Academic Insight
On the Cutting-Edge of CRISPR

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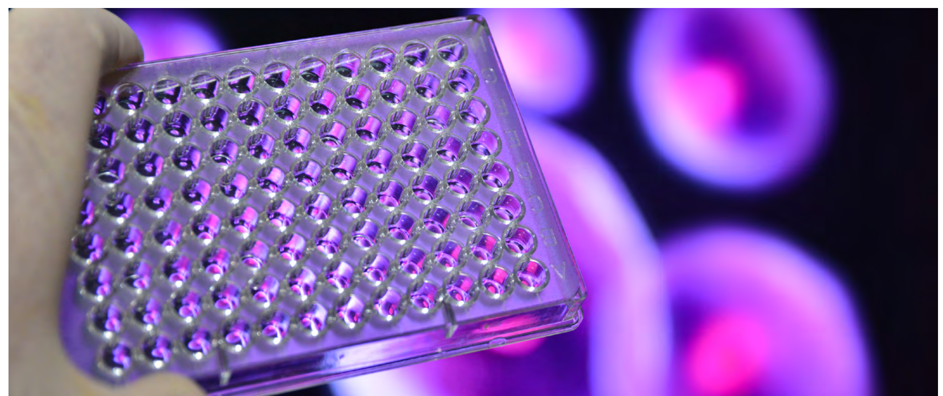
We spoke to Robin to find out about his work within CRISPR, insights on where the technique is heading and the field's exciting developments.

Robin Ketteler,
Group Lead MRC Laboratory for Molecular Biology
University College London

CRISPR Career

Robin and his team are using CRISPR for several applications. One to make individual knockouts for genes and cell lines. The other for high content and high throughput screening using CRISPR based libraries. The third application is to use CRISPR to tag endogenous genes with fluorescent proteins. In each, they have made about five or six knockouts in terms of screening. They are testing gRNA libraries and try to see if they can be used in high throughput screening, so they are in the early stage of developing protocols. In terms of GFE tagging, they have generated around a dozen tags, cell lines and are trying to see if that can be detected. Robin notes that the screening has seen many exciting developments with CRISPR at the moment. Coming from genomic screening, Robin and his team used siRNA libraries and cDNA libraries and so on. Now CRISPR opens up a whole new field. He added: "It seems to be so much easier to generate a proper knockout, compared to a knockdown, with RNA interference. I think at some point, we should be able to use CRISPR in a similar way, so that it's very easy to automate, and that's what is really exciting at the moment. We can do thousands of knockouts in the same time in one experiment."

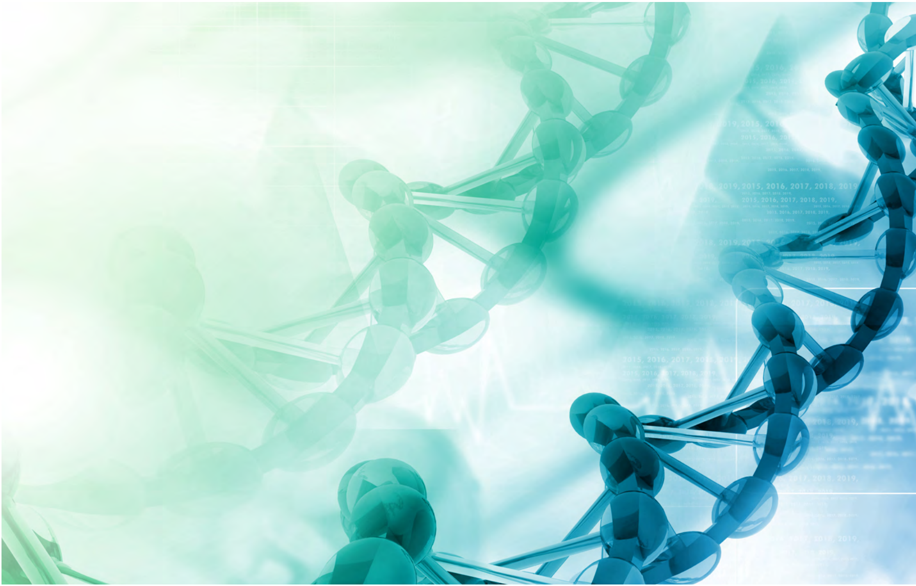


CRISPR Barriers

In regards to screening, a large blockade is the ability to quickly assess the editing and determine knockout efficiency. Outside of the applications that are mostly based on sequencing or protein western blotting, to speed up identifying knockout clones, Robin notes that techniques like digital droplets, PCR, could potentially be used in a much more highest throughput, which have not been attempted so far in the widespread industry.

Why CRISPR in the face of its drawbacks

In acknowledging some of the standing challenges with CRISPR, like efficiency, Robin rationalised that the buzz behind the technique is due to the speed in which a knockout for a single gene



can be achieved. It will take about three or four weeks, which is not possible with any other techniques. Also, the knockout is clean. A knockout could be achieved in a similar timeframe with an RNA interference experiment, but there would always be residual activity of the protein left, because only some of it is depleted. "With CRISPR it's so clean and quick, it's fantastic." He adds.

The future of CRISPR

Robin feels that over the next three years, things will start to slow down with CRISPR, with the field stopping to address problems and hurdles with the technique.

A few areas Robin Ketteler is keen to see progress are the inhibitors for CRISPR Cas9 which open up the possibility to use an inducible CRISPR based system and the tagging of proteins. "A lot of people try fluorescent proteins or some tags, but I believe any other modification can be tagged onto proteins and that could be done on a whole genome scale. I think that will be a big thing, if it works."

