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Targeted SNP Haplotype Analysis of Porcine Ovulation Rate QTL on SSC8

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Abstract

High-density SNP maps for chromosomal regions containing QTLs have proven successful in refining map positions. Haplotype-based SNP analysis is more informative than analysis based on individual SNPs and in analyzing associations with phenotypes. A QTL for ovulation rate has been previously located in the centromeric region of SSC8p (Wilkie *et al.,* 1999; Brauschweig *et al.,* 2001). To maximally refine genetic intervals containing QTL and facilitate positional cloning, we generated a pooled shotgun sub-library targeting the ovulation rate QTL defined by *SW205* and *SW206* with an interval of 7 Mb which corresponds to human genomic positions between 34 and 42 Mb on HSA4. Twenty-seven BAC clones representing a minimal tiling path of this region were used to generate a pooled shotgun sub-library. Skim sequencing of the pooled BAC sub-library was performed and the shotgun sequences were masked for repetitive elements and subjected to BLAST analysis for similarity to human genome sequences of HSA4. Sequences with significant BLAST hits between 34 and 42 Mb on HSA4 were then used for SNP discovery. We were able to isolate 340 SNP markers and 27 insertions/deletions using a panel of DNA from eight diversified pig breeds (Yorkshire, Meishan, Berkshire, Duroc, Hampshire, Landrace, Large White and Pietrain). SNPs heterozygous for the UIUC Resource Family F1 individuals are being used to genotype a commercial population using a high-throughput SNP genotyping platform for use in linkage/linkage disequilibrium (LD) analyses.

Introduction

During the last decade, significant resources have been focused on establishing appropriate divergent crosses of pigs to map quantitative traits loci (QTL). The global approach to map specific phenotypes in pigs has utilized divergent crosses between western breeds and exotic breeds. These breeds have extensive selection pressure for growth, carcass and reproductive traits. Of specific interest is the high reproductive characteristics of Meishan breed which has been used to map traits such as prolificacy, ovulation rates and age of puberty. In an attempt to map reproductive QTL, the University of Illinois created a divergent cross between Meishan and Yorkshire. Genome-wide significance for the number of corpora lutea (CL) was observed on SSC8 defined by microsatellites *S0086* and *SW1037* that accounted for 13.2% of the F2 phenotypic variance (Wilkie *et al.,* 1999). Many studies showed strong evidence for a QTL located on SSC8. In an effort to further resolve the SSC8 reproductive QTL interval, additional microsatellites were developed to refine the QTL region for ovulation rate (Braunschweig *et al.,* 2001). The QTL region was then refined to an interval of 7.1 cM defined by microsatellites *SW205* and *SW206*. Haplotype-based methods offer a powerful approach to mapping polygenic traits based on the association between causal mutations and ancestral haplotypes on which they arose (Gabriel *et al.,* 2002). The development of SNP-based LD maps in QTL regions could facilitate association studies, leading to more efficient detection of candidate genes.

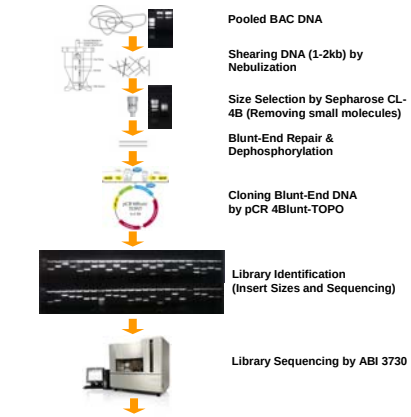
Objectives

The objectives of this project are (I) creating high resolution physical maps of the QTL region; (II) creating SNP haplotype maps for making linkage disequilibrium analysis of the QTL region; (III) determining LD for SNPs defining QTL region in commercial populations; (IV) evaluating positional candidate genes within the maximally refined QTL region for ovulation rate on SSC8.

