

DNA extraction from cartilage and milk samples for genotyping.

Application Note

**AUTOMATED DNA EXTRACTION FROM CARTILAGE AND BULK MILK SAMPLES
USING MACHERY-NAGEL'S NUCLEOSPIN™ TISSUE KIT**



INTRODUCTION

AGRANIS is a service laboratory in Laval, France, that specializes in milk sample analysis. The company has developed an innovative automated technique for determining the somatic cell count (SCC) of each cow in a herd from a bulk tank milk sample. The underlying protocol, GénoCellules® – for which Groupe Seenergi holds an international patent – represents a revolution in the world of agriculture.

The SCC is quantified as the number of cells/ml of milk, and is used as an indicator of quality, since a high SCC is typically the result of an increase in white blood cells in response to an infection. Generally, the SCC is determined by flow cytometry, requiring analysis of individual milk samples from each cow in a herd, which involves a large number of samples. However, prior genotyping of cartilage samples from each cow in a herd makes it possible to determine the SCC of each cow in a single bulk tank milk sample.

MATERIALS AND METHODS

AGRANIS uses the Nucleospin Tissue 96 kit (Macherey-Nagel) for DNA extraction of both cartilage and bulk milk samples.

This process is automated on a Fluent® Automation Workstation equipped with an eight-channel Air FCA™, a Robotic Gripper Arm™, a BioShake® 3000-T elm (Quantifoil Instruments) for heating, shaking and homogenization of samples, and a Te-VacS™ module to perform filtration steps without requiring an external centrifuge (Figure 1). The Te-VacS uses a customer-specific suction force profile to optimize the process.

The TouchTools™ interface allows any operator to preview the workflow, and walks users through each step of the automation set-up to minimize the potential for human error (Figure 2). This streamlined workflow permits reliable batch processing of 96 samples in two hours, and several plates per day.

DNA extraction from each matrix presents specific challenges; the cartilage samples are processed in a non-standard tube format, and the milk contains high amounts of protein and fat. Consequently, two separate protocols are used to allow each sample matrix to be handled appropriately.

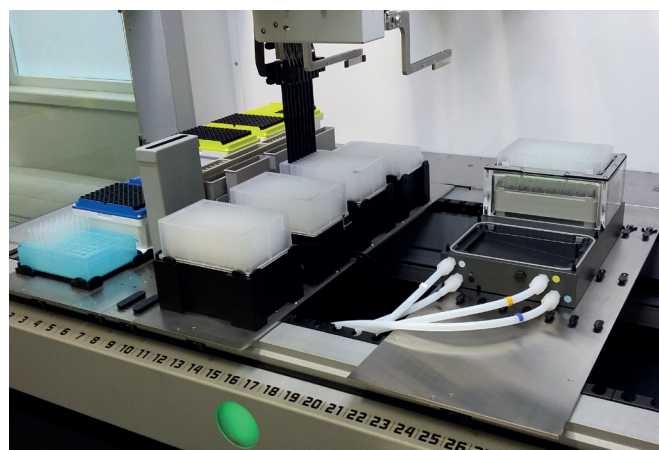


Figure 1: Worktable layout for the Fluent Automation Workstation installed at AGRANIS.

