AN OPTIMISED CHEMILUMINESCENT DETECTION SYSTEM: IS IT COMPATIBLE WITH DATA GENERATED USING ^{32}P AND A DIFFERENT LADDER?

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Introduction

Chemiluminescent detection systems are rapidly becoming the method of choice in VNTR analysis. Such systems offer considerable advantages over ^{32}P labelled probes in terms of speed and safety. However, many laboratories have constructed large population databases using ^{32}P techniques. It is important to verify that the results obtained using the chemiluminescent detection systems are directly compatible with those using ^{32}P. This is particularly relevant if, as in this laboratory, a different molecular weight ladder has been used with each methodology.

Previous studies comparing different ladders have shown some significant differences, notably where the protocol has also changed (1). A study involving the two ladders compared here (2) suggested no significant differences between them, but did not compare the effects of different methodologies.

This investigation attempts to clarify the situation using the combinations of ladder and methodology relevant to the changing protocols in many laboratories.

Methods

DNA samples from 26 individuals were analysed, Each gel was loaded with both ^{35}S Amersham SJ5000 ladder and BRL "NICE" ladder. The resulting blots were first probed with ^{32}P labelled probes (MS43a (Cellmark), YNH24, TBQ7 (Promega)) and sized with the ^{35}S Amersham ladder (Fig.1a). The blots were then reprobed for the same loci using alkaline phosphatase (AP) conjugated oligonucleotide probes ("NICE", Cellmark or GPL, Promega) and sized using the BRL "NICE" ladder. A total of 146 fragments were identified and sized using each method. Sizing was by manual measurement and a local reciprocal fit algorithm.

Detection using ^{32}P labelled probes was carried out as previously described (3).

The following protocol was used for generation of the Southern blots and chemiluminescent detection of AP labelled probes.

1μg samples of *Alu 1* digested DNA were electrophoresed in 25cm 1% agarose TAE gels (40V, 20hrs). 3 lanes of each ladder were loaded (2.5μl/lane BRL "NICE" ladder, 8μl/lane Amersham SJ5000 ladder). Gels were neutralised and blotted onto Promega GPL

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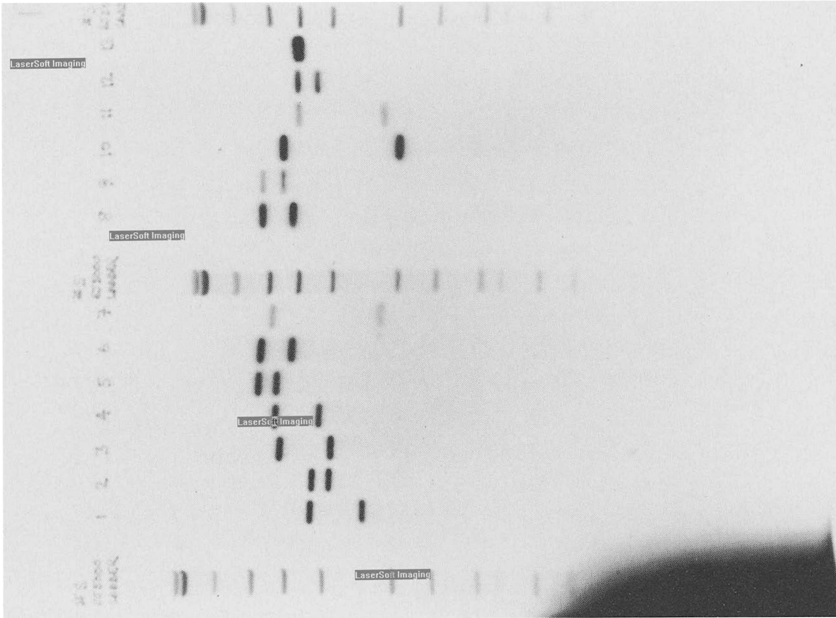

Fig 1a
Autoradiograph of blot probed with a ^{32}P-labelled probe, showing the ^{35}S-Amersham sizing ladder.

