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Comparison of two molecular weight markers used in DNA-profiling

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Summary. DNA-profiling was performed on DNA from human blood samples. Restriction was performed with *Hin*fI and the fragments were analysed with the single locus probes MS1, MS31, MS43a, and YNH24. Calculations of the sizes of DNA-fragments in the range from 1.4 to 22 kilobase pairs (kb) were performed with two different size markers: the Amersham marker SJ5000 and the Gibco BRL marker 4401SA. The standard deviation of the difference between duplicate determinations was significantly lower with the Gibco BRL marker than with the Amersham marker. Calculation of the fragment lengths with the two markers differed significantly, especially in the high molecular weight region (> 8 kb). Fragment lengths were 3–8% (kb) higher with the Amersham marker than with the Gibco BRL marker which corresponds to a difference of 1.0–1.8 mm in migration distance. The difference was enhanced in the presence of ethidium bromide. The consequences of the replacement of the Amersham marker by the Gibco BRL marker in practical casework is discussed.

Key words: DNA-profiling – Single locus probes – Molecular weight markers – Ethidium bromide

Zusammenfassung. DNA-Bestimmungen wurden an Extrakten von menschlichen Blutproben durchgeführt. Die Restriktion erfolgte mit Hinf-I und die Fragmente wurden mit den Singlelocus-Sonden MS1, MS31, MS43a und YNH24 bestimmt. Die Bestimmung der Größen der DNA-Fragmente im Bereich zwischen 1,4 and 22 Kilobasen-Paaren (kb) erfolgte mit 2 unterschiedlichen Standards: dem Amersham-Marker SJ5000 und dem Gibco BRL-Marker 4401SA. Die Standardabweichung der Differenz zwischen Doppelbestimmungen war signifikant geringer mit dem Gibco BRL-Marker als mit dem Amersham-Marker. Die Berechnung der Fragmentlängen mit den beiden Markern unterschied sich signifikant, speziell im Bereich der hohen Molekulargewichtsregion (> als 8 kb). Die Fragmentlängen waren 3–8% (kb) höher mit dem Amersham-Marker als mit dem Gibco-BRL-Marker, dies entspricht einer Differenz der Wanderungsstrecke von 1,0–1,8 mm. In Anwesenheit von Ethidiumbromid wurde die Differenz gesteigert. Die Konsequenzen des Ersatzes des Amersham-Markers durch den Gibco-BRL-Marker in den praktischen Arbeit wird diskutiert.

Schlüsselwörter: DNA-Profilierung – Singlelocus-Sonden – Molekulargewichtsmarkierer – Ethidiumbromid

Introduction

The molecular weight marker is an important part of a DNA-profiling protocol and may be an essential parameter in inter-laboratory standardization. Commercially available markers are used by most laboratories, and many European laboratories have used the ^{35}S labeled Amersham SJ5000 marker which was used in the collaborative excercises of the European DNA Profiling Group (EDNAP), (see Schneider et al. 1991 and Gill et al. 1992). This marker is characterized by 12 irregularly spaced DNA bands of varying intensity in the range from 1.4 to 22 kb. Other markers comprising more than 20 equally spaced bands of almost equal intensity in the same range are now available, e.g. the Gibco BRL 4401SA marker and the Promega DG1931 marker. It is expected that the measurement error can be reduced by replacement of the Amersham marker by one of these markers. Such a change, however, requires an investigation of possible differences in the determination of fragment lengths with the two marker systems. In this study we report on the results of comparative measurements of fragment lengths using the Amersham SJ5000 and the Gibco BRL 4401SA markers. The effect of the presence of ethidium bromide in the electrophoresis buffer and loading buffer was also investigated.

Methods

Unless otherwise stated DNA was prepared and analysed as described previously (Eriksen et al. 1992). Restriction was performed with HinfI (Boehringer). The single locus VNTR probes MS1, MS31, MS43a (Cellmark Diagnostics) and YNH24 (Promega Corporation) were used. Labeling was performed with cytidine 5'-[$\alpha^{32}P$] triphosphate using random priming before hybridisation. The electrophoresis buffer (TBE) and the loading buffer contained ethidium bromide (0.5 µg/ml) unless otherwise noted. Each plate comprised at least 3 lanes with the Amersham marker SJ5000 (approximately

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150 ng/lane) and 3 lanes with the Gibco BRL 4401SA marker (approximately 12 ng/lane). The Gibco BRL marker was visualized as recommended by the manufacturer using ^{32}P labeling of the probe included in the kit. In some experiments the amount of the Amersham marker was reduced to 12 ng/lane. In these cases the visualization of the marker fragments was accomplished by the use of ^{32}P labeled HindIII digested λ-DNA as probe. The migration distances from the application point were measured manually. Calculations of the fragment lengths were carried out with a computer programme based on the local form of the reciprocal hyperbolic method of Elder and Southern (1987) including a correction for transversal skewness of the migration using linear interpolation between corresponding pairs of marker bands.

