RNA extraction

Isolation of RNA from organoids and spheroids

Introduction

Cell culturing is an important technique in biomedical research and studies on basic cellular processes. Most research involving cell culture has traditionally been performed using two-dimensional (2D) culturing methods. Three-dimensional (3D) culturing techniques have recently gained the attention of the scientific community, because they make it possible to model *in vivo* natural systems [1]. 3D cell models include spheroids, organoids, scaffolds, hydrogels, 3D bioprinted tissues, and organs-on-chips [2].

Spheroids

Spheroids are 3D aggregates of one or more cell types. Compared to cells in 2D cultures, cells in 3D spheroids exhibit physiological responses that are more similar to those of cells in living organisms. Spheroids are established using cells from primary tissues or immortalized cell lines. Spheroids closely resemble the *in vivo* environment due to the enhanced adhesion capacity of cells in cellular aggregates. This important advantage over traditional monolayer cell cultures is having an impact in various areas of biomedical research, including cancer research, drug discovery, and toxicity testing, as well as research in the emerging fields of tissue engineering and regenerative medicine [3]. It should be noted that variability in the dimensions and shapes of spheroids can make it difficult to obtain reproducible data, so these parameters must be strictly controlled [4].

Organoids

Organoids consist of self-organized and differentiated functional cells derived from pluripotent stem cells (PSCs), neonatal stem cells, or adult stem cells [5]. Organoids bear a striking resemblance to their *in vivo* counterparts and recapitulate at least some functional characteristics of the derivative organs more closely than spheroids do [2,5,6]. Organoids derived from PSCs are significantly more complex than differentiated stem cells in 2D monolayer cultures, so they are likely to exhibit greater variability. Variability can arise from differences between starting cell lines or genotypes, as well as differences between batches of organoids derived from the same starting material. Variability can occur among organoids in the same culture or even in regions within an individual organoid. This variability must be minimized or at least controlled if organoid models are to be reliable enough for studying cellular physiology [7].

Nucleic acid extraction from 3D cultures for gene expression analysis

Gene expression in 3D cell cultures can be evaluated using many of the same techniques that are used for 2D cell cultures. Reverse transcription–quantitative PCR (RT-qPCR) is a technique commonly used to analyze gene expression in 3D cell cultures. One of the prerequisites for studying gene expression in 3D cultures is RNA isolation. Isolation of RNA from 3D cultures is similar to that of RNA from 2D cultures, but it has its unique challenges. The most commonly used extraction methods require the use of organic solvents like phenol and chloroform. Solid phase extraction techniques include column extraction, magnetic bead–based extraction, and hybrid organic and solid phase methods. Each method requires appropriate selection of reagents to achieve high extraction efficiency. While significant progress has been made in generating 3D cell cultures, nucleic acid extraction techniques that can be widely adopted for these sample types are needed.

In this study, we evaluated four methods for routine extraction of nucleic acid from 3D cell cultures. RNA was isolated from human spheroids and organoids using the column-based Invitrogen™ PureLink™ RNA Mini Kit, the Invitrogen™ TaqMan™ Fast Advanced Cells-to-C_T™ Kit, the magnetic bead–based Applied Biosystems™ MagMAX™ *mir*Vana™ Total RNA Isolation Kit, and Invitrogen™ TRIzol™ Reagent. Extraction performance was evaluated by performing RT-qPCR with the isolated RNA to measure and compare gene expression levels in the spheroids and organoids.

Materials and methods

Culture and storage of iPSC spheroids, primary hepatocyte spheroids, and human midbrain organoids

Induced pluripotent stem cell (iPSC) spheroids, primary hepatocyte spheroids from three donors (Hu8356, Hu8372, and Hu8373A), and 5-week-old human midbrain organoids were cultured and shipped to our laboratory by collaborators in Frederick, Maryland. The iPSC spheroids were shipped on ice in complete medium and stored at 4°C for 24 hours prior to nucleic acid extraction. The primary hepatocyte spheroids were cultured for 5 days, then washed three times with phosphate-buffered saline (PBS). They were then shipped on dry ice and stored at –80°C for 10 to 55 days prior to extraction.

Two human midbrain organoid samples designated 96W2K and 6WSSd3 were shipped on ice in Gibco™ Hibernate™-A Medium with Gibco™ B-27™ Supplement. The 96W2K organoids were cultured in a 96-well plate, and the 6WSSd3 organoids were cultured in a 6-well plate. The organoids were washed three times with PBS and transferred individually with 25 µL of residual PBS to microcentrifuge tubes, and then the residual PBS was removed. Nucleic acid was extracted from 96W2K and 6WSSd3 replicates on the same day, but extraction with TRIzol reagent was only performed with 6WSSd3 after 5 days of storage at 4°C.

Spheroid generation with HepG2 cells

HepG2 spheroids were generated at a biosafety level 2 facility in Austin, Texas. After thawing, the HepG2 cells were maintained in Thermo Scientific™ Nunclon™ Delta T-25 cell culture flasks in Gibco™ Dulbecco's Modified Eagle Medium (DMEM) with glucose and HEPES, supplemented with 10% Gibco™ fetal bovine serum (FBS). The HepG2 cells were maintained for two passages before seeding them for spheroid generation. The cells were stained with trypan blue and counted on the Invitrogen™ Countess™ II Automated Cell Counter, and cultures displaying more than 90% viability were selected for spheroid generation.

The cells were diluted in 10-fold dilution series, and the dilutions were pipetted in 100 µL aliquots into wells in Thermo Scientific™ Nunclon™ Sphera™ 96-well U-bottom microplates. Each well already contained 100 µL of complete DMEM, giving a final volume of 200 µL. The plates were centrifuged at 800 rpm for 5 minutes to aggregate the cells, and then placed in a 37°C incubator under 5% CO_2. Every few days, 100 µL of spent medium was aspirated from each well and replaced with 100 µL of fresh medium. The HepG2 spheroids were ready on day 5, 6, or 7 depending on how many cells were originally seeded. Images of the spheroids were recorded using the Invitrogen™ EVOS™ XL Core Imaging System.

