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Developmental Biology and Reproduction laboratory, INRA, Jouy-en-Josas, dir. Jean-Paul Renard

Name of SABRE partner : Gene Center of the Ludwig-Maximilian University

the name and position of the key contact person : Eckhard Wolf Supervisor

Financial support : Short-term placements awards 2007-2008

Location : Fachgruppe Chemie, Bergische Universität Wuppertal

Supervisor in the lab : Oliver J. Schmitz

Duration : 3 weeks (July, 16th – August, 1st 2007)

Title :

DNA methylation analysis of samples from nuclear transfer cows and twin cows

The collaboration between the 2 labs began in 2006 at the beginning of SABRE project as a result of several previous visits.

Stefan Hiendleder's departure to Australia modified the previous schedule. The coming of B. de Montera to Wuppertal was programmed in order to perform the experimental plan for the methylation part of Work Package 2 in SABRE.

Details of Project

In order to evaluate why adult cows with the same genotype may present phenotypical variations, we first controlled the genetic identity by initiating a comparison between genomic DNAs derived from muscle of 13 somatic cell nuclear transfer (SCNT) cows of the same genotype (2251) and between DNAs of 8 couples of twins cows (INRA) with the RDA (*Representational Difference Analysis*) subtraction technique. The group of SCNT cows contains 8 living adult cows and 5 perinatal aborted foetuses. The first results between the SCNT cows couples revealed that SCNT cow display different profiles.

In parallel, methylated cytosine measurements performed in E. Wolf's lab and Oliver Schmitz's lab (IMABB and University of Wuppertal) show the contribution of global DNA methylation level, particularly in liver, to phenotypical variations in SCNT and in vitro Fertilization Procedures bovine foetuses.

Therefore, once checked the genetic differences, we wanted to perform the *Capillary Electrophoresis* (CE) 5 methyl-Cytosine (5mC) measurements in genomic DNAs of 4 groups of SCNT cows of INRA derived from several tissues (muscle, blood, liver). The 5mC measurements in SCNT cows of INRA (Holstein breed) were to be compared with measurements in twin cows and cloned animals of the same breed (Simmental breed) from Eckhard Wolf's lab, in order to determine the part of global cytosine methylation in phenotypical variations.

This was made possible thanks to the invitation of Pr. Eckhard Wolf to learn the technique in his lab and in the lab of Dr. Oliver Schmitz in Wuppertal and thanks to the Short-term placement award from SABRE.

Funds were used for 1 travel to Wuppertal and for accommodation and consumable costs during the training period to learn the technique, and during the experimental period.

During the 3 weeks, there was an exchange of theoretical and experimental expertise between B. de Montera and Dalia El Zeihery. B. de Montera brought 35 genomic DNA already extracted but that needed to be precipitated to get rid of TE buffer. B. de Montera explained to D. El Zeihery the DNA precipitation protocol for small amounts of DNA using Ammonium Acetate. B. de Montera precipitated the 35 DNA samples successfully and put them in water.

These samples were then tested to see if they were in good enough conditions to be measured by CE. After the quality control test, Dalia El Zeihery explained the protocol to prepare the samples (digestion, derivatization, precipitation) in order to do the 5mC measurements. B. de Montera could make the measurement for one INRA sample and the control. The results showed that everything worked well.

This methylation project involved further exchange of biological samples (from liver) between INRA and IMABB (via Wuppertal) in January, 2008 by post, and in May, 2008 when Dalia El Zeihery was in Paris.

The obtained placement helped transferring the skills and experience in epigenetic profiling of tissue samples which an important technology, to Jean-Paul Renard's lab in INRA.

On the other hand, it allowed the transmission of the experience and knowledge in the genetical, physiological and clinical characterization of cloned animals of Jean-Paul Renard's lab to the labs of Eckhard Wolf and Oliver J. Schmitz.

This exchange of expertise was a further step to strengthen the already existing research links between INRA and IMABB.

Results and prospects :

The exchange of expertise led to 2 posters and a submitted article :

- **A strategy to determine the contribution of Epigenetics to phenotypical variations using adult cows with the same genotype**, B. de Montera, D. El Zeihery, J-F Oudin, J-P. Renard, E. Wolf, O. J. Schmitz, S. Hiendleder. International Embryogenomics meeting, October, 17-20th, 2007, Paris, France.

- **Highly variable epigenomes in healthy adult clones**, Béatrice de Montera¹, Dalia El Zeihery², Sigrid Müller³, Hélène Jammes¹, Gottfried Brem⁴, Horst-Dieter Reichenbach⁵, Fabian Scheipl⁶, Pascale Chavatte-Palmer¹, Valeri Zakhartchenko³, Oliver J. Schmitz², Eckhard Wolf³, Jean-Paul Renard¹, Stefan Hiendleder^{3,7}. 3rd SABRE Conference on Welfare and Quality Genomics, September, 10-11th, 2008, Foulum, Denmark.

- **Epigenetic plasticity of healthy adult cloned cattle**, Béatrice de Montera¹, Dalia El Zeihery², Sigrid Müller³, Hélène Jammes¹, Gottfried Brem⁴, Horst-Dieter Reichenbach⁵, Fabian Scheipl⁶, Pascale Chavatte-Palmer¹, Valeri Zakhartchenko³, Oliver J. Schmitz², Eckhard Wolf³, Jean-Paul Renard¹, Stefan Hiendleder^{3,7}. Submitted in Nature Biotechnology Journal, October, 2007

Such an exploration of genetic identity and global methylation variations in genomic DNA of adult animals of same genotype is a good strategy first, to help defining the real characteristics of cloned animals, then to determine epigenetic co-variables and evaluate their impact on quantitative trait loci in cattle which is a central issue of Work Package 2 in the SABRE project.

In the near future, this work will more generally allow an estimation of the contribution of Epigenetics in the phenotypic variation that is currently observed and used in genetic selection programmes.