

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

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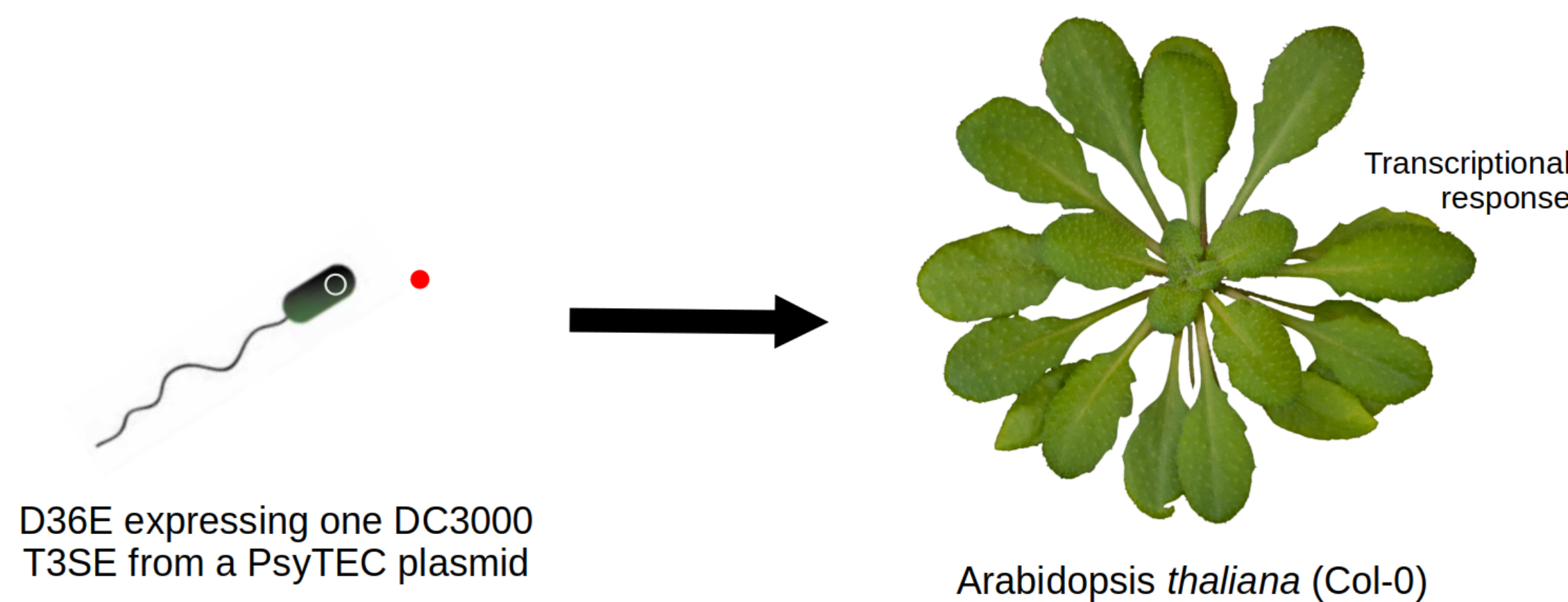
## Introduction

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> to answer that question:



Isolated effectors > overexpression

## Methods

### Infection:

- Pressure infiltrate the following well-characterised *P. syringae* effectors, suspended in 10 mM MgSO<sub>4</sub> at OD = 0.0002, in into *A. Thaliana* in biological triplicate:

