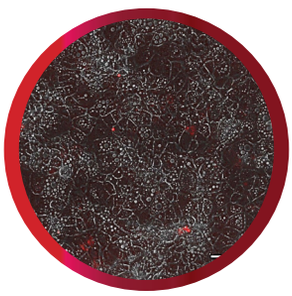
DefiniGEN

DefiniGEN human iPS-derived FH hepatocytes



Familial hypercholesterolemia (FH) is an autosomal dominant disorder of lipoprotein metabolism caused mainly by mutations in the low-density lipoprotein receptor (LDLR) gene. Disease modelled Familial hypercholesterolemia hepatocytes (Def-HEP FH) are highly functional human hepatocytes derived using human induced pluripotent stem cell technology.

For the production of Def-HEP FH cells, fibroblasts are first reprogrammed into iPSC using the Nobel Prize winning technology developed by Yamanaka and colleagues Horizon CRISPR gene-editing is then used to introduce a precise genetic mutation into the LDLR gene of an iPSC line. Def_HEP FH hepatocytes represent an optimized disease model for drug discovery applications and a principal tool for elucidating the underlying mechanisms of the disease.

Genetic validation and cell viability

Def-HEP FH cells have been validated and verified for the E101K genetic mutation in the LDLR gene. Typical viabilities of the thawed hepatocytes are >70% upon receipt.

Sequence data

cDNA sequencing (RT-PCR followed by TOPO cloning) was undertaken to determine the sequence of the cDNA expressed from the LDLR allele.

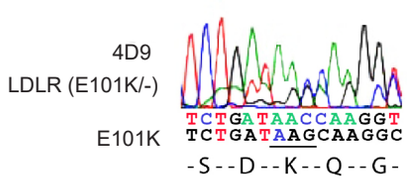


Figure 1. Sequence confirmation of LDLR E101K mutation in Def-HEP FH cells.

Advantages

Standardized cell product containing >98% human hepatocyte cells producing reproducible and biologically relevant data

Disease circuit verification LDL uptake is significantly impaired in Def-HEP FH cells

Optimized work flow we can deliver industrial quantities of cryopreserved cells to client specification

Functional test

The in vivo functional implications of LDL receptor deficiency are conserved in our model, as shown by immunostaining. The results demonstrate that Def-HEP FH hepatocytes have an impaired ability to incorporate LDL (Figure 2). Receptor-specific binding of Dil-LDL is followed by internalization of the bound complex and lysosomal hydrolysis of the ligand. An increase in the fluorescence intensity per cell is hence used as a measure of Dil-LDL uptake and, implicitly, as an indication of LDLR presence. These findings signify that CRISPR generated disease-specific human iPS cells can successfully be used to model Familial Hypercholesterolemia.

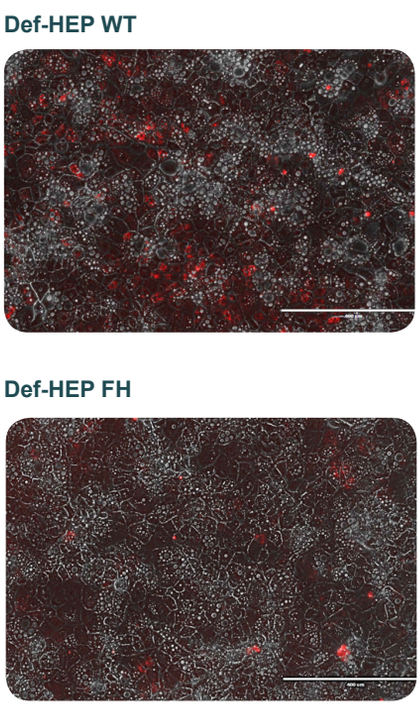


Figure 2. Quantification of intracellular A1AT polymers and intercellular total A1AT secretion in Def-HEP A1ATD cells. Primary human hepatocytes (PHH) and hepatocellular liver carcinoma cells (Hep G2) are used as internal controls.

LDL receptor analysis

Def-HEP FH cells demonstrate an impaired ability to take up LDL cholesterol relative to the WT isogenic control.

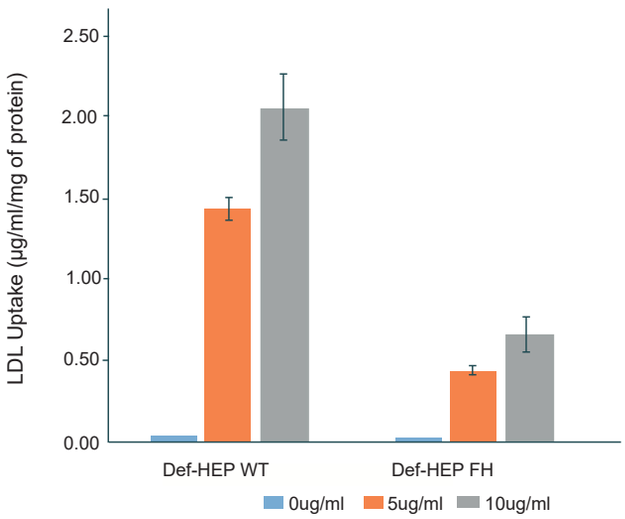


Figure 3. Quantitative LDL receptor assays basd on fluorescent Dil-LDL internalization have demonstrated that Def-HEP FH iPSC-derived hepatocytes have a significantly impaired ability to incorporate LDL relative to the isogenic control Def-HEP WT over a range of LDL substrate ranges.

References

Modelling Familial Hypercholesterolemia using human isogenic induced pluripotent stem cells. Diaz A, Soares F, Santos R, Jhaveri K, Schofield C, Lowe C, Yeo M. ISSCR 2016. June 22-25.

Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. Rashid ST, Lomas DA, Vallier L et al. J Clin Invest. 2010 Sep;120(9):3127-36.

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