

# The Alpaca Enters THE GENOMIC ERA

## Development of a Radiation-Hybrid Map

By Warren E. Johnson, PhD  
and Polina Perelman, PhD



In many livestock industries, an increasing array of DNA tests are being offered to enhance health and determine the physical appearance of their animals.

Will alpacas be next?



Helen, Pat Dishaw

Our appreciation and understanding of the impact of genetics on human health, appearance, and behavior has increased rapidly over the last decade, with several disciplines coalescing around the new field of genomics. The results of these advances are filtering into our lives in innumerable ways, some of which are obvious, but others of which are less evident. Perhaps one of the least appreciated aspects of this genomic evolution is the rate at which the associated technologies and sciences are being applied to other species, and how this focus on nonhuman subjects is, in turn, increasing the overall pace of discovery in the field. This synergy is especially apparent for members of the exclusive genomics club, consisting of species for which there is a detailed genomic map.

The alpaca, as one of the most recent entries into this genomics club, is already beginning to benefit from the added exposure and biomedical and scientific attention that membership accrues. And although it is uncertain exactly what the genomics era portends for the extended alpaca community, the experiences of other groups – such as

those centered around species like dogs, cats, horses, and cows – provide some context.

However, it is first useful to have a sense of what is a genetic map, how is it made, and what is its utility? The most detailed genomic maps

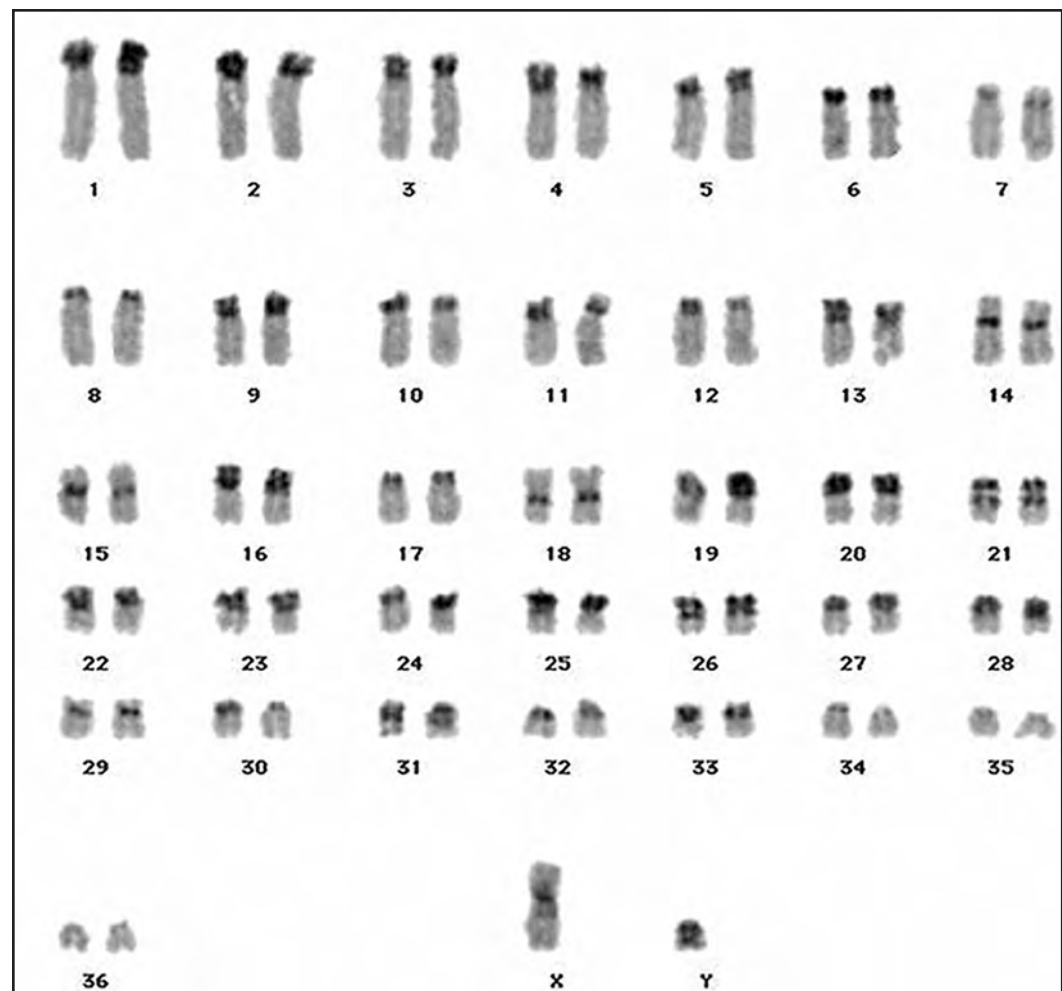
are physical maps. These serve both to orient genomic information along chromosomes, much as a road map, but equally as important, it serves as a way of linking landmarks and knowledge obtained from other species and their physical maps. These maps also have the desirable feature of being built to scale, not only providing the likely order of features along a chromosome, but also a good estimate of the relative distance between items of interest, such as regions coding for genes or specific mutations associated with different traits. Until recently, the only available physical maps were chromosomal or cytogenetic maps, often highlighted with different banding patterns with a light microscope (*Figure 1*) or with fluorescently labeled probes (*Figure 2*). Of much broader utility, however, and as such, a general prerequisite for entry into the genomic era, are radiation hybrid maps and sequence maps.



