

# SABRE's Cutting Edge

## CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

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### A 54-month project

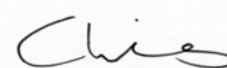
The completion of the first draft of the pig genome may be the most recent and eye-catching news about work that SABRE has been involved in, but the rest of the SABRE team have also been active, as can be seen from the articles in this newsletter.

We are very pleased and grateful to the European Commission who have agreed to our request for a 6-month extension to the project. With the project now finishing at the end of September 2010, the SABRE scientists will have the opportunity to thoroughly investigate the huge quantities of data that have been generated.

Genesis Faraday, the coordinator of SABRE, has merged with two UK knowledge transfer organisations and will continue to coordinate SABRE under a new name: Biosciences Knowledge Transfer Network.



Toine Roozen - SABRE Operations Manager



Chris Warkup - SABRE Co-ordinator

## SABRE contributes to 1<sup>st</sup> draft of Pig Genome Sequence

The completion of the first draft of the domesticated pig genome by an international team of scientists was celebrated at the third Pig Genome Conference on 2-4<sup>th</sup> November 2009.

The draft sequence is now about 98% complete and will allow researchers to pinpoint genes that are useful to pork production or are involved in immunity or other important physiological processes in the pig. It will enhance breeding practices, offer insight into diseases that afflict pigs - and, sometimes, also humans - and will help in efforts to preserve the global heritage of rare, endangered and wild pigs.

Professor Alan Archibald of The Roslin Institute and R(D)SVS, University of Edinburgh said that it was a "great day for the pig research community". "When we launched the international pig gene mapping project almost 20 years ago, few if any of us thought a pig genome sequence was attainable or affordable."

Professor Larry Schook (University of Illinois), who lead the team, said: "The pig genome sequence is an essential first tool that will allow scientists to delve into the health, science and natural history of the pig".

A female Duroc pig from a farm at the University of Illinois was used for sequencing project. At the conference it was also announced that further funding has been received to sequence the porcine Y-gene, which may shed further light on, for example, male fertility problems in mammalian species.

SABRE provided €1.6 million in funding towards the draft sequence of two chromosomes of the pig (SSC7 and SSC14), achieved by SABRE partner Wellcome Trust Sanger Institute (SI). Some residual funds from the SABRE budget for this activity have been allocated to improve the quality of the pig genome sequence in other areas of the genome relevant to traits being pursued in SABRE Work Packages (such as boar taint and gut health). The total cost of the sequencing project was approximately \$24.3 Million. Partly as a consequence of the SABRE investment in a European contribution to pig sequencing, the (American) National Institute of Food and Agriculture (NIFA) provided a further \$10m to SI for more pig sequencing. Additional support came from other American, Asian and European funding including individual contributions from SABRE partners Wageningen University; The Roslin Institute and R(D)SVS, University of Edinburgh; Institute for Pig Genetics (IPG); Wellcome Trust Sanger Institute; and Institut National de la Recherche Agronomique (INRA).

The findings by the team are all public, with no proprietary interests attached. The latest draft of the pig sequence can be found in Ensembl: [www.ensembl.org/Sus\\_scrofa](http://www.ensembl.org/Sus_scrofa)



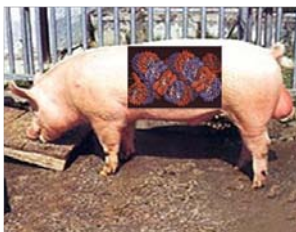


## SABRE WP2 (Epigenetics) Results

### Nutritional effects on expression profiles, epigenetic modifications and their inheritance in pigs

Klaus Wimmers (FBN Dummerstorf) and Martin Braunschweig (University of Berne)

