

Comparative genomics provides insights into the evolution of novel *Fusarium oxysporum* f. sp. *lactucae* race variants

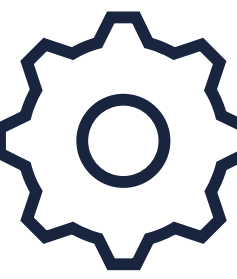
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1. Introduction

- Fusarium oxysporum* f. sp. *lactucae* (Fola) is a devastating soil-borne pathogen that causes Fusarium wilt of lettuce, leading to **crop losses** exceeding 50% worldwide.
- The recent emergence and spread of the **highly aggressive** Race 4 across Northern Europe poses a significant new threat to both protected and open field lettuce production.
- Initial molecular and pathological characterisation of field isolates **revealed unique variants (v)**, including a potential **new race (NR)** from France capable of breaking multiple sources of genetic resistance.



2. Methods

- We performed **Nanopore long-read sequencing** to generate high quality genome assemblies for 16 diverse Fola isolates, including variants (v) of Race 1 (R1) and 4 (R4), a potential new race (NR), and the first assemblies for Race 2 (R2) and 3 (R3) (**Figure 1**).
- Phylogenomic analysis** of 3,804 single-copy orthologous genes was conducted to resolve the evolutionary relationships between Fola races.
- Comparative genomics** and the **FoEC2 pipeline** were used to analyse the diversity of accessory regions and identify the repertoire of putative effector genes across all isolates.



3. Results

- Phylogenomic analysis reveals that Fola is **polyphyletic**. R1 and R4 cluster closely, but R2, R3, and the potential new race (NR) are distributed separately throughout the *F. oxysporum* phylogeny (**Figure 2**).
- The potential new race (NR) isolate possesses an **accessory genome (AG)** that is distantly related to the four previously described races and can overcome both R1 and R4 resistance in lettuce differentials (**Figure 2**).
- We identified a core set of 44 effectors common to all isolates (PanFola), but also **race-specific effectors**, with the new race (NR) isolate containing **4 unique effectors** not shared with any other Fola isolates (**Figure 3**).



4. Discussion

- The polyphyletic ancestry of Fola indicates that the ability to cause disease on lettuce has **evolved independently multiple times** within the *F. oxysporum* species complex.
- Distinct profiles of **putative effector genes**, especially those unique to specific isolates, likely underpin the observed differences in pathogenicity and the ability to overcome host resistance.
- The discovery of a potential new race with a distinct evolutionary origin highlights the pathogen's adaptive potential and stresses the urgent need for **ongoing disease surveillance and breeding for novel resistance**.

