Segregation analysis and determination of parentship by use of RFLP´s corresponding to genes of the MHC on chromosome 6 and the short arm of chromosome 11

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INTRODUCTION

Due to molecular cloning of a great variety of human DNA sequences the human genome is now accessible to a direct analysis at the genomic level. By use of restriction analysis, polymorphisms can be described with a panel of restriction endonucleases by use of Southern technique.
For segregation analysis and determination of parentship we have chosen gene probes corresponding to the human MHC gene region and the human insulin locus and the H-ras proto-onc-gene, both of which reside on chromosome 11. The MHC locus is known to be highly polymorphic at the product level. This is not the case for the ras and the insulin-gene locus. But this sequences contain so-called hypervariable segments either 5´or3´ to the corresponding coding sequences and permit therefore a series of useful RFLP´s.
Although DNA restriction analysis permits a segregation and thus also parentship analysis at the genomic level, series limitations of this technique have to be overcome before a routine use, most probably as an adjunct to conventional serotyping, is in sight.

MATERIALS AND METHODS

We will focus in this part on the quality standard when performing RFLP analysis.
DNA isolation:
5-1o ml of EDTA blood yields enough DNA (about 25 to 5oug) for a series of restriction analyses. Blood should not be anticoagulated with heparin since this basic protein inhibits several enzymes even after several purifications. For practicability EDTA blood can be shipped on dry ice and stored about 4 weeks at -2o°C before DNA is extracted. For a restriction analysis high molecular weight DNA (size > 4okb) should be preferred. Unspecifically degraded or mechanically sheared material gives high background or even loss of high molecular bands in Southern blots. DNA concentration is usually determined by two ways,a) DNA spectrofotometry and b) gel electrophoresis with phage lambda DNA as a reference.
Restriction enzyme digests:
Restriction analysis can be achieved most conveniently with a set of three buffers, low,medium and high salt. Spermidine at a final concentration of 1 mM can be added immediatly before the digest. It will improve digestability for medium and high salt conditions, whereas inhibition was observed under low

Advances in Forensic Haemogenetics 2
Edited by W.R. Mayr

salt conditions. Restriction endonucleases are added at three steps with a final concentration of about 5-1oU/µg of DNA. Digests are performed overnight. With TaqI, a final concentration of 5U/µg of DNA is used. Incubation lasts for 6-8 hrs only. Most important, TaqI is insensitive to inpurities in a DNA preparation and yields an extensive series of RFLP´s within human sequences.

Completeness of digestion:

Evaluation that the restriction endonucleases have cut all the possible recognition sites is the most crucial point in Southern technique when applied to segregation analysis. The evaluation of complete digestion depends on three complementary approaches. A) The high molecular weight DNA is broken into smaller pieces by the restriction enzymes and gives at control electrophoresis a homogenous smear with distinct bands which correspond to repetitive sequences. If low amounts of DNA, or unspecifically degraded DNA is loaded,repetitive sequences may no longer be visible.B) An aliquot of the digest-mixture is mixed with 1-2 µg of phage lambda DNA. Phage lambda DNA then gives a distinct pattern with a given enzyme which superimposes on the homogenous smear of genomic DNA. Note that phage lambda DNA can be preferentially digested while genomic DNA is still not restricted completely. C) After a complete digestion a Southern transfer is performed. This blot is hybridized with a non-polymorphic probe (Fig.2a). Only invariant bands should now be visible on the autoradiogram. Also efficacy of transfer of the DNA to the filter can be calculated with this approach. After having loaded the same amount of DNA, transfer is not identical for all the DNAś loaded. This fact renders the calculation of gene dosage a rather difficult task. D) Starting with a new sample, a second restriction digest should be prepared. The same pattern should be observed.

Southern transfer:

A great variety of transfer protocols do exist. Most convenient is the transfer on a nylon-membrane, which allows a series of up to 2o hybridizations.Transfer buffer should be either SSC or SSPE, whereas alkaline transfer gives good results for high molecular weight DNA only.

RESULTS AND DISCUSSION

By use of probes corresponding to the human MHC as well as the hypervariable regions of the human insulin and H-ras gene locus, a variety of informative restriction fragment length polymorphisms can be defined (Fig.1 &2).

