Certificate of Analysis

HZGHC-LIG4-Cas9 Stable; Human HAP1 LIG4 KO + Cas9 Stable

This cell line used Horizon Discovery HAP1 (haploid cell line) LIG4 knockout product (HZGHC000759c005) as starting material for the generation of Human HAP1 LIG4 KO + Cas9 stably expressing cell line.

PRODUCT INFORMATION			
Cell Line	LIG4 KO Cas9 stable cell line	**Genotype**	LIG4 (-) Cas9 (+)
Product ID	HD CLP-014	**Parental**	HAP1
Date of Manufacture	Jan 2024	**Passage**	17

PROPERTIES	
Total Cells	>0.5x10^6
Volume / Ampule	0.4 ml
Storage Conditions	Liquid nitrogen vapor phase

PRODUCT INFORMATION		
Test	**Test Method**	**Pass / Fail**
Cell Line Characterization	Sanger Sequence (DNA)	Pass
Cas9 Activity	PCR of target	Pass

GROWTH CONDITIONS	
Recommended Media	Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% FCS, 100 U/ml Penicillin and 100 µg/ml Streptomycin
Subculture	Cells are cultured as a monolayer at 37ºC in a humidified atmosphere with 5% CO2. Cells should be passaged every 2-3 days. Split at 70-75% confluency, approximately 1:10-1:15.
Cell Line Revival	Rapidly thaw cells in a 37ºC water bath. Transfer contents into a tube containing pre-warmed media. Centrifuge cells and seed into a 75cm^2 flask containing pre-warmed media
Recommended Freezing Media	IMDM + 20% FCS + 10% DMSO

Certificate of Analysis

HZGHC-LIG4-Cas9 Stable; Human HAP1 LIG4 KO + Cas9 Stable

CELL LINE CHARACTERIZATION (DNA)

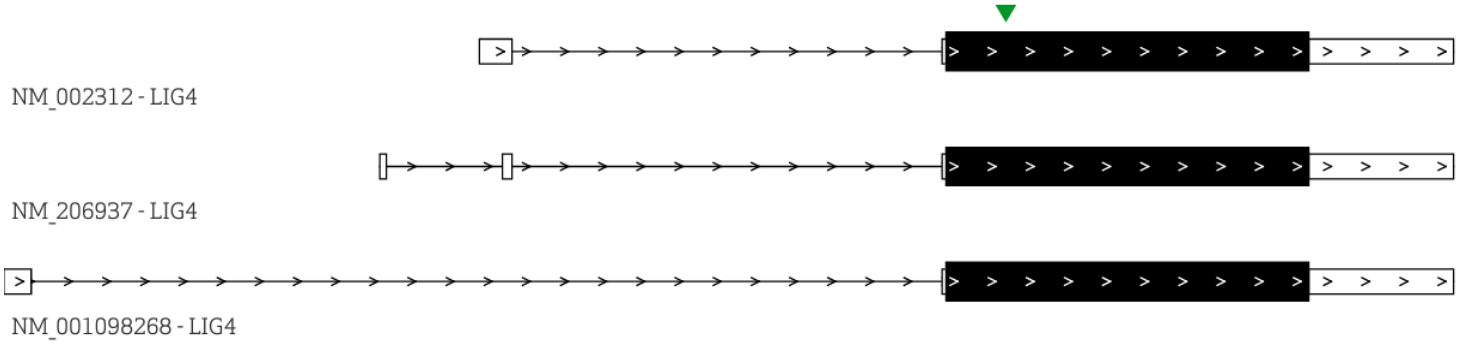
Target gene	LIG4	**Mutation**	10bp deletion in exon 2
Guide RNA Sequence	AAGGTCGTTTACTTGCTGTA	**Genomic location**	chr13:108210816
Target Transcript	NM_001098268	**Passage**	7

Forward Primer	Reverse Primer	Sequencing Primer
GTAGTGACATTATGCAACTCAGCAG	TAGAGATGGAAAAGATGCCCTCAAA	TAGAGATGGAAAAGATGCCCTCAAA

