

Functional genomics - a new tool for animal improvement explained

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Introduction

How are we going to continue to improve animals in the future? Scientists at a number of universities and USDA are using new tools to gain knowledge to help develop future improvements in health, reproduction and production. Functional genomics is this new science that is providing scientists the ability to study large numbers of genes that control important traits and physiological functions in livestock. Functional genomics involves techniques that link genes with their function. So now, scientists can see what genes are “turned on” or “off” as a result of a treatment or physiological condition. Many of the sets of genes being studied have been linked to specific pathways or functions such as the immune system, stress response and reproductive functions. This science presents a new way to investigate what makes a cow tick.

For centuries, man has attempted to use animal selection and management to improve cattle performance. In the past century much knowledge has been gained about the physiology and feeding of cattle for high production through hormone studies and feeding trials. And since the late 60's, genetic gain has contributed two thirds of the annual gain in milk production, a result of improved techniques in genetic evaluations of bulls and cows. More recently, scientists have been able to use quantitative trait loci (QTL) to identify segments of chromosomes that contain variants of genes that significantly influence production or other traits. Some of these are involved in known physiological pathways of animals.

The knowledge gained about gene expression and function using functional genomics is our next step. It will first help scientists better understand physiological functions and pathways, even at the cell level. From better understanding of function we will be able to enhance our management techniques and perhaps some day, regulate specific genes to improve animal performance and well-being. Then we may be able to select for sets of identified genes to improve animals genetically.

Production traits, genetics, and the environment.

A dairy cow's performance or phenotype is dependent upon the genes she receives from her parents, her physiological state, as well as the environment in which she resides and the conditions to which she is exposed. We can depict the performance of an animal as:

$$P = G + E$$

where: P is the observed performance or phenotype for a particular trait, such as milk production, G is the genetic ability (genotype) of the animal for a trait, and E is the sum of environmental and physiological factors that influence the trait. Environmental and physiological factors include internal and external factors, such as hormonal changes, nutrition, weather, disease, age, season, and stress. Many internal changes are a result of external changes. Others are influenced by disease such as immune cell response or normal physiological changes such as the estrus cycle.

The livestock industry’s interest has been to identify ways to improve important traits (P). To do this we select superior animals as parents to improve G and manage animals in an attempt to have a positive influence on E.

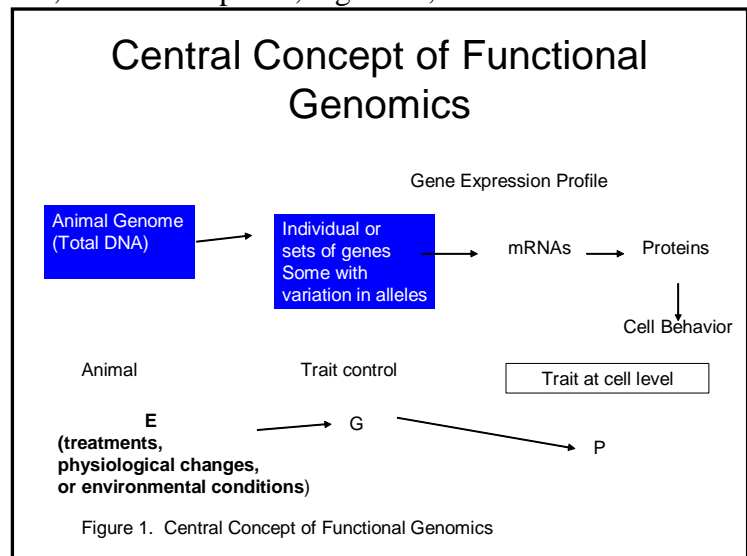
For years genetic ability (G) of an animal has been estimated for the collective effects of genes yielding values called Predicted Transmitting Abilities (PTA) for traits such as milk production and fat test. PTAs are computed without knowing what genes are involved, their function, or their DNA (Deoxyribonucleic Acid) sequence. Although the quantitative techniques used to estimate PTAs represent a “black-box” approach to genetic improvement, it has resulted in significant improvement for many important traits.

