

Mapping Gene Expression as Quantitative Traits for Growth



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Applied Biosystems Technologies

- Applied Biosystems 7500 Fast Real-Time PCR System
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Applications

- Gene Expression

To better understand how genetic variation regulates growth and obesity, Dr. Juan Medrano, Professor of Genetics, Department of Animal Science, University of California, Davis, and the five researchers in his lab have been employing a novel approach to the mapping of quantitative trait loci (QTL) in different mouse models using the 7500 Fast System and TaqMan® Gene Expression Assays. By defining levels of gene expression as quantitative traits, the researchers measure the expression of candidate genes associated with changes in levels of gene expression, and then map the DNA sequence variation that accounts for these traits as expression quantitative trait loci, or (eQTL).

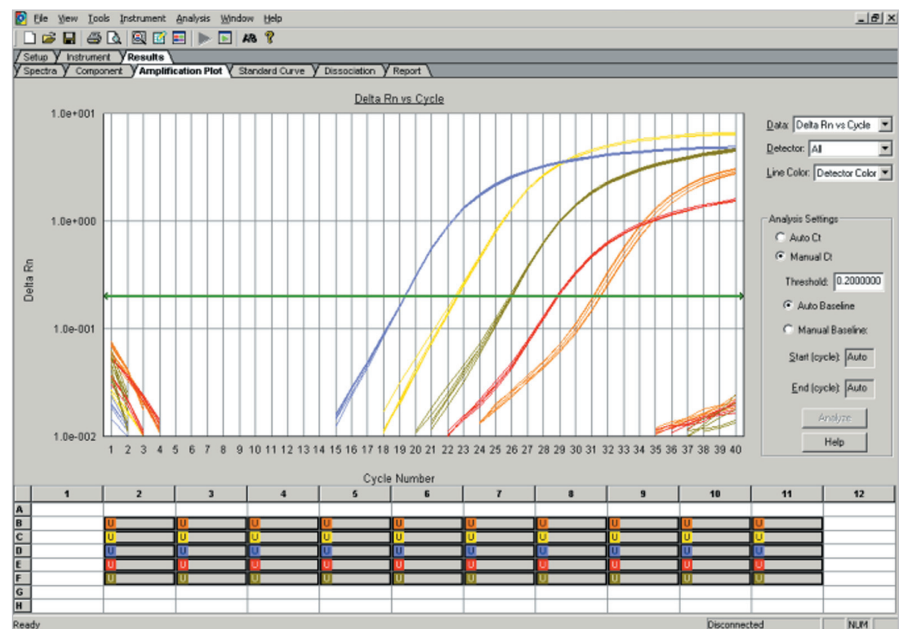


Figure 1. Performance of assays in fast mode on the 7500 Fast System using fast chemistry. Run time: 37 minutes.

Microarray analysis, followed by real-time PCR on the Applied Biosystems 7500 Fast Real-Time PCR System, has made it possible for the researchers to successfully map eQTL to specific mouse chromosomal locations. By mapping these eQTL, Dr. Medrano hopes to identify genetic variation associated with molecular phenotypes that have small effects on the complex pathways underlying the traits of growth, body composition, and obesity.

Complexity of Growth Traits

Many environmental and genetic factors contribute to complex diseases and conditions such as cancer, diabetes, and obesity. For the complex traits of growth and obesity, factors such as nutrition and exercise, combined with the actions of numerous genes that govern efficient

regulating growth and obesity in the mouse,” says Dr. Medrano.

Socs-2 Provides Clues as to How Genetic Variation Regulates Growth

In mice and man, understanding the molecular mechanisms regulating body size and weight means looking for DNA sequence variations that disrupt the homeostasis of growth, a state of equilibrium between molecular factors that promote and inhibit growth rates.

To uncover genetic clues that will better explain how disruptions in this equilibrium of different growth factors affects growth and body composition in mice, Dr. Medrano has been working with a strain of high growth mice that lack expression of the suppressor of cytokine signaling (Socs-2) gene. This

to enter the nucleus, where it initiates the transcription of genes that promote growth. The SOCS-2 negative regulator protein then feeds back into the receptor and stops signal transmission.