To obtain data with normally distributed measurement errors independent of the fragment length all measurements were transformed into normalized migration distances (Eriksen et al. 1992). The tranformation was accomplished by the function:

$$f(b) = m = 796/(3.7 + b^{1.5}) + 32.3$$

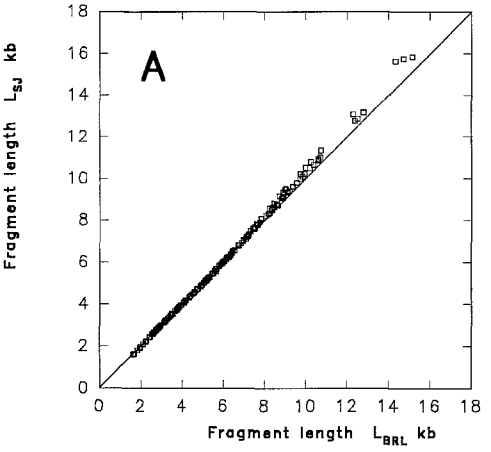
where b is the fragment length in units of kilobase pairs (kb) and m the normalized migration distance in millimetres.

Results

DNA from 30 blood samples was analyzed with the 4 probes in duplicate on different gels. Calculation of fragment length was performed with the Amersham and the Gibco BRL markers as size standards. Fragment lengths (kb) determined with the Amersham marker are designated as L_{SJ}, and those determined with the BRL marker as L_{BRL}. The fragment lengths were transformed into migration distance (mm) and designated as M_{SJ} and M_{BRL}, respectively.

The standard deviation of the difference between the first and the second measurement was 0.53 mm with the Amersham marker and 0.40 mm with the Gibco BRL marker.

In the following the mean values of duplicate measurements were used. The fragment lengths L_{SJ} in units of kb as calculated with the Amersham marker as size



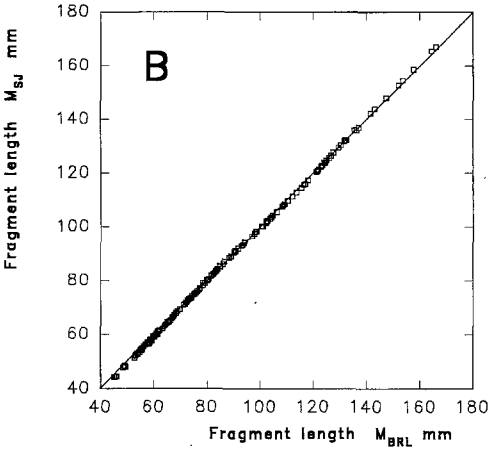
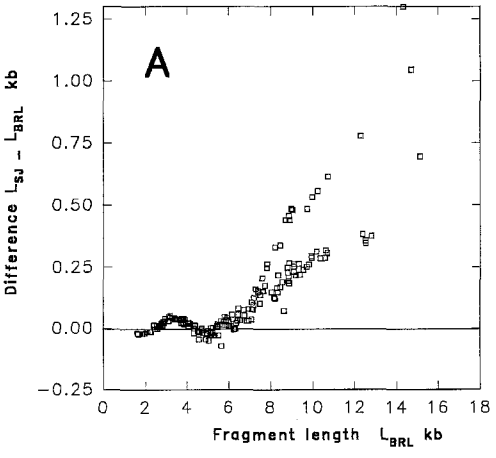


Fig. 1A, B. DNA from 30 blood samples digested with HinfI and analysed with MS1, MS31, MS43a, and YNH24 using two different size markers. **A** Fragment lengths L_{SJ} (kb) as calculated with the Amersham SJ5000 marker (150 ng/lane) plotted against the fragment lenghts L_{BRL} (kb) as calculated with the Gibco BRL marker 4401SA (12 ng/lane). **B** Fragment lengths after transformation of L_{SJ} into M_{SJ} (mm) as calculated with the Amersham marker plotted against fragment lengths M_{BRL} (mm) as calculated with the Gibco BRL marker



