ISAG CONFERENCE 2012, Cairns, Australia

Cattle Molecular Markers and Parentage Testing Workshop

STANDING COMMITTEES / WORKSHOPS Information will be posted online

Organised by a standing committee yes

Date and meeting time: 19th July 2012, 9:00 12:30

Chair : Romy Morrin O'Donnell (rmorrin@weatherbys.ie), Marie-Yvonne Boscher (marie-yvonne.boscher@jouy.inra.fr)

Agenda / programme attached
Comparison tests
Cattle STR comparison Test 2011-2012
Presentation by Duty Lab, evaluation results by Computer Lab STR
Cattle SNP comparison Tests (two) 2011-2012
Presentation and evaluation by Computer Lab SNP
Next Comparison Tests 2013, 2014
Selection of new Duty Labs and new Computer Labs
STR/SNP imputation panel
Presentation by Tad Sonstegard, (USDA) – invited speaker
Discussion on SNPs
Development of SNP panel suitable for parentage verification, recommended set of markers and strategies. Definition of a Minimum core set SNP? Additional panels or SNPs?SNP for STR imputation?
Different type of "recognition"
Role of ISAG (definition of panels, nomenclature, comparison test)
Possible expansion of comparison tests, nomenclature... to SNP chips used for selection in cattle?
Role of ICAR: Update about ICAR accreditation for SNP typing
Business
Election of Committee
Any other business
Number of participants at meeting: 83 people and 24 Institutional members (the only ones that are invited to vote)

Summary of the meeting including votes, decisions taken and plans for future conferences

1. ***STR/SNP Cattle STR comparison Test 2011-2012***

STR Duty laboratory: Weatherbys, Ireland

Samples consisted of 20 cattle DNA extracts at 30-100ng/µl and 50µl/sample. Sample 11 was the reference sample with genotypes provided for 12 ISAG STRs and 100 SNPs. DNA was extracted from 10 blood samples & 10 Nasal Swabs (DNA Genotek).

Total lab applications were 75 from 31 countries: 40 labs applied for STRs only, 34 labs for STR and SNP and 1 lab for SNP only. Most samples were shipped September 19 and 20 2011 to accommodate the 2nd ICAR/ISAG SNP comparison test. Deadlines for reports were November 1 2011 for SNP and March 31 2012 for STRs.

There were 5 late applications, 2 returned shipments due to inadequate documentation at point of entry, 2nd set shipped, 1 request for 2nd shipment due to reagent problem at receiving lab, 1 lab requested 2nd shipment of sample 20 only,1 lab requested 2nd shipment of samples 6, 10 and 20. Inadequate documentation was a problem for Brazil in particular. Receiving labs ensured they sent the correct courier information and active account number. Still, the Duty Lab received some invoices from couriers.

Sample 13 was a blood from a twin, sample 20 has probably contaminated by sample 19. Nevertheless, results were consistently good on those samples.

STR Computing Laboratory: IDENTITAS, Uruguay

Of 75 applications, 70 laboratories submitted results. The Computing Lab sent out Final Compilation to all reporting labs. Thirty countries were represented ranging from 1 to 8 labs each. Sixty-three labs reported all 12 markers in the ISAG panel, 6 reported 11 markers and 1 lab reported 10 markers. Additional markers reported included MGTG4B (22 labs), CSRM60 and SPS113 (21 labs), ILSTS6 (18 labs) and CSSM66 (17 labs).

STR CCT 2011-2012 Conclusions:

Almost all labs reported ISAG panel microsatellites

Genotype concordance was overall high with 10 markers ranking 98-100% and 2 markers showing more disagreement, with INRA23 (97%) and TGLA53 (91%) concordance rates. A summary of the absolute and relative performance evaluation is shown below. The average absolute accuracy for established labs was 97.4% and for new laboratories it was 93.5%.

