ISAG CONFERENCE 2016, Salt Lake City, USA

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: 25th July 2016 14:00-17:30

Chair, name and contact email: Romy Morrin O'Donnell (rmorrin@weatherbys.ie)

Agenda / programme:

- Welcoming Remarks .
- Cattle STR/SNP Comparison Test 2015-2016 .
    - Presentation by Duty Lab .-Jiansheng Qiu, Geneseek, Neogen, USA.
    - Presentation of the STR results .-Luis Cancela, Identitas, Uruguay.
    - Evaluation of results by the Chair .
    - Presentation of SNP results by the chair Chair.
    - SNP standardised nomenclature discussion .
- Invited Speaker:
    - *Genomic evaluations in dairy cattle, beef cattle and sheep in Ireland* . Presenter: Donagh. P. Berry-Teagasc Ireland
- Poster presentation:
    - *Effectively Managing Bovine Genetic Disease Risk via Genotyping the Irish National Herd.* Presenter: Matt McClure- ICBF Ireland
- Next Comparison Test 2017 .
- Election of Committee .
- AOB .
- Close.

Number of participants at meeting: 95 recorded.

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

The meeting commenced with welcome remarks from the chair.

Reminder of new procedures implemented by ISAG in relation to Comparison tests.

- Liability policy document-should be signed by an authorised representative of the institution.

- Rules for Comparison tests must be followed.
- FASS organises the courier for sending samples.
- On line submission of results.
    - Instructions must be followed.
- Compilation of results by FASS

New Reporting Procedure
FASS compiled results for the first time. As Luis Cancela has been the computer lab for many Bovine STR comparison tests, he reviewed the draft results to ensure that the new system compiled the results similar to previous CT's.

Luis also prepared a process flow to illustrate the "New" comparison test reporting procedure, see Figure 1.