On the other hand, much animal research has focused on understanding the effects of management and environmental factors (E) on physiological responses of animals (P), ignoring G. Functional genomics allows us to put it all together by looking at phenotypes at the cellular and molecular levels and linking genes with their function. The results will further help scientists understand animal biology and lead to new ways to improve health, well-being, and performance.

What is functional genomics?

Functional genomics provides a peek into the “black box” by linking a trait or characteristic (P), such as disease resistance or milk production, with specific genes, yielding new knowledge about physiological functions such as milk secretion, immune response, digestion, and metabolism at the cell level.

This is done by determining which genes are “turned on” or “off” in specific types of cells as a result of treatments, physiological changes, or environmental conditions. For example, we can determine differences in genes expressed in diseased tissue of “sick” animals and that of healthy animals. In a number of cases, sets of genes have been linked to pathways or conditions which trigger a pathway and impact physiological functions such as milk secretion. By studying gene expression profiles and known pathways, we can learn more about milk secretion, immune response, digestion, and numerous other physiological functions in the cow.



Cell behavior is influenced by the proteins generated in the cell (Figure1). These proteins are created by digital instructions called mRNA (messenger Ribonucleic Acid) which are produced by copying the DNA sequences of genes when genes are “turned on”. Genes are made of DNA and are “turned on” by certain stimuli or conditions. Many proteins created are hormones or hormone regulators. It is the collection of synthesized proteins which ultimately define the

behavior and function within a cell and are responsible for the biological responses that underlie phenotypic traits of an animal. For example, biological processes in cells result in products such as milk protein, fat globules, and muscle growth.

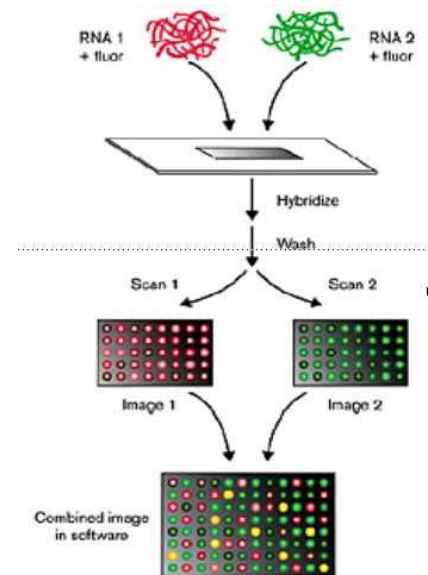
Each gene may come in several configurations or allele(s) with one allele received from each parent. A classic example of differing alleles is “R” and “r” for hair coat color in Holsteins. Different alleles can change the mRNA message or the amount of message and thereby change the amount or make up of the proteins generated thereby causing variation in cell behavior.

Conversely, when genes are “turned off” by specific stimuli, the DNA is not copied into RNA instructions and their corresponding proteins are not synthesized. This can modify the behavior and function of the cell as well. So genes or gene profiles are like a set of hundreds of switches with some “turned on” and others “turned off” at various times to regulate development or physiological functions. And these switches are influenced by various stimuli resulting from treatments, physiological status, the genetic make up of an animal, and environment.

What can we do with knowledge gained from functional genomics?

Identifying key genes in physiological pathways (2,3) is the main goal of functional genomics. Once scientists identify genes that cause a beneficial response to a change in environment or treatment, they may be able to develop methods to regulate the appropriate genes. For example, functional genomics can be used to investigate gene expression profiles (which switches are turned on and off) in diseased tissue in the mammary gland and white blood cells of infected versus healthy animals. Once scientists identify genes that cause a beneficial response to a change in environment or treatment, they may be able to develop methods to up regulate and/or down regulate the appropriate genes. Such methods could involve simple management changes, using new preventive vaccines, therapeutic drugs, nutritional supplements, or technologies to enhance or block gene expression.

Mentioned previously, some genes are polymorphic, i.e., they have different alleles inherited from parents (such as R and r) that significantly alter the expression of a trait. Using functional genomics, scientist can identify genes with variation in response caused by different alleles. This can lead to selecting animals as parents that have the desired allele.



How is functional genomics done?

