Electroporation

High-fidelity electroporation of primary human hepatocytes to support oligonucleotide therapeutic applications

Introduction

The liver is an important target organ for oligonucleotide therapeutics because of ease of delivery to it, and its involvement in several monogenic disorders. It is estimated that more than 400 rare monogenic disorders are associated with the liver [1]. Liver-directed therapeutic modalities that have been approved or are in clinical and preclinical stages of development include gene silencing utilizing siRNA or antisense oligonucleotides, gene supplementation using mRNA or plasmids, and various gene editing approaches, including CRISPR-Cas9. *In vitro* models assembled from primary human hepatocytes (PHH) help provide a physiologically relevant model to evaluate efficacy, toxicity, pharmacokinetics, and potential off-target effects of these therapeutic approaches. Given that these modalities encompass a wide range both in terms of the type and size of delivered therapeutics, a flexible method of delivery into PHH will facilitate ease of evaluation. Electroporation of PHH enables a relatively flexible route for introducing such wide-ranging novel therapeutics in cells and facilitates their independent evaluation not confounded by the effect of the delivery vehicle. However, during electroporation PHH are subjected to high-voltage pulses, and this requires careful selection of voltage programs to achieve efficient delivery while maintaining high viability and functionality of the cells.

The Invitrogen™ Neon™ NxT Electroporation System with 8-Channel Pipette is ideal for conducting electroporation experiments with hepatocytes. The system offers the flexibility of independently regulating voltage magnitude, pulse width, and number of pulses to identify electroporation parameters that result in efficacious delivery while maintaining cell viability and function. The 8-channel pipette, of which each pipette tip can be individually programmed, further augments its capability for screening a wide range of parameters in a relatively high-throughput manner. Leveraging these features, in this study we first establish a workflow for electroporating and plating PHH

that is compatible with the Neon NxT 8-Channel Pipette. Next, the Neon NxT system is used to deliver mCherry mRNA to the cells, which are cultured in a sandwich configuration between a collagen-coated plate and an overlay of Gibco™ Geltrex™ matrix. Fluorescence on days 2 and 5, along with ATP and CYP3A4 levels on day 5, are assessed to identify the optimal program. Additional experiments are conducted without the sandwich configuration, as this is the preferred workflow that is more compatible with other relevant *in vivo* delivery modalities such as lipid nanoparticles (LNPs) that may eventually be employed for evaluating the efficacy of a fully assembled system. Without the overlay, the cell culture medium needs to be supplemented with Gibco™ HepExtend™ Supplement for efficient delivery and to maintain high viability and function of the cells. Finally, the optimal program identified for delivering mCherry mRNA is used to deliver a Cas9–gRNA complex for editing of the *HPRT* gene in PHH.

Materials and methods

Experimental workflow for electroporation and plating of cells

A vial of PHH is thawed in a water bath and poured into a pre-warmed 50 mL conical tube containing Gibco™ Hepatocyte Thaw Medium (Figure 1). The tube is centrifuged at 100 x *g* for 10 minutes. The cell pellet is resuspended in 1 mL of plating medium or maintenance medium by gently rocking the tube back and forth. Next, 4 mL of plating medium or maintenance medium is added to bring the cell suspension volume to 5 mL. The tube is gently rocked back and forth to mix the cells before manually counting the cells using a hemocytometer. After counting the cells, 45 mL of PBS without calcium or magnesium (–/–) is added to the cell suspension, and the tube is gently inverted a couple of times to mix the contents. The tube is centrifuged at 76 x *g* for 6 minutes, and the resulting pellet is resuspended in R buffer* to a final concentration of 18.8 x 10^6 viable cells/mL, based on by the previous cell count.

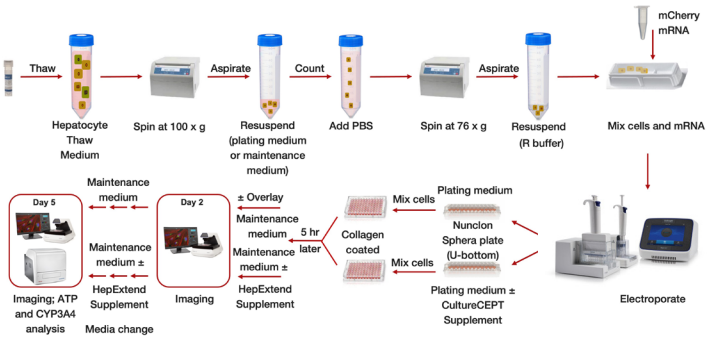

Figure 1. Overview of experimental workflow. Preparation and electroporation of PHH with mCherry mRNA are shown along with subsequent culture in a 96-well plate under different culture conditions. Transfection, viability, and metabolic function are assessed.