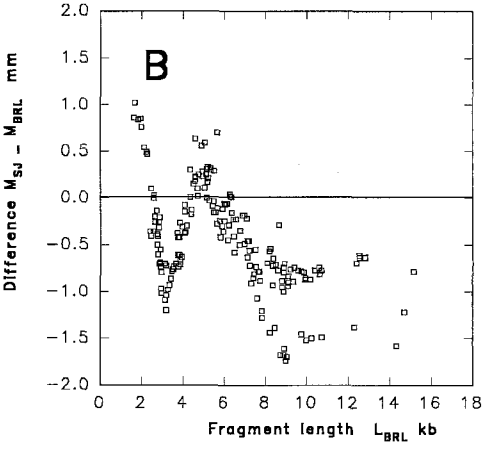
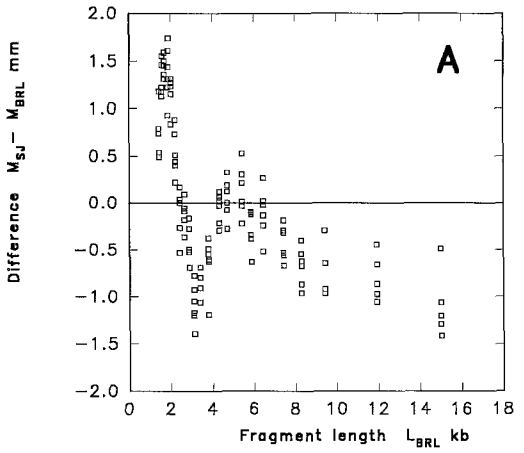


Fig. 2A, B. The differences between determinations of fragment lengths from 30 DNA-profiles. DNA was digested with HinfI and analysed with MS1, MS31, MS43a, and YNH24. Calculations were performed with two different size markers. **A** The differences $L_{SJ} - L_{BRL}$ between the fragment lengths (kb) as calculated with the Amersham SJ5000 marker and the Gibco BRL 4401SA marker plotted against the fragment length L_{BRL} as calculated with the Gibco BRL marker. **B** The differences $M_{SJ} - M_{BRL}$ between the transformed fragment lengths (mm) as calculated with the Amersham and the Gibco BRL marker plotted against the fragment lengths L_{BRL} as calculated with the Gibco BRL marker



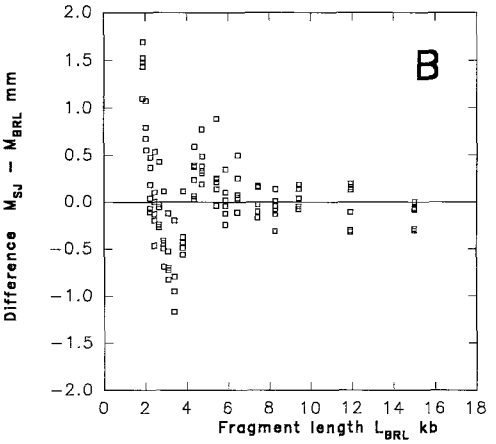


Fig. 3A, B. Differences between the determinations of the fragment lengths of the bands of the Gibco BRL 4401SA marker using the Amersham SJ5000 marker as a size marker in two different concentrations. Fragment lengths were used as transformed values (mm). The transformed fragment lengths of the bands of the Gibco BRL marker as calculated with the Amersham markers is designated as M_{SJ}. The fragment lengths L_{BRL} of the Gibco BRL marker bands were those given by the supplier. They were transformed and designated as M_{BRL}. The difference $M_{SJ} - M_{BRL}$ was plotted against L_{BRL}. The concentration of the Amersham marker SJ5000 was approximately 150 ng/lane (**A**) and 12 ng/lane (**B**)

standard were plotted against the corresponding values L_{BRL} as calculated with the Gibco BRL marker (Fig. 1A). Correspondingly, the transformed values M_{SJ} were plotted against M_{BRL} (Fig. 1B). In Fig. 1A pronounced deviations are seen above 8 kb. The fragment lengths determined with the Amersham marker were higher than those determined with the Gibco BRL marker. The corresponding plot of the transformed values (Fig. 1B) showed small deviations from a straight line in different regions.

