Size Calculations of Restriction Fragments: Comparison Between Two Laboratories

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Material and Methods

DNA samples from 45 unrelated Caucasians were digested with HinfI and after Southern blotting hybridized with probes MS1, MS31, MS43a, and G3. Altogether 334 restriction fragments were observed ranging from 1.6 to 29 kb.

The fragment sizes were calculated in Amsterdam by a computer aided system (TANGO) by using a digitizing tablet, and in Düsseldorf manually by using a semilogarithmic plotted curve. The following size markers were used: in Amsterdam the Lambda EcoRI/HindIII (Promega) und Lambda HindIII (BRL) and in Düsseldorf the Analytical Marker DNA Wide Range (Promega) (van Eede et al.,1991).

The results obtained in both laboratories were analysed by calculating the bias estimated by mean differences followed by the calculation of the estimated limits of agreement (Finney, 1978). The intralaboratory differences of repeated measurements of various samples were compared with the interlaboratory results.

For checking the influence of different marker systems on the estimation of fragment sizes, 60 fragments were calculated with both marker systems on the same filters.

Results

Intra-laboratory comparisons reveal a good reproducibility of fragment size measurements in each laboratory. In the range from 1.6 kb to 12 kb the standard deviation of the measurements is approximately 0.2 kb in one laboratory while the interlaboratory measurements show an increased standard deviation to approximately 0.35 kb for the same range.

Over the whole range from 1.6 to 30 kb the mean standard deviation is 0.7 kb (Fig. 1).

The data of interlaboratory comparison are shown in table 1.

Table 1: Differences (kb $size_{Düs}$ - kb $size_{Ams}$) and standard deviation in different fragment size ranges

Fragment size	1.6 - 3,9	4.0 - 5.9	6.0 - 7.9	8.0 - 9.9	10.0 - 11.9	12.0 - 30
Number of comparisons	44	87	108	64	31	18
Mean differences (kb)	0.091	-0.052	0.091	0.177	0.235	-1.92
Standard deviation	0.218	0.137	0.218	0.289	0.584	2.13

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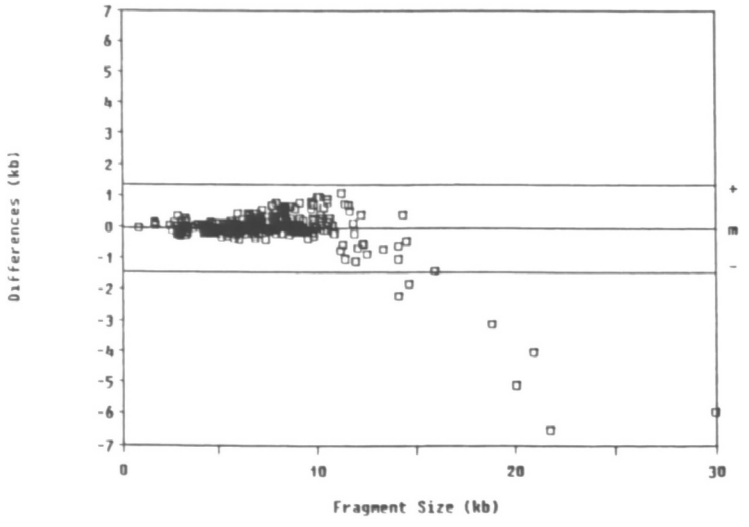

Fig. 1 Differences (kb size $_{Düs}$ - kb size $_{Ams}$) in fragment size of the same DNA samples processed in Amsterdam and Düsseldorf. Number of samples n = 352; m = mean difference; + = mean difference + $\alpha_{0.05}$* standard deviation; - = mean difference - $\alpha_{0.05}$* standard deviation

The influence of the different marker systems were analysed by calculating 60 fragments in the range of 3.8 kb to 20 kb with both marker systems (fig. 2). It was shown that the fragment size calculated with the Lambda markers were seemingly larger than the fragment size calculated using the Analytical Marker DNA Wide Range. The mean difference appeared to be -0.17 kb and a standard deviation of 0.10 kb. The mean diffferences increased with the fragment size.

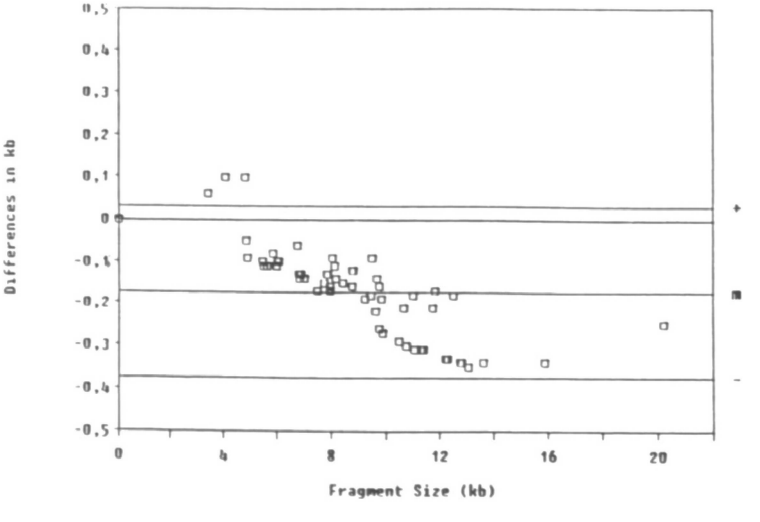

Fig. 2 Differences (kb size$_{Analytical\ Marker\ DNA}$ - kb size$_{Lambda\ Markers}$) in fragment size of the same fragments by using different markers. Number of samples n = 60; m = mean difference; + = mean difference + $\alpha_{0.05}$* standard deviation; - = mean difference - $\alpha_{0.05}$* standard deviation

Conclusions

Below a fragment size of 8 kb the results of the two laboratories appeared to be comparable. Fragments larger than 8 kb showed seemingly larger size in Amsterdam than in Düsseldorf.

This difference appeared to be at least partly due to the use of different marker systems. For pooling of data and for comparison of fragment sizes obtained in different laboratories it will be necessary to come to consenses about the marker systems.

References

Van Eede PH, Henke L, Fimmers R, Henke J and de Lange GG (1991) Size calculation of restriction enzyme *HaeIII*-generated fragments detected by probe YNH24 by comparison of data from two laboratories: The generation of fragment-size frequencies. For Sci Int 49:21-31

Finney DJ (1978) Statistical methods in biological assay, 3rd edn., Charles Griffin and Company Ltd., pp 316-348