In the meantime, a stock solution of CleanCap™ mCherry mRNA (TriLink BioTechnologies) at 1 mg/mL is diluted in R buffer to a final concentration of 100 µg/mL. The diluted mRNA is transferred to a reservoir, and an equal volume of the cells suspended in R buffer is added to the reservoir using a wide-bore pipette tip. The cell suspension is mixed with mRNA by moving the reservoir back and forth. Next, electroporation is conducted as per instructions for the Neon NxT system. Briefly, the 8-channel pipette is used for withdrawing 10 µL of cell suspension and docked in the 8-channel pipette station that already contains 2 mL of E10 buffer* in each of the chambers. Electroporation in the 8 channels is conducted according to the preset 24-well optimization protocol in the Neon NxT system. After electroporation, the 10 µL volume is added to a Thermo Scientific™ Nunclon™ Sphera™ 96-Well U-Shaped-Bottom Microplate that already contains 130 µL of the plating medium. A uniform cell suspension is obtained by mixing the volume twice with a wide-bore pipette tip, and then 120 µL of the cell suspension is seeded along the walls of a collagen-coated 96-well plate. The plate is left undisturbed in an incubator maintained at 37°C and 5% CO_2. After 5 hours, the cells are washed with maintenance medium and overlaid with 120 µL of cold Geltrex matrix diluted in maintenance medium at a concentration of 350 µg/mL. The maintenance medium is replaced daily until the end of the culture.

Variation in PHH culture conditions

In one of the other experiments in the previous workflow, plating medium is supplemented with Gibco™ CultureCEPT™ Supplement at a 1X dilution (Figure 1). CultureCEPT Supplement contains the CEPT cocktail molecules—chroman 1, emricasan, polyamines, and trans-ISRIB—that reduce cellular stress and provide improved cell viability [2]. Additionally, the cells are cultured without an overlay of Geltrex matrix. After washing the cells with maintenance medium on the day of plating, the medium is changed to maintenance medium with or without HepExtend Supplement. Subsequent, daily media changes are with maintenance medium with or without HepExtend Supplement.

Image acquisition and analysis

On days 2 and 5, phase and fluorescence (Texas Red™ dye channel) images are acquired using the Invitrogen™ EVOS™ M7000 Imaging System. The same fluorescence settings are used for acquisition on both days. For assessing differences in fluorescence level across different culture conditions and electroporation programs, average fluorescence intensity for each image is measured using ImageJ software. The average fluorescence level for background (no-electroporation control) is subtracted from each image to quantify average fluorescence intensity across different culture conditions. In some cases, transfection efficiency is assessed using Invitrogen™ EVOS™ analysis software, which initially determines confluency and then based on that calculates transfection efficiency. The same threshold is applied across all the images to calculate transfection efficiency.

Cas9-gRNA delivery for gene editing and detection of genomic cleavage

Guide RNA (gRNA) for the *HPRT* gene is complexed with Invitrogen™ TrueCut™ Cas9 Protein v2 in R buffer and incubated at room temperature (RT) for 20 minutes. First, 86.4 µL of R

* R and E10 buffers are supplied in the Invitrogen™ Neon™ NxT Electroporation System 10 µL Kit.

buffer is combined with 10 µL of TrueCut Cas9 Protein v2 (5 µg/µL) and mixed thoroughly. Next, 3.6 µL of gRNA (100 µM) is added, followed by mixing and incubation. Then, 100 µL of cells suspended in R buffer are added to the Cas9–gRNA complex and mixed once using a wide-bore pipette tip. The contents are transferred to a reservoir for conducting electroporation as described previously. For these experiments, the cells are maintained without an overlay of Geltrex matrix. The cells are recovered and analyzed for genome cleavage using the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit, with a slight modification for plated cells. Briefly, on day 4 the cells in culture are washed with 100 µL of PBS (–/–) once, and then 50 µL of Cell Lysis Buffer/Protein Degrader mix (prepared from kit components) is added to the well and incubated at RT for 2 minutes. The lysate is recovered by pipetting the contents of the well multiple times. The lysate is subjected to the temperature profile outlined in the lysis and extraction step described in the kit, and then stored at –20°C. The samples are PCR-amplified using the forward and reverse primers for the targeted loci in the *HPRT* gene, and 3 µL of PCR product is used for the cleavage reaction. The Invitrogen™ E-Gel™ Power Snap Electrophoresis System is used to run and image the PCR products and cleavage reaction on a gel. The remaining steps were performed according to the kit instructions.