The Alpaca Research Foundation encourages scientific research which benefits the North American alpaca industry, primarily in the areas of alpaca health, husbandry, genetics, and fiber.



Morris Animal Foundation is dedicated to improving the health and well-being of companion animals and wildlife by funding humane health studies and disseminating information about these studies.



**FIGURE 1.** C-banding pattern of alpaca chromosomes. The alpaca karyotype consists of 74 chromosomes, one of the highest numbers in mammals. The alpaca karyotype also has unusually large heterochromatic regions, represented here as dark bands. All camelid species have an almost identical set of chromosomes with the same diploid number, but with differences in their distribution of heterochromatin (consisting largely of repetitive DNA sequences).

At the Laboratory of Genomic Diversity at the National Cancer Institute, we recently completed a “first-generation” radiation hybrid map of the alpaca (Figure 4). The donor cell line was established from a white male huacaya named Limerick (Figure 3). This resource provides a way to localize almost any genetic marker, as well as other genomic fragments, to a defined map position, and can be essential to the rapid identification of genes or mutations that are involved in specific inherited diseases or physical traits (Figure 4).

As useful as a radiation hybrid map can be, even more informative are maps consisting of large amounts of direct sequencing of the genome, often referred to as whole genome sequences (WGS). These have been made possible by new technologies that have rapidly increased the speed and reduced the costs of sequencing every base pair of

a chosen genome. These WGS not only more directly identify the genetic markers, but also quite precisely measure the distance between these markers in base pairs.

