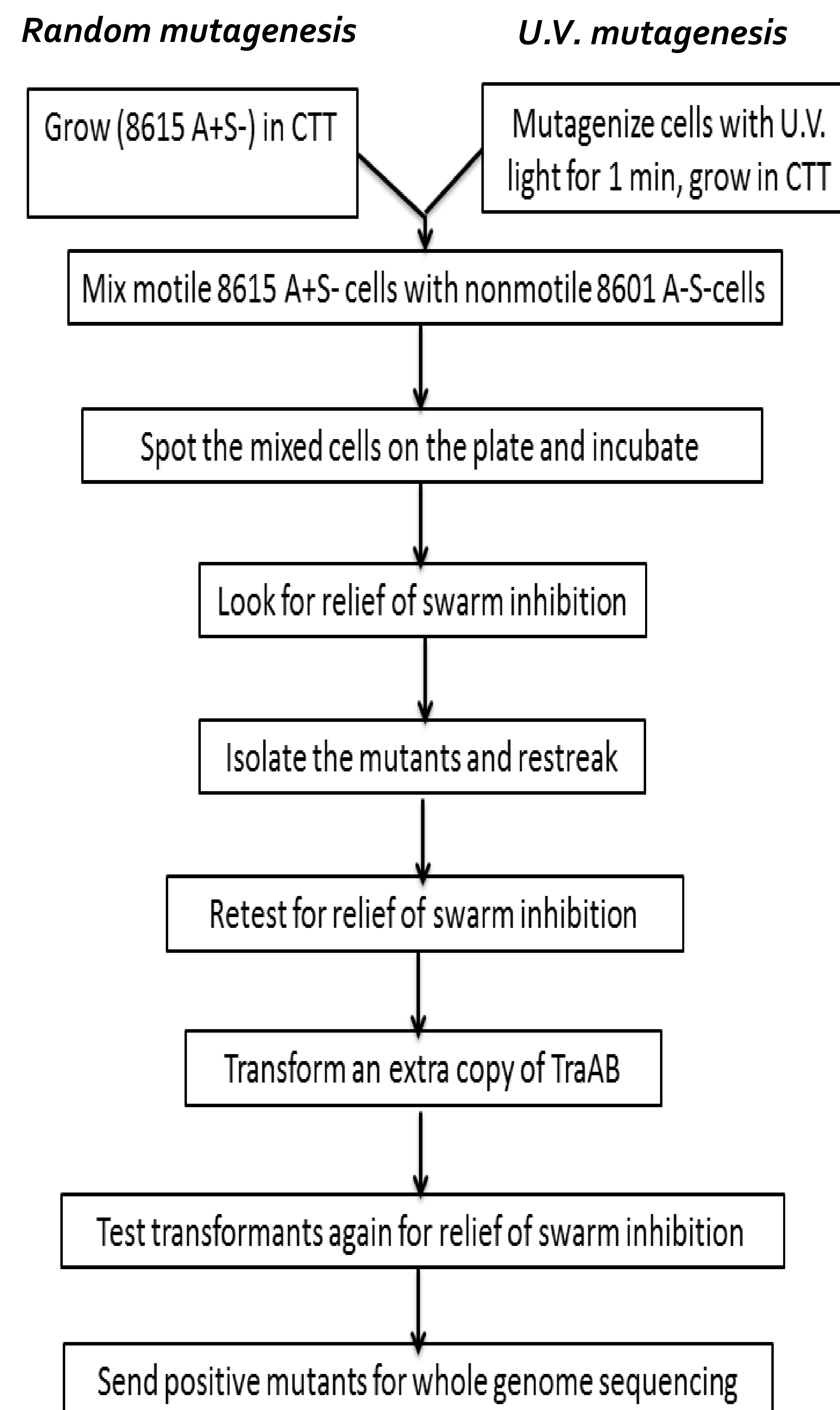


Summary: *M. xanthus* possesses two motility systems that control gliding motility, namely S- and A-motility. Cells use these motility systems to socially interact with their siblings. Interestingly, we discovered when motile cells are mixed with isogenic nonmotile cells (DK8601) the motile cells are inhibited from moving (Figure 1). This process is called swarm inhibition. In prior work we identified two key players – TraA and TraB involved in swarm inhibition and the fusion and exchange of outer membrane proteins [1]. To better understand how this cell-cell interaction works, our objective here was to identify additional mutants to test if other proteins are involved in this process. Both spontaneous and U.V. induced mutants were isolated in the motile strain that showed a relief of swarm inhibition. To do this motile cells were mixed with nonmotile cells in an equal ratio and placed on agar plates and incubated. Motile cells or flares that “broke out” from the spots were carefully streaked to isolate individual colonies and those cells were retested for relief of swarm inhibition. Once we confirmed their phenotype, an extra copy of the *traA* and *traB* genes were transformed into the mutant