ATP and CYP3A4 analysis

On day 4 or day 5 of culture, ATP levels and CYP3A4 function are assessed from the same population of cells using the CellTiter-Glo™ 2.0 Cell Viability Assay and the P450-Glo™ CYP3A4 Assay with luciferin-IPA (Promega). Briefly, the medium is aspirated and the cells are washed with 100 µL of HBSS with calcium and magnesium (+/+). The cells are incubated at 37°C for 1 hour with 50 µL of Luciferin-IPA substrate prepared in HBSS (+/+) at 3 µM (1:1,000 dilution). Next, 25 µL of buffer containing luciferin product is transferred to a 96-well opaque white-wall plate, and 25 µL of P450-Glo Luciferin Detection Reagent is added. After that, the plate is shaken for 1 minute at 600 RPM on an orbital shaker and incubated for 20 minutes at RT to allow the luminescence signal to stabilize. In the meantime, 25 µL of fresh HBSS (+/+) is added to the 25 µL of buffer remaining in the cell-containing well. Next, 50 µL of ATP reagent is added to the cell-containing plate. The plate is shaken on an orbital shaker at 600 RPM for 2 minutes to induce cell lysis, and the plate is incubated at RT for 10 minutes to stabilize the luminescence signal. This is followed by transfer of 50 µL to a 96-well opaque white-wall plate. For quantifying CYP3A4 function and ATP levels, luciferin and ATP standards are prepared in HBSS (+/+) and processed similarly to the cell samples. The luminescence signal is measured using a Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader.

Results

Sandwich culture of PHH electroporated with mCherry mRNA

PHH from one donor (HU8XXX) are electroporated with mCherry mRNA using the 24 electroporation programs listed in Figure 2A, where the voltage ranges from 850 to 1,700 V in conjunction with pulse width and number of pulses varying from 10–40 ms and 1–3, respectively. After electroporation, the cells are cultured in a sandwich configuration using standard plating and maintenance media. Figure 2B shows a heat map illustrating relative levels of fluorescence (F) on days 2 and 5 as well as ATP and CYP3A4 function on day 5, across various electroporation programs. The heat map indicates that electroporation using programs P11 (1,100/40/1) and P19 (1,050/30/2) results in the cells exhibiting high fluorescence while also maintaining high ATP levels and CYP3A4 function. Next, these two programs are used for electroporating mCherry mRNA in PHH from three donors.

A

Program ID	Parameters	Program ID	Parameters	Program ID	Parameters
P1	No electroporation	P9	1,400/30/1	P17	850/30/2
P2	1,400/20/1	P10	1,000/40/1	P18	950/30/2
P3	1,500/20/1	P11	1,100/40/1	P19	1,050/30/2
P4	1,600/20/1	P12	1,200/40/1	P20	1,150/30/2
P5	1,700/20/1	P13	1,100/20/2	P21	1,300/10/3
P6	1,100/30/1	P14	1,200/20/2	P22	1,400/10/3
P7	1,200/30/1	P15	1,300/20/2	P23	1,500/10/3
P8	1,300/30/1	P16	1,400/20/2	P24	1,600/10/3

B

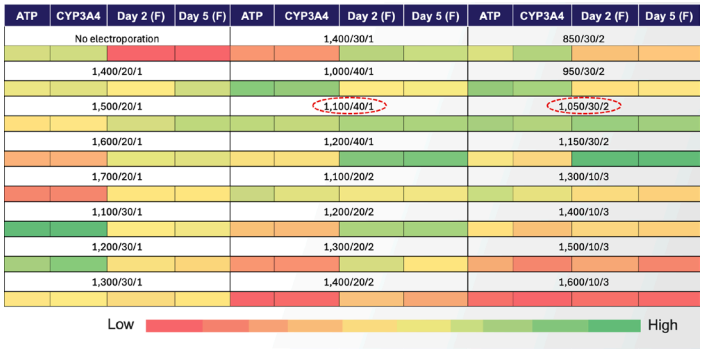

Figure 2. Identification of optimal electroporation parameters for transfection. (A) List of programs and associated parameters evaluated for electroporation. Electroporation parameters are given in the format of voltage (V)/pulse width (ms)/number of pulses. **(B)** PHH from donor HU8XXX are electroporated in triplicate using the Neon NxT Electroporation System and cultured in a sandwich configuration using standard plating and maintenance media. The fluorescence (F) level on days 2 and 5 is assessed based on an average of 9 images from 3 wells. The ATP level and CYP3A4 function on day 5 of culture are based on an average of 3 wells. The heat map shows variation in the average of these four measurements plotted independently across different programs. PHH electroporated with programs P11 (1,100/40/1) and P19 (1,050/30/2) show relatively high levels of fluorescence and ATP and CYP3A4 function, indicating suitability of these programs for transfection.

