

The Porcine Blastocyst Transcriptome Generated From 454-Sequencing Platform



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Abstract

Currently, our understanding of the gene expression profile for early development in the porcine embryo is limited. Therefore, the porcine embryonic transcriptome is being characterized. This information will eventually be used to establish a microarray for the study of the molecular mechanisms of preimplantation development under the activities of the EmbryoGENE NSERC Strategic Network. We have chosen the Ribo-SPIA® technology (NuGen) and the 454-Genome Sequencer FLX system (Roche) to generate and sequence cDNAs from pools of expanded/hatched blastocysts (day 5-6 porcine embryos) respectively. A total of 19,438 reads were generated from a 1/8 plate run using Titanium reagent kit, resulting in 18,338 quality ESTs after filtering. All ESTs were assembled using the SeqMan Pro module from Lasergene® 8.0 and Sequencher® 4.7, resulting in 978 contigs and 2,521 singletons. Of these unique transcripts, 54% (1,889 transcripts) had significant similarities to known genes searched by nucleotide Megablast selected against reference mRNA sequences (refseq_rna) database from NCBI. Approximately 10% (195 transcripts) was identified as unique non-redundant genes that were not found in porcine RefSeq_rna database. Fifteen of these are considered to be new genes, undiscovered previously in pigs that may be blastocyst specific. The remaining 180 uncharacterized transcripts had significant matches against the EST porcine database. We further annotated the transcripts using non-porcine mammalian protein database and Gene Ontology terms. This newly found EST resource should be valuable for embryo specific microarray identification and for comparative genome analysis. Further validation of the relative expression level of transcripts is on-going using microarray.

