ers GENOMICS

Your connection to CRISPR/Cas9

HOW CRISPR IS TRANSFORMING

HIGH-THROUGHOUT SCREENING

INTRODUCTION

CRISPR libraries offer huge advantages for high-throughput screening, including unprecedented editing efficiency, accuracy, and speed. This article walks through the fundamentals of CRISPR screening and its most exciting applications, including unpicking cellular signaling networks, discovering how viruses like COVID-19 infect our cells and finding new drug targets.

The advent of high-throughput DNA sequencing gave us more information than ever before about the genes that make up life. However, sequencing data is of little use without a complementary understanding of how that sequence generates function.

Genotype-phenotype studies, which involve altering the expression of specific genes and studying the effects on cells or organisms, have enabled us to understand the function of specific genes and ultimately entire genomes. This approach has been critical to the history of genetics, leading to important nsights into development and function across a range of species, including our own.

Early approaches to genotype-phenotype screening applied techniques – such as site-directed mutagenesis, homologous recombination and antisense RNA – to cause loss of function or alter gene expression. But each of these was hampered by limitations, including complicated protocols and high error rates.

More recently, RNA interference (RNAi) took over as the best way to knockdown genes on a genome-wide scale, with short interfering RNA (siRNA) emerging as a leading technology for large-scale phenotypic screens. However, this can still only achieve partial suppression of gene activity and comes with a high rate of off-target effects.

Enter CRISPR Screening

The development of CRISPR/Cas9, the Nobel-prize-winning gene editing system, changed all this. CRISPR/Cas9 screening is highly efficient and precise, without the off-target effects that plague RNAi screens. CRISPR is completely programmable, being targeted to a specific DNA sequence through the use of a single guide RNA (sgRNA).

The ability of CRISPR to precisely target sequences of the genome at scale – whether deleting, adding or changing DNA sequences – quickly saw it become the predominant system for genome-wide screens.

In addition to CRISPR knockout libraries, which target genes for complete loss of function across the genome, there are two other variations on this theme: CRISPR-a (activating) and CRISPR-i (inhibitory) libraries that can be used to tune the expression of target genes either up or down in a controlled way without altering the underlying protein coding DNA sequence.

Transfecting cells with pools of different sgRNAs can alter thousands of genes in a single experiment across the whole genome and have become a popular way for researchers to study the genetic basis of biological processes.

While custom sgRNA libraries can be created on a case-by-case basis, in practice this is a time-consuming and expensive process. For this reason, several companies are now offering off-the-shelf CRISPR libraries for high-throughput screening.

ADVANCES IN CRISPR SCREENING

Typical CRISPR screens aim to knock out genes by cutting a gene at a specific place within a coding region which is then imperfectly repaired by the cell's non-homologous end joining (NHEJ) system. This results in the loss or addition of a small section of DNA, generating a frameshift that stops the gene from working. While this technique usually works, the resulting changes can sometimes have no effect on gene function, adding a layer of unpredictability and uncertainty to the screen. Synthego, a California-based company offering CRISPR screening libraries licensed from ERS Genomics, overcomes this problem by using a multi-guide strategy. To enable a gene knockout in any given gene, it is often necessary to screen multiple guide RNAs and determine not only which are active but also which can generate a frameshift. This is a time-consuming exercise and can really hamper design efforts to generate high-throughput, arrayed gene knockout libraries," explains Kevin Holden, head of science at Synthego.

Synthego's strategy employs three separate sgRNAs that are designed to work together to ensure high cutting activity. This multi-guide approach generates a fragment deletion at the start of an exon that consistently results in a gene knockout, offering a new level of reproducibility and consistency.

"The multi-guide approach can save researchers a great deal of time by removing the evaluation, guesswork and worry associated with using individual sgRNAs to knockout a gene," Holden says.

