DefiniGEN

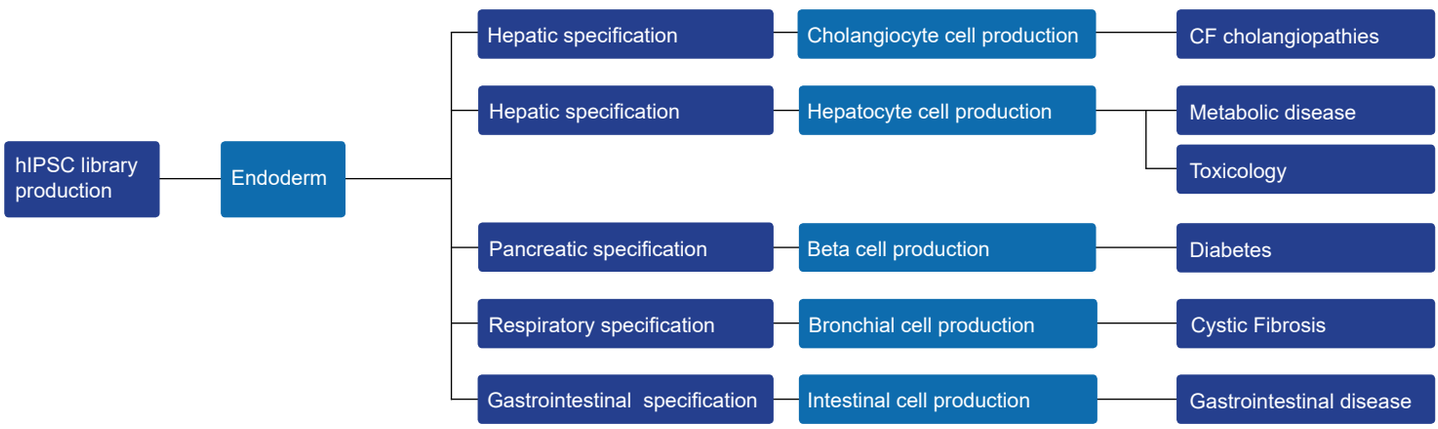
CELL PRODUCTS AND SERVICES

LIVER PANCREAS INTESTINAL CHOLANGIOCYTES CUSTOM DISEASE MODELS IPSC REPROGRAMMING IPSC DIFFERENTIATION

Contents

OptiDIFF Platform Technology

DefiniGEN are a University of Cambridge supported company whose mission statement is to provide optimized human cell products to the scientific community. DefiniGEN is focused on serving the growing need in the pharmaceutical industry for more precise ways to predict efficacy and toxicity in candidate drugs prior to clinical trials. The company has world-leading expertise in the area of iPSC production, differentiation, and metabolic disease modelling. The application of these technologies in drug discovery provides pharmaceutical companies with more predictive *in vitro* cell products enabling the development of safer and more effective treatments. Human Induced Pluripotent Stem Cell (hIPSC) production was first demonstrated in 2007 and has aroused great scientific, social, and economic interest earning Shinya Yamanaka the Nobel Prize in 2012. OptiDIFF™ our proprietary core technology is a world-leading production platform developed at the University of Cambridge for the generation of iPS cells, and their differentiation into commercially prioritized cell types, including liver, pancreas, intestinal, cholangiocytes, and lung cells. The platform uses GMP-compatible defined conditions which enables the exquisite quantitative and temporal process control required to produce standardized populations of terminally differentiated cell products. The OptiDIFF platform can be used to generate client-specified custom disease models for a variety of indications ranging from orphan liver and metabolic diseases to type 1 and type 2 patient-derived diabetes models.



Selected Platform References

Generation of Hepatocytes from Pluripotent Stem Cells for Drug Screening and Developmental Modeling. Gieseck RL 3rd, Vallier L, Hannan NR. **Methods Mol Biol**. 2015;1250:123-42.

Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation. Sampaziotis F, Vallier L et al **Nature Biotechnol**. 2015 Aug; 33(8): 845–852.

Generation of Distal Airway Epithelium from Multipotent Human Foregut Stem Cells. Hannan NR, Sampaziotis F, Segeritz CP, Hanley NA, Vallier L. **Stem Cells Dev**. 2015 Jul 15;24(14):1680-90.

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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.

Human Hepatocytes Wild Type

OVERVIEW

DefiniGEN's human hepatocyte cells are highly functional cryopreserved cell products displaying many of the characteristics of primary human cells. Key hepatocyte functions include albumin production, glycogen storage and A1AT secretion. CYP450 mRNA expression and catalytic activities are also observed at comparable levels to primary human hepatocytes (PHH).

Cell morphology

When thawed and plated as a monolayer, Def-HEP cells form hepatocytes with characteristic cobblestone morphology and tight cell junctions (Figure 1).

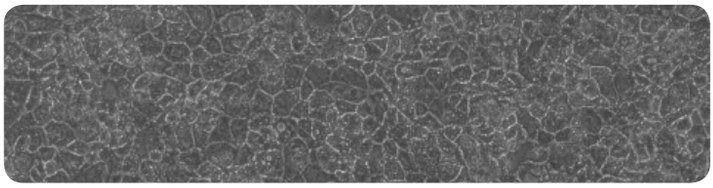

Figure 1. Overview of Def-HEP cell morphology. Def-HEP WT cells exhibit typical hepatocyte cobblestone morphology and bi-nucleation.

Product Specification	
Catalog Number	Def-HEP WT
Format	Cryopreserved
Cell Number	Typically 3-6 million cells
Viability	>70%
Disease	None (Healthy Donor)
Application	Research & Predictive Toxicology

Hepatocyte maturation markers

Def-HEP cells display the functional characteristics of primary human hepatocytes including albumin secretion, A1AT production, glycogen storage and LDL uptake (Figure 2).

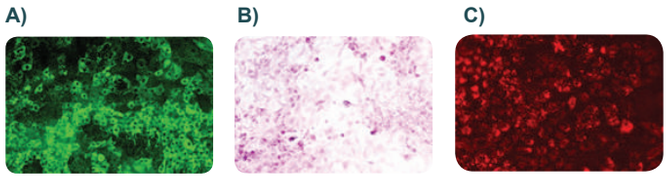

Figure 2. Functional analysis of Def-HEP WT hepatocytes. (A) Albumin secretion, 10x magnification (B) Glycogen storage shown by PAS staining (C) LDL cholesterol uptake shown by fluoresceinated LDL incorporation.

Gene expression profiles

Def-HEP cells express key hepatocyte markers including albumin, A1AT and HNF4a at similar levels to primary human hepatocytes. Significantly the observed AFP (Alpha-fetoprotein) levels are very low in the Def-HEP cells indicating they have progressed to a mature state comparable to PHH.

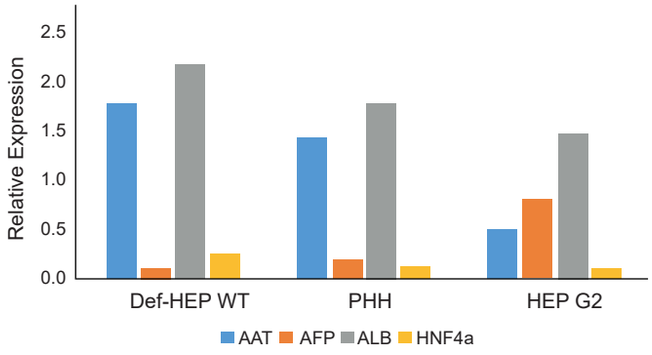

Figure 3. Gene expression analysis demonstrates that Def-HEP express key hepatocyte markers at similar levels to PHH. AFP levels are extremely low in Def-HEP indicating the cells have attained a functional mature status.

Human Hepatocytes Wild Type

In terms of general metabolism DefiniGEN hepatocytes generate ATP via mitochondrial oxidative phosphorylation and are thus suitable for mitochondrial toxicology applications. They do not exhibit the Crabtree Effect, the disadvantageous phenomenon observed in immortalized liver cell lines which generate their ATP via glycolysis.

Following a thaw and recovery protocol, DefiniGEN hepatocytes have a 15-20 day window of use making them effective models for hepatitis lifecycle studies. The cells express key hepatitis markers such as CD81, SR-B1, Claudin-1 and Occludin at similar levels to primary human hepatocytes.

Hepatitis marker analysis

Gene expression analysis demonstrates that multiple batches of Def-HEP cells reproducibly express key hepatitis markers including CD81, NTCP, SCAR-B1, Claudin-7 and Occludin. The levels of markers observed are extremely similar to PHH (HepG2 cells do not express all key hepatitis markers) (Figure 4).

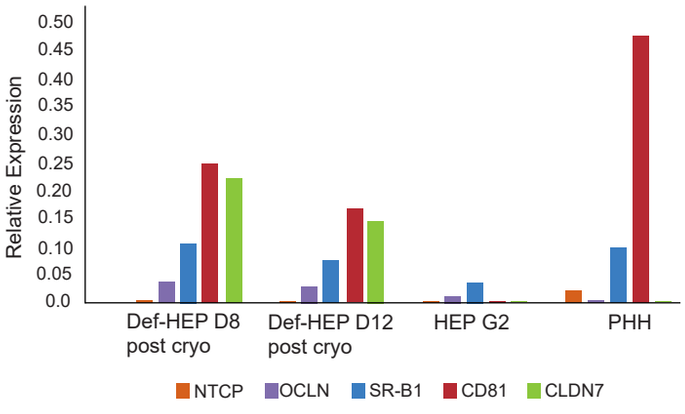

Figure 4. Gene expression analysis demonstrating the presence of key Hepatitis markers in Def-HEP including NTCP, Occludin, SR-B1, CD81 and CLDN7.

Multiple inducible CYP450 activities

Def-HEP cells display CYP450 induced activity profiles that are comparable to PHH (CYP1a2 EROD assay, inducer - omeprazole), (CYP3A4 PGlo assay, inducer - rifampicin) (Figure 5).

