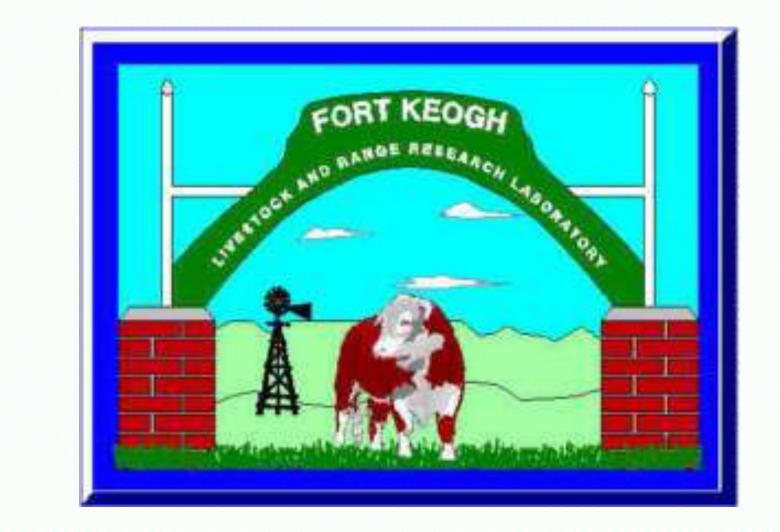


Genome Mapping and Genetics

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Goal

Identify genes and genetic interactions that characterize elite animals in order to improve production and sustainability of US livestock.

Current Objectives

To generate genomic maps that have the density of markers to RAPIDLY and efficiently define regions/gene(s) responsible for the phenotype.

To develop markers that accurately predict physical traits (phenotypes).

Long Term Objectives

Identify causal mutations in DNA responsible for the observed phenotype.

Development of diagnostic tests for detecting the specific DNA sequence.

Some Definitions

Genome—The genetic material of an organism. Can be Nuclear, Mitochondrial, or Chloroplast etc. We will refer to the nuclear DNA as the Genome.

Locus—a region of a chromosome, Loci (pl).

Allele— a variant of the DNA sequence at a locus. As the non-sex chromosomes come in pairs (one from each parent) an individual may have two different alleles.

Why Develop Maps for Livestock Species?

Genomes of higher animals are very complex. In humans the genome size is about 3.2 billion base pairs. Only 5% of the genome is thought to represent genes – About 22-25,000.

Available Avenues

Some traits are affected by well characterized genes and one can decipher what is happening in one organism by studying the same gene in a well defined system in another organism, e.g. some metabolic diseases.

However, if there is little or no information about a trait then it is necessary to start from the beginning. For example, birth weight, carcass measurements are traits not studied in humans or mice. In order to narrow down the regions affecting the trait of interest, it is necessary to generate a map showing the relationship of the trait to the genome.

How do we do this?

Primer pairs (see Figure 1) are designed against parts of the bovine genome. These are used in a reaction called Polymerase Chain Reaction (PCR, Figure 2). In this reaction we start off with 25 nanograms (1ng equals one billionth of a gram or 0.00000000035 of an ounce) of total genomic DNA. (In fact if the region we wish to examine is 150bp then the amount of starting target is 0.00000005 of 25ng) Thirty-one cycles of the PCR amplifies the target sequence 100 million fold.

The PCR products are separated by passing an electric current through a gel matrix onto which our DNA samples have been loaded. The process is summarized in Figure 3.

AGAAGATAGAAGGTTTCACATCATTGGTGGTTGTTGATTTTAGAAACAGT

TCTTCTATCTTCCAAAGTGTAGTAACCACCAACAACTAAAATCTTTGTCA

Figure 1. An example of a microsatellite marker.

This is a small stretch of bovine DNA. The letters in green is a microsatellite sequence. This is a simple repeat of alternating Ts and Gs. During DNA replication mistakes are often made leading to many alleles being present in a population. The different alleles vary in length. The different size alleles are then separated on the basis of length. The blue and red letters indicate where the synthetic DNA pieces (termed primer pairs) are designed to base pair with the animals DNA.