There was a good agreement for non-ISAG frequently used microsatellites

Absolute Genotyping Accuracy Total # labs: 70		**Relative Genotyping Accuracy** Total # labs: 70	
Rate	**% Labs**	**Rate**	**% Labs**
1: 100 – 98%	67	**1:** 100 – 98%	74
2: 98 – 95%	13	**2:** 98 – 95%	16
3: 95 – 90%	10	**3:** 95 – 90%	6
4: 90 – 80%	7	**4:** 90 – 80%	1
5: 80%	3	**5:** 80%	3

Low interest in non-microsatellites markers, very few laboratories reported results for genetic diagnostic tests: Sex determination (4), hereditary disorders (CITRULLINAEMIA, DUMPS, Mulefoot, FACTOR XI deficiency, BLAD, CVM and BRACHYSPINA) (3), Kappa-casein (4) and Red Factor (4), Beta-lactoglobulin (1), Beta-casein (1), DGAT1 (1).

Consensus in parentage inclusion and exclusion criteria

2. Cattle SNP comparison Tests (two) 2011-2012

Summary

Duty Laboratories: Maxxam Canada (1st), Weatherbys IRL (2nd)
Computing Lab: VGL-UC Davis, USA

In the 1st test, samples consisted of 3 references and 17 unknown.
In the 2nd test, samples were 1 reference and 19 unknown.

A total of 24 labs participated in both tests with 1st/2nd numbers being 21/17 (3 new), 18/15 labs reporting more than 90 markers, 1 lab reporting 74 markers and 2 labs reporting 20 and 27 markers each.

Genotyping platforms represented were: Primer extension/capil. electrophoresis (1 lab), Sequenom iPlex (5), Kbioscience KASPar (1), Illumina 50K V2 (11), Illumina 3K Golden Gate (5), sequencing (1), Fluidigm (2), ABI Snap-Shot (1), Open Array (1). A summary of markers not reported for the 2nd SNP test is shown below.

SNP CCT 2011-2012 Conclusions:

Genotyping concordance was overall high. For example, in the 2nd comparison test 63% of markers showed 100% concordance, 34% between 98-99.65% and 2% between 94-97.7%. Differences in marker performance between the two tests were small, with 57 markers having slightly increased consensus calls in the test (gain 0.2 – 4%), 14 markers with same rate of 100% in both tests and 29 markers with slightly lower consensus calls in 2nd test (loss 0.06 – 2.6%). Overall absolute and relative accuracy ratings are shown below.

In the 2nd comparison test, poor clustering pattern of DQ786766-rs29012070 was reported by 2 laboratories and 1 of these provided sequence information to show a motif substitution polymorphism near the target SNP site. The polymorphism affects assays for the Sequenom platform but the problem can be corrected by redesigning.

Overall, accuracy and concordance of SNP results were good. Use of different platforms had little impact on the results.

Absolute Genotyping Accuracy		**Relative Genotyping Accuracy**	
Rate	**%Labs**	**Rate**	**% Labs**
1: 100 – 98%	68	**1:** 100 – 98%	100
2: 98 – 95%	27	**2:** 98 – 95%	0
3: 95 – 90%	0	**3:** 95 – 90%	0
4: 90 – 80%	0	**4:** 90 – 80%	0
5: 80%	5	**5:** 80%	0

3. SNP Core Panel

Since lab performance showed Absolute Genotyping Accuracy superior to 99% in 2011-2012 SNP ISAG CT on the SNP panel tested, it is proposed to **define these 100 SNPs as the core panel**. The list of markers with all details is posted on the ISAG website.

4. Additional SNP panels

Lucie Genestout from Labogena (France) presented a pipeline designed to evaluate the minimal panel needed to provide the most accurate parentage. Two hundred SNPs, including the 100 used in the SNP comparison test, were checked on 20 French cattle breeds. The 100 extra SNPs were selected on LD/HD/50K Illumina bead chips and met the criteria of MAF mean of 0.4 and a minimum distance of 10cM on all Chr. The study used data from 4000 animals, with 20 to 675 animals per breed, analyzed on HD Illumina bead chip. The PE1 value of the panel reached 0.9999999 with 175 markers for all breeds. In order to define the minimum number of SNPs required for parentage, an extreme situation was modelled with 500 offspring, 200 of 500 total sires removed and dams not included.

Nb SNP	**200**	**175**	**150**	**125**	**100**	**75**	**50**
% Parentage assignment	100	100	99	99	99	99	91
% Correct parentage assigned	100	100	93	78	57	32	13

The number of mismatches tolerated for parentage with 1 parent or 2 parents have been checked by simulating thousands of correct parentages. For 100 SNP, below 2-3 mismatches, there is no risk to accept a parentage (1parent), 3-4 mismatches for two parents.