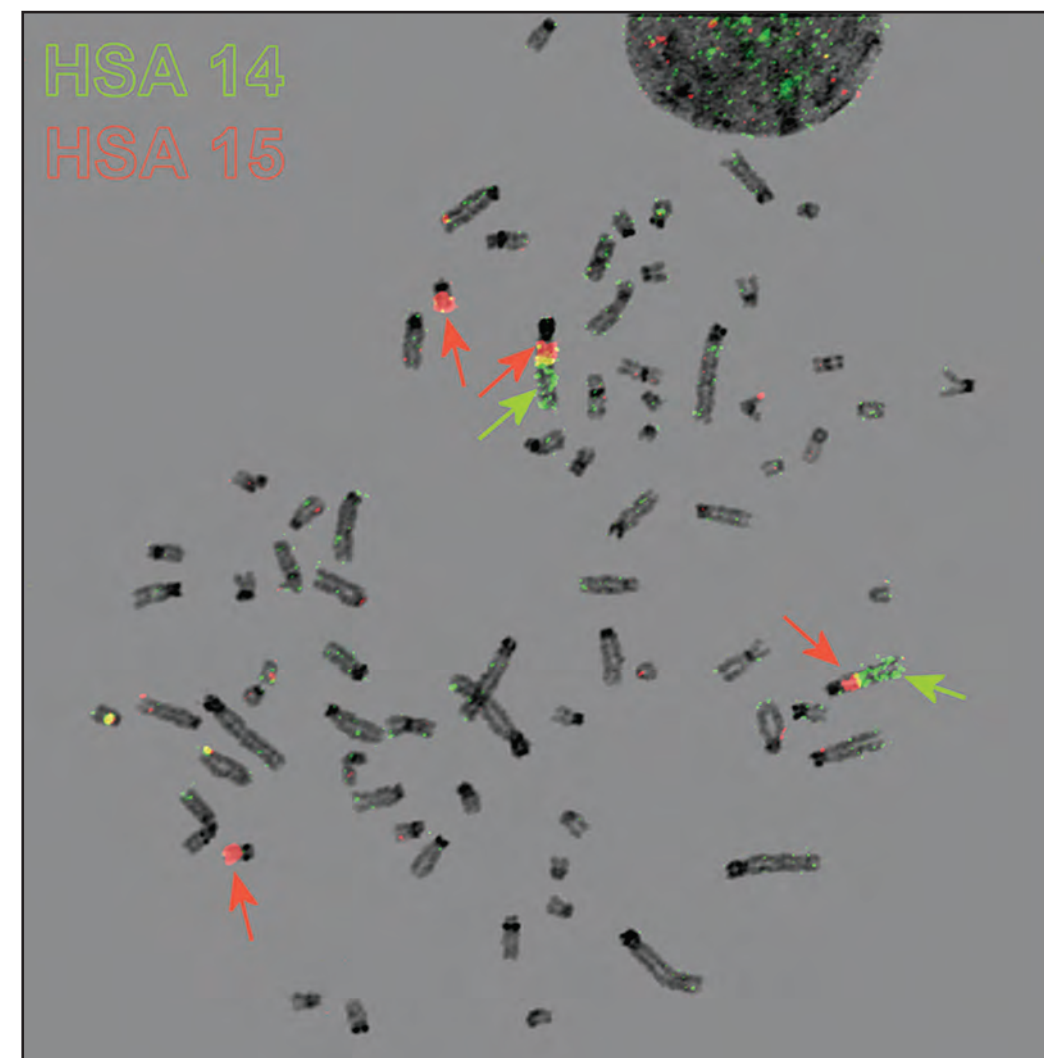
Although the initial focus of sequencing efforts was the human genome, the number of mammals for which there are whole genome sequences is growing at an increasing pace. These species are chosen for a variety of reasons, but generally selection has been based on their economic importance (e.g. cow, pig, horse, domestic dog), their use and potential as a bio-medical research model (e.g. mouse, rat, zebra fish, domestic cat), or for how their sequence will increase our understanding of human genome organization and function (e.g. baboon, macaque).

Last year, in large part because of the genomic resources that were already being developed for the alpaca, includ-

ing the radiation hybrid map, and with the support of researchers from the Laboratory of Genomic Diversity, the momentous decision was made by the National Human Genome Research Institute to nominate the alpaca for whole genome sequencing. Sequencing of the alpaca at the Genome Sequencing Center at the Washington University School of Medicine in St. Louis ([http://genome.wustl.edu/genome\\_group.cgi?GROUP=8](http://genome.wustl.edu/genome_group.cgi?GROUP=8)) is expected to be complete sometime around the end of 2007.

*The implications of the selection of the alpaca cannot be overestimated.* Once the sequence is assembled, integrated with the radiation hybrid map, and linked with other whole genome maps, it will be a tremendous catalyst for researchers from near and far a field. It will allow for the efficient identification of genes and mutations of functional importance to alpaca

**FIGURE 2.** Localization of two fluorescent painting probes of human chromosomes 14 (green) and 15 (red) onto alpaca metaphase chromosomes. The grouping of human chromosomes 14 and 15 is an ancestral association that is observed in most mammalian karyotypes and depicts how sections of the alpaca genome have similar patterns (homology) with other mammals, including humans.



breeders and researchers. The availability of comparative genomic information in closely related species (pig, horse, cow, dog, cat, human, mouse etc.) and a fuller description of patterns of similarity (homology) between the alpaca and other related mammals will also dramatically increase the utility of the alpaca map and will facilitate the transfer of discoveries from one species to another. There is already growing interest in the scientific community in the use of the alpaca as an animal model. By some estimates, of over eighty congenital defects identified in camelids, a third have similar inherited conditions in humans. This promises to lead to the further commitment of resources for alpaca research and to the broadening of the number of researchers embarking on camelid research projects worldwide. It also

bears emphasis that the vast majority of what is learned about the alpaca genome will be directly applicable to the llama, the two new world wild camelids, the guanaco and vicuña, and most likely, to the old-world Bactrian and dromedary camels, as well.