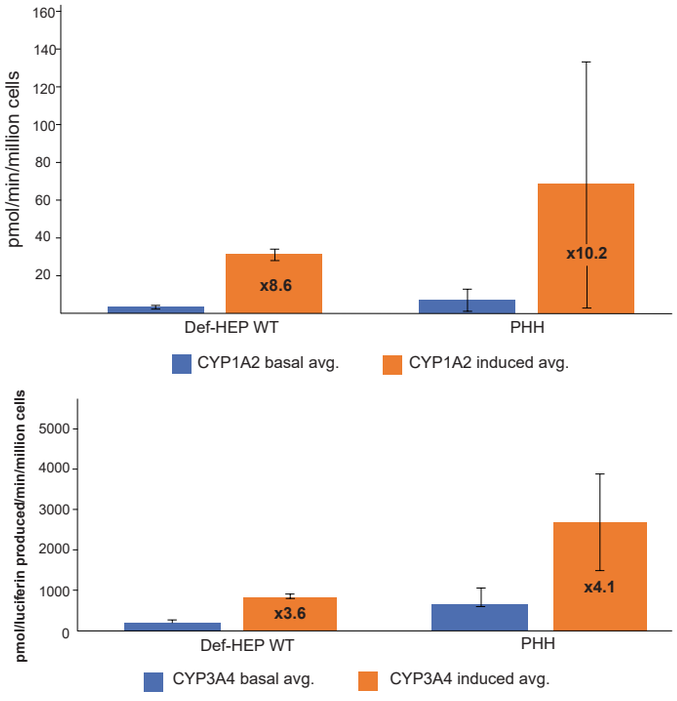

Figure 5. Def-HEP cells have comparable CYP activity to PHH and induced activity profiles that are highly similar to PHH (CYP1a2 EROD assay, inducer - Omeprazole), (CYP3A4 PGlo assay, inducer - rifampicin).

References

Generation of Hepatocytes from Pluripotent Stem Cells for Drug Screening and Developmental Modeling. Gieseck RL 3rd, Vallier L, Hannan NR. **Methods Mol Biol**. 2015;1250:123-42.

Maturation of induced pluripotent stem cell derived hepatocytes by 3D-culture. Gieseck RL 3rd, Hannan NR, Vallier L. **PLoS One**. 2014 Jan 22;9(1):e86372.

Production of hepatocyte-like cells from human pluripotent stem cells. Hannan NR, Vallier L et al. **Nature Protocols**. 2013 Feb;8(2):430-7.

Targeted gene correction of α1-antitrypsin deficiency in induced pluripotent stem cells. Yusa K, Rashid ST, Vallier L, et al. **Nature**. 2011 Oct 12;478(7369):391-4.

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.

Human Hepatocytes Non-Alcoholic Fatty Liver Disease

OVERVIEW

Non-alcoholic fatty liver disease (NAFLD) affects one-third of adults in developed countries. This disease is characterized by the accumulation of fat within the liver that can lead to inflammation, fibrosis, and hepatocellular carcinoma. The most significant NAFLD risk-associated genetic variant is I148M in the gene coding for Patatin-like phospholipase domain-containing protein 3 (PNPLA3). NAFLD hepatocytes contain this mutation and in combination with the isogenic control represent optimized disease model tools for drug discovery applications assisting the elucidation of the complex mechanisms underlying the condition.

Immunofluorescence analysis

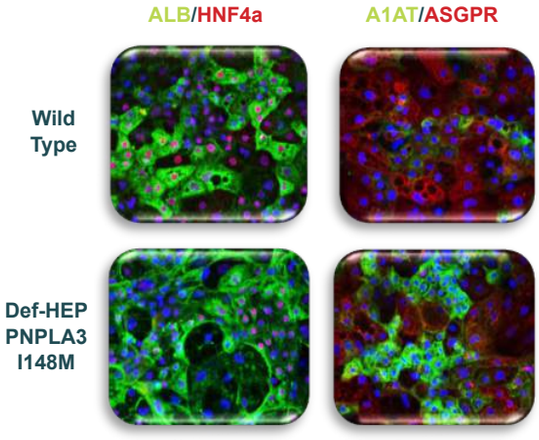

Figure 1. Immunofluorescence staining of Def-HEP PNPLA3 cells overviewing the expression of general hepatocyte maturation markers

Product Specification	
Catalog Number	Def-HEP PNPLA3-I148M Def-HEP PNPLA3-KO
Format	Cryopreserved
Cell Number	Typically 3-6 million cells
Genetic background	Non-alcoholic fatty liver disease
Viability	>70%

Disease circuit verification

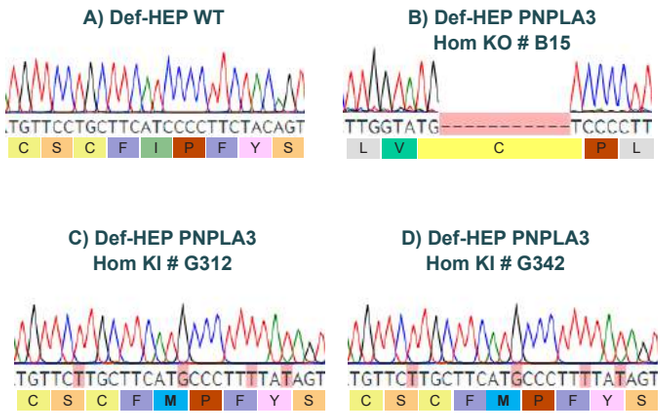

Figure 2. Sanger sequencing of edited PNPLA3 gene using CRISPR/Cas9 in hiPSC line. (A) PNPLA3 gene in wild-type line. (B) PNPLA3 homozygous knock-out clone with 11bp deletion causing a frameshift mutation. (C,D) PNPLA3 homozygous knock-in clones with I148M mutation (Isoleucine to methionine at position 148, exon 3 (I148M, rs738409).

Fatty acid accumulation

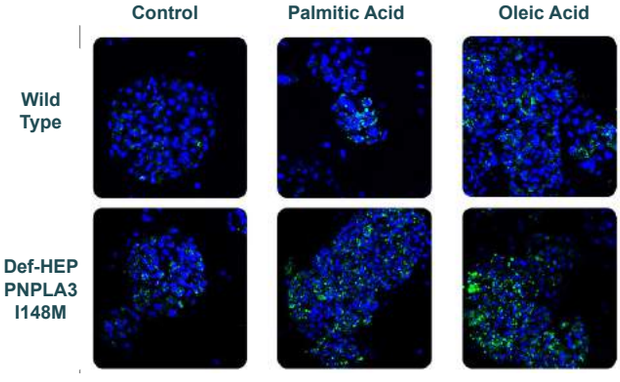

Figure 3. When cells are treated with 0.25 mM of either oleic acid or palmitic acid, the fatty acids are absorbed by the hepatocyte-like cells and accumulated into lipid droplets within the cells. After treatment, the lipid droplets were stained with bodipy. The I148M variant demonstrated increased fatty acid accumulation upon treatment with these fatty acids in comparison with the isogenic wild-type control.

Human Hepatocytes Non-Alcoholic Fatty Liver Disease

Key hepatocyte marker analysis

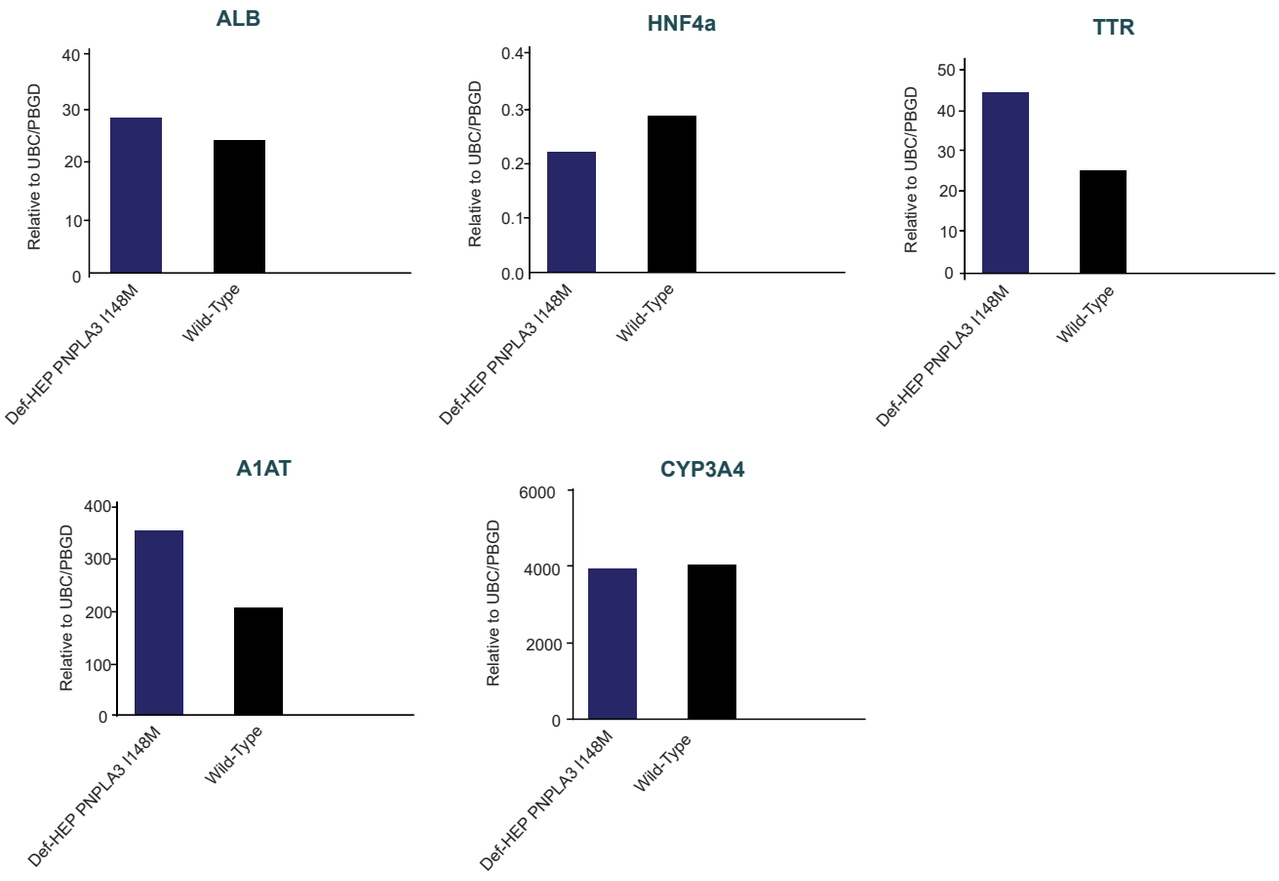

Figure 4. QPCR analysis indicates that the PNPLA3 knock-in hiPSCs can be successfully differentiated into hepatocyte-like cells that express key hepatocyte markers.

Complex disease - NAFLD

PNPLA3 rs738409 impaired hepatocellular triglycerides hydrolysis and increased lipogenesis associated to the 148 M allele

GCKR rs1260326 increased glycolysis favours an increase in triglyceride levels

TM6SF2 rs58542926 impaired mobilization of neutral lipids for very low-density lipoprotein (VLDL) assembly and secretion by the liver in E167K carriers

MBOAT7 rs641738 variant causing decreased MBOAT7 expression, predisposes to NAFLD/NASH by affecting the acyl remodeling of phosphatidylinositol in the liver

Human Hepatocytes Alpha-1 Antitrypsin Deficiency

OVERVIEW

Disease modelled alpha-1 antitrypsin deficiency hepatocytes (Def-HEP A1ATD) are highly functional patient-derived human hepatocytes. The cells are generated using dermal fibroblasts from an alpha-1 antitrypsin (A1AT) diseased patient carrying the 'Z' (E342K) point mutation in the A1AT gene SERPINA1. This mutation leads to the formation of mutant A1AT polymers that ultimately cause liver and lung damage.