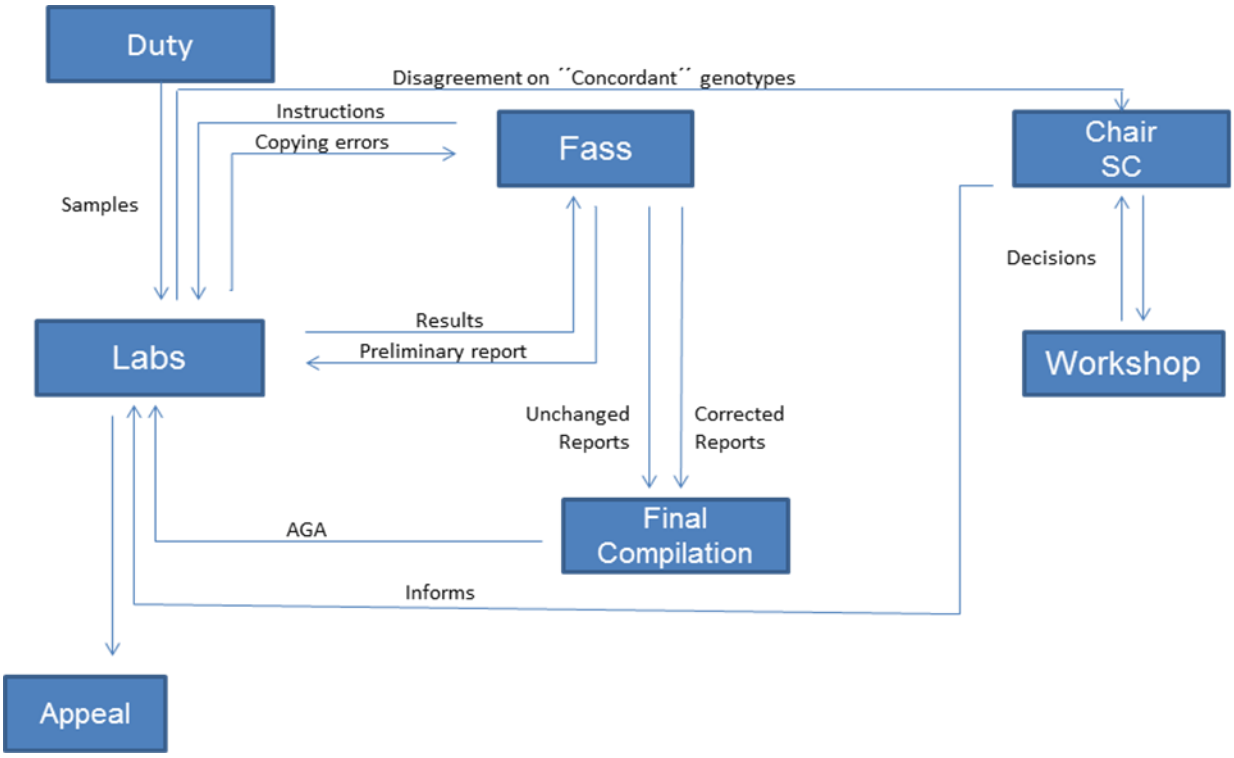

Figure 1. Process flow – "New" Comparison test reporting procedure.

- FASS sent a draft report of the compilation of the results to all participants prior to the conference. The non-concordant results were highlighted.
- Formatting errors, will be corrected by FASS (e.g. 125/ instead of 125/125).
- Some laboratories reported that they had clerical errors in their submission file and requested that the result be corrected for the final compilation.
  After discussion of this point, the following motion was voted on by the
  Work-shop participants:

Motion: Delegate the authority to the Standing Committee to review requests for correction of clerical errors that are properly documented by the participant and make the correction for the final compilation of results. Evidence to be provided by the participant.

Result: Passed by majority with one participant voting against.

- If a lab disagrees on "concordant" genotypes, they must submit their disagreement to the chair of the SC at least one week ahead of the conference, so a potential disagreement in concordance can be discussed in the workshop
- FASS will distribute the final compilation 7 weeks after the conference.

STR/SNP Comparison Test 2015/2016

Duty lab report (Jiansheng Qiu, GeneSeek)

- GeneSeek made 105 complete DNA kit
- 21 samples (1 reference)
- The samples were made from whole blood or semen from 19 diverse breeds, and the DNA purification was done by magnet beads
- Each sample 30µl with a 30ng/µl concentration
- 93 labs from 36 countries requested samples
    - 60 labs for STR CT
    - 3 labs only requested samples for the SNP CT
    - 30 labs requested samples for both STR & SNP CT
    - Nine additional kits were sent due to late applications or empty tubes.

- GeneSeek experienced the following issues:
    - Import permits not valid (date expired).
    - Undelivered sample kits were returned to the duty lab for the following reasons:
        - Additional documents needed by the lab
        - One Lab refused to pay additional "fees"
        - One Lab did not pick up the shipment
    - Some labs experienced that tube (s) were empty upon arrival. Replacements were sent. It was noted that some labs added water to the tubes and noted the DNA was good. Evaporation had occurred.
    - The duty lab had extensive email contacts with several participants to resolve document issues.
- Suggestions for future duty labs
    - Prepare extra kits
    - Thorough capping of the samples.
    - Use 3 samples as reference samples.
    - Contact previous duty labs for advice.

STR results (Luis Cancela, Identitas)

- 85 Laboratories reported results to FASS
  - 79 labs reported the 12 STR ISAG core panel
  - 6 labs reported 11 STR markers. 4 did not report BM2113 and 2 did not report TGLA53
  - 25% of labs reported an additional set of 6 markers:
    - SPS113
    - RM067
    - CSRM60
    - MGTG4B
    - CSSM66
    - ILSTS006
  - 15 additional markers were reported by 5-10 labs, this is consistent with previous CT's.