We prepared homogeneous HepG2 spheroid cultures containing 5,000 cells per well. The medium was removed from the wells after growing the spheroids for five days, and the spheroids were washed three times with PBS, leaving 25 µL of residual PBS in each well. Pools of two spheroids were prepared by transferring them to single wells that each contained 25 µL of residual PBS. Six replicates were prepared for each extraction protocol. We also generated HepG2 spheroids from 500, 1,000, 2,000, 4,000, 8,000, and 16,000 HepG2 cells to evaluate the effect of varying the number of starting cells. After growing the spheroids for 48 hours, the medium was removed from the wells. The spheroids were washed three times with PBS, and 25 µL of residual PBS was left in each well.

Gene expression in iPSC-derived episomal spheroids

We evaluated *ACTB, CDK4,* and *GNE* gene expression in spheroids generated from the Gibco™ Human Episomal iPSC Line, including an internal positive control (IPC) to compare the performance of the RNA isolation kits. The spheroids were generated in stem cell culture by our collaborators, cultured, and shipped to our laboratory in suspension medium in a 12-well plate.

RNA extraction

RNA was extracted from individual, multiple, and pooled spheroid and organoid test samples using the **TaqMan Fast Advanced Cells-to-C_T Kit**, the **PureLink RNA Mini Kit**, the **MagMAX mirVana Total RNA Isolation Kit**, or **TRIzol Reagent**, according to the manufacturer's instructions. Samples that were processed using TRIzol Reagent were flash-frozen in a mixture of dry ice and ethanol once lysis was complete, and stored at –80°C for RNA extraction on a different day. Purified total RNA was eluted in RNase-free water and stored at –20°C for downstream analysis.

Human episomal iPSC spheroids

The human episomal iPSC spheroids were washed three times with PBS, and individual spheroids were carefully transferred in 50 µL PBS to wells in a 96-well plate. Excess PBS was then removed from the wells. RNA was extracted from individual spheroids using each of the three extraction kits (8 spheroid replicates per extraction kit), following the standard kit protocols. The only modification made was to the protocol for the TaqMan Fast Advanced Cells-to-C_T Kit. To lyse the spheroids, samples were pipetted 10 times instead of 5 following the addition of 50 µL lysis buffer. The final volume of each isolate obtained with the TaqMan Cells-to-C_T kit was 55 µL, so the total RNA extracted using the PureLink RNA Mini Kit and the MagMAX *mir*Vana Total RNA Isolation Kit was eluted in 55 µL RNase-free water. Prior to RT-qPCR analysis, Applied Biosystems™ TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control was added to the samples.

HepG2 spheroids

Each HepG2 spheroid grown in homogeneous culture seeded with 5,000 cells was mixed with 1 µL DNase and 49 µL lysis solution in a 96-well plate for extraction with the TaqMan Fast Advanced Cells-to-C_T Kit. Slight modification to the cell lysis step was necessary to efficiently extract RNA with the TaqMan Cells-to-C_T kit. The HepG2 spheroids had to be pipetted 5 to 10 times in the lysis solution and incubated at room temperature for 7 minutes to completely lyse the cells. For RNA isolation using the PureLink RNA Mini Kit and the MagMAX *mir*Vana Total RNA Isolation Kit, 200 µL lysis buffer was added to each sample and mixed by pipetting. The lysates were transferred to microcentrifuge tubes with the remaining lysis buffer to ensure they were completely lysed according to the kit instructions. The total RNA was eluted in 80 µL RNase-free water. RT-qPCR was performed to analyze *CDK4, GNE,* and *GPI* expression and compare the RNA yields obtained with each method.

Two replicates were prepared for each HepG2 spheroid generated from 500, 1,000, 2,000, 4,000, 8,000, or 16,000 cells for each extraction by each method. For extraction with the TaqMan Cells-to-C_T kit, 49 µL lysis buffer and 1 µL DNase were added to each replicate in a 96-well plate according to the kit instructions. For extraction with the PureLink RNA Mini Kit and the MagMAX *mir*Vana kit, 200 µL of lysis buffer was added to each replicate and mixed with the sample. The lysates were then transferred to 1.5 mL microcentrifuge tubes in lysis buffer to completely lyse the cells according to the kit instructions. Each spheroid processed with TRIzol Reagent was mixed with 25 µL PBS, transferred to a microcentrifuge tube, and processed per the manufacturer's instructions. Sets of four spheroids grown from the same number of cells were also pooled and transferred to microcentrifuge tubes. Extraction and downstream processing were performed with duplicates in the same manner used to extract and process individual spheroids. The total RNA was eluted in 80 µL of RNase-free water prior to one-step RT-qPCR to analyze *CDK4* and *GNE* expression.

Primary hepatocyte spheroids

The primary hepatocyte spheroids were processed on day 55 using the TaqMan Fast Advanced Cells-to-C_T Kit, the PureLink kit, the MagMAX *mir*Vana kit, and TRIzol Reagent, following the recommended protocols. Three replicates of each primary hepatocyte spheroid were prepared for each extraction method. Samples processed using the TaqMan Cells-to-C_T kit were pipetted 10 times in the lysis buffer. Pools of 4 primary hepatocyte spheroids were also prepared and transferred to microcentrifuge tubes for extraction with TRIzol Reagent. The pooled spheroids were extracted and processed in the same manner as the individual spheroids extracted with TRIzol Reagent. The total volume of each isolate obtained with the TaqMan kit was 105 µL, so the total RNA isolated using the other two kits and TRIzol Reagent was eluted in 105 µL RNase-free water. RT-qPCR was then performed to analyze *CDK4* and *GNE* expression.

