



## **CASE STUDY: ENHANCING CELL PRESERVATION WITH ATELERIX TECHNOLOGY — A COLLABORATION WITH DEFINIGEN**

### **BACKGROUND AND CHALLENGES**

DefiniGEN, a leading provider of induced pluripotent stem cell (iPSC)-derived hepatocytes, faced a significant challenge in its cell preservation processes. The conventional method of cryopreservation was proving to be both invasive and potentially damaging to the cells, impacting their viability. DefiniGen's clients were required to thaw and plate cells upon receipt, a process that could introduce variability depending on how optimally the thawing was performed. This inconsistency in cell handling not only reduced cell viability but also hampered the ability to offer their customers truly assay-ready, high-quality cells.

Given these challenges, DefiniGEN sought an innovative solution that would enable them to ship live, viable plated cells to customers without subjecting them to cryopreservation. The goal was to ensure that clients could receive fresh cells, reducing cell death and maintaining the functional quality of the cells.

### **THE ATELERIX SOLUTION**

Atelerix's novel cell preservation technology provided an answer to these challenges. DefiniGen integrated Atelerix's WellReady™-96 in-plate preservation technology into their workflow, removing the cryopreservation step entirely. The process was straightforward: on the day of shipment, DefiniGEN would use WellReady™ to prepare their iPSC-derived Opti-HEP wild-type hepatocytes, allowing the cells to be shipped live to clients. This significant change meant that DefiniGen's clients would no longer need to thaw and plate cells, a step known to reduce cell viability.

Other aspects of DefiniGEN's workflow remained unchanged, making the implementation seamless. The ability to ship cells live in plates represented a dramatic improvement, streamlining processes and improving overall outcomes for clients.



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## METHODOLOGY AND RESULTS

DefiniGEN conducted several tests to evaluate the effectiveness of Atelerix's cell preservation products. Opti-HEP cells were cultured, and the cells were treated with the Atelerix gel for various durations (72h and 96h). After removing the gel, DefiniGEN assessed the cells using quantitative PCR (qPCR) to analyse hepatic markers such as Albumin, Alpha-1 antitrypsin, and HNF4 $\alpha$ . Additionally, cell viability was tested, with comparisons made between cells stored at room temperature and those stored under normoxic conditions.

The results were impressive. Cells stored using WellReady™ exhibited high viability and maintained their quality. Furthermore, the cells passed QC criteria for the qPCR tests, indicating that the use of Atelerix's technology did not compromise their function or quality. This solidified the decision to adopt the technology on a larger scale, as it allowed DefiniGEN to ensure the consistent quality of their products.

## TANGIBLE BENEFITS

Since adopting Atelerix's cell preservation technology, DefiniGEN has realised several tangible benefits. One of the most significant improvements has been the reduction in variability of cell viability, thanks to the elimination of cryopreservation. The ability to live-plate cells and ship them directly to clients has improved the overall consistency and quality of the cells received. For customers who are unable or unwilling to send small molecules for in-house testing, this advancement means that they can now receive high-quality Opti-HEP cells without the risk of damage caused by freezing and thawing.

Furthermore, this innovation has increased the flexibility of DefiniGEN's offering. Clients can now receive fresh, assay-ready plates, enhancing the precision of their functional assays and minimising logistical complications. The shift away from cryopreserved cells not only enhances the viability of the cells but also saves time and costs associated with cold chain logistics.



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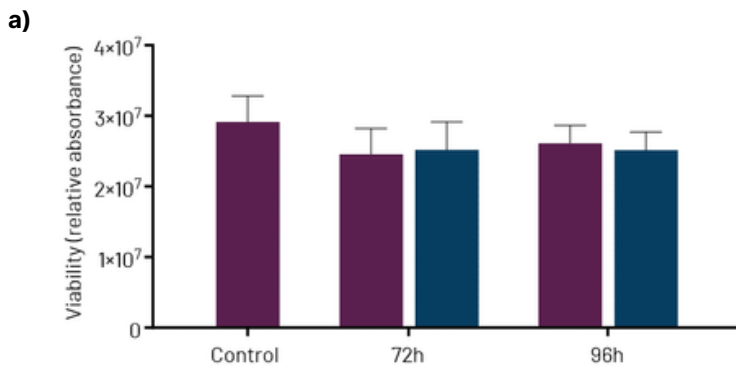


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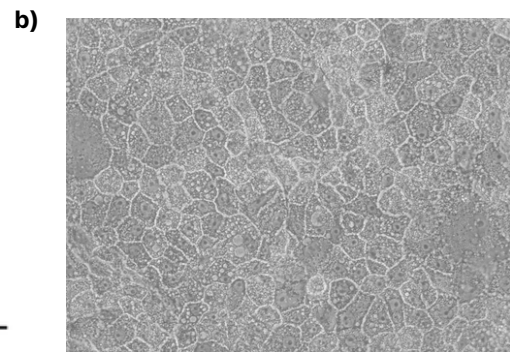
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## DEFINIGEN OPTI-HEP MAINTAIN THE COBBLESTONE MORPHOLOGY POST-ENCAPSULATION

