

The Alpaca Whole Genome Cytogenetic Map

Felipe Avila¹, Pranab J. Das¹, Polina Perelman², Warren E. Johnson², Terje Raudsepp¹

¹Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, USA;

²Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD 21702 USA

Corresponding author: Terje Raudsepp - (979)862-2879; traudsepp@cvm.tamu.edu

Key Words: Alpaca; Mapping; Chromosomes; Fluorescence *in situ* hybridization

The Alpaca Genome Project constitutes a starting point for camelid genomics, and includes whole genome (WG) sequencing, radiation hybrid (RH) mapping and human-camel comparative chromosome painting (Zoo-FISH). However, there was still no common platform that aligned these various maps and precisely assigns them to individual chromosomes. Additionally, because of a high diploid chromosome number ($2n=74$) and similar morphology and banding patterns between different chromosome pairs, the cytogenetic identification of individual chromosomes in alpacas and other camelids is difficult. Thus, the objective of this research was to develop a whole-genome cytogenetic map for the alpaca, in order to physically anchor the genome sequence and RH maps, and to generate molecular markers for chromosome identification and cytogenetic analysis, especially for the study of chromosomal aberrations.

In order to accomplish that, large insert clones from the alpaca genomic BAC library (CHORI-246) were isolated and hybridized to chromosome spreads by fluorescence *in situ* hybridization (FISH). The BACs were selected based on the available Zoo-FISH and RH map data to target evolutionarily conserved genes, candidate genes for traits of interest, and also to obtain a uniform distribution of markers throughout the alpaca genome. The BAC clones were isolated from the library using radioactive overgo hybridizations. So far, 200 markers have been assigned to 35 alpaca autosomes and the X chromosome. Of these, 100 markers anchor the alpaca RH map. Refined maps have been generated for the Major Histocompatibility Complex (MHC) — a genomic region found in most vertebrates that plays an important role in immune system and autoimmunity and which is located on chromosome 20, and the pseudoautosomal region (PAR) — a region of high sequence homology and pairing between the X and the Y chromosomes during male meiosis. Detailed maps were also constructed for alpaca homologs of human chromosomes 4 and 8 — a region corresponding to an ancestral segment in the protoeutherian karyotype, and that harbors genes for important alpaca traits. Additionally, targeted mapping was conducted for candidate genes for various congenital and developmental/reproduction-related disorders frequently found in alpacas such as choanal atresia, as well as for phenotypic traits like fiber color and texture. Ordering of closely located genes was done by multicolor FISH in metaphase and interphase chromosomes.

The cytogenetic map of the alpaca genome, the first constructed for a camelid species, effectively integrates the genome sequence and RH map with cytogenetic data. This information facilitates the discovery of genes of biological and economic interest, as well as provides tools for clinical molecular cytogenetics. Additionally, the collection of physically mapped BAC clones is a unique resource for targeted re-sequencing — aimed at improving the current sequence assembly — and for mutation discovery.