Human midbrain organoids

RNA was extracted from the human midbrain organoids on the same day using the PureLink, TaqMan Cells-to-C_T, and MagMAX *mir*Vana kits. 96W2K samples were prepared in triplicate for extraction with each kit, but extraction with TRIzol Reagent was only performed with 6WSSd3 samples. Eight replicates of individual 6WSSd3 organoids were prepared for extraction with each kit, and eight 6WSSd3 organoids were pooled for extraction with TRIzol Reagent. Modification of the lysis step was necessary in all cases. The organoid samples had to be vortexed in 1.5 mL tubes for 2–4 minutes to completely lyse them, and the lysis mixtures had to be incubated for 7–10 minutes for extraction with the TaqMan Cells-to-C_T kit. The total volume of each isolate obtained with the TaqMan Cells-to-C_T kit was 55 µL, so the total RNA was eluted in 55 µL of RNase-free water for each method. RT-qPCR was then performed to analyze *CDK4, GNE*, and *GPI* expression.

RT-qPCR analysis of gene expression

Gene expression levels were evaluated via one-step RT-qPCR with fast cycling conditions on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System equipped with a 384-well block. Briefly, 2 µL of purified nucleic acid extract was added to a mixture containing 5 µL of Applied Biosystems™ TaqMan™ Fast Virus 1-Step Master Mix, 1 µL of an Applied Biosystems™ TaqMan™ Gene Expression Assay formulation (20X), and 12 µL water. The following cycling conditions were applied: 1 RT cycle at 50°C for 5 min; 1 cycle of RT inactivation and initial denaturation at 95°C for 20 sec; and 40 cycles of activation at 95°C for 3 sec and amplification at 60°C for 30 sec. The qPCR reactions were prepared in duplicate, and the data were analyzed using automatic threshold and baseline settings.

Results

There was little difference in the measured RNA levels for each gene, in the episomal iPSC spheroids, when different extraction methods were compared; the threshold cycle (C_t) values for each gene target differed by <1.5 cycles, which meant that RNA recovery with all three kits was similar (Figure 1B). There was no variation between the kits in terms of the C_t of the Xeno RNA, which indicated there was no inhibition of RT-qPCR. The results suggested that RNA could be isolated from individual iPSC-derived spheroids using any of the extraction methods employed.

HepG2 spheroids seeded with the same number of cells

As expected, the C_t difference (ΔC_t) for the *CDK4*, *GNE*, and *GPI* expression gene targets expressed in individual spheroids or pools of two spheroids grown from 5,000 HepG2 cells was approximately 1 cycle after 5 days of culturing (Figure 2). The average ΔC_t values for the three genes' expression in the individual and pooled spheroids processed with the TaqMan Cells-to-C_T kit were 1.1, 0.9, and 0.9, respectively. The average ΔC_t values for the three genes' expression in the individual and pooled spheroids processed with the PureLink RNA Mini Kit were 0.8, 1.3, and 1.3, respectively. The average ΔC_t values for the three genes' expression in the individual and pooled spheroids processed with the MagMAX *mir*Vana kit were 0.9, 1.1, and 1.2, respectively. The ΔC_t values ranged from 0.8 to 1.3, which indicated that RNA recovery from spheroids grown from the same number of HepG2 cells was consistent across all three kits.

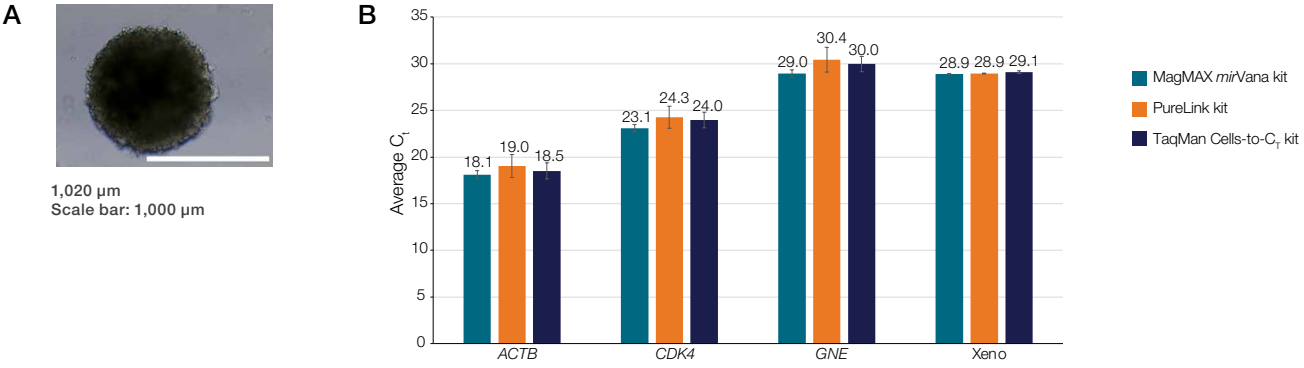

Figure 1. RT-qPCR analysis of episomal iPSC spheroid RNA extracted using the MagMAX *mir*Vana Total RNA Isolation Kit, the PureLink RNA Mini Kit, and the TaqMan Fast Advanced Cells-to-C_T Kit. (A) Image of an iPSC spheroid used for extraction. **(B)** Comparison of average C_t values for the *ACTB, CDK4,* and *GNE* gene targets and Xeno RNA control after RNA extraction from the iPSC spheroids (n = 8).

