

# *Pseudomonas syringae* Type-III Secreted Effectors Elicit Unique Transcriptional Responses in *Arabidopsis thaliana*

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## Introduction

The bacterium *Pseudomonas syringae* destroys millions of dollars of crops as a hemibiotrophic phytopathogen.

*P. syringae* enters the stomata and injects its type-III secreted effector proteins<sup>[1]</sup> into the symplast to disable host immunity. The plant responds with a transcriptional counter-attack (PAMP-triggered immunity, or *PTI*).

But does each effector induce a *unique* transcriptional response in the plant?

We've adapted a system developed by Wei et. al<sup>[2]</sup> and Laflamme et. al<sup>[3]</sup> to answer that question:

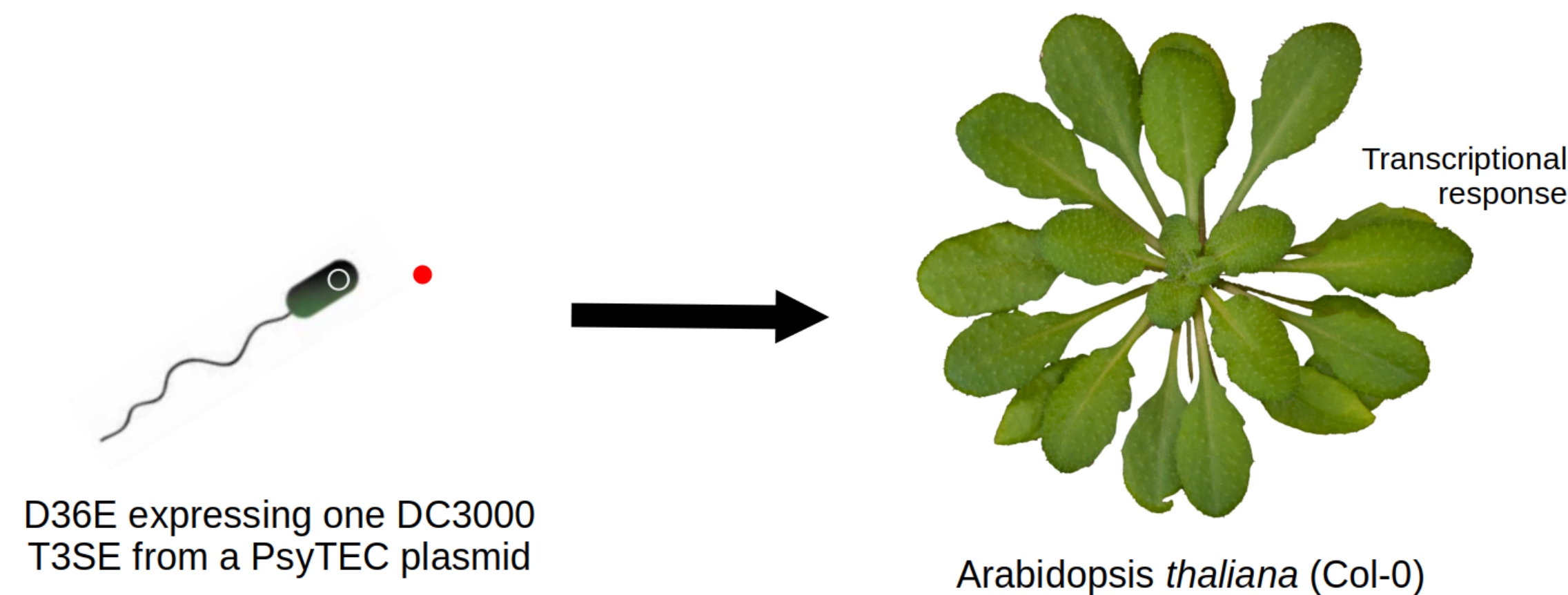


Figure 1: The model system we used to study individual effectors

## Methods

### Infection:

- Pressure infiltrate the following well-characterised *P. syringae* effectors, suspended in 10 mM MgSO<sub>4</sub> at OD<sub>600</sub> = 0.0002, in into *A. Thaliana* in biological triplicate:
  - D36E::**HopN1a**
    - Cleaves PsbQ in chloroplast's photosystem II to suppress SA signalling
  - D36E::**HopB1a**
    - Cleaves BAK1 at the membrane to suppresses PTI
  - D36E::**HopAB1j**
    - Ligates ubiquitin to FLS2 at membrane to suppress PTI
- With D36E::EV and 10 mM MgSO<sub>4</sub> as controls, leaves were frozen 1h and 8h post-infiltration.

