

# Sequencing the Agouti gene in SKC43 Mutant Mice Using a Random Shear BAC Library

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## Abstract

The mouse agouti gene locus is a very complex genomic region, and the physiological consequences of ectopic agouti gene expression include changes in coat color, hyperinsulinemia, obesity, stimulation of somatic growth and tumorigenesis. To search for the mutation in the agouti mutant, SKC43, we constructed a random shear bacterial artificial chromosome (BAC) library of the transgenic mice (*Mus musculus* strain C57Bl6J). The library contained 124,800 BAC clones with average insert size of 131 kb, representing an ~5x coverage of the transgenic mouse genome. We then designed overgo probes and PCR-primers at 50 kb intervals along the ~150 kb agouti locus. These probes were used to screen the BAC library by hybridization and PCR. Positive BACs containing the agouti genomic region were identified and characterized and among them, one positive BAC clone was subjected to shotgun sequencing. Sequence analysis revealed the capture of a 130-kb portion of the agouti SKC43 mutant allele.

## Background

The agouti gene locus in chromosome 2 regulates the differential production of black and yellow pigment granules that give rise to the agouti coat color of the mice. It functions within the microenvironment of the hair follicle. The agouti locus are also associated with essential developmental processes unrelated to pigmentation, such as embryonic lethality, obesity, diabetes, and the development of tumors in a wide variety of tissues. The agouti gene locus is one of the most complex and very large genomic regions in mice. Besides more than six alleles at the agouti locus, gene mutation, modification, regulation and interaction, phenotypic variations have been also observed due to epigenetic modification, retrotransposon insertion into >100kb upstream of the agouti gene (Figure 1). Unbiased large-insert random insert BAC libraries are ideal and powerful tools to study such a complex and large genomic regions and help to accelerate the animal mutant discovery.



Morgan et al. Nature Genetics 23:314, 1999

Figure 1. Isogenic C57BL/6 A<sup>vy/a</sup> mice show a continuum of phenotypes ranging from completely yellow, through degrees of yellow/agouti mottling, to completely agouti (termed pseudoagouti because the mice are isogenic with fully yellow mice and not genetically agouti).

## Methods and Results

**MATERIALS:** Transgenic mice (*Mus musculus* strain C57Bl6J) containing the agouti mutant, SKC43

**METHODS AND RESULTS:** Construct Random Shear BAC library 124,800 clones, average 131 kb, or ~5x genome coverage

Design overgo probes & PCR-primers at 50 kb intervals along the ~150 kb agouti locus

BAC library screening by overgo hybridization and PCR confirmation

Sequence analysis revealed capture of 130-kb portion of the agouti SKC43 mutant allele

## Random Shear BAC Library

Transgenic mouse genomic DNA was randomly sheared, size-selected, and cloned into the pSMART BAC vector. The BAC library contained 124,800 clones with average inserts of 131 kb (Figure 2), which equals ~5x genome coverage.

Transgenic mice (*Mus musculus* strain C57Bl6J) containing the agouti mutant, SKC43

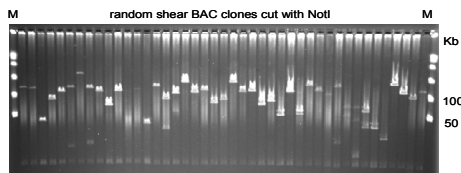


Figure 2. Genomic DNA was isolated from the transgenic mouse, randomly sheared, size-selected to >100 kb, and cloned into the pSMART-BAC vector. DNA from minipreps was digested with NotI to excise inserts. The pSMART-BAC vector band is visible at 7 kb.

## Overgo Oligo and Primer Design

The Agouti gene locus including ~50kb upstream of exon A is located on the chromosome 2 of *Mus musculus* strain C57Bl6:154726955 ~154876747. Four overgo oligo probes: 50K'-upstream, EXONA-intron, EXON1-intron, EXON4 and 4 PCR primer pairs: P-50k, P-Ea-I, PE1-I, P-E4 were designed with the OVERGO 1.02i program (<http://www.mouse-genome.bcm.tmc.edu/webovergo/Overgoinput.asp>) at the same positions of 50 kb intervals along the ~150 kb agouti locus (Figure 3).

