Dear Editors,

We would like to submit the attached manuscript entitled '*Analysis of allelic series with transcriptomic phenotypes*' for publication as a Brief Communication in Nature Methods.

Human genetics has shown that allelic variants of the same gene can have different disease severities or cause different diseases altogether. As a result, the broader biomedical community must now address the challenge of characterizing these medically identified variants. Transcriptomics (RNA-seq) has proven to be a useful and scalable way of defining cell or organismal phenotypes. We have developed an analytical framework to characterize allelic variants of the same gene using RNA-seq as the phenotype.

- Our framework incorporates complementation and dominance, concepts from classical genetics, and extends them to transcriptomic data.
- We define a transcriptome-wide dominance coefficient as a concise descriptor of allelic dominance.
- We have tested this framework using *C. elegans* by focusing on the gene encoding the ortholog of the Mediator subunit MED12 (*dpy-22*, also known as *mdt-12*).
- We sequenced homozygotes and *trans*-heterozygotes of two *dpy-22* alleles and identified dysregulated genes that fell into distinct categories that we called phenotypic classes.
- We developed an algorithm that systematically identifies and removes artefactual and confounding classes, which are the result of false positive and false negative hits.
- These phenotypic classes are analyzed using our dominance coefficient, which allows us to infer intragenic functional units affected by each allele, as well as their sequence requirements.
- Using transcriptomic signatures derived from other mutants, we can associate these phenotypic classes with physiological processes, such as body morphology or response to hypoxic stress.

Our results clearly demonstrate the utility of this analytical approach, which is widely applicable to genetic analyses performed using either single-cell or bulk RNA-seq. Because our approach is simple to implement, we believe it will be widely used, and thus our description of it is highly appropriate for Nature Methods.

Sincerely yours,
David Angeles & Paul Sternberg