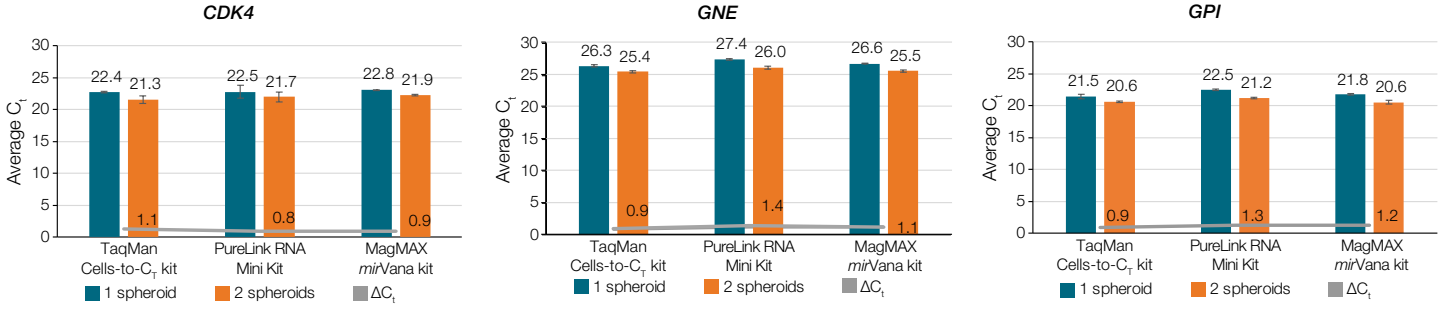

Figure 2. Comparison of average C_t values for *CDK4*, *GNE*, and *GPI* gene targets in HepG2 spheroids. RNA was extracted from the spheroids using the TaqMan Fast Advanced Cells-to-C_T Kit, the PureLink RNA Mini Kit, and the MagMAX *mir*Vana Total RNA Isolation Kit with inputs of one or two spheroids (n = 6).

HepG2 spheroids seeded with various quantities of cells

Images of the HepG2 spheroids generated with varying numbers of HepG2 cells are shown in Figure 3A. The average C_t values indicated that recovery of RNA from the spheroids with the PureLink RNA Mini and MagMAX *mir*Vana kits was linear (Figure 3B). While the C_t values for individual and pooled spheroids grown from the same number of cells and extracted with TRIzol Reagent differed by 2–4 cycles on average, the pooled spheroids grown from 500 cells generated significantly higher C_t values than individual spheroids grown from the same number of cells. This was most likely due to the loss of pooled spheroids during extraction, which resulted in a significantly lower RNA yield. There were differences between the RNA yields obtained with the TaqMan Fast Advanced Cells-to-C_T Kit and TRIzol Reagent, which suggested that the individual spheroids were heterogeneous. Extraction of RNA from pooled HepG2 spheroids appeared to compensate for this heterogeneity, although it could also be attributed to the age of the HepG2 spheroids.

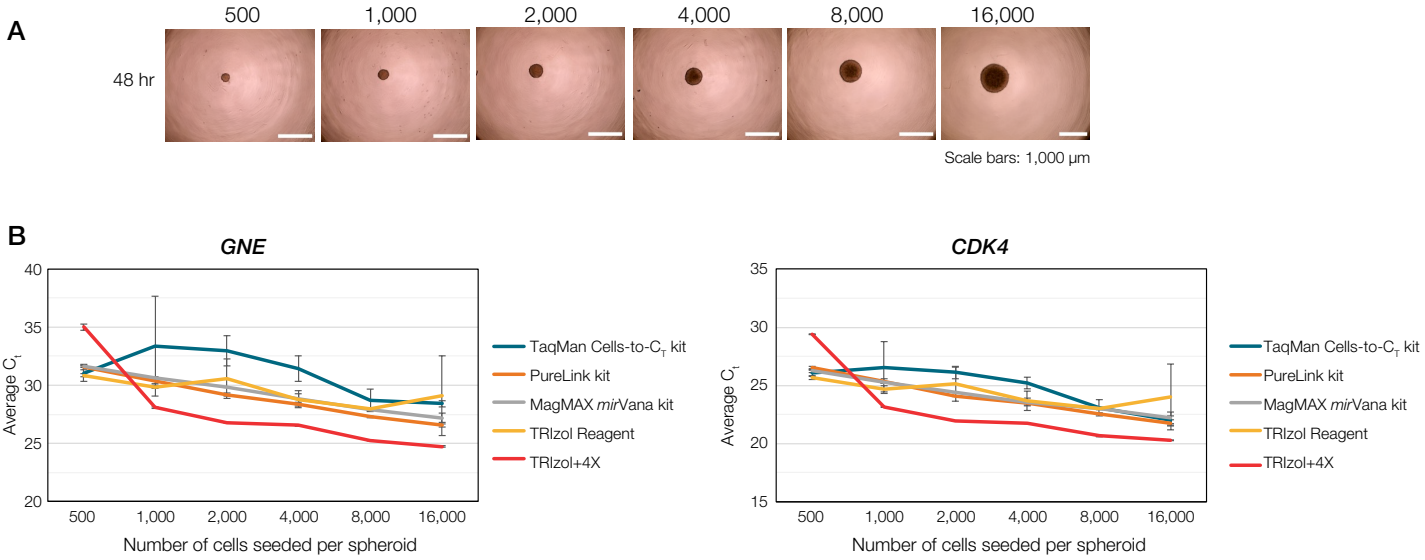

Figure 3. Comparison of C_t values after RNA extraction from HepG2 spheroids grown from various numbers of cells. (A) Representative images of HepG2 spheroids grown for 48 hours. **(B)** RT-qPCR results for *GNE* and *CDK4* expression in spheroids extracted using four different protocols.

We also compared gene expression in HepG2 spheroids seeded with various numbers of cells after allowing them to grow for 7 days. There was no difference between the average C_t values, regardless of how many cells were used to seed the spheroids (data not shown). Importantly, similar C_t values were consistently observed after extraction with the TaqMan Cells-to-C_T, PureLink, and MagMAX *mir*Vana kits. This could be attributed to the morphological features of spheroids generated by long-term culturing, deficient media, spheroid size, or even a lack of control over the dimensions of the spheroids [4]. When we compared the average diameters of the HepG2 spheroids over a period of one week, we noticed significant differences between their morphological features (Figure 4A). The spheroids grown from 500, 1,000, or 2,000 cells had a linear growth pattern for 4 days (Figure 4B). Inner cores of dead cells were evident in the spheroids seeded with 8,000 or 16,000 cells as early as the second day, but we did not observe necrotic cores in the spheroids seeded with 500, 1,000, or 2,000 cells until they had grown for 7 days. Seeding with different cell quantities could lead to variations in gene expression. The problems associated with spheroid generation must therefore be mitigated for optimal gene expression in HepG2 spheroids.