Results

Figure 1: DNA size range from Expanded and Hatched Blastocyst

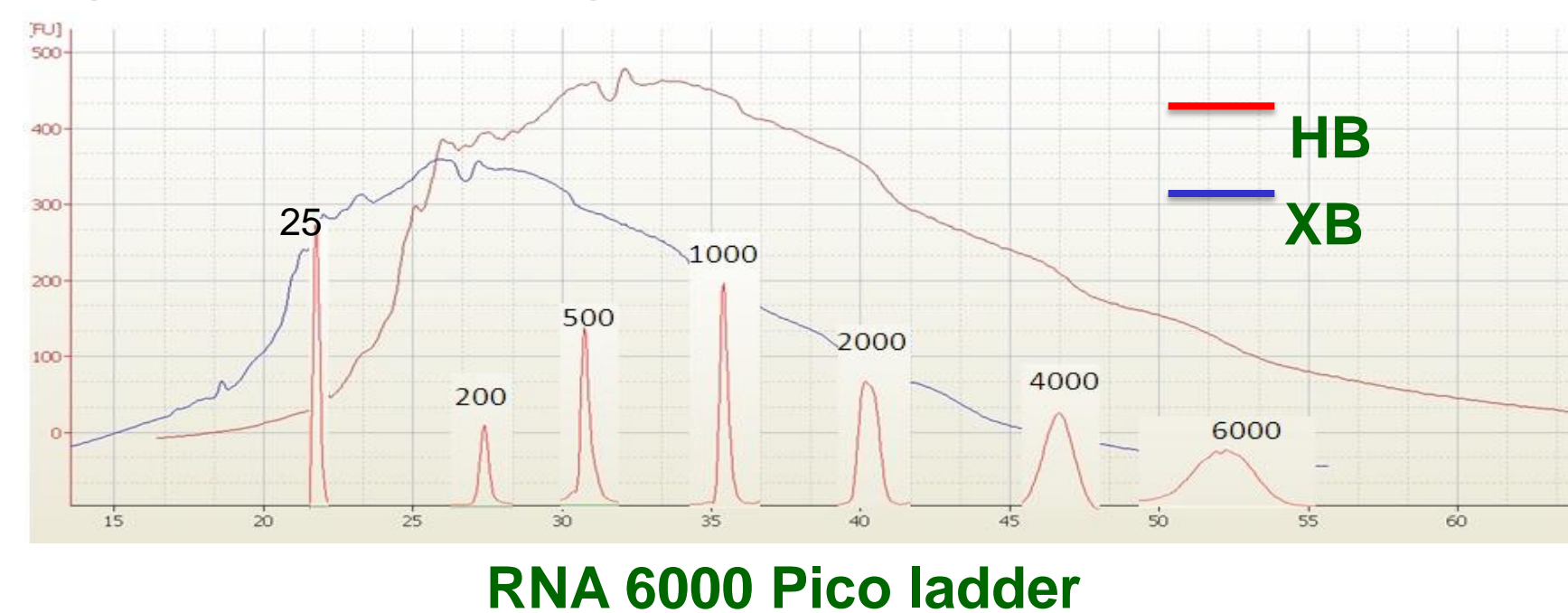


Figure 2: Sequence lengths for 18,338 sequences

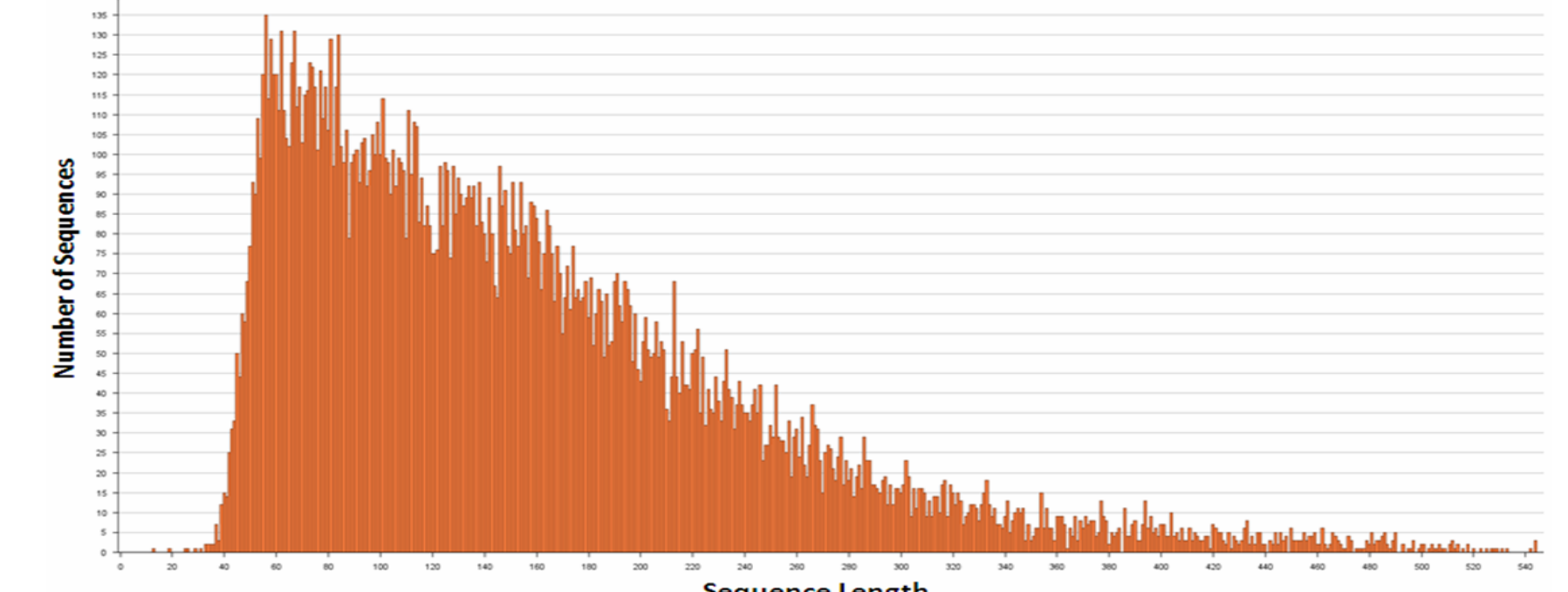


Table: 15 new genes identified by Blast⁵ from Porcine Blastocysts

Contig#	GBID	RefSeqID	Gene symbol	Description	E value	Max ident
unique_13504	133778126	NM_001083432.1	TMEM214	Bos taurus transmembrane protein 214 (TMEM214), mRNA	1.00E-73	92%
unique_18065	118197271	NM_018036.5	ATG2B	Homo sapiens ATG2 autophagy related 2 homolog B (S. cerevisiae) (ATG2B), mRNA	2.00E-44	95%
unique_12118	163838630	NM_001112736.1	C3orf63	Homo sapiens chromosome 3 open reading frame 63 (C3orf63), transcript variant 1, mRNA	2.00E-49	92%
Contig_10273	62821786	NM_014900.3	COBLL1	Homo sapiens COBL-like 1 (COBLL1), mRNA	2.00E-54	90%
Contig_10283	57222562	NM_014756.2	CKAP5	Homo sapiens cytoskeleton associated protein 5 (CKAP5), transcript variant 2, mRNA	1.00E-47	95%
unique_4695	148596983	NM_004487.3	GOLGB1	Homo sapiens golgin B1, golgi integral membrane protein (GOLGB1), mRNA	3.00E-18	92%
unique_5313	198041776	NM_001134779.1	IPO11	Homo sapiens importin 11 (IPO11), transcript variant 1, mRNA	2.00E-44	97%
