Patient-derived 3D Ready Organoids for high throughput screening

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Introduction

Industrial manufacture of patient-derived organoids

3D Patient-Derived Organoids (PDOs) have improved predictive power and have the potential to be used as a more clinically relevant model in applications that have typically relied on 2D cell lines, such as high-throughput screening (HTS), genetic engineering and co-culture assays. However, their use is currently limited due to the difficulty in producing PDOs in sufficient quantity, with the required quality and reproducibility.

Industrial manufacture of human-derived 3D organoids is now possible with 3D Ready™ Organoids and the 3D Ready Organoid Expansion Service from Molecular Devices. Our proprietary bioreactor system has been developed for the controlled production of standardized PDOs at scale. Organoids are cultured in a regulated environment to ensure the constant delivery of nutrients and growth factors to the culture, while preventing the accumulation of toxins that can lead to cell death. This approach enables the large-scale production of assay-ready organoids that are viable and consistently sized between batches. 3D Ready Organoids are supplied as cryopreserved vials ready for plating at your convenience.

Methods

3D Ready Organoid expansion, seeding & assay

Colorectal cancer (CRC) organoids (ISO49 & ISO68) and the duodenal organoid line DP41N2, were expanded using a patent-pending bioreactor system, harvested, and vialled according to established internal protocols.

For seeding assay plates, vials of 3D Ready Organoids were thawed, rinsed in media, and resuspended in Matrigel® (Corning). Organoids were then seeded into 384-well clear-bottom microplates in organoid growth medium containing ROCK inhibitor for 48 hours to ensure optimal recovery prior to any compound treatment. Following 48-hour recovery, organoids were treated with trametinib or DMSO control and incubated for a further 5 days. Treated organoids were assayed using CellTiter-Glo® 3D Cell Viability Assay (Promega), with results detected using a microplate reader.

3D Ready Organoid imaging & segmentation

To ensure optimum recovery of vialled PDOs, transmitted light (TL) imaging of organoids was performed daily using the ImageXpress Pico Automated Cell Imaging System. 2D projection images were acquired with a 4X objective and 50 µm focus step. Organoid growth media was changed every 2–3 days. We developed a custom analysis pipeline to assess organoid growth using 2D projection TL images. The SINAP module within IN Carta® Image Analysis Software was used to train a custom model to accurately segment TL images of organoids. SINAP relies on deep learning-based image analysis, resulting in robust segmentation for any biological structure. Custom analysis was performed to generate masks on the 2D projection of the TL channel to accurately segment organoids from background. Average organoid area was determined, and data was exported to determine fold growth over the culture period.

Results

3D Ready Organoid expansion

A semi-automated bioreactor system was used to manufacture batches of ISO68, ISO49 & DP41N2 organoid lines at scale (Figure 1). Assay-ready organoids were harvested and vialled at a set-density for use in a variety of downstream assays. Quality control was performed to ensure organoids were sterile and endotoxin-free.

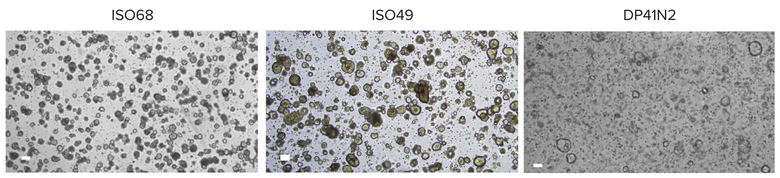


Figure 1. ISO68, ISO49 & DP41N2 3D Ready Organoids lines prior to harvest following bioreactor expansion (scale bar = 100 µm).

Results

3D Ready Organoid growth

The ability to monitor organoid growth in real-time and extract meaningful information is dependent on the robust segmentation of label-free TL images. Three vials per organoid line (ISO68, ISO49 & DP41N2) were thawed and plated in 384-well plates. Organoids were imaged over 7 days using the ImageXpress Pico Automated Cell Imaging System to acquire TL images. IN Carta Image Analysis Software's SINAP module deep learning analysis and model development was used to segment organoids and determine average organoid area at each imaging timepoint. All three 3D Ready Organoid lines proliferate over 7 days in culture, and the growth profiles remain consistent between vials from the same batch of 3D Ready Organoids.

