



**ISAG**  
**CONFERENCE 2014, Xi'an, China**

**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:** 28<sup>th</sup> July 2014, 2:00-5:00 pm

**Chair, name and contact email:** Leanne van de Goor (lgo@vhladmin.nl)

**Agenda / programme attached**

- Welcome
- STR/SNP Cattle Comparison Tests 2013-2014
  - Report Duty Lab 1st SNP CT, Marcela Martinez, Laboratory of Genética Aplicada, Argentina
  - Report Duty Lab STR + 2nd SNP CT, Rikke Vingborg, GenoScan, Denmark
  - Report Computer Lab STR CT, Luis Cancela, Identitas, Uruguay
  - Report Computer Lab 1st + 2nd SNP CT, Cecilia Penedo, UC-Davis, USA
- Next Comparison Tests 2015/2016 and 2017
- Additional ISAG SNP panel
- Imputation of STR profiles – Romy Morrin, Weatherbys, Ireland
- Genomics and Animal Recording, the road Ahead for Cattle – Brian Wickham, ICAR
- OMIA website
- Election of Committee & any other business
- Close

**Number of participants at meeting:** 109

**Summary of the meeting**

**1. STR/SNP Cattle Comparison Tests 2013-2014**

**Duty Lab 1<sup>st</sup> SNP ISAG/ICAR CT, Marcela Martinez, Laboratory of Genética Aplicada, Argentina**

Samples consisted of 12 cattle DNA extracts of 50ng/μl originating from blood of 6 breeds (B. taurus and indicus). 11 unknown samples and 1 reference sample. Genotypes of ISAG Core + Additional panel and 50K Illumina array were supplied for reference sample. This was the 1<sup>st</sup> time for participants to send the Consignment Forms by the ISAG Secretary. Total lab applications were 24. Samples were shipped March 4<sup>th</sup> 2013. Deadline for report was May 31<sup>st</sup> 2013. This is the 1<sup>st</sup> CT that requested to report the ISAG Additional Panel, 7K and 50K Illumina arrays, in addition to the Core Panel (only the core Panel will be ranked). Genotype reads from the Forward strand were required, following the reference sample. No Problems with

Consignment Forms, Shipment, Customs. No request for a second batch of samples were received.

**Duty Lab STR CT + 2<sup>nd</sup> SNP ISAG/ICAR CT, Rikke Vingborg, GenoScan, Denmark**

Samples consisted of 20 cattle DNA extracts of 50ng/µl and 20 µl per sample originating from nasal swabs or semen of 7 B. taurus breeds. 19 unknown samples and 1 reference sample. Genotypes of ISAG Core + Additional panel and Illumina Bovine LD Beadchip were supplied for reference sample. Total lab applications were 94 (four after the deadline), 11 labs ordered twice and one lab three times. 55 labs applied for STRs only, 27 labs for STR and SNP and 6 labs for SNP only. Samples were shipped in October 2013 and because of several reports that there was not enough DNA in some sample, all 20 samples were shipped again in December 2013. Deadline for report was May 31<sup>st</sup> 2014. Two sets of samples were returned and the Duty lab was not able to get in contact with 3 labs. It is proposed to use only one courier to ship the samples to avoid all kinds of problems with the shipment of the samples.

**Computer Lab STR CT, Luis Cancela, Identitas, Uruguay**

Of 86 applications, 82 laboratories submitted results. The Computing Lab sent out Final Compilation to all reporting labs. Seventy-one labs reported all 12 markers in the ISAG panel, 9 labs reported 11 markers, 1 lab reported 10 markers and 1 lab reported 9 markers. Genotype concordance among the established labs was overall high with 9 markers ranking 98-100% and 3 markers showing lower agreement: ETH225 (97%), ETH3 (96%) and SPS115 (95%). The lower concordance of marker SPS115 was caused by sample 17, the correct genotype is 248/261, however many labs reported 248/260. 25-30% of the labs reported an additional panel of 6 markers (MGTG4B, CSRM60, SPS113, ILSTS006, RM067 and CSSM66). There was a good agreement for the non-ISAG frequently used STRs. A summary of the absolute and relative performance evaluation is shown below.