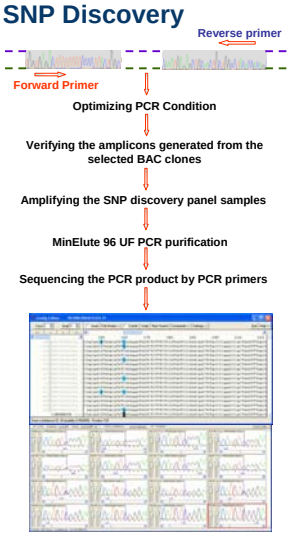
Materials and Methods

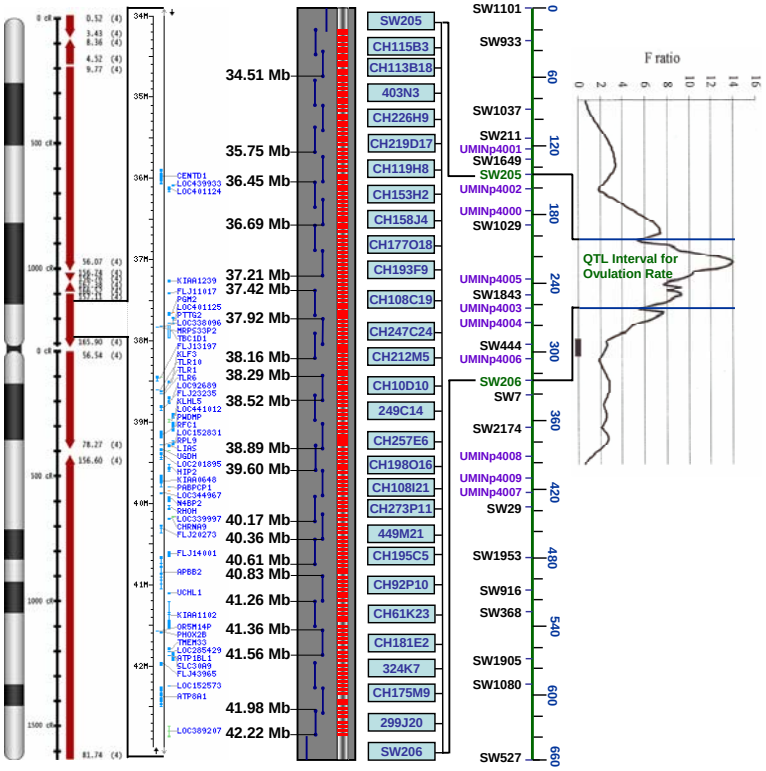
Twenty-seven BAC clones were selected from the RPCI-44 porcine BAC library (http://bacpac.chori.org/mporcine44.htm) and CHORI-242 porcine BAC library (http://bacpac.chori.org/porcine242.htm) to generate a pooled shotgun sub-library (Invitrogen). Skim sequencing of the pooled BAC sub-library was performed using ABI 3730 automated DNA sequencer. Shotgun sequences were assembled by phred/phrap/consed (http://www.phrap.org/phredphrap consed.html). All sequences were trimmed for vectors and *E.coli* DNA sequences. After quality assessment (Q>20), sequences were masked for repetitive elements (http://www.repeatmasker.org/), and subjected to BLAST analysis for similarity to human genome sequences of HSA4 using NCBI-BLASTn. An expectation value (E) of e^{-5} was used as the threshold. Sequences with significant BLAST hits between 34 and 42 Mb on HSA4 were then used for SNP discovery. Primers were designed using primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). SNP discovery was performed by directly sequencing approach, and sequence comparison was done by Phrap and Gap4 integration (http://staden.sourceforge.net/phrap.html).

Library Construction



SNP Discovery





Schema for Physical Map Construction and SNP Discovery for the Ovulation Rate QTL Interval on SSC8.

A. Represents porcine SSC8 cytogenetic map.
B. Comparative human-pig map showing HSA4 homologue for centromeric region of SSC8p (Meyers *et al.,* 2005).
C. HSA4 physical map (34 Mb to 42 Mb) and annotated genes corresponding to QTL interval.
D. Depicts RPCI-44 and CHORI-242 BAC clones selected for SNP discovery. Distances in human Mb as defined by BAC end-sequence blasts against Human Draft 34 (http://www.sanger.ac.uk/Projects/S_scrofa/mapping.shtml).
E. Red bars indicate location of selected SNPs for use in probe sets.
F. Selected BACs physically mapped between the QTL interval defined by *SW205* and *SW206*.
G. Location of microsatellites (cR) used to define the original QTL interval (Wilkie *et al.,* 1999; Brauschweig *et al.,* 2001).
H. QTL map showing Ovulation Rate QTL at genome-wide significance.

Results and Disscusion

340 SNP markers and 27 insertions/deletions using a panel of DNA from eight diversified pig breeds (Yorkshire, Meishan, Berkshire, Duroc, Hampshire, Landrace, Large White and Pietrain) were obtained from the targeted QTL region for ovulation rate in pig. SNPs heterozygous for the UIUC Resource Family F1 individuals are being used to genotype a commercial population using a high-throughput SNP genotyping platform for use in linkage/linkage disequilibrium analyses. The project provides a model for creating targeted SNP discovery in QTL regions but it also provides valuable information with respect to the structure of the pig genome.

References

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Acknowledgements

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