



Transcriptome Profiling of *A. fumigatus* $\Delta laeA$ in the Murine Lung

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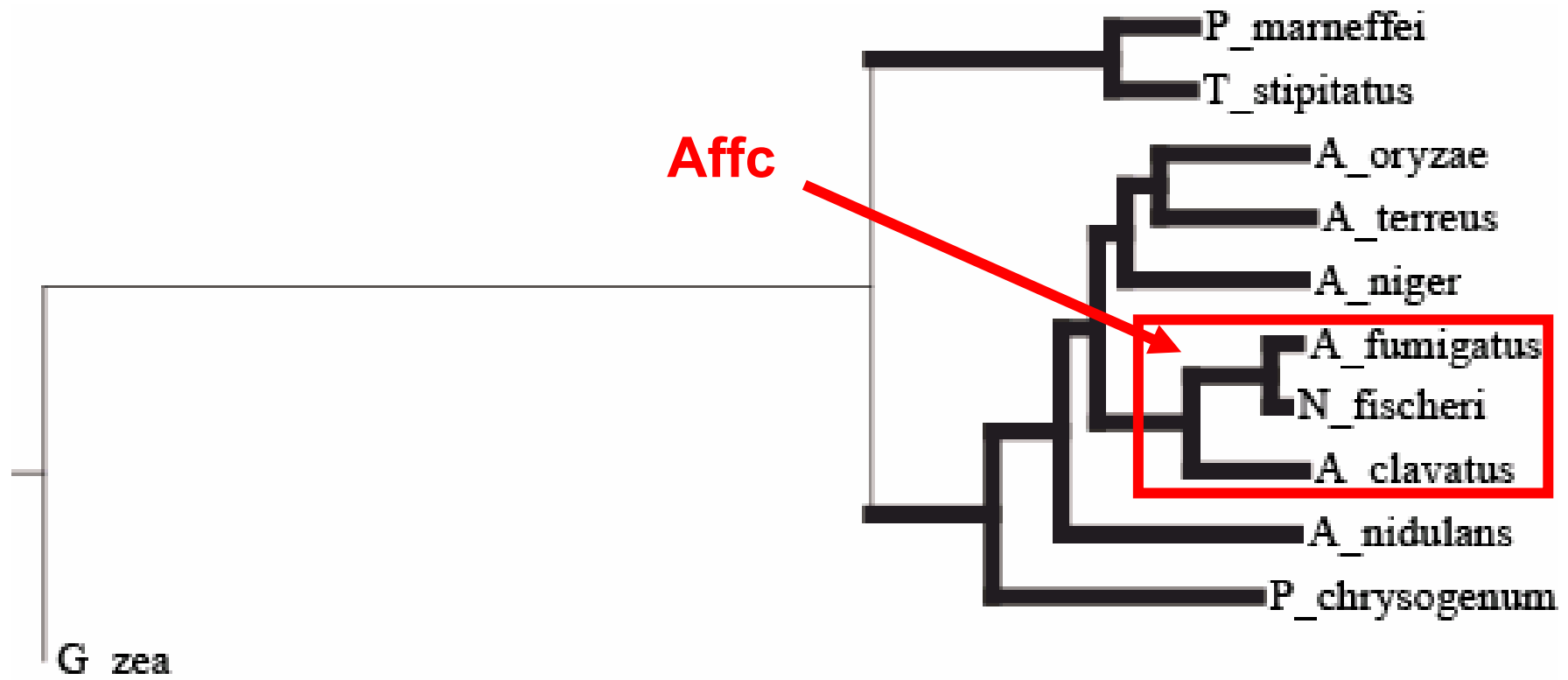
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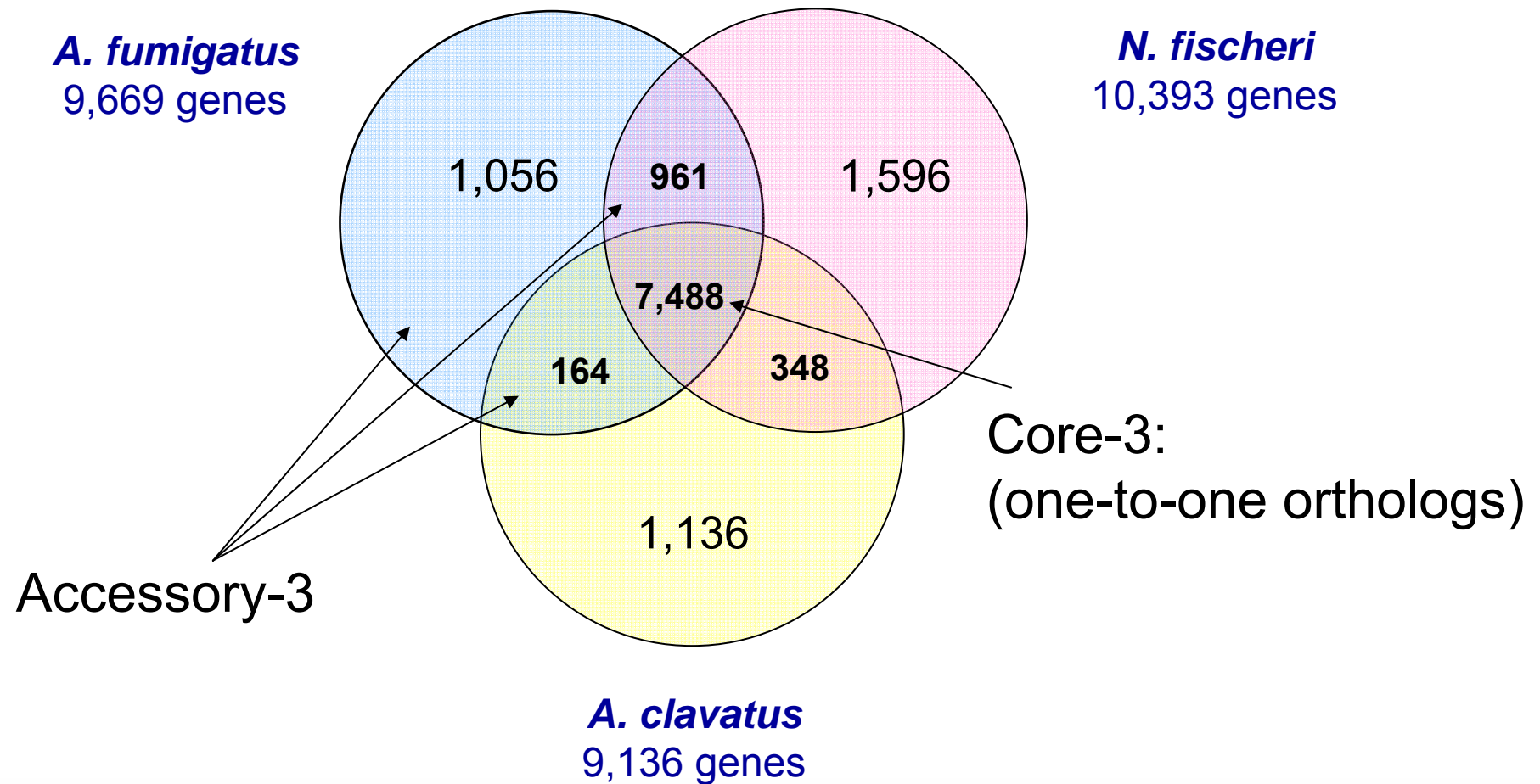
Role of LaeA in *A. fumigatus* Virulence