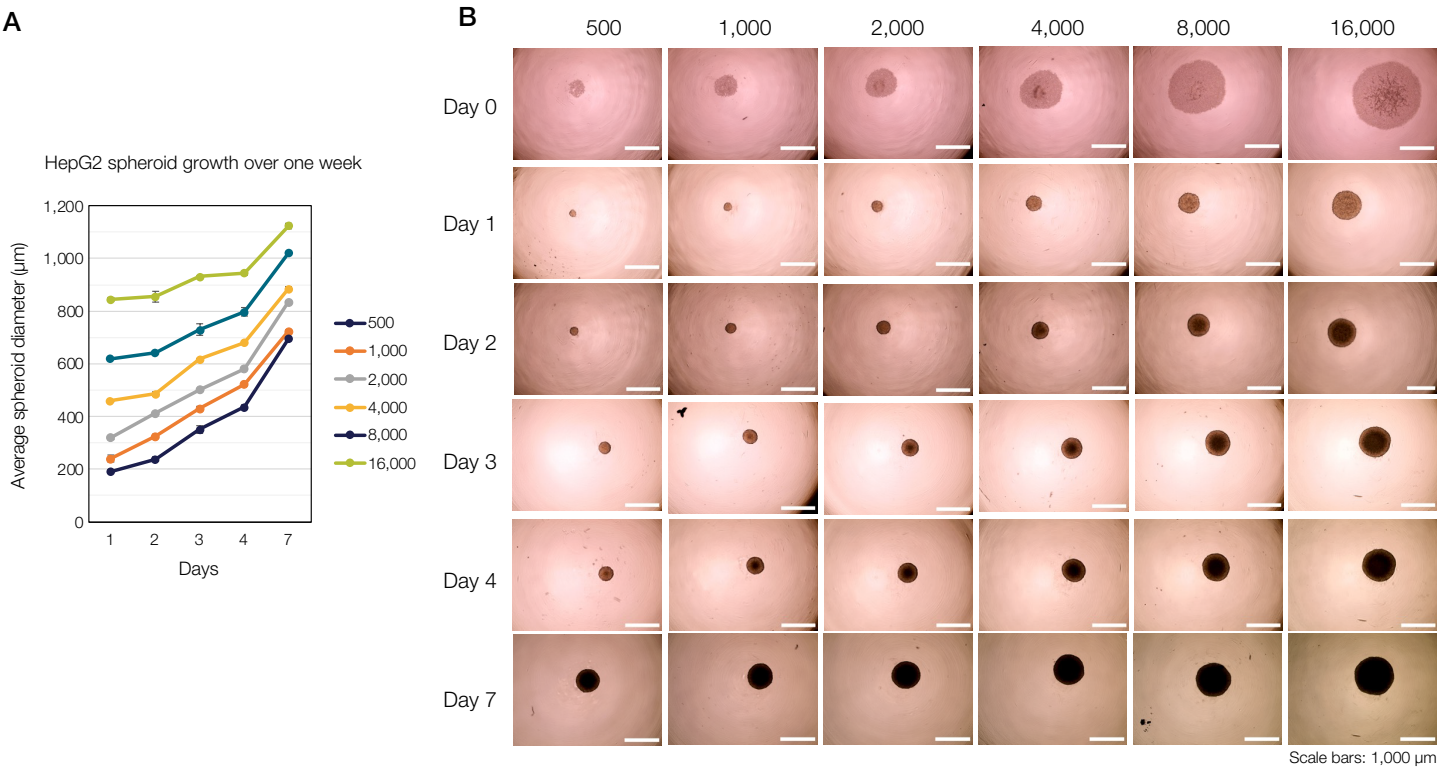

Figure 4. Growth patterns of HepG2 spheroids. (A) Average diameters of HepG2 spheroids seeded with various numbers of cells. **(B)** Representative images of HepG2 spheroids seeded with various numbers of cells after 7 days of growth.

Primary hepatocyte spheroids

The average diameter of primary hepatocyte spheroids grown from 1,500 cells for 5 days was approximately 280 µm (Figure 5A). The RNA extraction efficiency of the four methods varied, and there were significant differences between the average C_t values (Figure 5B). This may have been due to an excess of PBS (50 µL) in the original samples. To assess the effect of PBS volume on C_t, we extracted RNA from spheroid samples that contained 10 µL or 25 µL PBS, using the TaqMan Fast Advanced Cells-to-C_T Kit. The average C_t values for the *GPI* and *G6PD* targets were lower but less variable when the initial samples contained 10 µL PBS (Figure 6). This suggested that a larger starting volume of PBS in the samples influenced lysis efficiency. The samples were also frozen in PBS, which may have contributed to RNA degradation. We compared the C_t values for *ADH5* and *GPI* after extraction with the TaqMan Cells-to-C_T and PureLink kits and observed similar values (Figure 7). This indicated that long-term storage of the spheroids in PBS may have contributed to variable RNA recovery, which would result in inconsistent gene expression profiles. The smaller size of the primary hepatocyte spheroid cultures may have contributed to additional variability in extraction efficiency. This suggests that pooling smaller spheroid cultures may minimize heterogeneity.

