Generation of Bovine Genetic Markers by Representational Difference Analysis: a genome subtraction technique

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Summary and Implications

Representational Difference Analysis (RDA) is a genomic subtraction method to identify differences between two genomes. RDA was performed to isolate polymorphic cattle genetic markers that are potentially associated with carcass traits of interest. Sequence homology analysis indicated that some of the difference products had homology with known genes from cattle, human or mice, while most had no significant match. Over 20 different sequence tagged sites (STS), were generated. To evaluate the polymorphic nature of these RDA markers, genomic samples from five different cattle breeds were PCR amplified and sequenced. Polymorphisms in the form of single nucleotide polymorphisms (SNP) and base pair insertion were identified. Further, RDA generated STS markers were mapped to various bovine chromosomes. Thus, Genomic Representational Difference Analysis is suitable for generation of DNA markers and will help to increase the resolution of the bovine genomic map. In the future, we will evaluate the extent to which these marker may be used to predict phenotype. If successful, these marker may aid in the selection of breeding stock thereby improving the carcass composition of the end production of beef cattle production, high quality lean meat production.

Introduction

Representational Difference Analysis (RDA) is a powerful, versatile but complex procedure, which is based on the principle of subtractive hybridization coupled with the use of polymerase chain reaction (PCR). This allows efficient subtraction of complex eukaryotic genomes and helps to isolate genetic markers without prior knowledge of DNA sequence, location etc. Development of a dense framework genetic map is a pre-requisite for fine mapping of quantitative trait loci. Thus, techniques such as RDA can be applied to develop polymorphic genetic markers, which may also be potentially associated with traits of interest.

Materials and Methods

RDA was performed using genomic DNA from bulls with high and low EPDs for marbling following the original protocol of Lisitsyn and Wigler (1993) with modifications. The major steps of typical RDA techniques includes:

1. Adaptors and Primers for PCR
2. Representation
3. Subtractive/Kinetic Enrichment
4. Cloning difference product
5. Analysis of RDA markers

RDA was performed using *Bgl* II restriction endonuclease. A plasmid library was constructed of the second round difference products of which over 200 clones were isolated, DNA insert size was estimated, and 96 *Bgl* II difference products were sequenced.

Sequencing of Clones and Development of Marker:

Sequencing of cloned plasmid DNA was carried out by high-throughput automated DNA sequencing in 96 well format using M13 universal primer. All sequences of clones were compared with the GeneBank database to find similar sequence to that of the RDA difference products. The BLAST search tool (http://www.ncbi.nlm.nih.gov/BLAST/) was used to identify similarity with the nonredundant and/or human/mouse genome databases. Sequence Tagged Site (STS) specific primers were designed using Primer3 (http://www. genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

Results and Discussion

To isolate bovine genetic markers, RDA was performed using *Bgl* II, and after two rounds of subtractive hybridization, clear enrichment of difference products was observed. RDA difference product were cloned in pBluescript vector and sequenced. Over 50% redundancy was observed in sequence similarity between cloned difference products. Sequence homology analysis indicated that some of the difference products had homology with known genes from bovine (e.g. Msx), human or mouse, while most had no significant match. About 10% of the clones had known repeat elements. To generate sequence tagged sites (STS), oligonucleotide primers were designed for RDA difference product using sequence information and several STS (>20) were generated. To identify polymorphic nature of RDA markers, genomic samples from five cattle breeds were PCR amplified and sequenced. Polymorphisms in the form of SNPs and base pair insertion were identified. The RDA generated STS markers were mapped to various bovine chromosomes using Radiation Hybrid panel which will help to enrich the map.

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Table 1: Summary of bovine genetic markers developed by RDA

Marker	STS size(bp)	Number of SNPs
ISURDA 34	199	0
ISURDA 71	369	1
ISURDA 88	420	3
ISURDA 63	353	3
ISURDA 65	608	5
ISURDA 43	456	4
ISURDA 82	314	1
ISURDA 19	263	1
ISURDA 41	154	0
ISURDA 3	381	1
ISURDA 75	182	0
ISURDA 64	323	2
ISURDA 50	123	0
ISURDA 27	405	3
ISURDA 6	361	5
ISURDA 80	135	0
ISURDA 17	214	2
ISURDA 5	171	0
ISURDA 61	290	4
ISURDA 95	194	0
ISURDA 7	416	2

Figure 1: Example of SNP at nt-243 (T - C) for marker ISURDA 7