strains to test for complementation; i.e. were the mutations in *traA* or *traB* or different genes. The resulting transformed colonies were verified for their kanamycin resistance and retested for relief of swarm inhibition. From these experiments four positive mutants (swarm relief) were identified and sent for whole genome sequencing to identify SNP/INDELS and the new gene(s) candidates involved in swarm relief. The mutants were also confirmed for relief of swarm inhibition by mixing different ratio of motile and non motile cells (Figure 2). In contrast, 12 mutants were complemented by the *traAB* plasmid. The identification of new players or genes will help give us a better mechanistic understanding of how cell-cell signaling regulates swarming behavior in *M. xanthus*.

Methods: Mutations were generated randomly and through U.V. mutagenesis in the motile strain. The method are discussed in the below flow chart.



Results

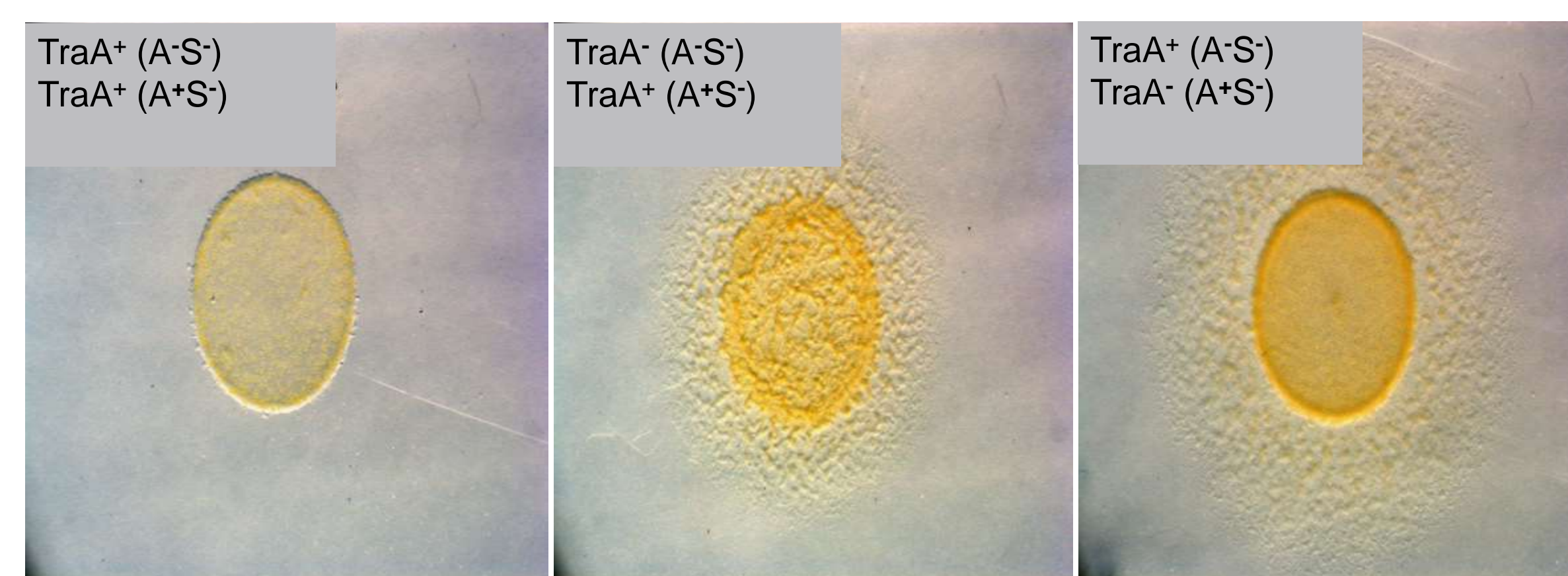


Fig. 1. Swarm inhibition by nonmotile strain mixtures. Top panels show swarm inhibition dependent on TraA. Bottom panels show select inhibition of environment strains.

Table 1. Three screens were conducted to isolate mutants that showed a relief of swarm inhibition.