The results showed that the power of a well chosen panel of about 100 SNP with a mean MAF of 0.3 is not affected by an error rate of 0.01 (this error rate needs to be taken in account depending on the technologies that are used).

Those results showed that the greater the number of SNPs, the lesser the impact of errors is.

The 100 additional markers which performed well on *B. Taurus* breeds need to be validated on *B. Indicus* and crosses. If these SNPs are found not to be appropriate, a *B. Indicus* panel of 100 SNPs will be selected with assistance from Tad Sonstegard from the USDA.

The list of the 100 additional SNPs (back up panel), including sex marker will be made available on the ISAG website.

5. Definition of guidelines for parentage verification based on SNP markers

The guidelines proposed are based on the results of the study performed by Labogena in 20 breeds. Results showed that with 75 SNPs, the PE1 is for almost all breeds > 0.99 and PE2 is for all 20 breeds > 0.999999.With 100 SNPs, PE1 is superior to 0.999 in all 20 breeds. The PE1 reaches 0.9999999 with 175 markers for all breeds. In this study simulated pedigrees were designed with 1% genotyping errors in the SNP profiles, in order to determine the number of mismatches that should be tolerated to qualify a supposed parentage. The CMMPT Committee is currently composing a suggested backup panel of 100 SNP markers.

GUIDELINES FOR PARENTAGE VERIFICATION BASED ON SNP MARKERS are,
SNP profile:
Minimum number of SNPs in panel: 100
Minimum number of SNPs available in profile: 95
(If less than 95 SNPs can be scored, retest the sample or request a new sample).

If mismatches occur in a supposed parentage, the general rule first is to retest the samples involved or request new samples to confirm the determined genotypes. If the genotypes are confirmed the following guidelines are suggested.

Case with offspring and one parent tested
Minimum number of corresponding SNPs in verification offspring: 90
Number of mismatches*: 0-1 -> parentage accepted
Number of mismatches*: 2-3 -> parentage doubtful, backup panel required**
Number of mismatches*: >3 -> parentage excluded.
*: example: offspring = GG, sire = AA
**: When the parentage is doubtful, first genotype the samples on the ISAG panel again and the backup panel then, if results confirm as doubtful, ask customer for the other given parent and for another candidate, if there are no other then qualify the parentage.

Case with offspring and both parents tested
Minimum number of corresponding SNPs in verification offspring: 85
Number of mismatches*: 0-2 -> parentage accepted
Number of mismatches*: 3-4 -> parentage doubtful, backup panel required**
Number of mismatches*: >4 -> parentage excluded.
*: example: offspring = AG, sire = AA, dam = AA
**: When the parentage is doubtful, first genotype the samples with both panels (ISAG panel and the backup) and then, if results remain doubtful, ask customer for other possible parents, if there are no other then qualify the parents.

In any case, it is recommended that samples be retested if there is a parentage exclusion with ISAG panel and/or back up panel.
These guidelines were approved by voting (24 for)

6. ISAG certificates of participation

The ISAG certificate of STR cattle comparison test participation will be changed to display only the absolute genotype accuracy. This will be effective for 2013-2014 comparison test. This motion was ***approved by voting (20 in favour, 4 against)***

A certificate of participation for 2011-2012 for both STR and SNP with Aga and Rga will be provided by ISAG.

It is reminded that this certificate is the only document concerning comparison tests that can be provided to third parties (e.g., customers). Data concerning other laboratories are confidential and cannot in any case be provided to other parties that have not participated in the comparison test. It is specially the case for the reports, comments and evaluations provided by computing lab.

7. SNP for STR conversion

USDA's work was presented by Tad Sonstegard: *Imputation of Microsatellite Alleles from Dense SNP Genotypes for Parentage Verification*. A paper by McClure *et al*. is available

from Frontiers in LivestockGenomics (Front. Gene. 3:140), it details those results. A call for data on more breeds was made during the workshop (deadline 1st August 2012) to allow an update of this panel on more beef breeds and *Bos.indicus* to be available by the beginning of next year.

