GENETICS

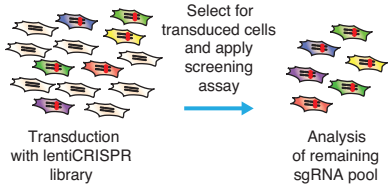
Screening CRISPly in human cells

Two groups demonstrate the use of the CRISPR-Cas9 system for genetic screens in human cells.

The discovery that the Cas9 endonuclease, a component of bacterial immunity, can in principle be targeted to any gene of interest in any genome has opened up new landscapes of possibility in genetic analysis. As is true for the zinc-finger and transcription activator–like effector nucleases, Cas9 cleavage can trigger an imperfect repair process, leading to mutagenesis at the site. Unique to the clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 system, however, targeting can be achieved simply with a small RNA complementary to the target sequence, termed the single guide RNA (sgRNA). It thus becomes possible to synthesize a library of sgRNAs and systematically target the Cas9 endonuclease genome wide. The groups of Feng Zhang at the Broad Institute and of Eric Lander and David Sabatini at the Broad and Whitehead Institutes, respectively, now demonstrate experimentally that CRISPR-Cas9–based genetic screens are feasible in cultured human cells.

Zhang and colleagues deliver the gene encoding Cas9, the sgRNA and an antibiotic-resistance gene via a single lentiviral vector into target cells. Sabatini, Lander and colleagues instead take the approach of generating a stable Cas9-expressing cell line and then virally delivering the sgRNA and an antibiotic-resistance gene into that line. Both groups design libraries that target thousands of genes (with several sgRNAs per gene), deliver the library in a pooled configuration and use deep sequencing to identify enriched or depleted sgRNAs after selecting the cells for the phenotype of interest.

Both research groups demonstrate that CRISPR-Cas9 can be used for negative selection screens to identify essential genes that are required for cell viability or robust proliferation; one would expect sgRNAs targeting these genes to be depleted from a population of proliferating cells. For the method to work for this purpose, single-copy integrated sgRNAs must knock out the target gene in most cells that harbor them, and must furthermore do so biallelically for screens in diploid cells. Indeed, both groups report the reproducible depletion of sgRNAs targeting categories of genes known to be important for cell survival.



Pooled screens of an sgRNA library in cells. Figure reproduced from *Science* (Shalem *et al.*, 2013) with permission from AAAS.

Zhang and colleagues also went on to screen an sgRNA library targeting about 18,000 genes for those that, when mutated, would confer resistance to vemurafenib, a BRAF kinase inhibitor, in a human melanoma cell line. They identified both previously known resistance genes and new candidates.

Sabatini, Lander and colleagues conducted similar positive selection screens, in their case with an sgRNA library targeting about 7,100 genes, with at least ten sgRNAs per gene. They used this library to identify all genes necessary for DNA mismatch repair (mutation of which confers resistance to 6-thioguanine) in the near-haploid KBM7 cell line. In a separate screen conducted also in diploid cells, they screened for genes whose loss confers resistance to the DNA topoisomerase II poison etoposide. In both cases, they report that cells expressing sgRNAs targeting the expected genes were highly enriched in cultures grown in the relevant toxic compounds.

Although not directly demonstrated in these reports, an exciting aspect of this approach is "the ability to interrogate regions in the genome currently inaccessible by other techniques," says Tim Wang, first author on one of the papers. "For example, one can study the functional significance of *cis*-regulatory elements, which would be impossible with RNAi."

Recent work has raised concerns about the fidelity of the CRISPR-Cas9 system and has indicated that, at least in some contexts, off-target cleavage could be substantial. Will off-targets—which have bedeviled RNA interference (RNAi) screens, the main genetic analysis platform in mammalian cells—prove problematic for the CRISPR-Cas9 methodology as well?

Neither of the groups reporting these proof-of-principle screens thinks so, although both report low levels of off-target modification. "I'm not too concerned," says Zhang. "The consistency across multiple sgRNAs targeting the same gene and the validation rate of the hits discovered is very high." Lander, Sabatini and colleagues also argue that, at least for their drug-resistance screens, the expected genes are so strongly enriched that off-target effects do not appear to make much of a contribution. Nonetheless, potential users should note that the sgRNAs used by both groups are filtered for potential off-target cleavage, and that it will remain critical, as for RNAi screening, to test the effects of several sgRNAs per gene and to independently validate hits in secondary experiments.

Systematic knockout of one or more genes in yeast and bacteria has been productively used to understand the way in which genotype directs phenotype in these microorganisms. The development of the CRISPR-Cas9 platform, as well as of single-gene-knockout collections of human cells (*Nat. Methods* **10**, 965–971, 2013), will enhance the power of genetics also in human systems.

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RESEARCH PAPERS

Shalem, O. *et al.* Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**, 84–87 (2014).

Wang, T. *et al.* Genetic screens in human cells using the CRISPR-Cas9 system. *Science* **343**, 80–84 (2014).