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Review

Functional genomics in farm animals – Microarray analysis

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Abstract

The rapid progression of farm animal genomics has introduced novel technologies capable of presenting global descriptions of biological systems at the level of gene and protein expression and protein interaction. To fully benefit from these developments, experimental designs have to be adapted to these new technologies, and important considerations must be made in the choice of technologies and methods of analysis to be used. This paper addresses practical issues in the use of microarray based methods for gene-expression analysis in farm animals, and provides an overview of different array-platforms as well as a presentation of methods and software for the analysis of array data. Experimental design and the selection of animals and samples for microarray studies in farm animals present novel challenges, which are often overlooked. In particular, the frequent use of half sibs and full sibs in animal studies increases the risk of falsely identifying genes as being differentially expressed, due to genetic linkage of the gene to a QTL or a major gene affecting the trait in question.

Keywords: Microarray; Review; Farm animals; cDNA array; Oligo array; Array analysis

Contents

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1. Introduction

Microarray technology facilitates quantitative assessment of gene expression levels for several thousand genes simultaneously. Patrick Brown's group at Stanford was the first to print arrays of PCR fragments amplified from cDNA libraries on a glass surface the size of a standard microscope slide using a robotic printing device (DeRisi et al., 1996). The technology remains essentially the same today and is referred to as cDNA microarrays. To conduct a microarray analysis of gene expression, RNA is purified from tissues or cells of interest and labeled with fluorescent dyes. After hybridization of the labeled RNA to the array, the slides are scanned and the fluorescent signal in each cDNA element on the slide provides a measure of the expression of the corresponding gene (Duggan, Bittner, Chen, Meltzer, & Trent, 1999). The different steps used in a microarray experiments are described in more detail in the following sections. In addition to PCR-fragments amplified from cDNA clones, it is possible to print arrays using long synthetic oligonucleotides, giving increased flexibility in design and the potential to increase the specificity of the hybridization (Chou, Hsia, Mooney, & Schnable, 2004; Hessner et al., 2004; Hornshoj, Stengaard, Panitz, & Bendixen, 2004). Typically, oligonucleotides of 50–70 bp in length are used for printing, each representing a unique sequence close to the 3′-end of a particular transcript. A special type of oligonucleotide arrays is the high-density array produced by the use of photolithographic technology to chemically synthesize short oligonucleotides directly on the surface of the chip, a technology pioneered by Affymetrix.

2. Farm animal microarray resources

To conduct microarray experiments on farm animals a number of both commercial (Table 1) and custom-made arrays are available (see Section 5). An increasing number of vendors offer to print arrays from customer-provided clone collections or to synthesize sets of oligonucleotides based on customer-provided sequence information. However, many research groups and institutions have developed their own microarray facilities. Many of these focused initially on tissue specific or otherwise specialized arrays, made by printing cDNAs fragments expressed in the tissue of interest (Bai et al., 2003; Nobis et al., 2003; van Hemert, Ebbelaar, Smits, & Rebel, 2003). Construction of genome wide cDNA arrays requires access to cDNA resources from many tissues and developmental stages in order to obtain adequate gene representation. Currently there is a critical lack of availability of genome-wide farm animal cDNA arrays. Strategies to collect cDNA libraries from many different laboratories are hampered by the use of different vector systems resulting in the need for different sets of vector primers. In addition, logistic problems in assembling appropriate cDNA-panels, amplifying and purifying PCR fragments have resulted in numerous groups to focus on the development and use of long oligonucleotide arrays, which is made feasible due to the increased availability of genomic sequences.

The decision to print homemade arrays or to use commercially available arrays requires careful consideration. Custom-made arrays require investments in expensive equipment, like robotic printing devices, and rely on the availability of cDNA libraries for the production of cDNA microarrays or on access to panels of synthetic oligonucleotides for the printing of oligonucleotide arrays. This option is most suitable for research groups and institutions with preexisting genome research facilities. However, use of oligonucleotide arrays provides the freedom to optimize array-design and to easily include new genes as more sequences become available. The use of commercial arrays requires investments in less equipment (vs. custom-made arrays), but the price per array tends to be higher. Hence, the use of in-house or commercial arrays is highly dependent

Table 1
Selected commercial available microarray resources for farm animals

Name	Array type	No. spots/probe	No. genes	Vendor[c]
GeneChip® Porcine Genome Array	High density oligo	23,937	20,201	Affymetrix
GeneChip®Chicken Genome Array[a]	High density oligo		28,000	Affymetrix
GeneChip® Bovine Genome Array	High density oligo	24,072	23,000	Affymetrix
Pig Genome Oligo Set ver. 1.0	Array ready oligos	10,665	10,665	Operon
Pig Genome Oligo Extension Set ver. 1.0	Array ready oligos	2632	2632	Operon
Pig Immune Array	cDNA microarray		2860	ARK genomics
Chicken Embryo Array	cDNA microarray		1152	ARK genomics
Chicken Immune Array	cDNA microarray		5000	ARK genomics
Chicken Neuroendocrine Array	cDNA microarray		4800	ARK genomics
Pig Oligo array[b]	Oligo microarray	13,297	13,297	ARK genomics

[a] This array also contains 689 probe sets for detecting 684 transcripts from 17 avian viruses.
[b] Qiagen Pig Genome Oligo Set Version 1.0 and the Pig Genome Oligo Extension Set Version 1.0 printed on one slide.
[c] Affymetrix (http://www.affymetrix.com), Operon (http://www.operon.com), ARK Genomics (http://www.ark-genomics.org).