- Association with *A. fumigatus* lineage specific/subtelomeric region genes expressed *in vivo* relative to *in vitro* culture (with Elaine Bignell).
- Association with gliotoxin and other putative virulence factors *in vitro* profiles WT Afu vs. $\Delta laeA$ (with Nancy Keller).
- Associations *in vivo* WT Afu vs. $\Delta laeA$ (with Elaine Bignell and Nancy Keller).

Aspergillus fumigatus relatives Tree Based on 90 Concatenated Proteins



Core-3 and Lineage Specific-3 Genes

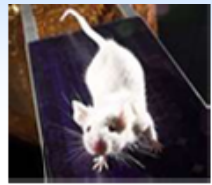


Genome Organization of *Aspergillus fumigatus*

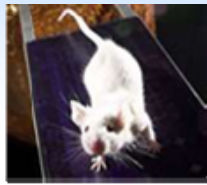
- 8.5% of *A. fumigatus* genes are species-specific.
- These genes are often found clustered together along chromosomes.
- Most are found within 300 Kb from telomere ends

Fedorova *et. al.* 2008 PLoS Genetics

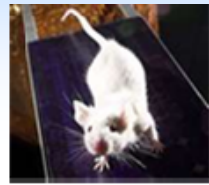
A. fumigatus Expression Analysis from the Neutropenic Murine Lung



