pixlbio

CASE STUDY

Decoding biology.
Pixel by pixel.

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Modelling Familial Hypercholesterolemia (FH) through CRISPR-derived LDLR pixHep

Familial hypercholesterolemia (FH) is a common inherited genetic disease with an estimated prevalence of 1 in 250 individuals. It is caused by pathogenic variants regulating the low-density lipoprotein (LDL) cholesterol plasma levels, with the majority of them identified at the low-density lipoprotein receptor (LDLR) genomic locus. Elevated LDL cholesterol accumulates in tissues, often leading to atherosclerosis and cardiovascular disease from a young age. Although certain drugs (e.g., statins, bile acid sequestrants) and lifestyle modification can alleviate some of the FH symptoms, a significant number of patients still cannot achieve optimal LDL levels. At pixlbio we have developed a novel iPSC derived hepatocyte system that recapitulates the human FH phenotype in-a-dish, offering an effective pre-clinical disease model for the large-scale screening of novel FH-related therapies.

Advantages
Functional cholesterol transport pathways with high expression levels of LDLR
Disease circuit verified carrying the E101K mutation in the ***LDLR*** gene
Optimized ApoB **secretion assays** as key endpoint in pixHep
Suitable in vitro platform for screening of compound and gene therapy systems
Standardized cell products containing iPSC-derived human hepatocytes producing reproducible and biologically relevant data

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pixlbio CRISPR-derived LDLR iPSCs carry the E101K mutation without any effect in pluripotency status

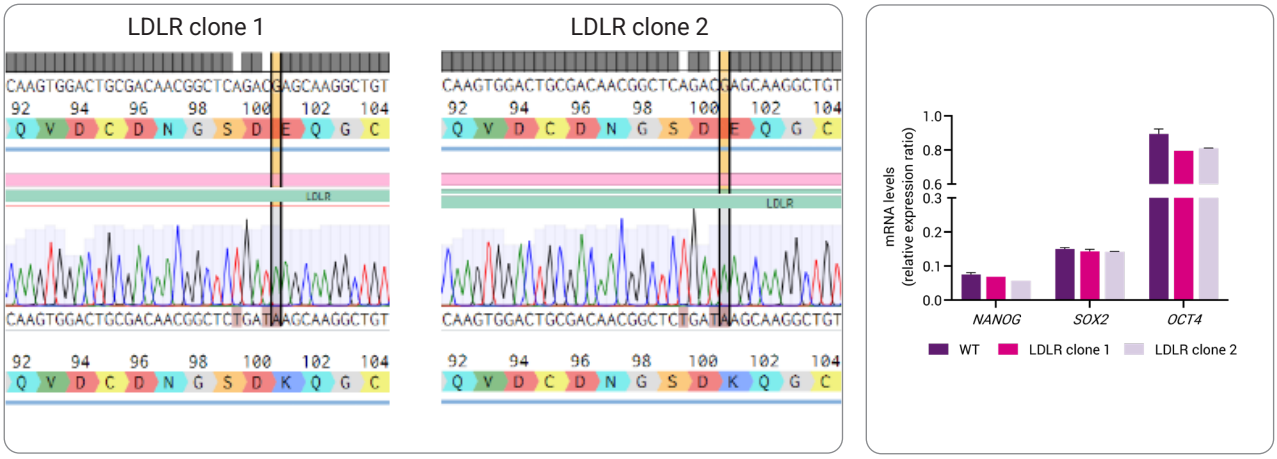

Figure 1. Sanger sequencing showing wild-type (top sequence) and mutated iPSCs (bottom sequence) in two clones carrying the E101K mutation (GAG>AAG) in the LDLR gene (homozygous). The codon change is highlighted with yellow (left). mRNA expression levels of the key pluripotency markers NANOG, SOX2, and OCT4 in wild-type (WT) and two homozygous CRISPR-derived LDLR iPSC clones. mRNA data were normalized to GAPDH and are presented as mean±SEM of n=2 technical replicates (right).

