Characterization of a pig skeletal muscle microarray to study pork quality: the GenmascqChip 15K

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Abstract
Pork quality is highly variable and availability of relevant predictors of fresh meat quality at slaughter is critical to optimize carcass use and improve its valuation. High throughput gene expression studies have been widely used to describe biological mechanisms underlying variation of several traits but data are scarce regarding their use as tools for development of biomarkers. The GENMASCQ (GENomics enabled Marker Assisted Selection and Certification of Quality in Pork products) research program was set up to select, using high-throughput gene expression screening, markers of the biological variation mostly appropriate to describe, and ultimately forecast, the sensory quality of an important meat product, accounting for nearly 40% of meat protein sources in human food. This report describes the characterization of a porcine skeletal muscle microarray, the GenmascqChip 15K, as a new tool to study pig meat quality. This microarray is annotated at nearly 90% and permits to explore a list of 9169 unique genes.

Introduction
Meat quality is a complex trait which strongly depends on various physiological criteria (fibers characteristics, intramuscular fat content, type of collagen, proteolysis intensity...) affected by genetic, nutritional or environmental regulation factors. Moreover, if major genes have already been identified for two defaults of meat quality (Pale Soft and Exsudative meat and acid meat), most problems are still poorly understood (Bidanel and Rothschild, 2002) and variability in pork quality has to be better understood. Moreover, the global nature of genomics technology could be an advantage for elucidating the complex physiological control of meat quality, which is likely mediated through multiple biochemical and molecular mechanisms. To this end, the GENMASCQ (GENomics enabled Marker Assisted Selection and Certification of Quality in Pork products) research program has been launched to better describe and predict pig meat quality using functional genomics tools (both proteomics and transcriptomics tools). In a previous work, we used an heterologous microarray designed from human and murine genes (MyoChips,
http://cardioserve.nantes.inserm.fr/ptf-puce/spip.php?article18) to study gene expression in skeletal muscle of two groups of pigs with contrasting levels of intramuscular fat (Liu et al., 2009). However, the modest number of differentially expressed genes prompted us to design a most sensitive and powerful microarray for pork quality research. This 15K microarray, called GenmascqChip is described in this report.

Material and methods

**GenmascqChip 15K design**

To quantify the widest possible number of transcripts known to be expressed in skeletal muscle, around 30 000 consensus sequences were selected from 12 skeletal muscle and 1 adipose tissue libraries in the PEDE database (Pig EST Data Explorer; http://pede.dna.affrc.go.jp/), microarray hybridization results using NRSP8-Qiagen array (Zhao et al., 2005) and microarray hybridization results using pig AGENAE 9K microarray (Bonnet et al., 2008). Then, the corresponding Sigenae (http://www.sigenae.org/) Sus Scrofa contigs sequences were used to create a 44K pig microarray (one or two probes per contig) using the eArray Agilent web design application (https://earray.chem.agilent.com/earray/). After hybridization of this 44K microarray with skeletal muscle RNA from 24 pigs with contrasting redness (a*) measurements, 15198 probes were selected as significantly expressed in pig skeletal muscle (i.e. signal to noise ratio higher than 2). Finally, an 8×15 K oligo-microarray Agilent format was chosen and therefore one probe per microarray and eight microarrays were fitted in each slide. This microarray platform has been deposited into National Center for Biotechnology Information Gene Express Omnibus (GEO) website (http://www.ncbi.nlm.nih.gov/geo/), and is publicly available through GEO Platform accession no. GPL11016.

**Microarray annotation**

An annotation file was produces using BLAST 2.2.23+ (Camacho et al. 2009) for megaBLAST analysis against ENSEMBL cDNA and NCBI refSeq mammal databases. Annotation was based on similarity and quality criteria (Casel et al., 2009). Among the 15198 probes of the GenmascqChip, 12939 probes (i.e. 85 % of the microarray oligonucleotides) have been linked to a unique annotated sequence and to 9169 unique genes (i.e. 30% of redundancy).

**Results**

This microarray is informative and permits to explore a list of 9169 unique genes. The hybridization results in the framework of the GENMASCQ program, i.e. 325 animals analyzed (Cherel et al., 2011), showed that about 85% of the probes pass quality control criteria indicating that corresponding genes were expressed in pig skeletal muscle. At last, amongst these 9169 genes, 8638 have human Entrez Gene ID facilitating functional analysis using frequently human orthologs. Thus, the GenmascqChip is annotated at nearly 90 %. To compare, amongst the 43603 probes from the commercial pangenomic porcine 44K chip from Agilent, 15628 probes have been annotated by SIGENAE team. The WEB-based GEne SeT AnaLysis Toolkit (WebGestalt, http://bioinfo.vanderbilt.edu/webgestalt/ ) was used for the categorization of the GO-slim (i.e. representing high-level GO) terms. As shown in Figure 1.A, these 8638 genes, expressed in skeletal muscle, encoded proteins implicated in 13 biological processes. With more than 50 % of genes, the metabolic process category is
the most important ones whereas growth category account for less than 5 % of genes. For cellular component (Figure 1.B), membrane and nucleus are the two highest categories (36 and 32 %, respectively). Last (Figure 1.C), binding activity for protein, ion and nucleic acid appears as the most important molecular function category (52, 22 and 18%, respectively). For the 3 GO-slim terms (i.e. Biological Process, Cellular Component and Molecular Function), around 20 % of genes were unclassified.

Conclusion

We described here a porcine skeletal muscle microarray with a large covering of gene expression in this tissue. In fact, this microarray comprises more genes than the MyoChips (8638 human genes for GenmascqChip versus 6681 for the MyoChips) and is thus more appropriate to study gene expression in pig skeletal muscle. Moreover, with 9169 genes it should be as powerful as the multi-tissue microarrays available now, either publicly (Steibel et al., 2009) or commercially 44K (Agilent, Affymetrix). This tool is really promising since we found already that the microarray data correlate to QPCR data for most genes detected to be differentially expressed using the GenmascqChip in several experiments.

References


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Figure 1. Microarray GO slim classification. 
A/ Biological Process, B/ Cellular Component and C/ Molecular Function. Each GO term category is represented by a bar. The height of the bar represents the percentage of genes observed in the category. The number of genes per category is indicated upon the bars.
C/ Molecular Function

- protein binding
- ion binding
- nucleic acid binding
- nucleotid binding
- hydrolase activity
- transferase activity
- transcription regulator activity
- molecular transducer activity
- transporter activity
- enzyme regulator activity
- structural molecule activity
- lipid binding
- carbohydrate binding
- electron carrier activity
- chromatin binding
- molecular adaptor activity
- antioxidant activity
- translation regulator activity
- oxygen binding
- protein tag
- nutrient reservoir activity
- unclassified