Molecular approaches for the construction of integrated physical and genetic maps

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Abstract

This study reports the development of chromosome-specific libraries by chromosome microdissection and microcloning and its utility in developing high density linkage map for particular chromosomes. Chromosome-specific painting probes were prepared for bovine (Bos taurus) autosomes 11 and 23 using two different translocation cell lines. Chromosome painting probe for swine chromosome 6 was developed using chromosomes from primary swine fibroblast cultures. The purity and specificity of the painting probes was verified by fluorescent in situ hybridization (FISH) on bovine and swine metaphase chromosomes. Bovine painting probes were used on sheep (Ovis aries) and goat (Capra hircus) metaphases to identify their corresponding homologs.^ BTA 11 and SSA 6-specific DNA fragments were cloned in Lambda Zap Express vector to develop high titer chromosome-specific libraries. BTA 11 library was screened for microsatellite-containing clones using (AC)$\sb{12}$ oligos. Primer pairs developed for 17 microsatellites yielded successful amplifications with bovine genomic DNA. Three markers were binned on Illinois Reference/Resource family (IRRF) and 14 were mapped on the USDA-MARC resource family. Two point analysis was done on MARC population to generate a preliminary linkage map for BTA 11.^ A bovine YAC library was screened with BTA 11-specific microsatellite primers. Four YACs were identified and physically mapped by FISH. Two YAC clones that were mapped to BTA 11 by linkage and by FISH helped to orient and anchor the linkage map on bovine chromosome 11.^ BTA 11-specific DNA was subjected to subtractive hybridization using bovine Cot4 DNA and the subtracted product was used to screen a bovine cosmid library. Positive clones were pooled and mapped to bovine metaphases by FISH. All the clones showed very strong hybridization on the centromeres of all autosomes in the bovine chromosome complement. However, they failed to hybridize to the sex chromosome centromeres suggesting that the sequences are specific to autosomal centromeres. The same probes failed to hybridize to sheep and goat metaphases suggesting species specificity of these probes. An answer to the exact function of these DNA sequences need to be investigated. ^

Subject Area

Molecular biology|Veterinary science

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