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AFLP™ technique used for genetic characterization of rat inbred strains

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Introduction

Inbred strains of rat (*Rattus norvegicus*) are widely used in biomedical research (*Gill et al.* 1989). The importance of genetic quality control of inbred strains has been demonstrated by *Festing* (1982), *Groen* (1977), *Hoffmann* (1978). The penalty for not conducting a careful genetic monitoring program has recently been reported (*Lezin et al.* 1994). The development of an efficient genetic monitoring program involving the establishment of a unique genetic profile for every strain, is a prerequisite for the responsable use of genetically defined animals. Until now biochemical and immunogenetic markers, have proved to be of great value (*Hedrich & Kluge* 1985, *Kluge & Hedrich* 1985, *Gill et al.* 1989, *Hedrich* 1990, *Bender et al.* 1994). However, only a limited number of these markers is available, and many of these require the application of laborious techniques, several markers require the animal to be killed.

The introduction of DNA markers and in particular of microsatellite markers has had a major impact on the genetic characterization of inbred strains. Recently, *Otsen et al.* (1995) presented a study on the genetic characterization of 61 rat inbred strains, using 37 microsatellite markers. Each of the 61 strains could be provided with a unique genetic profile using these 37 microsatellite markers. However, for microsatellite typing, information about the genomic nucleotide sequences is necessary. Here we describe the use of the AFLP™ technique for characterization of rat inbred strains (AFLP™ is a trade-mark filed by Keygene N.V., Wageningen, The Netherlands). The technique was originally developed for use in plant species, but it was shown that the technique could be applied to almost any DNA (*Zabeau & Vos* 1993).

The AFLP technique is based on the combined use of restriction enzymes and selective PCR primers. Adapters with known sequences are ligated to the ends of the restriction fragments. The sequences of the adapters serve as primer binding sites for subsequent PCR. Because of selective nucleotides which are added to the 3′ ends of the PCR primer, only a subset of the restriction fragments will be recognized for amplification. Both the restriction enzymes and the selective nucleotides at the 3′ end of the primers can be varied. Therefore a huge amount of new genetic markers can be generated. This technique has a wide potential application. In this study, we describe the genetic characterization of 12 rat inbred strains, using four enzyme/primer combinations. The genetic profiles are also used for the estimation of genetic relationship between the 12 strains.

Materials and methods

DNA of animals from 12 different rat inbred strains was isolated from liver using a standard isolation procedure (*Ausubel et al.* 1987). The origin of the inbred strains and the collection of the samples are described by *Otsen et al.* (1995). In the present study, we used the original template preparation protocol described by *Zabeau & Vos* (1993). Total genomic DNA was digested using two restriction enzymes: SseI (CCTGCA/GG), and MseI (T/TAA). Adapters were ligated to the ends of the restriction fragments. The SseI adapter carries a biotin label at the 5′ end. The adapters are designed as follows:

Ssel adapter:

5′-biotin- CTCGTAGACTGCGTACATGCA
CATCTGACGCATGT - 5′

Msel adapter:

5′- GACGATGAGTCCTGAG
TACTCAGGACTCAT - 5′

After ligation of the adapters, the complexity of the fragment pool was reduced by isolating the biotinylated fragments (Ssel-Msel and Ssel-Ssel fragments) using streptavidin coated Dynabeads (Dynal), according to the manufacturers protocol.

The fragment amplication took place in two consecutive steps. The first amplification step (preamplification) was a non-radioactive PCR. Both primers contained only one selective nucleotide

Ssel primer:

5′- GACTGCGTACATGCAGG | . - 3′

S01: A

Msel primer:

5′- GATGAGTCCTGAGTAA | . - 3′

M02: C

PCR reactions were performed in a PE-9600 thermal cycler (Perkin Elmer) and were started at a relatively high annealing temperature (65°C), to obtain optimal primer selectivity. In the following steps the annealing temperature was gradually lowered to a temperature at which efficient primer binding occurs (56°C). This temperature was maintained for the remaining cycles.

For the second amplification step one of the primers was labelled with γ-^{33}P-ATP. We had two different Ssel primers each with one extra selective nucleotide added to the 3′ end and three different Msel primers each with two extra selective nucleotides.

Ssel primers:

5′- GACTGCGTACATGCAGG | . . - 3′

S12: AC

S13: AG

Msel primers:

5′- GATGAGTCCTGAGTAA | . . . - 3′

M51: CCA

M61: CTG

M62: CTT

Four different Ssel-Msel primer combinations were made out of these five primers. The PCR program used for the second step amplification was identical to that used for preamplification.