Sequencing Results

TAGAGATGGANAAGATGCCCTCAAACTTTTAAACTACAGAACACCCACTGGAACTCATGGAGATGCTGGAGACTTTGC
AATGATTGCATATTTTGTGTTGAAGCCAAGATGTTTACAGAAAGGAAGTAGCAAGTAAACGACCTTTTAGACTCAATTG
CCAGCAATAATTCTGCTAAAAGAAAAGACCTAATAAAAAAGAGCCTTCTTCAACTTATAACTCAGAGTTCAGCACTTGA
GCAAAAGTGGCTTATACGGAT GATCATAAAGGATTTAAAGCTTGGTGTTAGTCAGCAAACTNTCT

Transcript Plot



Sequence Alignment

```
TAGAGATGGANAAGATGCCCTCAAACTTTTAAACTACAGAACACCCACTGGAACTCATGGAGATGCTGGAGACTTTGCAATGATT
||||||||||.||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TAGAGATGGAAAAGATGCCCTCAAACTTTTAAACTACAGAACACCCACTGGAACTCATGGAGATGCTGGAGACTTTGCAATGATT

GCATATTTTGTGTTGAAGCCAAGATGTTTACAGAAAGGAAGT----------AGCAAGTAAACGACCTTTTAGACTCAATTGCCA
|||||||||||||||||||||||||||||||||||||||||          ||||||||||||||||||||||||||||||||||
GCATATTTTGTGTTGAAGCCAAGATGTTTACAGAAAGGAAGTTTAACCATACAGCAAGTAAACGACCTTTTAGACTCAATTGCCA

GCAATAATTCTGCTAAAAGAAAAGACCTAATAAAAAAGAGCCTTCTTCAACTTATAACTCAGAGTTCAGCACTTGAGCAAAAGTG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCAATAATTCTGCTAAAAGAAAAGACCTAATAAAAAAGAGCCTTCTTCAACTTATAACTCAGAGTTCAGCACTTGAGCAAAAGTG

GCTTATACGGATGATCATAAAGGATTTAAAGCTTGGTGTTAGTCAGCAAACTNTCT
|||||||||||||||||||||||||||||||||||||||||||||||||||.|||
GCTTATACGGATGATCATAAAGGATTTAAAGCTTGGTGTTAGTCAGCAAACTATCT
```

Wellcome Sanger Institute Datapack

HZGHC-LIG4-Cas9 Stable; Human HAP1 LIG4 KO + Cas9 Stable

The Hap1 cell line is a highly desirable platform for genetic research due to its near-haploid genotype. Having only a single copy of any given genomic locus means that any phenotype observed as a result of genome editing can be attributed to a single specific genetic alteration, avoiding the risk of masking or compensation from additional unedited alleles.

It has been previously demonstrated (*REF 1*) that the inactivation of the LIG4 gene in HAP1 cells can bias CRISPR/CAS9 genome editing events away from non-homologous end joining, and in the presence of a homology template, towards Homology Dependent Repair-based incorporation of exogenous replacement loci. This desirable trait allows for **high editing efficiencies at some of the highest rates available in a cellular model**.

Previous work using the HAP1^{LIG4-} background involved the co-transfection of plasmids containing a CAS9 expression cassette, an sgRNA expression cassette, and an HDR template library (*REF 1*). However, subsequent experiments at the Wellcome Sanger Institute determined that the inefficient transfection of CAS9 expressing plasmids, due to their large size (the CAS9 CDS alone is over 4kb), reduced the efficiency of CAS9 delivery and subsequent genome editing. To overcome this limitation, researchers at the Wellcome Sanger Institute generated a stably transduced CAS9-expressing population of Hap1^{LIG4-} cells (parental line: Horizon product #HZGHC000759c005), using lentivirus derived from plasmid pKLV2-EF1a-Cas9Bsd-W (Addgene plasmid# 68343), followed by Blasticidin selection. The resulting stably CAS9-expressing population was found to edit at higher rates, likely due to the smaller plasmid sizes of the sgRNA expressing plasmid and HDR template plasmids that remained to be transfected. Using this pooled CAS9-expressing population, researchers at the Wellcome Sanger Institute successfully performed SGE (Saturation Genome Editing) to generate a whole gene variant effect map for the intellectual disability-associated gene DDX3X (*REF 2*).