unique_15075	145275203	NM_024635.3	MAK10	Homo sapiens MAK10 homolog, amino-acid N-acetyltransferase subunit (S. cerevisiae) (MAK10), mRNA	2.00E-34	95%
unique_9626	223468614	NM_015135.2	NUP205	Homo sapiens nucleoporin 205kDa (NUP205), mRNA	1.00E-127	89%
unique_12913	145046268	NM_173630.3	RTTN	Homo sapiens rotatin (RTTN), mRNA	5.00E-66	88%
unique_4583	30578409	NM_178862.1	STT3B	Homo sapiens STT3, subunit of the oligosaccharyltransferase complex, homolog B (S. cerevisiae) (STT3B), mRNA	5.00E-41	93%
unique_15986	194222464	XM_001503615.2	ORC2L	PREDICTED: Equus caballus origin recognition complex, subunit 2-like (yeast) (ORC2L), mRNA	5.00E-44	95%
unique_7808	149693662	XM_001493358.1	STIL	PREDICTED: Equus caballus SCL/TAL1 interrupting locus (STIL), mRNA	2.00E-46	93%
unique_6159	194210181	XM_001914879.1	LOC100146148	PREDICTED: Equus caballus similar to RNA binding motif protein 33 (LOC100146148), mRNA	4.00E-31	86%
unique_16183	194220760	XM_001498516.2	LOC100068692	PREDICTED: Equus caballus similar to S1 RNA-binding domain-containing protein 1 (LOC100068692), mRNA	2.00E-42	96%

