### Computer Assisted Labelling in Mutagenicity Testing: I. The *Drosophila Melanogaster* Sex-Linked Recessive Lethal Assay

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**ABSTRACT:**
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COMPUTER-ASSISTED LABELLING IN MUTAGENICITY TESTING

I. The Drosophila Melanogaster Sex-Linked Recessive Lethal Assay

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(Signature and date)
PREFACE

This is the first in a series of technical notes on the utilization of the computer facilities at Letterman Army Institute of Research to assist in mutagenicity testing as part of the Institute's toxicology program. These reports will detail the use of the computer in labelling articles, recording, storing and retrieving data, and data analysis. Both the Salmonella mutagenicity and the Drosophila melanogaster sex-linked recessive lethal mutagenicity assays will be covered.
ACKNOWLEDGMENTS

The authors express their appreciation to the Information Sciences Group for making computer facilities readily available and to all those who have made suggestions for improving the utility of the program described herein.
The mutagenicity testing of materials in compliance with Federal regulations (1) requires the implementation of an extensive labelling system which must be integrated with data collection, storage, data retrieval and analysis. The Drosophila melanogaster sex-linked recessive lethal assay is an often used mutagenicity test which requires the unique numbering of the test flies and their progeny, dilutions of test and control materials, and their multiple replicates. The detection of test materials with low mutagenic activity requires the concurrent testing of 7,000-10,000 X-chromosomes, depending on the spontaneous mutation frequency of the stock colonies (2,3), from both negative control and test material treated flies. Due to space and personnel limitations, we currently divide the testing of a single compound into four runs of approximately 2,500 X-chromosomes each for the negative control and test material treated flies as well as 500 X-chromosomes from positive control treated flies (4). This requires the generation of 880 unique, sequential labels and brood cards for testing a single compound. These items are currently hand labelled in most laboratories. We have designed and implemented a FORTRAN V program for the rapid generation of the large numbers of uniquely identified labels and cards. The use of this system has greatly reduced the time spent generating these materials, eliminated errors in numbering, and ensured continuity from the initiation to the termination of the assay.

PROGRAM DESCRIPTION

The program (See Appendix A - SLRLABELS.FR) generates the labels and cards for the individual male flies that will be exposed to negative control, positive control, and test compounds.

As seen from the sample run (Figure 1), the program displays the following information (the underlined characters were entered at the time of the program execution by the user): The number of the last run for which labels and cards were prepared and the sequence number of the last male fly for which labels and cards were generated.

As can also be seen in the sample run (Figure 1) and the sample labels and cards (Figures 2-5), the identification number of the male is composed of two fields: the first designates the nature of the compound (C - negative control, P - positive control, and T - test compound) and which of the series of compounds it is by an integer.
designator from 1 to 5. In the case of test substances, a repetition number is also printed. The second field of the male identification is the integer sequence number of the fly.

The program requests the following information from the user (refer to Figure 1):

- The initial sequence number to be used for the flies of this run.
- The maximum sequence number to be used for the flies of this run. If the maximum sequence number, minus the minimum sequence number, is greater than 100, then the user must re-enter both values. This feature is utilized because experience has shown that 25 is a reasonable practical limit for setting up the assay in our laboratory.
- The new run number.
- The number of negative control compounds (a maximum of five is allowed) for which labels and cards are to be prepared to permit the concurrent testing of several control compounds.
- The codes for each of the negative control compounds. The user is allowed up to six characters of any type for the designation of each compound.
- The number of positive control compounds (a maximum of five is allowed) for which labels and cards are to be prepared.
- The maximum sequence number for the positive control compounds (usually, there is no need for as many flies to be exposed to the positive control as to the negative control and test compounds).
- The codes for the positive control compounds. The user is allowed up to six characters of any type for the designation of each compound.
- The number of test compounds (a maximum of five is allowed).
- The codes for the test compounds. Again, the user is allowed up to six characters of any type for the designation of each compound.
- The repetition number for each test compound. As is illustrated in Figure 1, no number greater than 99 is allowed.
- The date for each of the four broods.

