The ILINK program performs iterative maximum likelihood estimations in general

pedigrees.

LINKMAP	The LINKMAP program calculates location scores in general pedigrees.
UNKNOWN	The UNKNOWN program infers possible genotypes for unknown individuals. This program prepares input files for the ILINK, LINKMAP, MLINK, and LODSCORE programs.
CILINK	The CILINK program performs iterative maximum likelihood estimations in CEPH type pedigrees (nuclear families with the possible addition of grandparents).
CMAP	The CMAP program calculates likelihoods for genetic locations in CEPH pedigrees.
CLODScore	The CLODScore does for CEPH type pedigrees what LODSCORE does for general pedigrees.
CFACTOR	The CFACTOR program applies factorization and transformation rules to genotypes in CEPH pedigrees prior to analysis with the CMAP and CILINK programs.
TLINKAGE	is a modified version of the LINKAGE programs allowing for two loci to jointly cause disease (Lathrop and Ott 1990). This set of programs is not discussed here.

## 1.2 Conventions

The following notational conventions are used throughout this manual:

Convention	Meaning
CTRL/x	The control “x” character, where x can be any designated letter. The command is generated by holding down the control key (Ctrl) and pressing the “x” key while the Ctrl key is held down. For example: CTRL/D is generated by holding down the control key and pressing the “D” key.
^x	Alternative notation for CTRL/x.

## Chapter 2: Linkage Control Program

### 2.0 Introduction

The Linkage Control Program (LCP) is an interactive menu-driven program for building command files to perform linkage analysis. LCP is designed to aid the user in the analysis phase of a linkage problem. Figure 2-1 gives an overview of the analysis phase.

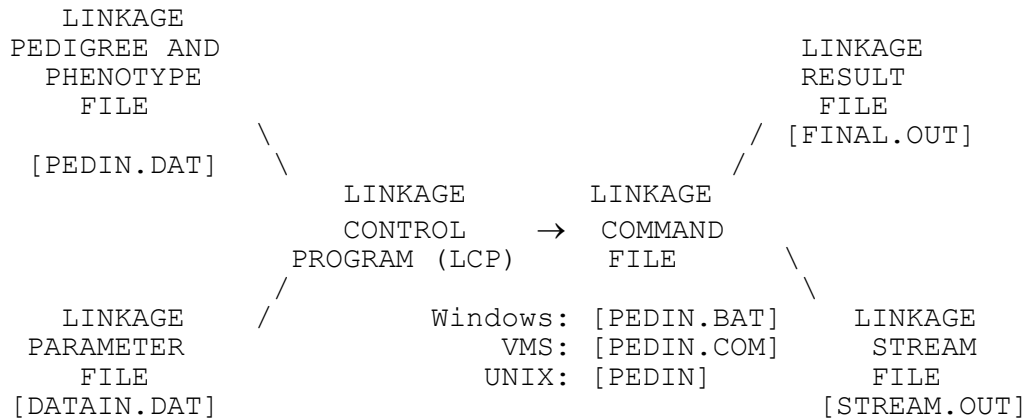


Figure 2-1

LCP distinguishes primary and secondary files. This distinction may be useful if you have two pedigree files with identical individuals in exactly the same order but different loci are given in the two files. In practice you usually work only with primary files.

### 2.1 LCP Concepts

LCP is organized internally as a hierarchical “decision” tree. Traversing this tree is invisible to the user. The “decision nodes” of the tree are typically represented by “select” screens, and “terminal nodes” are represented as “entry” or “action” screens (identified by the words “Command screen” in inverse video). Entering the “next screen” command (see CTRL/\) from a decision node will cause the user to “descend” to the next node in the tree. Entering the “next screen” command from a terminal node will cause two events. The first event generates some “action” or “commands” in the specified command file. The second event resets the user “back” into the decision tree. This design enables the user to continue building commands by means of the “next screen” command; in addition, it makes the “tree traversal” invisible. Entering the “previous screen” command (see CTRL/) from any node will cause the users to “ascend” to the previous node. The user **MUST ENTER THE “NEXT SCREEN” COMMAND ON TERMINAL NODES** if he/she wants commands to be generated for that screen. A “previous screen” command from a terminal node generates **NO** commands in the specified command file.

Built into each screen entry is a single-line “editing window.” LCP provides several commands for maneuvering and editing entries in this window. IF the entry gets larger than the “display window”, the entry line is scrolled in the appropriate direction. LCP displays a “<” symbol (to the left of the entry line) if the editing window has scrolled to the left, and a “>” symbol (to the right of the entry line) if the editing window has scrolled to the right. This feature enables the user to enter, delete, and modify long lines.

