Dr. John Pham, Editor-in-Chief*, Cell*

March 14, 2019

Dear Dr. Pham,

We submit the accompanying manuscript, entitled “**Systematic Dissection of a Complex Trait using High-Order Genetic Analysis**”, for your consideration as an Article in *Cell*.

It is becoming increasingly clear that most traits, including common human genetic diseases, are complex. Understanding complex traits, which rely on multiple genes functioning together, requires phenotypic study of multiple variant combinations. Currently, such combinatorial genetic analysis can be done exhaustively for two-gene combinations. In yeast, for example, two-gene synthetic genetic array analysis (SGA) has mapped genetic interactions systematically to derive a global functional relationship network.

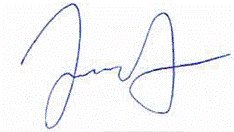
Unfortunately, many functional relationships can only be revealed by simultaneous perturbation of three or more genes. The lack of systematic studies on such ‘high-order’ genetic interactions has fundamentally limited our ability to map genotype to phenotype and thus model gene and pathway function.

To address this gap, we implemented an ‘X-gene’ genetic analysis (‘XGA’). XGA works by engineering and profiling many combinations of multiple gene variants to recover surprising combinatorial effects, which can be exploited for functional understanding. We demonstrate XGA on the complete set of 16 yeast ABC transporters (efflux pumps) which have been implicated in multidrug resistance. We generated a set of >5,000 strains carrying different combinations of multi-gene knockouts. Using a combination of next-generation barcode-sequencing technologies, every strain was genotyped and phenotyped for resistance to a set of 16 bioactive compounds (‘drugs’), yielding 85,632 genotype-to-resistance relationships.

XGA analysis revealed many complex drug resistance interactions amongst ABC transporters which would have been missed by one- and two-gene approaches. We used an interpretable neural network to objectively model functional relationships from XGA data. This systematically revealed many examples of multiple transporters effluxing a drug in parallel, and more suprisingly, we also identified six pairs of ABC transporters for which one repressed the other’s activity. For example, we found a quadruple knockout which showed increased drug resistance via de-repression of a fifth gene, resulting in high-order genetic interactions. For this trait, neural network modeling guided follow-up experiments suggesting that this repression is happening via both direct and indirect mechanisms.

Given the pressing need to better understand complex traits, and the potential general applicability of our approach towards other traits and model organisms, we believe that our manuscript will benefit the broad readership of Cell. We appreciate your time and consideration. Please feel free to contact us for discussion.

Sincerely,



Frederick P. (Fritz) Roth on behalf of all authors