Figure 3: Contigs covered the entire full length of three highly expressed genes

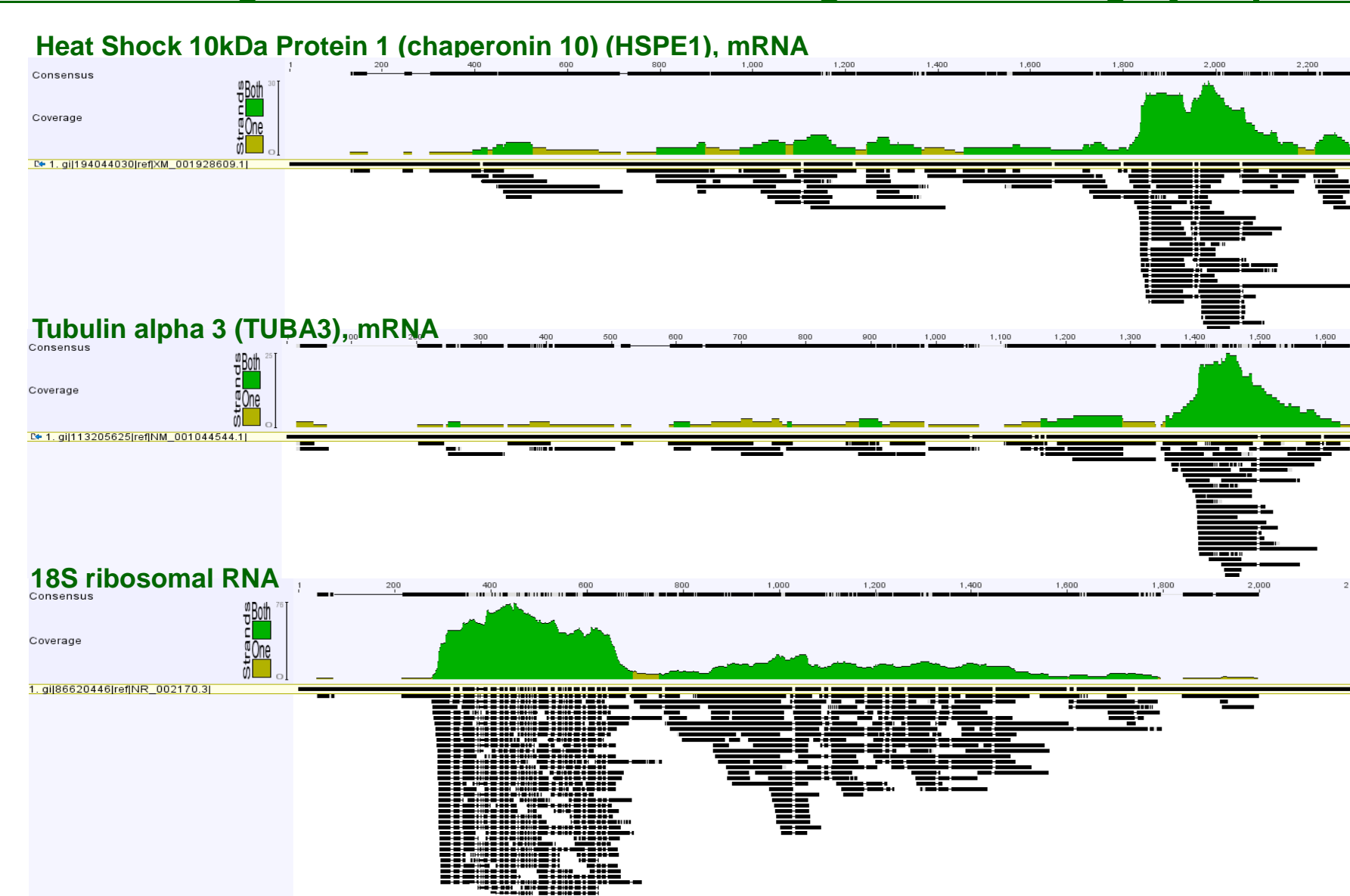


Figure 5: Possible Signaling Pathways predicated using 454 sequencing data from Day 5-6 porcine blastocysts