“In a mouse model we are currently using, SOCS-2 is not produced, so the negative regulator is not there, explains Dr. Medrano. “As a result, there is an extended transmission of this signal to the nucleus of the cell.”

Dr. Medrano’s lab is now trying to identify other genetic variations in the Socs-2 (hg) mouse model that may either be involved in the SOCS-2 signal transduction pathway, or other—as yet unknown—growth regulation pathways. Any such discoveries will serve as entry points for studying the network of gene interactions regulating growth in the mouse.

Mapping of Global Gene Expression

Using the high growth mouse model, Dr. Medrano and five researchers in his lab are now mapping gene expression QTL or eQTL.

“This approach of using eQTL is something very new for us. By mapping the expression of a gene to a chromosomal position, we can say that something has happened at this chromosomal location to affect the level of expression of that specific gene,” says Dr. Medrano.

“The high growth mouse is about 40 percent larger than a normal mouse, and is also more efficient in utilizing food. The mouse is not obese and has a higher lean mass, so it’s an excellent model for domestic animal breeding,” says Dr. Medrano.

To identify and map eQTL, the researchers in Dr. Medrano’s group initially characterize regions of the mouse genome they know contain

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utilization of nutrients and control of appetite give rise to quantitative traits that describe their phenotypes.

“The genetic complexity of quantitative traits related to growth is due to the combined action of genes that have both large and small effects on the overall phenotype,” explains Dr. Medrano.

In their search for gene loci that influence these large and small effects on resulting growth phenotypes, the researchers in Dr. Medrano’s lab are examining quantitative trait loci (QTL) to dissect the underlying genetic architecture comprised of networks of molecular interactions.

“Our lab is primarily involved in the identification of genetic variation

mutant strain of mice was identified and characterized at UC Davis in 1984.

According to Dr. Medrano, in the Socs-2 mouse model, a chromosomal deletion results in a loss of expression of a gene that normally serves as an important negative regulator of growth.

The Socs-2 gene is involved in signal transduction of growth hormone and other cytokines. So deletion of the gene involved in the regulation of growth hormone results in higher expression of genes that promote growth, resulting in a much larger mouse.

In normal mice, growth hormone binds to a cell-surface receptor. This sends a signal to phosphorylate a transcription factor, which enables the protein factor

QTL for growth and obesity. This process begins with the study of congenic strains of mice, produced by repeated backcrosses to an inbred strain selecting chromosome segments from a donor strain.

The congenic strains of mice contain regions of the chromosome from one strain that have been introduced onto the high growth genetic background of another strain. According to Dr. Medrano, this affords researchers a clean genetic background that allows them to more clearly see the effects of a phenotype of interest.

The congenic strains have been designed on high growth genetic background to study QTL, which interact with this mutation. A cross is then performed using the congenic mice, creating a segregating population



A normal mouse (left) and a high growth mouse (right). The high growth mouse is about 40 percent larger than the normal mouse, but is not obese.



According to Alma Islas, Staff Research Associate in Dr. Medrano's lab, the 7500 system is fast, easy to use, and has improved graphing capabilities compared with earlier models of sequence detection systems.

“For our mapping studies, we find the assays we need on the Applied Biosystems web page, and directly order the TaqMan® Assays. Two days later, the assays arrive here, and one day later the experiments are done.”

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of recombinant mice, which can then be used to map eQTL and QTL for growth and obesity traits.

To explore the influence of networks of genes on growth phenotypes, researchers in Dr. Medrano's lab first survey global gene expression in different mouse tissue samples using DNA microarrays. They then use the 7500 Fast Real-Time PCR System to both validate microarray data, and to measure expression of candidate genes in mouse tissue samples in an effort to map the genetic variations that play a role in regulating the observed differences in gene expression.