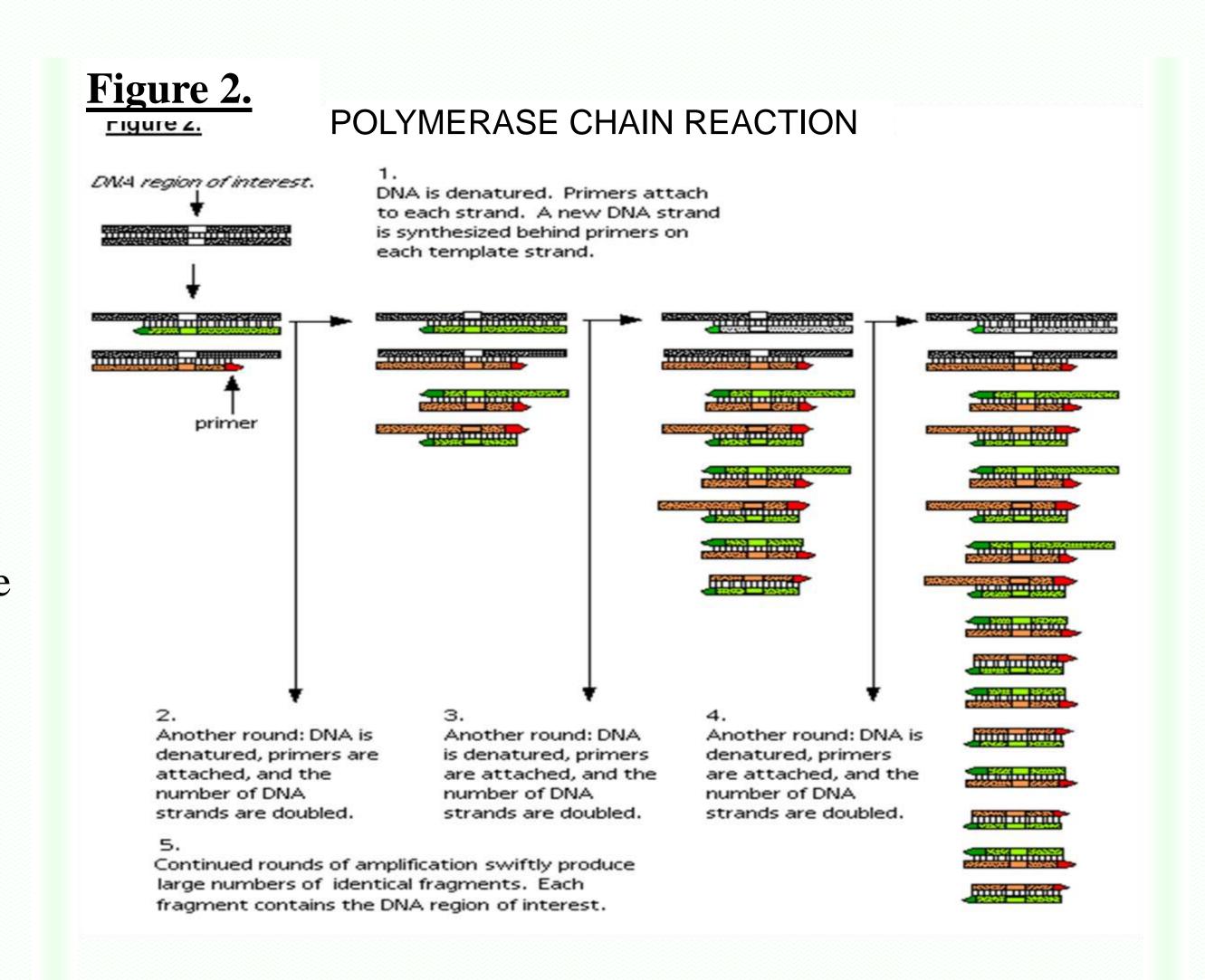