Another interesting and emerging field is stem cell work, particularly anything to do with X chromosome in activation. Robin adds that when trying to make a knockout of an X linked gene, using male cells, on entering the stem cell stage it seems that there is reactivation of the locals, which creates a serious challenge for making a CRISPR knockout.

Wish to continue the discussion? Reserve your place at CRISPR 2017 today.

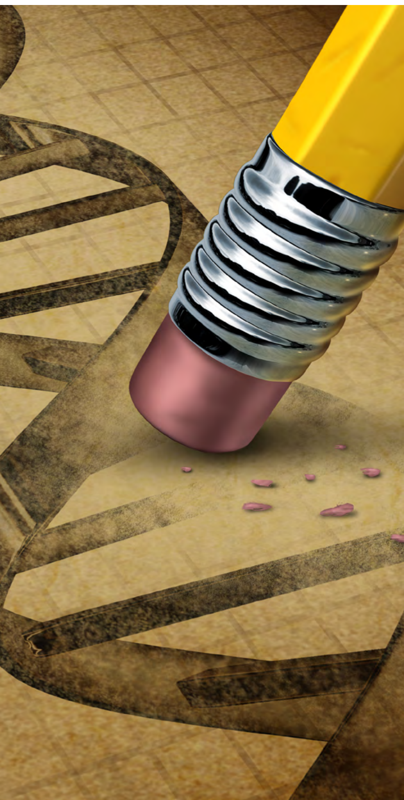
We also spoke to Lorena to find out about her work within CRISPR, insights on where the technique is heading and the field's exciting developments.

Lorena Benedetti
Division of Cancer Studies
King's College London

The Francis Crick Institute

CRISPR Career

Lorena started working with CRISPR around two years ago. She now works in a computational group studying cancer genomes, where CRISPR is used to try to mimic the mutations found in tumour cell lines for different projects. In regards to how CRISPR contributes to understanding human diseases, Lorena notes: "It's very exciting because now we have a very powerful tool to edit human genomes and basically correct them in case there is something wrong that is causing a very serious disease. I think all the advances on that front are very exciting."



CRISPR Barriers

Despite the buzz surrounding the strengths of CRISPR there are some hurdles to overcome for example off-targets and efficiency issues.

Controlling the pathway sufficiently from end to end stands as a challenge for the technique's delivery. For instance, some primary cells cannot be passaged for too long and cannot be generated into the stable clones. Only partial knockout in the cell population might not be sufficient to answer the question of whether the knockout is actually critical or not. These primary cells also do not expand very long - living for only three or four passages.

Some experts have noted that a number of issues are presented by the size of Cas9. Due to it being quite a large nuclease, expressing and packaging it into viruses can be quite challenging. This limits feasibility when one or two or multiple gRNAs need to be packaged in the same virus.

Lorena highlighted another limitation of Cas9 is that target region should be surrounded by a PAM sequence (NGG) therefore limiting the regions of the genome that can be edited. New nucleases like CPF1 have different requirements and therefore allow the editing of more regions.

Why CRISPR in the face of its drawbacks

Lorena acknowledges that at the basic level where she works the off-target effects and editing inefficiencies of CRISPR are critical considerations. However, she maintains that the benefits of using it outweigh the disadvantages. For example: the technique is relatively easy to set up. She says that CRISPR gives very interesting results if caution and controls are applied regarding the off target effects through

monitoring the areas of the genome which are edited but are not targets.

She adds that with editing efficiency 'you can try to enrich your edited population either by single-cell cloning or [via] another technique that can select cells which have been edited.'

The future of CRISPR

The CRISPR field has moved at lightning speed over recent years - as a key tool to accelerate drug discovery, crop improvement, disease treatment and vaccine treatment.

Lorena pinpoints a recent combination of CRISPR and Gene Drive to try to control diseases transmitted by mosquitoes. Although, she notes that some have labelled this as risky and promising results are yet to realize from editing mosquitoes.

Some have noted the promise behind the synthetic biology approach, especially with new forms of Cas9-based approaches. So not the DNA repair itself, but the use of Cas9 as a docking system and bringing new types of modification that can act as methylation or demethylation activators: therefore acting as repressors of expression. Lorena adds that a modified Cas9 without the nuclease function combined with VP64 and MS2-p65-HSF1 can be used to activate gene expression.

Lorena is aware of many Cas 9 variations being tested for improvements to reduce off-target effects and increase effectiveness to edit more cells. These mutants have been earmarked for their potential to contribute to the field's progression.

CRISPR is predicted to contribute most in screening and identifying new targets, helping the quality the research become better and faster. Even simplifying small molecule screening, for instance when revisiting targets and making sure the desired pathway is present or when modifying counter-pathways.

Wish to continue the discussion? Reserve your place at CRISPR 2017 today. Lorena will be present to discuss her research in more detail.

17th-18th May 2017
London, UK

Unlocking The True Capabilities of CRISPR

4 Reasons to Attend:

- Learn from **AstraZeneca and Novartis on how to overcome precision challenges in cell line generation and pre clinical mouse** models to further understand the development of therapeutic application.

- Understand how the **right application for your modification will decrease unwanted immunogenic responses**, with presentations from ZeClinics and Wellcome Trust Sanger Institute.

- **Discover how genome screening with CRISPR can determine target genes** and gain clarity on how best to identify cell type modifications with presentations on how bioinformatics can be used as a tool for in depth evaluation of CRISPR targets

- Gain **insight into the screening capabilities and uncover how to ensure the best CRISPR library design** as well as hearing how this tool can be used to identify both resistant or sensitive genes in cells in your research.