Genotype Concordance

- Concordant genotypes are the most frequently reported genotypes, but concordant genotypes are not always the correct genotypes.

$$MC = \frac{concordant\ \ genotypes}{reported\ \ genotypes} \cdot *100$$

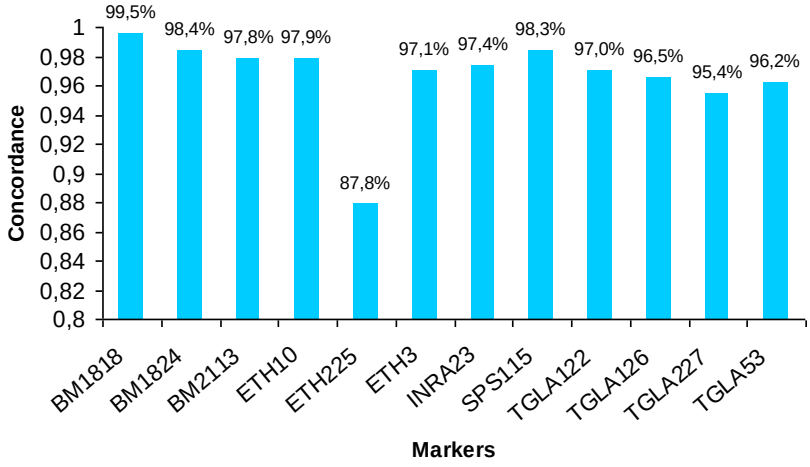

Figure 2. 2015-2016 Bovine STR marker concordance.

ETH225 was an issue in this comparison test due to one allele being reported as, 158,159,160 and 161. This allele was a problem in the 2008 CT. It had been agreed in 2008 that the correct allele name was 158 but sequencing was to be carried out to confirm this. The allele has since been sequenced by both Cecilia Penedo and Leanne Van de Goor and this has confirmed that the true allele is 158. In this CT the allele 160 was the concensus genotype but the correct allele is 158.

- ---------+---------+---------+---------+---------+---------+---------+
- 10 20 30 40 50 60 70
- ---------+---------+---------+---------+---------+---------+---------+
- 1 GATCACCTTGCCACTATTTCCTCCAACATATGTGTGTGCGTGCACA----------
-CACACACACACA ETH225 1-146
- 1
GATCACCTTGCCACTATTTCCTCCAACATATGTGTGTGCATGCACAGACACAT
ACACACACACACACACACA ETH225 1-158
- 1
GATCACCTTGCCACTATTTCCTCCAACATATGTGTGTGCATGCACAGACACAT
ACACACACACACACACACA ETH225 17-158
- ---------+---------+---------+---------+---------+---------+---------+
- 80 90 100 110 120 130 140
- ---------+---------+---------+---------+---------+---------+---------+
- 59
CACACACACACACACATGATAGCCACTCCTTTCTCTAATGCCACAGAATTACA
CAGTCAACTTCTCTAGT ETH225 1-146
- 71
CACACACACACACACACGATAGCCACTCCTTTCTCTAATACCACAGAATTACA
CAGTCAACTTCTCTAGT ETH225 1-158
- 71
CACACACACACACACACGATAGCCACTCCTTTCTCTAATACCACAGAATTACA
CAGTCAACTTCTCTAGT ETH225 17-158
- ---------+--------
- 150
- ---------+--------
- 129 AGCAGCTGGCTGTCATGT ETH225 1-146
- 141 AGCAGCTGGCTGTCATGT ETH225 1-158
- 141 AGCAGCTGGCTGTCATGT ETH225 17-158

$(TG)_4\ CG\ (TG)(CA)_n$

22 repeats – 146

28 repeats – 158

Figure 3, Sequence for ETH225 alleles 148 and 158 from Cecilia Penedo

Veterinary Genetics Laboratory – UC Davis

Table 1. STR nomenclature based on repeat number-nomenclature

Conversion of ISAG nomenclature to repeat-number nomenclature				
	Repeat number	ISAG ETH225		
	11			
	12			
	13			
	14			
	15			
	16			
	17			
	18			
	19	140		
	20	142		
	21	144		
	22	146		
	23	148		
	24	150		
	25	152		
	26		*154*	
	27		*156*	
	27.1		*158*	
	28	*	*160*	
	29			
	30			
	31			

Adapted from:
Leanne van de Goor et al.
Dr. Van Haeringen Laboratorium
A proposal for standardization in forensic bovine DNA typing: allele nomenclature of 16 cattle-specific short tandem repeat loci. ***Animal Genetics, 40, 630–636***

Table 2: Shows the number of laboratories per sample that reported the different nomenclature for the same allele for the marker ETH225.