<b>Absolute genotyping Accuracy</b>		<b>Relative genotyping Accuracy</b>	
Total # labs: 82		Total # labs: 82	
<b>Rate</b>	<b>% Labs</b>	<b>Rate</b>	<b>% Labs</b>
<b>1: 100 – 98%</b>	67	<b>1: 100 – 98%</b>	76
<b>2: 98 – 95%</b>	13	<b>2: 98 – 95%</b>	15
<b>3: 95 – 90%</b>	6	<b>3: 95 – 90%</b>	1
<b>4: 90 – 80%</b>	6	<b>4: 90 – 80%</b>	2
<b>5: 80%</b>	7	<b>5: 80%</b>	6

There was good agreement for parentage verification questions. Interest in genetic diagnostic markers is low, with very few laboratories reporting results: 7 labs reported Sex determination and only 1-3 labs reported other genetic variants (Citruilinaemia, DUMPS, Mulefoot, Factor XIdiciency, BLAD, CVM, Kappa Casein, Red Factor, Beta-lactoglobulin, Beta-casein and DGAT1).

**Computer Lab 1st + 2nd SNP CT, Cecilia Penedo, UC-Davis, USA**

In the 1<sup>st</sup> test, samples consisted of 1 reference and 11 unknown. In the 2<sup>nd</sup> test, samples consisted of 1 reference and 19 unknown. A total of 12 labs participated in both tests with 1<sup>st</sup>/2<sup>nd</sup> numbers being 18/19.

Genotyping platforms represented were: KASPar, Illumina, Fluidigm, Sequenom, USDASNP96. Open Array and NGS-based platform. Several labs reported results in wrong orientation which was corrected during compilation. The overall Absolute Genotyping Accuracy (aga) of both CTs combined for the core Panel is 95.5% and for the Additional Panel 99.9%. The additional Panel was only reported by labs using the Illumina platform and one lab using the Fluidigm platform. For the core Panel the average aga among the labs using the Illumina platform was also above 99.9%. Thirteen markers in the core Panel show an average consensus < 99%:

Marker Name	1 <sup>st</sup>	2 <sup>nd</sup>	Average
ARS-USMARC-Parent-DQ786764-no-rs	85.64	99.72	92.68
ARS-USMARC-Parent-DQ786766-rs29012070	96.08	90.22	93.15
ARS-USMARC-Parent-AY761135-rs29003723	92.59	97.22	94.91
ARS-USMARC-Parent-DQ846691-rs29019814	98.44	96.20	97.32
ARS-USMARC-Parent-EF034087-no-rs	95.10	100.00	97.55
ARS-USMARC-Parent-AY851163-rs17871661	100.00	95.24	97.62
ARS-USMARC-Parent-DQ990834-rs29013727	97.20	99.72	98.46
ARS-USMARC-Parent-EF034081-rs29009668	97.54	99.72	98.63
ARS-USMARC-Parent-DQ647187-rs29010510	97.56	99.72	98.64
ARS-USMARC-Parent-EF042090-no-rs	97.37	100.00	98.69
ARS-USMARC-Parent-DQ839235-rs29012691	100.00	97.50	98.75
ARS-USMARC-Parent-EF034082-rs29013532	97.74	100.00	98.87
ARS-USMARC-Parent-AY842474-rs29003226	98.15	99.72	98.94

It is suggested to look into the problems in more detail and decide if adjustments to the core Panel are necessary. There are only results available from a few CTs so far. Some of the above markers seem to have a problem with only one or a few platforms.

A summary of the absolute and relative performance evaluation is shown below (1<sup>st</sup>/2<sup>nd</sup>).