So what are the implications for alpacas and other camelids? Breeders of many animals, including horses and dogs, are already pioneering the use of genetic research to exert more control over the gene pool, using an increasing array of DNA tests being offered by companies to enhance health and determine the physical appearance of their animals. Candidate genes, or genes controlling the expression of many of the traits of interest to alpaca and llama owners, have already been identified in other species. Once tested in camelids, genetic tests will quickly

become available. Eventually more complex traits, such as those associated with behavior, will also be linked with genetic variation. By screening the DNA of their animals for desirable and undesirable traits that might appear in their offspring, breeders will be able to make more informed decisions about which animals to mate. This management will greatly enhance the economic value of these populations and will improve animal health and husbandry. As has been amply demonstrated with other domestic species, genetic management is an important step in herd improvement.

The alpaca is one of the most recent entries into the genomics club, due largely to the inspiration and financial support of members of the camelid community, through the Alpaca Research Foundation and Morris

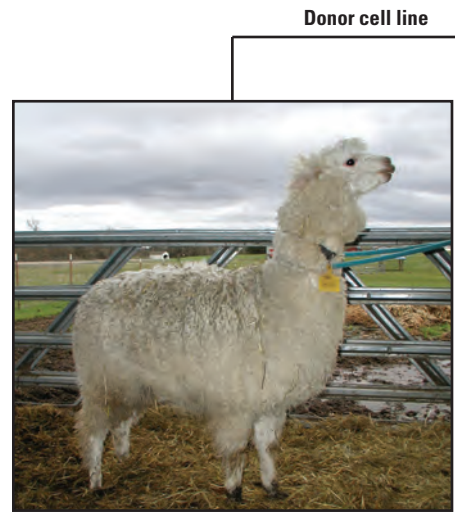


FIGURE 3. Limerick, a male huacaya white alpaca from the Oregon State University research herd, was the donor for the alpaca radiation hybrid map.

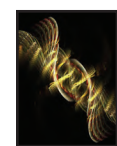
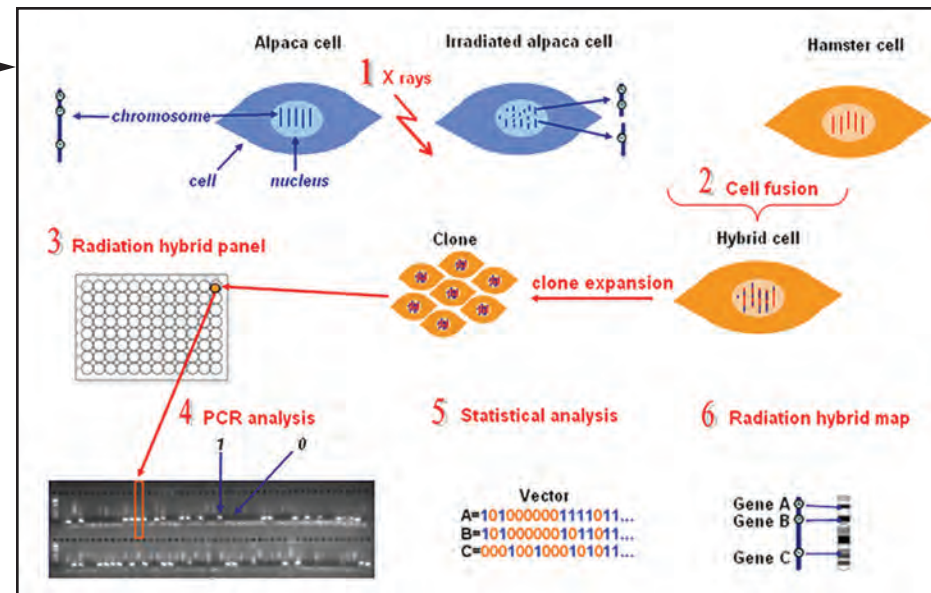


FIGURE 4. Construction of a Radiation Hybrid Map is based on the assumption that the closer the genes are on the chromosome, the more likely they are to be in the same fragment.

1. Radiation randomly breaks chromosomes into small fragments. Close genes will stay in one fragment with higher probability than genes that are far apart on the chromosome. Irradiated alpaca cells will not survive by themselves, that is why they are fused with non-irradiated hamster cells.
2. Fragments of alpaca chromosomes can survive inside of hamster cells. Some chromosome fragments will be retained by one hamster cell and some by another cell. This will create diversity among clones.
3. Radiation hybrid panel consist of 92 different hybrid clones and control DNA.
4. PCR analysis will show if the hybrid clone retained the particular gene. Bright band on the agarose gel shows the presence of the gene in the clone. Presence of the signal for a particular clone is scored as 1, absence as 0.
5. The vector is created for each gene and it contains information about presence or absence of the gene in all 92 clones.
6. Statistical difference between vectors is an indication of the distance between genes on the chromosome. Genes A and B have similar distribution of positive signals, so they are situated on the chromosome closely. The vector of marker C is quite different. It had a big distance between genes A and B.