To determine which genes are “turned on” or “off” by a condition or treatment, a tool called a **microarray** is used (Figure 2). A microarray is a glass slide holding thousands of genes from a species such as the dairy cow. To develop a “standard” microarray, tissues are first collected from animals at different states of health,

Figure 2. Microarray slides with mRNA from genes “turned on” labeled red in the control animal (Image 1) and green from the treated animal (Image 2). When combined the red spots indicate genes that were “on” in the controls and “off” in the treated samples, green indicates genes that were “turned on” in treated but “off” in control samples. The yellow spots indicate genes that did not change their expression between treated and control samples.

nutrition, production, stress, etc. From these samples, DNA sequences for individual genes are spotted on glass microarray slides forming a grid of dots (Figure 3). The Center of Animal Functional Genomics (CAFG) at Michigan State University, in collaboration with the U.S. National Bovine Functional Genomics Consortium, has developed one of the world's largest bovine DNA microarrays containing DNA sequences for 18,263 genes in the bovine genome (5).

To use microarray slides, scientists then collect cells or tissue samples from “treated” and control animals and extract the mRNA present to make comparison between mRNA generated in “treated” vs. control animals. The mRNA from the control animals, in our example in Figure 2, is labeled “red” and that from treated animals is labeled “green”. A scanner with a laser then detects the degree of redness and greenness of each spot which is a measure of the amount of mRNA message produced by the treated and control animals. Once processed, these images are laid on top of each other to produce a final image of red, green and yellow spots. This combined image is the gene expression profile.

The red spots indicate genes that were “turned on” in the control samples but not in the treated samples, the green spots are genes that were “turned on” in the treated samples but not the control samples, and the yellow spots represent genes that were “turned on” or “off” to the same extent in both samples, and thus unaffected by the treatment or condition. It is the genes with red and green spots that are of interest in understanding what the treatment did to gene expression in the tissue. Then knowing what pathways these genes control helps us understand what is going on when a cow is treated or influenced by a physiological condition. It's this knowledge that can lead to development of new management tools mentioned above.

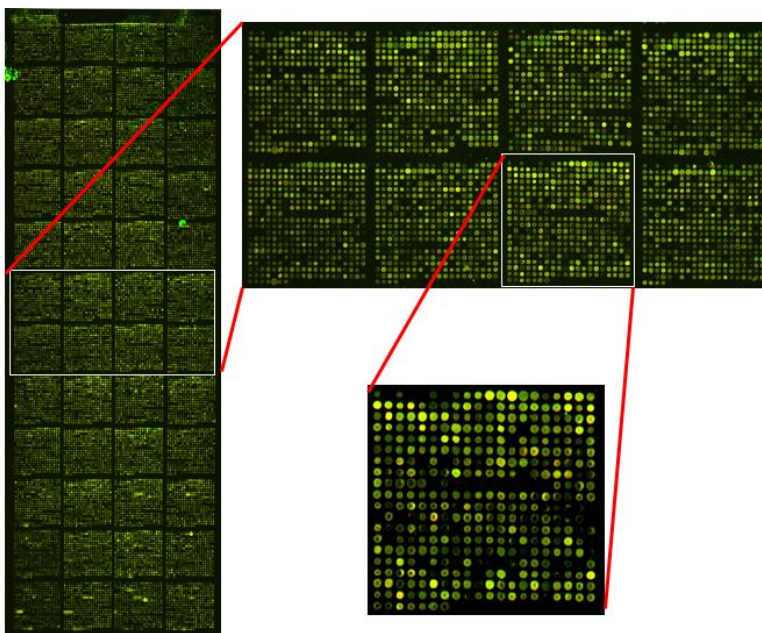


Figure 3. A microarray slide. The NBFGC microarray, developed through a CSREES-funded collaboration between MSU's Center for Animal Functional Genomics and the National Bovine Functional Genomics Consortium, is currently the largest bovine microarray available and contains DNA spots for more than 18,000 genes.