Cell morphology

The Def-HEP A1ATD cells are patient representative human hepatocytes. When thawed and plated Def-HEP A1ATD cells form a monolayer with typical hepatocyte cobblestone morphology (Figure 1).

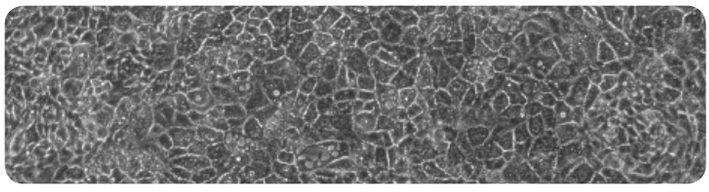

Figure 1. Clear cobblestone morphology of Def HEP A1ATD cells post-thaw. Cell seeding: 0.5x10^6 cells/well in collagen type 1 coated 24 well plate. Confluent monolayer. Magnification level: x10.

Product Specification	
Catalog Number	Def-HEP A1ATD
Format	Cryopreserved
Cell Number	3-6 million cells
Viability	>70%
Disease	Alpha-1 Antitrypsin Deficiency
Application	Disease Modelling & Drug Discovery

General hepatocyte maturation markers

Def-HEP A1ATD are functionally mature human hepatocyte cells. Therefore in addition to their specific disease circuit they display general hepatocyte maturation markers such as albumin, A1AT, CK18 and HNF4a (Figure 2).

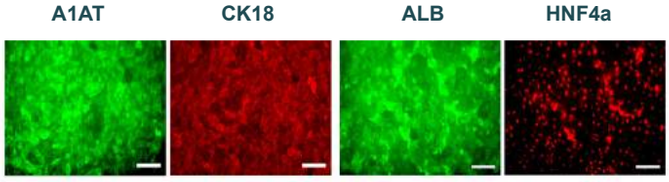

Figure 2. Immunostaining of Def-HEP A1ATD cells overviewing the expression of general hepatocyte maturation markers.

Advantages

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

Normal human genetics - patient donor genetics and karotype verified

Disease circuit verification - ZZ mutation in SERPINA1 gene leads to build up of mutant A1AT polymer in the cells

Optimized work flow - we can deliver industrial quantities of cryopreserved cell products to fit client specification

Human Hepatocytes Alpha-1 Antitrypsin Deficiency

For production of the Def-HEP A1ATD cells, A1ATD patient fibroblasts are first reprogrammed into iPSC using the Nobel Prize winning technology developed by Yamanaka and colleagues. These iPSC are then differentiated into liver hepatocytes using the OptiDIFF protocol developed at the University of Cambridge-Laboratory for Regenerative Medicine. Def-HEP A1ATD patient derived hepatocytes represent an optimized disease model for drug discovery applications and are an effective tool for elucidating the underlying mechanisms of the disease.

Detection of disease markers via ELISA

The A1AT disease marker can be quantified using an ELISA for mutant polymer and wild-type secreted A1AT. The assay utilizes antibodies specific for A1AT polymers (2C1mAb, top) or all conformers of A1AT (bottom) (Figure 3).

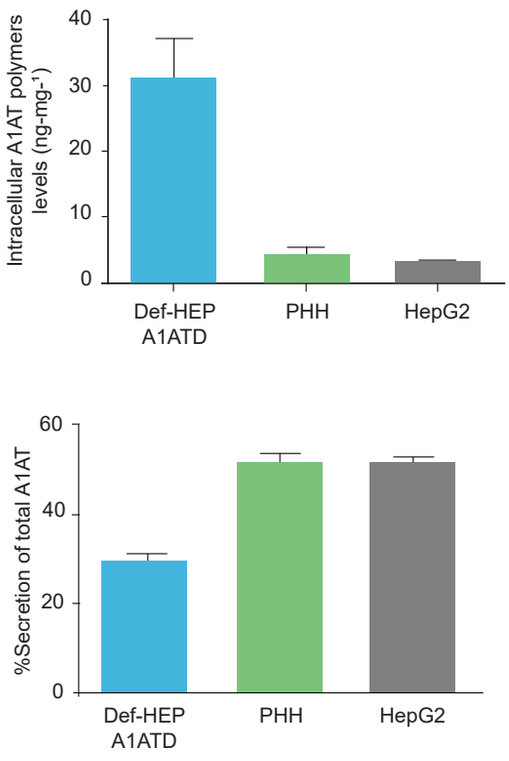

Figure 3. Quantification of intracellular A1AT polymers and intercellular total A1AT secretion in Def-HEP A1ATD cells. Primary human hepatocytes (PHH) and hepatocellular liver carcinoma cells (Hep G2) are used as internal controls.

Immunocytochemistry analysis of A1AT mutant polymer

Previous studies have shown that the Z allele (Glu342Lys) results in the formation of ordered polymers of α1-antitrypsin that are retained within the ER. This pathway of α1-antitrypsin polymerization is central to the clinical phenotype. We therefore used the 2C1 polymer specific monoclonal antibody to detect polymers within Def-HEP A1ATD hepatocytes. Polymers were detected by immunostaining (Figure 4). The immunocytochemistry data show that accumulation of α1-antitrypsin polymers only occurs in disease-specific human Def-HEP hepatocytes from individuals with A1ATD; no polymers are present in human iPS cell–derived hepatocytes from control subjects.

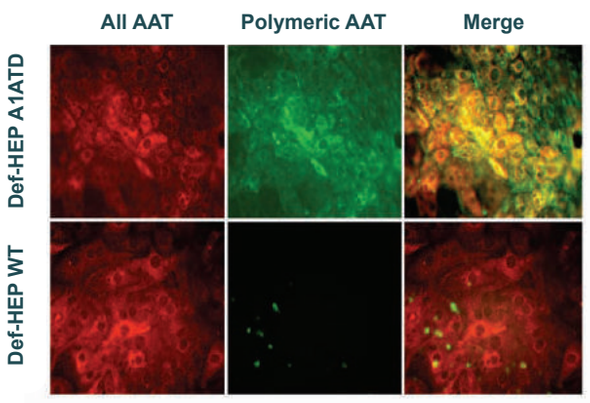

Figure 4. Immunostaining analyses for expression of misfolded polymeric α1-antitrypsin using the polymer- specific 2C1 antibody (green) or an antibody that detects all forms of α1-antitrypsin (red) in Def-HEP A1ATD disease modelled cells and control human iPS cell–derived hepatocytes. Merged images are shown at right.

References

Targeted gene correction of α1-antitrypsin deficiency in induced pluripotent stem cells. Yusa K, Rashid ST, Vallier L, et al. **Nature**. 2011 Oct 12;478(7369):391-4.

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.

Human Hepatocytes Glycogen Storage Disease

OVERVIEW

DefiniGEN's GSD1a human hepatocytes effectively model glycogen storage disease type1a the most common of the glycogen storage diseases. This genetic disease results from a deficiency in the glucose-6-phosphatase (G6P) enzyme which impairs the ability of the liver to produce free glucose from glycogen and gluconeogenesis. These cell products display the disease phenotype in combination with general hepatocyte functions in a similar manner to human primary hepatocytes.

Phenotypic analysis of the cells using periodic acid/diastase staining has demonstrated that the cells excessively accumulate glycogen as the glucose-6-phosphatase enzyme is dysfunctional. Accordingly these cell products can offer disease modelling and drug discovery researchers a novel tool for dissecting the underlying mechanisms of this and similar lysosomal storage diseases.

Product Specification	
Catalog Number	Def-HEP GSD1a
Format	Cryopreserved
Cell Number	3-6 million cells
Viability	>70%
Disease	Glycogen Storage Disease
Application	Disease Modelling & Drug Discovery

Advantages

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

Human hepatocyte function - Def-HEP are functionally mature hepatocytes which can be plated and maintain function over a twenty day window

Optimized work flow - we can deliver industrial quantities of cryopreserved cell products to fit client specification

References

Generation of Hepatocytes from Pluripotent Stem Cells for Drug Screening and Developmental Modeling. Gieseck RL 3rd, Vallier L, Hannan NR. **Methods Mol Biol**. 2015;1250:123-42.

Maturation of induced pluripotent stem cell derived hepatocytes by 3D-culture. GieseckRL 3rd, Hannan NR, Vallier L. **PLoS One**. 2014 Jan 22;9(1):e86372.

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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.

Functional analysis

Period acid-Schiff (PAS) staining has revealed Def-HEP GSD1a hepatocytes accumulated substantially greater amounts of intracellular glycogen compared with iPSC derived hepatocytes from control subjects (Figure 1A); in addition BODIPY staining showed excessive production of intracellular lipids in Def-HEP GSD1a hepatocytes (Figure 1B); and Def-HEP GSD1a hepatocytes secrete more lactate compared with iPSC derived hepatocytes from control subjects, as assessed by ELISA analysis of a 24-hour collection of cell culture medium.

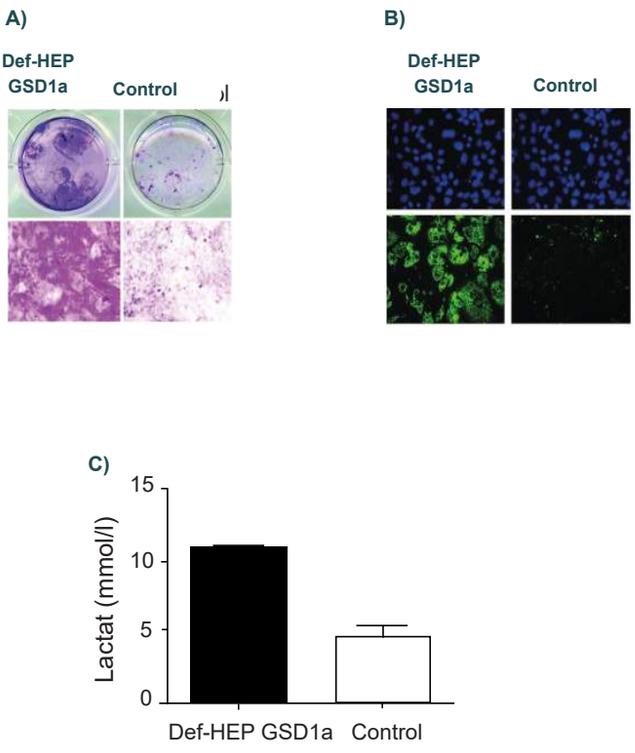


Figure 1. a) Period acid-Schiff (PAS) staining revealed Def-HEP GSDIa hepatocytes accumulated substantially greater amounts of intracellular glycogen than did those of controls (Figure 1A) and showed excessive production of lipid (Figure 1B) and lactic acid (Figure 1C) confirming the cellular disease phenotype.