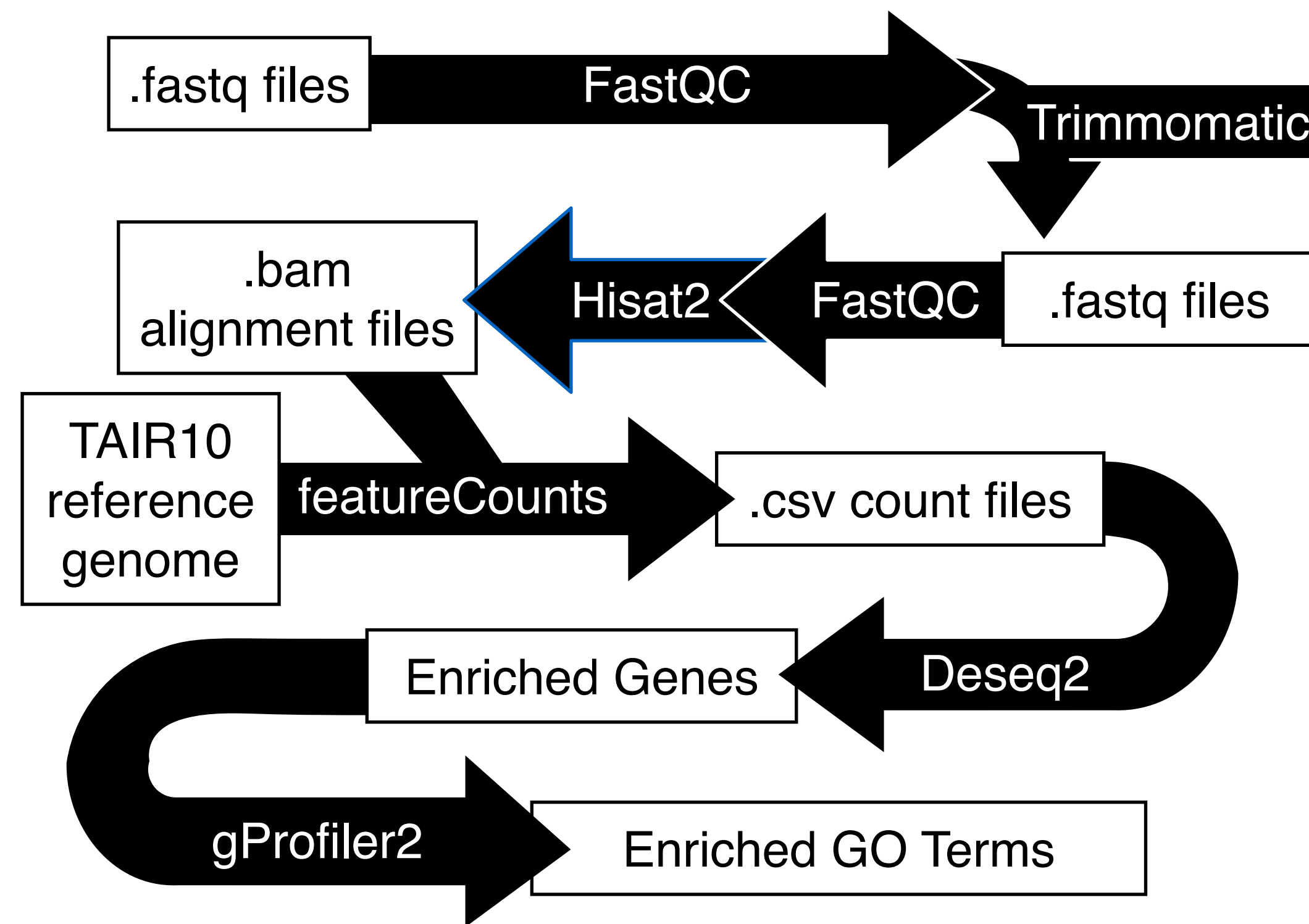
- D36E::**HopN1a**
  - Cleaves PsbQ in chloroplast's photosystem II to suppress SA signalling
- D36E::**HopB1a**
  - Cleaves BAK1 at the membrane to suppresses PTI
- D36E::**HopAB1j**
  - Ligates ubiquitin to FLS2 at membrane to suppress PTI

- With D36E::EV and 10 mM MgSO<sub>4</sub> as controls, leaves were frozen 1h and 8h post-infiltration.