Screen number	Random	U.V.	Random mutants transformed with pTraAB	U.V. mutants transformed with pTraAB	Mutants not complemented and sent for sequencing
Screen 1	10	-	10	-	3
Screen 2	4	3	4	3	1
Screen 3	4	7	3	7	0

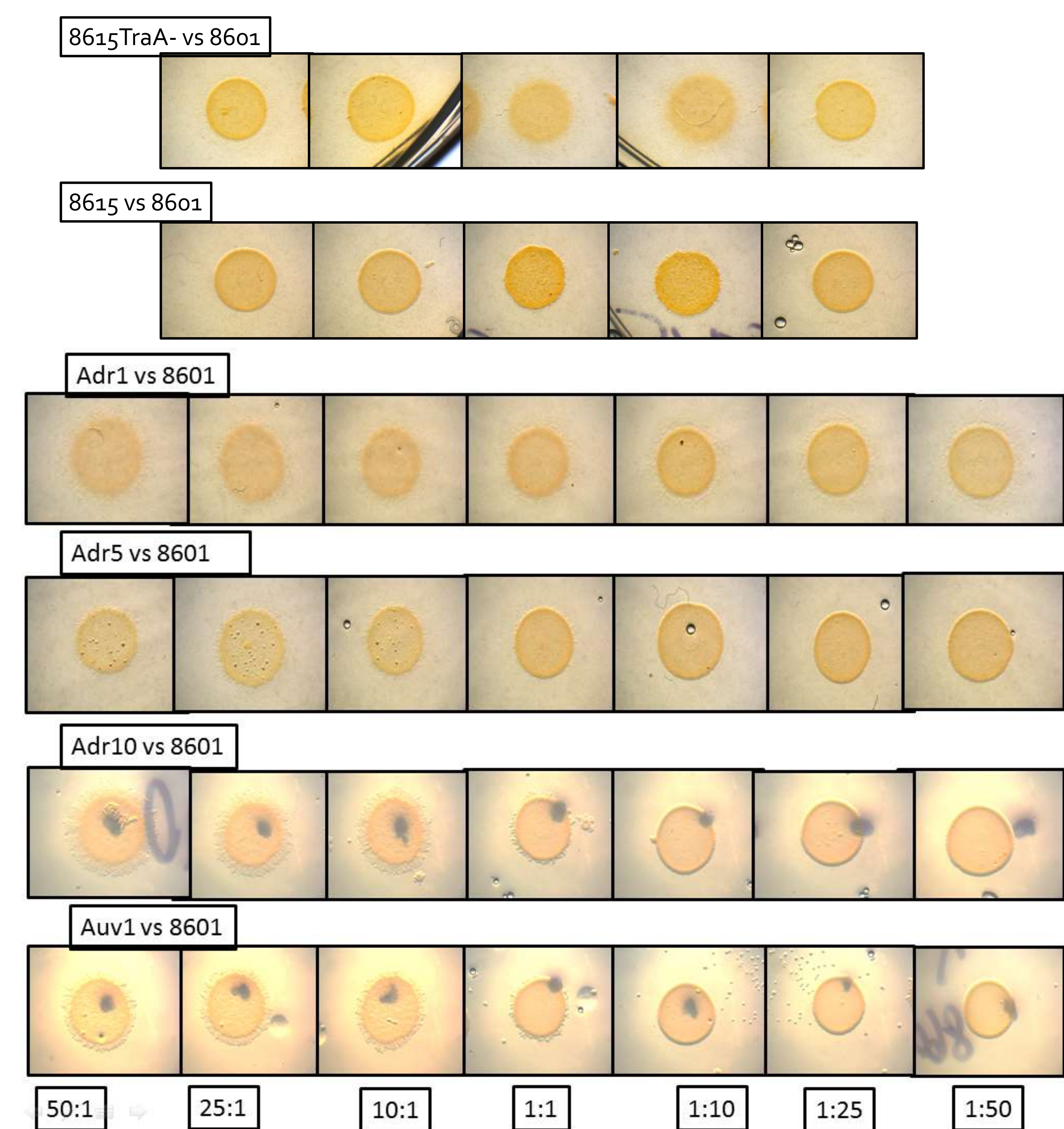


Fig 2. Motile and nonmotile cells were mixed in different ratios. Wild-type motile and nonmotile cells produce swarm inhibition at different ratio whereas the TraA⁻ mutant produces swarm relief. Four mutants showed varying degrees of relief. Mutants and controls are spotted with nonmotile cells at different ratios.

Discussion: Four colonies that were found to relieve swarm inhibition and were sent for whole genome sequencing. These findings should help us identify down proteins that we hypothesize are involved in a signal transduction pathway involved in regulating *M. xanthus* behavior.

Acknowledgements

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References

- 1) Pathak, D.T. Wei, X. Bucuvalas, A. Haft, D.H. Gerloff, D.L. Wall, D. 2012. Cell contact dependent outer membrane exchange in myxobacteria: genetic determinants and mechanism. *PLoS Genetics*. 2012 Apr; 8(4): e1002626.