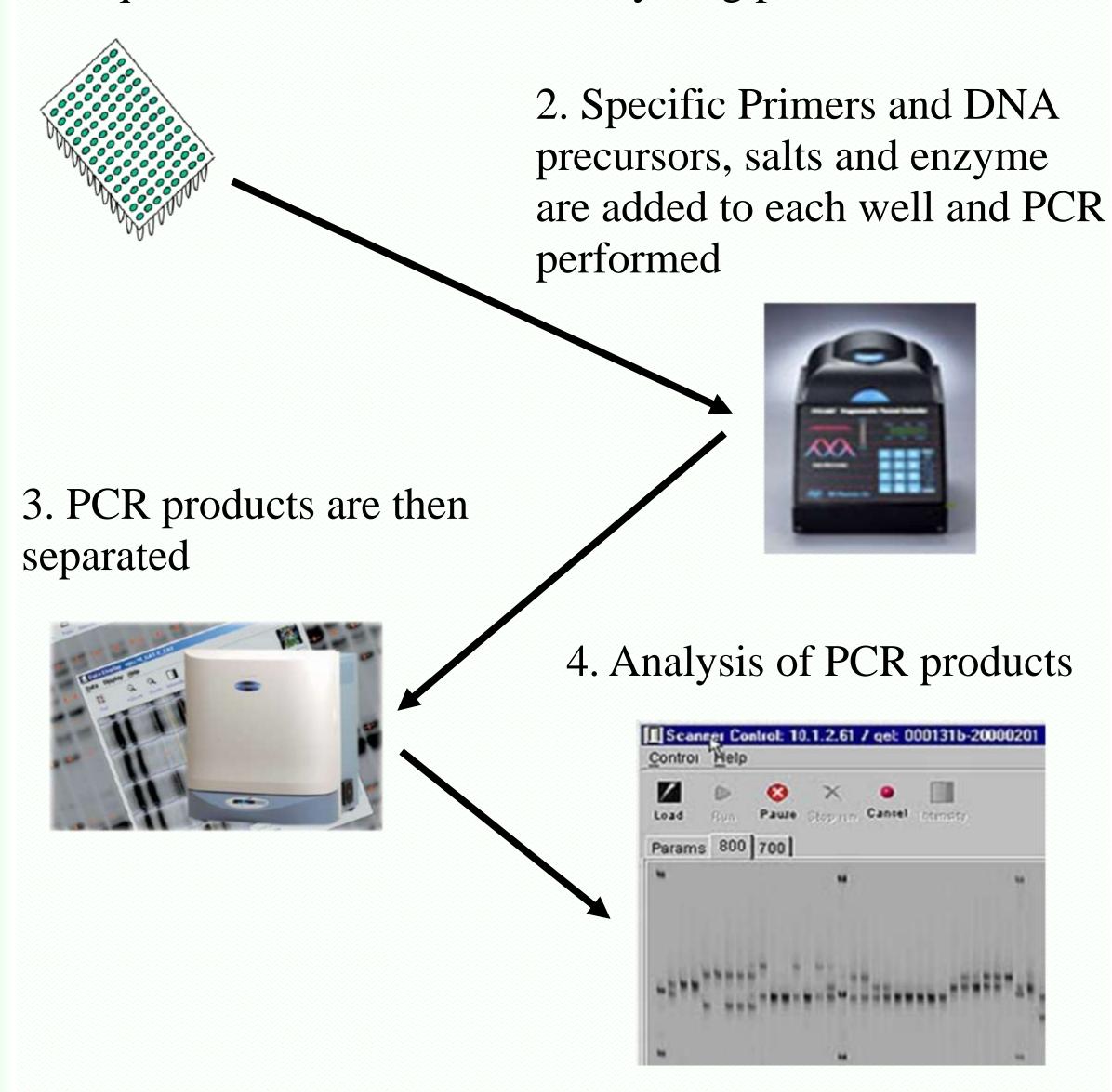