PAS/Diastase functional test

Period acid-Schiff (PAS) is a staining method used to detect glycogen storage in tissue and in the diagnosis of GSDs. Diastase is an alpha-amylase enzyme that breaks down glycogen and is used in combination with a PAS stain to specifically identify glycogen granules. PAS staining revealed that Def-HEP GSD1a hepatocytes accumulated significantly higher amounts of intracellular glycogen than the negative control (Figure 2).

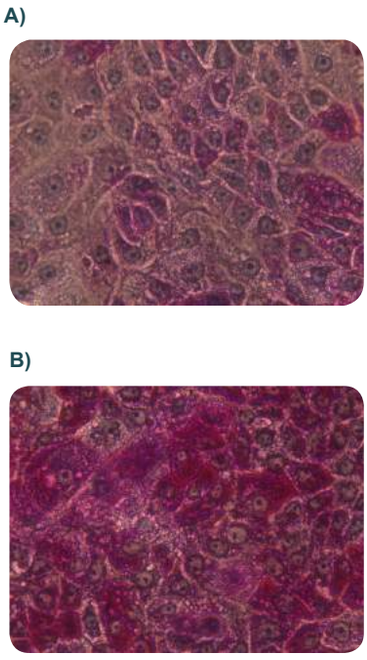


Figure 2. PAS/Diastase staining of cryopreserved Def-HEP GSD1a. Magnification level: x400. (A) PAS/Diastase staining showing breakdown of accumulated glycogen in the cells. (B) PAS staining showing the accumulation of glycogen in the cells.

Human Hepatocytes Familial Hypercholesterolemia

OVERVIEW

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.

Product Specification	
Catalog Number	Def-HEP FH
Format	Cryopreserved
Cell Number	3-6 million cells
Viability	>70%
Disease	Familial Hypercholesterolemia
Application	Disease Modelling & Drug Discovery

Powered By horizon

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 cell products to fit client specification

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 was undertaken to determine the sequence of the cDNA expressed from the LDLR allele.

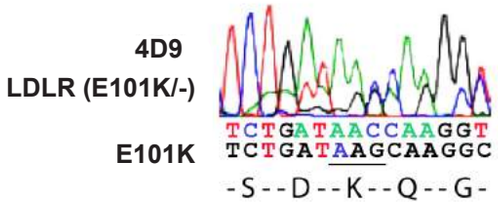


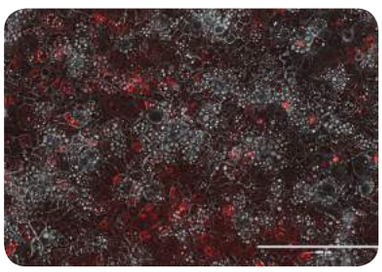
Figure 1. Sequence confirmation of LDLR E101K mutation in Def-HEP FH cells.

Human Hepatocytes Familial Hypercholesterolemia

Functional test

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

Def-HEP WT



Def-HEP FH

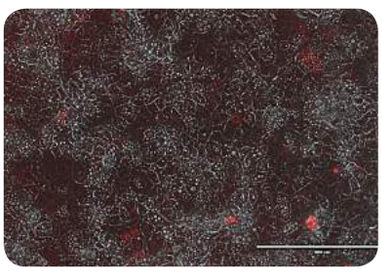


Figure 2. Microscopy images demonstrating that uptake of LDL was impaired in the LDLR mutant line (Def-HEP FH) in comparison to WT control (Def-HEP WT).

LDL receptor analysis

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

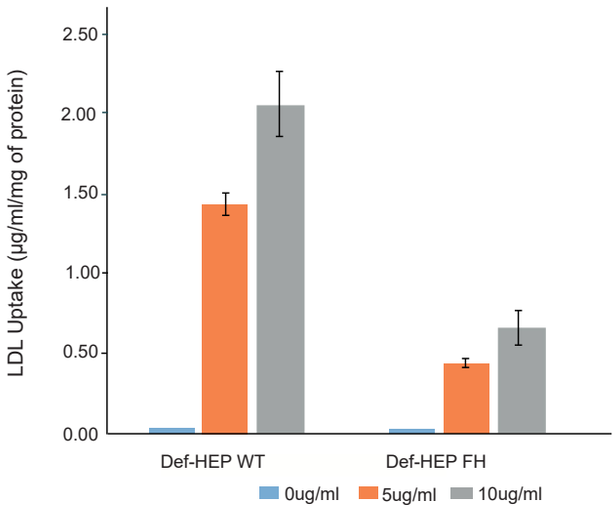


Figure 3. Quantitative LDL receptor assays based on fluorescent DiI-LDL internalization have demonstrated that Def-HEP FH iPSC-derived hepatocytes have a significantly impaired ability to incorporate LDL relative to the isogenic control Def-HEP WT over a range of LDL substrate ranges.

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.

Human Pancreatic Beta Cells Wild Type

OVERVIEW

DefiniGEN's wild-type beta cells are generated using human Induced Pluripotent Stem Cell (hIPSC) technology. The resulting cells display a robust Glucose Sensitive Insulin Secretion (GSIS) response over a range of physiologically relevant glucose concentrations and exhibit an elevated GSIS response to key reference drugs in a similar manner to primary human pancreatic islets.

Quantification of the pancreatic insulin gene expression marker by qPCR

Def-PANC cells express the insulin gene at very similar levels to primary human pancreatic islets.

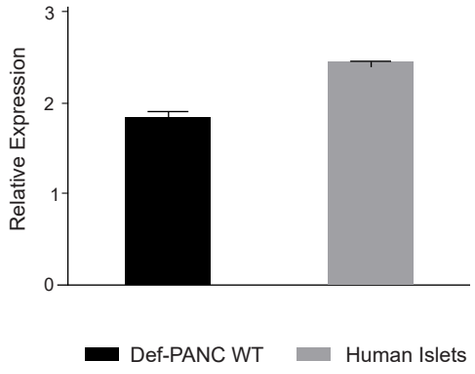


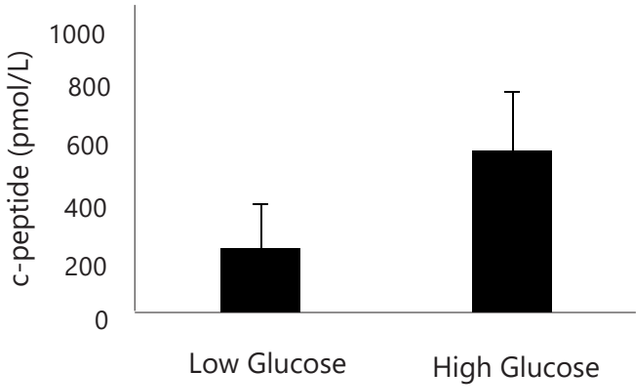
Figure 1. Insulin gene expression marker analysis observed in Def-PANC cells thawed from a cryopreserved vial in 96 well low adherent plates and cultured as microislets.

Product Specification	
Catalog Number	Def-PANC WT
Format	Cryopreserved
Cell Number	10 million cell format
Viability	>70%
Disease	None (Healthy Donor)
Application	Research & Drug Discovery

Glucose stimulated insulin secretion assay

A robust GSIS response is observed in Def-PANC cells when thawed from a cryopreserved vial in 96 well low adherent plates and grown as islet-like structures. They have also demonstrated a dose-dependent response to well-known secretagogues such as GLP-1 and Exenatide.

(A)



(B)

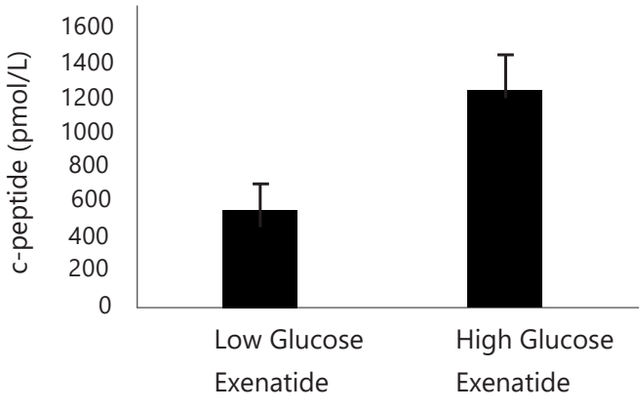


Figure 2. Panel (A) shows GSIS assay results for Def-PANC cells stimulated with low and high concentrations of glucose only. Panel **(B)** overviews the GSIS response of Def-PANC cells stimulated with low and high concentrations of glucose and exenatide. low glucose concentration 1.6mM, high glucose concentration 16.7mM, exenatide concentration 25nM.

Human Pancreatic Beta Cells Wild Type

Def-PANC WT cells can be grown in monolayer or they can be conveniently cultured as microislets which resemble primary human pancreatic islets in structure and function. The cells are available for key applications including drug discovery and diabetes research. The continual supply of Def-PANC WT cells enabled by iPSC technology ensures that clients do not encounter supply issues often associated with fresh human beta cells.

Monolayer culture

The Def-PANC cell products are highly functional iPS-derived pancreatic cells. Yamanaka iPSC technology in combination with fully defined differentiation conditions enables the generation of standardized populations of pancreatic cell products. Through a 25 day differentiation process the cells proceed through key developmental stages ultimately producing functional pancreatic cells (Figure 3).

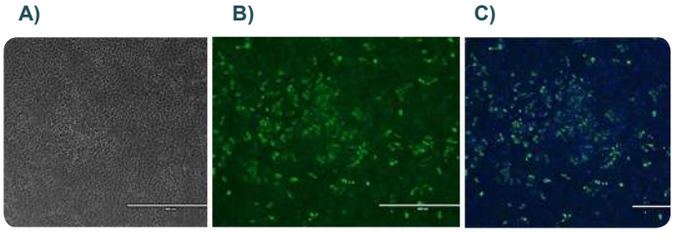


Figure 3. When grown in standard laboratory 96-well plates Def-PANC cells show typically tight-packed pancreatic cell morphology (A) and a high proportion of C-peptide secretion from beta cells (green) (B). Panel C depicts DAPI staining of nuclear DNA (blue) as well as C-peptide (green).

Advantages

Standardized cell product - containing >97% pancreatic cells providing reproducible and biologically relevant data

Normal human genetics - wild-type donor genetics and karotype verified in contrast to immortalized lines

Microislet cluster formation - similar to primary human pancreatic islets

Microislet formation

When grown on 96-well low adherent plates the Def-PANC cells aggregate and form microislet structures of similar size to primary human islets (Figure 4).