Generation of a clonal Hap1^{LIG4-}CAS9+ line

To further optimize the use of the HAP1 line in their work on SGE, it was proposed that a clonal population of the higher expressing cells from the above pooled population might provide even higher editing rates: with variegated expression of CAS9 integrated at numerous different loci, it was suspected that the editing efficiencies would be following an equally variegated efficiency.

The polyclonal population of **Hap1^{LIG4-}CAS9+** cells was therefore subcloned by low density plating under Blasticidin selection, followed by colony picking, to generate a number of independent clonal isolates. A number of these were karyotyped and assessed for Cas9 activity using a Cas9 reporter assay. Two clones, A1 and A5, showed high Cas9 activity and no significant karyotypic abnormalities, of which one was selected for further work (A5).

When tested in HDR assays typical of SGE experiments, researchers found that editing rates were extremely high (panel 1, "Programmed edit"), and importantly the rate of residual wildtype cells approached zero (Panel 1, "Wildtype"): this is in contrast to the polyclonal population ("Pooled HAP1 line"), in which 30-50% of cells can typically remain wildtype due to a lack of CAS9 cutting.

This clonal line, due to its high efficiency of editing and ease of transfection, has been used in a growing number of SGE screens (*REF 3*, and manuscripts in submission) and is the mainstay of routine production of SGE data at the Wellcome Sanger Institute in their flagship SGE pipeline.

METHODS

CULTURING CELLS

Culture conditions for Hap1^{LIG4-}CAS9+ cells are as described for HAP1 cells, in IMDM basal media supplemented with 10% FBS and 1% Glutamine/Penstrep. Cells were typically split (using TrypLE) 1:10-1:15 every 2-3 days and kept in culture lower than 80% confluency to maintain karyotypic stability.

HAPLOIDY

As haploid HAP1 cells grow more slowly than diploid revertants, it is important to regularly FACS-purify haploid populations during line maintenance and banking, and just prior to starting experiments relying on haploidy. For methods, please see (*REF 2*).

BLASTICIDIN SELECTION

Selection for CAS9/Bsd expressing cells is routinely carried out to ensure continued CAS9 expression in the cell line. For this, Blasticidin is simply added to the routine culture media at a final concentration of 10 microgrammes per ml, for a few days.