Pregnancy is a period of rapid growth and cell differentiation. It is also a period when mother and offspring are vulnerable to changes in dietary supply. The impact of maternal nutrition during pregnancy on gene expression profiles and phenotypes of offspring has been shown experimentally and in epidemiological studies. These effects may accumulate resulting in so called metabolic or nutritional or foetal programming. One potential mechanism that could bring about long-term changes in gene expression is altered DNA methylation. Because DNA methylation patterns are largely established in utero, the embryonic and foetal environment may alter DNA methylation, inducing stable changes in gene expression that may be sustained throughout the life span of an individual. There is also a growing body of evidence in mice and man that nutrition can induce such epigenetic modifications that are not completely erased in the germline and thus transmitted to the next generations. Using the pig model, the SABRE study conducted at the FBN Dummerstorf (FBN) aimed at demonstrating foetal programming on the level of gene expression, listing genes sensitive to foetal programming, and evidencing and evaluating the role of DNA methylation in this phenomenon. In the SABRE project at the University of Berne (UBERN) it is hypothesized that the nutrition related transgenerational effects are epigenetic in nature and the transmission is followed through the paternal line. This implies that nutrition induced epimutations are not reprogrammed in gametogenesis or in the early embryo resulting in transgenerational epigenetic inheritance. Therefore, at the FBN German Landrace (DL, n =18) and Pietrain (PI, n =18) sows were fed either a control diet (CON) or a methyl-supplemented diet (MET) consisting of cofactors and methyl donors required for the one-carbon metabolism. Diets were fed starting 10 days before insemination until slaughter for tissue sampling. In order to investigate whether differential nutrition of F0 Swiss Large White (LW) boars affects gene expression and carcass traits in the next but one F2 generation at UBERN two groups of eight F0 boars received either a control diet (CON) or a methyl-supplemented diet (MET). F0 boars were mated to LW sows to produce the F1 male generation which was fed the control diet. These F1 boars produced 36 (MET group) and 24 (CON group) F2 pigs, respectively.



The gestation diets fed to DL and PI sows affected the global hepatic DNA methylation and foetal growth in a breed- and stage-specific manner. Liver samples of fetuses of three developmental stages (d35, d63, d91 post-conception) were subsequently monitored for diet-dependent differential gene expression by using Affymetrix micorarrays (3 sows (=litters) per breed, per stage, per diet; 2 pools of 6 fetuses each per litter; total of 72 hybridisations). Statistic and bioinformatic evaluation of the expression profiles revealed 1811, 562 and 2089 transcripts differentially regulated between CON and MET in DL foetal liver tissue samples of 35, 63 and 91 dpc (days post-conception), respectively. For PI microarrays showed 552,

1350 and 1448 transcripts differentially regulated at 35, 63 and 91 dpc, respectively. Genes of pathways related to metabolism of lipids, amino acids, carbohydrates, cell growth, proliferation, differentiation, and communication were differentially expressed. In DL and PI top ranked biological functions enriched among the lists of differentially expressed genes include methionine metabolism pathway, IGF-1 signaling pathway and BMP signaling pathway at 35, 63 and 91 dpc, respectively. Even at peripubertal age persistent alteration of expression of about 200 genes of tissue development, cellular growth and proliferation, gene expression, and protein DNA interaction was found. Growth of PI offspring of sows receiving the MET diet was reduced throughout postnatal development. Ongoing evaluation of peripubertal expression profiles and phenotypes and the quantification of the global and locus specific DNA methylation will shed light on the role of this epigenetic mechanism as the molecular basis of genotype-environment (diet) interactions.

In order to address transgenerational epigenetic inheritance IGF2 and IGF2R gene expression was measured in muscle, kidney and liver tissues of F0 and F1 boars as well as in liver and muscle of F2 pigs. The imprinting status of the IGF2 gene in these tissues was analyzed by means of the microsatellite SWC9. DNA methylation patterns were determined in differentially methylated regions (DMR), in a CpG island and in the QTN region of the IGF2 gene. No difference was found in gene expression of IGF2 and IGF2R genes in liver, kidney and muscle of F0 and F1 boars or in the tissues of the F2 pigs. Different global DNA methylation content was found in the liver of F0 boars. No differences were found in the differentially methylated regions at the IGF2 locus in muscle and liver tissues of F0 and F1 boars. Methylation-sensitive representational differential analysis revealed more than 20 CpG sites in liver that are potentially differentially methylated between the two F2 groups. Interestingly we found indications that the methyl-supplemented diet affects the percentage of backfat, adipose tissue and fat thickness in the next-but-one F2 generation. The challenge now is to separate the genetic from the epigenetic effects and to demonstrate that epimutations induced by the diet have occurred.

Knowledge of how nutrients target specific regulatory genes and thereby influence the overall energy homeostasis enables elucidating molecular routes of genotype-environmental (nutritional)-interaction and provides more precise diet recommendations and appropriate treatments of metabolic diseases. Furthermore the outcome of our studies adds to a better understanding of the impact of nutrition on epigenetic modifications and their inheritance and might contribute to more efficient and sustainable pork production.