The deviations are more easily seen in difference plots. The differences (kb) between the fragment lengths as determined with the Amersham marker and that determined with the Gibco BRL marker ($L_{SJ} - L_{BRL}$) are shown as a function of the fragment length L_{BRL} (Fig. 2A). Correspondingly, the differences calculated as mm migration distance $M_{SJ} - M_{BRL}$ are shown as a function of the fragment length L_{BRL} in Fig. 2B. Pronounced deviations were seen for fragment lengths above 8 kb together with minor deviations at 5, 3, and below 2 kb. In Fig. 2B the same relationship is given in units of mm. It is seen that all deviations fell within a range from 0.5 to 1.8 mm, including those below 2 kb.

Similar results were obtained in experiments where the Amersham and the Gibco BRL markers were loaded alternately on 4 gels. In 2 of the gels the amount of the Amersham marker was approximately 150 ng/lane, and in the other 2 gels approximately 12 ng/lane. The fragment lengths of the bands of the Gibco BRL marker were calculated with the Amersham marker as size standard. Figure 3A shows the results derived from duplicate determinations obtained with the high concentration of the Amersham marker, and Fig. 3B those obtained with the low concentration. With the high concentration of the Amersham marker the deviations were similar to those seen in Fig. 2B. With the lower concentration the marked deviation above 8 kb was absent. This may indicate that 'overloading' causes the inaccuracies involved in the determination of the position of the high molecular weight bands of the Amersham marker.

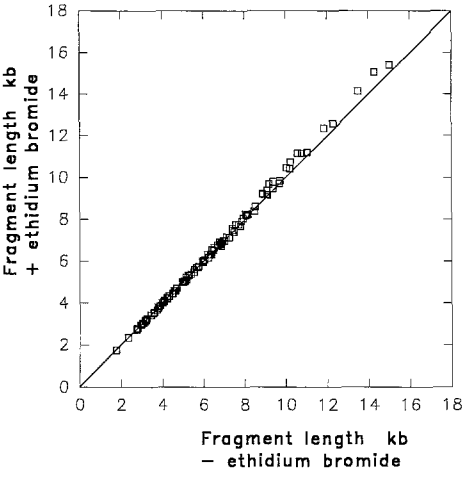


Fig. 4. The effect of ethidium bromide on the determination of fragment length. DNA from 15 blood samples was analysed in duplicate in the presence of ethidium bromide (0.5 µg/ml) and in its absence. DNA was digested with HinfI and MS1, MS31, MS43a, and YNH24 were used as probes. The Amersham marker was used as size marker (approximately 150 ng/lane). Fragment lengths (kb) determined in the presence of ethidium bromide were plotted against the fragment lengths (kb) determined in its absence

We have also investigated the effect of ethidium bromide on the size measurements. DNA from 15 blood samples was analysed in duplicate on 2 different gels with ethidium bromide in the loading and electrophoresis buffers, and in duplicate on 2 other gels without ethidium bromide. The Amersham marker was used in the high concentration. The fragment lengths as determined in the presence of ethidium bromide were plotted against those determined without ethidium bromide (Fig. 4). It is evident that the measurements in the high molecular weight region were higher in the presence of ethidium bromide than in its absence.

Ethidium bromide exerted a marked effect on the migration of the DNA fragments of the Amersham marker

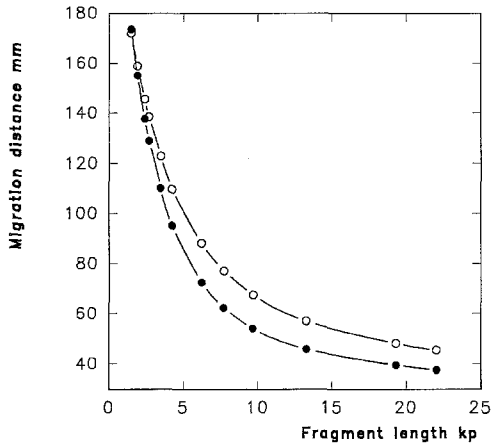


Fig. 5. The effect of ethidium bromide (0.5 µg/ml) on the migration distances of the bands of the Amersham SJ5000 marker. The concentration of SJ5000 was approximately 150 ng/lane. In two experiments, one without and one with ethidium bromide, the migration distances of the bands were measured in 10 lanes. The values were normalized and the average plotted against the fragment lengths (kb) as given by the supplier. (●) Ethidium bromide present. (○) Ethidium bromide absent