As the text for the labels and cards is generated by brood, a message is displayed at the user's terminal to indicate what has been prepared and the files in which they are stored.
After the text for all the labels and cards have been placed in the files DLABELS and BCARDS, the user is so notified and may print the labels and cards out on an appropriate device (usually a printer with a tractor feed). The program is formatted so that the labels and cards should be printed on continuous-feed single-width stock material. These may be obtained from MISCO, 936 Holmdel Keyport Road, Box 399, Holmdel, N.J. 07733, or other distributor of computer related items. The labels are the standard 1 X 3.5 inches and the cards are standard 3 x 5 inches.

All cards and labels are generated so that they are uniquely identified and parallel one another. They are prepared in the sequence in which they will be used. The cards provide permanent records and greatly facilitate data entry into permanent data files on the computer.

DISCUSSION

By utilizing the program and subroutines presented in the report we have realized a significant savings in time for the preparation of materials for the Drosophila melanogaster sex-linked recessive lethal mutagenicity assay. The labels and cards have also been of great help in maintaining order and uniformity while running this assay.

CONCLUSION

None.

RECOMMENDATION

None.
REFERENCES


Figure 1. Sample run of SLRLABELS.FR
Figure 1. Sample run of SLRLABELS.FR
(Continued)
LABELS AND CARDS FOR T1-T-ONE ARE IN 'DLABELS' AND 'BCARDS'.
LABELS AND CARDS FOR T2-T-TWO ARE IN 'DLABELS' AND 'BCARDS'.

BROOD: 4

LABELS AND CARDS FOR C1-NEGONE ARE IN 'DLABELS' AND 'BCARDS'.
LABELS AND CARDS FOR P1-POSONE ARE IN 'DLABELS' AND 'BCARDS'.
LABELS AND CARDS FOR T1-T-ONE ARE IN 'DLABELS' AND 'BCARDS'.
LABELS AND CARDS FOR T2-T-TWO ARE IN 'DLABELS' AND 'BCARDS'.

ALL LABELS AND CARDS ARE READY IN 'DLABELS' AND 'BCARDS'.

Figure 1. Sample run of SLR LABELS.FR
(Continued)

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Figure 7. Sample Negative Control Label and Card
Figure 3. Sample Positive Control Label and Card
GLP STUDY NO. 12345
RUN: 33 REP. #: 2
TI: 810 BR: 1 99DEC99
COMPOUND CODE: T-ONE
NOTES:

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEDIAN BATCH #</th>
<th>INITIALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Sample Test Compound Label and Card
GLP STUDY NO. 12345  
RUN: 33  REP. #: 45  
T2- 810 BR: 1 99DEC99  
COMPOUND CODE: T-TWO  
NOTES:  

GLP STUDY NO. 12345  REP. #: 45  
T2- 810 BR: 1 99DEC99  RUN: 33  

-------------------------------------------  
F2 CROSS  MEDIUM BATCH #:   INITIALS   
DATE:   INITIALS   
FAILURES  LETHALS  NONLETHALS  
-------------------------------------------  
F3 CROSS  MEDIUM BATCH #:   INITIALS   
DATE:   INITIALS   
FAILURES  LETHALS  NONLETHALS  
-------------------------------------------  
NOTES:  

Figure 5. Sample Test Compound  
Label and Card  

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APPENDIX A

'SUBLABELS.FR

--- PROGRAM NAME: SUBLABELS.FR
--- AUTHOR: W. J. Jedenberg
--- SUBROUTINES: NISLABELS.FR, NTLABELS.FR
--- INPUT:
--- AT TIME OF EXECUTION:
--- RANGE OF LABELS, RUN NUMBER, BROOD DATES
--- COMPOUND CODES, COMPOUND NUMBERS
--- FORMAT FOR INPUT: CRT OR DASHER
--- FORMAT TO GENERATE LABELS AND CARDS FOR THE
--- SEX-LINKED RECESSIVE LETHAL DROSOPHILA ASSAY.
--- THIS PROGRAM AND SUBROUTINES GENERATE THE LABELS FOR
--- THE NEGATIVE CONTROL COMPOUND(S), THE POSITIVE CONTROL
--- COMPOUND(S), AND THE TEST COMPOUND(S) IN ORDER OF BROOD.
--- A MAXIMUM SEQUENCE OF 100 IS ALLOWED AND UP TO 5 OF EACH
--- COMPOUND. THE LABELS ARE STORED IN 'DLABELS'.
--- THE SUBROUTINES NISLABELS.FR AND NTLABELS.FR ARE USED.
--- INPUT CODE, P, PCOM, TCOM, IR, UR, E, TL, IDATE
--- DIMENSION CODE(6,5), PCOM(6,5), TCOM(6,5), IDATE(7,4), NREP(5)