### Viability of Stored Cells



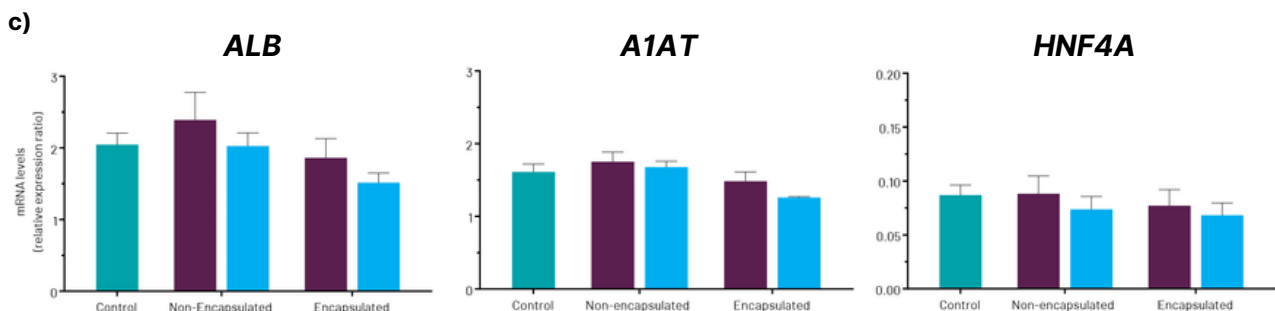
### Morphology Post-Encapsulation



a) Viability of non-encapsulated (purple) and encapsulated (blue) DefiniGEN Opti-HEP cultures stored at normothermic temperatures. Culture plates prepared and sealed using WellReady™ were released following 72 and 96 hours of encapsulation. Wild-type Opti-HEP cultured in standard conditions with no additional manipulations were also used as control. Viability data are presented as mean±SEM of n=3 biological replicates.

b) Representative brightfield images of DefiniGEN Opti-HEP presenting the characteristic cobblestone-like morphology after release from WellReady™.

## DEFINIGEN OPTI-HEP GENE EXPRESSION IS NOT AFFECTED BY CELL ENCAPSULATION



c) Relative mRNA expression of hepatic maturity markers albumin (ALB), alpha-1-antitrypsin (A1AT), and hepatocyte nuclear factor 4A (HNF4A) in DefiniGEN Opti-HEP stored for 72 Hours (purple) and 96 hours (blue) at normothermic conditions, also illustrated is the Control (Green). Wild-type Opti-HEP cultured in standard conditions with no additional manipulations were also used as control. mRNA data were normalised to *PPIA* and presented as mean±SEM of n=3 biological replicates



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## **FUTURE IMPLICATIONS**

DefiniGEN's adoption of Atelerix's cell preservation technology opens up new avenues for research and product development. The ability to ship live, ready-to-use cells expands the possibilities for client collaborations, particularly with those who prefer to manage their own small molecule screening but still need access to high-quality cells. The enhanced flexibility of the process is set to strengthen DefiniGEN's competitive advantage, enabling them to cater to a broader range of customer needs in the global market.

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## **LESSONS LEARNED**

For other organisations considering the implementation of Atelerix's cell preservation technology, DefiniGEN's experience offers clear insights. The integration process was straightforward, and Atelerix proved to be a highly collaborative partner, willing to work closely to tailor the solution to meet specific needs. The ability to ship cells live, without the variability introduced by cryopreservation, marks a significant advancement in cell-based assay preparation and delivery.



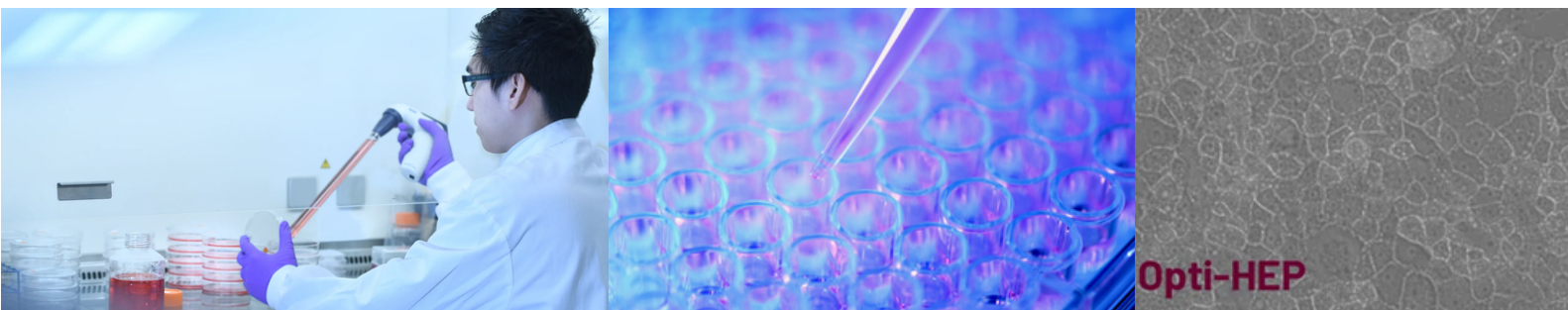
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As Celine Gomez, Head of Operations at DefiniGEN, sums up

“

We are incredibly proud of our ability to ship *in vitro* liver models from the UK to the US without freezing or cryopreserving by partnering with Atelerix Ltd. This represents a watershed moment, since we can now ship our iPSC-derived hepatocytes fresh to customers in assay-ready plates for screening and analysis, enhancing the quality of functional assays. This saves research time, as cells do not need to be thawed, and avoids the need for cold chain logistics.

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Atelerix CEO, Alastair Carrington added

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Our technology for shelf-stable science solutions has proven to be a game-changer in the field of biopreservation. This partnership demonstrates the efficacy of Atelerix's preservation technology, exceeding expectations with what is deemed one of the most sensitive and often problematic cell types, to ship fresh and still maintain functionality. Working alongside DefiniGEN, we are excited to be at the forefront of reducing the industry's carbon footprint, improving the logistics of biological materials while moving towards reducing or even replacing the use of animals in drug testing.

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