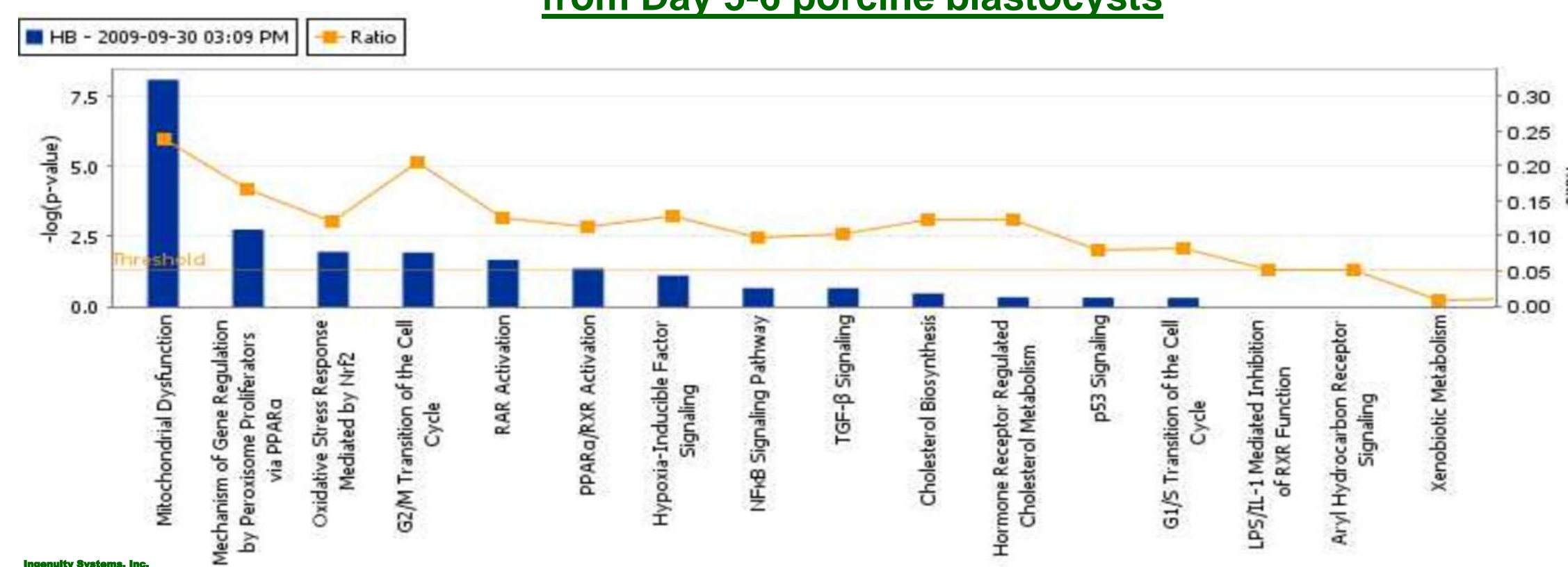


Figure 4: Genes identified by DAVID which involved in porcine blastocyst development