	ETH225			
Sample/allele	**158**	**159**	**160**	**161**
02	29	4	49	1
03	29	4	50	1

04	29	3	51	1
05	29	4	50	1
15	29	4	48	1

The following motion was proposed as there was still some ambiguity about this allele prior to this recent comparison test.

Motion: Accept ETH 225 allele 158,159,160,161 for samples 2,3,4,5 & 15 for this CT 2015-2016. Allele 158 is the correct allele and for future CTs penalties will apply if it is not reported correctly.

Result: Unanimous acceptance

Table 3: Illustrates that for Marker ETH225 in samples 2,3,4,5 and 15 the following genotypes are to be counted as correct for the ranking process.

Sample 02	Sample 03	Sample 04	Sample 05	Sample 15	Comment
150/158	158/	148/158	150/158	144/158	Correct genotype
150/159	159/	148/159	150/159	144/159	
150/160	160/	148/160	150/160	144/160	Consensus Genotype
150/161	161/	148/161	150/161	144/161	

Parentage Question

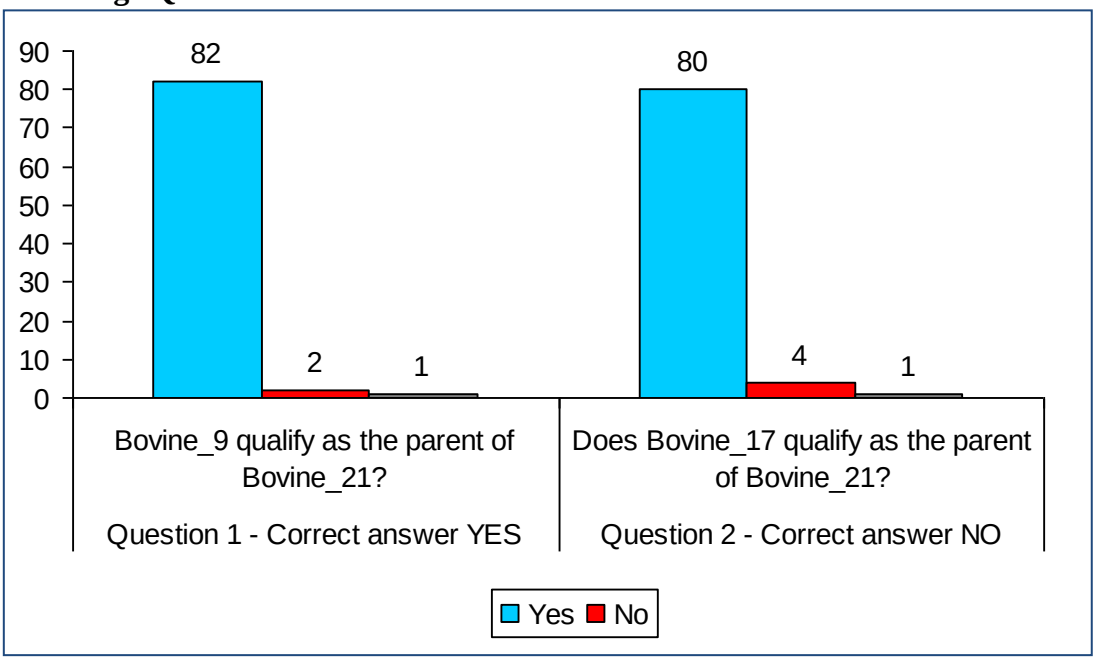

Figure 4, illustrates the results of the parentage verification questions.

SNP results

23 laboratories returned results out of 33 applications. Table 4 shows the performance of the laboratories. Overall SNP concordance was excellent. Genotyping accuracy calculated using the core 100 markers on 18 samples (3 references not included)

One marker in the additional panel was an issue –Hapmap 46653-BTA47447

Table 4: Illustrates the overall results for the Absolute and Relative Genotyping accuracy for the SNP CT.