This panel will be helpful for the switch from STR to SNP without the need of retesting parents. The challenge is to obtain the minimum panel to save money and be competitive compared with the SNP typing of parents.

8. Update about ICAR approval for SNP typing (Wim Van Haeringen)

ICAR promotes the development and improvement of performance recording and evaluation of farm livestock. ICAR establishes rules and standards and specific guidelines for animal identification and parentage recording. The current Work Group on DNA Analysis, chaired by Wim van Haeringen, is updating ICAR guidelines for parentage verification by STR and SNP testing and laboratory accreditation. ICAR has been working with ISAG to organize comparison tests for SNPs and takes in account the results obtained on the comparison tests for lab accreditation. The ICAR Work Group on DNA Analysis considers lab accreditation for commercial chip tests (e.g. LD, 50K). It also considers standardisation in; Reporting and Nomenclature, Together with other Working Groups of ICAR (Interbull, Parentage recording), Guidelines on SNP Parentage checks procedures, together with ISAG. Interested laboratories are encouraged to consult ICAR's website (http://www.icar.org/) for current information and application.

9. Extend the cattle SNP comparison test to chip micro arrays used for genomic evaluation

Genetic evaluation enhanced with molecular information requires high quality SNP data, obtained with standardized procedures and normalized nomenclature.

A system of laboratory recognition could be defined, using procedures similar to those implemented for parentage verifications. ISAG can organize CT and deliver certificate, ICAR can approve laboratories, a laboratory approved by ICAR could send its genotypes worldwide for genomic evaluation.

A proposal to allow use of commercial genomic evaluation SNP chips in the next cattle comparison test was made at the workshop.

It was approved that the next comparison test will allow use of SNP microarrays and that a subset of test samples will be selected specifically for this purpose.

10. Format Report to exchange SNP data

A file format will be provided with all details (on ISAG WEB site). The file will include lab data (name, country, Institutional member ID), animal information (international ID number, breed, sex; birth date), sample data (analysis date, sample tissue, technology used, list of markers) and results (format provided).

11. Next SNP comparison test 2012-2013

The ISAG Executive Committee (ISAG EC) has agreed to support the reimbursement of an additional comparison test to be held in 2012-2013.This test will give opportunity to labs to improve their experience and, for those who want to begin with SNP testing, to acquire some experience using the core or alternative panels (parentage testing and STR/SNP conversion panel, commercial chips for genomic selection). The consignment form will ask for the different options chosen.

The Duty will be SRA (Argentina), contact person: Marcela Martinez
TheComputer Labs will be for SNPs: VGL (USA), contact person Cecilia Penedo and for STRs: Identitas (Uruguay), contact person Luis Cancela

Schedule will be as follows: Consignment form by November 2012. Samples shipped in March 2013, results in May 2013 and the report in July 2013. It will be asked that the ISAG EC provide a certificate of participation for this test.

12. STR/SNP comparison test 2013-2014

A combined test for STR and SNP will be organised in 2013-2014.The consignment form for comparison tests will be available on ISAG web site.

The Duty will be Lab GenoSkan A/S (Denmark), contact person Rikke Vingborg.
The SNP-Computer Lab will be VGL (USA), contact person Cecilia Penedo and the STR-Computer Lab will be Identitas (Uruguay), contact person Luis Cancela.

13. Other Business

Labs were asked to contact the chair of the CMMPT committee if they haven't yet received their certificate of participation for the 2009-2010.

The workshop concluded with a reminder that, until the next ISAG conference, either STR or SNP can be used for parentage testing and that results for either can be exchanged.

Committee members

Leanne Van De Goor (NL) chair, re-elected in 2010 for a 2nd term (lgo@vhladmin.nl).
Cecilia Penedo (USA) re-elected in 2010 for a 2nd term.
Daniela Imartino (IT) was elected in 2010, so remains for next 2 years.
Romy Morrin O'Donnell (IR), Marcela Martinez (AR) & Marie-Yvonne Boscher (FR) all elected in 2008, are elected for a 2nd term.
Emily Piper from the University of Queensland (AU) is elected.
Computer lab'srepresentative, Luis Cancela (UR).
Duty lab's representative: Rikke Vingborg (DK)

SIGNATURES

Chairs **Duty laboratory** **Computing laboratories**