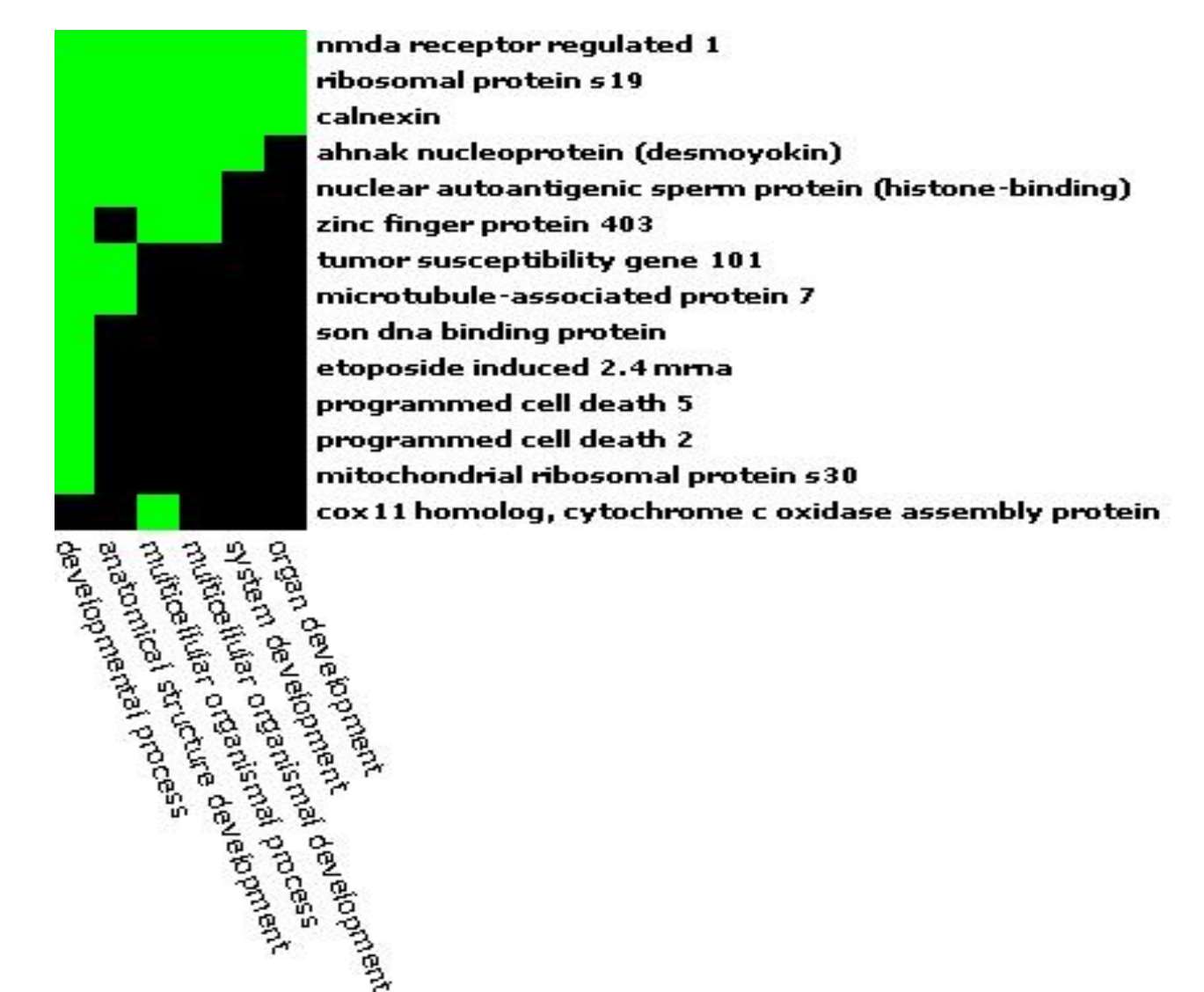
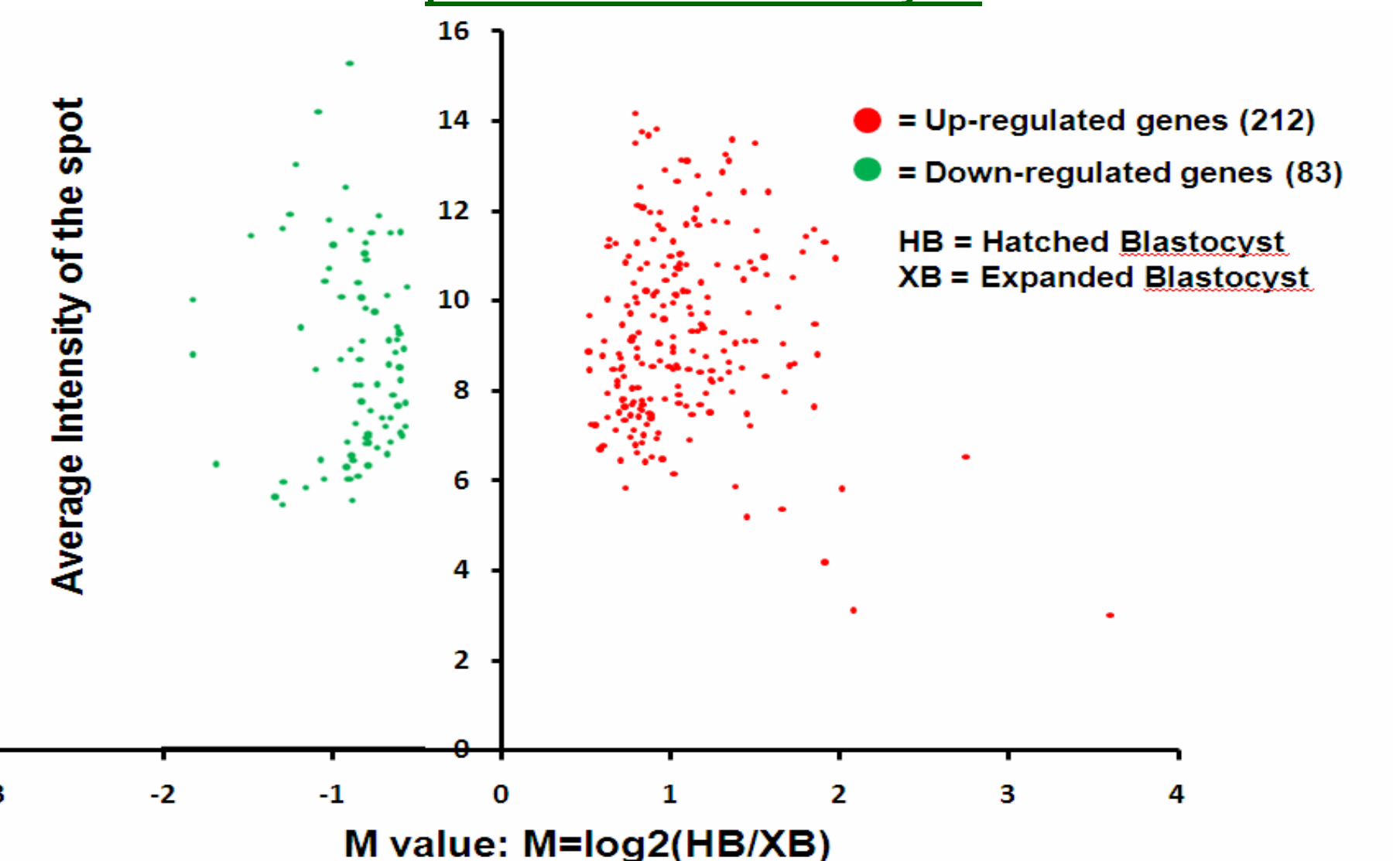


