Generating a clonal HAP1LIG4(-) Cas9(+) cell line – an expert interview

DNA double-strand breaks (DSBs) are common events that occur as the result of normal cellular processes but can also arise following exposure to DNA-damaging chemicals or irradiation. DSBs can trigger cell death if left unrepaired, while incorrect repair can lead to genomic instability and the introduction of cancer-promoting genomic abnormalities. Once detected, DSBs are typically repaired through one of two pathways: homologous recombination (HR) or non-homologous end joining (NHEJ). While NHEJ is more error-prone than HR, it represents the primary DSB repair pathway in both proliferating and non-dividing cells due to its greater efficiency and mechanistic flexibility.

A key component of NHEJ is DNA ligase 4, an ATP-dependent ligase encoded by LIG4 which reconnects the broken DNA strands following a DSB. A growing body of literature has demonstrated that inhibition of DNA ligase 4 or knockout of LIG4 significantly increases the efficiency of CRISPR/Cas9-mediated gene-editing, the latter of which requires the introduction of a DSB.

This consideration led scientists at the Wellcome Sanger Institute to turn to Revvity's (formerly Horizon Discovery) HAP1 LIG4 knockout cell line (HAP1 LIG4(-)). This haploid human cell line lacks LIG4 and therefore DNA ligase 4 activity, effectively disabling the NHEJ pathway while enhancing HR. The Sanger Institute aims to map the consequences of all sequence variants of disease-related genes and elements, an undertaking that requires highly efficient gene editing. By incorporating a stably expressed Cas9 nuclease into Revvity's HAP1 LIG4(-) cell line, the Sanger Institute has generated a cell line that is highly amenable to efficient CRISPR/Cas9-mediated genomic modifications.

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Dr. Sebastian Gerety is currently in charge of animal and cellular modeling, and applies his background and expertise in vertebrate genetics and model organisms, to elucidate the links between candidate disease mutations and the phenotypes observed in affected patients. Since joining in 2011, Dr. Gerety spearheaded the application of CRISPR/CAS9 in zebrafish and mice at the Sanger Institute, generating their first mutant animals using this technique.

Dr. Gerety completed his Ph.D. in Developmental Biology at the California Institute of Technology under the HHMI investigator Dr. David Anderson. Afterward, he joined a Human Frontiers Science Program-funded post-doctoral fellowship under Dr. David Wilkinson at the National Institute for Medical Research, in London.

We sat down with Dr. Sebastian Gerety, Principal Staff Scientist in the Human Genetics Program at the Sanger Institute, to discuss how leveraging the HAP1 LIG4(-) cell line accelerates the Institute's research goals and how he envisions this new cell line impacting scientific discovery.

Q Revvity: Why did you opt for the HAP1 cell line in your research and how does it enhance your objectives?

A Dr. Gerety: The Sanger Institute is a founding member of the Atlas of Variant Effects (AVE) Alliance. In short, the goal of the consortium is to build an atlas of the impact of all possible sequence variants for disease-related genes and functional elements to greatly accelerate patient diagnosis and triage for treatment. At the Sanger Institute, we have unique capabilities in building high-throughput platforms. The HAP1 cell line is at the center of one of those pipelines, one we have built to explore the effects of single nucleotide variants (SNVs), using Saturation Genome Editing (SGE).[1] The technique, originally created by Greg Findlay in the Shendure Lab, employed HAP1 cells, and our work builds upon those foundational experiments.

A key feature of the HAP1 cell line is that it is haploid. In a normal diploid background, the interpretation of experiments is confounded by the presence of two alleles, both potentially subject to editing. Using a haploid background allows us to cleanly assess a single allele for variant function. The second important feature of the HAP1 line is its high transfection efficiency, allowing us to achieve extremely high rates of Cas9-mediated editing.

Q Revvity: What unmet need in the scientific community prompted you to generate a stably expressing Cas9 cell line in a LIG4 null background?

A Dr. Gerety: As part of our efforts to contribute to the mission of the AVE Alliance, we needed an efficient cellular platform for SGE.[1] Homology-directed repair and HR in cell lines is typically inefficient. The experiments that we perform (SGE at high-throughput scale) require extremely high efficiencies of HR. The combination of the LIG4 mutant background and the stable Cas9 expression increases the efficiency of HDR and HR by three to four-fold compared to the alternative approaches. These cells provide an amazing platform to perform cellular experiments requiring high rates of precise gene editing, not just the SGE we are undertaking.

At the Sanger Institute, we are very keen to share the cell lines that we generate with the scientific community. We hope that collective efforts in studying disease-associated genes and functional elements will lead to advances in diagnosing and treating human disease and understanding genes, gene products, and their regulation.

Q Revvity: What challenges did you face during the development of this cell line?

A Dr. Gerety: The biggest challenge we encountered while working with HAP1-derived lines is maintaining the haploid state. They tend to revert to diploid state over weeks in culture (up to 20% diploid within three weeks). The growth advantage of the diploid revertants means that they often outcompete the haploid cells. To avoid this, we use a FACS (fluorescence-activated cell sorting) based approach to enrich haploid cells at regular intervals, and especially at the start of experiments.[2,3]

Q Revvity: How are other teams at the Sanger Institute utilizing this cell line and which research programs is it currently supporting?

A Dr. Gerety: The line is currently being used in a large-scale pipeline that supports the AVE Alliance, as mentioned previously, where we perform SGE of disease genes across both cancer and neurodevelopmental disorders to assess variant function. Leveraging the HAP1 line, we have been able to generate high-quality variant effect maps, four of which are published or are under review for publication, which should transform the diagnosis of patients carrying variants in those genes.[2,3]

Q Revvity: With this tool now available, what types of questions are you hoping the scientific community will try to answer now that this tool is available?

A Dr. Gerety: We're hoping that by disseminating this line, we will enable other groups across the world to perform SGE as efficiently as we've been able to at the Sanger Institute. For our work, this allows us to generate very high-quality variant effect maps, which will assist with diagnosis, prognosis, and treatment of disease. More broadly, having a haploid, Cas9-expressing line such as this will allow the research community to perform better screens with higher efficiency, wherever HDR and Cas9 activity are required.

Access the Wellcome Sanger Institute's HAP1 LIG4(-) Cas9(+) cell line here.

1. Findlay, G.M., Daza, R.M., Martin, B. et al. Accurate classification of BRCA1 variants with saturation genome editing. Nature 562, 217–222 (2018). https://doi.org/10.1038/s41586-018-0461-z
2. Radford, E.J., Tan, HK., Andersson, M.H.L. et al. Saturation genome editing of DDX3X clarifies pathogenicity of germline and somatic variation. Nat *Commun* **14**, 7702 (2023). https://doi.org/10.1038/s41467-023-43041-4
3. Waters et al, Saturation genome editing of BAP1 functionally classifies somatic and germline variants, Nature Genetics, 2024, in press.

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