--- LOG UI.HDL
--- TYPE "null-drosophilA LABELS . . . . . . ."
--- TYPE

--- GET THE LAST RUN AND SEQUENCE NUMBERS
OPEN 2, "DLABEL", ATT="SB"
READ(2,1) N, NL
READ (2, 1(X, 14)) NCOL, (10, Y) NCOL
FORMAT (1X, /, "THE LAST RUN WAS: ", 1X, 14,
/1X, "THE LAST SEQUENCE NUMBER WAS: ", 1X, 14)
CLOSE 2

--- GET THE GLP STUDY NUMBER
100 CONTINUE
--- ACCEPT "GLP STUDY No. ? (15) ", CSH
IF (CSH. GT. 99999) GO TO 100

---

--- INPUT AND CHECK THE SEQUENCE RANGE
100 CONTINUE
--- TYPE

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APPENDIX A
(CONTINUED)

ACCEPT "INITIAL SEQUENCE NUMBER?",Ih
ACCEPT "MAXIMUM SEQUENCE NUMBER?",Ur

C --- CHECK RANGE
TL = Ur-Ih
IF (TL.LE.100) GO TO 300
TYPE
TYPE "************Error************"
TYPE "MAXIMUM - INITIAL > 100"
Go TO 700
300 CONTINUE

C ---

C ** INPUT NEW RUN NUMBER
TYPE
ACCEPT "WHAT IS THE NEW RUN NUMBER?",Nh

C ---

C ** INPUT COMPOUND CODES
C --- NEGATIVE CONTROL COMPOUNDS
400 CONTINUE
ACCEPT "HOW MANY NEGATIVE CONTROL COMPOUNDS? (MAX.-5) ",Nnc
IF (Nnc.GT.5) GO TO 400
TYPE
DO 90 I=1,Nnc
WRITE (10,3) I
3 FORMAT (1X,"WHAT IS THE CODE FOR THE NEGATIVE CONTROL #:",1X,I1,1.X
1"? (AN/6)")
READ (11,4) (CCod(K,I) K=1,6)
4 FORMAT (6Ai)
90 CONTINUE

C ---

C --- POSITIVE CONTROL COMPOUNDS
500 CONTINUE
ACCEPT "HOW MANY POSITIVE CONTROL COMPOUNDS? (MAX.-5) ",Npc
IF (Npc.GT.5) GO TO 500

C *** GET NUMBER FOR POSITIVE CONTROL SEQUENCE
900 CONTINUE
ACCEPT "MAXIMUM SEQUENCE NUMBER FOR POSITIVE CONTROLS? ",Ptl
Ptl = Ptl - 1h
IF (Ptl.LE.100) GO TO 800
TYPE
TYPE "************Error************"
TYPE "MAXIMUM - INITIAL > 100"
GO TO 900
800 CONTINUE

C ---
APPENDIX A
(CONTINUED)