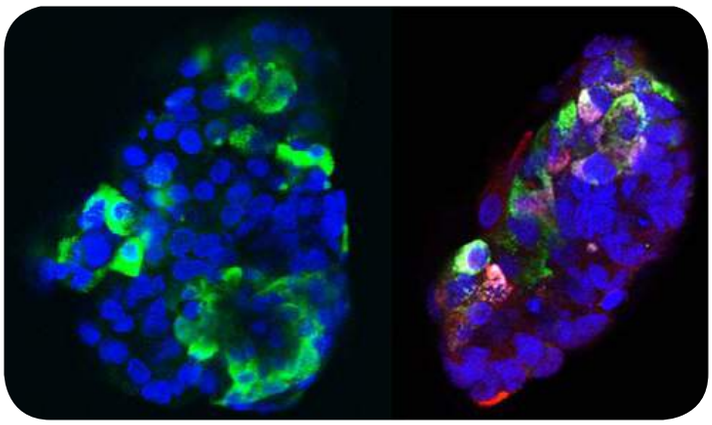


Figure 4. Microislet formation of Def-PANC in low adherent plates. Insulin secreting beta cells (green) are the dominant cell population observed alongside lower populations of glucagon and somatostatin-expressing cells (size 100-150 µm).

References

Modelling Neonatal and MODY Diabetes in Vitro Using IPS Cell-derived human Pancreatic Beta Cells. Soares F, Santos R, Schofield C, Lowe C, Vallier L. **ISSCR 2016** June 22-25.

TEAD and YAP regulate the enhancer network of human embryonic pancreatic progenitors. Cebola I, Vallier L, Ferrer J et al. **Nature Cell Biology**. 2015 May;17(5):615-26.

Generation of multipotent foregut stem cells from human pluripotent stem cells. Hannan NR, Vallier L. **Stem Cell Reports**. 2013 Oct 10; 1(4):293-306.

Inhibition of activin/nodal signalling is necessary for pancreatic differentiation of human pluripotent stem cells. Cho CH, Hannan NR, Vallier L et al **Diabetologia**, 2012 Dec;55(12): 3284-95.

Human Pancreatic Beta Cells MODY3 Diabetes

OVERVIEW

DefiniGEN MODY3 diabetes human pancreatic cells display a mutation in the HNF1-alpha transcription factor gene, specifically an insertion of C at codon 872 producing a frameshift mutation. MODY3 (also known as HNF1A-MODY) is caused by mutations in the HNF1-alpha gene which is a key regulatory transcription factor controlling the downstream regulation of multiple genes involved in the differentiation of beta cells. These cell products can offer disease modelling and drug discovery researchers unique tools for elucidating the mechanistic basis of MODY. Our custom services can also engineer bespoke mutations for additional forms of the eleven known types of MODY.

Cell morphology

In contrast to Def-PANC WT cells which display normal tightly packed pancreatic cell morphology, Def-PANC MODY3 cells display aberrant morphology expected from developmental retardation caused by the HNF1a mutation.

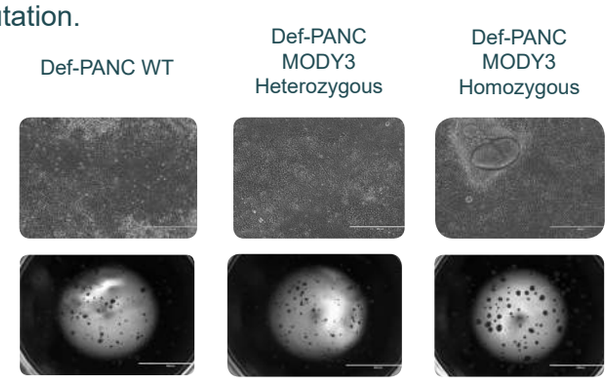


Figure 1. Def-PANC MODY3 cells display aberrant morphology in both monolayer and microislet culture.

Product Specification	
Catalog Number	Def-PANC MODY3
Format	Cryopreserved
Cell Number	10 million cell format
Viability	>70%
Disease	Monogenic Diabetes (HNF1a)
Application	Research & Drug Discovery

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Pancreatic key marker analysis

The expression of crucial pancreatic genes is sequentially reduced in disease modelled Def-PANC cells.

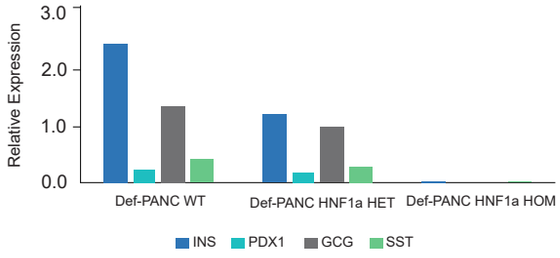

Figure 2. Key marker analysis by qPCR shows the expected reduction in key pancreatic cell markers in the Def-PANC disease models.

Disease circuit verification

The MODY3 pancreatic disease model has a mutation in the HNF1-alpha transcription factor gene – insertion of C at codon 872 producing frameshift mutation – isogenic control available.

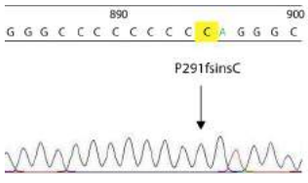

Figure 3. Sanger sequencing showing heterozygous HNF1a mutation with single base insertion of a C in a polyC tract around codon 291 for Proline.

GLUT-2 + PKLR expression analysis

The expected down regulation of pancreatic cell function gene sets is observed in Def-PANC MODY3 cells.

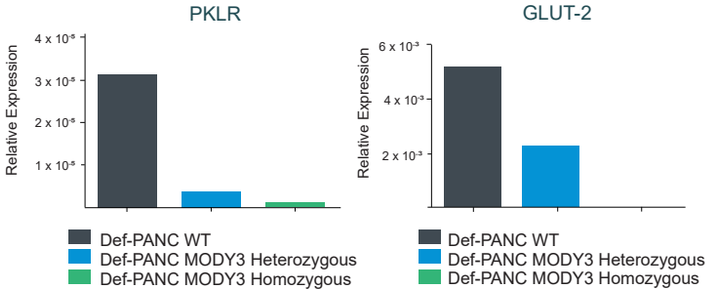

Figure 4. Heterozygous and homozygous Def-PANC MODY3 cells show progressive gene change effects in key HNF1a gene regulated genes including PKLR and GLUT-2.

Human Pancreatic Beta Cells Neonatal Diabetes

OVERVIEW

DefiniGEN's neonatal diabetes human pancreatic cells are an effective model of this form of monogenic diabetes. Neonatal diabetes mellitus (NDM) is a rare but potentially devastating metabolic disorder characterized by hyperglycemia combined with low levels of insulin. The neonatal diabetes cell products display the disease phenotype in combination with general function comparable to human primary pancreatic islets.

Cell morphology

Def-PANC WT and Neonatal disease model variants display typical pancreatic cell morphology.

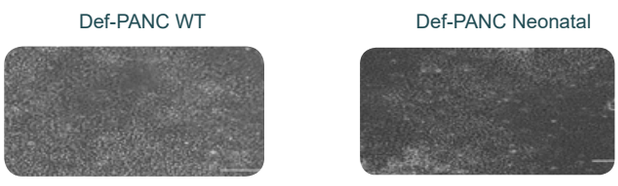

Figure 1. Morphology of pancreatic cells grown as a monolayer.

Disease circuit verification

Neonatal diabetes disease model with confirmed mutation in KCNJ11 gene encoding Kir6.2 subunit of potassium channels – CAT>CGT at codon 201 – isogenic control available.

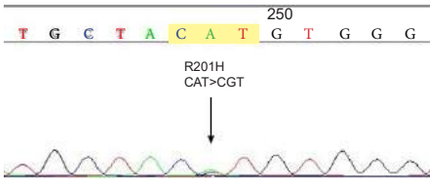

Figure 2. Sanger sequencing showing heterozygous KCNJ11 mutation with single base change (CAT>CGT) at codon 201 in the gene encoding for the ATP-sensitive potassium channel subunit Kir6.2.

Product Specification	
Catalog Number	Def-PANC Neonatal
Format	Cryopreserved
Cell Number	10 million cell format
Viability	>70%
Disease	Neonatal Diabetes (KCNJ11)
Application	Research & Drug Discovery

Glucose stimulated insulin secretion assay

Def-PANC Neonatal disease modelled cell products display dysfunctional GSIS results in contrast to wild type Def-PANC cells which exhibit an effective GSIS response.

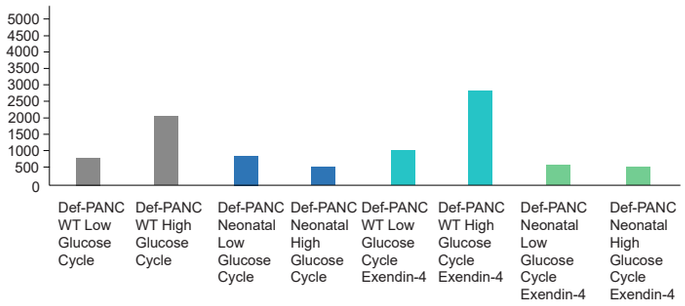

Figure 3. Def-PANC WT isogenic control shows expected glucose cycling GSIS response at low and high glucose concentrations. The Def-PANC Neonatal disease model shows dysfunction in its glucose responsive insulin production in contrast to the isogenic WT control. Genetic information and phenotypic GSIS response confirms that Def-PANC Neonatal cells are an effective model of neonatal diabetes.

References

Modelling Neonatal and MODY Diabetes in Vitro Using IPS Cell-derived human Pancreatic Beta Cells. Soares F, Santos R, Schofield C, Lowe C, Vallier L. **ISSCR 2016** June 22-25.

TEAD and YAP regulate the enhancer network of human embryonic pancreatic progenitors. Cebola I, Vallier L, Ferrer J et al. **Nature Cell Biology**. 2015 May;17(5):615-26.

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Type 2 diabetes disease model development

Currently although over 120 Type 2 Diabetes-associated loci identified, it has proven challenging to identify causal genes. iPSC-derived pancreatic T2D cell models can help to elucidate cellular mechanisms and identify / validate causal mutations. DefiniGEN's mature and functional beta cell phenotype and marker profile provides a human primary-like cell and a more predictive preclinical model for the study of diabetes.

