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AN IMPORTANT TOOL FOR GENETIC STUDIES**

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A GENETIC MAP OF THE RABBIT: AN IMPORTANT TOOL FOR GENETIC STUDIES

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ABSTRACT

A genetic map of the rabbit is an important tool to localize and identify QTLs involved in production traits and diseases like hypercholesterolemia and atherosclerosis. Our goal is to establish a detailed genetic map of the rabbit using DNA markers. For this purpose we generated 280 AFLP markers, and developed SSLP markers in or close to genes (Type I loci) and SSLP markers from flow-sorted chromosomes. The markers were tested for the degree of polymorphism using a testpanel containing different rabbit strains and breeds. Markers which were polymorphic between the AX/JU and IIIVO/JU inbred strains were used for analysis on an F2 intercross of these strains. A preliminary map was constructed with 23 linkage groups. The present map provides a framework for future genetic studies.

INTRODUCTION

The rabbit is used for the production of meat, wool and fur and as an animal model for human diseases like atherosclerosis and hypercholesterolemia. In the past, several rabbit genetic studies on the heritability of production and disease traits have been published. However, a detailed genetic map of the rabbit genome, an important tool to localize and identify the QTLs which control these traits, is still missing. When compared to other mammalian species that are used as a production or laboratory animal, the genetic map of the rabbit is underdeveloped. The current map covers only a small part of the genome. At present, nine biochemical, 12 immunological and 13 morphological markers are included into 10 autosomal linkage groups and only one of these linkage groups is assigned to a chromosome (Fox, 1994). Our goal is to establish a detailed genetic map of the rabbit genome using markers at the DNA level (AFLP and SSLP markers) and assign a linkage group to each of the 21 autosomes.

MATERIAL AND METHODS

AFLP markers

The AFLP™ technique was originally developed for plant breeding (Vos et al., 1995) but it is shown to work also on DNA of mammalian origin (e.g. Otsen et al., 1996). The main steps of the AFLP™ marker technology have been described by Vos et al. (1995) A detailed description of the procedure has been described by Vos & Kuiper (1996). Briefly, genomic DNA is digested with one or two restriction endonucleases resulting in 'sticky ends', which

are then ligated to suitable double-stranded DNA adapters. The sequences of adapters and adjacent restriction sites serve as primer binding sites for subsequent PCR. Because of selective nucleotides which are added to the 3' ends of the PCR primers, only a subset of the restriction fragments will be amplified. The polymorphism of these amplified DNA fragments is resolved by a sequencing gel. The results are high-density 'fingerprints'. By variation in the choice of the restriction enzymes and the selective nucleotides at the 3' ends of the primers many new genetic markers can be generated.

SSLP markers in gene sequences

Microsatellites are simple sequences consisting of short stretches of repeating nucleotides. These regions show extensive polymorphism due to the variation in repeat number (Simple Sequence Length Polymorphism) and therefore are excellent for usage as a DNA marker. Searching for these SSLPs in (or close to) genes gives us the possibility to use such markers as Type I anchor loci (O'Brien et al., 1993). Therefore, the EMBL nucleotide sequence database was screened for rabbit sequences containing microsatellite repeats (Van Lith & Van Zutphen, 1996). Primers were developed to amplify the region of interest and analysed for polymorphisms (Van Haeringen et al., 1997).

Chromosome-specific SSLP markers

Linkage groups can be assigned to chromosomes by incorporating DNA markers of which the chromosomal origin is known. Chromosome-specific DNA libraries were produced using flowsorted rabbit chromosomes (Korstanje et al., 1999). These libraries were enriched for DNA fragments containing CA-repeats. Fragments were sequenced and primers designed.

Marker analysis and genetic mapping

The degree of polymorphism of the SSLP and AFLP markers is tested using a panel of DNA samples prepared from male and female rabbits of different breeds (table 1).

At the department of Laboratory Animal Science in Utrecht two inbred strains (AX/JU and IIIVO/JU) are available which have been used to produce F2-intercross progeny. The DNA of these animals is used for analysis of polymorphic markers and the construction of linkage maps.

RESULTS AND DISCUSSION

A total of 280 AFLP markers were found polymorphic between the two inbred strains and suitable for linkage analysis. Polymorphism has been detected for 22 SSLPs extracted from the EMBL databases (Type I anchor loci). Eight of these markers were polymorphic between the two inbred strains and could be linked to the AFLP markers.

From ten chromosome-specific DNA libraries (chromosomes 1, 2, 5, 6, 7, 12, 15, 18, 19 and 21)

clones containing CA-repeats were sequenced, primers developed and analyzed for polymorphism between the AX/JU and IIIVO/JU rabbits. Polymorphic markers were analyzed on the F2 progeny. We mapped the AFLP and SSLP markers in 23 linkage groups. Ten of these linkage groups were assigned to chromosomes. The existence of more linkage groups than there are autosomes indicates that some of these groups belong to the same chromosome. Generating more SSLP markers from the chromosome-specific libraries will

resolve this problem in the near future. With the construction of the present map we provide a framework for future genetic studies. It enables mapping of genes and quantitative traits both in biomedical and agricultural research.

Table 1 Rabbit breeds used for testing DNA markers (n=40)

(Partially) inbred strains:

AX/JU; IIIVO/JU (Utrecht University, The Netherlands)

OS/J; WH/J; X/J (The Jackson Laboratory, USA)

Random-bred rabbits/outbred strains:

ELCO (Outbred strain; Bilthoven The Netherlands)

WHHL-HH and WHHL-Hh (homozygous and heterozygous

Watanabe

heritable hyperlipaemic rabbits; Nijmegen, The Netherlands)

NZW (New Zealand White; 4 different local rabbitries)

CAL (Californian; 1 local rabbitry)

F1-hybrids:

(IIIVO/JU x AX/JU)F1

Wild Rabbits:

European wild rabbit (Texel, The Netherlands)

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