

Llama and Alpaca Working Group - Comparison test workshop

Working Group Chair: Cecilia Penedo, VGL, UC Davis, USA

Approximate number of attendees: 30

Background information: The Llama and Alpaca Working Group first met during the ISAG 2008 Conference because of the growing number, particularly in Europe, of laboratories performing STR typing for identification and parentage verification of llamas and alpacas, and the clear need for genotyping standardization among laboratories. The group's goal was to identify an STR panel and to standardize nomenclature of alleles to be achieved by means of comparison tests. The initial comparison test was expedited for results to be available early in 2009. Participants were asked to submit lists of markers for inclusion in the test, with four laboratories contributing material. Dr. Ilona Gunsser from Llamas and Alpacas Registries Europe (LAREU) from Germany volunteered to provide blood samples from llamas and alpacas and the Veterinary Genetics Laboratory, University of California, Davis, volunteered to organize the test and compile results.

DNA samples were shipped in November-December, 2008 with results due on March 31, 2009. The test comprised 40 samples (22 alpacas and 18 llamas). Primer sequences for 36 STRs and two reference genotypes for each marker were provided. In addition to reporting genotypes, laboratories were asked to answer two parentage questions and to identify X-linked marker(s) included in the test. Genotyping for a common set of 14 markers was encouraged. Samples were shipped to 18 laboratories, with 16 laboratories reporting results. Results were compiled and returned to participants in June 2009 via email.

ISAG 2010 Workshop: Formal discussion of the 2008-2009 comparison test was held in conjunction with the Cattle Molecular Markers and Parentage Testing Workshop of ISAG's 2010 conference in Edinburgh. Representatives from LAREU (Drs. Ilona Gunsser and Christian Kiesling) attended the llama/alpaca meeting.

Results were presented by Cecilia Penedo. Markers reported ranged from 8 to 33. Results were evaluated on the basis of genotyping concordance among laboratories. Overall, the error rate (genotyping discrepancy) among laboratories was low: 10 (63%) had an error rate of 5% or less with 3 (19%) showing no disagreement. The majority of discrepancies were explained by differences in allele sizing. Relative to other species' comparison tests, and taking into account that this was the first camelid comparison test, the overall results were very good.

One of the outcomes of the workshop was the identification of a “**minimum 14-marker panel**” for llamas and alpacas. These markers were chosen based on polymorphism, ease of genotyping and common usage among laboratories for identification and parentage testing. Details of the panel are provided below. Participating laboratories, especially those from Europe where most record exchange occurs, were encouraged to begin using the standardized nomenclature immediately. Another topic of discussion raised by LAREU pertained to parentage analyses performed by registries and to management of discrepant results when genotype records of parents and offspring were produced by different laboratories. The consensus and recommendation of the workshop were that a) parentage comparisons should be performed by genotyping laboratories, b) the laboratory testing the offspring should contact those that provided the parental records for review and resolution of genotypes involved in apparent incompatibilities, c) as needed, DNA samples could be exchanged among laboratories for additional testing to resolve questionable cases

Participants requested that another comparison test be held in 2011-2012. Eberhard Manz (Germany) volunteered Generatio to be Duty Lab. Cecilia Penedo volunteered the VGL to be Computer Lab. The participants also proposed that, given the interest for continued exchange of information and comparison tests, the group be organized as a formal Standing Committee of the Society with members Cecilia Penedo (Chair), Eberhard Manz (Germany) and Deanne Waine (Australia). This proposal will be presented to ISAG’s Executive Committee.

ISAG RECOMMENDED MINIMUM PANEL - Llamas and Alpacas			
Locus	Forward	Reverse	Size Range
LCA5	GTGGTTTTTGCCCAAGCTC	ACCTCCAGTCTGGGGATTTC	178-218
LCA8	GCTGAACCACAATGCAAAGA	AATGCAGATGTGCCTCAGTT	211-261
LCA19	TAAGTCCAGCCCCACACTCA	GGTGAAGGGGCTTGATCTTC	80-122
LCA24	ACTCACGGGTGACATACAGTG	GAGCAGTGTTTGGTTTGATT	104-134
LCA37	AAACCTAATTACCTCCCCCA	CCATGTAGTTGCAGGACACG	124-174
LCA65	TTTTTCCCCTGTGGTTGAAT	AACTCAGCTGTTGTCAGGGG	159-193
LCA66	GTGCAGCGTCCAAATAGTCA	CCAGCATCGTCCAGTATTCA	216-266
LCA94	GTCCATTCATCCAGCACAGG	ACATTTGGCAATCTCTGGAGAA	187-213
LCA99	CAGGTATCAGGAGACGGGCT	AGCATTTATCAAGGAACACCAGC	263-297
YWLL29	GAAGGCAGGAGAAAAGGTAG	CAGAGGCTTAATAACTTGACAG	210-232
YWLL40	CACATGACCATGTCCCCTTAT	CCAGTGACAGTGTGACTAAGA	176-190
YWLL44	CTCAACAATGCTAGACCTTGG	GAGAACACAGGCTGGTGAATA	84-136
LCA56	ATGGTGTTTACAGGGCGTTG	GCATTAAGTAAAAGCCCAGG	133-171
LGU49	TCTAGGTCCATCCCTGTTGC	GTGCTGGAATAGTGCCAGT	219-249