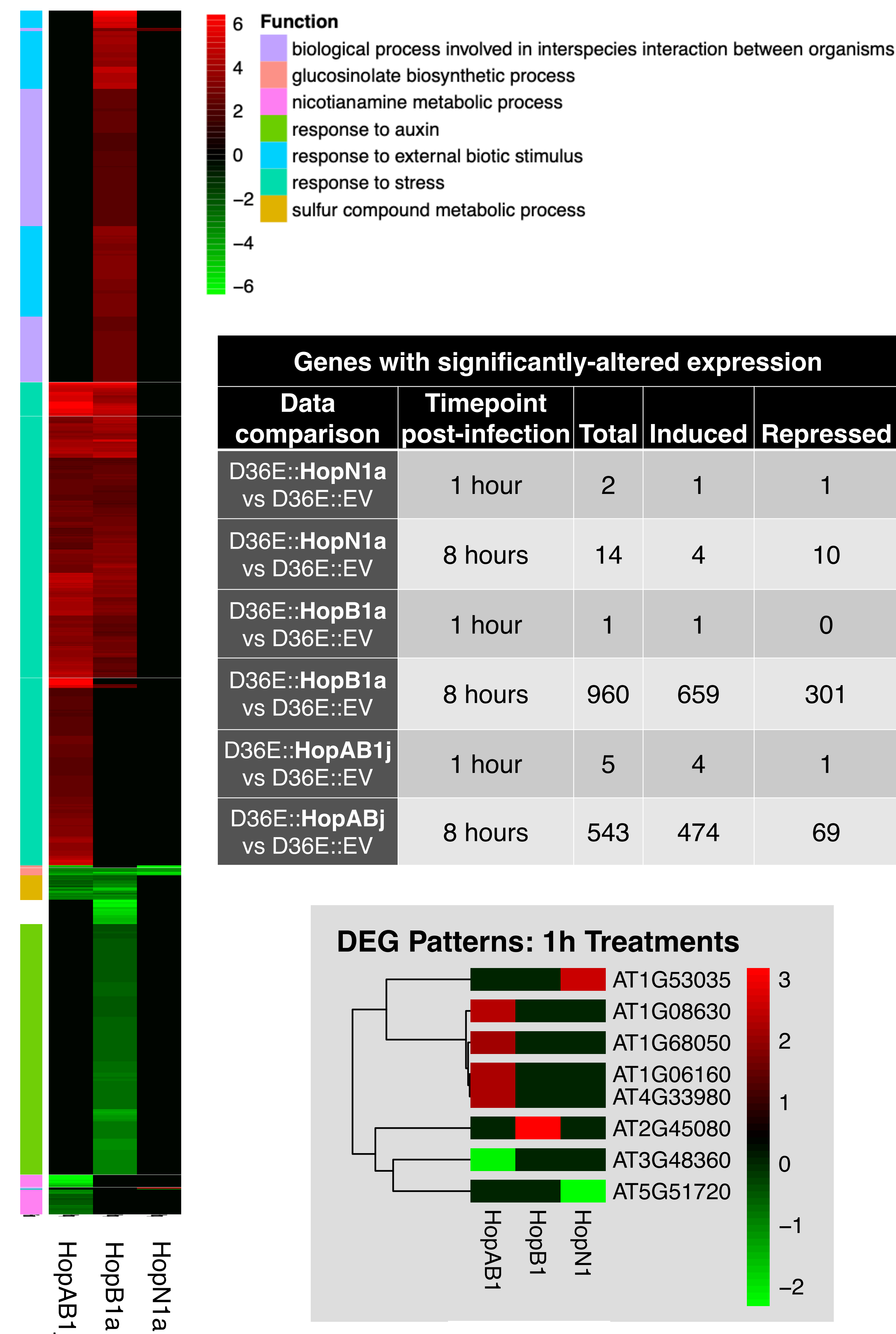
### RNA Extraction & Sequencing:

- 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



### DEG Patterns: 8h Treatments

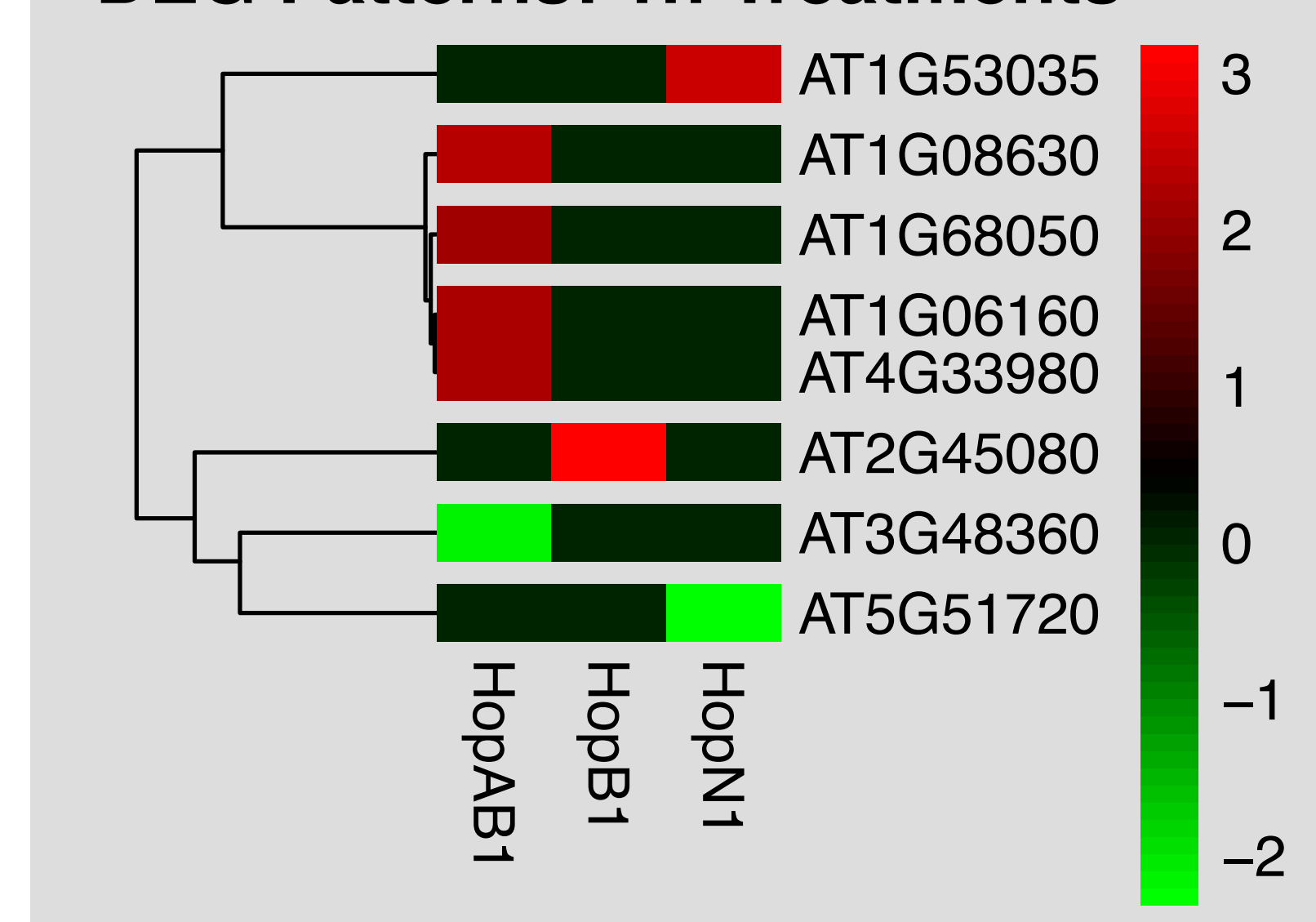


## Results

### Genes with significantly-altered expression

Data comparison	Timepoint post-infection	Total	Induced	Repressed
D36E:: <b>HopN1a</b> vs D36E::EV	1 hour	2	1	1
D36E:: <b>HopN1a</b> vs D36E::EV	8 hours	14	4	10
D36E:: <b>HopB1a</b> vs D36E::EV	1 hour	1	1	0
D36E:: <b>HopB1a</b> vs D36E::EV	8 hours	960	659	301
D36E:: <b>HopAB1j</b> vs D36E::EV	1 hour	5	4	1
D36E:: <b>HopABj</b> vs D36E::EV	8 hours	543	474	69

### DEG Patterns: 1h Treatments



## Results cont.

DEGs at 1 hour post-infection by effector treatment				
Effector	Locus	Locale	Regulation	Product
HopN1a	AT1G53035	Chloroplast	Up	Transmembrane protein
HopN1a	AT5G51720	Chloroplast	Down	<b>NEET</b> , 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 <b>SCF uquititin ligase</b> complex
HopAB1j	AT1G06160	Nucleus	Up	<b>ORA59</b> , master regulator of JA pathway
HopAB1j	AT4G33980	Nucleus	Up	COR28
HopAB1j	AT3G48360	Nucleus	Down	BT2, part of TAC1-mediated telomerase pathway

## 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.

### References

1. Xin X, Kvitko B, He SY. “*Pseudomonas syringae*: what it takes to be a pathogen”. Nat Rev Microbiol, 16, 5, 2018, pp. 316-318. 10.1038/nrmicro.2018.17

2. Wei H, Chakravarthy S, Mathieu J, Swingle B, Martin G, Collmer A. “*Pseudomonas syringae* pv. *tomato* DC3000 Type III Secretory Effector Polymutants Reveal an Interplay between HopAD1 and AvrPtoB”. Cell Host & Microbe, 17, 2015, pp. 752-762. 10.1016/j.chom.2015.05.007

