D383
Evaluation of probability of exclusion of 16 microsatellites from Brazilian Mangalarga Marchador equine breed.

KATIA T. SOUZA, MARCIA C. B. N. CAMPOS, ELISANGELA A. SILVA, PATRICIA P. MACHADO, CECILIA TRANCOSO, VALERIA M. P. CALIJORNE, CRISTIANE L. OLIVEIRA & CARLA L. P. DE MENEZES.

Linhagen Produtos e Serviços em Biotecnologia LTDA, Belo Horizonte, Brasil.
E-mail:ktsouza@linhagen.com.br

In these past few years the efficacy and reliability of DNA tests have made it the choice for human genetic identification and paternity tests. This approach has also being used for animal identification . In this study 1500 paternity tests cases have been analized using a set of 16 microsatellites, including the nine loci recommendedby the international standard panel – ISAG (AHT4, AHT5, ASB2, HMS3, HMS6, HMS7, HTG4, HTG10 AND VHL20). DNA was purified from total blood and hair roots. The material was amplified in two multiplex PCR reactions. Each reaction contained 1x of Buffer 10x (500mM KCl, 100mM Tris –HCl and 15mM MgCl2), 2mM of each dNTP, 0,5 U of Platinum Taq Polymerase (Invitrogen), working solutions of primers in variable concentrations (1 to 5uM/sample) and 50ng of genomic DNA. The PCR products were analised by capillay electrophoresis using a MEGABACE 1000 sequencer (GE HealthCare). Among the 1500 cases we have found 123 cases where the offspring was not confirmed, whether sire- offspring or dam-offspring. Based on these data the combined probability exclusion of our multiplex have been analised resulting an estimated CPE= 0,9999998. The estimated CPE of the international standard panel is 0,999648. Another result is that the microsatellites with the lowest PE in our multiplex is HTG6, and the microsatellite with the highest PE is ASB2. These results confirm that the multiplexes are efficient and recommended to be used in Mangalarga Marchador breed genetic identification and parentage testing.

D390
Genetic analyses of six stocks of tilapia (*Oreochromis spp*) using microsatellite markers

DANIELA C. MELO[1], DENISE A. A. OLIVEIRA[1], LINCOLN P. RIBEIRO[1]; CLÁUDIA S.TEIXEIRA[1], ALEXANDRE B. SOUSA[1]; LILIAN VIANA TEIXEIRA[1], EDUARDO G. A. COELHO[1], DANIEL V. CREPALDI[1]

[1]*Federal University of Minas Gerais, Veterinary College, Belo Horizonte, Brazil*
e-mail: danichemim@bol.com.br

The production of Tilapia in the state of Minas Gerais has grown in the last years, trying to attend the lack of the consumer's market. Thus, the genetic identification of stocks is extremely important, once the genetic variability is the base for the commercial success, as well as for the implantation of any program for commercial selective breeding. In this study, 235 individuals from six commercial stocks of tilapias (Ceará, Chitralada, Israel, Nilótica, Taiwan and Red) from the Southeast region of Brazil were genetically characterized using five microsatelittes loci. Analyzing the stocks it was possible to identify the existence of genetic differences among stocks, estimated through the fixation allele index (Fst = 0,3263), and that a considerable loss of heterozigosity is occurring in almost all the stocks, according to the inside population inbreeding coefficient (Fis=0,0486). The stocks Israel and Nilótica were the most genetically similar (Ig=0,6663), while Chitralada and Taiwan were the ones that presented less genes in common (Ig=0,2463). The stock named Red was the most distinct among all of them. Differences in the identity matrix were observed between results from the present study and the literature, regarding origin of stocks. These results indicate that without a better genetic control of the stocks, is not possible to conduct effective programs of genetic improvement of Tilapias.