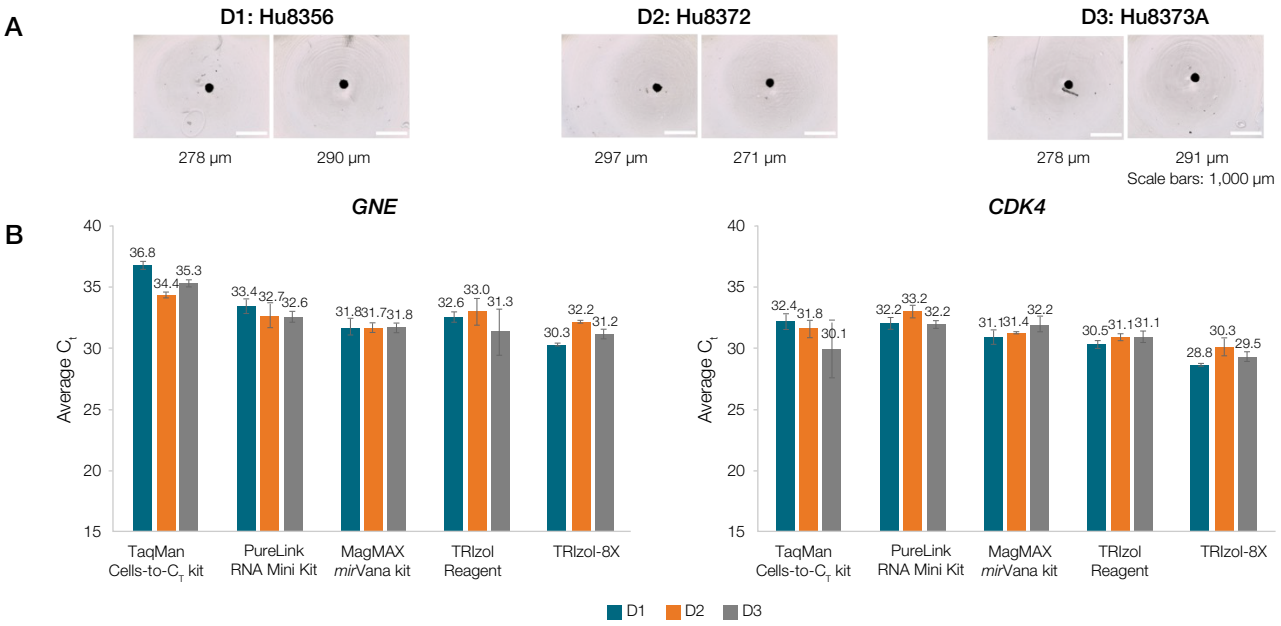

Figure 5. Results of RNA extraction from human primary hepatocyte spheroids seeded with cells from three different donors. **(A)** Representative images of spheroids grown for 5 days after seeding with 1,500 cells per spheroid. **(B)** RT-qPCR analysis of *GNE* and *CDK4* expression. C_t is reported as the average of three replicates (n = 3). TRIzol-8X: pools of eight primary hepatocyte spheroids extracted with TRIzol Reagent.

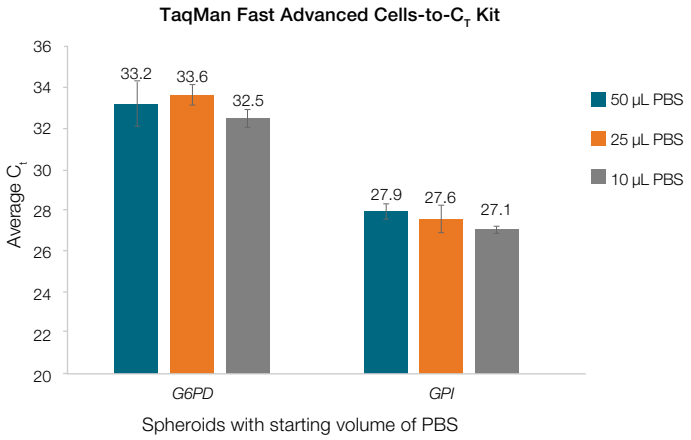

Figure 6. RT-qPCR analysis of *G6PD* and *GPI* gene expression in primary hepatocyte spheroids. RNA was isolated from spheroid samples containing different amounts of PBS using the TaqMan Fast Advanced Cells-to-C_T Kit, and extractions were performed in triplicate. C_t for each volume of PBS is reported as the average of all C_t values (n = 9).

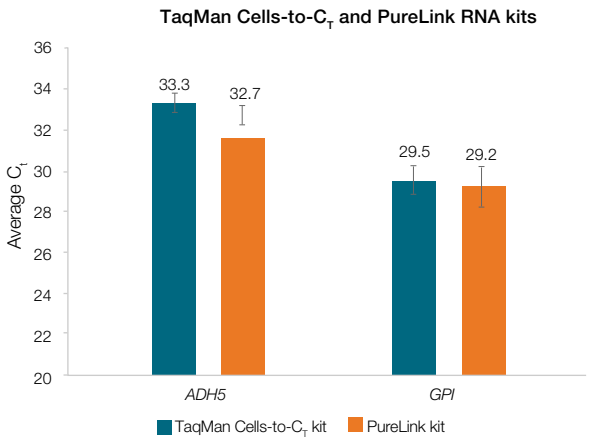

Figure 7. RT-qPCR analysis of *ADH5* and *GPI* gene expression in primary hepatocyte spheroids. RNA was extracted on day 4 using the TaqMan Fast Advanced Cells-to-C_T Kit and the PureLink RNA Mini Kit. Reactions were prepared by adding 1 µL of nucleic acid isolate to 5 µL of TaqMan Fast Virus 1-Step Master Mix, 1 µL of the TaqMan Gene Expression Assay formulation (20X), and 13 µL water.

Human midbrain organoids cultured in a 96-well plate (96W2K)

The average C_t values obtained after RNA extraction from 96W2K organoids (Figure 8A) with the PureLink and MagMAX *mir*Vana kits differed by <1 cycle for each gene (Figure 8B). Extraction with the TaqMan Fast Advanced Cells-to-C_T Kit resulted in similar but higher average C_t values for all three genes. This may have been because the TaqMan Cells-to-C_T kit had a less efficient lysis buffer than the other kits. Differences in organoid size, as well as variability between regions within individual organoids, may also have contributed to variable gene expression profiles.

