Evolution of Gene Regulation in Mammalian Tissues

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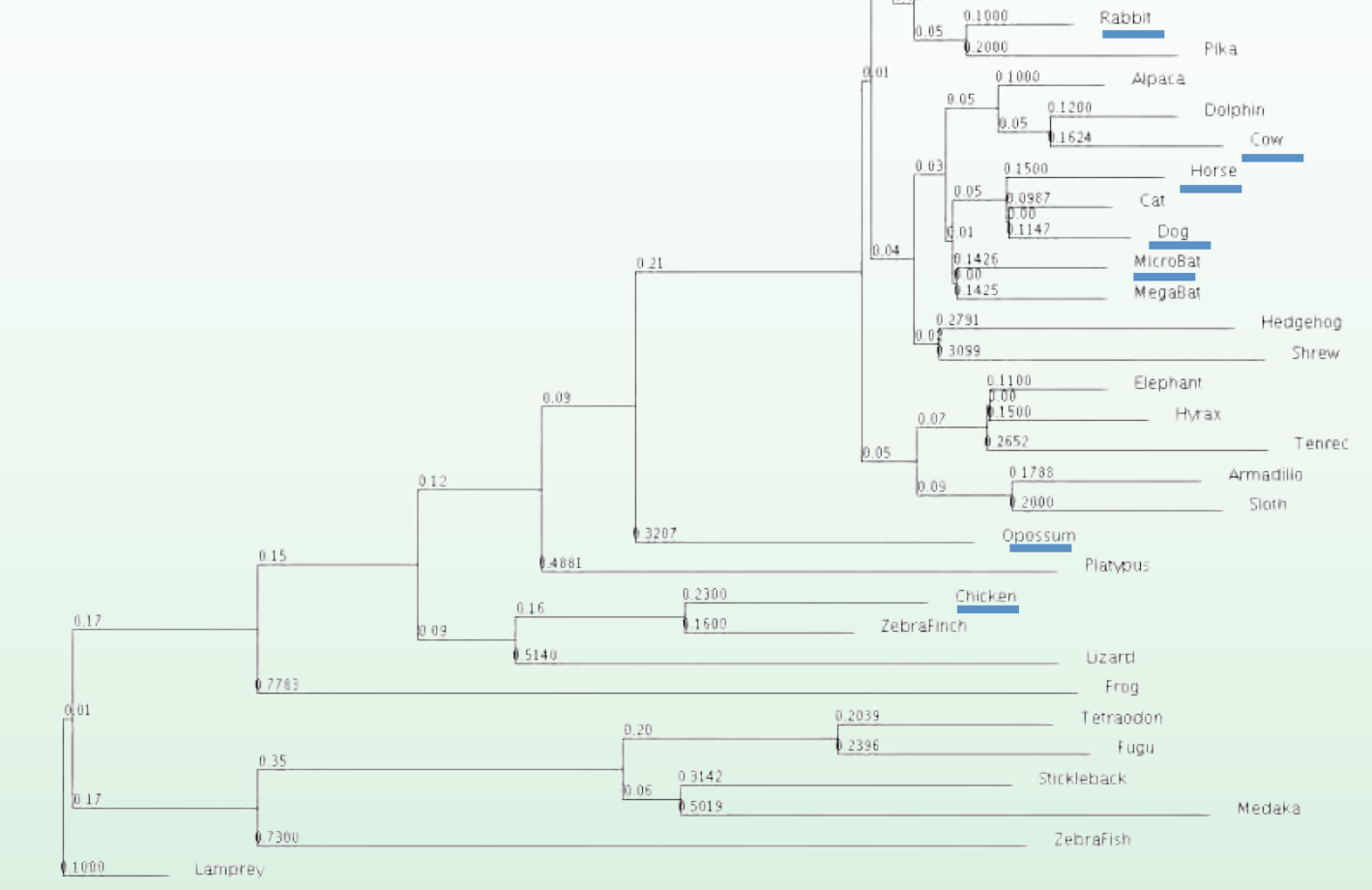### Broad Institute, Cambridge, MA, USA

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Introduction

Phylogenetic Tree of 44 Vertebrates
Source: UCSC

☐ = part of my project
— = part of future project

Variation in genotype does not play a solitary role in the diversity of mammals; instead, it has been hypothesized that differences in gene expression contribute significantly to the diversity of life [1]. Differentially expressed genes can explain the divergence in an organism's phenotype. Meanwhile, conserved expression profiles among various species may indicate that the precise expression level of a certain gene or module is under purifying selection [2,3], thus indicating the evolutionary importance of these gene regulatory networks.