Animal Foundation. The speed with which genomic techniques are translated into herd improvements and more active management will depend greatly on the continued interest and support of this community, both in terms of financial support, but also as measured by open access to information and biological samples from their animals.

#### Acknowledgements

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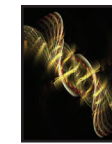
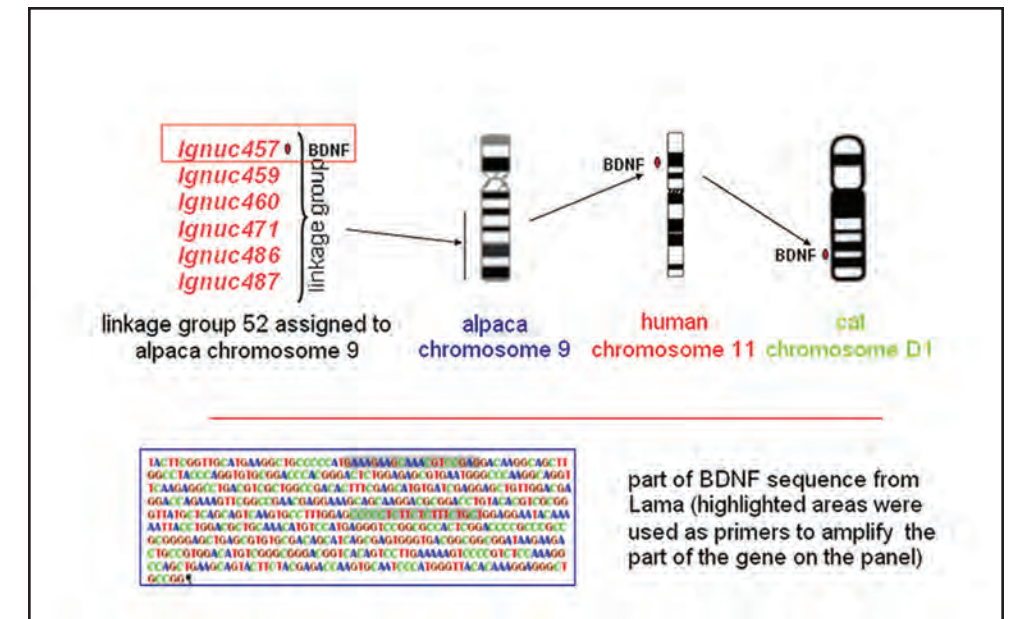


FIGURE 5. Genes with similar vectors are combined in linkage groups. Linkage groups are assigned to a particular chromosome based on the comparative sequence analysis and the chromosome painting data. For example, gene BDNF is a brain-derived neurotrophic factor that is essential for the survival of neurons in the brain. The link between changes in this gene and behavioral and neurodegenerative syndromes (like memory impairment, obsessive-compulsive disorder, and hypoventilation syndrome) was shown both in human and mouse. Maker Ignuc457 was designed from a Lama sequence of the BDNF gene. This gene is known to be on chromosome 11 in humans and on chromosome D1 in cats. Chromosome painting data show that alpaca chromosome 9 and human chromosome 11 have very similar DNA content. So we can safely assign whole linkage groups with BDNF to alpaca chromosome 9. This may be checked by direct localization of this gene on the alpaca chromosomes. The radiation hybrid map of alpaca allows one to determine not only the chromosome, but also precise location of the gene on this chromosome.



**By screening the DNA of their animals for desirable and undesirable traits that might appear in their offspring, breeders will be able to make more informed decisions about which animals to mate.**

## Definitions

**Genome:** An organism's complete genetic make up, its complete nucleotide sequence of DNA, which provides the instructions that are used to make an entire organism, and guide its growth and development.

**Genomics:** The study of the relationships between genetic features and biological function in organisms. Genomics is a broad discipline that has arisen as a new science that studies the whole genome by integrating traditional genetic disciplines such as molecular, population, and quantitative genetics with new technologies in molecular biology, DNA analysis, bioinformatics, and automated robotic systems.

**Genetic markers:** An observable variation that results from an alteration or mutation at a single genetic locus. Markers may be used as landmarks on a genomic map if it is passed from parent to offspring following standard rules of inheritance. Markers can be linked with genes that code for traits of interest or be located within non-coding regions in unique regions of a chromosome.