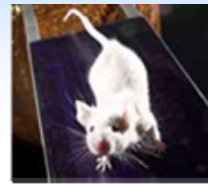
24 mice



24 mice



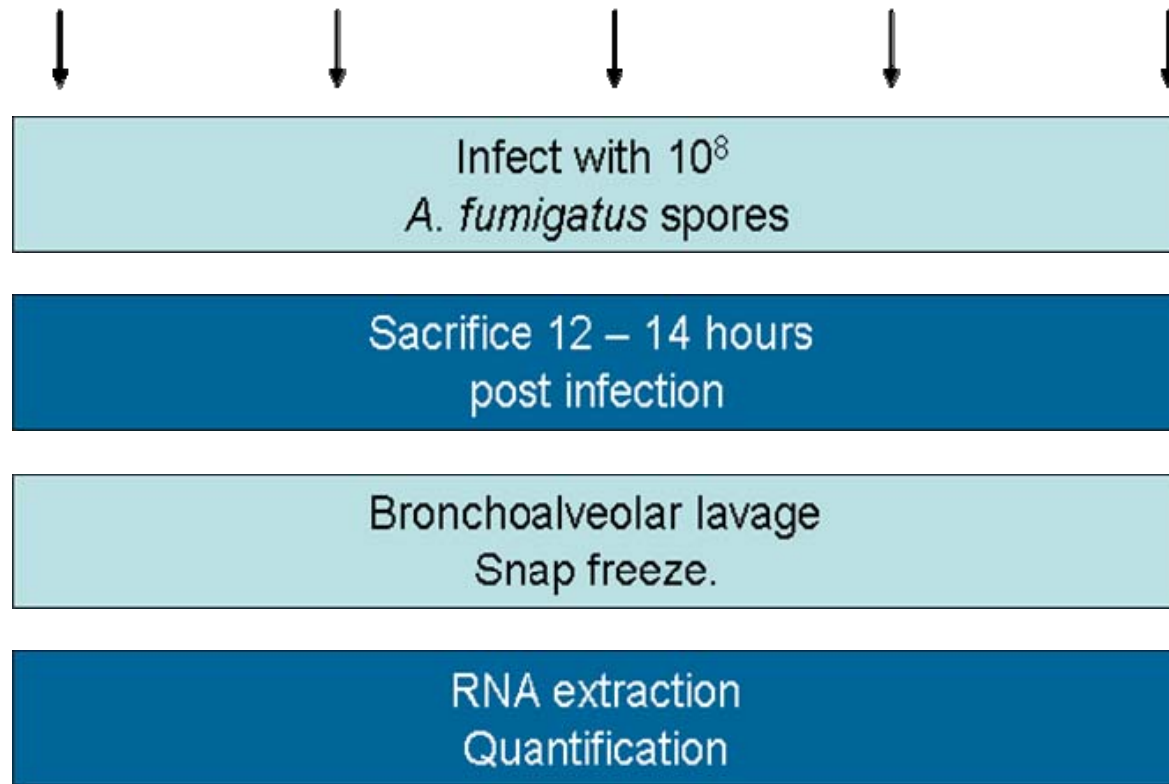
24 mice



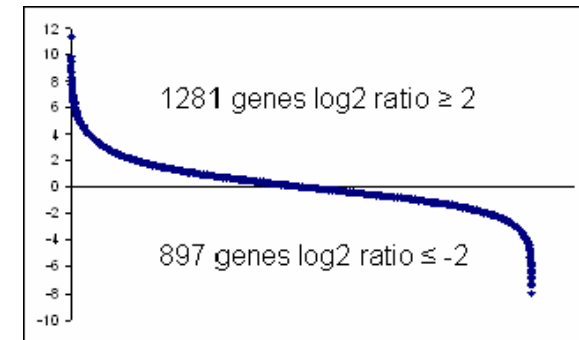
24 mice



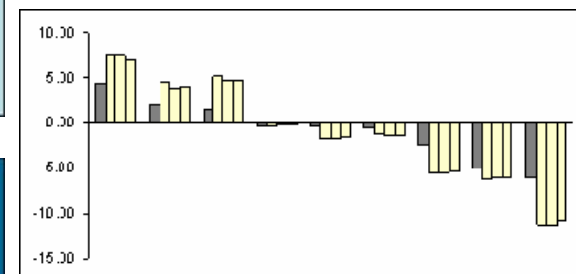
24 mice



Log₂ ratio (red-green)