D394
Mapping and polymorphism of bovine ghrelin gene

FREDERIC G. COLINET[1], ANDRE EGGEN[2], NICOLAS GENGLER[3], CAROLINE HALLEUX[1], VALERIE ARNOULD[1], DANIEL PORTETELLE[1] & ROBERT RENAVILLE[1]

[1] *Animal and microbial Biology Unit, Gembloux Agricultural University, Belgium.*
[2] *Laboratoire de Génétique biochimique et de Cytogénétique, INRA-CRJ, Jouy-en-Josas, France.*
[3] *National Fund for Scientific Research, Animal Science Unit, Gembloux Agricultural University, Belgium.*
E-mail: colinet.f@fsagx.ac.be

Bovine ghrelin, a 27-amino-acid peptide has been identified in bovine oxyntic glands of the abomasum. It is an endogenous growth hormone secretagogue. Total mRNA was extracted from abomasum and complete ghrelin mRNA was sequenced by rapid amplification of cDNA ends. The gene contains five exons and four introns with a short noncoding first exon of 17 bp similar to mouse and human ghrelin gene. Using a radiation hybrid panel, the gene was mapped to chromosome 22 near microsatellite markers UWCA49, BM4102, BMS1932, BM2613 and URB035 with good LOD Score. Some studies detected different QTLs near these markers like for milk fat percent, milk protein percent and somatic cell score. So, it would be interesting to study the polymorphism on the bovine ghrelin gene. Screening for polymorphisms in the five exons and the introns II and IV on ten Belgian Blue bulls, ten Holsteins bulls and ten Limousin bulls revealed a total of three single nucleotide polymorphisms. In order to evaluate if ghrelin could be involved in genetic variation for milk fat percent, milk protein percent and somatic cell score an association study between SNPs on ghrelin gene and these traits could be performed in a major cattle population.

D397
Genetic relatedness of Caribbean hair sheep (Preliminary results and analysis)

LEROY MCCLEAN[1] , LYNDON D. WATERMAN[2] , CYRIL A. ROBERTS[3]

[1,2]*Department of Biological and Chemical Sciences, University of The West Indies, Cave Hill Campus, P.O. Box 64, Bridgetown, Barbados*
[3]*Caribbean Agricultural Research & Development Institute (CARDI), Barbados Unit, Cave Hill, St. Michael, Barbados*
Email: leroy.mcclean@cavehill.uwi.edu

Highly variable loci such as microsatellites provide a large amount of genetic information based on individual genotypes, permitting alternative approaches to the traditional ways of investigating and clarifying the genetic relationships between populations or breeds (Arranz *et al*., 2001). Microsatellite DNA polymorphisms were used to study genetic relationships between hair sheep breeds and to identify genetic markers for the Barbados Blackbelly sheep. Breeds investigated were the Barbados Blackbelly (Barbados population and St. Croix, US Virgin Islands, population), West African (Barbados), 'Mixed' breeds (Barbados), "Sugarlands Black" , a black off-type of the Blackbelly (Barbados), the St. Croix White (St. Croix, US Virgin Islands) and the Dorper (US Virgin Islands) as an out group. Fifteen *ovine* and *bovine* primers recommended by the International Livestock Research Institute (ILRI), Kenya were used and all showed polymorphism. These were *ILSTS017, OarFCB20, SR-CRSP-5, MAF214, ILSTSO19, BM1818, OarAE129, OarFCB304, MAF209, MAF035, TGLA53, BM827, ILSTS049, HSC and OarJMP29.* Preliminary results indicate that thirty alleles show frequencies ranging from 0.4 to 1 in the Barbados Blackbelly sheep and are potentially markers for the identification of the Barbados Blackbelly sheep breed. Five markers amplified showed frequencies ranging from 0.54 to 0.86 in both sub-populations of the Barbados Blackbelly sheep. These were: {*SR-CRSP-5* (157bp), *ILSTS019* (183bp), *BM1818* (231bp), *BM827* (220bp) and *OarAE129* (155bp)}. Calculation of average heterozygosity, Wright's F_{ST} and dendrograms constructed, using Microsoft Office Excel® and Minitab® Version 13.1, showed that the Barbados Blackbelly was genetically more similar to the mixed sheep found in Barbados than to the West African and the Sugarlands Black. Variation within the Barbados Blackbelly population supported the expected results based on the calculated heterozygosity within the sub-populations. The degree of genetic differentiation within the sub-populations ranged from significantly high in the Barbados Blackbelly sheep to very little in the mixed sheep and the off-type black sheep found in Barbados.