How does the microarray work? - An example

In the example shown in Figure 4, mRNA is collected from white blood cells (WBC) of lactating cows who serve as controls and from cows at the time of calving (“treated” cows). The goal is to determine which immune response genes may be affected in their expression, i.e., “turned on” or “off” by calving. Determining this may help explain the heightened disease susceptibility in

fresh cows. The control mRNA is labeled with red dye and mRNA from treated cows is labeled with green dye (Figure 4). The genes with greater expression (produced more mRNA) in WBC of lactating cows and thus repressed in calving cows show up as red spots. Those with greater expression in WBC of calving cows and thus repressed in lactating cows show up as green spots. The genes that were equally expressed in both cow groups show up as yellow spots. So again, it is the genes that are expressed differently in treated and control cows that we are looking for, i.e., the dark red and green spots.

In the case demonstrated in Figure 4, the microarray analysis has shown multiple gene families whose expression profiles are affected by calving and lead to heightened inflammatory potential of the cells at calving (2,4). Knowing what pathways these identified genes control will ultimately help us understand why some cows suffer more severe infections and inflammatory diseases in the early postpartum period compared to later lactation.

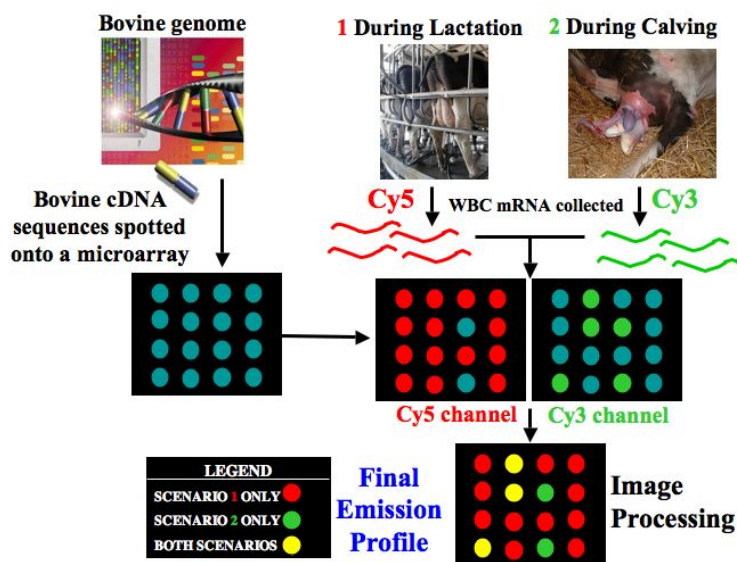


Figure 4. Microarray experiments are basically genome-scale surveys of the collection of genes expressed in a given cell type during a defined physiological change (lactation versus calving shown here), environmental insult, or treatment scenario. The combined pattern of red, green and yellow spots on the microarray reveal which genes are turned “on” (green), “off” (red) or unchanged (yellow) by a “treatment”.

Adapted From Burton et al., 2001 Acta Vet. Scand.

Where is this technology being used today?

A large group of scientists using functional genomics has formed the National Bovine Functional Genomics Consortium (NBFGC). Members of the NBFGC are using functional genomics to learn more about (1,2,3,4,5):

- nutrient partitioning during transition period
- fertility
- partition-induced suppression of immune system
- milk composition and yield
- heat stress
- transportation stress
- pathogenesis of disease

Members of NBFGC include scientists at:

- Michigan State University
- Cornell University
- University of Arizona
- University of Idaho
- University of California at Davis
- University of Missouri
- USDA-MARC – Clay Center, NE
- USDA-BARC – Beltsville, MD

SUMMARY

For years, genetic evaluations have been estimated using quantitative methods to compute PTAs for dairy sires and cows without considering genes involved. Today functional genomics tools allow scientists to link genes with function and further the understanding of animal biology. This is done by answering:

- Which genes are “turned on” (expressed) or “off” (repressed) as a result of a condition or treatment?
- Which genes might be involved in a pathway?
- What conditions trigger a pathway?

With a better understanding of biology and what genes are involved in pathways, we will be able to develop methods to enhance or turn off the expression appropriate genes. In addition, we may be able to select for sets of appropriate genes to change G, particularly in traits of low heritability.

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