

Appendix for

An Hepatitis B and D Virus Infection Model Using Human Pluripotent Stem Cell-Derived Hepatocytes

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Appendix Table S1

Name	Sequence (5'-3')	Target	Reference
HDV-Ferns-F	GCGCCGGCYGGGCAAC	Total HDV RNA	(Ferns <i>et al</i> , 2012)
HDV-Ferns-R	TTCCTCTTCGGGTCGGCATG		
HDV-Ferns-Probe	FAM-CGCGGTCCGACCTGGGCATCCG-BHQ1		
Tag1-G-RT Taylor	TCTCCCCAGAGTTGTCGACCCCAGTGAATAAAGCGGGTTTCCACTCACAG	HDV G	
Tag1-G-F Taylor	GATTCTCCCTCTGAGTGCTACT	HDV G	
Tag2-AG-RT Taylor	TTCTTCTTTGTCTTCCGGAGGTCTCTCTCGAGTTCTCTAACTTCTTTCTTCC	HDV AG	
Tag2-AG-F Taylor	AGAGGGGACGAGTGAGGCTTATC	HDV AG	
AG-R-1	GTCCAGCAGTCTCCTCTTTACAG	HDV AG	(Harichandran <i>et al</i> , 2019)
HDV (951-970)-F	TATTCAGTGGGGTCGACAAC	Total HDV RNA	(Verrier <i>et al</i> , 2022)
HDV (1082-1101)-R	CTTCGTCCCCAATCTGCAG	Total HDV RNA	
HDV-Probe-Non-Edited	FAM-CCTATGGAAATCCCTGGTTTCCCCTGATG-BHQ1	Total HDV RNA	
HDV-Probe-Edited	HEX-CCCATGGAAATCCCTGGTTTCCCCTGATG-BHQ1	Total HDV RNA	
RPS11-F	GCCGAGACTATCTGCACTAC	RPS11	(Dao Thi <i>et al</i> , 2020)
RPS11-R	ATGTCCAGCCTCAGAACTTC		
NTCP-F	AAGGACAAGGTGCCCTATAAAGG	NTCP	
NTCP-R	TTGAGGACGATCCCTATGGTG		
AFP-F	TGGGACCCGAACCTTTCCA	AFP	
AFP-R	GGCCACATCCAGGACTAGTTTC		
ALB-F	GGTGTTGATTGCCTTTGCTC	ALB	
ALB-R	CCCTTCATCCCGAAGTTCAT		
CAV1-F	GCGACCCTAAACACCTCAAC	CAV1	(Spandido <i>s et al</i> , 2009)
CAV1-R	ATGCCGTCAAACTGTGTGTC		
CAV2-F	AAGACCTGCCTAATGGTTCTGC	CAV2	
CAV2-R	CTCGTACACAATGGAGCAATGAT		
EGFR-F	AGGCACGAGTAACAAGCTCAC	EGFR	
EGFR-R	ATGAGGACATAACCAGCCACC		
LAMP1-F	TCTCAGTGAACCTACGACACCA	LAMP1	
LAMP1-R	AGTGTATGTCCTCTTCCAAAAGC		
SCARB1-F	CCTATCCCCTTCTATCTCTCCG	SCARB1	
SCARB1-R	GGATGTTGGGCATGACGATGT		
SCARB2-F	AGATGGAGATTCTTTTCACCCAC	SCARB2	
SCARB2-R	CAGGAACTTTATACCGAAAGGCA		
ITGA2-F	CCTACAATGTTGGTCTCCCAGA	ITGa1	
ITGA2-R	AGTAACCAGTTGCCTTTTGGATT		

ITGB1-F	CCTACTTCTGCACGATGTGATG	ITGβ1	
ITGB1-R	CCTTTGCTACGGTTGGTTACATT		
LDLR-F	TCTGCAACATGGCTAGAGACT	LDLR	
LDLR-R	TCCAAGCATTCGTTGGTCCC		
SDC2-F	AAATGGACCCAGCCGAAGAG	SDC2	
SDC2-R	TGGTTTGCGTTCTCCAAGGT		
GPC5-F	AGACGCTTGCCAACAGAAGA	GPC5	
GPC5-R	CCATTTCCAACCACACGCTG		
POLR2A-F	CCCCAACCTCTCCATTGACC	POLR2A	
POLR2A-R	CCCTGCGCACTAGTTCTTGA		
CAD-F	GCGGTGCTGCTATGAATGTG	CAD	
CAD-R	ATGCTCAGAGATGGCGATGG		
MOV10-F	TGCTCCCCATGCTTCTTCAG	MOV10	
MOV10-R	GTGCCGGATATCATGCTCCA		
CD63-F	CAGTGGTCATCATCGCAGTG	CD63	
CD63-R	ATCGAAGCAGTGTGGTTGTTT		
HBV cccDNA-F	CCGTGTGCACTTCGCTTCA	HBV cccDNA	(Allweiss et al, 2023)
HBV cccDNA-R	GCACAGCTTGGAGGCTTGA		
HBV cccDNA- probe	FAM-CATGGAGACCACCGTGAACGCCC- BHQ1		
HBV-F	GTTGCCCGTTTGTCTCTAATTC	Total HBV DNA	(Qu et al, 2018)
HBV-R	GGAGGGATACATAGAGGTTCTTGA		
β-globin-F	AGGTACGGCTGTCATCACTTAGA	β-globin	
β-globin-R	CATGGTGTCTGTTTGAGGTTGCTA		