The amplification products were analyzed on standard 5 % denaturing polyacrylamide gel. The AFLP patterns were visualized using a phosphor-imager (Fuji Bas 2000)

Table 1. Strain distribution pattern of the polymorphic bands of the four primer combinations tested in 12 rat inbred strains. X stands for presence of a specific band (indicated by their length in bp), while an empty compartment denotes absence of that band.

	S12M51						S12M62							
STRAIN	260	248	220	156	125	84	380	245	243	240	229	157	133	116
ACI/A		X	X	X	X	X	X			X			X	
AGUS/OlaHsd	X			X		X	X				X		X	
AUG/OlaHsd			X		X		X			X				
BN/M		X	X		X	X				X		X	X	
BUF/Han			X	X	X		X	X			X	X	X	
COP/OlaHsd				X	X	X	X			X			X	X
DA/Han		X	X	X	X	X	X	X	X	X			X	
F344/Han			X	X	X		X			x	x	X	X	
LEW/M		X	X	X			X				X		X	
PVG/OlaHsd			X	X	X		X			X			X	
WF/Han		X	X	X		X	X				X	X	X	
WKY/OlaHsd				X	X						X	X	X	

	S13M51												S13M61												
STRAIN	450	330	308	292	284	220	186	155	147	124	82	76	354	320	297	290	237	235	163	157	141	137	136	134	100
ACI/A	X						X		X	X		X			X	X		X	X				X		
AGUS/OlaHsd		X	X	X				X		X		X		X		X	X		X				X		
AUG/OlaHsd	X				X		X		X			X			X	X			X	X			X	X	
BN/M	X		X	X	X				X	X	X	X		X		X	X			X	X		X		
BUF/Han									X		X	X			X				X	X		X		X	
COP/OlaHsd	X		X	X					X	X	X	X	X	X		X		X			X	X	X	X	X
DA/Han			X						X	X		X			X	X			X				X		
F344/Han					X		X	X	X		X	X			X	X			X	X		X		X	
LEW/M			X	X	X	X	X		X	X	X				X	X					X				
PVG/OlaHsd			X	X	X			X	X	X	X				X	X			X	X			X		
WF/Han	X	X	X	X				X	X	X	X	X			X	X	X				X		X	X	
WKY/OlaHsd	X		X	X	X		X		X	X	X	X	X	X		X	X				X		X	X	

after o/n exposure of the gel to a phosphoimaging screen.
Genetic similarities between inbred strains were calculated using the NTSYS computer-package of Applied Biostatistics. The construction of the rat inbred strains dendrogram is based on the UPGMA method (*Nei* 1987, *Rohlf* 1989).

Results and discussion

The four different primer combinations used in this study are: Ssel+AC/Msel+CCA (S12/M51); Ssel+AC/Msel+CTT (S12/M62); Ssel+AG/Msel+CCA (S13/M51); Ssel+AG/Msel+CTG (S13/M61). Selection procedures resulted in AFLP-prints which all contained 50 to 100 bands within the range of 450 to 75 bases. The polymorphisms were predominantly based on the presence/absence of a specific band, which is typical for dominant markers. The polymorphisms are scored for all four primer combinations tested and the results are summarized in Table 1. Markers are indicated by the symbols of their primer combinations and lengths (in bp) of the specific bands. Polymorphisms are indicated with an X for presence and an empty compartment for absence.
All four primer combinations revealed identical patterns when tested in animals of the same strain, thus indicating the stability of AFLP-markers within strains. The high degree of inter-strain variation and the fact that the patterns are highly reproducible indicate that this type of marker provides a useful contribution to the genetic quality control programs of rat inbred strains.
The AFLP profiles were used to estimate the genetic relationship between the 12 rat inbred strains. In Table 2 the similarity matrix between all 12 strains is given, whereas Figure 1 shows a relationship dendrogram of these inbred strains. Knowledge of such information can be helpful in selecting strains for comparative testings. The NTSYS computerprogram (*Rohlf* 1989) was used to calculate genetic similarities, which are indicated as percentages of sharing alleles between pairs of strains (Table 2). The rat inbred strain dendrogram as presented in Figure 1 is based on the unweighted pair group method with arithmethic mean (UPGMA).

Table 2. Similarity matrix between 12 rat inbred strains. The calculated percentages are based on 39 polymorphic AFLP loci.