The images and transfection plots in Figures 3A–3C indicate that despite using the same program (P19, 1,050/30/2), transfection efficiency varies from donor to donor. On day 2, the highest transfection efficiency (~95%) is obtained with donor HU8XXX followed by donor HU8YYY (~85%) and then donor HU8ZZZ (~50%). Additionally, for the donors HU8XXX and HU8YYY, the fluorescence level increases from day 2 to day 5, indicative of the transfection efficiency reaching close to 100%, suggesting sustained or enhanced mRNA translation. For electroporation using P19 (1,050/30/2), both HU8XXX and HU8YYY retain ATPlevel and CYP3A4 function within 75% of the no-electroporation control (Figure 3D). By contrast, there is a dramatic reduction in both ATP level and CYP3A4 function with the donor HU8ZZZ. Comparing P11 (1,100/40/1) to P19 (1,050/30/2), in general, electroporation using program P11 results in slightly higher ATP level and CYP3A4 function, although that is accompanied by somewhat lower transfection efficiency. Taken together, these results indicate that for two out of three donor samples electroporated using program P19, PHH can be robustly transfected with mRNA while retaining high viability and functionality.

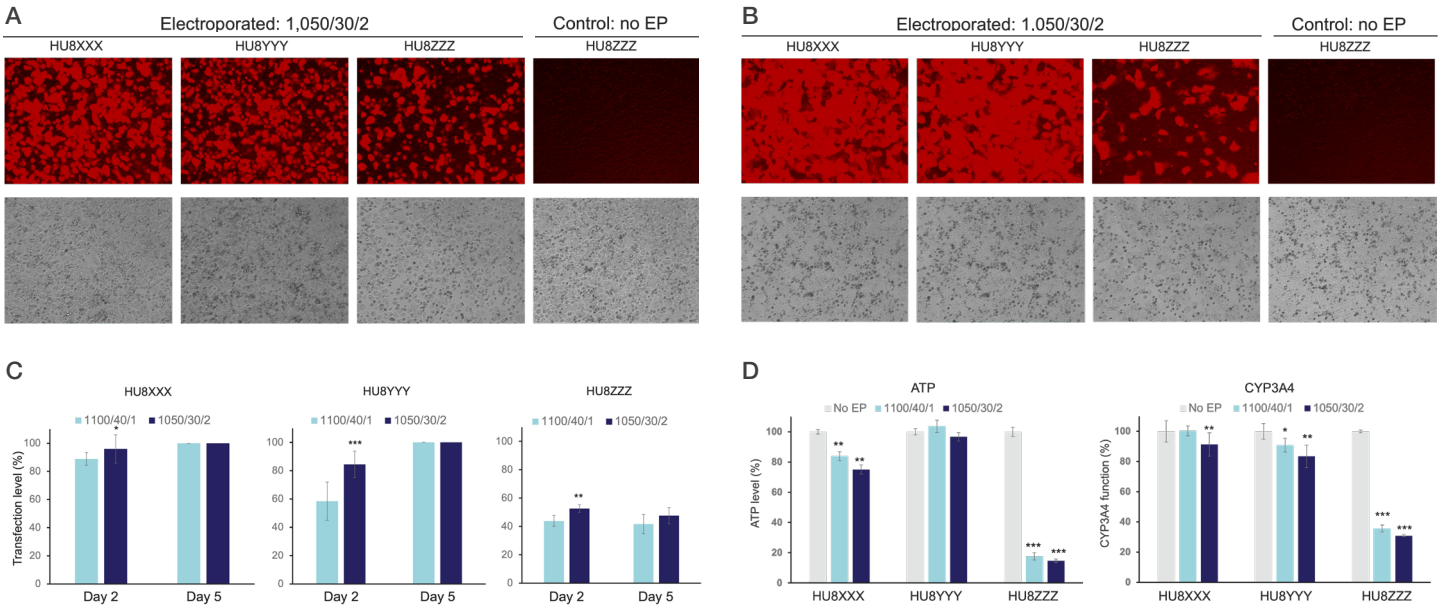

Figure 3. Sandwich culture of PHH electroporated with mCherry mRNA. (A) Day 2 and **(B)** day 5 representative fluorescence and phase-contrast images of PHH electroporated with mCherry mRNA using program P19 (1,050/30/2) for the three donors. For reference, images of the no-electroporation (no EP) control are included for one of the donors (HU8ZZZ). **(C)** Percent transfection level for the three donors on days 2 and 5. Transfection level is calculated using EVOS analysis software, where the threshold is set such that for the no-EP control, the transfection level is less than 0.2%. The transfection level is based on an average of 9 images from 3 wells for each condition. Differences compared to parameters of 1,100/40/1 are indicated as * p <0.05, ** p <0.01, and *** p <0.001. **(D)** Normalized ATP level and CYP3A4 function for the electroporated PHH derived from three donors on day 5 of sandwich culture. The levels are normalized to the no-EP control. Differences compared to the no-EP control are indicated as * p <0.05, ** p <0.01, and *** p <0.001. All differences are calculated using a paired two-tailed t-test.