Absolute genotyping Accuracy		Relative genotyping Accuracy	
Total # labs: 18/19		Total # labs: 18/19	
Rate	% Labs	Rate	% Labs
1: 100 – 98%	44/74	1: 100 – 98%	88/89
2: 98 – 95%	33/16	2: 98 – 95%	6/11
3: 95 – 90%	6/0	3: 95 – 90%	6/0
4: 90 – 80%	6/10	4: 90 – 80%	0/0
5: 80%	11/0	5: 80%	0/0

## **2. Next Comparison Tests 2015/2016 and 2017**

The ISAG conferences will change from even years to odd years, starting in 2017. The next three conference years will be 2016, 2017 and 2019. It was decided by the representatives of the institutional members in the workshop that both CTs need to be organized in 2016 and 2017.

2016

Duty Lab: Geneseek, USA, contact person Jiansheng Qiu

Computer Lab STR: Identitas, Uruguay, contact person Luis Cancela

Computer Lab SNP: VGL, USA, contact person Cecilia Penedo

2017

Duty Lab: Labogena, France, contactperson Lucie Genestout

Computer Lab STR: Identitas, Uruguay, contact person Luis Cancela

Computer Lab SNP: VGL, USA, contact person Cecilia Penedo

ISAG/FASS will provide more support to organizing future comparison tests, e.g. consignment online, reporting/uploading results online, analysis of results together with computer labs, downloading of certificates online.

## **3. Additional ISAG SNP panel**

During ISAG 2012 conference preliminary results of 100 additional markers were presented. The panel performed well on B. taurus breeds. B. indicus and crosses needed to be validated. Now B. indicus breeds have been validated. Data on B. indicus are lower than in B. taurus but the additional panel increases the power of the 100 SNPs in the core panel. The SNPs in the additional panel are present on the LD/HD/50K Illumina beadchips and met the criteria of MAF mean of 0.4 and a minimum distance of 10cM on all chromosomes. The list of the 100 additional SNPs (back up panel), is available on the ISAG website with performances in a study conducted by Lucie Genestout (Labogena) including more than 4000 animals, with 20 to 675 animals per breed (see <http://www.isag.us/committees.asp> click on "ISAG cattle core + additional SNP panel 2013"). The results of the ISAG SNP CT showed good concordance between the labs for those markers. It was decided by the representatives of the institutional members in the workshop that these 100 additional SNPs are the ISAG back-up Panel for Parentage purposes.

## **4. The application of Microsatellite Imputation, Romy Morrin, Weatherbys, Ireland**

Ireland imputation is necessary where parents have been genotyped for STR profiles only and offspring are being genotyped for SNPs only. The SNP chips used are the International Dairy and Beef (IDB) SNP Chip (v1 is 17K chip and v2 is a 18K chip). The STR genotypes for the offspring are imputed in order to verify parentage without genotyping the offspring for STRs. 17% STR-SNP haplotypes are identical across  $\geq 2$  breeds. For parentage cases involving imputed STR profiles, no mismatches or a single mismatch are accepted for parentage qualification. If multiple mismatches are present, all animals involved are genotyped on STRs. In about 20% of the cases with a single mismatch, the marker involved is TGLA53. The accuracy with imputed STR profiles for the 12-marker panel is >96%. After removing TGLA53, the accuracy improves to >97%.

## **5. Genomics and Animal Recording, the road Ahead for Cattle, Brian Wickham, ICAR**

ICAR stands for International Committee for Animal Recording ([www.ICAR.org](http://www.ICAR.org)). ICAR has 105 members in 54 countries and provides guidelines & standards for all aspects of animal recording (Genetic analysis, Parentage determination, Genetic evaluations). It provides services

for international evaluations (dairy & beef), shared data repository (GENOEX) and quality assurance. It organizes Interbull/Interbeef workshops and annual meetings. GENOEX stands for International Genotype Exchange Platform. The services of GENOEX are differentiated into three categories: Parentage SNP exchange service (PSE), genomic data exchange service (GDE) and customized genomic repository service (CGR). A step-wise implementation process will be adopted, starting by PSE followed by GDE and CGR. ICAR and ISAG can collaborate by

- recognition of respective strengths and common interest
- overlap in people in relevant ICAR Groups and ISAG committees
- adoption of relevant standards & guidelines from each other
- link to each others communication networks

ICAR and ISAG need to find ways of strengthening future collaboration and cooperation.