Figure 3.

1. Bovine DNA from different animals is aliquoted into 96 well thermal cycling plates.



These data are then analyzed by various computer programs and the frequency that alleles are co-inherited is calculated. During meiosis—the process where ova and sperm are generated—chromosomes undergo rearrangements called meiotic recombination/crossing over. This has the effect of creating genetic diversity by having different alleles being inherited in the next generation. Figure 4. We started with the pattern ABC and abc on each of the parents chromosomal pair. In this example patterns that may be passed onto the offspring are ABC, abc abC, Abc aBc, or AbC. Remember only one chromosome of each pair is transmitted.

Meiotic recombination and crossing over

The closer the markers the less chance there is of rearrangement. If there is only one rearrangement in 100 meioses then we say the two markers are one centiMorgan (cM) apart (1% recombination). The cM is the unit of genetic distance we use. We then construct a map of each of the chromosomes (Figure 5).

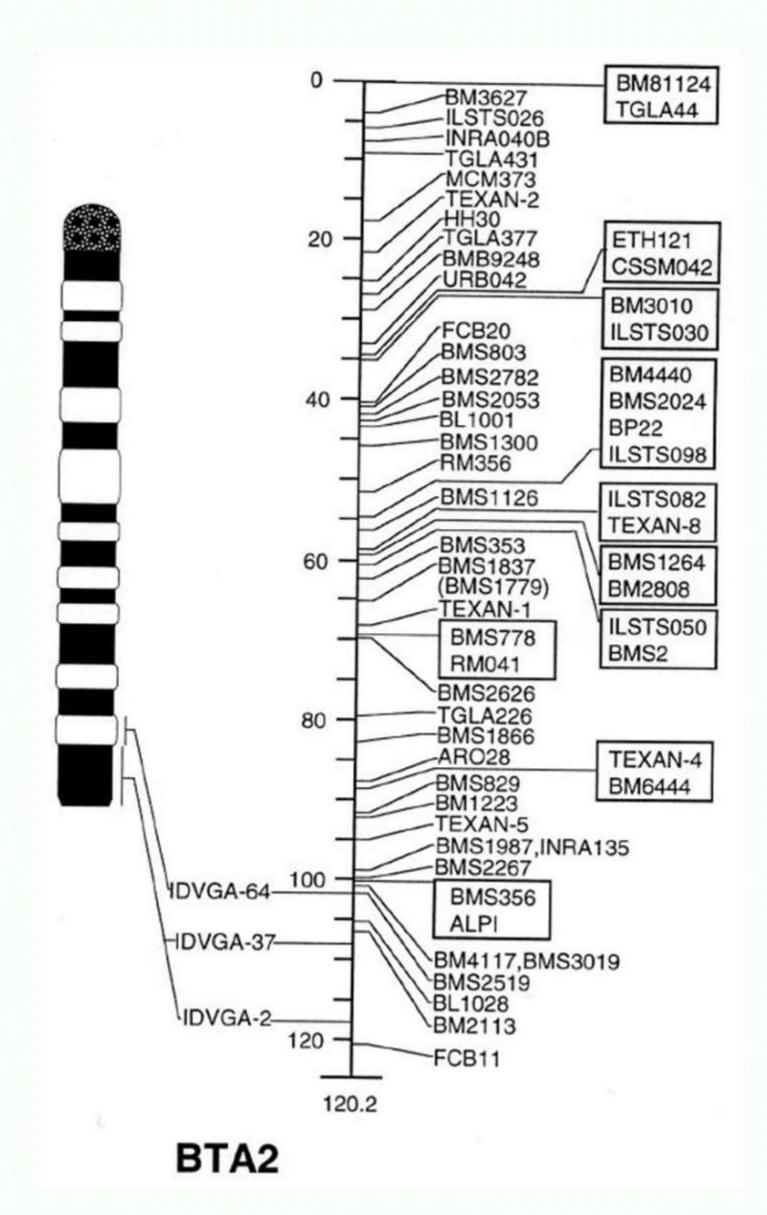


Figure 5. This shows the chromosomal map of cattle chromosome 2. The units are in centiMorgans

