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Computer programs for accurate determination of size of DNA fragments in agarose gels

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The use of plasmid profiling for epidemiological studies of bacterial infections is an accurate and reliable method of typing, especially where no other typing scheme is available.¹ Restriction endonuclease digestion of whole DNA has also been used in fingerprinting strains of the same bacterial species.^{2,3} All these studies require accurate estimation of the size of DNA fragments. Usually, molecular size of these fragments is determined by subjecting the DNA samples to electrophoresis on agarose, or polyacrylamide gels. The molecular size of DNA is inversely proportional to the distance travelled and may be calculated by comparison with the migration of known standards on the same gel.⁴ The logarithm of size in megadaltons is plotted against the logarithm of relative mobility for each of the standards. The size of the unknown DNA fragments, which is on the same gel as the standards, is read off from the curve of best fit. Such a standard curve is usually concave upward, and only a portion of it is roughly linear. The relative mobility of DNA fragments is calculated by

following formula:

$$\frac{10}{X} \times Y,$$

where X = distance travelled by one of the standards

Y = distance travelled by the DNA fragment of which the relative mobility is to be calculated

This method is very inaccurate, however, if the curve of best fit is drawn subjectively. More accurate methods have been devised that relate migration and molecular size on the basis of standard mathematical formulae. These complex equations usually require computer analysis for their solution.⁵ Such programs are available in BASIC^{6,7} and FORTRAN.⁵

We have written two computer programs in MBASIC that will run on Apple II/II+ /IIE computers, and these may be easily adapted for other microcomputers. The programs are relatively short and produce two solutions, one fitting a straight line and the second a parabola to results from a set of standards (figs 1 and 2). They are written based on the following equations:

$$y = mx + c \text{ where}$$

$$\begin{array}{lll} y = \text{molecular weight of DNA} & > \\ x = \text{relative mobility of DNA} & > \text{for linear} \\ m \text{ and } c = \text{constants} & > \text{relation} \end{array}$$

$$y = a + bx + cx^2 \text{ where}$$

$$\begin{array}{lll} y = \text{molecular weight of DNA} & > \text{for} \\ x = \text{relative mobility of DNA} & > \text{parabolic} \\ a, b, \text{ and } c = \text{constants} & > \text{relation} \end{array}$$

The migration distance of each standard from individual gels and its size are entered into the program. The migration of one standard is chosen and entered as "STANDARD MIGRATION" when prompted. The relative mobility will be calculated by the program. Once data are entered, results are produced almost instantaneously.

Fig 1 Computer program for calculation of size of DNA using linear relation of its size and relative mobility on agarose gel.

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1 REM ***LING32APPLE***MAR**1986***
5 REM ***MOBILITY/MOLECULAR WEIGHT-LINEAR RELATIONSHIP***
10 HOME: CLEAR: VTAB (5)
15 REM X(A) = DISTANCE TRAVELLED BY DNA, Y(A) = LOGe(MOLECULAR WEIGHT OF DNA)
20 INPUT "NUMBER OF STANDARDS = "; A: DIM X(A), Y(A)
30 W = 1: WW = 1: VTAB (10)
35 REM B = STANDARD MIGRATION, DISTANCE TRAVELLED BY A DNA MOLECULE OF KNOWN SIZE
  FROM WHICH RELATIVE MOBILITIES ARE CALCULATED
40 INPUT "DISTANCE OF STANDARD MIGRATION = "; B: HOME
50 FOR N = 1 TO A
60 IF N = W THEN GOSUB 500
65 REM C = MOLECULAR WEIGHT OF DNA
70 INPUT C: Y(N) = LOG (C): VTAB (2 + WW): WW = WW + 1
80 PRINT SPC( 5)C SPC( 15-LEN( STR$(C)));
85 REM D = DISTANCE TRAVELLED BY DNA WITH MOLECULAR WEIGHT C
90 INPUT D: X(N) = D * 10 / B
100 NEXT
200 FOR N = 1 TO A
205 REM CALCULATE R(CORRELATION COEFFICIENT), M(GRADIENT), C(INTERCEPT)
210 S = S + X(N): SY = SY + Y(N): SZ = SZ + (X(N) * Y(N))
220 SS = SS + X(N) ^ 2: YY = YY + Y(N) ^ 2
230 NEXT
240 RR = (SZ - (S * SY) / A)
250 R = RR / ( SQR ((SS - (S ^ 2 / A)) * (YY - (SY ^ 2 / A))))
260 M = RR / (SS - ((S ^ 2) / A))
270 C = (SY / A) - M * (S / A)
300 HOME: W = 1: WW = 1: VTAB (10)
310 PRINT "NUMBER OF BANDS"
315 REM E = NUMBER OF DNA BANDS FOR SIZE ESTIMATION
320 INPUT "FOR ESTIMATIONS = "; E: HOME
330 FOR N = 1 TO E
340 IF N = W THEN GOSUB 600
345 REM F1 = DISTANCE TRAVELLED BY DNA MOLECULE, F = RELATIVE MOBILITY OF DNA
350 INPUT F1: F = F1 * 10 / B: VTAB (2 + WW): WW = WW + 1
355 REM G = MOLECULAR WEIGHT OF DNA CALCULATED FROM M AND C VALUES
360 G = INT ((EXP (M * F + C)) * 100 + .5) / 100
370 GOSUB 700
380 PRINT SPC( 5)F1 SPC( 15 - LEN (STR$( F1))) SPC( 5 - I)G;
390 PRINT : NEXT
400 PRINT "PRESS ANY KEY"
410 GET B$: IF B$ = " " THEN 410
420 HOME: VTAB (10)
425 REM FOR MORE CALCULATIONS USING THE SAME M AND C VALUES
430 PRINT "MORE CALCULATIONS USING"
440 INPUT "EXISTING DATAS (Y/N) ? "; A$
450 IF LEFT$(A$,1) = "Y" THEN GOTO 300
460 HOME: END
500 IF N = 1 THEN 530
510 PRINT "PRESS ANY KEY": WW = 1
515 REM PRINT TITLES ON SCREEN: MW OF BAND (INPUT STANDARD VALUES); BAND DISTANCE
  (INPUT CORRESPONDING DISTANCE TRAVELLED)
520 GET B$: IF B$ = " " THEN 520
530 HOME
540 PRINT SPC( 4) "MW OF BAND" SPC( 3) "BAND DISTANCE"
550 GOSUB 650
560 RETURN
600 IF N = 1 THEN 630
610 PRINT "PRESS ANY KEY": WW = 1
615 REM PRINT TITLES ON SCREEN: BAND DISTANCE (INPUT DISTANCE TRAVELLED BY SAMPLE
  DNA); MW OF BAND (MOLECULAR WEIGHT OF SAMPLE DNA CALCULATED TO 2 DECIMAL
  POINTS)