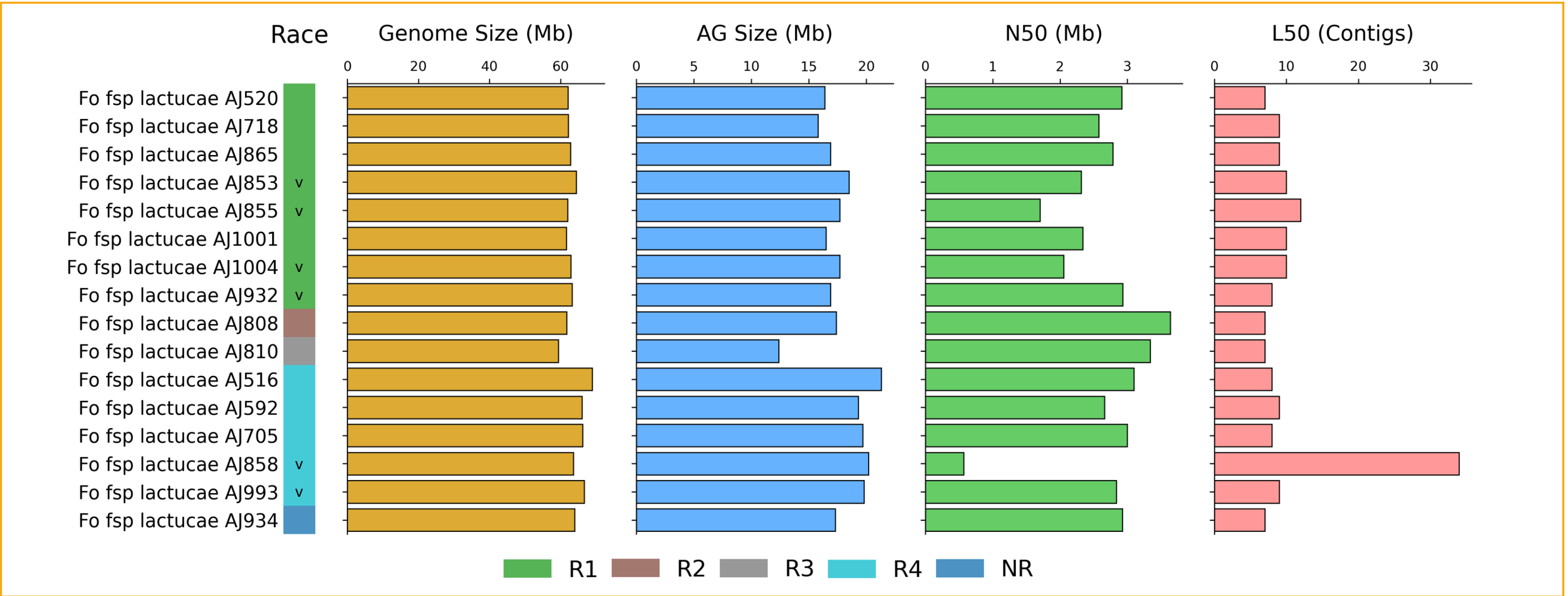


Figure 1. Total Genome and Accessory Genome (AG) sizes, and Assembly Metrics.

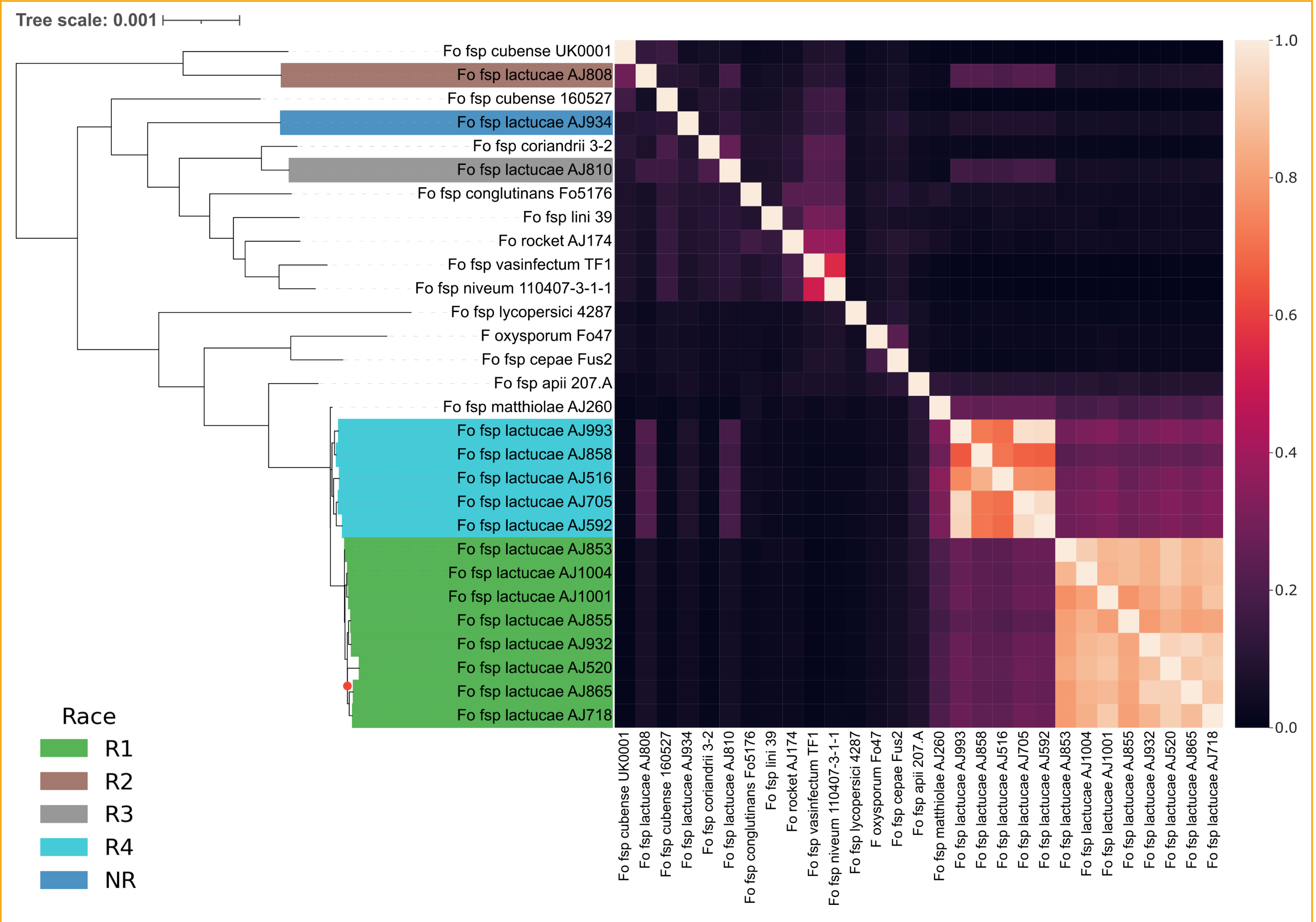


Figure 2. Single Copy Ortholog Phylogeny and AG Similarity Heatmap.