### 2.2 Invoking LCP

At the operating system prompt type the following command and press RETURN:

LCP

LCP will issue the message:

Copyright (C) Howard Hughes Medical Institute 1987, 1988.  
All rights reserved.  
Initializing...

After a brief time, LCP will clear the screen and put up the first menu. Use the LCP commands to maneuver through the various screens. Use the CTRL/A or the CTRL/Z commands to exit LCP.

Note: Under Windows, LCP requires the ANSI.SYS driver to be loaded (a line similar to DEVICE=C:\WINNT\SYSTEM32\ANSI.SYS must be present in the CONFIG.NT file). For detailed information, see <http://www.genemapping.cn/linkhelp.htm>.

### 2.3 LCP Commands

LCP commands can be divided into several categories:

#### Window Control Characters

- ^J - Move the cursor to the left
- ^L - Move the cursor to the right.
- ^B - Move the cursor to the beginning of the line.
- ^E - Move the cursor to the end of the line.
- <- - Delete the character to the left of the cursor.
- ^X - Delete the character above the cursor.
- ^U - Delete the entire line.
- ^D - Display the default entry.
- ^F - Display the last entry.

#### Locus and Recombination Control Characters

- Display all loci from the primary file.
- Display all loci from the secondary file.
- Display all loci from both the primary and secondary files
- Repeat the current recombination rate the appropriate number of times.

#### Entry Control Characters

- ^I - Move (up) to the previous entry.
- ^K - Move (down) to the next entry.
- ^M - Move (down) to the next entry (if screen is an entry screen).

#### Screen Control Characters

- Move (up) to the previous screen.
- ^N - Move (down) to the next screen.
- ^M - Move (down) to the next screen (if screen is an entry screen).

## Session Control Characters

^H - Display the help screen.

^V - Display the version and operating system (OS) configuration of LCP.

^W - Refresh the screen.

^A - Abort the current session.

^Z - Exit the current session.

The following pages contain a detailed description of the LCP commands, in alphabetic order. These descriptions include alternate keys and restrictions, if any, for use of the command.

### N O T E (PC VERSION ONLY)

Some of the alternate keys for LCP commands will NOT work correctly if "NUM LOCK" is on. Check that the "NUM LOCK" key is in the OFF state before invoking LCP.

#### Abort current session - CTRL/A

##### Description:

CTRL/A aborts the current session. No command file is generated. This command should only be used if the user has made a serious mistake and wants to abort the program.

#### Move to the beginning of the line - CTRL/B

##### Description:

CTRL/B will move the cursor to the beginning of the current line. IF the cursor is already at the beginning of the line, this command is ignored. This command is ignored on select screens.

#### Display the default entry - CTRL/D

##### Description:

CTRL/D will ERASE THE CURRENT ENTRY and display the default value, if one exists, following the prompt. The default value also appears within brackets. This command is ignored on select screens.

#### Move the cursor to the end of the line - CTRL/E

##### Description:

CTRL/E will move the cursor to the end of the current line. IF the cursor is already at the end of the line, this command is ignored. This command is ignored on select screens.

### Display the last entry - CTRL/F

#### Description:

CTRL/F will ERASE THE CURRENT ENTRY and display the last entered value for this entry if one exists. The last entered value for the entry is also displayed on the entry line when the screen is first displayed. This command is ignored on select screens.

### Display loci from the primary and secondary files - CTRL/G

#### Description:

CTRL/G will ERASE THE CURRENT ENTRY and display all the loci from the primary and secondary files. If no secondary file has been specified, then only the primary loci are displayed (see CTRL/O). Loci from the primary file will be prefixed with 'p' and loci from the secondary file will be prefixed with 's'. Primary loci are displayed first. This command is only allowed on entries that are expecting loci. An error message is issued on other entries. CTRL/G is ignored on select screens.

### Display the HELP screens - CTRL/H

#### Description:

CTRL/H displays the LCP help screens. Press any character to display the next help screen. After the last help screen, the user is returned to the current screen.

### Move to the previous entry - CTRL/I

#### Description:

CTRL/I moves the cursor up to the previous entry on the screen. IF the cursor is on the first entry, this command moves it down to the last entry on the screen.

### Move the cursor to the left - CTRL/J

#### Description:

CTRL/J moves the cursor one character to the left. If the cursor is already at the beginning of the line, this command is ignored. This command is ignored on select screens.

### Move to the next entry - CTRL/K

#### Description:

CTRL/K moves the cursor down to the next entry on the screen. IF the cursor is on the last entry, this command moves it up to the first entry on the screen.