Fig.1 gives an example of an RFLP which resides within the DQß locus. The gel allows a resolution of bands from 24kb down to 2kb. Fig.2 gives an example for parentship analysis. In the first part (A), hybridization is given with a non-polymorphic probe. Part B to E shows the successive hybridizations with probes corresponding to the MHC and the chromosome 11. Note that the probe corresponding to the 5´-HVR of the human insulin gene -ins310- gives an exclusion for

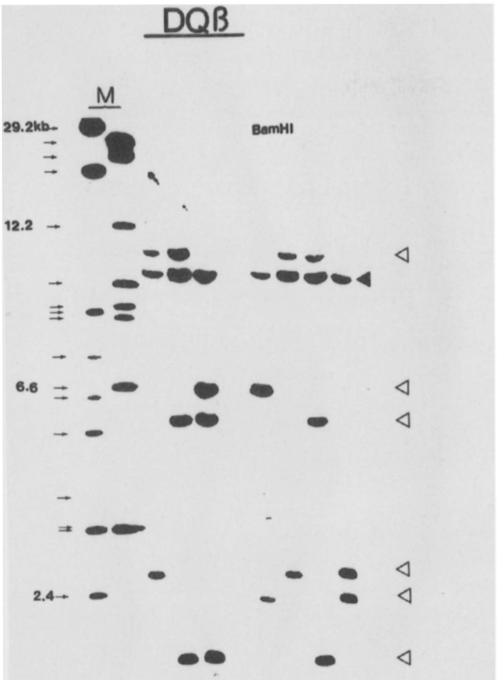

Figure 1

Figure 1 shows BamHI restricted genomic DNA hybridized with a HLA-DQß probe.
Open triangles mark polymorphic DQß specific fragments.

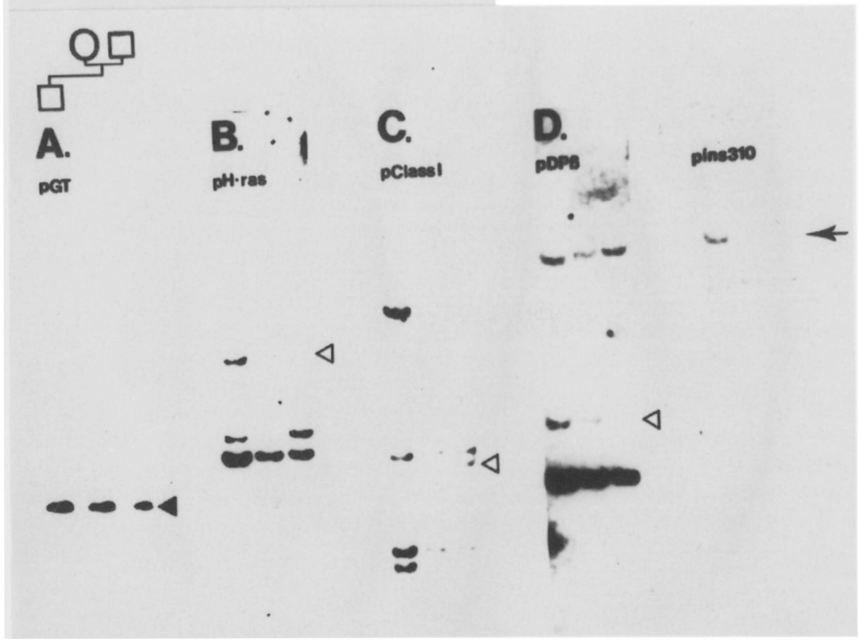

Figure 2. Figure 2 shows segregation of various HLA and chromosome 11 specific restriction fragments. In part A a non-polymorphic probe was used in order to proof complete digestion.

parentship, whereas all other probes show segregation of parental bands. Since recombination frequency has been calculated to be around 1o% in the 5´insulin gene region the reported RFLP can not be calculated in the segregation analysis.

Table 1. Problems in view of practical application of "DNA"-tests in paternity testing

Sample:	quantity	5-1o ml of uncoagulated blood
	quality	DNA should best be prepared from fresh leucocyte sample
		high molecular weight DNA (> 4okb)
Costs:	equipment	costs around 15.ooo - 2o.ooo US$
	fixed costs	1oo.ooo US$
	cost per analysis	(1 individual, 1 enzyme, 3 probes) 15o US$
Time:	DNA preparation	- 2days
	RE-digest	- 1-2 days
	Electrophoreis	- 3 days
	Southern transfer	- overnight
	Hybridization	- 2-3 days
	Autoradiogram	- 1-3 days
Skill:	highly standardized lab	
	sterile work, if possible	

DNA technology is at present clearly second to the classical serological methods concerning practicability and reproducibility, since DNA analysis requires more hands-on time, and is considerably more expensive (Tbl.1). The goals of the molecular approaches to the human genome are both to obtain an exhaustive description of the human genome, and to be able to rapidly and inexpensively compare the genomes of many individuals, which will then provide new data on population genetics and will be the basis for parentship analysis. But at present, the number of persons screened as well as the number of familiy data are much to small in order to allow the calculation of recombinatorial events.

Acknowledgements.
This work was supported by grants from the Deutsche Forschungsgemeinschaft Bo 829/1-1 and Ro 649/1-1.