Absolute Genotyping Accuracy Errors and Blanks counted			**Relative Genotyping accuracy Blanks not counted**		
Rank %		**# of Labs**	**Rank %**		**# of Labs**
1	**100-98**	**18 (78.3%)**	**1**	**100-98**	**19 (82.6%)**
2	**97.9-95**	**3 (13%)**	**2**	**97.9-95**	**3 (13%)**
3	**94.9-90**	**0**	**3**	**94.9-90**	**1 (4.4%)**
4	**89.9-80**	**0**	**4**	**89.9-80**	**0**
5	**Below 80**	**2 (8.7%)**	**5**	**Below 80**	**0**

SNP Standardised Nomenclature Discussion

The reference sample results sent out for the comparison test were not in the ISAG standard nomenclature of Forward direction but were sent in TOP format. This was noted after the samples had been sent to participants. Subsequently the reference samples were re- sent in ISAG nomenclature. Participants were unhappy about the changes and had expressed their concern prior to the conference by e-mail to FASS and the chair of the SC. The SC discussed this issue and as the volume of SNP data in the public domain is generated for Genomic analysis and reported in TOP Format it was decided to discuss at the workshop changing the ISAG SNP nomenclature to TOP Format.

SNP orientation and symmetry issues also created some problems with the reference samples.

The chair explained that ICAR/Interbull are working on a Format for parentage SNP exchange for the GenoEX PSE and they can use the TOP format too. The GenoEX-PSE is expected to be launched in January 2017.

It was noted that Genotyping by Sequencing (GBS) is being reported in TOP format.

A vote was required between members to change the reporting format.

Motion: Change ISAG standard Nomenclature to TOP format with effect for the next comparison test in 2017.

Result: Unanimous acceptance

For the next CT, the duty lab will send out 20 test samples and three reference samples to cover alleles.

- The reference samples form 2015-2016 SNP CT will be published on the ISAG website in TOP format.
- The SC will provide a paper to describe the TOP format, forward and AB formats publish on the web site

Draft format for SNP exchange for ICAR/Interbull GenoEx-PSE

The chair showed the draft format for data exchange provided by the ICAR/Interbull expert group for the GenoEx PSE data base. There are 2 documents. One document with the information relating to the animal and sample details and a second document with the SNP results. Please see figures 5 and 6.

GenoEx-PSE File Exchange Formats

Draft 4

22 July 2016

Authorized Services Users with a signed Service Agreement submitted to ICAR and the Interbull Centre will have access for downloading SNP genotypes and associated data to the GenoEx-PSE database and will have the obligation to routinely upload required data to the GenoEx-PSE database . The SNP genotypes and related information submitted (uploaded) to GenoEx-PSE is categorized in two groups:

1) Information related to a group of animals with a SNP genotype to upload;
2) Information related to the actual SNP genotypes for the animals in 1).

At the time of each upload from a Service User, both files must be submitted at the same time. Data submitted in one file will not be processed until both files are available.

Information Related to a Group of Animals

The following table describes the file format to be used for uploading general information for the group of animals for which SNP genotypes will be submitted in the associated second file (see below). This format will also be used when downloading data from GenoEx-PSE. This file will be exchanged as a variable length, comma delimited file in .csv format and include a single record for each animal for which the SNP genotype details will be included in the second file. For example, if the second file (File 704-AB or 704-TOP) includes SNP genotype results for 100 animals, this files will have 100 records.

Field Name	Column Size	Example	Description
Record Type	3	702	Numeric
Service User	6	INRA	Alphanumeric
Source Country of Animals	3	FRA	Alpha
Animal ID - Breed Code	3	BSW	Alpha
Animal ID - Nation Code	3	AUS	Alphanumeric (USA, 840, CAN, ...)
Animal ID - Sex Code	1	M	Alpha (M or F)
Animal ID - Registration	18	A12345	Alphanumeric
Lab ID	12	GeneSeek	Alphanumeric
Sample ID	15	R1234567890	Alphanumeric
Scan Date	8	yyyymmdd	Numeric
Platform	1	1=Illumina 2=Affymetrix	SNP Chip Platform
No. SNPs in Genotype	8	55647	Total number of SNPs in the animal's full genotype used to create the SNP record for GenoEx-PSE
Genotype Call Rate	4	99.9	Percent call rate of the animal's full genotype used to create the SNP record for GenoEx-PSE
Maximum Record Length	**85**		

Figure 5, ICAR/Interbull GenoEx PSE - Draft of File exchange format- for sample and animal information.

