

# Chromosome mapping of five differently expressed miRNAs in porcine skeletal muscle development (Brief Report)

## Kartierung von fünf miRNA mit differenzieller Expression während der Skelettmuskelentwicklung beim Schwein (Brief Report)

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### Background

MicroRNAs (miRNAs) are a class of short, non-coding regulatory RNAs, which are approximately 22 nucleotides in length. Typically, miRNAs negatively regulate gene expression by binding with the 3' untranslated region (UTR) of its regulatory target mRNAs. MicroRNAs are known to play diverse roles in fundamental biological processes, such as proliferation, differentiation and apoptosis (BARTEL 2004, 2009). It has been reported that *miR-1*, *miR-133*, *miR-181* and *miR-206* play important roles in skeletal muscle proliferation and hypertrophy (CALLIS *et al.* 2007, McCARTHY-ESSER 2007). We have detected porcine miRNA expression profiles during different stage of skeletal muscle development and a total of 140 miRNAs were differentially expressed (HUANG *et al.* 2008). In this study, we mapped five differentially expressed miRNAs (*mir-29c*, *mir-103-1*, *mir-127*, *mir-193b* and *mir-218-1*) using the radiation hybrid (IMpRH) panel (YERLE *et al.* 1998).

### Procedures

#### Primer design

Pre-miRNA sequences of the five miRNAs of human were collected from miRBase (<http://microrna.sanger.ac.uk/sequences/>) firstly. Then the porcine orthologues of human pre-miRNAs (identity >90%) were collected by comparing with all sequences available in the porcine Traces-other database using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=9823>). The primers were designed according to the DNA sequence containing the porcine pre-miRNAs. The primer sequences are as follows:

*miR-29c*-F: 5'-AGC AGC CAC CAG CCG TAA G-3'

*miR-103-1*-F: 5'-GGA TGT TCG GGT AGT TTG CAG-3'

*miR-127*-F: 5'-CCA GCT TTG TGA ACC ACG TAG-3'

*miR-193b*-F: 5'-GGG TGG CCT TTC CAG AAC-3'

*miR-218-1*-F: 5'-CAG TTC TGA GGA ACG TTG GAG-3'

*miR-29c*-R: 5'-TGC CCA TCC ATC TTC CAG-3'

*miR-103-1*-R: 5'-AGG TCA ATG CAG CAG AAC ATG-3'

*miR-127*-R: 5'-GGA GGT CGA CTG GGT CAA AG-3'

*miR-193b*-R: 5'-GAG GCT GAG CTG CTG AAT G-3'

*miR-218-1*-R: 5'-GGC AAA TAG ATG GAC TCA GGT AG-3'

### RH mapping

The PCR was performed in a 10  $\mu$ L mixture containing 1 $\times$ buffer, 20 ng panel DNA, 0.3  $\mu$ M each primer, 75  $\mu$ M dNTP, and 1.0U Taq DNA polymerase (TaKaRa, Dalian, P. R. C.). The PCR profile consisted of 4 min at 94  $^{\circ}$ C, 35 cycles of 30 s at 94  $^{\circ}$ C, 30 s at 60~63  $^{\circ}$ C, 30 s at 72  $^{\circ}$ C, and a final 5 min extension at 72  $^{\circ}$ C. The PCR results were submitted and analysed using the chromosome mapping tool available on INRA website <http://imprh.toulouse.inra.fr/>.

## Results

The mapping results of all the five miRNA genes are presented in Table 1. According to the mapping results, all the porcine miRNA genes are significantly linked to markers in the RH mapping database. Moreover according to the comparative map, the porcine miRNA mapping positions are consistent with human miRNA locations (<http://www.toulouse.inra.fr/lgc/pig/compare/compare.htm>) (GOUREAU *et al.* 1996).

Table 1

The mapping results of the miRNAs

*Ergebnisse der Kartierung der miRNAs*

Gene Symbol	Human chromosome mapping	Closely linked marker	RH mapping results			
			location of the marker	Distance (CR)	LOD score	Retention fraction, %
<i>miR-29c</i>	1q32.2	<i>SW1651</i>	9q2.6	68	5.27	19
<i>miR-103-1</i>	5q35.1	<i>SW977</i>	16q	53	7.4	37
<i>miR-127</i>	14q32.31	<i>SSC12B09</i>	7q2.6	15	15.89	24
<i>miR-193b</i>	16p13.12	<i>SW487</i>	3p	81	3.76	14
<i>miR-218-1</i>	4p15.31	<i>SW268</i>	8p	24	12.93	23

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