Editorial



# Simultaneous profiling of multiple genomic variations using a *CRISPR-Cas9* high-throughput strategy

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*Comment on:* Guo X, Chavez A, Tung A, *et al.* High-throughput creation and functional profiling of DNA sequence variant libraries using CRISPR-Cas9 in yeast. Nat Biotechnol 2018;36:540-6.

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In the paper untitled "high-throughput creation and functional profiling of DNA sequence variant libraries using CRISP-Cas9 in yeast" (1) published online in May 2018 in Nature Biotechnology, George M. Church, an eminent expert in CRISPR-Cas9 biology and technology, makes, yet another, giant step in the field of genomics.

Measuring the functional effects of genomic variants in a high-throughput manner has been a challenge for researchers for decades. In the recent past, genes had to be deleted one by one, even in so-called easy to manipulate yeasts, often with additional undesired "left behind" sequence modifications, that eventually polluted functional read-outs. In this work, Guo and colleagues empowered CRISPR-Cas9 that can be precisely addressed to sequences of interest in DNA using small guide RNAs (sgRNA) to interrogate known and unknown genes function. Cell-based, homology-directed recombination (HDR) comes next on line to repair crisperized genes following target sequence interruption, but for this, HDR relies on donor template sequence encoding for sequences of interest.

Pr Church's colleagues have scored a major "*coup*" by combining this donor template with the guiding sequences for the genetic scissors in one stable and heritable extra-chromosomal DNA molecule (guide + donor). This strategy offers the tremendous promise of specificity without disturbing neighboring genes, while allowing the study of candidate genes manipulation within their cellular context. Using this unique methodology, Harvard based researchers altered with pristine precision, and with up to 80–100% efficiency, hundreds of different genes reproducing single point mutations often seen in human genes. They also went one step further by identifying gene alterations that can either induce or prevent specific behaviors in modified cells.

After optimizing their process through linearization of the guide + donor plasmid, Guo and colleagues focused on the DNA helicase and repair enzyme SGS1, as a proof-of-concept target. Remarkably, DNA toxic agent treatment and SGS1 multiple genetic alterations using Cas9 help uncloak residues in the helicase sequence that are mandatory for proper DNA repair of damaged DNA and cell survival. The next step was to apply this broad genetic screening to genuine discovery programs, exemplified by the targeting of small open reading frames (smORFs) that are dispersed throughout the genome, and for which functional information are lacking. By analyzing how smORFs targeting alters yeast survival following exposure to various stresses, the authors highlighted for the first-time essential functions to specific smORFs, for discovering new functions out of genes and to better understand gene regulation and chromosome biology.

Similar attempts of genome-wide investigation using CRISPR-Cas9 technology have been made by other group quasi concomitantly; indeed, a team from the University of California reported in the same journal one month earlier the creation of a similar CRISPR library to engineer variants on a genomic scale, combined to high-throughput

profiling (2). In the latter study, they used CRISPR Cas9 technology to investigate the functional consequences of targeting premature-termination codons (PTCs) in essential genes in yeast. They discovered that most PTCs are highly deleterious, with two main exceptions: (I) if they occur close to the 3' end of the gene and (II) if they don't affect an annotated protein domain. Surprisingly, they also found that some genes that were previously thought to be essential were actually dispensable, at least in part(s). The methodology used by these researchers is highly similar to the one described earlier; they physically connected gRNA targeting sequences with matching repair DNA templates *in cis*, on oligonucleotides that were generated in bulk using high-throughput synthesis, that were latter delivered into yeast cells using pooling plasmids. In another study also recently published in Nature Biotechnology (3), researchers from University of Illinois developed a strategy named CHAnGE, corresponding to CRISPR-Cas9- and HDR-assisted genome-scale engineering method. By pairing CRISPR guide sequence and HDR templates in single oligonucleotides, this strategy can rapidly generate myriads of specific genetic variants in yeast, with 98% of efficacy and an average frequency of 82%. In validation studies, they generated a genome-wide gene disruption collection, with the objective of improving tolerance to growth inhibitors. This approach is not only extremely precise and efficient, but is notably low-cost, with short time-to-result. Remarkably, Zhao's group offers to the scientific community libraries of single gene knockout for *S. cerevisiae*, against a minimal $50 handling fee. Now they want to expand their technology to other types of yeast, including strains that can have a voice in industrial applications such as lubricants and biofuels production.

In conclusion, several groups are currently experimenting large genetic variant libraries in yeast to better understand genome function, to investigate fundamental biological questions in a high-throughput manner with exquisite precision. It is highly probable that the next step will be to engineer yeast strains with industrially relevant abilities or to investigate pathological yeast strains for the discovery of genes and gene functions that affect their infectious properties. On a more general note, such scientific endeavor may not only help better understand or identify gene function in an unbiased way but may also stem for the discovery of the most intimate molecular mechanisms by which cells regulate their physiology and growth, and may provide new insights on infection processes and human diseases.

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## Footnote

*Conflicts of Interest:* The author has no conflicts of interest to declare.

## References

1. Guo X, Chavez A, Tung A, et al. High-throughput creation and functional profiling of DNA sequence variant libraries using CRISPR-Cas9 in yeast. Nat Biotechnol 2018;36:540-6.
2. Sadhu MJ, Bloom JS, Day L, et al. Highly parallel genome variant engineering with CRISPR-Cas9. Nat Genet 2018;50:510-4.
3. Bao Z, HamediRad M, Xue P, et al. Genome-scale engineering of Saccharomyces cerevisiae with single-nucleotide precision. Nat Biotechnol 2018;36:505-8.