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## SABRE Demonstration Projects

We are pleased to announce that the following SABRE Demonstration Projects have been awarded funding:

- ♦ Aviagen (UK) - High density genomic selection for breeder traits using sparse marker panels in commercial poultry
- ♦ France Hybrid (FR) - Accuracy of predicted Genomic Breeding values for feed efficiency within a pig sire line, using a medium throughput SNP set
- ♦ Viking Genetics (DK) - Exploiting linkage disequilibrium across breeds to improve mastitis resistance in dairy cattle
- ♦ PTP (IT) - SCAN IT—Tackling the genetic components of disease resistance in Italian commercial swine populations

## SABRE Training Funds Update

Throughout the SABRE project we have awarded training funds for (i) SABRE scientists to carry out activities to promote integration within the SABRE project ('Integrating' Awards); (ii) Early Stage Researcher (ESR) short-term placements; and (iii) New EU Member State and INCO scientist short-term placements. At the end of their placements, recipients write short reports; extracts from a selection of these reports can be found below.

### Software for Data Simulation and Genetic Evaluation

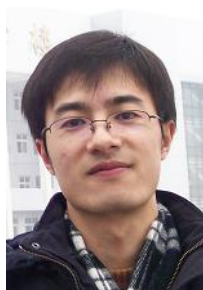
**Ricardo da Fonseca** from the Federal University of Vicosa in Brazil received a SABRE **Integrating award** to fund visits to the Roslin Institute (University of Edinburgh) in Scotland and the Institute for Pig Genetics (IPG) in the Netherlands, in Spring 2009.

At the Roslin Institute, Ricardo studied the theory of QTL mapping, association techniques using SNP markers, and the theory of genomic selection under the guidance of DJ de Koning. During his stay he also learned how to use the GenAble Software and ran some association analyses using simulated SNP data.

During his visit to IPG, Egbert Knol explained their boar taint research and introduced him to the IPG SNP database. With additional help from Naomi Duijvesteijn, he became familiar with the software that IPG uses to map QTLs, and he was involved in developing an R script to extract data from the SNP database.

Ricardo will now use this knowledge to develop routines for the data simulator that the Federal University of Vicosa is developing as part of its contribution to SABRE.

### Candidate genes for immune response and anti-viral activity



**XiangDong Liu** of Huazhong Agricultural University (HZAU) in China received an **INCO placement award** to visit the Parco Tecnologico Padano (PTP) in Italy for 7 weeks in the

summer of 2009, at the invitation of John Williams and Elisabetta Giuffra.

The objective of this placement was to test the variations of the candidate genes identified by HZAU in PTP samples from pigs with PRRSV infection and in controls, and also to establish a long-term collaboration between PTP and HZAU.

HZAU has focused on the selection of candidate genes which have functions related to immune response and anti-viral activity. These genes have been sequenced from a range of pig breeds, and polymorphisms have been identified within them. For the placement project, six candidate genes were selected: CAV1, GBP2, CD163, MHC2TA, RNASE6 and MiR29C. These genes were genotyped using PCR-RFLP and sequencing in Landrace parent samples from the MISAGEN project population. The primary results showed that CAV1, CD163 and MHC2TA are polymorphic at the tested locus in the MISAGEN population.

Additional animals will now be genotyped for these genes to evaluate putative associations between the candidate gene and PRRSV disease susceptibility.

### Candidate genes affecting embryo viability in cattle



**Natalia Renska** (Poznan University of Life Sciences) was awarded SABRE **ESR funding** to visit MTT Agrifood Research Finland for 3 months in the summer of 2009, to work with Johanna

Vilkki, Jaana Peippo, Kati Korhonen and Mervi Rätty.

The aim of her visit was to familiarise herself with a qPCR procedure suitable for embryo sample gene expression studies and to learn how to evaluate morphology of bovine embryos during *in vitro* culture. Natalia participated in the validation of selected candidate genes for embryo viability discovered in SABRE's WP6 (Fertility and Reproduction) and studied the expression of 6 genes in *in vitro* produced embryos derived from three different IVM media (PVA, PVA+GF, FBS+GF). The genes selected for validation were among the up-regulated genes identified by cDNA microarray technology in the *in vivo* part of this project. Some genes known to be correlated with calf delivery in *in vitro* embryos (El-sayed *et al.*, 2006) were also analysed to assess the quality of these *in vitro* embryos. They optimised the qPCR conditions for these genes, and made the plasmids of the gene products to be used for the standard curve, and then performed the qPCR runs of these selected genes from the embryo samples. The results of this work were presented at the 4<sup>th</sup> SABRE conference by Kati Korhonen.