Quantitative PCR



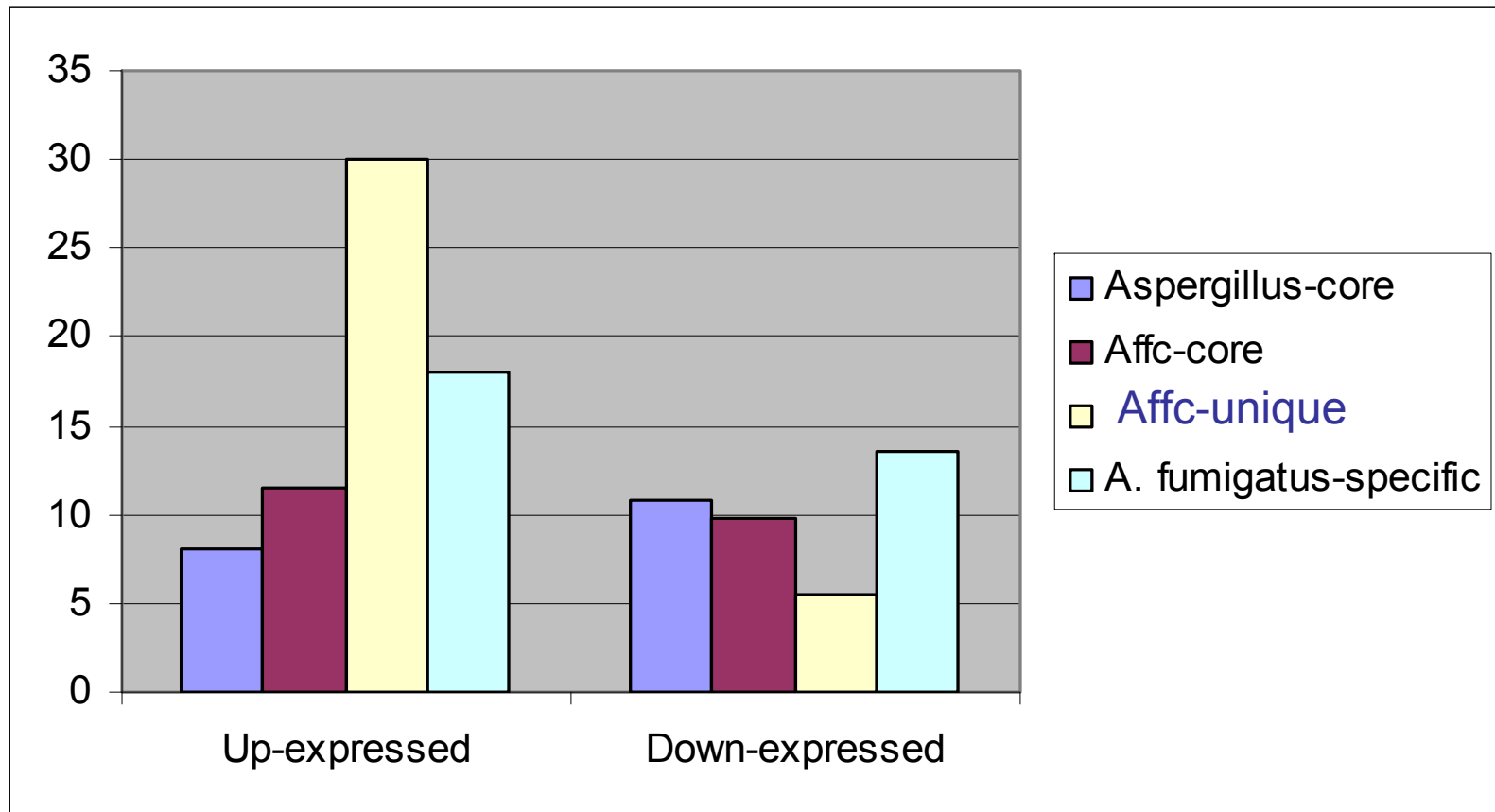
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Bignell early infection model