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620 GET BS: IF BS = "" THEN 620
630 HOME
640 PRINT SPC(3) "BAND DISTANCE" SPC(4) "MW OF BAND"
650 PRINT : HLINE 0,39 AT 2
660 W = W + 20
670 RETURN
700 XS = STR$(G)
705 REM TO LINE UP ALL THE DECIMAL POINTS TO THE SAME POSITION ON SCREEN
710 FOR I = 1 TO 4
720 IF MID$(XS,I,1) < > "." THEN IF MID$(XS,I,1) < > "" THEN NEXT I
730 RETURN

```

Fig 2 Computer program for calculation of size of DNA using parabolic relation of its size and relative mobility on agarose gel.

```

1 REM ***LING33APPLE***JUN**1986***
5 REM ***MOBILITY/MOLECULAR WEIGHT-PARABOLIC RELATIONSHIP***
10 HOME: CLEAR: VTAB(5)
15 REM X(A) = DISTANCE TRAVELLED BY DNA, Y(A) = LOGe(MOLECULAR WEIGHT OF DNA)
20 INPUT "NUMBER OF STANDARDS = "; A: DIM X(A), Y(A)
30 W = 1: WW = 1: VTAB(10)
35 REM B = STANDARD MIGRATION, DISTANCE TRAVELLED BY A DNA MOLECULE OF KNOWN SIZE
  FROM WHICH RELATIVE MOBILITIES ARE CALCULATED
40 INPUT "DISTANCE OF INT STD MIGRATION = "; B: HOME
50 FOR N = 1 TO A
60 IF N = W THEN GOSUB 500
65 REM C = MOLECULAR WEIGHT OF DNA
70 INPUT C: Y(N) = LOG(C): VTAB(2 + WW): WW = WW + 1
80 PRINT SPC(5) C SPC(15 - LEN(STR$(C)));
85 REM D = DISTANCE TRAVELLED BY DNA WITH MOLECULAR WEIGHT C
90 INPUT D: X(N) = D * 10 / B
100 NEXT
200 FOR N = 1 TO A
205 REM CALCULATE CONSTANTS AA, BB, CC FOR THE PARABOLIC EQUATION
210 S = S + X(N): S2 = S2 + X(N) ^ 2: S3 = S3 + X(N) ^ 3: S4 = S4 + X(N) ^ 4
220 SZ = SZ + (X(N) * Y(N)): XY = XY + ((X(N) ^ 2) * Y(N)): SY = SY + Y(N)
230 NEXT
240 MX = S / A: MU = S2 / A: MY = SY / A
250 X2 = S2 - A * (MX ^ 2): X3 = S3 - A * MX * MU: XX = S4 - A * (MU ^ 2)
260 Y1 = SZ - A * MX * MY: Y2 = XY - A * MU * MY
270 CC = ((X3 * Y1) - (X2 * Y2)) / ((X3 ^ 2) - (X2 * XX))
280 BB = (Y1 / X2) - (CC * (X3 / X2))
290 AA = MY - BB * MX - CC * MU
300 HOME: W = 1: WW = 1: VTAB(10)
310 PRINT "NUMBER OF BANDS"
315 REM E = NUMBER OF DNA BANDS FOR SIZE ESTIMATION
320 INPUT "FOR ESTIMATIONS = "; E: HOME
330 FOR N = 1 TO E
340 IF N = W THEN GOSUB 600
345 REM F1 = DISTANCE TRAVELLED BY DNA MOLECULE, F = RELATIVE MOBILITY OF DNA
350 INPUT F1: F = F1 * 10 / B: VTAB(2 + WW): WW = WW + 1
355 REM G = MOLECULAR WEIGHT OF DNA CALCULATED FROM AA, BB AND CC CONSTANTS
360 G = INT((EXP(AA + BB * F + CC * F ^ 2)) * 100 + .5) / 100
370 GOSUB 700
380 PRINT SPC(5) F1 SPC(15 - LEN(STR$(F1))) SPC(5 - I) G;
390 PRINT : NEXT
400 PRINT "PRESS ANY KEY"
410 GET BS: IF BS = "" THEN 410
420 HOME: VTAB(10)
425 REM FOR MORE CALCULATIONS USING THE SAME AA, BB AND CC CONSTANTS
430 PRINT "MORE CALCULATIONS USING"
440 INPUT "EXISTING DATAS (Y/N) ? "; AS
450 IF LEFT$(AS,1) = "Y" THEN GO TO 300