More placement reports are available on the SABRE website at [www.sabre-eu.eu](http://www.sabre-eu.eu) > Get Involved > Training. Funding for placements is still available. Please contact [training@sabre-eu.eu](mailto:training@sabre-eu.eu) for further information.

## 4<sup>th</sup> SABRE Conference and EAAP 2009

The 4<sup>th</sup> SABRE Conference was integrated with the 60<sup>th</sup> Annual Meeting of the European Association for Animal Production (EAAP) in Barcelona from 24<sup>th</sup>-27<sup>th</sup> August 2009.

There were 21 theatre presentations and 13 posters arising from SABRE research, distributed throughout the four days of the conference, and there was also a dedicated EADGENE and SABRE session on "Molecular tools for disease resistance." This year's EAAP meeting was attended by over 1,500 delegates, providing a wider dissemination of SABRE's results than we could reach at our usual annual conferences. The proceedings, which include SABRE abstracts, have now been downloaded from the EAAP website more than 15,000 times!

A list of all SABRE presentations at this conference is available on the SABRE website at [www.sabre-eu.eu](http://www.sabre-eu.eu) > News and Events > 4<sup>th</sup> SABRE Conference. Further information and the proceedings can be downloaded from [www.eaap.org](http://www.eaap.org).

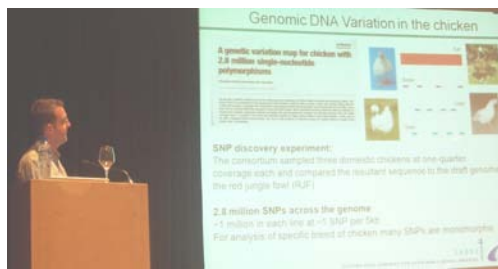


Photo: Mark Fife presenting SABRE results at the SABRE Conference/EAAP

## SABRE at 1<sup>st</sup> European Food Science Day

SABRE Coordinator Chris Warkup recently presented the results of SABRE's WP7 on Product Safety to MEPs, European Officials, and other stakeholders in a talk entitled "Improving egg safety through genetics."

Cracked or broken eggshells provide a route for pathogen contamination of eggs by salmonella and other zoonotic infections which may cause food poisoning. Work within the SABRE project has identified key genetic factors that could be used to improve egg shell quality through the selective breeding of laying hens, resulting in a decreased incidence of food contamination through cracked or broken eggs.

The European Food Science Day (18<sup>th</sup> November 2009, Brussels), was organised by CommNet, which is a network of communications managers in EU-funded research projects about food quality and safety. CommNet has members from nearly 30 projects, including SABRE and EADGENE.

The event consisted of a workshop during which the results of 9 EU funded projects were presented, followed by a Q&A session with EU Policy Makers, and a cocktail reception where networking was encouraged.

For further information and to view the presentations please visit the COMMNET website at: [www.commnet.eu](http://www.commnet.eu)



Photos: Chris Warkup presenting WP7 results and the audience at the European Food Science Day (photos courtesy of Christian Colmer, ttz Bremerhaven)



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## Upcoming Events

The following Dissemination Events are scheduled for 2010. Further details will follow in due course.

### ◆ Boar Taint Workshop

A whole-chain industry workshop addressing both genetic and non-genetic factors and solutions to boar taint will be organised in Brussels in the spring of 2010.

### ◆ SABRE Industry Training

Two Industry training events will be organised to update industry on the impact that the SABRE outcomes may have on industrial activities and results.

### ◆ 5<sup>th</sup> SABRE Conference

The 5<sup>th</sup> SABRE conference will take place as part of the 32<sup>nd</sup> ISAG Conference in Edinburgh, 26-30<sup>th</sup> July 2010.

### ◆ 5<sup>th</sup> SABRE Consortium Mtg

The fifth and final SABRE Consortium Meeting will be held in Edinburgh in conjunction with the 5<sup>th</sup> SABRE conference. The focus of the meeting will be on Dissemination of Results and the completion of the Final Report.

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Your comments and contributions are welcome.

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Food Quality and Safety