At present, over 30 mammalian genomes are available, many of them recently sequenced. In order to gain further insight into variation of gene expression throughout evolution, we have chosen to use microarray technology. Microarrays are the method of choice in most large-scale whole-transcriptome studies because they allow analysis of an entire genome in a single experiment through use of high density oligo-nucleotide based arrays.
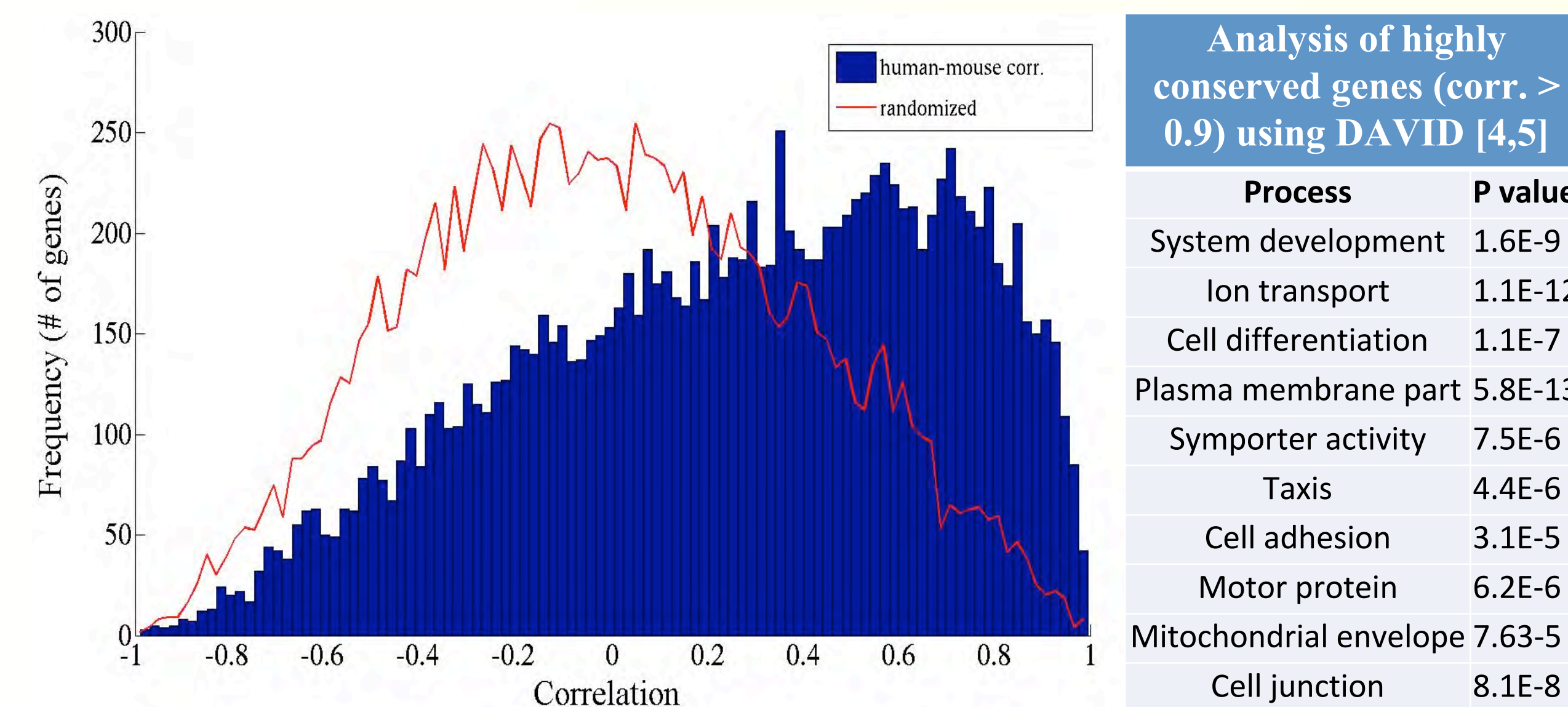
Currently, a large scale comparative gene expression study is in progress to evaluate conserved and diverged gene regulatory systems within twelve vertebrates: ten eutherian mammals, one marsupial (the opossum) and one non-mammalian vertebrate (the chicken). Numerous tissues are being investigated in order to gain a fuller understanding of evolutionary patterns of gene expression.

Vs.