References

- Alexa A, Rahnenfuhrer J (2016) topGO: enrichment analysis for Gene Ontology. R Packag. version 2.26.0. *R Package version 2260*
- Allweiss L, Testoni B, Yu M, Lucifora J, Ko C, Qu B, Lütgehetmann M, Guo H, Urban S, Fletcher SP (2023) Quantification of the hepatitis B virus cccDNA: evidence-based guidelines for monitoring the key obstacle of HBV cure. *Gut* 72: 972-983
- Börner K, Kienle E, Huang LY, Weinmann J, Sacher A, Bayer P, Stüllein C, Fakhiri J, Zimmermann L, Westhaus A *et al* (2020) Pre-arrayed Pan-AAV Peptide Display Libraries for Rapid Single-Round Screening. *Molecular Therapy* 28: 1016-1032
- Dao Thi VL, Wu X, Belote RL, Andreo U, Takacs CN, Fernandez JP, Vale-Silva LA, Prallet S, Decker CC, Fu RM *et al* (2020) Stem cell-derived polarized hepatocytes. *Nature Communications* 11: 1677-1677
- Ferns RB, Nastouli E, Garson JA (2012) Quantitation of hepatitis delta virus using a single-step internally controlled real-time RT-qPCR and a full-length genomic RNA calibration standard. *Journal of Virological Methods* 179: 189-194
- Gripon P, Rumin S, Urban S, Le Seyec J, Glaise D, Cannie I, Guyomard C, Lucas J, Trepo C, Guguen-Guillouzo C (2002) Infection of a human hepatoma cell line by hepatitis B virus. *Proceedings of the National Academy of Sciences of the United States of America* 99: 15655-15660
- Gudima S, Chang J, Moraleta G, Azvolinsky A, Taylor J (2002) Parameters of Human Hepatitis Delta Virus Genome Replication: the Quantity, Quality, and Intracellular Distribution of Viral Proteins and RNA. *Journal of Virology* 76: 3709-3719
- Harichandran K, Shen Y, Stephenson Tsois S, Lee S-C, Casey JL (2019) Hepatitis delta antigen regulates mRNA and antigenome RNA levels during hepatitis delta virus replication. *Journal of Virology* 93: 10.1128/jvi. 01989-01918
- Lempp FA, Schlund F, Rieble L, Nussbaum L, Link C, Zhang Z, Ni Y, Urban S (2019) Recapitulation of HDV infection in a fully permissive hepatoma cell line allows efficient drug evaluation. *Nature Communications* 10: 1-11
- Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15: 1-21
- Maurer L, Andari JE, Rapti K, Spreyer L, Steinmann E, Grimm D, Thi VLD (2022) Induction of Hepatitis E Virus Anti-ORF3 Antibodies from Systemic Administration of a Muscle-Specific Adeno-Associated Virus (AAV) Vector. *Viruses* 14: 266-266
- Ni Y, Lempp FA, Mehrle S, Nkongolo S, Kaufman C, Fälth M, Stindt J, Königer C, Nassal M, Kubitz R *et al* (2014) Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. *Gastroenterology* 146: 1070-1083
- Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C (2017) Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods* 14: 417-419
- Qu B, Ni Y, Lempp FA, Vondran FW, Urban S (2018) T5 exonuclease hydrolysis of hepatitis B virus replicative intermediates allows reliable quantification and fast drug efficacy testing of covalently closed circular DNA by PCR. *Journal of virology* 92: 10.1128/jvi. 01117-01118
- Soneson C, Love MI, Robinson MD (2015) Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Research* 4
- Spandidos A, Wang X, Wang H, Seed B (2009) PrimerBank: A resource of human and mouse PCR primer pairs for gene expression detection and quantification. *Nucleic Acids Research* 38: D792-D799
- Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. *science* 282: 1145-1147
- Verrier ER, Salvetti A, Pons C, Michelet M, Rivoire M, Baumert TF, Durantel D, Lucifora J (2022) Loss of hepatitis D virus infectivity upon farnesyl transferase inhibitor treatment associates with increasing RNA editing rates revealed by a new RT-ddPCR method. *Antiviral Research* 198: 105250

Wang W, Lempp FA, Schlund F, Walter L, Decker CC, Zhang Z, Ni Y, Urban S (2021) Assembly and infection efficacy of hepatitis B virus surface protein exchanges in 8 hepatitis D virus genotype isolates. *Journal of Hepatology* 75: 311-323

Wolff G, Taranko AE, Meln I, Weinmann J, Sijmonsma T, Lerch S, Heide D, Billeter AT, Tews D, Kronic D *et al* (2019) Diet-dependent function of the extracellular matrix proteoglycan Lumican in obesity and glucose homeostasis. *Molecular Metabolism* 19: 97-106