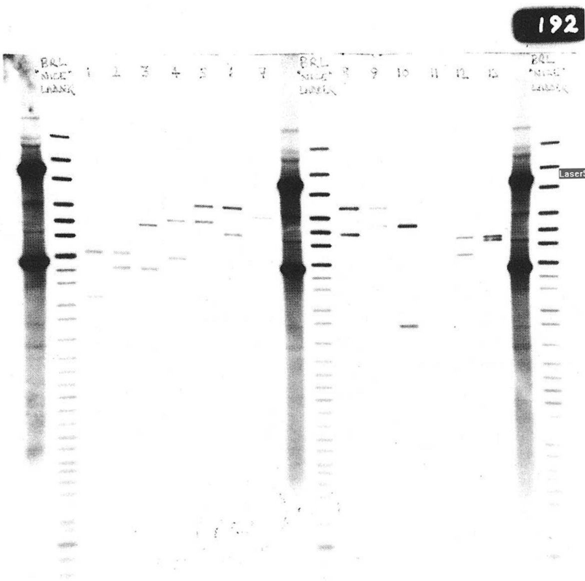

Fig 1b
Autoradiograph of blot reprobed with alkaline phosphatase conjugated oligonucleotide probe, showing the BRL size ladder.

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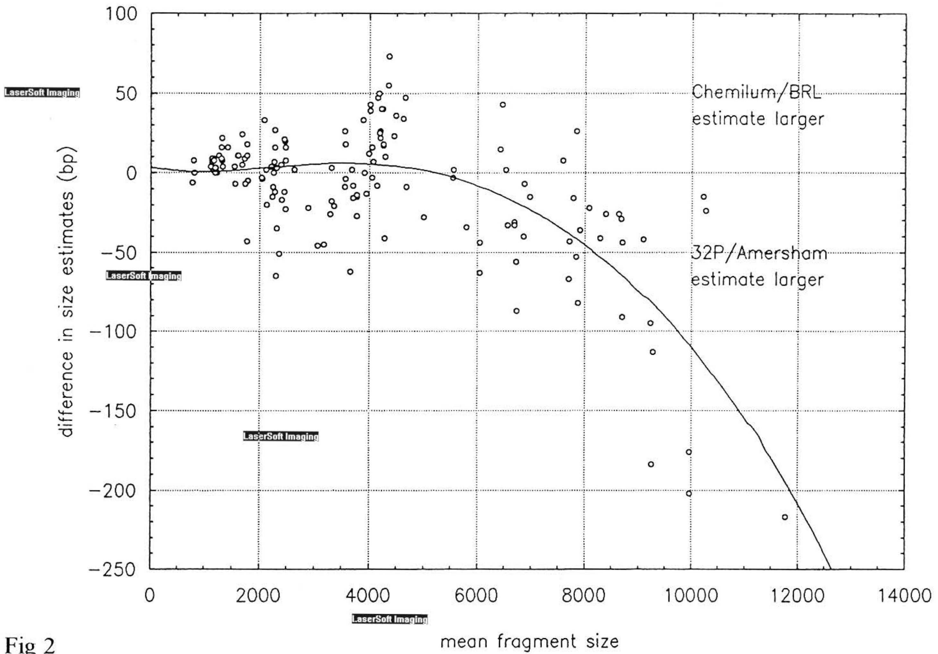

Fig 2

Size differences observed in measurements made after radiolabelling, using the Amersham size ladder, and after chemiluminescent detection of fragments using the BRL sizing ladder

