THE EFFECTS OF USING DIFFERENT MOLECULAR WEIGHT MARKERS IN DNA PROFILING

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INTRODUCTION

In forensic single locus probe analysis DNA molecular weight markers are used as a series of controls of known size. This enables bands in the profile of a blood or stain extract to be measured. By reference to a database an estimate of their rarity can then be obtained. The ability to store profiles in a numerical form is also useful as it enables comparisons to be made between crimes and suspects from different laboratories.

In order that a laboratory may use frequency data or crime intelligence data from another source it is essential that the protocols are comparable. Much of the work of the European DNA Profiling Group (EDNAP) has been directed to this end and the results of trials have shown that where common protocols are used results are directly comparable.

In this study the effects of using four different commercially available size markers are investigated.

EXPERIMENTAL

The four markers used in this study were:		catalogue No.
1)	Amersham DNA analysis marker.	SJ5000
2)	BRL-Life technology DNA analysis system.	4401SA
3)	Promega wide range DNA marker.	DB1391
4)	BRL 1KB ladder.	5615SA

The BRL 1KB ladder is not suitable for sizing bands in our system as it only extends to 12 KB and using the probes MS1,MS31 and MS43A with the restriction enzyme Hinf1, bands of a greater size are frequently encountered. It is included in this study to show the differences found when marker DNA from a different species is used.

COMPARISON OF THE AMERSHAM AND BRL-LIFE TECHNOLOGY MARKERS

Both of these marker systems are based on λ virus DNA. Our data collection had been prepared using the Amersham system and we were primarily interested in seeing if this could be used with the BRL markers.

An experiment was performed in which a gel was prepared with several samples of each marker. The band weights of each type of marker were measured using the other as control (table 1) and the logarithm of each band weight was plotted against distance migrated (Fig. 1).

As can be seen in Fig.1 the migration distances of the fragments from both marker systems are very similar. The values obtained for the band sizes of the Amersham marker using the BRL marker as standard are also close to the known sizes.

In a further study semen samples from 2 individuals were repeatedly profiled using both

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Table 1. Amersham DNA marker measured using the BRL-Life Technology system (all values in kilobase)

KNOWN VALUE	MEASURED VALUE	% DIFFERENCE
22.01	*	
19.32	*	
13.29	13.36	-0.5
9.69	9.78	-0.9
7.74	7.76	-0.3
6.22	6.24	-0.3
4.25	4.27	-0.5
3.47	3.45	0.6
2.69	2.69	0
2.39	2.38	0.4
1.88	1.90	-1.1
1.48	1.49	-0.7

* out of normal calibration range.

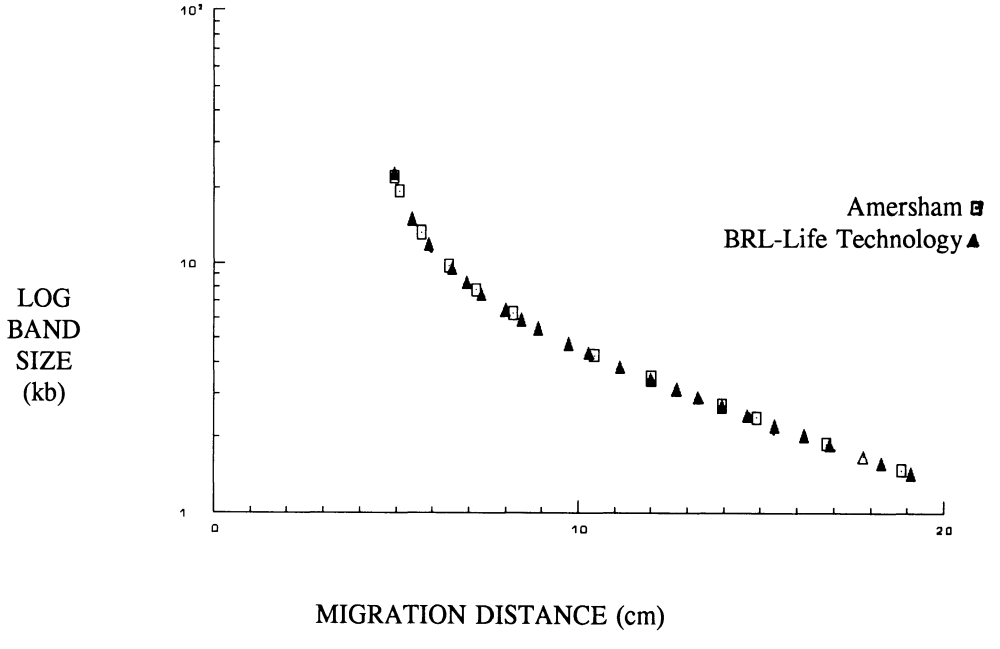

Fig 1. Amersham and BRL-Life Technology markers
log of fragment size vs migration distance

marker systems. A total of 12 bands were obtained ranging in size from 1.7 kb to 13.5 kb. The largest difference between values obtained using the different systems was less than 1.4%.(1 SD of the measurement at this point was 1.1%). There is no overall trend to the differences ie. the Amersham values are not always larger or smaller than those from BRL.

COMPARISON OF AMERSHAM MARKER AND PROMEGA MARKER

A similar set of experiments has been performed with the Promega wide range marker and the results (not shown here) indicate that comparable band sizes can be obtained using this system.

COMPARISON OF AMERSHAM MARKER AND BRL 1KB LADDER.

Table 2. BRL 1KB ladder measured using Amersham DNA size marker (all values in kilobase)

KNOWN VALUE	MEASURED VALUE	%DIFFERENCE
12.22	12.72	-4.1
11.20	11.51	-2.8
10.18	10.45	-2.7
9.16	9.35	-2.1
8.14	8.28	-1.7
7.13	7.21	-1.1
6.11	6.15	-0.7
5.09	5.10	-0.2
4.07	4.09	-0.5
3.05	3.09	-1.3
2.04	2.02	1.0
1.64	1.62	1.2

The results in table 2. show considerable differences in the mobility of the BRL 1kb ladder which is derived from yeast DNA. This may be due to the different GC content of eucaryotic DNA.

DISCUSSION

The differences between the band sizes obtained using the Amersham and BRL-Life technology markers are small and it is reasonable to exchange information gathered from protocols using either of these systems. It is also likely that the Promega wide range marker is compatible. All of these systems are largely based on λ DNA fragments (some ΦX174 fragments are present in the Promega marker) and hence it is not surprising that the mobility of the fragments is very similar.

With suitable validation it may be possible to change marker systems as long as the DNA originates from the same species.

It is important to remember that forensic scientists are using a λ virus standard to measure the molecular weight of human DNA and therefore the values that we obtain are relative and not absolute.