Spleen   Heart   Brain   Kidney   Liver   Testes   Ovary   Thymus
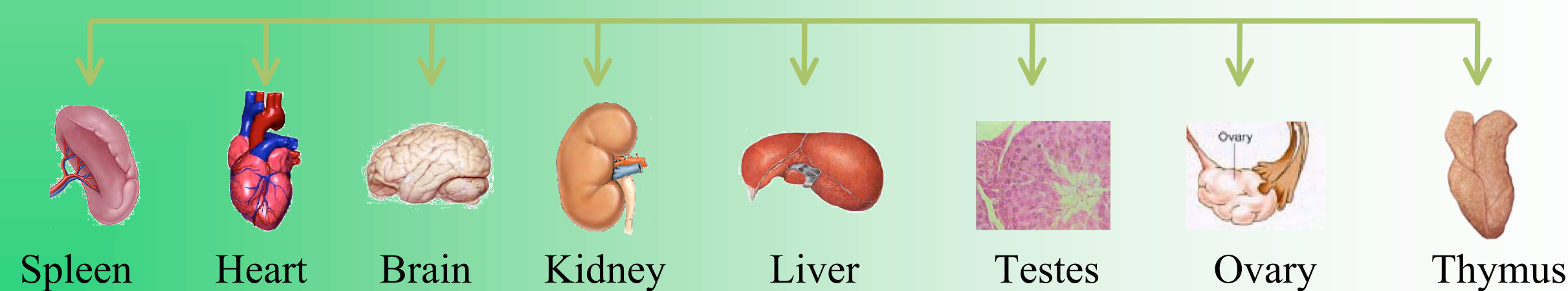
My goal is to compare gene expression between human and mouse in eight tissues:
spleen, heart, brain, kidney, liver, testes, ovary and thymus.

Results

Gene correlation analysis shows conserved expression between human and mouse

Analysis of highly conserved genes (corr. > 0.9) using DAVID [4,5]	
Process	**P value**
System development	1.6E-9
Ion transport	1.1E-12
Cell differentiation	1.1E-7
Plasma membrane part	5.8E-13
Symporter activity	7.5E-6
Taxis	4.4E-6
Cell adhesion	3.1E-5
Motor protein	6.2E-6
Mitochondrial envelope	7.63-5
Cell junction	8.1E-8

• Many genes exhibit a high correlation in relative expression across all tissues, as is indicated in the general shift to the right compared to the randomized correlation.