HepExtend Supplement supports electroporated PHH cultured without overlay or sandwich configuration

In vitro evaluation of *in vivo* delivery modalities such as LNPs or direct conjugation of oligonucleotides with N-acetylgalactosamine (GalNAc) can be challenging in sandwich configuration because the overlay matrix can potentially impede access of delivery material to the cells. To simplify *in vitro* evaluation of delivered material across different methods, electroporated PHH are cultured without an overlay, which is a more compatible culture configuration with other delivery methods. Figure 4A shows that for the donor HU8XXX, the ATP levels drop by almost 40% as the cells are cultured in maintenance medium without the overlay. Further, a reduction in the ATP level occurs for the electroporated cells cultured similarly. Exposure to maintenance medium plus HepExtend Supplement for both no-EP control and electroporated (1,050/30/2) cells leads to recovery ATP levels similar to those observed with the sandwich configuration (Figures 3D and 4A). Prior exposure to CultureCEPT Supplement included in plating medium marginally increases the ATP level in comparison to exposure with HepExtend Supplement alone. Similar ATP recovery results are obtained for the donor HU8YYY. With the donor HU8ZZZ, exposure to HepExtend Supplement is not able to rescue the electroporated cells, but with the no-EP control, like with other donors, the ATP level recovers in the cells cultured without overlay.

For CYP3A4 function, addition of HepExtend Supplement to cells cultured without the overlay leads to a much more dramatic increase beyond what is observed for sandwich culture (Figure 4B). Also, CYP3A4 function of the cells across different culture configurations and electroporation parameters follows the same trend as observed for ATP levels for all three donors. Figures 4C and 4D show fluorescence images of electroporated cells from donors HU8XXX and HU8YYY, cultured with and without overlay. On day 2 of culture, similar fluorescence levels are observed for the cells cultured in a sandwich configuration and those maintained in HepExtend Supplement without the overlay. Additionally, the fluorescence levels increase from day 2 to day 5 of culture. Taken together, the results indicate that use of HepExtend Supplement during maintenance of PHH cultures without overlay is a practical strategy for electroporating cells and maintaining viability and CYP3A4 function. There is some flexibility when cells need to be exposed to HepExtend Supplement, as Figure 4E shows that even if HepExtend Supplement is delayed by one day, much of the benefit in terms of ATP levels and CYP3A4 function is still observed.