The OptiDIFF platform can produce effective T2D models using 2 methodologies:

- Patient-derived from T2D patients – recapitulating patient genetics and phenotype in the cell model, complete with patient clinical data
- CRISPR-edited model - systematically introducing mutations into specified T2D associated-loci in Def-PANC cells and providing the isogenic control for screening programmes (manipulation at multiple loci can also be achieved)

Type 2 diabetes genes

Melatonin receptor 1B gene (MTNR1B)

Zinc transporter (ZnT8) SLC30A8

Zinc finger MIZ domain-containing protein 1 (ZMIZ1)

ADP-ribosylation factor-like 15 (ARL15)

Thyroid adenoma (THADA)

Sprouty homolog 2 (Spry2)

StAR-related lipid transfer protein 10 (STARD10)

References

Modelling Neonatal and MODY Diabetes in Vitro Using IPS Cell-derived human Pancreatic Beta Cells. Soares F, Santos R, Schofield C, Lowe C, Vallier L. **ISSCR 2016** June 22-25.

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Rare Disease Models - Monogenic and Complex Diseases

The OptiDIFF platform can produce disease models for a range of inherited genetic diseases ranging from cystic fibrosis to orphan liver diseases such as alpha-1 antitrypsin deficiency. In addition to the monogenic diseases mentioned below complex diseases such as NAFLD can also be modelled by sourcing patient derived donors and where specified, systematically reversing disease implicated nucleotides using CRISPR gene-editing to provide isogenic controls.

Monogenic diseases

- Gaucher disease
- Genetic cholestasis (PFIC, TGP2, and Alagille syndrome)
- Wilson's disease
- Hereditary hemochromatosis
- Tyrosinemia type 1
- Alpha-1 antitrypsin deficiency
- Argininosuccinic aciduria (ASL)
- Glycogen storage disease (GSD) type I
- Urea cycle disorders (except ASL)
- Crigler-Najjar syndrome
- Familial amyloid polyneuropathy
- Atypical haemolytic uremic syndrome-1
- Primary hyperoxaluria type 1
- Maple syrup urine disease (MSUD)
- Acute intermittent porphyria
- Familial hypercholesterolemia
- Organic acidurias (except MSUD)
- Cystic fibrosis
- Erythropoietic protoporphyria
- MCAD deficiency
- D- bifunctional protein deficiency
- Galactosemia Type 1
- Citrullinemia

Complex disease - NAFLD

PNPLA3 rs738409 impaired hepatocellular triglycerides hydrolysis and increased lipogenesis associated to the 148 M allele

GCKR rs1260326 increased glycolysis favours an increase in triglyceride levels

TM6SF2 rs58542926 impaired mobilization of neutral lipids for very low-density lipoprotein (VLDL) assembly and secretion by the liver in E167K carriers

MBOAT7 rs641738 variant causing decreased MBOAT7 expression, predisposes to NAFLD/NASH by affecting the acyl remodeling of phosphatidylinositol in the liver

Human Intestinal Cells Wild Type

OVERVIEW

DefiniGEN intestinal organoids provide a unique *in vitro* system to model the human intestine. The organoids harbor a mixture of cell types normally present in the primary intestinal epithelial *in vivo*, including goblet cells, Paneth cells, enterocytes, and enteroendocrine cells. Multiple CYP450s and transporters such as SLCO2B1 and ABCB1 are also expressed in the cells. The cells can be used for drug absorption, metabolism, induction of transporters, and the modelling of infectious disease.

Cell morphology

Typical intestinal organoid morphology is observed in Def-INTESTINAL cells.

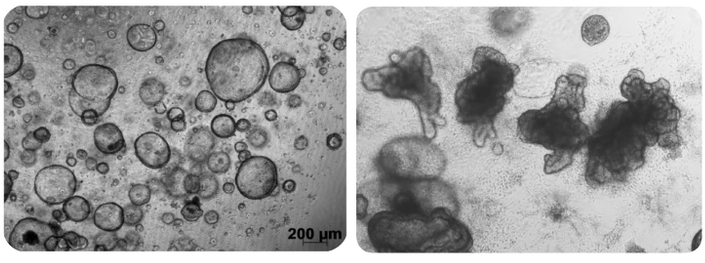


Figure 1. Typical intestinal morphology is observed in Def-INTESTINAL cells. Organoids grown encapsulated in matrigel in a 24-well plate.

Product Specification	
Catalog Number	Def-Intestinal WT
Format	Cryopreserved
Cell Number	200-300 organoids per vial
Viability	>70%
Disease	None (Healthy Donor)
Application	Research & Drug Discovery

Immunocytochemistry analysis

Immunocytochemistry analysis has demonstrated that the Def-INTESTINAL organoids display a polarized epithelium and are composed of differentiated cell types with distinct morphologies. The organoids display a polarized epithelium containing absorptive enterocytes as well as the major secretory lineages (including Paneth cells, goblet cells, and enteroendocrine cells).

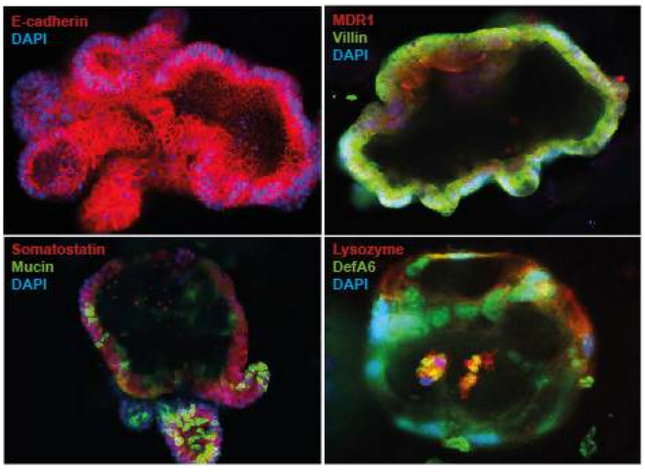


Figure 2. Organoids display positive staining of key intestinal cell markers including; epithelial cells (E-cadherin), enterocytes (villin), goblet cells (mucin), enteroendocrine (somatostatin), and Paneth cells (lysozyme).

References

Derivation of Intestinal Organoids from Human Induced Pluripotent Stem Cells for Use as an Infection System. Forbester JL, Hannan N, Vallier L, Dougan G. **Methods Mol Biol.** 2016 Aug 31.

Interaction of Salmonella enterica Serovar Typhimurium with Intestinal Organoids Derived from Human Induced Pluripotent Stem Cells. Forbester JL, Goulding D, Vallier L, Hannan N, Hale C, Pickard D, Mukhopadhyay S, Dougan G. **Infect Immun.** 2015 Jul;83(7):2926-34.

Human Intestinal Cells Wild Type

Key intestinal cell marker analysis

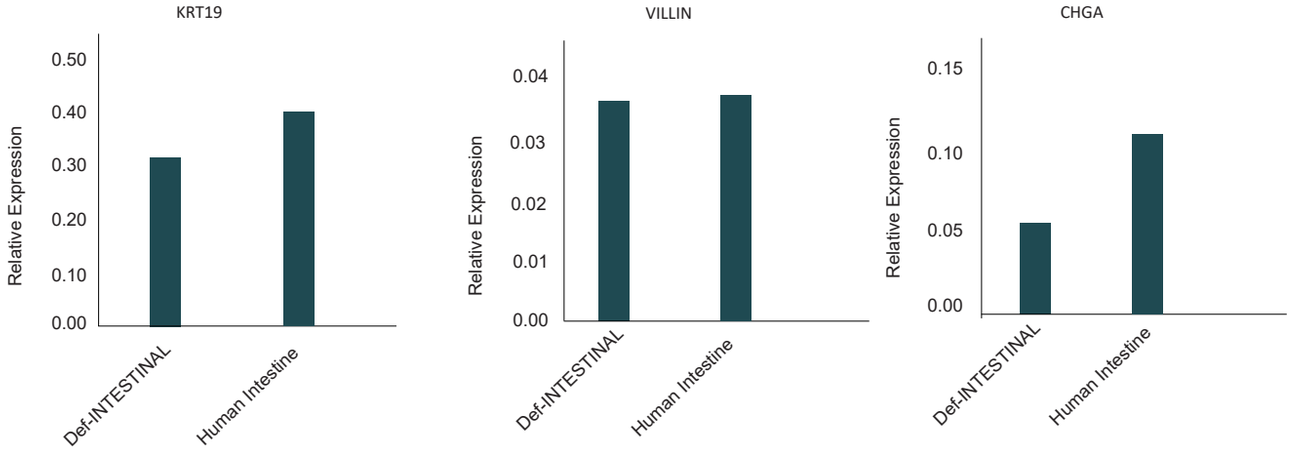


Figure 3. Def-INTESTINAL organoids have been demonstrated to display many key gut markers. Gene expression analysis shows key intestinal markers KRT19, Villin, and CHGA expression profiles relative against primary human control relative to GAPDH.