Information Related to the SNP Genotypes of Animals

The following table describes the file format to be used for uploading the specific SNP genotypes to the GenoEx-PSE database. The same file format will be used for producing the data files to be downloaded from the GenoEx-PSE database. The Service User may select to upload and/or download SNP genotype data in either the "AB" or "TOP" allele designations, which determines the content of the first field, namely Record Type, in the following file format (i.e.: File 704-AB versus File 704-TOP, respectively). This file will be exchanged as a variable length, comma delimited file in .csv format and include a single record for each SNP included for each animal. For example, if the first file (File 702) includes 100 animals and each SNP genotype include the 200 SNPs recommended by ISAG for Parentage Verification then this second file will include 20,000 records (100 animals x 200 SNPs each).

Field Name	Column Size	Example	Description
Record Type	7	704-AB (or 704-TOP)	Alphanumeric
Animal ID - Breed Code	3	BSW	Alpha
Animal ID - Nation Code	3	AUS	Alphanumeric (USA, 840, CAN, ...)
Animal ID - Sex Code	1	M	Alpha (M or F)
Animal ID - Registration	18	A12345	Alphanumeric
SNP name	40		Alphanumeric
Allele 1	1	A/B or A/C/G/T	Alphabetic (letters only)
Allele 2	1	A/B or A/C/G/T	Alphabetic (letters only)
Maximum Record Length	74		

Figure 6, ICAR/Interbull GenoEx PSE - Draft of File exchange format- for SNP results
Two documents with genotypes (both AB and TOP formats) will be accepted in the DB.

Comparison test 2017:

- Duty lab volunteered at ISAG 2014 in China.
  - Duty lab for 2017 is Labogena France.
- Tentative deadlines
  - CT application September 15th 2016-Late applications will not be considered
  - Invoices out for shipping Oct 1st
  - Payment by Oct 15th
  - Ship samples Dec 1st
  - 2nd sample request Feb 1st 2017
  - Samples sent Feb 15th 2017
  - Results returned April 1st 2017

Election of the Standing Committee

The current committee is willing to serve another year to bring the service time back on track to the new conference schedule.
Vote: Agreed unanimously.

AOB
For your information

- Luis Cancela from Identitas, maintains an archive of past comparison test samples, so new labs are able to make an interim testing between workshops. No certificates would be issued.
- Labs using the Illumina beadchips
  - There are 3 SNPs with clustering issues, when you have many breeds in one project
  - 1 core panel SNP
    - ARS-USMARC-Parent-DQ837645-rs29015870
  - 2 additional panel SNP's
    - ARS-BGFL-NGS-76191
    - BTA-100621-no-rs

- STR: ETH3 low frequency allele 113, will be a part of the official db. www.cstl.nist.gov/strbase/cattleSTRs.htm

Committee members (the new committee)

Chair	*term of service*	*E mail address:*
Romy Morrin O'Donnell (IRE)	Elected in 2012 for 2nd term	rmorrin@weatherbys.ie
Co Chair		
Rikke Vingborg (DK)	Elected in 2014 for 1st term	rkv@genoskan.dk

Other members	*term of service*	*E mail address*:
Marcela Marinez (AR)	Elected in 2012 for 2nd term	mmartines@sra.org.ar
Amparo Martinez (ES)	Elected in 2014 for 1st term	amparomartinezuco@gmail.com
Jiansheng Qiu (USA)	Duty lab 2015-2016	JQiu@neogen.com
Cecilia Penedo (USA)	SNP computer lab advisor	mctorrespenedo@ucdavis.edu
Luis Cancela (UR)	STR computer lab advisor	lcancela@chasque.net

COMPARISON TEST (2016-2017) **YES**

Duty laboratory

Lucie Genestout (FR)	Duty lab 2016-2017	lucie.genestout@labogena.fr

SIGNATURES

Chair **Duty laboratory**