### Move the cursor to the right - CTRL/L

#### Description:

CTRL/L moves the cursor one character to the right. If the cursor is already at the end of the line, this command is ignored. This command is ignored on select screens.

### Move the cursor to the next entry - CTRL/M

#### Description:

CTRL/M behaves differently according to the type of the current screen. If the current screen is a select screen, ^M is interpreted as a CTRL/N (next screen) command, and the user is moved to the next screen defined by the current entry. If the current screen is an entry screen, ^M is interpreted as a CTRL/K (next entry), and the cursor moves down to the next entry on the screen. IF the cursor is on the last entry, this command moves it up to the first entry on the screen.

### Move to the next screen - CTRL/N

#### Description:

CTRL/N moves the user to the next screen defined by the current entry. NOTE: The user MUST enter this command in terminal nodes to have commands generated in the command file.

### Display loci from the primary file - CTRL/O

#### Description:

CTRL/O will ERASE THE CURRENT ENTRY and display all the loci from the primary file. Loci from the primary file will be prefixed with 'p'. This command is only allowed on entries that are expecting loci. An error message is issued on other entries. CTRL/O is ignored on select screens.

### Move to the previous screen - CTRL/P

#### Description:

CTRL/P moves the user to the previous screen. This command enables the user to 'back out' of the current screen. If no previous screen exists, CTRL/P is ignored. NOTE: No command file entries are generated for the current screen if this command is issued.

### Repeat the recombination rate - CTRL/R

#### Description:

CTRL/R assumes that there is one valid recombination rate for the current entry, and repeats that value for the appropriate number of locus intervals. If more than one recombination rate is in the current entry or the recombination rate is not valid, the CTRL/R command will issue an error

message. This command is allowed only on entries that expect recombination rates. An error message is issued on other entries. CTRL/R is ignored on select screens.

#### Display loci from the secondary file - CTRL/T

##### Description:

CTRL/T will ERASE THE CURRENT ENTRY and display all the loci from the secondary file. If no secondary file was specified, an error message will be displayed. Loci from the secondary file will be prefixed with 's'. This command is allowed only on entries that are expecting loci. An error message is issued on other entries. CTRL/T is ignored on select screens.

#### Delete the line - CTRL/U

##### Description:

CTRL/U erases the current entry line. IF the current entry is already empty, this command is ignored. This command is ignored on select menus.

#### Display the version and OS configuration of LCP - CTRL/V

##### Description:

Displays the version, version date, and operating system (OS) configuration of LCP. The OS configuration is displayed as the host OS followed by the target OS. The host OS is the operating system under which LCP runs. The target OS is the operating system for which LCP generates the command file. For example:

DOS/DOS - Runs under DOS and generates a Windows ("DOS") command file.  
DOS/VMS - Runs under DOS and generates a VMS command file.

#### Refresh the screen - CTRL/W

##### Description:

CTRL/W rewrites the screen. This command is for "repainting" the screen if it becomes distorted for any reason.

#### Delete (forward) character - CTRL/X

##### Description:

CTRL/X deletes the character at the cursor position. If the cursor is already at the end of the line, this command is ignored. This command is ignored on select screens.

#### Exit the session - CTRL/Z

##### Description:

CTRL/Z saves the command file and then exits the current session of LCP.

Delete (backward) character - Backspace

Description:

Pressing the backspace key deletes the character to the left of the cursor. If the cursor is already at the beginning of the line, this command is ignored. This command is ignored on select screens.

*2.4 Invoking the LCP Command File*

When LCP exits normally (CTRL Z) it creates a “native” command file. This file can be edited, renamed, or copied before running. To execute this command file, follow the syntax in the table below for your specific operating system.

OS	Syntax	Default Command File Name	Example
Windows	<command_file_name>	PEDIN.BAT	PEDIN
UNIX	<command_file_name>	PEDIN	PEDIN
VMS	@<command_file_name>	PEDIN.COM	@PEDIN

The LCP command file will take several minutes to several hours to complete, depending on the type of analysis requested and the type of computer. During the execution of this command file, output will be sent to the screen informing the users as to the calculations being performed. The following is an example of output produced by an ILINK run with three loci (on Windows):

```

*
*
*****
Run 1 - ILINK : p2 p4 p6"
*****
*
Program UNKNOWN version 5.20
The following maximum values are in effect:
  20 loci
  120 single locus genotypes
  15 alleles at a single locus
  500 individuals in one pedigree
  3 marriage(s) for one male
  3 quantitative factor(s) at a single locus
  20 liability classes
  17 binary codes at a single locus
Opening DATAFILE.DAT
Opening PEDFILE.DAT
YOU ARE USING LINKAGE (V5.20) WITH 3-POINT AUTOSOMAL DATA
Ped. 774
Ped. 1076
Ped. 1378
Ped. 1380
Ped. 1400
Ped. 1407
Ped. 1409
Ped. 1412