### RNA Extraction & Sequencing:

- Frozen leaves were ground in LN<sub>2</sub> via mortar & pestle, then suspended in TRIzol.
- Centrifuged then supernatant was mixed with chloroform
- Centrifuged again and span down through RNEasy spin column kit
- Samples were stored at -80°C
- Samples were sequenced on an Illumina NextSeq 2000

## Computational Pipeline

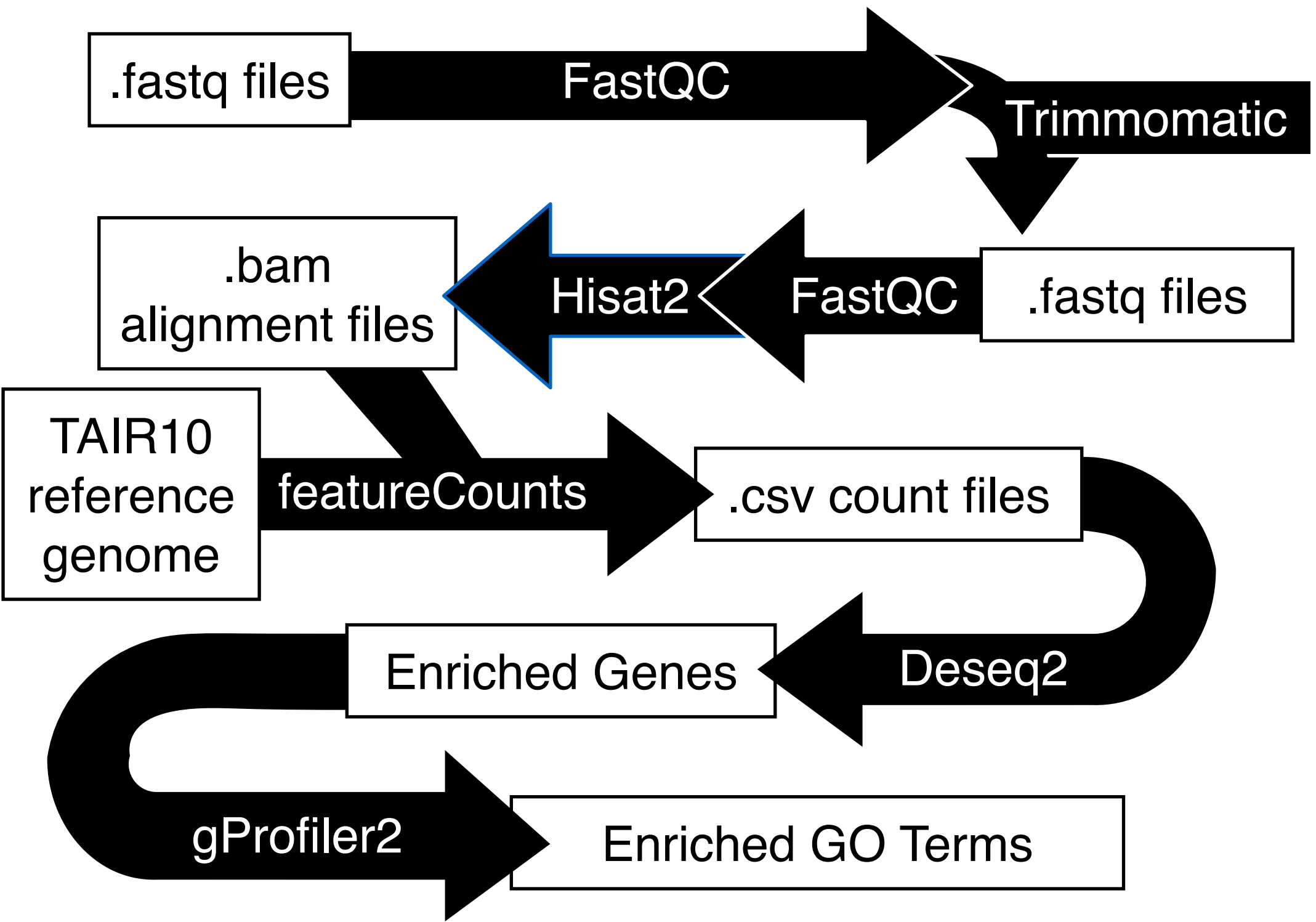


Figure 2: DEG patterns by treatment 8 hours post-infection

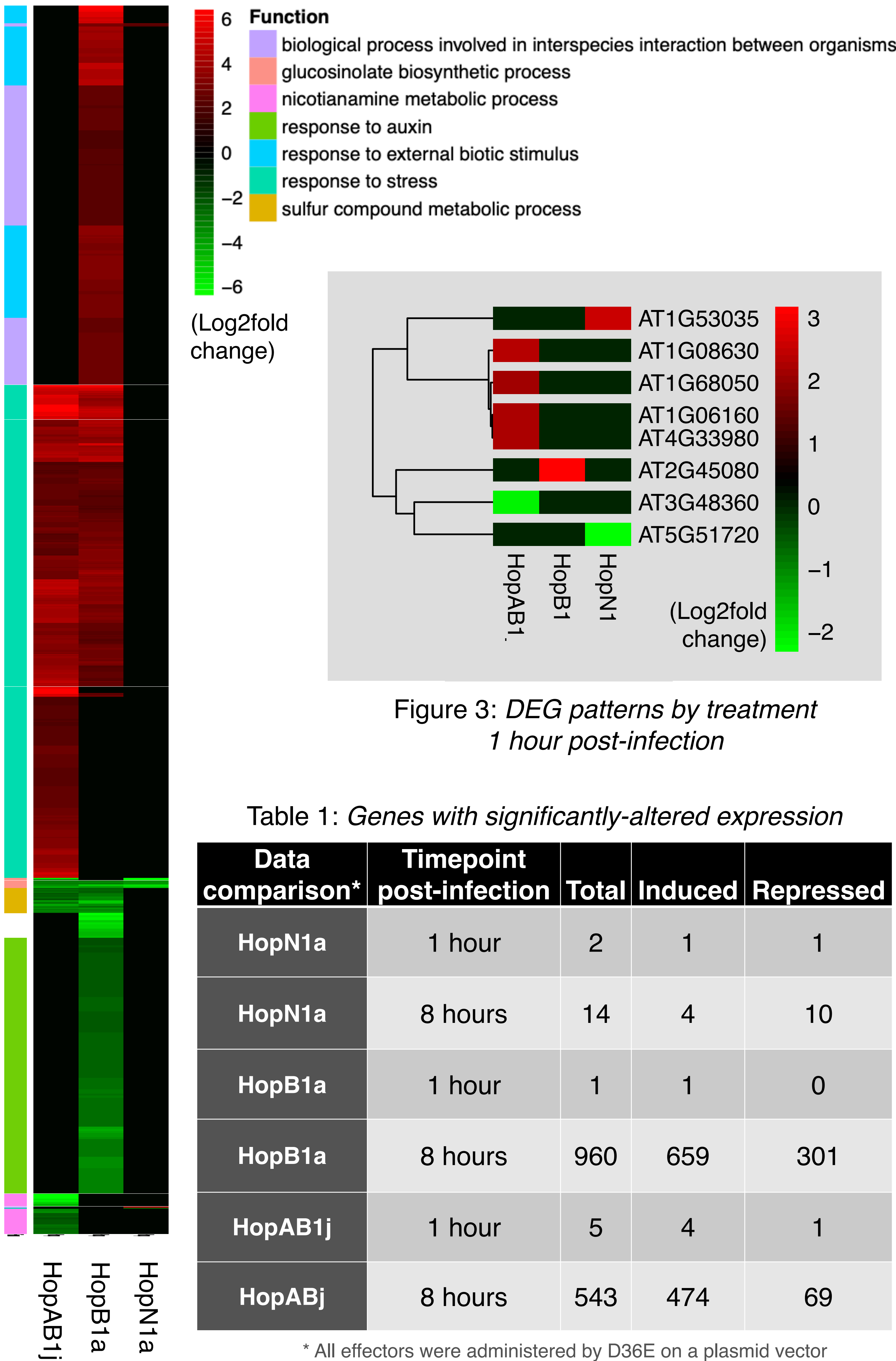


Table 1: Genes with significantly-altered expression

Data comparison*	Timepoint post-infection	Total	Induced	Repressed
HopN1a	1 hour	2	1	1
HopN1a	8 hours	14	4	10
HopB1a	1 hour	1	1	0
HopB1a	8 hours	960	659	301
HopAB1j	1 hour	5	4	1
HopABj	8 hours	543	474	69

\* All effectors were administered by D36E on a plasmid vector

## Results cont.

Table 2: DEGs at 1 hour post-infection by effector treatment. Red rows are up-regulated and green genes are down-regulated.