We then take measurements from the cattle we used to generate the map and look for associations between the alleles each animal has and the phenotype of the animal. This enables us to narrow down the region(s) of the genome responsible the differences between the traits of the animals under study (Figure 6). The next step is to identify which genes reside in this area and which is the causal gene. This is a laborious process if we don't know the DNA sequence of this area as there are many genes in these regions. We currently examine the equivalent region in humans and mice. However, there are many rearrangements between human, mice, and cattle. These rearrangements complicate the analysis.

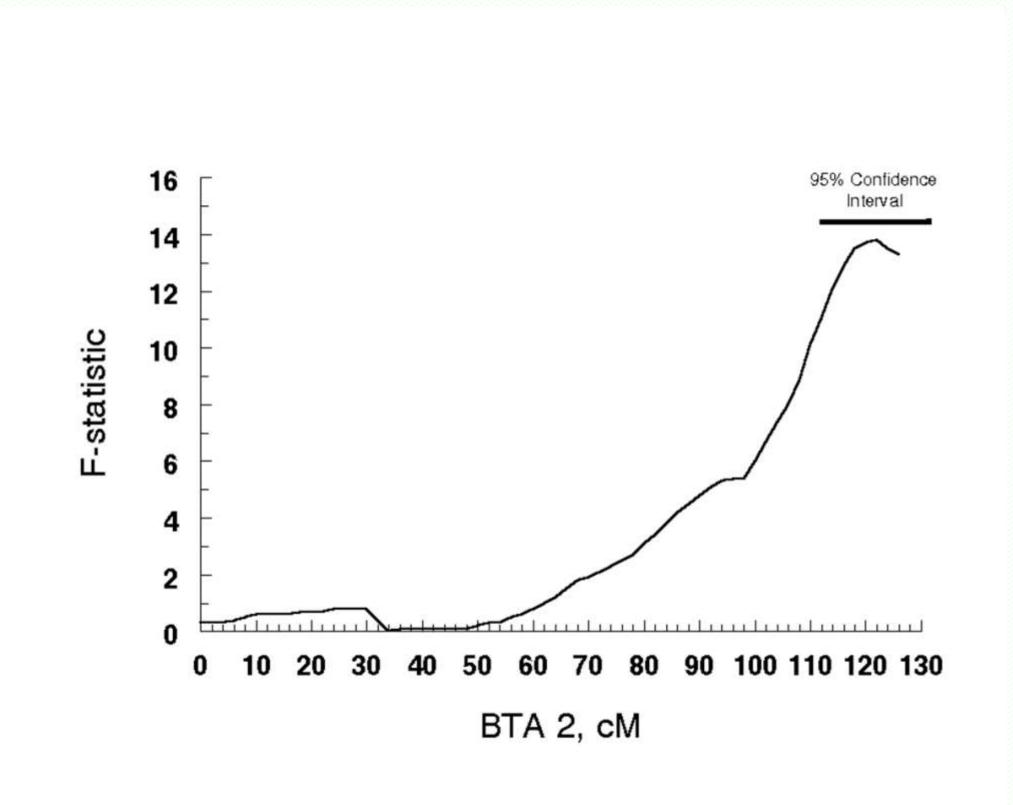


Figure 6. This shows the genomic location of a region of cattle chromosome 2 which affects Birth Weight. The F-statistic is an indicator of the probability that the observation is real.

Ideally, we would like to have the genome sequence of cattle available. This has been published April 2009. The one animal in the world that was the basis of the sequence is a Line 1 Hereford cow—L1 Dominette 01449. She is here at Fort Keogh (figure 7). Now that we have the genome sequence we can now use the next generation of markers called Single Nucleotide Polymorphism (SNP—Pronounced SNIP). These are single base changes and are very prevalent throughout the genome.



Figure 7. Line 1 Hereford cow L1 Dominette 01449