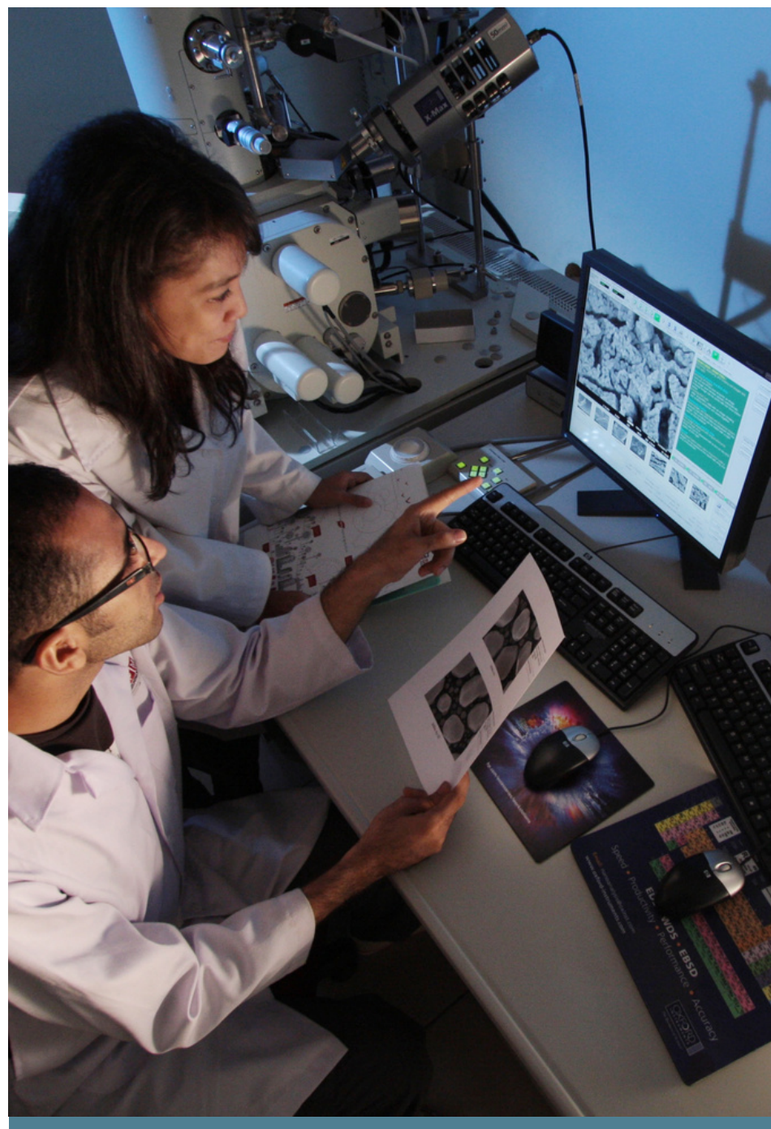
- Advances in liquid handling technology are bringing a shift from pooled CRISPR libraries to arrayed libraries in which each well of a microtitre plate contains a set of sgRNAs designed to knock out or modulate a single gene, which would significantly simplify the task of target deconvolution.

- Another development is phenotypic selection screens. This involves infecting a large pool of cells with a lentiviral-encoded CRISPR library and then subjecting them to a selection challenge, such as viral infection or drug resistance. Any surviving cells can then be analyzed via high-throughput sequencing to identify the guide sequences present in the population and reveal the genes involved.

- There are also moves to introduce novel methods of CRISPR-based gene perturbation, new models like 3D organoids, and integration with other technologies like single-cell RNA sequencing. Holden foresees an expansion of the regions of the genome targeted by these libraries too.

"I think it's highly likely that we'll see CRISPR screening libraries starting to target other interesting areas of genomes as well, beyond just coding regions, including promoter or enhancer region libraries, intron libraries and so on."

POWERING DRUG DISCOVERY AND DEVELOPMENT INTO THE FUTURE



Rapid deployment of high-throughput CRISPR screening methodologies is transforming the drug development pipeline.

In the target discovery phase, CRISPR libraries can be used to knock out large arrays of individual genes in cells and identify phenotypic changes. One common application is in synthetic lethality assays, which aim to identify new drugs that kill cancer cells by interacting with mutations within them.

CRISPR screening can also be deployed in the earlier stages of research, including understanding the mechanisms of disease. CRISPR screening has been fundamental in our understanding of signaling in cancer cells, for example, helping to identify some of the key drivers of cancer development.

Another major application of CRISPR screening is understanding how viruses infect their host cells. During the COVID-19 pandemic, CRISPR screening helped to identify the key proteins needed for SARS-CoV-2 to enter human cells and identified several proteins that could be developed into novel COVID-19 therapeutics. Through the SARS CRISPR screens website, researchers can browse the results of SARS-CoV-2 CRISPR screens to date, look at their quality control measures and even compare the results of multiple experiments – a powerful example of the research community coming together to utilize the benefits of CRISPR screening for global health.

As tools for large-scale high-throughput CRISPR screening continue to develop at pace, the applications of this powerful technology are limited only by the imaginations of researchers hunting for the next generation of targets and therapeutics.

As the global licensing leader for CRISPR/Cas9, ERS Genomics is the first port of call when developing a commercial or research application using CRISPR/Cas9. This applies whether you're a new biotech start-up or an established life sciences organisation.

We have already completed more than 100 licence agreements across a range of life science sectors and make patent rights available in more than 80 countries – the most comprehensive collection of proprietary rights to CRISPR/Cas9 available.

Talk to us today to discuss your licensing needs and let our experienced team help you to leverage the power of CRISPR/Cas9.

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