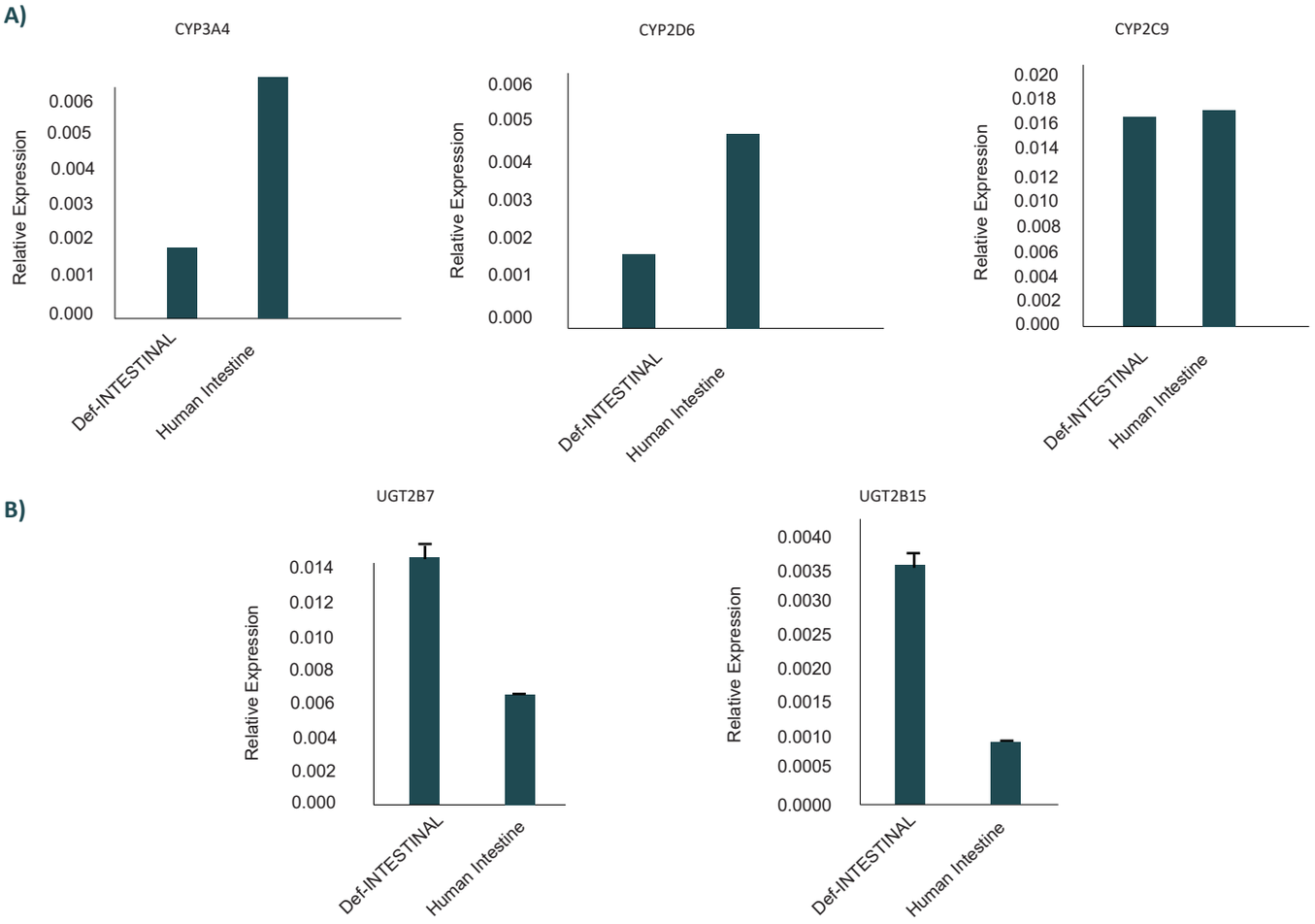


Figure 4. A range of cytochrome P450 and UGT enzymes have been identified in Def-INTESTINAL organoids. Gene expression analysis shows (A) cytochromes CYP3A4, CYP2D6, CYP2C9 and (B) UGT enzymes UGT2B7 and UGT2B15 expression against primary human control.

Human Intestinal Cells Wild Type

Intestinal structure

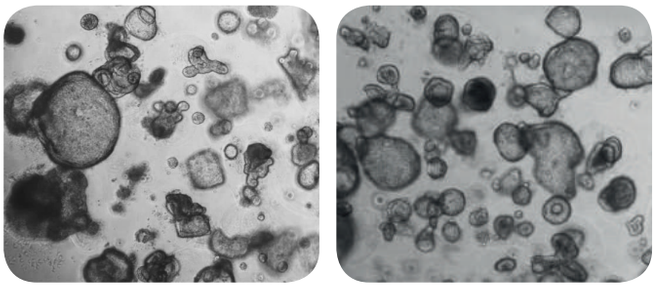


Figure 5. Typical intestinal organoid morphology observed in Def-INTESTINAL cells.

Immunocytochemistry analysis

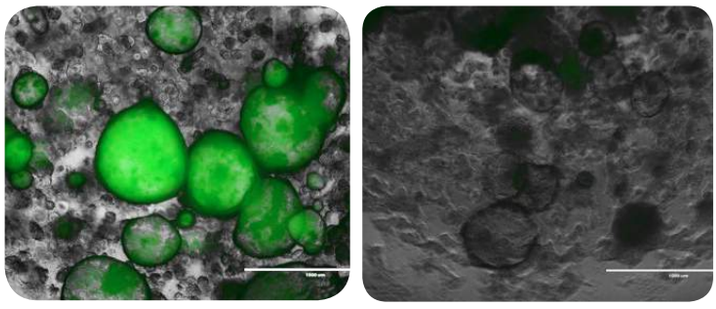


Figure 6. Def-INTESTINAL organoids can transport Rhodamine 123, a specific substrate of MDR1. MDR1 activity is inhibted by Verapamil, a specific inhibitor.

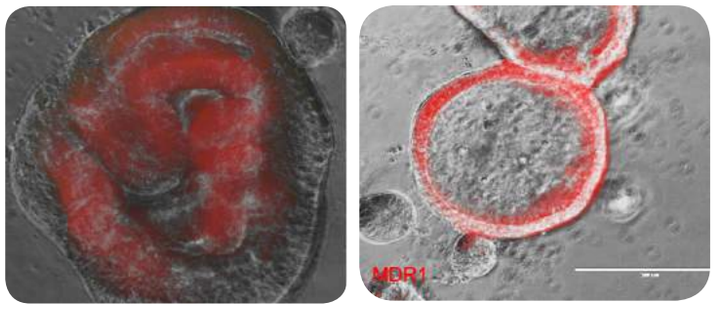


Figure 7. Immunostaining of intestinal organoids showing localization of the MDR1 transporter protein within highly folded crypt structures.

Drug transporter analysis

Down-regulation or inhibition of ABC efflux transporters in the intestine can be used as a strategy to improve oral drug bioavailability of known substrates as these transporters prevent drug molecules from being absorbed.

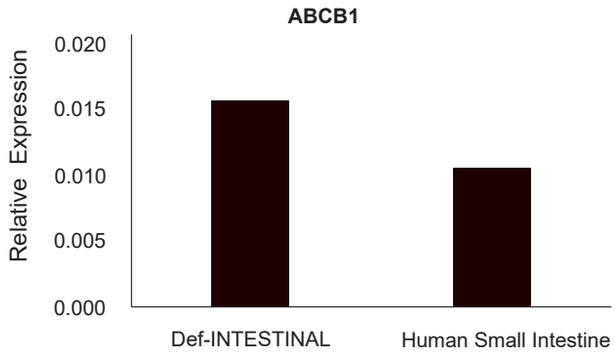


Figure 8. Gene expression analysis shows transporter ABCB1 expression profiles against primary control.

The SLC (solute carrier) family have an important role in physiological processes ranging from the cellular uptake of nutrients to the absorption of drugs and other xenobiotics. SLCs are primarily involved in the uptake of small molecules into cells.

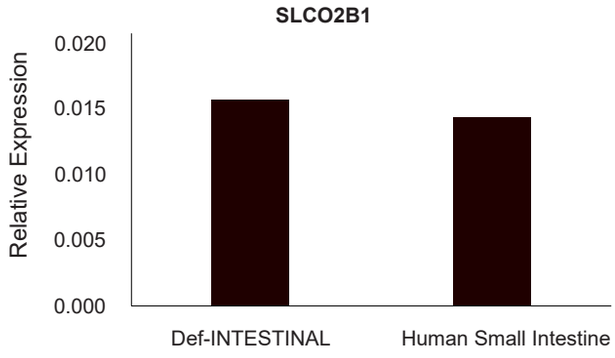


Figure 9. Gene expression analysis shows transporter SLCO2B1 expression profiles against primary control.

Human Cholangiocytes

OVERVIEW

Def-CHOLANGIOCYTES are a specialized cell product that grow as cystic organoids and branching tubular structures. They display cilia mimicking physiological biliary development, generating cell populations that closely resemble primary human cholangicoytes at the transcriptional and functional level. The cells display the functional characteristics of cholangiocytes including bile acids transfer, alkaline phosphatase activity, glutamyltranspeptidase activity and physiological responses to secretin somatostatin and vascular endothelial growth factor.

The products can be used as an optimized *in vitro* system to model key features of Alagille syndrome, polycystic liver disease and cystic fibrosis (CF)-associated cholangiopathy. For liver disease modelling wild-type cholangiocytes can be generated from healthy individuals and patients. Polycystic liver disease can be modelled effectively with patient cholangiocyte organoids reducing in size when the cyst-reducing drugs verapamil and octreotide are applied. Regarding Cystic fibrosis (CF) it has also been demonstrated that experimental small molecule CF drugs can rescue the disease phenotype of CF cholangiopathy *in vitro*.

Product Specification	
Catalog Number	Def-CHOLANGIOCYTE WT and CF
Format	Fresh
Application	Research & Drug Discovery

References

Directed differentiation of human induced pluripotent stem cells into functional cholangiocyte-like cells. Sampaziotis F, de Brito MC, Geti I, Bertero A, Hannan NR, Vallier L. **Nature Protocols**. 2017 March 12;1(4):814-827.

Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation. Sampaziotis, F., Cardoso de Brito, M., Madrigal, P., Bertero, A., Saeb-Parsy, K., Soares, F., Schrumpf, E., Melum, E., Karlsen, T., Bradley, J., Gelson, W., Davies, S., Baker, A., Kaser, A., Alexander, G., Hannan, N. and Vallier, L. **Nature Biotechnology**. 2015 Aug 33(8): 845-852.

Organoid System

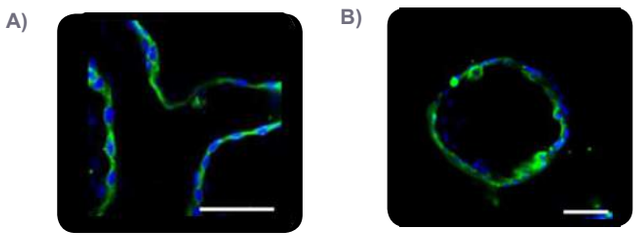


Figure 1. Immunofluorescence images of Cholangiocyte organoids demonstrating the formation of cystic (a) and branching (arrows) tubular structures (b). Scale bars, 100um.

Cystic fibrosis transmembrane regulator (CFTR) activity

Def-CHOLANGIOCYTES offer an effective model for preclinical screening of potential cystic fibrosis therapeutics with the expression of CFTR activity. We have demonstrated the experimental CF drug VX809 rescues the disease phenotype of CF cholangiopathy *in vitro* in our cholangiocyte organoids validating the capacity of the system to function as a cystic fibrosis drug screening platform.

Incubation of CF-CLCs with VX809 for 48hr increased CFTR function analyzed by MQAE to a level similar to what of WT-CLCs. This effect was negated by CFTR inhibitor 172, confirming that the phenotype rescue of CF-CLCs by VX8-9 depended on improved CFTR function.