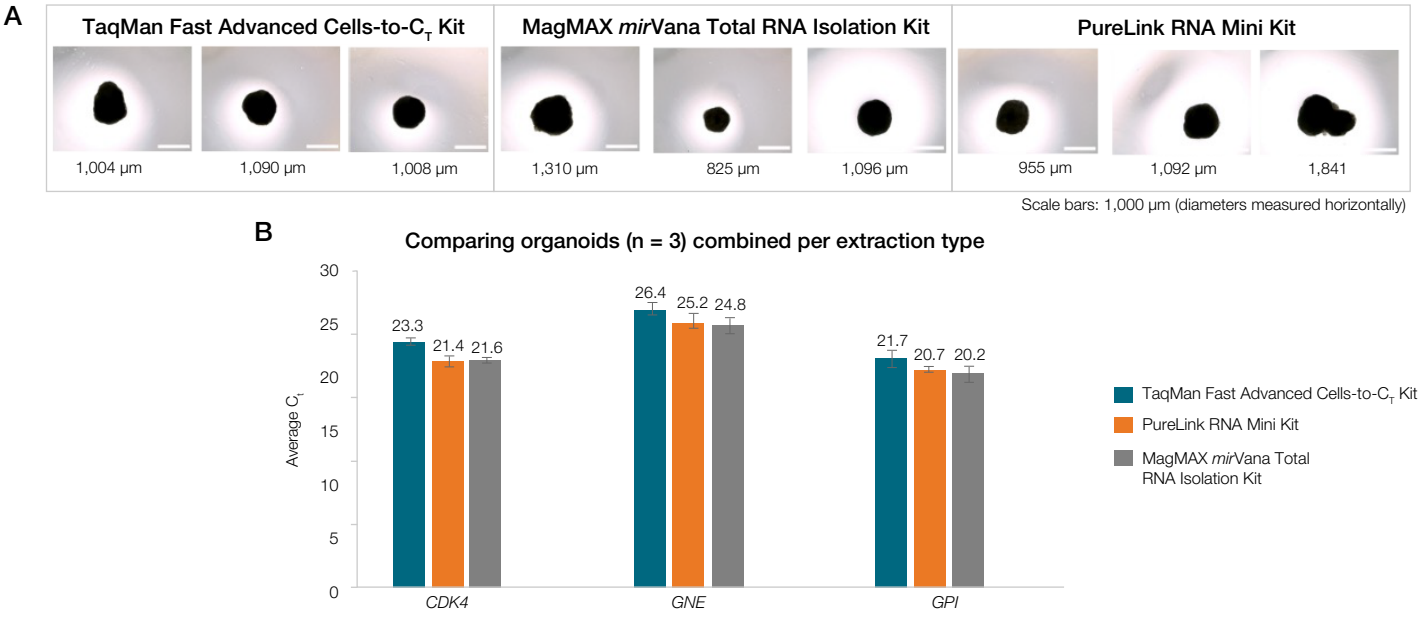

Figure 8. Analysis of five-week-old human midbrain organoids cultured in a 96-well plate. (A) Images of the organoids used for RNA extraction. **(B)** RT-qPCR results after extraction with the TaqMan Fast Advanced Cells-to-C_T Kit, the MagMAX *mir*Vana Total RNA Isolation Kit, and the PureLink RNA Mini Kit (n = 3 per kit).

Human midbrain organoids cultured in a 6-well plate (6WSSd3)

The average C_t values for each of the *GNE* and *CDK4* targets in these organoids (Figure 9A) differed by <1.5 cycles after RNA extraction by all four methods (Figure 9B), which indicated comparable extraction efficiency. The difference between the C_t values for individual organoids and pools of eight organoids processed using TRIzol Reagent was approximately 3 cycles. This suggested that it might be possible to obtain gene expression profiles with individual organoids using any of the extraction methods tested. The 6WSSd3 organoids were stored at 4°C before processing on day 4, while the 96W2K organoids were processed fresh. The 6WSSd3 organoids may have begun to disintegrate during storage, which would have made lysis with the TaqMan Fast Advanced Cells-to-C_T Kit's lysis solution more efficient. The samples were also incubated in the lysis solution for 10 minutes at room temperature, while the 96W2K samples were only incubated for 7 minutes.

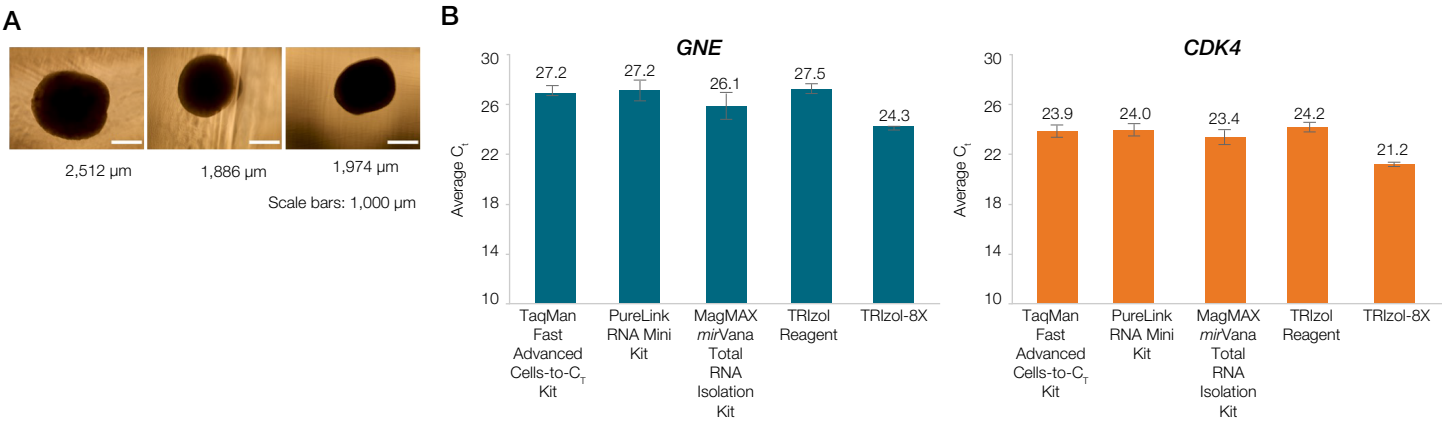

Figure 9. Comparing RNA extraction methods with five-week-old human midbrain organoids cultured in a 6-well plate. (A) Representative images of 6WSSd3 organoids used for RNA extraction. **(B)** Average C_t values for *GNE* and *CDK4* targets after extraction of RNA from 6WSSd3 organoids using four different methods. TRIzol-8X: eight organoids pooled for extraction with TRIzol Reagent.