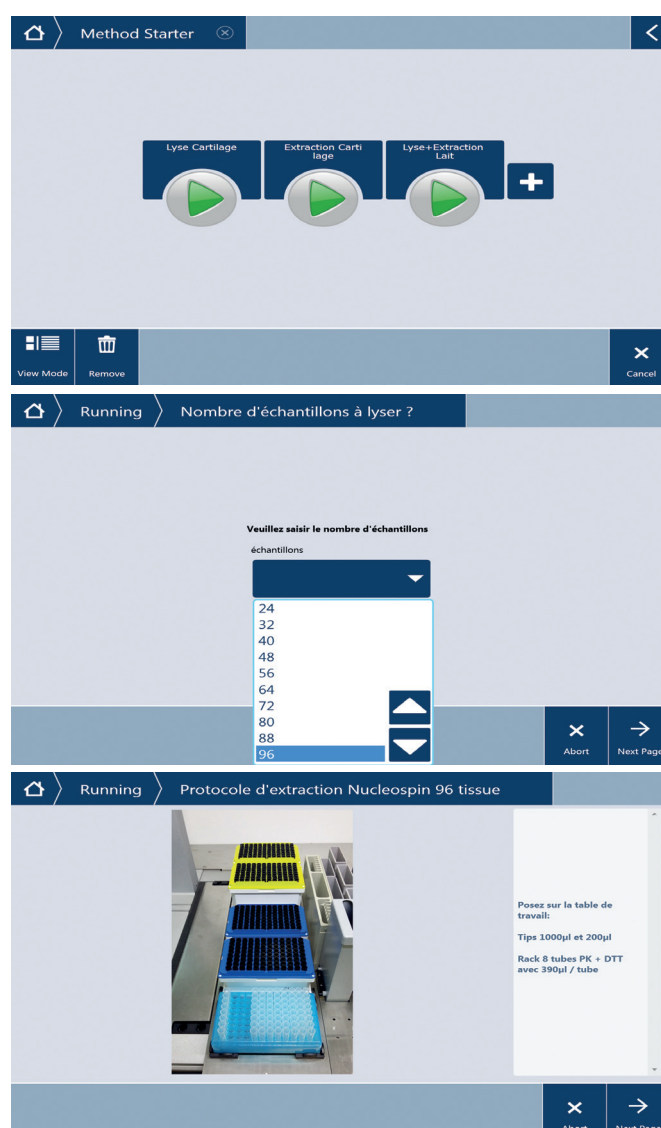


Figure 2: The TouchTools user interface allows simple selection of protocol steps.



DATA ANALYSIS AND RESULTS

DNA was extracted from cartilage samples for a herd of 52 cows using the protocol described. After genotyping all animals in the herd using a BovineSNP50 v3 DNA Analysis BeadChip protocol (Illumina), DNA from the herd's bulk tank milk sample was extracted and genotyped using the same method.

The information obtained from DNA extraction and genotyping was used to determine the SCC of each cow in the herd, and this data was compared with flow cytometric measurements made with a Fossomatic™ FC analyzer (Foss). Strong agreement between the two techniques demonstrated the robustness of the automated GénoCellules protocol on the Fluent system for the determination of SCCs from a single bulk tank milk sample (Figure 3).

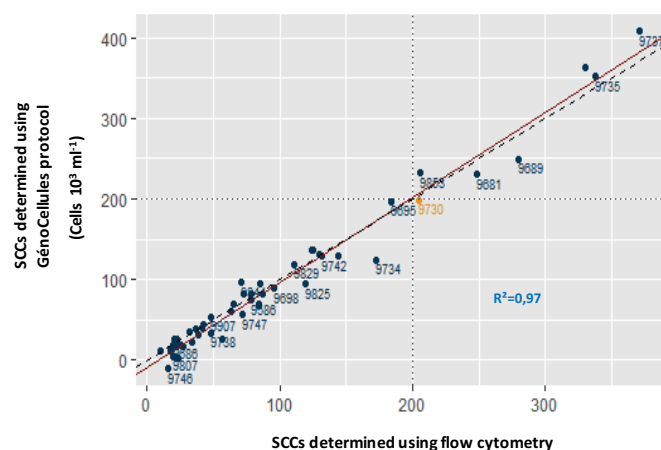


Figure 3: Comparison of SCC data obtained using flow cytometry and the GénoCellules protocol.

SUMMARY

The Fluent Automation Workstation allows AGRANIS to perform DNA extraction and genotyping using the same kit for both cartilage and milk samples, overcoming the specific challenges associated with each sample matrix. The integrated and easy-to-use TouchTools interface allows users to preview the entire work plan, and guides them through worktable loading to limit the risk of error.

The combination of precise pipetting and efficient mixing and heating leads to an increase in the overall DNA yield, and the integrated Te-Vacs removed the requirement for external centrifugation steps. In addition, true parallel arm movements allow the extraction to take place alongside further analyses required by AGRANIS.

The automated protocol has enabled AGRANIS to develop an innovative approach to herd management. Today, it is possible to rapidly genotype each cow of a herd and determine individual SCCs from a bulk tank milk sample, using the GénoCellules protocol. This is beneficial to breeders and dairy farmers alike, as they only need to supply AGRANIS with a single bulk tank milk sample, and the company can carry out twice as many analyses in the same time. This enhanced speed enables reliable batch processing of 96 samples in just two hours, and several plates per day.

LEARN MORE

To learn more about nucleic acid purification solutions, contact your Tecan sales representative or visit www.tecan.com/NAP.

ACKNOWLEDGEMENTS

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- Magali Foucher, assistant director, quality manager and specialist in milk recording
- Marie-Françoise Quesnel, laboratory manager, specialist in free fatty acids and inhibitor detection
- Nathalie Taupin and Marie Collet, molecular biology technicians

Additional data was provided by Pierre Lenormand, research and development engineer at Groupe Seenergi.



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Dr Florent Perrin is the genomic manager at AGRANIS. He studied molecular biology and genetics at the University of Angers and the French National Institute for Agricultural Research in Rennes. During his PhD, he focused on understanding molecular mechanisms of plant defense against biotic and abiotic stresses, and worked on a CRISPR/Cas9 genome editing method applied to potatoes. He joined AGRANIS in 2017, concentrating on the management and development of genomics and molecular biology activities.



Dr Beatrice Marg-Haufe is a product manager at Tecan Switzerland, with over 10 years' experience in assay development and product management. She studied biochemistry at the University of Bielefeld, Germany, and at Harvard Medical School, USA. Beatrice focused on cancer research during her PhD in biochemistry at the MPI, Munich, Germany. She joined Tecan in 2009 to work on applications for the agriculture and genomics market.

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