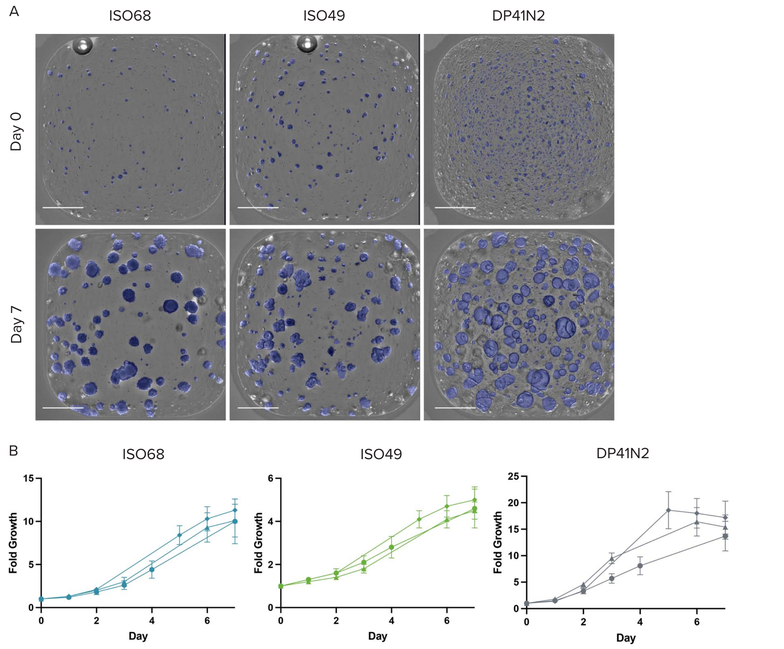


Figure 2. (A) IN Carta SINAP Organoid Segmentation of ISO68, ISO49 and DP41N2 3D Ready Organoid lines on day 0 and day 7. (B) 3D Ready Organoid growth curves. Scale bar = 700 µm.

3D Ready Organoid assay variability

In order to assess the feasibility of the use of 3D Ready Organoids in a HTS assay, an assay validation experiment was conducted to assess the variability of assay signal outputs. Here ISO49 3D Ready Organoids were manually seeded in 3 plates per day over 3 days (9 plates total) with varying plate layouts (Figure 3). After 48 hours recovery, organoids were treated with previously calculated trametinib IC50 and IC90 concentrations alongside DMSO control, corresponding to medium (mid), low and high output signal, respectively. After 5 days treatment, organoids were assayed using CellTiter-Glo® 3D Cell Viability Assay (Promega).

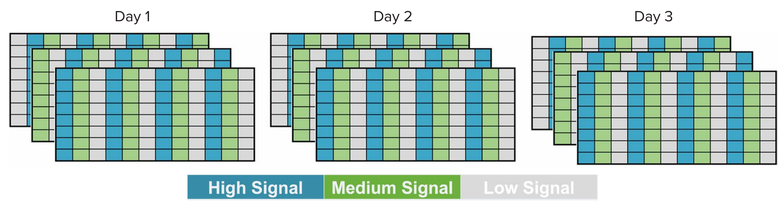
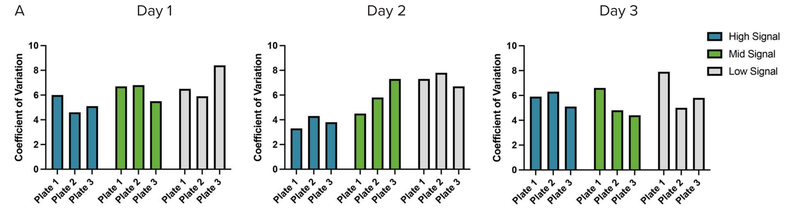