```

Created using Turbo Pascal, Copyright (C) Borland International 1987, 1988  
Program I LINK version 5.20

The program constants are set to the following maxima:

```
5 loci in mapping problem
5 alleles at a single locus
250 recombination probabilities (maxneed)
-25000 maximum of censoring array (maxcensor)
32 haplotypes = n1 x n2 x ... where ni = current # alleles at locus i
528 joint genotypes for a female (maxfem)
528 joint genotypes for a male (maxmal)
800 individuals in all pedigrees combined (maxind)
180 pedigrees (maxped)
1 quantitative factor(s) at a single locus (maxtrait)
5 liability classes (maxliab)
5 binary codes at a single locus (maxfact)
52 bits mantissa length (variable)
3.00 base scaling factor for likelihood (scale)
2.00 scale multiplier for each locus (scalemult)
0.00000 frequency for elimination of heterozygotes (minfreq)
Length of real variables = 8 bytes
```

YOU ARE USING LINKAGE (V5.20) WITH 3-POINT AUTOSOMAL DATA

Maxneed can be reduced to 32

Maxcensor can be reduced to -32763

ITERATION	1	T =	0.100	NFE =	3	F =	3.61586198107041E+0002
ITERATION	2	T =	0.004	NFE =	7	F =	3.59980683832184E+0002
ITERATION	3	T =	0.004	NFE =	11	F =	3.59427965210044E+0002
ITERATION	4	T =	0.000	NFE =	15	F =	3.59427965191670E+0002
ITERATION	5	T =	0.000	NFE =	18	F =	3.59427965191670E+0002
ITERATION	6	T =	0.089	NFE =	22	F =	3.59216156774292E+0002
ITERATION	7	T =	1.000	NFE =	26	F =	3.58664261012508E+0002
ITERATION	8	T =	1.000	NFE =	30	F =	3.58607827828390E+0002
ITERATION	9	T =	1.000	NFE =	34	F =	3.58603672090897E+0002
ITERATION	10	T =	1.000	NFE =	38	F =	3.58603639374483E+0002

Maxneed can be reduced to 32

Maxneed can be reduced to 32

The LCP-generated command files can be run in batch queues in VMS or as a background process in UNIX. This feature is useful for long runs or when other interactive work is to be performed. See your specific operating system documentation on how to accomplish this.

### 2.5 The LCP Command File Output

When the LCP command file finishes executing, two output files are created. The first ([FINAL.OUT]) contains a summary of the linkage results in a descriptive or annotated format; this file can be examined or printed. The second ([STREAM.OUT]) contains the complete linkage results, in a compressed "stream" format. This file can also be examined or printed, but it contains NO descriptive information. The stream file is intended to be read by other LINKAGE and user programs. This stream file is used as input to the Linkage Report Program (LRP), which is described in the next section.

## Chapter 3: Linkage Report Program

### 3.0 Introduction

The linkage Report Program (LRP) is an interactive, menu-driven program for displaying and formatting the stream output of the linkage programs. LRP is designed to aid the user in the report phase of a linkage problem.

### 3.1 LRP Concepts

LRP employs the same concepts that are used in LCP. See section 2.1 for a description of these concepts.

### 3.2 Invoking LRP

At the operating system prompt, type the following command and press RETURN:

```
LRP
```

LRP will issue the message:

```
Copyright (C) Howard Hughes Medical Institute 1987, 1988.  
All rights reserved.  
Initializing...
```

After a brief time, LRP will clear the screen and put up the first menu. Use the LRP commands to maneuver through the various screens. Use the CTRL/A or the CTRL/Z commands to exit LRP.

Note: Under Windows, the ANSI.SYS driver must be loaded for proper operation of LRP. See <http://www.genemapping.cn/linkhelp.htm>

### 3.3 LRP Commands

The LRP commands are the same as those described in section 2.3, with the exception that the CTRL/O, CTRL/T, CTRL/G, and CTRL/R commands have no meaning in LRP.

### 3.4 LRP Output Files

If requested, LRP will output reports to a file. These report files can be examined or printed. These files may contain form feeds.

