Enhanced CRISPR lets scientists explore all steps of health and disease in every cell type

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Wellcome Trust Sanger Institute and University of Cambridge researchers have created sOPTiKO, a more efficient and controllable CRISPR genome editing platform. Today, in the journal *Development*, they describe how the freely available single-step system works in every cell in the body and at every stage of development. This new approach will aid researchers in developmental biology, tissue regeneration and cancer.

Two complementary methods were developed. sOPiTKO is a knock-out system that turns off genes by disrupting the DNA. sOPTiKD is a knock-down system that silences the action of genes by disrupting the RNA. Using these two methods, scientists can inducibly turn off or silence genes, in any cell type, at any stage of a cell's development from stem cell to fully differentiated adult cell. These systems will allow researchers world wide to rapidly and accurately explore the changing role of genes as the cells develop into tissues such as liver, skin or heart, and discover how this contributes to health and disease.

The body contains approximately 37 trillion cells, yet the human genome only contains roughly 20,000 genes. So, to produce every tissue and cell type in the body, different combinations of genes must operate at different moments in the development of an organ or tissue. Being able to turn off genes at specific moments in a cell's development allows their changing roles to be investigated.

Professor Ludovic Vallier, one of the senior authors of the study from

the Wellcome Trust–Medical Research Council Cambridge Stem Cell Institute at the University of Cambridge and the Sanger Institute, said: "As a cell develops from being stem cell to being a fully differentiated adult cell the genes within it take on different roles. Before, if we knocked out a gene, we could only see what effect this had at the very first step. By allowing the gene to operate during the cell's development and then knocking it out with sOPTiKO at a later developmental step, we can investigate exactly what it is doing at that stage."

The sOPTiKO and sOPTIKD methods allow scientists to silence the activity of more than one gene at a time, so researchers have the possibility to now investigate the role of whole families of related genes by knocking down the activity of all of them at once.

In addition, the freely available system allows experiments to be carried out far more rapidly and cheaply. sOPTiKO is highly flexible so that it can be used in every tissue in the body without needing to create a new system each time. sOPiTKD allows vast improvements in efficiency: it can be used to knock down more than one gene at a time. Before, to silence the activity of three genes, researchers had to knock down one gene, grow the cell line, and repeat for the next gene, and again for the next. Now it can do it all in one step, cutting a nine-month process down to just one to two months.

Dr Alessandro Bertero, one of the first authors of the study from the Cambridge Stem Cell Institute, said: "Two key advantages of using sOPTiKO/sOPTIKD over other CRISPR editing systems are that it is truly inducible and can work in almost any cell type. In the past we have been hampered by the fact we could study a gene's function only in a specific tissue. Now you can knock out the same gene in parallel in a diversity of cell type with different functions."

More information: Bertero A et al. (2016) Optimized inducible

shRNA and CRISPR/Cas9 platforms for in vitro studies of human development using hPSCs. *Development* 143: 4405-4418. DOI: 10.1242/dev.138081