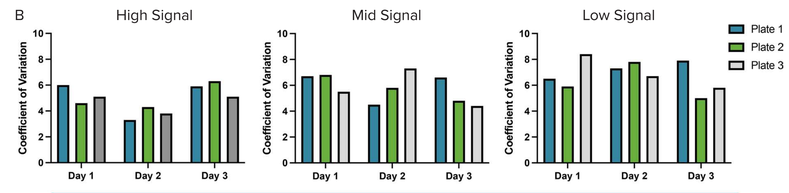
Figure 3. Standard assay validation protocol. Organoids are seeded, 3 plates per day with varying plate layouts and treated with compounds that mimic high, medium (or mid) and low assay signal readouts.

Results

3D Ready Organoid inter- and intra-assay variability



Inter-Assay Average Coefficient of Variation (%)		
Day 1	Day 2	Day 3
6.16%	5.64%	5.76%



Intra-Assay Average Coefficient of Variation (%)		
High Signal	Mid Signal	Low Signal
4.93%	5.78%	6.81%

Figure 4. (A) Inter-and (B) intra-assay low, mid (medium), and high signal variability of treated ISO49 3D Ready Organoids over days 1 to 3. To validate a high-throughput screening assay, coefficient of variation is required to be less than 20%.

For an assay to be used for HTS, the data collected from a 3-day validation experiment needs to meet minimum quality requirements set in the HTS assay validation guidelines[1]. One such requirement from this criteria is the coefficient of variation (CV) values of the raw high, medium (mid), and low signals need to be less than 20% in all 9 plates.

Results from our organoid variability assay were analyzed and show the inter-assay CVs were consistently <10% over days 1, 2, and 3 of the assay (Figure 4A). Similarly, the average intra-assay CVs for the high, mid, and low signals were all <10% (Figure 4B). These results show 3D Ready Organoids produce reliable and reproducible outputs for HTS assays.

Conclusion

Prior limitations to the widespread integration of organoids into HTS assays have included limited scalability and reproducibility due to manually intensive and costly culture processes.

Our patent-pending bioprocess technology now enables the large-scale expansion of reproducible, validated batches of 3D Ready Organoids compatible with high throughout screening applications.

- Reduce organoid culture time by utilizing the 3D Ready Organoids and bespoke 3D Ready Organoid Expansion Service from Molecular Devices
- Scale up screening capacity for organoid-based assays to increase throughput in drug discovery.
- Full protocols and technical support available.

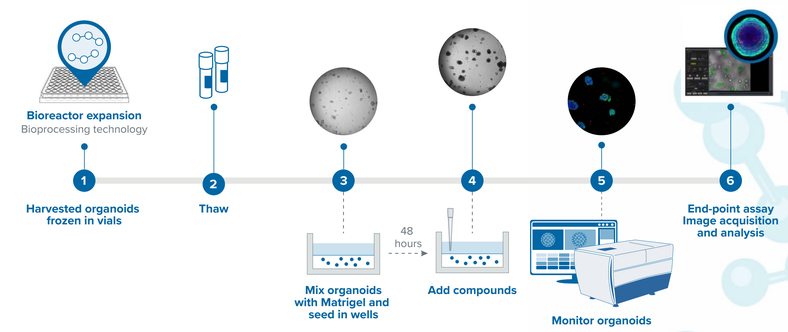


Figure 5. Example workflow for using 3D Ready Organoids for drug discovery from organoid seeding in Matrigel® (day 0) and compound addition (day 2), to analysis of results by end-point assay and/or image acquisition and analysis (day 7 – 10).

Reference

1. Iversen PW et al. HTS Assay Validation. 2012 May 1 [Updated 2012 Oct 1]. In: Markossian S, Grossman A, Arkin M, et al., editors. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004. Available from: https://www.ncbi.nlm.nih.gov/books/NBK83783/

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