30% of Affc-Unique Genes Are Up-expressed *In vivo* vs. *In vitro*

% of altered-expressed genes in a category

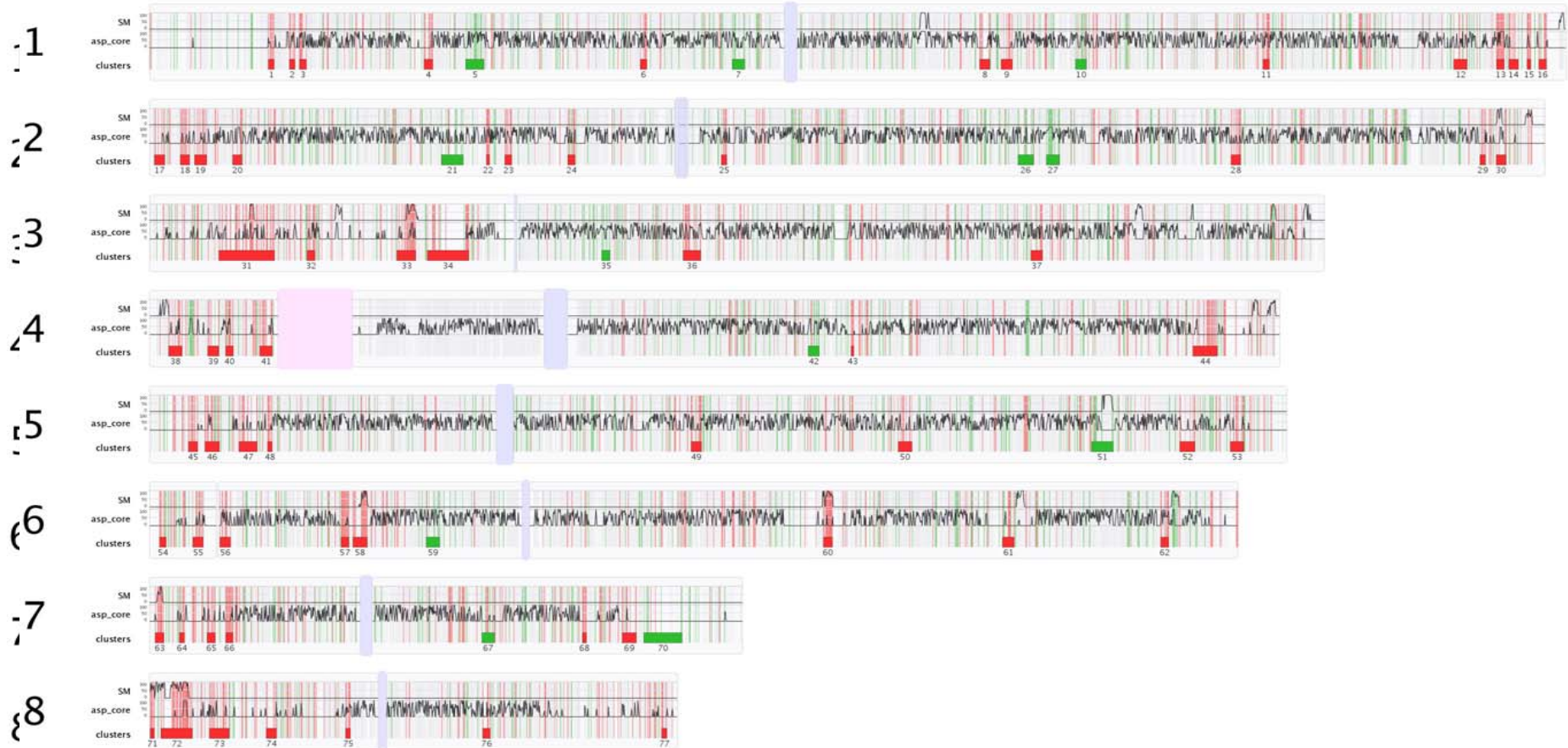


In vivo Up-expressed Genes Are Clustered on Chromosomes

- **Most (51%) up-expressed genes are found in 68 contiguous gene clusters (5 - 30 genes)**
- **Including 7 secondary metabolite biosynthesis clusters (e.g. gliotoxin, siderophore) and 61 unknown metabolic clusters**

