

# CERTIFICATE OF ANALYSIS

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## Cell Line Details

**Product code:** HEP-003-PNPLA3-c

**Product name:** iPSC-derived Human Hepatocytes: CRISPR-engineered Homozygous Non-Alcoholic Fatty Liver Disease; PNPLA3 I148M

**Lot number:** XXXXXX

**Storage conditions:** Store at less than -130°C

**QC completion date:** XXXXXX

## Cell Quality Controls

Test	Method	Specification	Result
Virus test for original iPSC clone (HIV1, HIV2, Hepatitis A, HBV, HCV, HTLV-1, HTLV-2)	PCR	Not detected	Pass
Post thaw viability	Automated cell counter	≥ 70% viable	Pass
Viable cells per vial	Automated cell counter	≥ 5.0 x 10 <sup>6</sup>	Pass
Cell morphology	Visual check	N/A	Pass (Fig.1)
Key hepatocyte maturity markers ( <i>ALB</i> , <i>A1AT</i> , <i>HNF4a</i> )	qPCR	Present	Pass (Fig.2)
Disease confirmation	Sanger sequencing	Mutation present	Pass (Fig.3)

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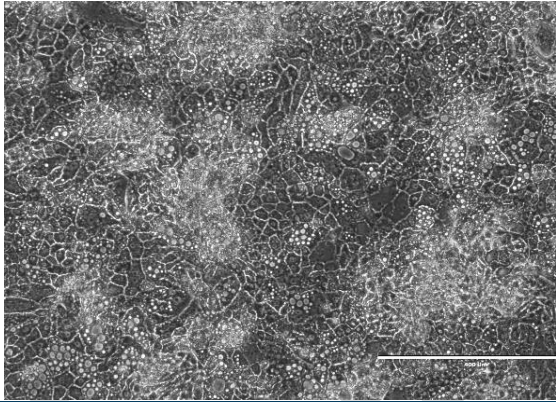
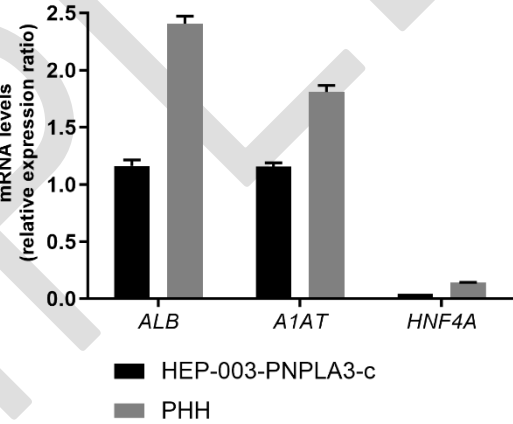
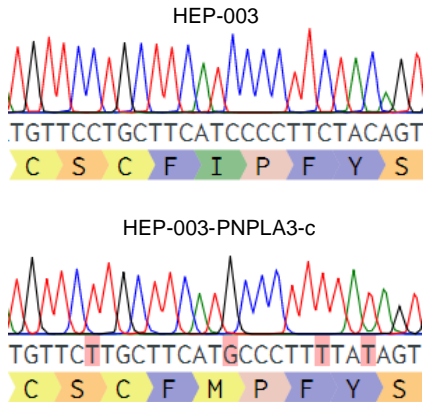
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## Appendix

<p><b>1. Cell morphology</b></p> <p><b>Figure 1.</b> Morphology of cryopreserved hepatocyte-like cells, 14 days post-thaw. Brightfield picture, magnification: 100x.</p>													
<p><b>2. Detection of hepatocyte maturity markers via qPCR</b></p> <p><b>Figure 2.</b> mRNA expression of the key hepatocyte maturity markers <i>ALB</i> (Albumin), <i>A1AT</i> (Alpha-1 Antitrypsin) and <i>HNF4A</i> (Hepatocyte Nuclear Factor-4) in cryopreserved hepatocyte-like cells (black bars) and primary human hepatocytes (PHH, grey bars), 14 days post-thaw. mRNA data are normalized to endogenous <i>PPIA</i> expression, and are presented as mean±SD of n=3 technical replicates.</p>	 <table border="1"> <caption>mRNA levels (relative expression ratio)</caption> <thead> <tr> <th>Marker</th> <th>HEP-003-PNPLA3-c</th> <th>PHH</th> </tr> </thead> <tbody> <tr> <td>ALB</td> <td>~1.2</td> <td>~2.4</td> </tr> <tr> <td>A1AT</td> <td>~1.2</td> <td>~1.8</td> </tr> <tr> <td>HNF4A</td> <td>~0.1</td> <td>~0.2</td> </tr> </tbody> </table>	Marker	HEP-003-PNPLA3-c	PHH	ALB	~1.2	~2.4	A1AT	~1.2	~1.8	HNF4A	~0.1	~0.2
Marker	HEP-003-PNPLA3-c	PHH											
ALB	~1.2	~2.4											
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HNF4A	~0.1	~0.2											
<p><b>3. Disease confirmation via Sanger sequencing</b></p> <p><b>Figure 3.</b> Sanger sequencing of the <i>PNPLA3</i> gene in the isogenic control line (top panel) and the mutant cell line (bottom panel) showing the homozygous I148M mutation (ATC&gt;ATG).</p>	 <p>HEP-003    TGTTCCTGCTTCATCCCCTTCTACAGT    C S C F I P F Y S</p> <p>HEP-003-PNPLA3-c    TGTTCCTGCTTCATGCCCTTITATAGT    C S C F M P F Y S</p>												

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Checked by,

*signature*

QC Scientist

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