#### **6. GENOEX as platform for exchange of SNP profiles between labs**

Within the Standing committee several formats for exchange of SNP data have been discussed. One of the tools of the GENOEX database is the exchange of parentage SNP profiles. The details of who will have access to the database need to be worked out, including agreements to protect the data. It was decided by the representatives of the institutional members in the workshop that ISAG will follow the development of GENOEX and decide in the future if GENOEX will be accepted as the platform to exchange SNP profiles between laboratories.

#### **7. OMIA Website**

The Online Mendelian Inheritance in Animals (OMIA) website is a catalogue/compendium of inherited disorders, other (single-locus) traits, and genes in 215 animal species (other than human, mouse and rats, which have their own resources) authored by Professor Frank Nicholas of the University of Sydney, Australia. This website ([omia.angis.org.au](http://omia.angis.org.au)) can be a handy tool to find sequence information.

#### **8. New guidelines**

New draft guidelines for standing committees are available on the ISAG website. [guidelines](#). Address comments to the Chair of the Standing committee before October 1<sup>st</sup> 2014. Also, new draft guidelines for comparison tests are available on the ISAG website. The comparison test guidelines include a paragraph about liabilities. A document describing the procedure of the comparison will be generated to add to the guidelines. Participants of future comparison test must accept the new guidelines.

#### **Committee members (the new committee)**

<i>Chair</i>	<i>term of service</i>	<i>E mail address:</i>
Romy Morrin (IR)	elected in 2012 for 2 <sup>nd</sup> term	<a href="mailto:rmorrin@weatherbys.ie">rmorrin@weatherbys.ie</a>
<i>Other members</i>	<i>term of service</i>	<i>E mail address:</i>
Marcela Martinez (AR)	elected in 2012 for 2 <sup>nd</sup> term	<a href="mailto:mmartinez@sra.org.ar">mmartinez@sra.org.ar</a>
Rikke Vingborg (DK)	elected in 2014 for 1 <sup>st</sup> term	<a href="mailto:rkv@genoskan.dk">rkv@genoskan.dk</a>
Amparo Martinez (ES)	elected in 2014 for 1 <sup>st</sup> term	<a href="mailto:amparomartinezuco@gmail.com">amparomartinezuco@gmail.com</a>
Félicie Lahalle (FR)	elected in 2014 for 1 <sup>st</sup> term	<a href="mailto:felicie.lahalle-faucon@jouy.inra.fr">felicie.lahalle-faucon@jouy.inra.fr</a>
Jiansheng Qiu (USA)	representative Duty lab	<a href="mailto:JQiu@neogen.com">JQiu@neogen.com</a>
Cecilia Penedo (USA)	representative Computer lab SNP	<a href="mailto:mctorrespenedo@ucdavis.edu">mctorrespenedo@ucdavis.edu</a>
Luis Cancela (UR)	representative Computer lab STR	<a href="mailto:lcancela@chasque.net">lcancela@chasque.net</a>

**COMPARISON TEST (2013-2014)**

yes

**STR**

Number of enquiries – requests for consignment forms: 86

Number of participants receiving samples: 86

Number of samples: 20

Number of participants reporting results: 82

**1<sup>st</sup> SNP**

Number of enquiries – requests for consignment forms: 24

Number of participants receiving samples: 24

Number of samples: 12

Number of participants reporting results: 18

**2<sup>nd</sup> SNP**

Number of enquiries – requests for consignment forms: 33

Number of participants receiving samples: 33

Number of samples: 20

Number of participants reporting results: 19

**Duty laboratory**

1st SNP CT: Laboratory of Genética Aplicada, Argentina, Marcela Martinez,  
mmartinez@sra.org.ar