Figure 6: Microarray analysis of differentially expressed genes of porcine hatched blastocysts



Introduction

Currently, the developmental stage at which porcine embryos are most commonly manipulated and transferred is the blastocyst and the quality of the embryos transferred is critical to the success of this reproductive technology. However, our ability to determine blastocyst quality based on morphological criteria is limited. Therefore, characterization of the gene expression profile for the developing porcine embryo is being performed to identify genetic markers of embryonic quality. Identification of genes that are transcribed at this stage during early porcine embryogenesis will allow us to define the regulatory elements that are related to embryonic competence. Although recent EST sequencing of project from blastocyst has identified a limited number of developmentally regulated genes^[1], a full description of novel genes expressed at this stage is still needed. The purpose of this study was to identify additional genes that are expressed by porcine embryos at the expanded blastocyst (pre-hatching) and hatched stages of development using the 454-sequencing platform. We also compared the sequencing data to somatic microarray results to identify embryo specific genes.

Materials & Methods

Gilt management and blastocyst collection were performed according to previously described methods^[2]. RNA was first extracted using PicoPure™ RNA kit from 10 expanded and 5 hatched blastocysts. A total 10 ng of RNA was pooled from individuals with high quality (RIN>8.0) for cDNA synthesis using WT-Ovation Pico RNA amplification system and WT-Ovation Exon Module. A total of 10 µg of purified cDNA was generated for 454 sequencing using Titanium reagent kit. Artificially replicated sequences in 454 data were first removed using a 454Replicate Filter^[3] according to the default settings. Contigs assembly and gene annotation were performed by Sequencher® 4.7 and SeqMan NGen™ v2.0 respectively, with refseq_rna and pig UniGene as a reference-guided database downloaded from NCBI. Ingenuity Pathway Analysis Software was used to predicate the possible pathways using 195 well annotated genes from 454 sequencing data. The cDNA probes were synthesized from expanded and hatched blastocysts, labeled with Cy3 and Cy5 fluorescent dyes, and used to hybridize Pig-Oligo Array (USDA Pig Genome Consortium) containing 20,000 genes. Microarray data extraction was performed using WebArray (<http://webarraydb.org/webarray/index.html>) and DAVID^[4] (<http://david.abcc.ncifcrf.gov/tools.jsp>)

Summary

- The 454 sequencing data indicated that cDNA generated by Ribo-SPIA® technology (NuGen) covered the full length of the mRNA for some highly expressed genes (e.g. HSPE1 and others).
- Among the 195 unique non-redundant genes identified in the porcine blastocyst, 14 are related to embryonic development and 15 new genes were found with strong similarity to other mammalian sequences.
- Numerous genes associated with pathways involved in energy/metabolism, growth, differentiation and maintenance of developing tissue were identified and may be the focus of future projects.
- Microarray analysis identified 295 differentially expressed genes in hatched blastocyst. Most of the up-regulated genes involved in oxidative phosphorylation and ribosome function were also found in high-abundant transcripts from the 454 sequencing data.
- Future experiments on the pathways involved in cellular differentiation and proliferation during the hatching process may help to define embryonic competence and quality in the pig.

References

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