McDonagh et al. PLoS Pathogens 2008

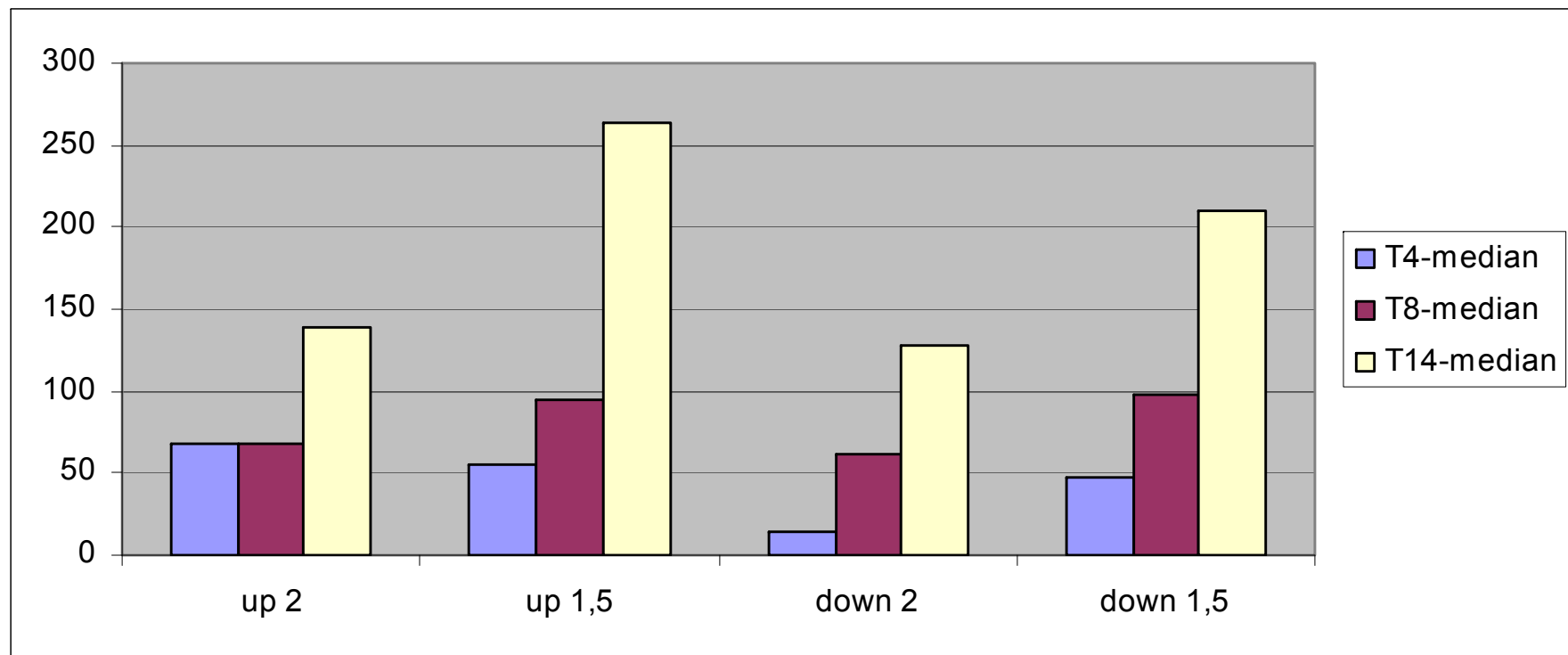
A transcriptional snapshot of *A. fumigatus* gene expression during initiation of mammalian infection



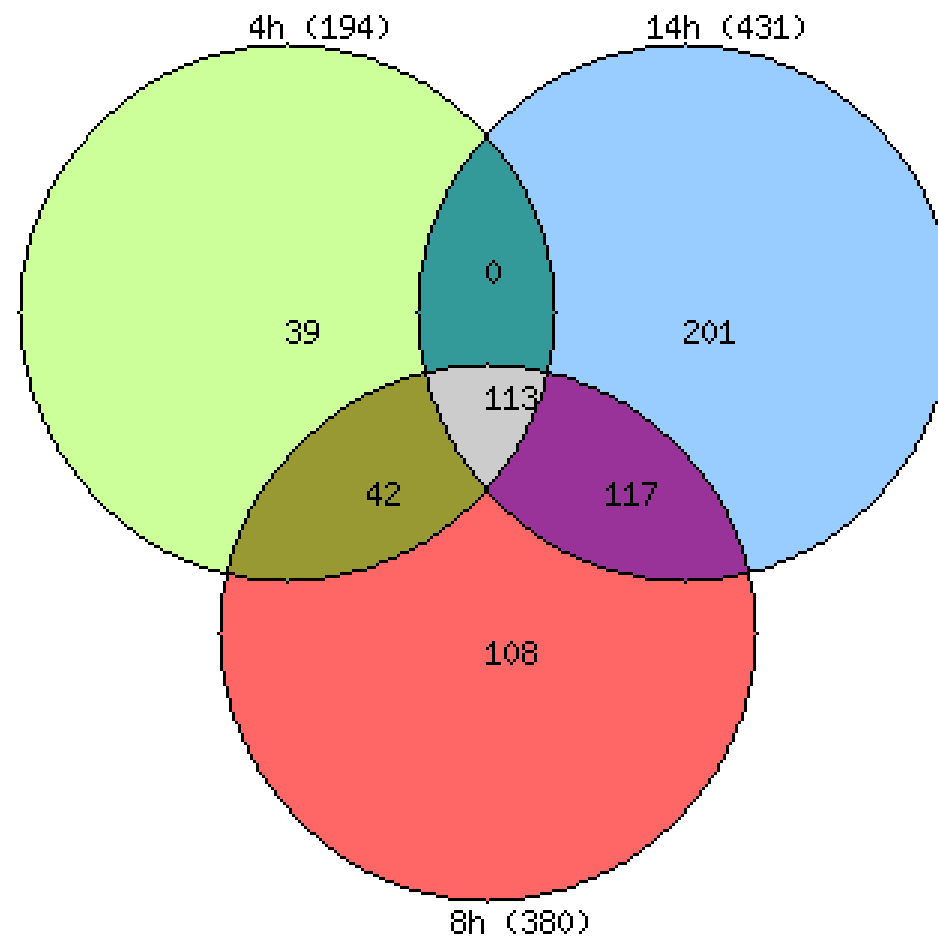
Expression Profiling of *A. fumigatus* WT vs. $\Delta laeA$ *In vivo*