TYPE
DO 20 I = 1,NPC
WRITE (10,5) I
5 FORMAT (1X,"WHAT IS THE CODE FOR THE POSITIVE CONTROL #:",1X,I1,I1,I1,
1"? (AN/B)"")
READ (11,4) (PCOM(K,I) K=1,6)
20 CONTINUE
TYPE
TYPE
C --- TEST COMPOUND(S)
600 CONTINUE
ACCEPT " HOW MANY TEST COMPOUNDS ? (MAX-5) ",NTC
1 = (NTC .GT. 5) GO TO 600
DO 50 I = 1,NTC
TYPE
50 CONTINUE
6 FORMAT(1X,"WHAT IS THE CODE FOR TEST COMPOUND #:",1X,I1,I1,I1,
1"? (AN/B)"")
READ (11,4) (TCOM(K,I) K=1,6)
C --- GET THE REPEITION NUMBER
123 CONTINUE
ACCEPT " WHAT IS THE REPEITION NUMBER FOR THIS COMPOUND ? (I2) ",
IREP1
1 IF (IREP1 .LE. 99) GO TO 123
C ---
50 CONTINUE
TYPE
TYPE
C --- INPUT BLOOD DATES
TYPE
TYPE " INPUT BLOOD DATES"
DO 40 B = 1,4
WRITE (10,7) B
7 FORMAT (1X,"DATE FOR BLOOD:",1X,I1,I1,I1,"? (XXMXX)")
READ (11,8) (IBDATE (K,B) K=1,7)
40 CONTINUE
C ---
C *** MESSAGE
TYPE
TYPE " WAIT FOR OUTPUT . . . . . . . . "
C ---
**APPENDIX A**

(CONTINUED)

```plaintext
*** MAKE LABELS FOR EACH BOARD

OPEN FILE 'DLABELS'
DELETE 'DLABELS'
OPEN 1, 'DLABELS', ATT = "SOF"
DELETE 'BCARDS'
OPEN 2, 'BCARDS', ATT = "SOF"

*** OUTPUT RUN NUMBER

WRITE (10,9) NA
FORMAT (/10X,"RUN:"1X,14,/)

*** LABELS MADE BY BOARD

DO 50 R = 1,4
WRITE (10,10) R
FORMAT (/10X,"BOARD:"1X,11)

!!! RELATIVE CONTROL LABELS

K = "C"
DO 60 J = 1,K,C
CALL HBLABELS (GSN,Mr,K,1,1,GR,0.04,1,1,,ILAB(1,J))
50 CONTINUE

!!! POSITIVE CONTROL LABELS

K = "P"
DO 70 J = 1,K,P
CALL HBLABELS (GSN,Mr,K,1,1,FO,POUR(1,1),0,200,J,JFT(J))
70 CONTINUE

!!! TEST COMPASS LABELS

K = "T"
DO 80 J = 1,K,T
CALL HBLABELS (GSN,Mr,K,1,1,TS,TCOM(1,1),0,DATE(1,J))
80 CONTINUE

!!! TYPE

50 CONTINUE

!!! CLASSES

CLASS 1
CLASS 3

*** END MESSAGE

TYPE
TYPE "ALL LABELS AND CARDS ARE READY IN 'DLABELS' AND 'BCARDS'"

END
```
APPENDIX B

NBLABELS.FR

C +++ SUBROUTINE NAME: NBLABELS.FR
C +++ WRITTEN BY: Warren Jederling, CUTANEOUS HAZARDS
C +++ SUBROUTINES: NONE
C +++ INPUT: GSN, Na, K, P, L, N, LCUM, N, N, N
C +++ GSN = GLP STUDY NUMBER
C +++ Na = RUN NUMBER
C +++ K = "C", "P"
C +++ L = INITIAL VALUE
C +++ M = MAXIMUM VALUE
C +++ LCUM = COMPOUND CODE
C +++ N = BROOD #
C +++ P = SUBGROUP
C +++ IDATE = PROPER DATE PER BROOD
C +++ FORMAT FOR INPUT: SEE SLABELS.FR
C +++ PURPOSE: GENERATES LABELS AND CARDS FOR THE NEGATIVE AND
C ++ POSITIVE CONTROLS FOR THE SRIL DROSOPHILA ASSAY. SUPPORTS THE
C ++ MAIN PROGRAM SLLABELS.FR ALONG WITH NBLABELS.FR.
C +++
SUBROUTINE NBLABELS (GSN, Na, K, P, L, N, LCUM, N, IDATE)
INTEGER I, P
DIMENSION IDATE(7), LCUM(6)
DO 10 I=1, N
WRITE (1,3) GSN, Na, K, P, I, N, IDATE, LCUM