Tissue Correlation Analysis shows inter-species and intra-species conservation patterns
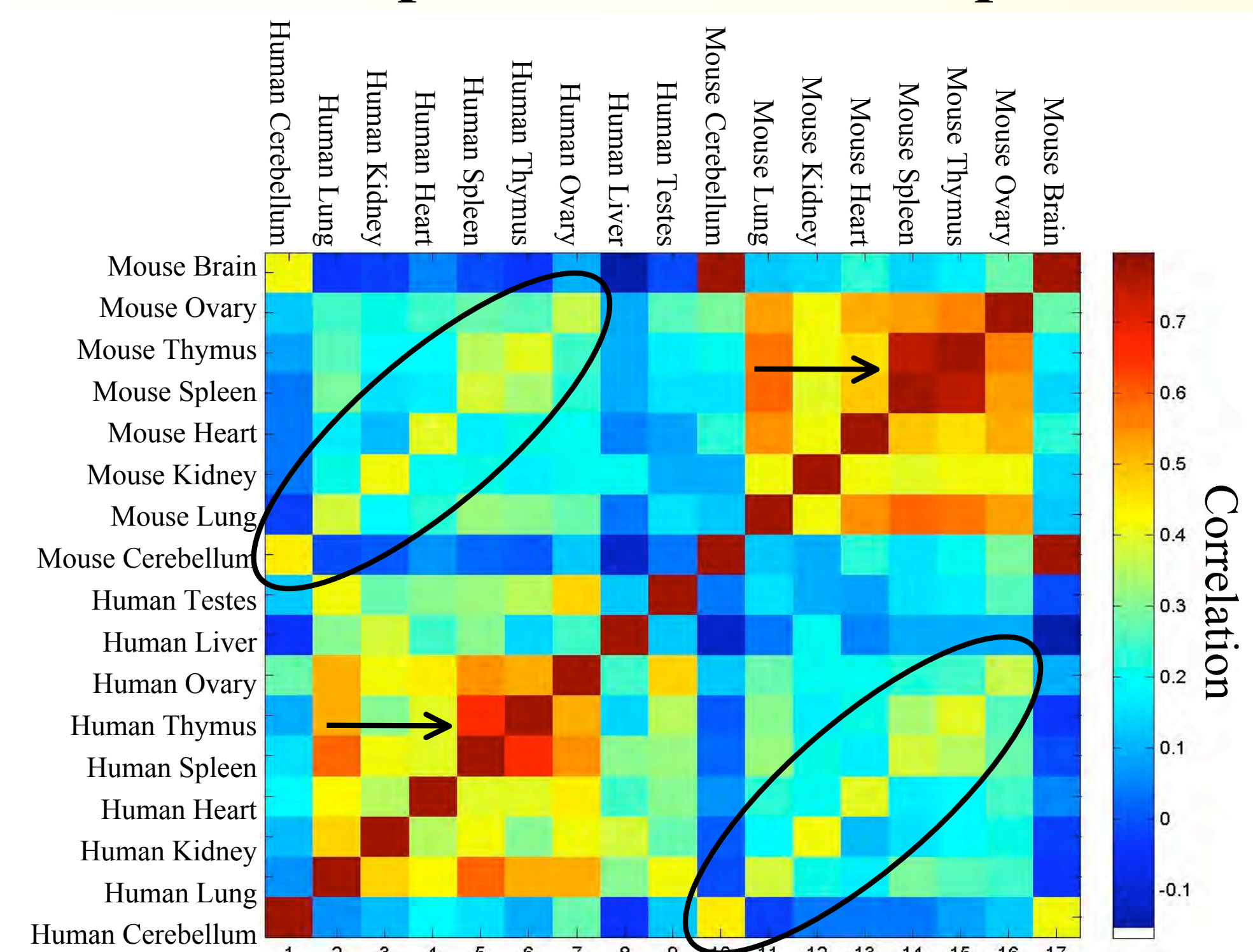
• Greater similarity of expression within a species (colored quadrants, upper right and lower left)
• High correlation between thymus and spleen, especially in mouse (arrows)
• Brain and cerebellum highly correlated to each other, but not to other tissues (blue perimeter)
• Correlation between corresponding tissues among mouse and human (circled)

Clustered Expression Heat Map shows conserved and diverged sets of genes

Corresponding tissues between species are clustered together, with the exception of spleen and thymus, and cerebellum and brain.
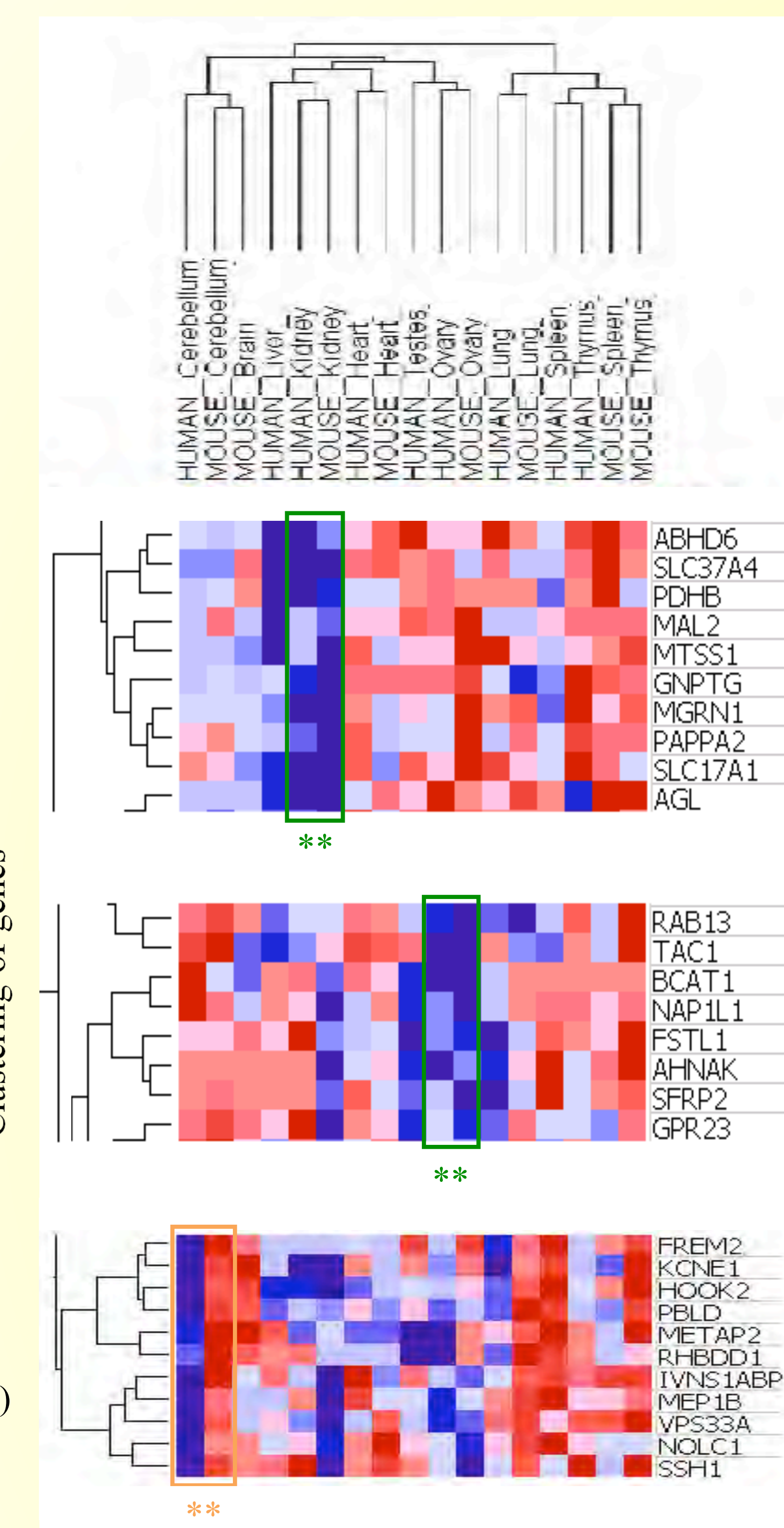
KEY to Clustered Expression Heat Map [6]
☐ = conserved expression
☐ = diverged expression
■ = down regulated
■ = up regulated