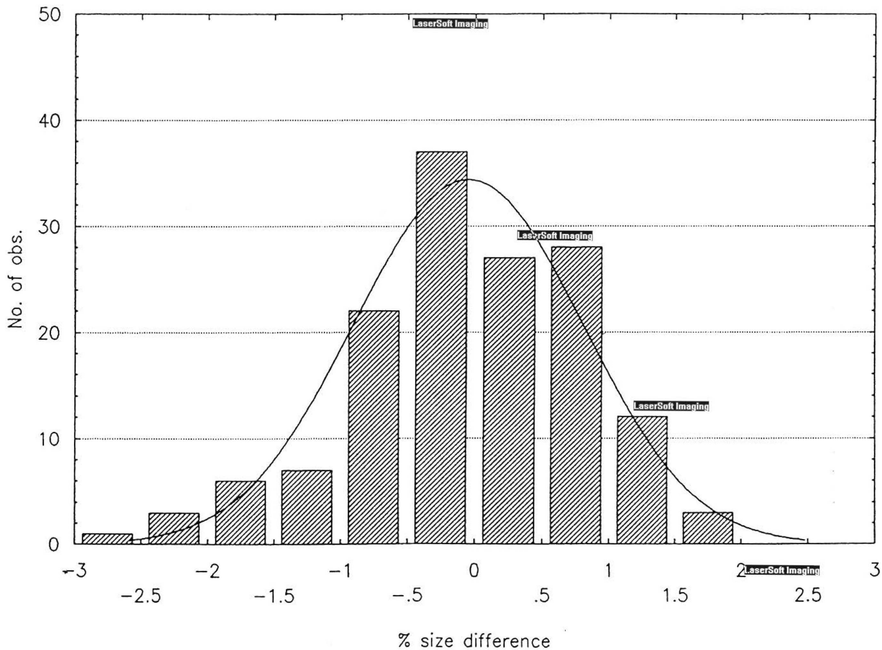

Fig. 3

Percentage size differences observed between the radio labelled and chemiluminescent labelled fragments

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membrane and UV fixed for 30sec. Prehybridisation in Promega blocking reagent was for 45min at RT in sandwich boxes. Hybridisation was carried out in a rotisserie oven for 45min at 55°C. To 10ml hybridisation buffer (1% ficoll, 1% PVP, 1% BSA, 0.75M NaCl, 0.75M NaCit, 1% N-lauryl-sarcosine, 0.2% NaAzide) per membrane was added 0.25μl/ml MW100 probe (Cellmark) and 0.5μl/ml "NICE" MS43a (Cellmark) or 4μl/ml YNH24 or 8μl/ml TBQ7 (Promega). Washing was performed in sandwich boxes (2x10min 0.5xSSC 1% SDS 65°C, 1x10min 0.5xSSC RT, 1x10min equilibration buffer (Promega) pH9.6 RT). All washing solutions were filtered through 0.4μ filters prior to use.

Results

The results shown in fig.2 show marked differences in estimated fragment sizes using the two methodologies.

For fragments <6kb there is no significant trend away from zero difference (r=0.115, p=0.232), but at higher molecular weights the ^{32}P/Amersham ladder size estimates are significantly larger than the chemiluminescent/BRL ladder size estimates.

Despite this systematic size difference, Fig.3 shows that the overall magnitude of the differences is small (3sd = 2.5%)

Discussion

Size differences observed using various different ladders have been observed previously (1,2). It has been suggested that the species origin of the ladder DNA, and the use of different protocols may both contribute to variations in different ladders' mobilities. A study comparing the two ladders used here, but with the same detection system for each showed no significant differences between them.

The reasons for the differences observed in this study are not clear. Both ladders are composed primarily of lambda DNA and would be expected to behave similarly under electrophoresis. A possible factor which may contribute to this effect is the characteristic slight distortion of the high molecular weight ^{35}S ladder bands (see fig.1a), which could cause problems with band position assignment during measuring. It is also possible that the incorporation of ^{35}S in the Amersham ladder may slightly alter its mobility. It is noted that a linear shift of 0.4mm would produce a difference of about 200bp at 12kb (1.7%) but just 18bp at 2kb (0.9%) (data not shown).

Overall, the differences presented here are relatively small and well within this laboratory's +/- 5% window used to extract frequencies from databases. It is concluded that it is thus reasonable and safe to use our existing databases with this new methodology.

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