

# **Cattle Molecular Markers and Parentage Testing**

STANDING COMMITTEES / WORKSHOPS

Information will be posted online

Organised by a standing committee yes

Date and meeting time: Tuesday, July 18 2.30PM

Chair, name and contact email: Romy Morrin-O'Donnell, Weatherbys Ireland Ltd. rmorrin@weatherbys.ie

Agenda / programme attached

Number of participants at meeting: 68

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

#### Duty lab report by Alexandre Vasilescu, Labogena, France

Number of participants in the CT

8 from North America

16 from South America

4 from Africa

56 from Europe

6 from Asia

2 from Oceania

92 participants in total

The kits were shipped out on the 8<sup>th</sup> of Feb. 2017, and additional 18 were shipped on the 27<sup>th</sup> of March.

Two topics were reported

- Caps didn't tighten the tubes, and labels didn't stick to the tubes.
- Problems with the import permits.

		2013-2014	2015-2016	2016-2017
STR	Applicants	91	91	92
	Submitted	82	85	84
	Delta	9	6	8
	Submission %	90 %	93 %	91 %
SNP	Applicants	37	33	42
	Submitted	18	23	38

Delta	19	10	4
Submission %	49%	70 %	90 %

- For the next CT, if evaporation occurs, re-suspend the DNA with TE (or water) as the DNA is still present. If it does not work, then ask for a new kit.
- Tubes should be labelled horizontal.

# Computer lab report STR (Luis Cancela, Identitas, Uruguay)

FASS compiled results from 85 labs of the 92 applicants.

Additional set of markers, reported

Marker	Nº labs
MGTG4B	19
CSRM60	18
SPS113	17
RM67	15
ILSTS6	13
CSSM66	12

Concordant genotypes are the most frequently reported genotypes. It does not mean necessarily correct genotypes.

Two markers were identified with problems:

	SPS115 (2016)			SPS115 (2013)			
Sample	260/	260/261	260/262	Sample	248/260	248/261	248/262
7	32	43	6	17	40	32	8
12	32	43	5				

• Rare intermediate allele

• Sequenced by Leanne van de Goor, see the at www.cstl.nist.gov/strbase/cattleSTRs.htm

SPS115 (261) is a one basepair allele. It is a rare intermediate allele, but it has been sequenced by VHL.

Absolute Genotyping Accuracy				
Rate	Labs	%		
1: 100 – 98%	60	71%		
2: 98 – 95%	13	15%		
3: 95 – 90%	5	6%		
4: 90 – 80%	5	6%		
5: 80%	2	2%		
Total	85	100%		

Repository samples, are stored at Identitas, do contact Luis Cancela (<u>lcancela@identitas.com.uy</u>) for samples. No ISAG certificates will be issued, but data can be evaluated.

## SNP comparison (Cecilia Penedo, UCDavis)

25 labs participated.

# SNPs reported: 92-200

Average consensus primary panel:  $99.7 \pm 0.64$ 

Average consensus extra panel:  $99.7 \pm 0.01$ 

Reference data were delivered in TOP format.

Data were reported from 7 different genotyping platforms: Illumina, MassArray, IonTorrent S5 (GBS), BioRad RealTime PCR, Z-Chip by Deoxi ( 30k)/ IScan Illumina, Biomark HD (Fluidigm) and Fluidigm

The compiled data were reported in the old format. The results will be delivered in the correct format.

Most of the labs had no problems with the markers.

Marker performance, an overall good performance

Marker: ARS-USMARC-Parent-DQ786766-rs29012070 is known to cause issues, and this marker also shows discrepancy between genotyping platforms. In the Illumina design this marker has a split heterozygote pattern, compared to the Mass Array platform, where the clustering is fine.

We have received this answer from Illumina regarding the marker: *SNP rs29012070,... I was not* expecting to see as many as **10 underlying SNPs**, as well as one deletion. I would definitely think, that this can be responsible for the pattern observed on the AB cluster. GeneSeek has sequenced 1300 samples for this SNP, which shows all the genotypes reported. Suggestion was made to remove the marker from the panel.

Looking at other platforms, there are markers, where it could be interesting to look at the data to ensure correct genotyping of the samples.

We have been looking at the sequence around the target SNPs, to see if there are additional SNPs causing the problems. There are SNPs in the area around target SNPs, so this might be worth looking into.