**Conserved expression: Genes are down regulated in human and mouse kidneys.

**Conserved expression: Genes are down regulated in human and mouse ovaries.

**Diverged expression: Genes are up regulated in mouse cerebellum and down regulated in human cerebellum.

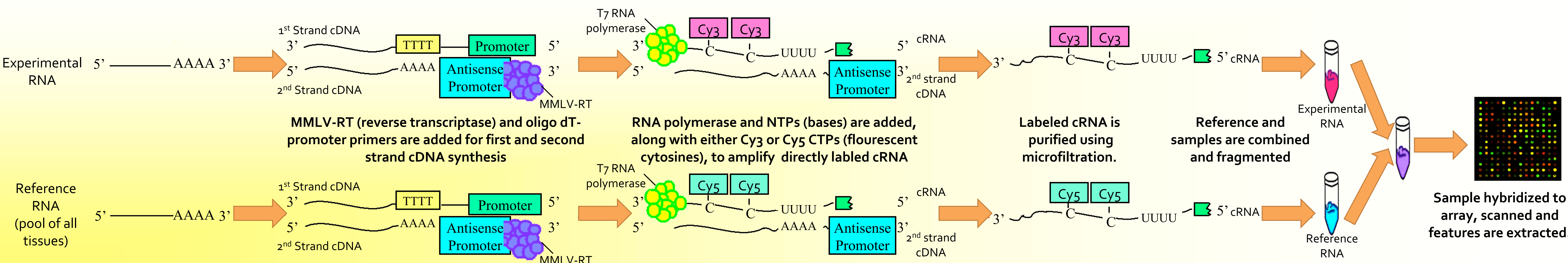### Gene Set Analysis reveals differences in expression between species