TRANSFECTION METHODS

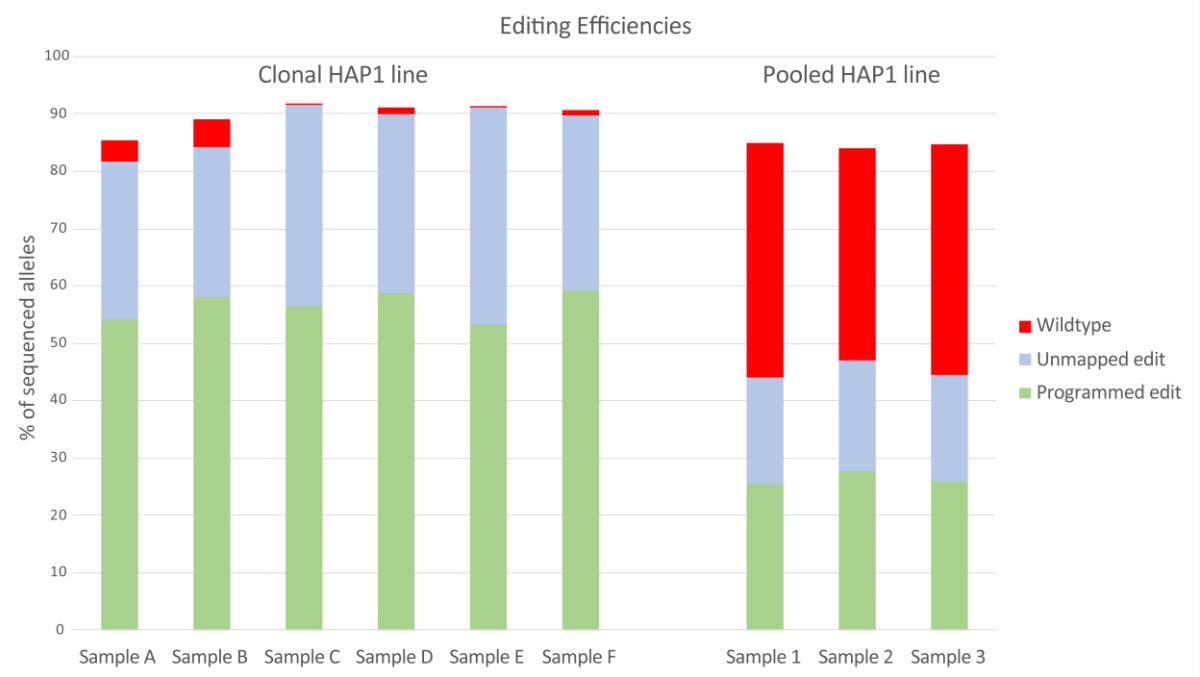
Wellcome Sanger researchers find that Xfect Transfection Reagent (Takara Bio) is very effective at transfecting HAP1 cells with plasmid DNA(*REFS 2,3*), achieving up to 80% efficiency when using GFP expressing plasmids and FACS-based quantification. More recent testing using electroporation-based methods can achieve 85%-98% transfection efficiencies using the Neon NxT system (Thermofisher, 100uL tip, 1x10^6 cells, Buffer R, 1600-1700V, 30mS pulse).

REFERENCES

1. Findlay et al, Accurate classification of BRCA1 variants with saturation genome editing, Nature, 2018 Oct;562(7726):217-222. doi: 10.1038/s41586-018-0461-z (PMID: 30209399)

2. Radford and Tan et al, Saturation genome editing of DDX3X clarifies pathogenicity of germline and somatic variation, Nat Communications, 2023 Dec 6;14(1):7702. doi: 10.1038/s41467-023-43041-4 (PMID: 38057330)

3. Waters et al, Saturation genome editing of BAP1 functionally classifies somatic and germline variants, Nature Genetics, 2024, *in press*



Panel 1: HDR Editing rates using clonal (Clonal HAP1 line) versus the pooled parental HAP1 line (Pooled HAP1 line). After co-transfection of sgRNA expression (with Puro cassette) and HDR library plasmids, cells were grown under Puromycin selection to select for transfected cells. After 4 days in culture, amplicon sequencing of the targeted loci revealed high editing rates, with lower residual wildtype loci in the Clonal HAP1 line.

The information provided in this document is for reference purposes only and may not be all-inclusive. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume liability for the accuracy or completeness of the information contained herein. Users should exercise caution when handling materials as they may present unknown hazards. Revvity shall not be liable for any damages or losses resulting from handling or contact with the product, as Revvity cannot control actual methods, volumes, or conditions of use. Users are responsible for ensuring the product's suitability for their specific application. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF.

The Wellcome Sanger Institute is a world leading genomics research centre. We undertake large-scale research that forms the foundations of knowledge in biology and medicine. We are open and collaborative; our data, results, tools and technologies are shared across the globe to advance science. Our ambition is vast – we take on projects that are not possible anywhere else. We use the power of genome sequencing to understand and harness the information in DNA. Funded by Wellcome, we have the freedom and support to push the boundaries of genomics. Our findings are used to improve health and to understand life on Earth. Find out more at www.sanger.ac.uk or follow us on Twitter, Facebook, LinkedIn and on our Blog.