CONSTRUCTION OF A BOVINE BACTERIAL ARTIFICIAL CHROMOSOME LIBRARY

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SUMMARY

We have constructed a bacterial artificial chromosome (BAC) library for bovine use in genome mapping. Currently, the library consists of 14,000 clones which achieves a 50% probability (P=0.5) of the library containing a specific unique DNA sequence. Sixty thousand clones or about three haploid bovine genomes will be required to achieve a 95% probability (P=0.95) of containing a unique sequence. An average insert size of 142kb was estimated from the analysis of 68 randomly selected BAC clones. Polymerase chain reaction (PCR) was used to screen the exist bovine BAC library. For three microsatellite markers and three anchor locus markers, positive clones were found for one microsatellite marker (ETH225) and two anchor locus markers (Glucocerebrocidase and 3β-hydroxy-5-ene steroid dehydrogenase). Characterization of the BAC library, in terms of the percentage of chimeric clones and the stability of inserts in BAC clones is in progress.

INTRODUCTION

Cloning large DNA fragments is required to efficiently obtain an accurate high resolution physical map of eukaryote genomes. Yeast artificial chromosome (YAC) technology was developed to take large DNA insertions (BURKE, CARLE and OLSON, 1987) and a bovine YAC library with an average insert size of 750kb has been constructed (LIBERT *et al.* 1993). However, there are several difficulties that are encountered in the manipulation of a YAC library. First, cloning efficiencies are low (SMITH, SMYTH and MOIR, 1990). Second, it is difficult to isolate cloned DNA, because yeast cells have a rigid cell wall and large linear DNA molecules are easily sheared during extraction from the yeast cells. Third, YAC clones are often chimeric, consisting of fragments of DNA from different regions of the target genome (GREEN and OLSON, 1990a). The larger the insert, the more often it is chimeric (ANDERSON, 1993). Finally, deletions are often a problem of YACs (ANDERSON, 1993). Chimeras and deletions cause serious problems when chromosome walking and jumping are performed, as walks will be led in the wrong direction by chimeras or stopped by deletions.

One of the most promising solutions to the problems with YACs are bacterial artificial chromosomes (BACs) that can clone and stably maintain genomic DNA fragments of >300kb (SHIZUYA *et al.* 1992). The BAC system vector is based on the *Escherichia coli* F factor. The F factor has the ability to be stably maintained and inherited among the cells of a bacterial population. The F factor not only codes for genes to replicate itself, but also includes two genes, *parA* and *parB*, that control its copy number at a low level, usually 1-2 copies per cell (WILLETTS and SKURRAY, 1987). The low copy number per cell reduces the chance of recombination between plasmids and preliminary studies show that the level of chimerism in BAC clones is much less than in YACs. The F factor is suitable for cloning large DNA fragments since it can carry and maintain inserted bacterial DNA up to 1 megabase in length (LOW, 1987). Data from a human BAC library show that transformation is 10-100 times more efficient than in yeast. The manipulation of cloned DNA in BACs is easier than in YACs because supercoiled, circular plasmid DNA is resistant to

shearing. Finally, human DNA has been shown to be quite stable in a BAC vector after 100 generations (SHIZUYA *et al.* 1992). The BAC system promises to be a useful system for cloning large DNA fragments for genome mapping.

MATERIALS AND METHODS

Construction of BAC library: To construct a bovine BAC library, blood samples were taken from a purebred Angus bull. White blood cells were separated and embedded into agarose beads. DNA extraction included lysis, proteinase K digestion and washing of the DNA embedded in the beads (OVERHAUSER and RADIC, 1987). High molecular weight DNA was partially digested by a restriction enzyme within the agarose beads (IMAI and OLSON, 1990). Optimal digestion conditions were determined by pulsed field gel electrophoresis and appropriate DNA fragments were excised from the gel to construct the BAC library. The DNA was released from the agarose by GELase (SHIZUYA *et al.* 1992). Two different restriction enzymes were used to construct the BAC library: *Hin* dIII and *Bam* HI. Size selected DNA was ligated to the pBeloBACII vector (SHIZUYA *et al.* 1992) and transformed into ElectroMAX DH10B^{TM} cells (GIBCO BRL) by electroporation. Blue-white colony selection was used to recognize the colonies which contained plasmid DNA with insertions. Positive BAC colonies were stored in 96-well microtiter plates at -80°C.

Characterization of BAC library: To estimate the average BAC insert size, 68 randomly chosen clones were selected, grown and miniprepped. Inserts were recovered using *Not* I, which had recognition sequences flanking the *Hin* dIII and *Bam* HI cloning sites of pBeloBACII. Charactrization of the stablilty of inserts and the percentage of chimeric clones are in progress. To test the insert stability, positive clones will be grown for at least 100 generations and rare cutting restriction enzyme maps will be compared from clones before and after growing. To estimate the percentage of chimeric clones, clones which lack *Not* I recognition site in the insert DNA will be used as probes to hybridize to genomic DNA which has also been cut by *Not* I. Chimeric clones will hybridize to at least two *Not* I fragments while nonchimeric clones should hybridize to only one *Not* I fragment.

PCR-based screening: The PCR systematic screening method (GREEN and OLSON, 1990b) was used to screen the BAC library for clones containing three microsatellite markers and three anchor locus markers. Single plate DNA pools were prepared by combining the cell cultures of 96 BAC clones. Super DNA pools which contained 384 BAC colonies DNA were made by pooling 5 single plate DNA pools. First, the super DNA pools were screened for each marker. Then, single DNA pools from the positive super pool were screened to locate the positive clone to a single 96 well microtiter plate. Eight row and twelve column DNA pools were made for the positive single plate. After row-column screening, positive clones containing each marker were found. PCR was performed for the positive clone DNA to confirm the screening result.

RESULTS

We have produced 14,000 bovine BAC clones that defined a library with a 50% probability of containing a unique DNA fragment. The average insert size of clones produced from DNA following a single round of size selection is about 142±70kb (95% interval) as determined from 44 randomly chosen clones. The insert size ranges from 40kb to 300kb. Following a second round of size selection the average insert size of clones remained 142kb from 24 randomly chosen clones, but the insert size range was from 120kb to 210kb. After the second size selection, small DNA fragments were eliminated and the resulting insert size was more uniform.

Three microsatellite markers and three anchor locus markers were used to screen the 14,000 clone bovine BAC library. Two BAC colonies were found containing microstellite marker ETH225 (BARENDSE *et al*, in press). Also, two BAC colonies containing the Glucocerebrocidase gene and one BAC clone containing the 3β-hydroxy-5-ene steroid dehydrogenase gene were isolated. Therefore, positive colonies were located for three out of six markers, which is consistent with the estimated 50% probability of this library containing a unique DNA sequence.

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