Effector*	Locus	Locale	Regulation	Product
HopN1a	AT1G53035	Chloroplast	Up	Transmembrane protein
HopN1a	AT5G51720	Chloroplast	Down	NEET, involved in ROS homeostasis
HopB1a	AT2G45080	Cytoplasm	Up	Cyclin P3, enables protein kinase binding
HopAB1j	AT1G08630	Cytosol	Up	THA1, degrades Thr → Gly
HopAB1j	AT1G68050	Cytosol & nucleus	Up	Part of SCF uquitutin ligase complex
HopAB1j	AT1G06160	Nucleus	Up	ORA59, master regulator of JA pathway
HopAB1j	AT4G33980	Nucleus	Up	COR28
HopAB1j	AT3G48360	Nucleus	Down	BT2, part of TAC1-mediated telomerase pathway

\* All effectors were administered by D36E on a plasmid vector

## Discussion

- 1 hour post-infection**
  - HopN1 demonstrates locale specificity, HopAB1 & HopB1 less so
  - HopN1 NEET and HopAB1j ORA59, SCF demonstrate functional specificity, HopB1 less so
- 8 hour post-infection**
  - Unique expression patterns visible across all treatments
  - Stress response activation and auxin

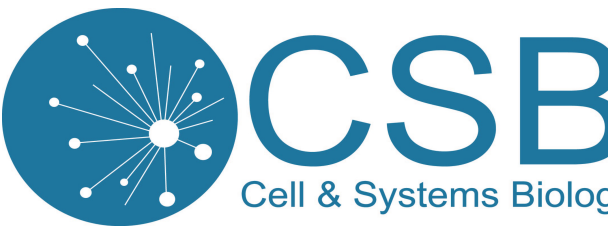
## Conclusion

D36E::HopAB1j, D36E::HopB1a, and D36E::HopN1a induce unique transcriptional responses in *A. Thaliana*.

Their “transcriptional fingerprints” are partially capable of characterising localisation and/or functional outcomes.



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## References

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- Wei H, Chakravarthy S, Mathieu J, Swingle B, Martin G, Collmer A. “*Pseudomonas syringae* pv. *tomato* DC3000 Type III Secretion Effector Polymutants Reveal an Interplay between HopAD1 and AvrPtoB”. Cell Host & Microbe, 17, 2015, pp. 752-762. 10.1016/j.chom.2015.05.007
- Laflamme B, Dillon M, Martel A, Almeida R, Desveaux D, Guttman D. “The pan-genome effector-triggered immunity landscape of a host-pathogen interaction”. Science, 367(6479), 2020, pp. 763-768. 6/science.aax4079



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