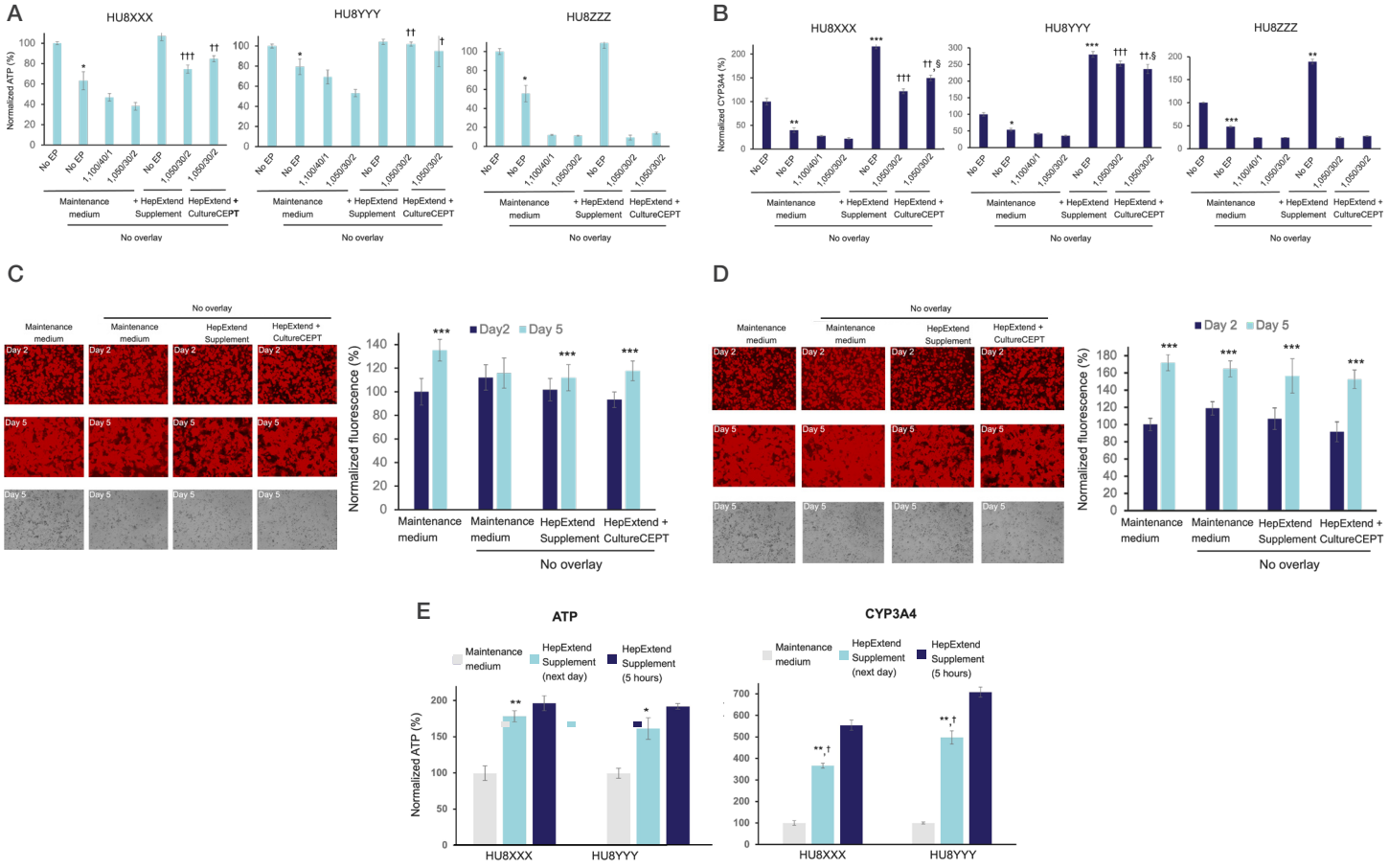

Figure 4. HepExtend Supplement better supports electroporated PHH cultured without overlay. (A) ATP level and **(B)** CYP3A4 function for three donors electroporated with mCherry mRNA on day 5 of culture. The cells are cultured without overlay and maintained in maintenance medium or maintenance medium plus HepExtend Supplement. Additionally, for program P19 (1,050/30/2), the cells are plated in plating medium plus CultureCEPT Supplement. The ATP and CYP3A4 levels for the three donors are normalized to no-EP control PHH cultured in sandwich configuration using standard plating and maintenance medium. Differences compared to the condition with overlay, no-EP, and maintenance medium are indicated as * p <0.05, ** p <0.01, and *** p <0.001, while those compared to the condition with no overlay, parameters 1,050/30/2, and maintenance medium are indicated as † p <0.05, †† p <0.01, and ††† p <0.001. Additionally, § p <0.05 indicates differences compared to the condition with no overlay, parameters 1,050/30/2, and added HepExtend Supplement. **(C)** Donor HU8XXX and **(D)** donor HU8YYY fluorescence and phase-contrast images for the cells electroporated using program P19 (1,050/30/2). Average fluorescence intensity for each condition is normalized to the day 2 fluorescence observed in electroporated PHH cultured in sandwich configuration. The HepExtend + CultureCEPT condition refers to when CultureCEPT Supplement is added to the plating medium, and HepExtend Supplement to the maintenance medium during cell culture. Differences compared to the day 2 condition are indicated as *** p <0.001. **(E)** Normalized ATP and CYP3A4 levels for the two donors comparing the effect of delaying HepExtend Supplement exposure to the electroporated (1,050/30/2) cells by one day in no-overlay cultures. HepExtend Supplement (5 hours) refers to the condition where the cells are exposed to maintenance medium with HepExtend Supplement beginning 5 hours after plating of the cells. In the HepExtend Supplement (next day) condition, the exposure to HepExtend Supplement begins from the next day and continues for the rest of the duration of the culture. The levels are normalized to the condition where the cells are maintained in maintenance medium without any HepExtend Supplement. Differences compared to this condition are indicated as * p <0.05 and ** p <0.01, while those compared to the HepExtend Supplement (5 hours) condition are indicated as † p <0.05. All differences are calculated using a paired two-tailed t-test.