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Technical methods

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460 HOME : END
500 IF N = 1 THEN 530
510 PRINT "PRESS ANY KEY":WW = 1
515 REM PRINT TITLES ON SCREEN: MW OF BAND (INPUT STANDARD VALUES); BAND DISTANCE
    (INPUT CORRESPONDING DISTANCE TRAVELLED)
520 GET B$: IF B$ = "" THEN 520
530 HOME
540 PRINT SPC(4) "MW OF BAND" SPC(3) "BAND DISTANCE"
550 GOSUB 650
560 RETURN
600 IF N = 1 THEN 630
610 PRINT "PRESS ANY KEY":WW = 1
615 REM PRINT TITLES ON SCREEN: BAND DISTANCE (INPUT DISTANCE TRAVELLED BY SAMPLE
    DNA); MW OF BAND (MOLECULAR WEIGHT OF SAMPLE DNA CALCULATED TO 2 DECIMAL POINTS)
620 GET B$: IF B$ = "" THEN 620
630 HOME
640 PRINT SPC(3) "BAND DISTANCE" SPC(4) "MW OF BAND"
650 PRINT : H$ = "0.39 AT 2"
660 W = W + 20
670 RETURN
700 X$ = STR$(G)
705 REM TO LINE UP ALL THE DECIMAL POINTS TO THE SAME POSITION ON SCREEN
710 FOR I = 1 TO 4
720 IF MID$(X$,I,1) < > "." THEN IF MID$(X$,I,1) < > "" THEN NEXT I
730 RETURN

```

Both computer programs have been shown to be accurate in estimating the size of DNA fragments. Tables 1 and 2 show the actual molecular weights and the molecular weights estimated by the linear and parabolic programs of *Hind* III digests of phage λ , and of standard plasmids of known size, respectively.

It should be noted that for the greatest accuracy, standards of similar sizes to the unknowns should be used for both linear and parabolic programs. Where

high molecular weight unknowns are to be estimated, the parabolic relation seems to be more accurate. Meyers *et al*⁴ showed that for high molecular weight DNA, there is a less linear relation between size and mobility. The parabolic program is also recommended for unknowns covering a wide range of sizes, especially when large sized standards are not available.

Table 1 Estimation (standard error of mean) of sizes of *Hind* III fragments of phage λ

Fragment size (Mdal)	Estimated fragment size (Mdal) by program	
	Linear	Parabolic
6.08	5.76 (0.22)	6.08 (0.04)
4.23	4.29 (0.06)	4.22 (0.06)
2.82	3.01 (0.16)	2.82 (0.05)
1.5	1.53 (0.03)	1.50 (0.04)
1.31	1.26 (0.02)	1.31 (0.03)

Table 2 Estimation (standard error of mean) of sizes of standard plasmids

Plasmid No	Plasmid size (Mdal)	Estimated plasmid size (Mdal) by program	
		Linear	Parabolic
TP 129	77.6	74.29 (3.83)	76.23 (2.63)
TP 125	64.0	64.73 (2.10)	65.58 (2.44)
Δ	59.4	57.78 (1.55)	57.72 (1.80)
x	40.0	42.48 (1.12)	41.45 (1.23)
RP 1	37.8	39.20 (1.32)	37.91 (1.77)
TP 120	31.7	32.25 (1.51)	31.49 (0.81)
Sa	25.9	24.56 (1.20)	24.71 (0.92)
TP 193	20.2	20.35 (0.85)	20.96 (0.58)

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