pixlbio CRISPR-derived LDLR iPSCs successfully differentiate to pixHep

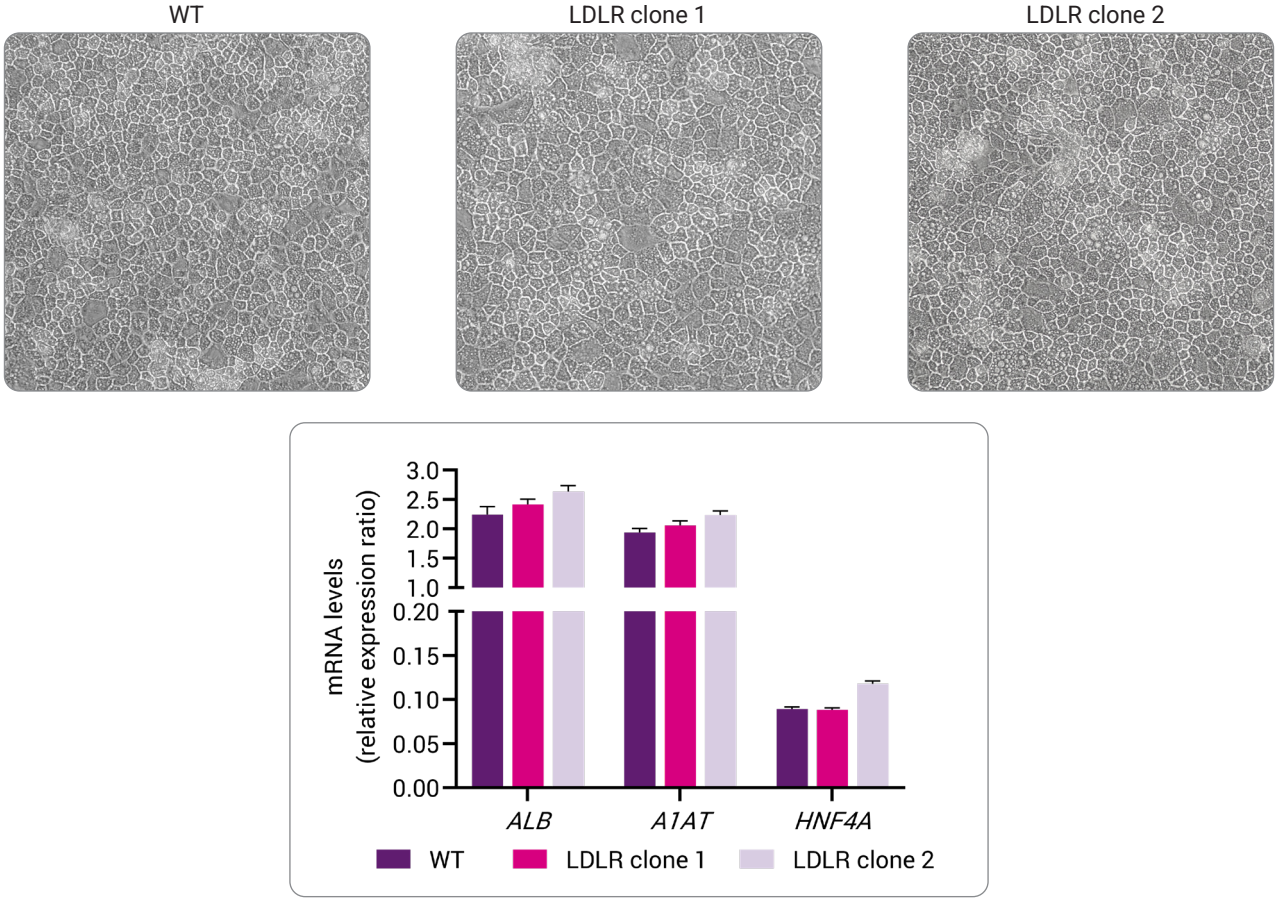

Figure 2. Representative images demonstrating the characteristic hepatocyte cobblestone morphology in wild-type (WT) and 2 LDLR-mutated pixHep clones (upper). mRNA expression levels of the hepatocyte maturity markers albumin (ALB), alpha-1-antitrypsin (A1AT), and hepatocyte nuclear factor 4A (HNF4A) in wild-type (WT) and 2 LDLR-mutated pixHep clones. mRNA data were normalized to PPIA and are presented as mean±SEM of n=3 biological replicates (lower).

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pixlbio CRISPR-derived LDLR pixHep demonstrate increased ApoB secretion levels without any difference in LDLR expression

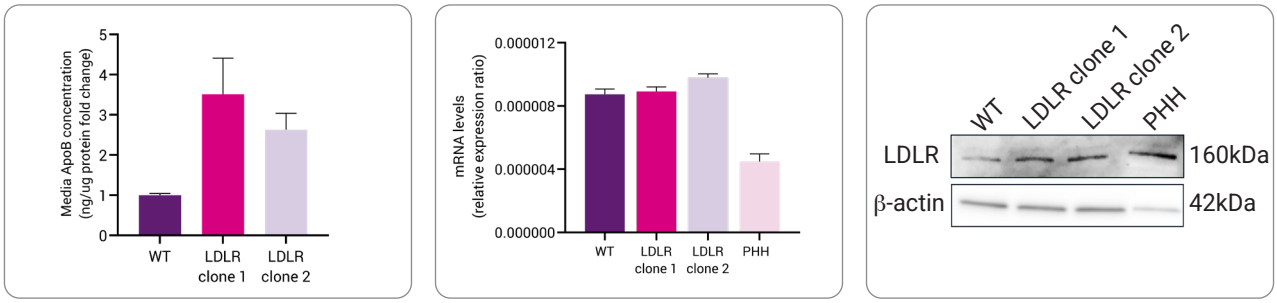

Figure 3. ApoB secretion in wild-type pixHep and 2 LDLR-mutated pixHep clones following 48 hours of treatment with lipidfree pixHep media (left). mRNA expression levels of LDLR gene in wild-type pixHep, 2 LDLR-mutated pixHep clones, and primary human hepatocytes (PHH) (middle). C) Protein expression levels of LDLR in wild-type pixHep, 2 LDLR-mutated pixHep clones, and PHH. Data are presented as mean±SEM of n=2 independent experiments. mRNA expression data were normalized to housekeeping gene PPIA. ApoB secretion data were normalized to total protein.

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