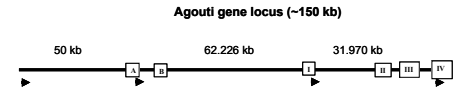


Figure 3. Scheme of ~150 kb Agouti gene locus and positions of overgo oligo probes and PCR primers designed. Arrowhead indicates 4 overgo oligo probes and PCR primer pairs: 50K'-upstream, EXONA-intron, EXON1-intron, EXON4.

## BAC Library Screening by Overgo Oligos and PCR

The above 4 overgos: 50K'-upstream, EXONA-intron, EXON1-intron, EXON4 were labeled with <sup>32</sup>P-ATP and <sup>32</sup>P-dCTP separately according to a common overgo labeling protocol and pooled together as a mixed overgo probe. Seven high density colony filters, containing the 5X covered Random Shear mouse BAC library, were screened with the overgo oligonucleotide probes. Total 51 overgo-positive clones were identified and 15 BACs showed the strongest signal (Figure 4A). Positive BAC clones, which were hybridized with at least one of the four overgo probes, were tested as colony PCR individually against the 4 PCR primer pairs: P-50k, P-Ea-I, PE1-I, P-E4; we found that the BAC clone 118I24 (clone #5) was confirmed by all 4 primer pairs (Figure 4B).

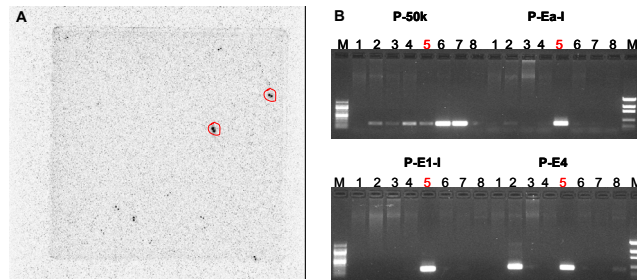


Figure 4. An example of BAC library screening results by mixed 4-overgo oligo hybridization and PCR confirmation. A) One of the seven high-density colony filters containing the entire mouse BAC library. The red circles indicate the strongest signals of BAC clones that are hybridized with at least one of the four overgo probes. B) PCR confirmation of positive clones. M, markers; 1, negative control; 2-8, positive clones by overgo oligos. Only clone #5 produces PCR products by all 4 primer pairs: P-50k, P-Ea-I, P-E1-I and P-E4.

## Sequence Analysis

The BAC clone 118I24 was subcloned and shotgun-sequenced by the Sanger method. Contig assembly was performed with the Sequencher program (Figure 5). As a result we have discovered a BAC clone covering more than a 130 kb portion of the agouti SKC43 mutant allele.

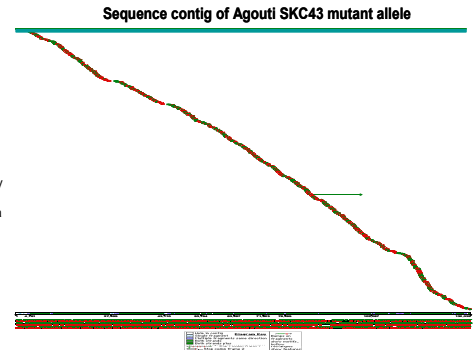


Figure 5. Diagram of sequence contig assembly of BAC clone 118I24 by the Sequencher program. The sequencing data showed this BAC clone covers more than 130 kb portion of the agouti SKC43 mutant allele.

## Conclusions

The results show that the combination of a Random Shear BAC library, overgo oligo hybridization-PCR, and DNA sequencing can be rapidly adapted to sequence a large and complex gene locus and dissect the mutant from the transgenic mice. Because Random Shear BAC libraries are unbiased, 5x genome coverage may be sufficient for most purposes of genomic analysis and efficient genome discovery. Future work will characterize more BACs, discover potential multiple mutant alleles, and analyze their functions. The unbiased Random Shear BAC techniques could be efficiently and effectively applied to many other genomic studies.

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