- 3 time points: 4, 8 and 14 hours
- 3 replicates: 2 biological and 2 technical
- Using the JCVI amplicon *A. fumigatus* array

More Genes Are Differentially Expressed in WT vs. $\Delta laeA$ *In vivo* at 14 h than at 4 or 8 h



Differentially Expressed in WT vs. $\Delta laeA$ *In vivo* at 4, 8 and 14 hours



Selected Clusters Up/Down-Regulated in WT vs. $\Delta laeA$

- Gliotoxin cluster: up at 8 and 14h
- 2 arsenic detox clusters: up at T4/8/14
- Quinate utilization cluster: 3 genes down T8
- 17-gene unknown cluster: up at T4/8/14

Selected Clusters Up-Regulated in WT vs. $\Delta laeA$ *In vivo* and *In vitro*

- 4-gene iron assimilation cluster:
 - MFS siderochrome iron transporter MirB
 - GNAT family acetyltransferase
 - siderophore esterase IroE-like
 - ABC multidrug transporter

- 9-gene gliotoxin cluster

Selected Genes Up-Regulated in *WT vs. ΔlaeA In vivo* - 1

- Virulence factors (4 total):
 - α -1,3-glucan synthase Ags3 (T4/8/14)
 - Lysophospholipase Plb2 (putative; T4/8/14)
 - Sensor histidine kinase Fos-1/TcsA (T8/14)
 - N5-oxigenase SidA (T8/14)
 - conidial pigment polyketide synthase PksP/Alb1 – down T14
- Protection against H₂O₂:
 - Spore-specific catalase CatA (T4/8/14)
 - Mn superoxide dismutase SodB (T4)
- Stress response proteins (13 total):
 - Hsp30/42 and Hsp9/Hsp12 (T4/8/14)
 - Heat shock transcription factor Hsf1 (T8/14)
 - Ca²⁺/calmodulin dependent kinase (T8/14)

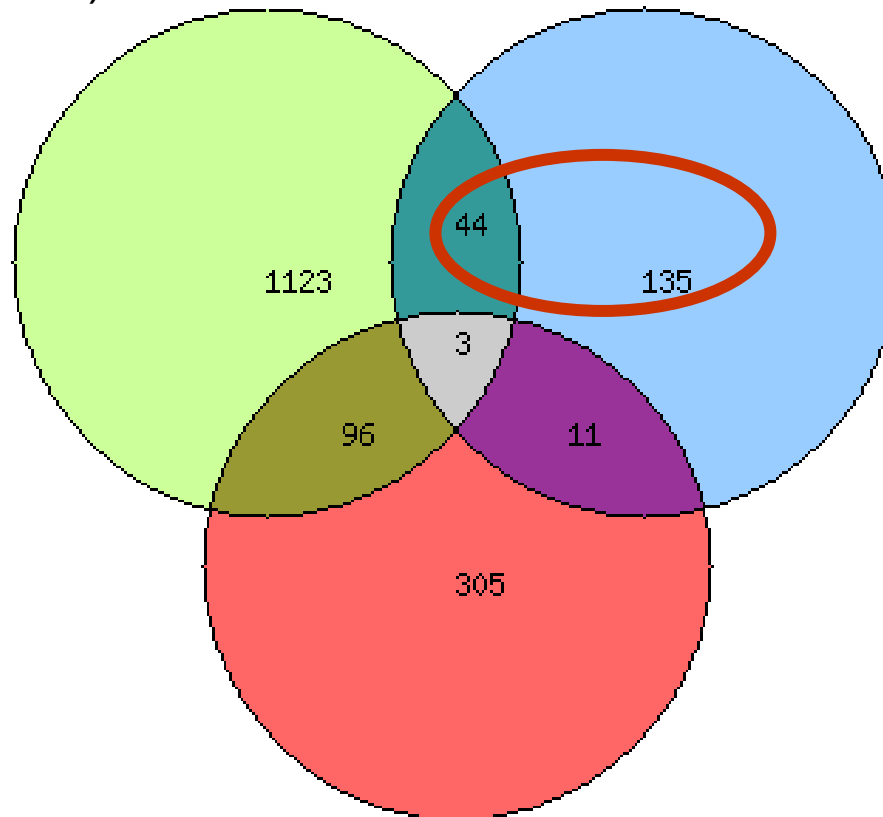
Selected Genes Up-Regulated in *WT vs. ΔlaeA In vivo - 2*

