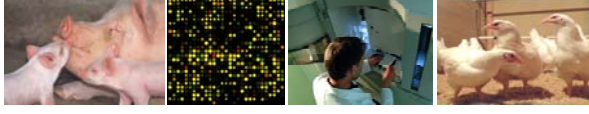


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Pathway results from the chicken data set using several softwares

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**Methods**

- 3 lists of regulated genes from :
  - MM8.PM8 (single or double infection effect),
  - MM8.MA8 (strain effect),
  - MM8.MM24 (time effect)
- Annotation using WP1 work
- GOTM software
- Pathway Studio software
- Ingenuity software

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**1. GOTM software Annotation**

- 11530 genes with human abbreviation
- among them, 10451 HUGO validated

	MM8-MA8	MM8-MM24	MM8-PM8
Number of genes	85	800	1736
Validated HUGO	38	354	931* (in 2 sets, since 500 max)

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**Test for enriched biological process GO terms**

Gene ontology analyses of clusters were performed using the Gene Ontology Tree Machine (GOTM) software [Zhang et al., *BMC Bioinformatics* 2004 ].  
HUGO = Entry key  
<http://bioinfo.vanderbilt.edu/gotm/frame.php>

Hypergeometric test was used as the statistical method to select enriched biological process GO terms for each cluster compared to the GO terms of the annotated genes present on the microarray (10451 genes).  
The biological process GO terms were considered as enriched when pvalue < 0.01.

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**4 biological processes for time, 2 for strain**

	Time effect	Strain effect
- <b>organelle organization and biogenesis</b> :	MM8-MM24	& MM8-MA8
-O=30;E=18.57;R=1.62;P=0.006		
-O=6;E=1.88;R=3.19;P=0.009		
- <b>gluconeogenesis</b> :	MM8-MM24	& MM8-MA8
-O=3;E=0.45;R=6.67;P=0.009		
-O=2;E=0.05;R=40;P=0.001		
- <b>glutamine biosynthesis</b> :	MM8-MM24	
-O=2;E=0.13;R=15.38;P=0.006		
- <b>nervous system development</b> :	MM8-MM24	
-O=20;E=10.74;R=1.86;P=0.005		

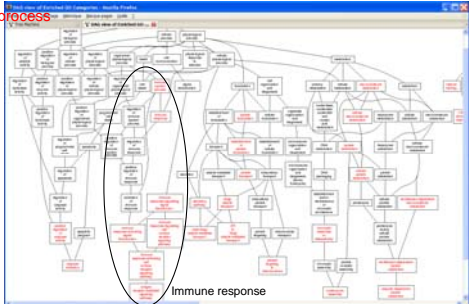
O=number of genes observed, E=expected, R=O/E=ratio of enrichment, P=pvalue

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**Single vs double infection, up-regulated**



biological process

Immune response

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Single vs double infection, down-regulated

- G-protein signaling, coupled to IP3 second messenger (phospholipase C activating):  
-O=6;E=1.81;R=3.31;P=0.009
- UDP-N-acetylglucosamine metabolism:  
-O=2;E=0.14;R=14.29;P=0.007
- regulation of DNA replication:  
-O=3;E=0.47;R=6.38;P=0.009
- endocytosis:  
-O=9;E=3.26;R=2.76;P=0.005
- tissue development:  
-O=10;E=3.77;R=2.65;P=0.004
- ectoderm development:  
-O=6;E=1.49;R=4.03;P=0.0034

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2. Pathway studio  
Sub-network enrichment analysis

- About 10% more genes identified wrt GOTM
- Demo version: 15% information of the database
- Test the over representation of sub networks from differential genes (input) wrt networks in the database (built from bibliography mining)

option=expression targets  
p-value=0.05 (H0: input genes put at random in database networks)

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4 networks

- Four networks : expression targets of
  - JUN (transcription factor),
  - SP1 (transcription factor),
  - CD8A (CD8 antigen, link with immunity),
  - IL13 (interleukine, link with immunity)
- Union of selected networks is presented (red =up-regulated in MA8, blue=down-regulated)

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Strain effect:  
only one regulated gene in the network

Protein **GNPDA1** (glucosamine-6-phosphate deaminase 1) is upregulated in MA8.

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Time effect:  
4 genes/proteins regulated in the network

Protein **GNPDA1** (glucosamine-6-phosphate deaminase 1) .  
Protein **COL1A2** (collagen, type I, alpha 2),  
Protein **OGG1** (8-oxoguanine DNA glycosylase) and  
Protein **SOX9** (SRY (sex determining region Y)-box 9  
are down-regulated in MM24.

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Double injection effect:  
protective immunity  
(memory of the cells towards infection)

All these results suggest a transcription activation during the first injection of Eimeria maxima and a role of IL13. Since CD8-alpha molecules promote the survival and differentiation of activated lymphocytes into memory CD8 T cells, it can be assumed that a protective immunity is generated.

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CD8-alpha-Madakamutti et al. (2004)

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### 3. Ingenuity

- From a list of expressed genes to a biological information
  - Gene networks
    - Biblio mining and sub-network enrichment test as in Pathway studio  
Score =  $-\log(p\text{-value})$
  - Biological functions
    - Like GOTM
  - Canonical pathways
    - Known pathways, e.g. glycogen pathway

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### Whole microarray vs differential genes

- 100 networks for the microarray genes (score max = 18)
- 22 significant networks from the 3 gene lists (score 19-55)

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### Secondary / primo infection effect: immune and inflammatory response metabolism/proliferation

14 networks involved in significant biological functions compared to microarray representation

Immune and inflammatory response

Metabolism/proliferation

- Immune function
- Immune response
- Energy production
- Inflammatory response
- Nucleic acid metabolism

■ microarray  
■ Secondary/primary infection

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### Genes of this network are immunity genes: coherent result

**Merged immunity networks:**

- Show the down expression of a majority of the genes expressed mainly in CD4+ and CD8+ T cells
- Suggest a decrease of the immune response during the secondary infection compared to the primo infection

○ Genes expressed in human thymus

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### Modulation of the protective immune response during the secondary infection

**Canonical pathway:  
Down expression of genes involved in inflammatory cytokines signalisation**

Interleukin 4      Interferon

in accordance with results of Kim et al (2008).

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### Time effect: change in cell morphology

3 networks (out of 7) and canonical pathways results show mostly an over expression of the genes involved in the actin polymerisation

Suggests a change in cell morphology that should support a decrease of the T cells migration at 24 H

Actin regulation

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### Strain effect: few differences between the 2 Eimeria strains

- 1 significant network with down expressed genes involved in inflammatory diseases in Eimeria acervulina strain compared to Eimeria maxima

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### Conclusions: technical constraints

- Number of genes taken into account are higher with Ingenuity > GOTM (limited to 500)
- Number of genes in a network limited to 50 by default in Ingenuity.
- Pathway studio: only 15% information in the demo version

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### Conclusion: biology

- Only primo vs secondary infection gene list gave significant biological interpretation with the 3 softwares: immune function and inflammatory response were much higher in primary infections (immune-system related processes)
- Nevertheless, the use of different softwares gave complementary information
- Immunologists are here alone able to identify new and original information

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### Acknowledgements

- Fabrice Laurent, INRA Tours

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