Electroporation of Cas9-gRNA for gene editing in PHH

Since the electroporation of PHH using program P19 (1,050/30/2) results in robust transfection of mCherry mRNA, the same program is used for delivering the Cas9–gRNA complex to the cells. gRNA-directed cleavage in the *HPRT* gene and the resulting insertion or deletion (indel) are detected using the GeneArt Genomic Cleavage Detection Kit. The kit relies on PCR-based amplification of the specific region in the gene where a double-strand DNA break is directed by gRNA and indels are created. Subsequently, a detection enzyme cleaves the indel-mediated mismatch in the PCR-amplified product, resulting in generation of lower molecular weight products that can be separated from the original product on an agarose gel. The agarose gel images in Figure 5A show that electroporation using program P19 (1,050/30/2) results in generation of additional lower molecular weight bands when the detection enzyme (E) is added, while for the no-EP control there is only one high molecular weight band detected in the presence of enzyme. As expected, only one high molecular weight band is detected when detection enzyme is not added. These results from two donors indicate that the parameters of 1,050/30/2 can be used for delivering a Cas9–gRNA complex and generating indels. Similar to the day 5 viability and function results when mCherry mRNA is delivered, exposure to HepExtend Supplement in this case enhances ATP and CYP3A4 function on day 4 of culture (Figure 5B). Again, the inclusion of CultureCEPT Supplement has only a marginal effect.

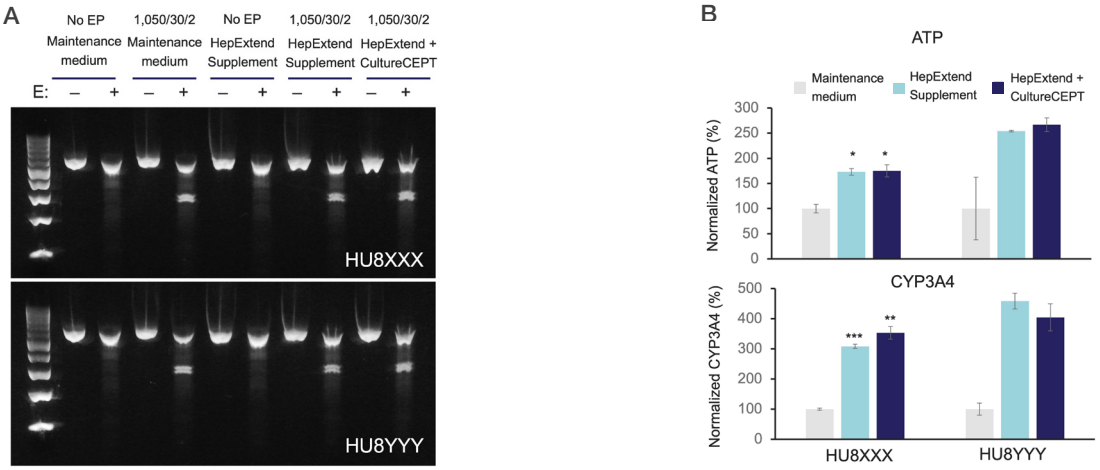

Figure 5. Electroporation of Cas9–gRNA for gene editing in PHH. (A) Agarose gel images showing additional lower molecular weight bands (with addition of detection enzyme (E) under program P19 parameters 1,050/30/2) corresponding to an indel-induced mismatch in a specific region of the *HPRT* gene. PHH from two donors are electroporated using program P19 (1,050/30/2) and a no-EP control with Cas9–gRNA complex directed towards the *HPRT* gene. The cells are cultured without overlay under different media conditions whereby the HepExtend Supplement condition corresponds to maintenance medium with HepExtend Supplement and the HepExtend + CultureCEPT condition relates to plating medium with CultureCEPT Supplement. The genomic cleavage detection assay is conducted on the cells recovered on day 4 of culture. **(B)** ATP and CYP3A4 levels on day 4 of culture of PHH electroporated using program P19 (1,050/30/2). The ATP and CYP3A4 levels are normalized to electroporated PHH cultured without overlay in maintenance medium. Differences compared to cells maintained in maintenance medium are indicated as * p <0.05, ** p <0.01, and *** p <0.001 (paired two-tailed t-test).