For each tissue and gene set, we tested for change in regulation between human and mouse. Shown are the gene sets with significant change in expression.
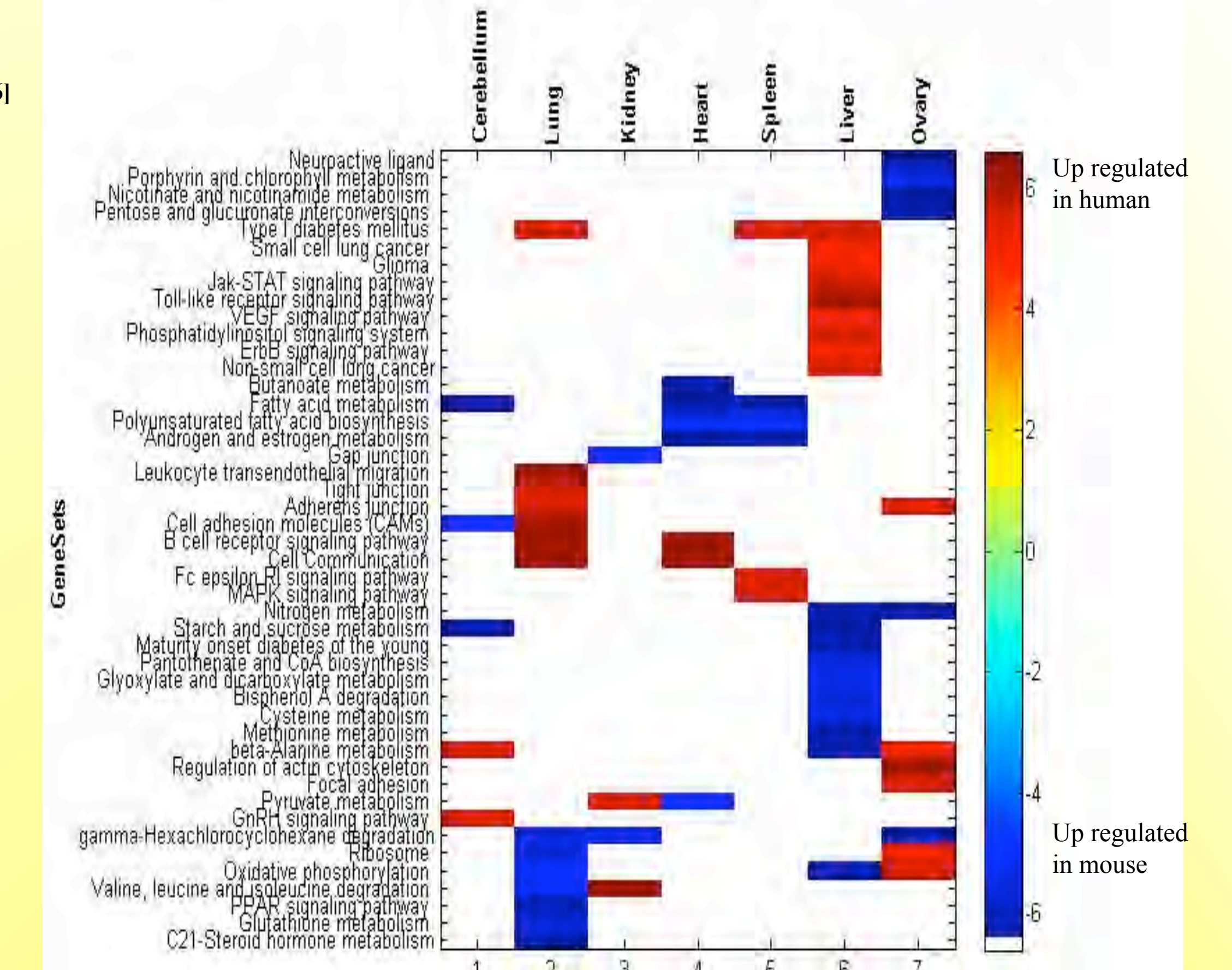
Conclusions

• The gene and tissue correlation analyses demonstrate high conservation of expression between corresponding tissues in human and mouse.
• We have identified sets of genes which exhibit high conservation of expression and have shown that they are enriched in developmental genes, ion transport and other important processes.
• We have discovered gene sets which show a consistent difference in expression between human and mouse in certain tissues. These genes will be followed up on using experimental validation.
• Further profiling in other mammalian species will be performed in order to track down the evolutionary changes in these genes' expression patterns.

Methods

MMLV-RT (reverse transcriptase) and oligo dT-promoter primers are added for first and second strand cDNA synthesis

RNA polymerase and NTPs (bases) are added, along with either Cy3 or Cy5 CTPs (flourescent cytosines), to amplify directly labled cRNA

Labeled cRNA is purified using microfiltration.

Reference and samples are combined and fragmented

Sample hybridized to array, scanned and features are extracted.

References

[1] King, M.C. & Wilson, A. C. Evolution at two levels in humans and chimpanzees. *Science* **188**, 107-116 (1975).
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[4] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protoc* **4**(1), 44-57 (2009).
[5] Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* **4**(5), P3. (2003)
[6] Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP (2006) GenePattern 2.0 *Nature Genetics* **38** no. 5 , 500-501 (2006). doi:10.1038/ng0506-500

Acknowledgements