		1	2	3	4	5	6	7	8	9	10	11	12
1	ACI/A	*											
2	AGUS/OlaHsd	65	*										
3	AUG/OlaHsd	81	52	*									
4	BN/M	72	65	68	*								
5	BUF/Han	67	55	71	59	*							
6	COP/OlaHsd	73	67	62	76	60	*						
7	DA/Han	88	69	72	72	71	69	*					
8	F344/Han	73	59	82	65	88	63	69	*				
9	LEW/M	69	63	61	69	64	63	69	71	*			
10	PVG/OlaHsd	76	69	77	75	71	69	80	81	78	*		
11	WF/Han	73	78	62	79	68	73	73	70	78	73	*	
12	WKY/OlaHsd	64	69	64	82	63	79	60	69	73	68	79	*

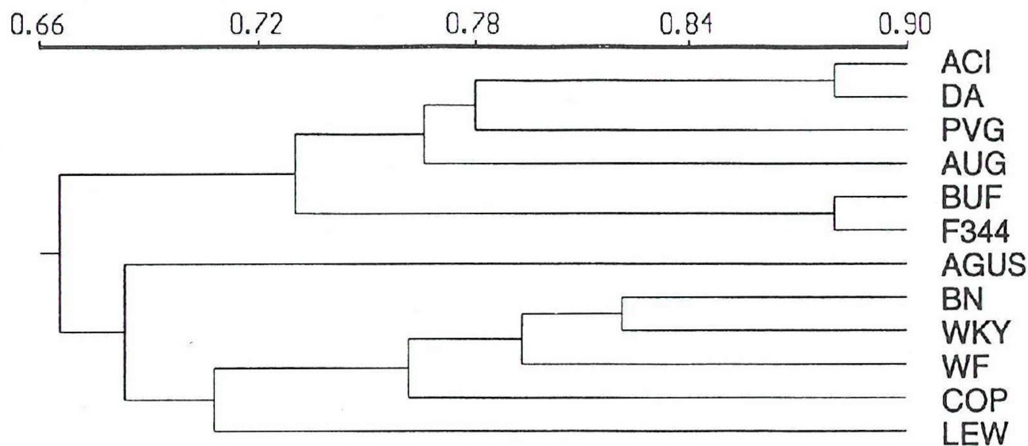

Figure 1. Dendrogram illustrating the relationships between 12 rat inbred strains based on alleles at the 39 polymorphic AFLP loci.

This method employs a sequential clustering algorithm in which local topological relationships are identified in order of similarity (*Nei* 1987). As yet we do not know the chromosomal locations of the AFLP markers used in this study. Therefore the present data on strain relationships need still to be interpreted with some caution. A linkage study for the genetic mapping of the AFLP markers is in progress.

In conclusion, the AFLP technique seems to be most suitable for genetic characterization of rat inbred strains. The technique is efficient because each primerset generates various polymorphic loci simultaneously. Furthermore, sequences for primers need not to be determined in advance. No database searches or scanning of genomic libraries is necessary. AFLP also provides an effective tool for the study of genetic relationships and for the construction of a phylogenetic tree.

Summary
The AFLP™ technique is a novel DNA technology which generates AFLP markers. The technique is based on the combined use of restriction fragments and selective PCR primers. Because both the restriction fragments and the selective primers can be varied, many new genetic markers will become available. We have used four enzyme/primer combinations for the genetic characterization of 12 rat inbred strains. All these strains could be provided with their own unique genetic profile, using these four combinations. No variation was found within the individuals of the same strain. This indicates that AFLP markers are stable and useful for genetic monitoring of strains and for linkage analysis.

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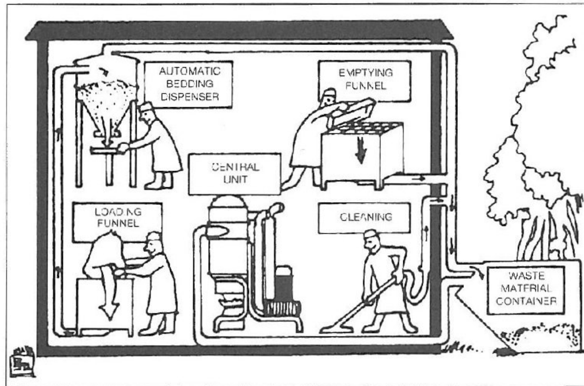

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