when it was applied in the high concentration (approximately 150 ng/lane). The positions of the marker bands as measured from the points of application were determined in 10 lanes run in the presence of ethidium bromide, and in 10 lanes run without ethidium bromide. For each lane the positions of the bands were normalized by multiplication by the factor $100/(d_{10}-d_1)$ where d_{10} and d_1 are the migration distances for band 10 (2.4 kb) and band 1 (22 kb), respectively. For each series the normalized mean values of the migration distances were plotted against the fragment lengths given by the supplier (Fig. 5). The migration rate was markedly lower for higher fragment lengths in the presence of ethidium bromide than without ethidium bromide. This effect was most pronounced in the region of 8 kb. At a lower concentration of the Amersham marker (approximately 12 ng/lane) the effect of ethidium bromide on the migration rate was not significant.

Discussion

When differences in fragment lengths over a wide range are to be evaluated it is preferable to operate with measurement errors which are independent of the fragment length. Measurement errors in units of kb are highly dependent on the fragment length. After transformation of fragment lengths into normalized migration distances (mm) the measurement errors are independent of the fragment length (Eriksen et al. 1992). The determinations of fragment lengths using the Amersham marker and the Gibco BRL marker as size standards exhibited significant differences, especially in the high molecular weight region above 8 kb, but also at 5, 3, and below 2 kb. It appears that the deviations range from 0.5 to 1.8 mm which in our system corresponds to 1–4 standard deviations. Deviations exceeding 3 standard deviations may be considered unacceptable when results from different laboratories are compared. It is obvious that the choice of size marker as well as the use of ethidium bromide are essential parameters in the standardization process. It is also obvious that the replacement of the Amersham marker by the Gibco BRL marker may present some problems in practical casework. Comparisons between future and previously determined DNA-profiles may be complicated by the need for corrections, especially for high molecular weight bands. It may be necessary to carry out a number of parallel analyses according to both protocols in order to obtain sufficiently robust correction factors which can be used in cases where actual and previous results are to be compared. We do not expect significant deviations in the frequency estimates as a result of using the existing database. In practical casework we have estimated the allele frequencies by counting in 6 mm sliding windows. A displacement of 1–2 mm corresponding to the differences observed in this study does not significantly affect the frequency estimates in the more or less continuous ranges of the frequency distributions. In the upper and lower end of the distributions, and in other regions with low frequencies larger differences may result from the displacement. However, with low frequency bands the frequency of a profile will be low, and in absolute terms the difference caused by the displacement will be low and without practical significance.

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References

Elder JK, Southern EM (1987) Computer-aided analysis of one-dimensional restriction fragment gel. In: Bishop MJ, Rawlings CJ (eds) Nucleic acid and protein sequence analysis. IRL Press, Oxford, pp 165–171

Eriksen B, Bertelsen A, Svensmark O (1992) Statistical analysis of the measurement errors in the determination of fragment length in DNA-RFLP analysis. Forensic Sci Int 52:181–191

Gill P, Woodroffe S, Bär W, Brinkmann B, Carracedo A, Eriksen B, Jones S, Kloosterman AD, Ludes B, Mevåg B, Pascali VL, Rudler M, Schmitter H, Schneider PM, Thomson JA (1992) A report of an international collaborative experiment to demonstrate the uniformity obtainable using DNA profiling techniques. Forensic Sci Int 53:29–43

Schneider PM, Fimmers R, Woodroffe S, Werrett DJ, Bär W, Brinkmann B, Eriksen B, Jones S, Kloosterman AD, Mevåg B, Pascali VL, Rittner C, Schmitter H, Thomson JA, Gill P (1991) Report on a European collaborative exercise comparing DNA typing results using a single locus VNTR probe. Forensic Sci Int 49:1–15

Note added in proof. After completion of this study a paper on the comparison of the Amersham and the Gibco BRL size markers was published (Greenhalgh 1992).

Reference

Greenhalgh MJ (1992) The effects of using different molecular weight markers in DNA profiling. In: Rittner C, Schneider PM (eds) Advances in forensic haemogenetics, vol 4. Springer, Berlin Heidelberg New York, pp 140–142