

REPORT OF SHORT-STAY VISIT (ONE MONTH) TO THE ROSLIN INSTITUTE THROUGH THE AUSPICES OF SABRE

The aims of my placement were:

1. Training in gene expression (qRT-PCR method) on the milk cell samples from a Polish cattle population.
2. Research on lactoferrin gene expression in milk cell samples from Polish cows of various alleles. To verify the hypothesis that polymorphisms of lactoferrin promoter region have an impact on gene expression in milk cells and are associated with differences in somatic cell count in milk (measure of udder inflammation).

During my short-stay visit through the auspices of SABRE I have worked under supervision of Elizabeth Glass, PhD, Group Leader and Kirsty Jensen, PhD, in Department of Genetics and Genomics of Roslin Institute. I have learned methods of real-time quantitative PCR reaction and been trained in qPCR method using samples of cow leukocytes from dairy herd belonging to Polish Institute of Genetics and Animal Breeding (Table 1), and samples of mammary tissue kindly provided by Professor H.-M. Seyfert, stored at Roslin Institute.

Table 1. Characteristics of animals, samples used in qPCR experiment.

Cow number	Lactoferrin genotype	Health status	Days of lactation	Relative lactoferrin gene expression (control sample 827)
813	AA	Healthy	70	0,94
834	AG	Healthy	426	1,14
854	AG	Unhealthy	270	2,44
798	AA	Unhealthy	149	0,63
827	AG	Unhealthy	490	1,01
819	AG	Unhealthy	554	3,37
775	AA	Unhealthy	30	0,27
701	AA	Healthy	55	0,5

I learned how to extract RNA, to design primers for qPCR, reverse transcribe RNA, optimize primer concentration for qPCR reaction, conduct qPCR reaction and calculate and interpret the results.

Samples of RNA were extracted from epithelial cells, where lactoferrin is expressed in amounts many times larger than in milk cells. Initially I designed two pairs of primers, for bovine lactoferrin gene using Primer3 software. Then I verified primers effectiveness for qPCR reaction using NetPrimer software. The next step was to synthesize cDNA from RNA from mammary tissue. Then with the use of mentioned cDNA and designed primers I optimized primer concentration for qPCR reaction. Then, I was able to conduct qPCR reaction, using GAPDH gene as housekeeping gene for reaction optimization. During the experiment it turned out that GAPDH gene is probably not suitable as HG

gene for experiments made on material extracted from neutrophils. Its expression in neutrophils seems to be not stable enough. For further work I tend to use beta actin gene as reference gene as it is claimed to be suitable in gene expression studies of neutrophils (Zhang *et al.* 2005). Results of qPCR experiment made on mammary tissue samples show clearly that lactoferrin expression in mammary epithelial cell varies among samples from cows treated with different pathogens in different time periods.

Apart from working on mammary tissue samples I was trying to extract RNA from my frozen samples. 16 samples of milk cell pellet were sent from Poland in dry ice, preserved with RNA – later ICE for further RNA extraction. Each sample was harvested from different dairy cow. Pellet was obtained by spinning fresh milk with PBS.

Under supervision of Kirsty Jensen, PhD, I have learned RNA extraction with use of Qiagen RNeasy Kit. This method, however, was not suitable for cell pellet. There had been almost no RNA or RNA of very poor quality and quantity obtained from cell pellet. I was also trained to extract RNA using Trizol and the latter method was better for RNA extraction from leukocytes than method with use of kit. I discussed with Kirsty on methods for harvesting cells from milk. In my previous experiments, there were a lot of problems with determining methods of milk leukocytes collection and RNA extraction. We found that some of the extracted RNA samples were contaminated with DNA, and some of them were degrading dramatically fast. On current stage of work it is difficult to find if the RNA degradation was caused by not appropriate extraction method or by using RNA later – ICE for conservation of cell pellet. I also learned how to purify RNA (DNase digestion and purification using some reagents from Qiagen Kit). From half of the batch of samples send from Poland I managed to extract RNA of quantity suitable for reverse transcription and qPCR. In addition I extracted RNA from buffy coat of white blood cells for investigation of the presence of lactoferrin transcripts in blood neutrophils. Unfortunately there are almost no transcripts of lactoferrin in blood, which is consistent with paper by Pffafel *et al.* (2003). Despite very small quantity of RNA extracted from 8 of samples, I managed to synthesize cDNA and conduct qPCR reaction. Unfortunately the rest of samples were of such poor quality that it was impossible to extract enough quantity of not degraded RNA for reverse transcription.