Summary of the SNP comparison:

- High overall concordance
- 85/100 SNPs in primary panel > 99.8% concordance
- 83/96 SNPs in extra panel >99.7% concordance

Comments from the committee

- Only clerical errors will be corrected
- Although four labs commented on the genotyping of SPS115, it will not be corrected
- Samples for comparison test are selected by the duty lab, and will represent the breeds from that area/country => unknown/rare alleles might be genotyped

Short presentation of ICAR. Made by Romy Morrin-O'Donnell, Weatherbys Ireland.

# CT 2018/19

Dutylab

- Volunteer
  - Animal Breeding Consulting from Spain (Vincenzo Landi) 2019
  - o Università degli Studi di Milano from Italy (Maria Longeri) 2021

### Time schedule for the next CT

- Apply for participation July 2018
- Invoices from FASS will be sent out on the 15<sup>th</sup> of August 2018
- Invoices must be paid by the 15<sup>th</sup> of September 2018
- Samples will be sent out on the 15<sup>th</sup> of November 2018
- Application for a second set of samples on the 15<sup>th</sup> of December 2018
- Upload of results to FASS on the 31<sup>st</sup> of March 2019

Proposal from the Executive committee will be discussed on the business meeting Friday: When samples are prepared, the Duty lab should send the samples to a core lab. The core lab then distributes the samples for all comparison tests to all labs that have applied for participating in the CT. Van Haeringen Lab in the Netherlands is being proposed as the core lab. The advantage of this is

- Reduction in price for FASS
- Samples are send in 96 well format

Comments from the audience:

- Uncertain that costs are reduced
- ISAG should have a protocol for the duty lab regarding the sampling for the CT

Proposal from the Bovine Committee regarding the underperforming SNPs in the core panel Remove the underperforming SNPs. Replace them with markers from the secondary/additional panel. The new list will be published on the website on the 1<sup>st</sup> of February 2018, and distributed to all institutional members by email.

Votes: In favour: 19, against: 0

Presentations

- Next-generation targeted sequencing panel for verification of bovine parentage. A. Burrell\*1, P. Siddavatam1, A. Allred1, C. Willis1, R. Ferretti2, and A. Raeber1, 1 Thermo Fisher Scientific; Neogen GeneSeek Operations.
- SNP data quality control in a national beef and dairy cattle system and highly accurate SNP-based parentage verification and identification. M. McClure\*1, J. McCarthy1, R. Weld2, P. Flynn2, M. Kean1, K. O'Connell1, and J. Kearney1, 1 Irish Cattle Breeding Federation, Bandon, Cork, Ireland; 2 Weatherbys Ireland, Johnstown, Kildare, Ireland.
- High cross-platform genotyping concordance of Axiom high-density microarrays and Eureka low-density targeted NGS assays. M. A. Patil\* and A. Pirani, Thermo Fisher Scientific, Santa Clara, CA, USA.

**Committee members** (the new committee)

Chair: Rikke Vingborg, GenoSkan A/S, (2014-2019), rikkevingborg@eurofins.dk

Amparo Martinez, Laboratorio de Genética Molecular Aplicada (2014-2019), amparomartinezuco@gmail.com Jiansheng Qiu, GeneSeek, (2014-2019), JQiu@neogen.com Leslie A Bickel, UCDavis (2017-2021) <u>labickel@ucdavis.edu</u> Alexandre Vasilescu, Labogena,(2016-2021), <u>alexandre.vasilescu@labogena.fr</u> Luis Cancela, Identitas, <u>lcancela@identitas.com.uy</u>

Ex officio members Representative for ICAR: Romy Morrin-O'Donnell, Weatherbys, <u>morrin@weatherbys.ie</u>

### COMPARISON TEST (2016-2017) yes

Duty laboratory Labogena, France

http://www.labogena.fr/ Labogena Domaine de Vilvert – Bât 224 CS 80009 78353 Jouy en Josas cedex

**Comments** (issues rising) See above

List of recommended markers with primer information, please see the website

### Duty laboratory for the next comparison test with contact details

Laboratorio de Genética Molecular Aplicada (LGMA) Animal Breeding Consulting SL Departamento de Genética, Universidad de Córdoba Edificio C5 Gregor Mendel, Planta Baja Campus Universitario de Rabanales 14071-Córdoba (Spain) https://www.abcgenetica.com/

e-mail: <u>animalbreedingconsulting@gmail.com</u> Tel.: +34957218708, +34630639439 fax: +34957218706 Contact person: Vincenzo Landi and Amparo Martínez

SIGNATURES

Chair

**Duty laboratory** 

Likke Vingborg

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