ISAG Conference 2008, Amsterdam, The Netherlands

Cattle Molecular Markers and Parentage Testing Workshop

Chair: ***Leanne van de Goor***, VHL Laboratories, The Netherlands

Around 100 people attended the workshop (92 registered participants).

Report on any business conducted:

The Cattle Molecular Markers and Parentage Testing (CMMPT) workshop was held on July 21st 2008, and focused on discussion of DNA typing results for the 2007-2008 Comparison Test (CT), the selection of additional ISAG recommended markers and use of SNPs for Parentage Testing or Identification.

Comparison Test (CT)

The Duty lab for the DNA Typing CT was Dr. Maria Marcela Cerruti (Argentina).

DNA was extracted from twenty blood samples (originating from Holstein, Blonde D'Aquitaine, Brangus, Black Red Simmental, Charolais, Braford, Angus and several crossbreds). A reference DNA sample with genotypes for 28 STR markers was provided. The 20 animals selected included animals with variation in their genotypes for CVM and red factor. The extracted DNA was send to 79 labs in aliquots of approximately 25 ng/µl. The Duty lab received many Consignment Forms that were incomplete or incorrectly filled out (courier or membership data absent or not updated). Many Health and Import Permits were not sent with the forms and had to be asked for before shipment. Those issues were discussed during the workshop and participants for the next CT were asked to make sure that they provide correct and complete information to the Duty lab. This is the responsibility of the participating lab and not of the Duty lab. A consequence of incomplete or incorrect information can be that the participating lab will not receive the samples. Furthermore many requests for technical information about PCR conditions, primer sequences, STR-allele nomenclature, etc. were received by the Duty lab. Dr. Marcela Martinez (Argentina) commented about adding this information to the ISAG website. Some labs reported problems with Customs to import samples (for instance Brazilian, Italian, Canadian Labs). DNA amplification problems were reported by 8 labs that could be attributed to non-standardized PCR conditions or DNA in bad condition due to extended permanence in customs. New sets of samples were sent in all cases and because requests for new samples were received very late, the results-reporting deadline was extended. For the next CT, participating labs were asked to test the samples as soon as possible after arrival. A suggestion was made for the next CT to set a new sample request deadline, to facilitate work by the Duty lab and to avoid extending the reporting deadline. The Duty lab explained genotyping issues in three markers: null-alleles in INRA023 (sample 2 and 17) and TGLA122 (sample 14 and 20) depending on the primer-sequences and/or PCR-conditions used, and two alleles differing by a single base in ETH225 (sample 18, heterozygous based on pedigree information).

The computer work (Compilation of data and comments, calculating lab/marker concordance) was performed by Dr. Luis Cancela (Uruguay).

Results were received from 72 participating labs of which 22 were identified as first time participating labs and in total 37 countries were represented. Because not all labs reported the numerical ISAG lab code (= Blackwell lab Customer ID), identification sometimes was difficult. Genotypes were reported for

a total of 81 STRs, ranging from 1 to 72 labs per marker. The 9 STRs in the ISAG recommended panel (BM1824, INRA23, BM2113, SPS115, ETH10, TGLA122, ETH225, TGLA126 and TGLA227) were reported by 72 labs. Among the remaining markers, three markers were reported by far more labs than the other microsatellites markers, BM1818 (26), ETH003 (57), TGLA053 (51). Results for diagnostic markers for 13 traits (e.g. CVM and BLAD) were reported, ranging from 1 to 7 labs per marker.

The overall concordance among labs for the nine ISAG recommended markers was good (>93%). To resolve nomenclature for one ETH225 allele (160 vs 158) for which there were more disagreements, Dr. Cecilia Penedo volunteered to provide sequencing information. After the sequence information is available the correct call for this allele can be determined.

Both Parentage questions included a possible null-allele in either marker INRA023 or marker TGLA122 depending on the primer-sequences and/or PCR-conditions used. In case those null-alleles were not amplified by a laboratory both parentage cases revealed a one-marker mismatch, which is not sufficient to exclude a parentage. Nevertheless several labs reported that the parentage could be excluded (question 1: 27 labs, question 2: 17 labs). This issue was discussed during the workshop. Since results for diagnostic trait assays were reported by only 7 laboratories the workshop agreed that the Duty lab for the next CT does not have to select samples with variation in the genotypes for Non-microsatellite markers. For the 2007-2008 CT, Dr. Tara McParland (Canada) volunteered to serve as Duty Lab. Dr. Luis Cancela (Uruguay) volunteered to serve again as the Computer Lab.

Announcements Executive committee

Dr. Cecilia Penedo made on behalf of the Executive committee from ISAG several announcements.

1. The guidelines for the CTs will be reconsidered, Standing Committees are asked to come up with suggestions for new guidelines for conduct of CTs, protocol for Duty labs and standardized report format for all species.

2. Implementing a rating of the results from the CTs, the ISAG Executive Committee invites all Standing Committees to come up with proposals and/or suggestions of what such a rating should be. The ISAG Executive Committee will take all feedback into account and make a final decision on a uniform rating system.

3. Developing CTs using SNPs. The ISAG Executive Committee calls for volunteers to participate in the development of SNP panels suitable for parentage verification (based on already existing panels, suggestion as to the number, type of SNPs …). Once a collection of SNP markers is identified, a CT will be organized. Labs interested in participating in this SNP CT can express their interest to the chair Leanne van de Goor (lgo@vhladmin.nl)

Selection of additional ISAG recommended markers

By voting, a majority of attendees agreed to add three markers (BM1818, ETH3 and TGLA53) to the ISAG recommended panel thus increasing the minimum number of markers to 12. The resulting increase in the Power of Exclusion of the new panel will benefit labs using exchanged records for parentage verification .

Use of SNPs for Parentage Testing or Identification

Brent Woodward from Merial Limited (USA) gave a presentation about SNPs for Parentage Testing, Individual Identification, and Traceability. Steps will be taken to set up a CT using SNPs (See Announcements Executive committee).

ICAR accreditation

At the moment 14 labs are accredited by ICAR (International Committee for Animal Recording) for parenthood recording. More information about applying for accreditation is available at www.icar.org/pages/working_groups/wg_genetic_analyis.htm.

Parentage verification in Water Buffalo

Dr. Daniela Iamartino (Italy) gave a presentation about STR typing in Water Buffalo. If enough labs are interested, Dr. Iamartino is willing to be the Duty Lab for a CT in Water Buffalo.

Election of standing committee

Three members of the Standing Committee stepped down: Dianne Vankan, Luis Cancela and John Flynn. Three new members were elected during the workshop: Romy Morrin-O'Donnell, Marcela Martinez and Marie-Yvonne Boscher.

The new committee *is composed of:* Leanne van de Goor (Chair), Elena Genzini, Cecilia Penedo, Romy Morrin-O'Donnell, Marcela Martinez and Marie-Yvonne Boscher. Tara McParland joins the committee as Duty lab and Luis Cancela as Computer Lab.