From the results, which are shown on a figure 1, and in Table 2, one can conclude that there were differences between lactoferrin expression of individuals, but for low number of animals, included in experiment, results need verification and continuation, because results were on the edge of significance.

Figure 1. Differences in lactoferrin gene expression of animals with different lactoferrin genotype (A->G SNP, localized in promoter region) and of different udder health status.

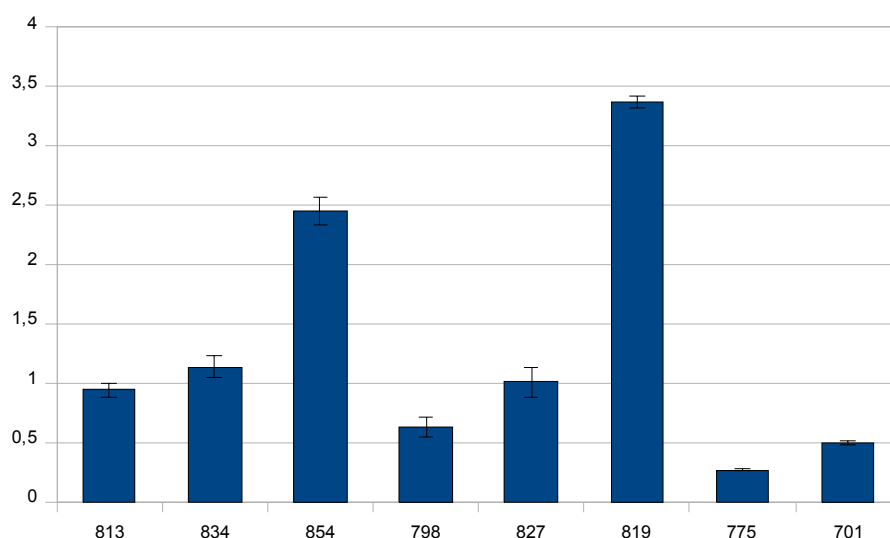


Table 2. Associations between expression and lactoferrin genotype.

Lactoferrin genotype	Expression (LSMEAN)	Pr> t
AA	0,585±0,41	0,051
AG	1,99±0,41	

Apart from training in qPCR method I was permitted to familiarize myself with microarray experiments at the Genomics and Bioinformatics Department of Roslin Institute, where I was shadowing Alison Downing at her work on microarrays.

I have been shown Affymetrix 3' IVT Express Labeling, Fragmentation, Hybridization and Staining of 3' IVT Express Labeled Arrays as well as fragmentation, Hybridization and Staining of Agilent one colour array experiments. I have learned to use Bioanalyzer for assaying RNA quality.

References:

1. Pfaffl M. W., 2001, A new mathematical model for relative quantification in real -time PCR, Nucleic Acid Research, 2001, Vol. 29 No. 9.00, p. 2002-2007
2. Pfaffl M. W., Wittmann S. L. , Meyer H. H. D., Bruckmaier R. M. Gene Expression of Immunologically Important Factors in Blood Cells, Milk Cells, and Mammary Tissue of Cows, 2003 Journal of Dairy Science, 86:538-545
3. Zhang X., Ding L., Sandford A. J., Selection of reference genes for gene expression studies in human neutrophils by real-time PCR, 2005, BMC Molecular Biology, 6:4