- Cell wall organization:
 - Chitosanase CsnC (T4/8/14)
 - α -1,3-glucan synthase Ags3 (T4/8/14)
- Sexual cycle (5 total):
 - α -Receptor PreA (T4/8/14)
 - Prenyl proteinase Rce1 (T4)
 - Mating locus protein (T8/14)
 - Transcription factor SteA (T8/14)
 - G-protein complex subunit α GpaA/FadA (T8/14)
- Transposases (T4)

32% of Genes Up-expressed *In vivo* WT vs. $\Delta laeA$ at 4 hours are Up-Expressed *In vivo* vs. *In vitro*

In vivo vs. *In vitro* (1266)

In vivo WT vs. $\Delta laeA$ at T4 (193)

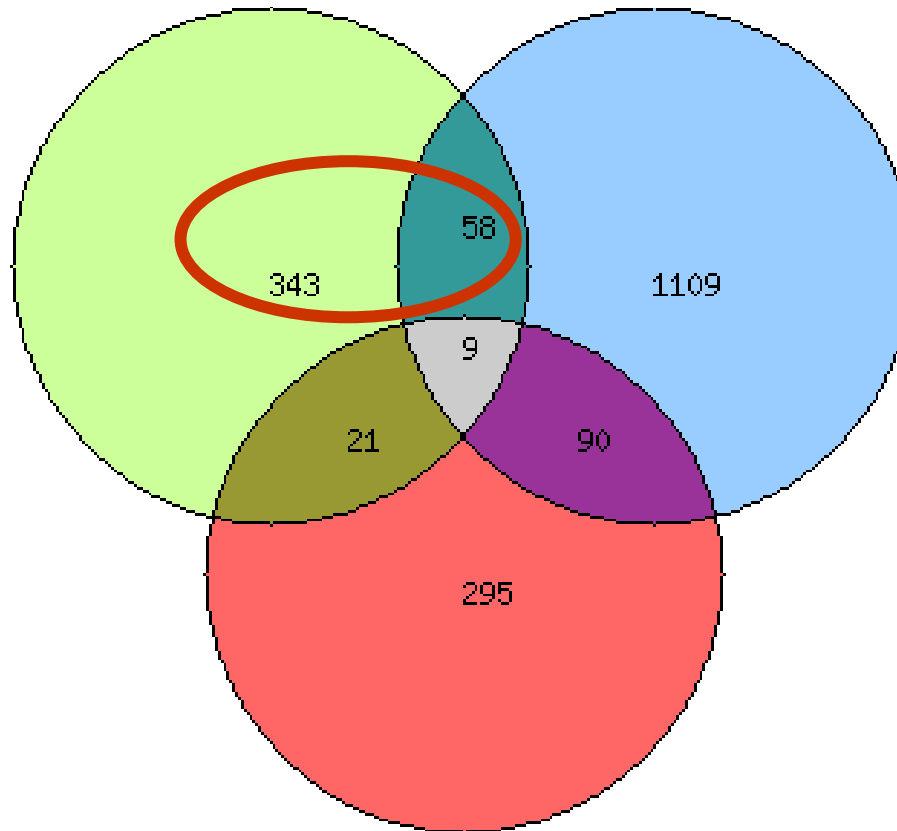


In vitro WT vs. $\Delta laeA$ (415)

17% of Genes Up-expressed *In vivo* WT vs. $\Delta laeA$ at 14 hours are Up-Expressed *In vivo* vs. *In vitro*

In vivo WT vs. $\Delta laeA$ at T14 (431)

In vivo vs. *In vitro* (1266)



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In vitro WT vs. $\Delta laeA$ (415)

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Conclusions/Speculations

- **Secondary metabolism clusters :**
 - **7** clusters are up-expressed in *in vivo vs. in vitro*
 - **13** clusters are up-expressed in *WT vs. $\Delta laeA$ in vitro*
 - **1** cluster (gliotoxin) is up-expressed in *WT vs. $\Delta laeA$ in vivo*
- **Genes up-expressed *in vivo* WT vs. $\Delta laeA$ are also up-expressed *in vivo vs. in vitro* :**
 - **32%** at 4 hours
 - **17%** at 8 and 14 hours
- **4 virulence genes up-expressed *in vivo* WT vs. $\Delta laeA$**
- **Some *In vivo vs. in vitro* and *in vivo* WT vs. $\Delta laeA$ up-expressed genes are clustered on chromosomes and are subtelomeric**

J. Craig Venter

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