C +++ PREPARE CORRESPONDING CARD
WRITE (3,7) GSN,LCUM,K,P,I,N, IDATE,Na

82X,"P3 CROSS MEDIUM BATCH #: __________/ 92X,"DATE: ________ INITIALS/ 12X,"FAILURES ______ LETHALS ______ NONLETHALS ______/}
22X,"--------------------------/
APPENDIX B
(CONTINUED)

32X, "NOTE: 

CALL CONTINUE
C --- STORE LAST SEQUENCE # IN "LABEL"
LL = 1-1
OPEN 2, "LABEL", ATT = "SO"
WRITE (2,5) RA, LL
FORMAT (2(1X,I4))
CLOSE 2
C --- USER  NOTE
WRITE (10,6) K,P,LCOM
FORMAT (/1X, "LABELS AND CARDS FOR", 1X,A1,I1, "="
REAL1,1X,'AND','SCARDS'")
RETURN
END
APPENDIX C

NTLABELS.FR

C ----- SUBROUTINE NAME: NTTLABELS.FR
C ----- WRITTEN BY: WALTER J. EBERHART, COTTON MILL HAZARDS
C ----- SUBROUTINES: NONE
C ----- INPUT: GSN, N, K, P, L, LOC, N, L, E
C ----- GSN = GLP STUDY NUMBER
C ----- N = NO. NONE...
C ----- LOC = REPEATION NUMBER
C ----- K = "T"
C ----- L = INITIAL VALUE
C ----- N = MAXIMUM VALUE
C ----- LOC = COMPOUND CODE
C ----- N = BOND #
C ----- P = SUBGROUP
C ----- I11 = Format for Bond
C ----- FORTRAN FOR INPUT: SEE NTTLABELS.FR
C ----- PURPOSE: SUBROUTINE TO GENERATE LABELS AND CARDS FOR THE
C ----- TEST COMPOUNDS USED IN THE GLP PHOSPHINA ARRAY. SUPPORT THE
C ----- PROBLEM SUBPROGRAMS ALONG WITH NTTLABELS.FR.
C ----- SUBROUTINE NTTLABELS (GSN, N, K, P, L, LOC, N, L, E)
INTEGER I, P
DIMENSION I(17,7), LOC(6)
DO 10 I=1,N
WRITE (1,3) GSN, N, K, P, L, LOC, N, L, E, LOC
  3 FORMAT (1X,"GLP STUDY NO.",1A,15,\,)
     1X,"COMPOUND: ",1A,14,5X,"REPEAT: ",1X,15,1X,1X,11,11,=",14,CA,14,\,)
     1X,11,2A,//,\,)
     1X,COMPOUND CODE: ",2A,6A,/,1X,"NO. L:",/)
  10 FORMAT (3,7) GSN,N,K,P,L,LOC,N,L,E

1 FORMAT (1X,"GLP STUDY NO.",1A,15,4X,"REPE: ",1A,15,\,)
52X,"-----------------------------",/)
42X,"F2 CROSS MATCHED BOND #: ",/)
52X,"DATE: ",/)
52X,"FAILURE: ",/)
42X,"NO. ANALYS ",/)
52X,"SHRT: ",/)
52X,"LONG: ",/)
52X,"MID: ",/)
52X,"F3 CROSS: ",/)

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APPENDIX C
(CONTINUED)

"CA,"DATE:_________INITIALS______",,"//
"DX,"Diagnosis:__LETHALS____NONLETHALS____",,"//
"EX,"--------------------------",,"//
"FX,"NOTICE":",,"//

TO CONTINUE

--- STORE LAST SEQUENCE # IN "DLLABEL"
   LL = 1:1
OPEN 2,"DLLABEL", ATT = "SO"
WRITE (2,5) LL,LL
5 FORMAT (2(I4,I4))
CLOSE 2
--- DATA NOTE
WRITE (10,6) K,P,LCOM
6 FORMAT (/I4,"LABELS AND CARDS FOR",I4,A1,I1,"-",
       I4,I1,"ARE IN 'DLABELS' AND 'BCARDS'"))

END
END

DATE

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