Discussion

Utilization of the Neon NxT Electroporation System with 8-Channel Pipette facilitated screening of different programs for electroporating PHH and simplified the identification of program P19 (1,050/30/2) for robustly transfecting PHH with mRNA. There is donor-to-donor variability that can be addressed by prescreening for donors, or the alternative option is evaluating other “gentler” programs that may be more compatible for a particular donor. Since the system allows the user to vary voltage, pulse width, and number of pulses independently, it may be possible to identify donor-specific optimal electroporation programs. The overall workflow includes resuspending the cells in R buffer prior to electroporation. To minimize the time that cells are in R buffer after thaw, the cells are resuspended in plating or maintenance medium for counting. For initial electroporation screening, the cells are resuspended in plating medium. However, the plating medium contains FBS, which may not be fully removed when the cells are subsequently resuspended in the R buffer, and may degrade mRNA. Thus, after initial screening, in subsequent experiments the cells are resuspended in maintenance medium for counting. Another consideration in the workflow is mixing of the cells after electroporation and prior to plating. This is important for a 96-well plate format, as it may be difficult to evenly distribute the cells by directly adding the 10 µL electroporated cell volume to the medium contained in the collagen-coated plate. We anticipate

that this will not be an issue while using a 100 µL volume for electroporation and plating in a 24-well plate, because in that plate format an even distribution of the cells can be achieved by shaking the plate after adding the cells.

The Neon NxT system allows for electroporation using voltages up to 2,500 V. In this work, R buffer is used, since the voltages do not exceed 1,900 V in any of the programs evaluated. Typically for using voltages higher than 1,900 V, T buffer is used since it is a lower-conductivity buffer. It is possible that use of T buffer during electroporation can facilitate using a higher voltage without compromising the performance of PHH. This may be especially relevant for introducing a payload such as a high molecular weight plasmid that may require relatively high voltage for to achieve high transfection.

Exposure of PHH to maintenance medium with HepExtend Supplement dramatically enhances the viability and metabolic function of cells cultured without the overlay matrix. This greatly simplifies the culture workflow and makes it compatible with other delivery methods. The ATP level recovers to the level observed in the cells cultured in the sandwich configuration, while CYP3A4 function even exceeds that. The positive effect of HepExtend Supplement on PHH viability and function is not confined to PHH cultured without the overlay. Previous work with the sandwich culture configuration indicates that HepExtend Supplement dramatically improves viability, metabolic function, and transporter function of PHH and promotes maintenance of function closer to that observed in suspension immediately after thawing the cells [3]. The effect of CultureCEPT Supplement is marginal when used in a workflow that includes HepExtend Supplement; however, it is plausible that in the cultures without HepExtend Supplement, a plating medium that includes CultureCEPT Supplement has a more dramatic positive effect on the viability and metabolic function of the cells than one without it. We observed that the fluorescence level in the cells electroporated with mCherry mRNA increases from day 2 to day 5, but it is not clear if a change in morphology of the cells from day 2 to day 5 contributed to this increase. Other plausible contributing factors to consider include relative stability of mCherry mRNA and perhaps an increase in translation activity over time in hepatocyte culture, as has been reported for albumin [4]. In addition to mRNA, the program P19 (1,050/30/2) can be used for delivering Cas9–gRNA and introducing indels in the specific region of a gene, as demonstrated for the *HPRT* gene in PHH derived from two donors. Just as in the case of mRNA delivery, HepExtend Supplement promoted maintenance of viability and metabolic function of PHH delivered with gene-editing material. In summary, the Neon NxT Electroporation System along with HepExtend Supplement helps provide a high-fidelity system for evaluating novel therapeutics in PHH that retain high viability and functionality.

References

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4. Dunn JC, Tompkins RG, Yarmush ML (1992) Hepatocytes in collagen sandwich: evidence for transcriptional and translational regulation. *J Cell Biol* 116(4): 1043–1053.

Ordering information

Product	Cat. No.
Cell culture plastics	
96-Well Plate, Collagen I Coated Surface	152038
Nunclon Sphera 96-Well, Nunclon Sphera-Treated, U-Shaped-Bottom Microplate	174925
Divided Reagent Reservoir	8096-11
Pierce 96-Well Polystyrene Plates, White Opaque	15042
Genome editing and transfection reagents	
TrueCut Cas9 Protein v2	A36498
TrueGuide sgRNA Positive Control, HPRT1 (human)	A35524
GeneArt Genomic Cleavage Detection Kit	A24372
Neon NxT Electroporation System 10 µL Kit with 8-Channel Tubes	N1096-8
Media, buffers, and supplements	
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Hepatocyte Thaw Medium	CM7500
Primary Hepatocyte Thawing and Plating Supplements	CM3000
Primary Hepatocyte Maintenance Supplements	CM4000
HepExtend Supplement (50X)	A2737501
Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A1413201
CultureCEPT Supplement (1,000X)	A56800
PBS, pH 7.4	10010023
HBSS, calcium, magnesium, no phenol red	14025092
Instruments	
Neon NxT Electroporation System with 1-Channel and 8-Channel Pipettes	NEON18S
EVOS M7000 Imaging System	AMF7000
Varioskan LUX Multimode Microplate Reader	VLBL00GD0

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