Comparative genomics of the lettuce wilt pathogen reveals a potential **new race** with a unique evolutionary origin and effector profile.

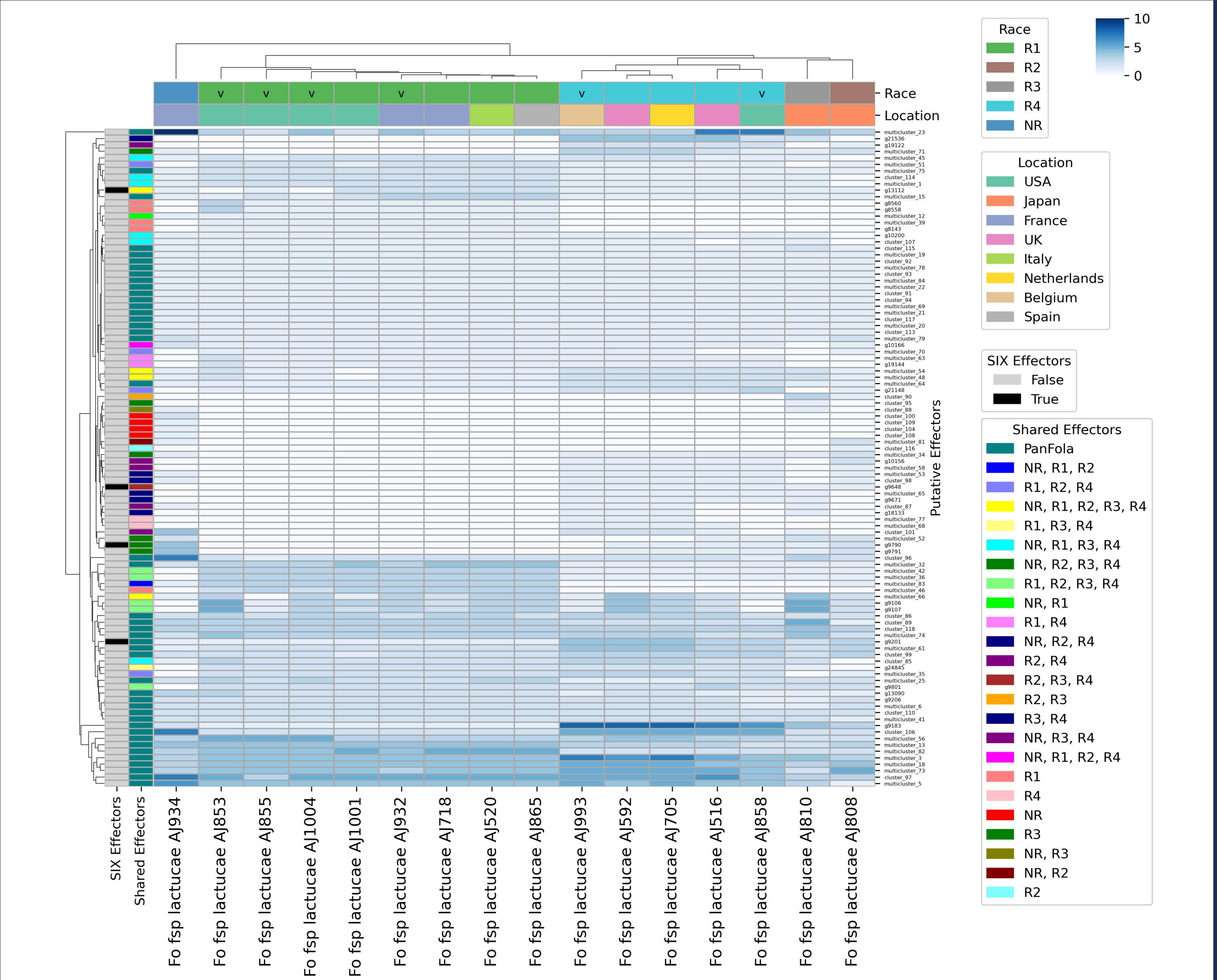


Figure 3. Putative Effector Analysis Heatmap.