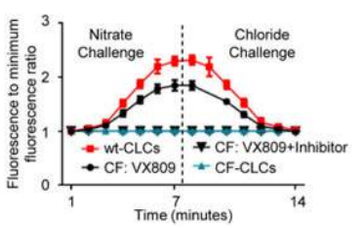
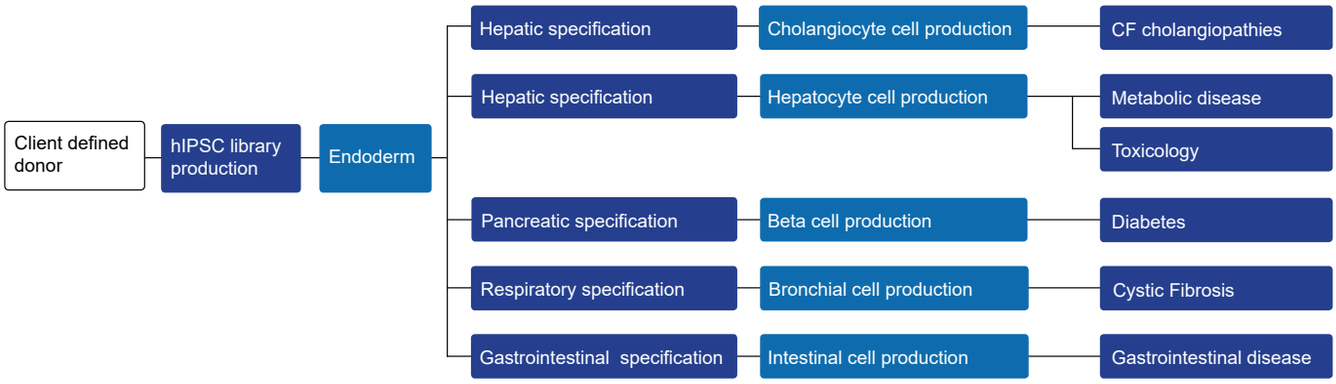


Figure 2. Immunofluorescence images of Cholangiocyte organoids demonstrating the formation of cystic (a) and branching (arrows) tubular structures (b). Scale bars, 100um.

Custom Disease Models - Patient Derived

It is known that genetic variability in donors can influence the capacity of donor material to generate truly pluripotent lines that can effectively differentiate into “end” cell types. DefiniGEN projects are structured to ensure the client recieves their desired custom differentiated cell disease model by using multiple donors and clonal lines with the lines being assessed and ranked at each stage to ensure that the best line is used in large scale custom cell model production.

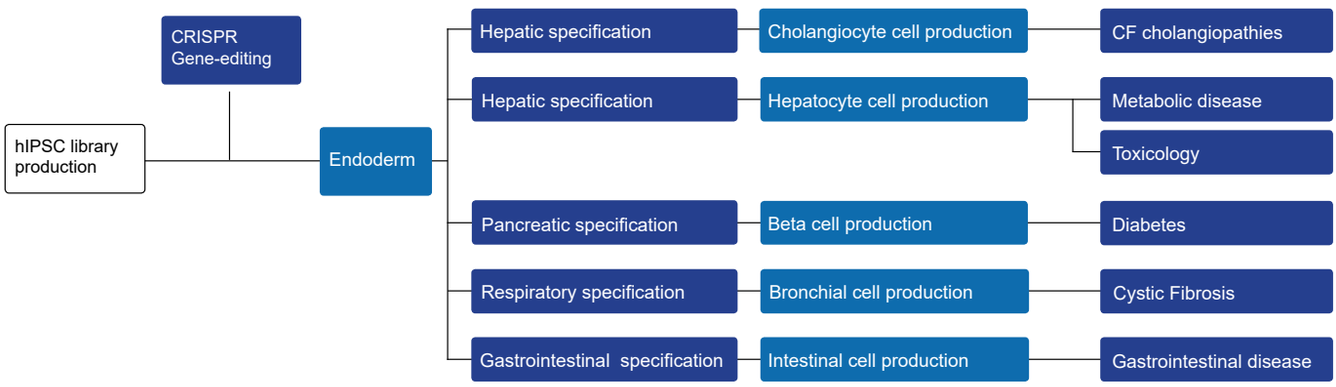
- DefiniGEN can source a disease patient sample for iPSC line generation or use an existing iPSC line from cell banks such as EBiSC (the European Bank for iPSC) and additional cell banks.
- The client recieves fully differentiated rigorously quality controlled cell products which are genotype and phenotype verified in a highly relevant physiological background.



Patient sample sourcing:	3x disease patient blood/fibroblast sample from an accredited bank
iPSC generation:	3x clonal lines from each donor reprogramming, screening, verification, expansion and banking
iPSC differentiation screen:	3x small scale OptiDIFF differentiation run of a clonal line from each donor - Use multiple seeding densities to establish the optimal number of cells required for the production differentiation - Lines ranked and best ranked line used in large scale production differentiation
Differentiation:	Using the OptiDIFF platform to genrate a range of cell types - Large scale T-flask generation of specified number of cells
Cryopreservation and banking:	Of differentiated cell products - Using our proprietary cryopreservation technology
Product validation:	Post-thaw viability verification, genotype, and cell marker analysis - ship to client

Custom Disease Models - CRISPR Gene-edited

The OptiDIFF technology was developed from world-leading research undertaken at the University of Cambridge and has been demonstrated on a range of cell types including liver, pancreatic and lung cell types. This platform has been combined with the market-leading CRISPR gene-editing technology provided by Horizon Discovery Ltd, to offer a unique resource for custom cell line development. This enables the precise introduction of any desired mutation and the rapid production of optimized disease models of monogenic and complex diseases including Diabetes and NAFLD. Ultimately the client receives fully differentiated, rigorously quality controlled cell products which are genotype and phenotype verified in a highly relevant physiological background.



6 step process for the generation of custom liver and pancreatic disease models powered by Horizon Discovery CRISPR gene-editing technology

CRISPR design and validation:	Vector design, synthesis and validation - Sequence verified KO/KI via CRISPR-Cas9 - Gene-editing in client provided iPSC line - Screening of up to 100 clones - Screening of 5 sgRNAs - Mycoplasma Hoechst and culture isolation testing - Full characterization and optimization of cell line - Alkaline phosphatase staining assessment of pluripotency
CRISPR iPSC generation:	Electroporation of iPSC lines, screening, verification, expansion, and banking
iPSC differentiation screen:	3x small scale OptiDIFF differentiation run of a clonal line from each donor - Use multiple seeding densities to establish the optimal number of cells required for the production differentiation - Lines ranked and best ranked line used in large scale production differentiation
Differentiation:	Using the OptiDIFF platform to genrate a range of cell types - Large scale T-flask generation of specified number of cells
Cryopreservation and banking:	Of differentiated cell products - Using our proprietary cryopreservation technology
Product validation:	Post-thaw viability verification, genotype, and cell marker analysis - ship to client

Powered By horizon

iPSC Reprogramming Service

DefiniGEN's founders have previously established the Cambridge hIPSC Core Facility and the Sanger iPSC platform – generating hundreds of lines for international projects. Our iPSC reprogramming platform is modelled on these high performance facilities. DefiniGEN are a partner in the European stem cell bank (EBISC) validating iPSC lines for both pharma and academia. We use integrative and non-integrative methods to reprogram a range of human blood or fibroblast samples generating iPS cells which are fully quality controlled.

Client defined donor → Reprogramming → Induced Pluripotent Stem Cells

5 step process for the generation of your iPSC lines

Source specific donor samples:	Fibroblasts, blood cells
iPSC reprogramming:	Using integrating and non-integrative systems
iPSC validation:	Pluripotency, karyotype sequence analysis
Cryopreservation:	Using our proprietary technology
Client delivery:	Of iPS lines with relevant QC pluripotency data

References

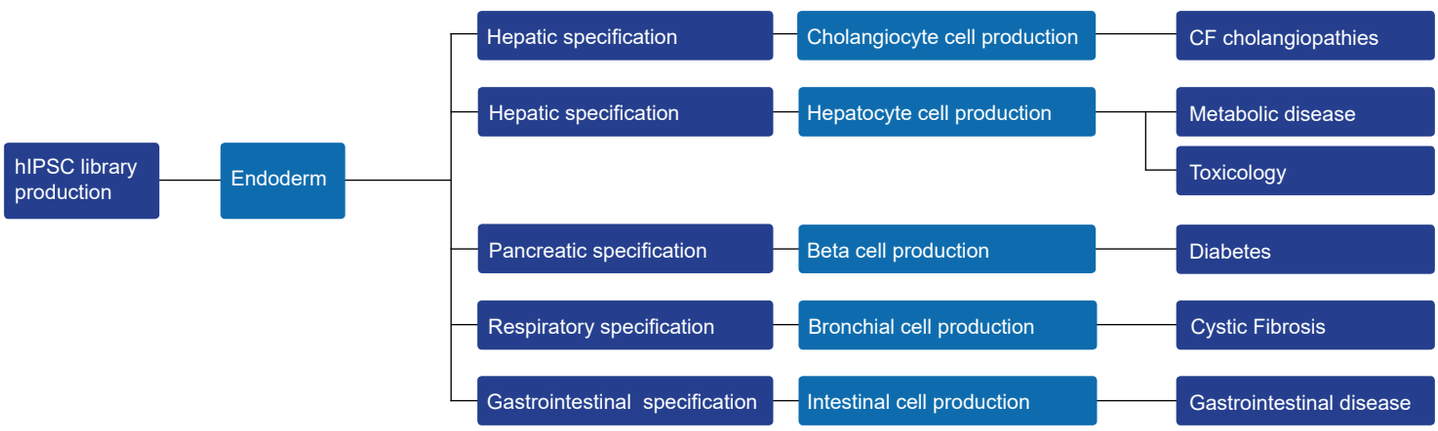
Generation of Human Induced Pluripotent Stem Cells from Peripheral Blood Mononuclear Cells Using Sendai Virus. Soares FA, Pedersen RA, Vallier L. **Methods Mol Biol**. 2016;1357:23-31

Genetic background drives transcriptional variation in human induced pluripotent stem cells. Rouhani F, Kumasaka N, de Brito MC, Bradley A, Vallier L, Gaffney D. **PLoS Genet**. 2014 Jun 5;10(6):e1004432

iPSC Differentiation Service

DefiniGEN's iPSC differentiation service utilizes our proprietary OptiDIFF platform to generate high functionality terminally differentiated custom cell products to fit client specifications. The OptiDIFF platform was developed from world-leading research undertaken at the University of Cambridge and has been demonstrated on a range of cell types. The client receives fully differentiated rigorously quality controlled cell products which are genotype and phenotype verified in a highly relevant physiological background.

OptiDIFF Platform



5 step process for iPSC differentiation using OptiDIFF platform

iPSC Reprogramming or sourcing:	iPSC generation of client provision of iPSC
Differentiation:	Using OptiDIFF platform
Cryopreservation:	Banking of the differentiated cells
Product validation:	Genotype, phenotype and cell marker analysis
Client delivery:	Differentiated cryopreserved cells to client with >60% post-thaw viability

DefiniGEN

LIVER PANCREAS INTESTINAL CHOLANGIOCYTES CUSTOM DISEASE MODELS IPSC REPROGRAMMING IPSC DIFFERENTIATION

Address

DefiniGEN Limited | Moneta B280 | Babraham Research Campus | Cambridge | United Kingdom | CB22 3AT

US phone

617-674-3260

International phone

+44-1223-497-106

Email

info@definigen.com

Website

www.definigen.com

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