“We're mapping QTL for growth and obesity and then doing fine-mapping in that chromosomal region, which includes eQTL mapping to determine what are the genes that may be affecting the phenotype. We have no preconceptions when we look for candidate genes,” says Dr. Medrano.

For researchers in Dr. Medrano's lab, the use of the Applied Biosystems 7500 Fast Real-Time PCR System coupled with TaqMan® Gene Expression Assays has bolstered their analysis of complex traits.

“The 7500 System has greatly increased our ability to unravel the complex genetics controlling growth and obesity,” notes Dr. Medrano.

Mapping eQTL

Equipped with genotype data from the congenic strains, Dr Medrano's group searches for instances of genetic variation within the mouse genome

that account for phenotypic differences in growth rate and body composition among the different strains. One type of phenotypic difference that they explore is varying levels of gene expression found in different mouse tissue samples. Real-time PCR applications allow precise quantitative measurements of even minute increases or decreases in levels of gene expression.

“The 7500 System, used together with TaqMan® Gene Expression Assays, allow us to do quantitative measurements of gene expression very precisely and very reproducibly. It really adds a new component to our phenotyping compared to how we measured gene expression several years ago when we used Northern blotting. With Northern, we can only measure very large differences in gene expression, they require more initial sample and the process is significantly slower,” notes Dr. Medrano.

Dr. Medrano's lab has found primers and probes for about 80 percent of the target candidate genes through Applied Biosystems TaqMan® Gene Expression Assays. If assays for a target gene are not available as part of the inventoried assays, the lab orders them through Applied Biosystems Custom TaqMan® Gene Expression Assays service.

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Recently, in a body composition study using microarray analysis followed by real-time PCR, the researchers in Dr. Medrano's lab were able to map genetic variation that regulates observed differences in gene expression of a functional candidate gene.

The researchers first performed microarray analysis and found the expression of a candidate gene to be upregulated by 2.9 fold. To validate these results they used the Applied Biosystems 7500 Fast Real-Time PCR System and TaqMan® Gene Expression Assay specific for this particular candidate gene.

To map eQTL for the candidate gene, the researchers turned to the 7500 Fast Real-Time PCR System.

Using standard protocols, the researchers isolated total brain RNA from forty-five recombinant F2 mice and converted it to cDNA. Each sample was assayed for the target and endogenous control gene using the Applied Biosystems 7500 Fast Real-Time PCR System. The relative abundance of the target gene was determined for each sample after correcting for control gene expression.

Using this data in combination with individual mouse genotypes, the

researchers subsequently mapped eQTL controlling the difference in gene expression to a specific chromosomal location which also contained QTL for growth and obesity traits.

To determine if the genetic variation controlling the eQTL also influences the obesity QTL will require further functional assays, such as creating a transgenic mouse that would mimic the observed phenotype.

From Mice to Man

Using the eQTL approach for growth studies with mouse models may eventually lead to the discovery of genes with similar functions in humans. In fact, Dr. Medrano's lab has already found QTL for obesity on mouse chromosome 2 that is syntenic with a location on human chromosome 20, a location that has previously been linked to obesity and body mass in several human studies.

"We are fortunate now that we have the completed sequence of the human genome and mouse genome, so by comparative mapping and sequence analysis we can identify comparable genes in humans," says Dr. Medrano.

However, Dr. Medrano notes that finding human genes that are homologous to newly identified mouse genes will first require the completion of population studies in humans, followed by large association studies.

Even if the homologous genes in humans do not influence obesity, identifying genes that contribute to growth rates and body composition in mice will give researchers a more detailed understanding of molecular pathways that may also be involved in traits related to obesity in humans. These pathways may be targets for anti-obesity therapeutics.

Overall, as Dr. Juan Medrano notes, to fully understand the phenotype of growth and obesity will require dissecting an entire network of gene actions that contribute to the overall phenotype. While this task sounds daunting, by using eQTL and the 7500 Fast Real-Time PCR System to study the genetics of complex traits, Dr. Medrano and his lab are following an approach they hope will lead them down a path to a better understanding of growth and obesity.

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