STR + 2nd SNP CT: GenoScan, Denmark, Rikke Vingborg, rk@genoskan.dk

**Computing Laboratory**

STR: Identitas, Uruguay, Luis Cancela, lcancela@chasque.net

SNP: VGL, USA, Cecilia Penedo, mctorrespenedo@ucdavis.edu

**List of recommended markers with primer information**

Locus	Chromosomal location	Repeat structure	Repeat sequence	Original reference	Primer sequences (Forward and Reverse)	Amplicon length (bp)
BM1818	D23S21	simple	(TG) <sub>n</sub>	Bishop et al. (1994)	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	253-277
BM1824	D1S34	simple	(GT) <sub>n</sub>	Barendse et al. (1994)	F: GAGCAAGGTGTTTTTCCAATC R: CATTCTCCAACCTGCTTCCTTG	176-188
BM2113	D2S26	simple	(CA) <sub>n</sub>	Sunden et al. (1993)	F: GCTGCCTTCTACCAAATACCC R: CTCCTGAGAGAAGCAACACC	124-146
ETH3	D19S2	compound	(GT) <sub>n</sub> AC(GT) <sub>6</sub>	Sofinas-Toldo et al. (1993)	F: GAACCTGCTCTCTCTGCATTGG R: ACTCTGCCTGTGGCCAAGTAGG	100-128
ETH10	D5S3	simple	(AC) <sub>n</sub>	Sofinas-Toldo et al. (1993)	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTCTCTCTCTC	206-222
ETH225	D9S2	compound	(TG) <sub>4</sub> CG(TG)(CA) <sub>n</sub>	Steffen et al. (1993)	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	139-157
INRA023	D3S10	simple	(AC) <sub>n</sub>	Vaiman et al. (1994)	F: GAGTAGAGCTACAAGATAAACTTC R: TAACTACAGGGTGTTAGATGAACCTC	201-225
SPS115	D15	compound	(CA) <sub>n</sub> TA(CA) <sub>6</sub>	Baylor College of Medicine Human Genome Sequencing Center (2006)	F: AAAGTGACACAACAGCTTCACCAG R: AACCGAGTGTCTTAGTTGGCTGTG	247-261
TGLA53	D16S3	compound	(TG) <sub>6</sub> CG(TG) <sub>4</sub> (TA) <sub>n</sub>	Georges & Massey (1992)	F: GCTTTCAGAAATAGTTTGCATTCA R: ATCTTCACATGATATTACAGCAGA	151-187
TGLA122	D21S6	compound	(AC) <sub>n</sub> (AT) <sub>n</sub>	Georges & Massey (1992)	F: AATCACATGGCAAATAAGTACATAC R: CCTCCTCCAGGTAAATCAGC	136-182
TGLA126	D20S1	simple	(TG) <sub>n</sub>	Georges & Massey (1992)	F: CTAATTTAGAAATGAGAGAGGCTTCT R: TTGGTCCCTATTCTCTGAATATTCC	111-127
TGLA227	D18S1	simple	(TG) <sub>n</sub>	Georges & Massey (1992)	F: GGAATTCCAAATCTGTAAATTTGCT R: ACAGACAGAACTCAATGAAAGCA	76-104

Information provided by Leanne van de Goor (<http://www.csl.nist.gov/strbase/cattleSTRs.htm>)

**Duty laboratory for the next comparison test**

Geneseek, USA, Jiansheng Qiu, JQiu@neogen.com

**Computing laboratory for the next comparison test**

STR: Identitas, Uruguay, Luis Cancela, lcancela@chasque.net

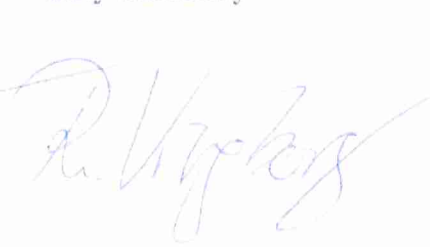
SNP: VGL, USA, Cecilia Penedo, mctorrespenedo@ucdavis.edu

**SIGNATURES**

Chair



Duty laboratory



Computing laboratory

