LINKING THE PORCINE LINKAGE AND PHYSICAL MAP

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SUMMARY

In order to anchor the linkage map to the physical map we constructed a porcine cosmid library. A total of 2400 clones were picked and gridded and subsequently screened by a $[GT]_{10}$ oligonucleotide yielding a total of 850 GT-positive clones. The chromosomal localization of 103 $[GT]_n$-containing clones as well as three cosmids showing VNTR polymorphism were determined by fluorescence in situ hybridization. A total of 86 $[GT]_n$-containing cosmids and the three VNTRs showed a unique hybridization site. Cosmids mapped by fluorescence in situ hybridization and shown to be in areas of interest was sequenced to determine the sequences flanking the microsatellite in order to make PCR-primers. Genotyping was performed in the reference families established for the European effort to make genetic maps of the porcine genome (PiGMaP).

INTRODUCTION

Good genetic markers, for which the chromosomal localization is known, is a prerequisite for comparing genetic and physical distances and for establishing chromosomal end points in a linkage map.

The usefulness of a particular linkage map depends on how many markers there are on the map and the number of alleles a marker exhibits. The more alleles a locus has, the more likely it is that any given individual will be heterozygous. A high degree of heterozygosity increases the likelihood of getting linkage information from family studies since genotypic information will be obtained from most meiosis in any sample set.

Two types of markers that has been shown to be extremely polymorphic with many alleles are microsatellites (Weber and May 1989) and VNTR's (Variable Number of Tandem Repeats) (Nakamura, Leppert et al. 1987), both consisting of a variable number of copies of a tandemly repeated core sequence and both showing high number of alleles.

We decided to use microsatellites as our main source for polymorphism and to develop cosmids for this purpose. The advantage of using cosmids in genetic mapping is the high potential for finding polymorphisms for use in linkage studies as well as their versatility for use in fluorescent in situ hybridization (FISH).

MATERIALS AND METHODS

A cosmid library from swine was constructed by partially digesting high molecular weight porcine DNA with Sau3A and using a partial fill in method to avoid insert or vector religation. The vector was Triple Helix Cosmid Vector from Stratagene modified by inserting a XhoI cloning site into the original BamHI cloning site.

From this library 2400 clones were picked and gridded in microtiterplates and subsequently screened for microsatellites by a $[GT]_{10}$-oligonucleotide. The oligonucleotide was endlabeled using radioactive gamma-ATP and polynucleotide kinase. Hybridization was performed by standard methods and signals were detected by autoradiography.

Fluorescence in situ hybridization was done by labeling cosmid DNA with biotin-11-dUTP by nick translation. The labeled cosmid was hybridized to male pig metaphase spreads by standard methods and R-banding or Q-banding were used to identify chromosomes.

Since the average insert in cosmids is about 40kb, sequencing the microsatellite area would involve several rounds of subcloning to get a size suitable for conventional sequencing. A method described by Yuille et al (1991), where internal primers are used to sequence the areas flanking microsatellites, were used to sequence the cosmids containing a GT-sequence.

Genotyping of families was done using PCR by standard methods on 25 nanogram of DNA. One primer was endlabeled using radioactive gamma-ATP and polynucleotide kinase prior to the PCR reaction. The resulting PCR-product was run on a sequencing gel and the signal was detected by autoradiography.

RESULTS AND DISCUSSION

Screening with the $[GT]_{10}$-oligonucleotide resulted in a total of 850 positive clones representing 35% of the total number of clones. In order to estimate the average number of GT-repeats in the cosmids 132 GT-containing cosmids were digested with restriction endonucleases. The fragments were separated on agarose gels, blotted onto Hybond N+ membranes and reprobed with the $[GT]_{10}$-oligonucleotide. A total number of 165 GT-repeats were found in the 132 cosmids indicating an average of 1.25 GT-repeats per cosmid. Since the average insert in cosmids is about 40kb this gives an average of one GT-repeat per 32 kb in the porcine genome.

The chromosomal localization of 103 $[GT]_n$-containing clones as well as three cosmids showing VNTR polymorphism were determined by hybridization of biotinylated cosmids to male pig metaphases in situ. Most metaphases showed a signal appearing as symmetrical pin-point spots on both chromatids of at least one of the two homologous of a particular chromosome as identified by Q-banding or the R-band like pattern generated by propidium iodide in alkaline mountant. A total of 86 $[GT]_n$-containing cosmids and the three VNTRs showed a unique hybridization site. All chromosomes except chromosome 12 and the Y chromosome were covered by one or more cosmids.

A total of 27 cosmids were selected for sequencing by the method of Yuille et al (1991). The method was modified so that only the sequence flanking one side was initially determined by this method and a unique primer was constructed to sequence back through the microsatellite. Twenty of the cosmids have been sequenced and two of them gave more than one sequence with the primers designed by Yuille et al. The remaining seven cosmids have been difficult to sequence by this method and will be subcloned before new sequencing is performed.

Primers have been selected from all 20 cosmids giving sequence information and all have been shown to be polymorphic in a panel consisting of the founder animals used for the European PiGMaP project. The number of alleles ranged from two to eleven with an average of six alleles. Seven of the markers have been used to genotype the PiGMaP reference families and will be included in the linkage map emerging from this collaboration. Preliminary results from the linkage analysis confirms the physical assignments done by fluorescence in situ hybridization.

We have shown that using fluorescence in situ hybridization to physically localize cosmids and subsequently sequencing them by using the method of Yuille et al. is a way to link the linkage map with the physical map. The method has also shown to be useful in detecting cosmids containing GT-repeats that physically is localized to areas of particular interest such as areas where there are no other markers or to the proterminal region thereby providing end-point markers to the linkage map.

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