High-quality RNA is essential for downstream applications like gene expression studies, whole transcriptome analysis, microarray analysis, and next-generation sequencing (NGS). To ensure the extraction protocols applied in this study did not reduce RNA integrity, we analyzed the quality of RNA isolated from individual organoids using the Agilent™ RNA 6000 Pico Kit on the Agilent™ 2100 Bioanalyzer. The representative electropherograms in Figure 10 were obtained after extraction with the PureLink RNA Mini Kit, the MagMAX *mir*Vana Total RNA Isolation Kit, and TRIzol Reagent. The data indicated that high-quality RNA could be isolated with minimal loss of integrity with all three methods. Total RNA, including small RNA, was more efficiently isolated with the MagMAX *mir*Vana kit and TRIzol Reagent than with the PureLink kit.

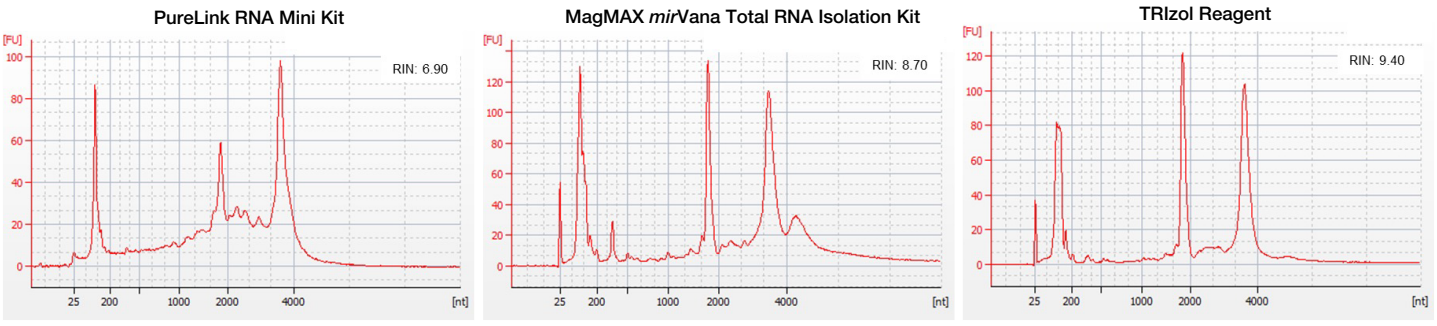

Figure 10. Representative electropherograms and RNA integrity numbers (RINs) for RNA isolated from single organoids using three different methods.

Conclusion

We have successfully applied four distinct extraction methods to extract RNA from spheroids and organoids. Our results showed that with slight modification, efficient RNA recovery from 3D cultures could be achieved with all four methods. Completely removing PBS from the samples significantly increased lysis efficiency, particularly the efficiency of the TaqMan Fast Advanced Cells-to-C_T Kit's lysis solution. Cell viability in spheroids decreased as the spheroids grew larger during long-term culture, so spheroid size did not equate to RNA expression. Thus, controlling the growth characteristics of spheroids is important for obtaining comparable gene expression profiles. We also found that extracting RNA from fresh spheroids and organoids rather than frozen ones resulted in better RNA recovery and less variation in gene expression profiles.

Selection of an appropriate extraction method depends on end-user requirements for a particular experiment and whether a manual, low-throughput, or semi-automated workflow is desired. The TaqMan Fast Advanced Cells-to-C_T Kit would be ideal for high-throughput users who need to process individual spheroid and organoid samples for gene expression studies. However, residual PBS should be removed prior to extraction to ensure complete lysis of spheroids. The lysis solution of the TaqMan Cells-to-C_T kit is not optimal for organoid lysis. The PureLink RNA Mini Kit might be preferable to users who process <24 samples at once. The MagMAX *mir*Vana Total RNA Isolation Kit is compatible with high-throughput purification of RNA and DNA, so it is favored by end users with NGS and qPCR workflows. Extraction with TRIzol Reagent may be the best option if multiple spheroids or organoids have to be pooled for downstream analysis.

Each of the methods tested in this study can be used for RNA extraction from 3D cultures. However, the TaqMan Fast Advanced Cells-to-C_T Kit and the MagMAX *mir*Vana Total RNA Isolation Kit may be more scalable and amenable to high-throughput sample processing than the PureLink RNA Mini Kit or TRIzol Reagent. This work was not intended to be an exhaustive study of extraction methods, but rather to demonstrate methods that can be employed successfully to isolate RNA from organoids and spheroids.

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Ordering information

Description	Quantity	Cat. No.
TaqMan Fast Advanced Cells-to-C_T Kit	40 reactions	A35374
	100 reactions	A35377
	400 reactions	A35378
PureLink RNA Mini Kit	10 preps	12183020
	50 preps	12183018A
	250 preps	12183025
MagMAX *mir*Vana Total RNA Isolation Kit	96 reactions	A27828
TRIzol Reagent	200 mL	15596018
	100 mL	15596026
TaqMan Gene Expression Assay	variable	4331182
TaqMan Fast Virus 1-Step Master Mix	1 x 1 mL	4444432
	5 x 1 mL	4444434
	1 x 10 mL	4444436

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