## Chapter 4: Linkage MAKEPED Program

### 4.0 Introduction

In many studies data is presented in the form of pedigree number, id number, father id, mother id, and phenotype data. The MAKEPED program converts pedigree files of this format to one which includes offspring and sib-pointers. It also breaks loops interactively.

This program varies in several ways from previous versions (PEDPOINT) which you may be familiar with. This includes:

- A. Pedigree and individual id's can now be strings or integers. The current program version treats all these identities as strings.
- B. Selecting phenotype sub-sets is no longer carried out by this program. This function is performed by the program LSP.
- C. The following integrity checks are performed:
  - 1. Sex checking

If an individual's parents are both listed as the same sex the program aborts.
  - 2. Checking for loops:

Under Windows, MAKEPED is a batch program that first runs MAKEPED1 to insert additional pointers and break loops. It then runs the LOOP program to verify that all loops have been resolved by the user and no loops are left undetected. The original program (without checking for loops) may be invoked by typing MAKEPED1.
  - 3. No data for individual:

If an individual is listed as a parent or sibling but is not provided a record of their own, the program aborts.
  - 4. No family:

If an individual has no parents and no children then the program aborts.
- D. Proband and loop definitions can now be stored in a file and reused in subsequent runs.
- E. Under Windows the input and output filenames must be specified on the command line, for example:

```
makeped pedin.dat pedout.dat
```

## 4.1 Program Usage

Start the program by typing MAKEPED followed by the names of the input and output files. Optionally, these file names may be followed by the letter 'n', which, if present, tells MAKEPED that no loops are in the pedigrees and it should not prompt the user for loops.

You are then asked if your pedigree file contains any loops. If it does answer 'y', if not answer 'n'. If you answer 'y' you will be asked if you have a file with loop definitions. (This refers to a file you may have created in previous runs of MAKEPED.) If you do then answer 'y' and enter the file name when prompted for it. If not answer 'n'. You will be prompted for the pedigree and individual id's where you would like loops defined and be given the chance to save these responses in a file for future use. You can select as many loops as you wish. When you have finished enter 0 (zero) at the next pedigree id prompt and the program will stop asking you for more. Once you have entered all of your loop definitions you are asked if you would like to save the selections in a file. If you think you might be running this program on the same pedigree file in the near future and if you made more loop selections than you would care to re-type you should answer 'y' and give the file name where you would like these selections saved. Otherwise, answer 'n'.

After you have finished loop definitions you are asked if you would like probands selected automatically. If you answer 'y' the program will search each pedigree for the first male with no parents and define each to be a proband. If you answer 'n' you are allowed to select your own probands in one of two ways. The first is used if you need to set a proband for every pedigree. If this method is selected the program will prompt you for an individual's id for each pedigree found in the file. The second method is used if you would like to select only a few probands and let the program assign the rest. This method requires that you enter both the pedigree id and the individual id. As in loop selection, you enter a pedigree id of 0 when you are finished. The choice of methods is selected by answering 'y' or 'n' when the program asks if you would like to select all probands.

Once all probands have been selected the program proceeds with the conversion and places the results in the output file. The main reason for selecting probands is to designate that person for whom risks are to be calculated.

## 4.2 Example Session

```
>MAKEPED
Pedigree file -> pedin.dat      \      or provide these names
Output file   -> ped.out       /      on the command line
Does your pedigree file contain any loops?      (y/n) -> y
Do you have a file of loop assignments?        (y/n) -> n

      Enter identifiers for each pedigree and person...
      enter pedigree 0 when finished.

Pedigree      -> 1
Person        -> 5
Pedigree      -> 0
```

Do you want these selections saved for later use? (y/n) -> y  
Enter filename -> loops.dat  
Do you want probands selected automatically? (y/n) -> n  
Do you have a file of proband assignments? (y/n) -> n  
Do you want to select all probands? (y/n) -> y

Enter the identifier of the person who is to be the proband for...

Pedigree	-> 1	(The program typed the 1. You type)
Person	-> 2	(your selection for pedigree 1.) (In this example there was only) (one pedigree in the file.)

Do you want these selections saved for later use? (y/n) -> n

## References

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. *Proc Nat Acad Sci USA* **81**, 3443-3446

Lathrop GM, Ott J (1990) Analysis of complex diseases under oligogenic models and intrafamilial heterogeneity by the LINKAGE programs. *Am J Hum Genet* **47**, A188

Ott J (1999) *Analysis of Human Genetic Linkage*, 3<sup>rd</sup> edition. Johns Hopkins University Press, Baltimore

Terwilliger JD, Ott J (1994) *Handbook for Human Genetic Linkage*. Johns Hopkins University Press, Baltimore