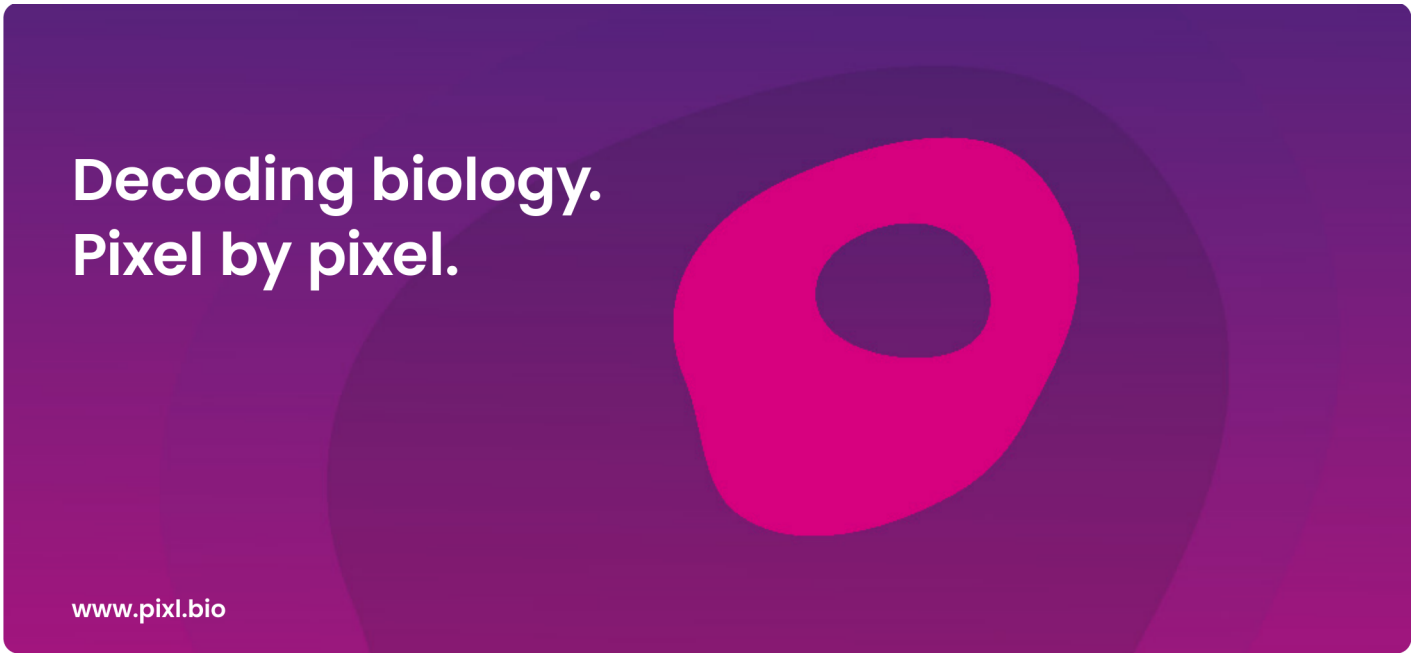
pixlbio



Cryopreserved pixHep™ retain the identity, metabolic function and transcriptome of live cells

Cryopreservation is a critical enabler of a practical and scalable hepatocyte product, allowing cells to be manufactured and banked in advance, distributed globally, and recovered on demand, rather than requiring use within a limited window following preparation. However, the freeze-thaw process represents a substantial biological stressor in its own right. It may preferentially compromise highly metabolically active cells, attenuate cytochrome P450 activity, and perturb the maintenance of a mature hepatocyte phenotype. Accordingly, for any hepatocyte product intended to provide a robust alternative to freshly prepared cells, the critical benchmark is not merely post-thaw viability, but the preservation of cellular identity and function following recovery. At pixlbio, we performed a direct comparison of cryopreserved pixHep™ with matched, non-cryopreserved pixHep™ cultures using three orthogonal assessment modalities: hepatocyte identity, metabolic competence, and genome-wide transcriptomic profiling.

Cryopreserved pixHep™ retain hepatocyte identity markers

By immunofluorescence, cryopreserved pixHep™ displayed the same hepatocyte marker profile as live pixHep™. Cytoplasmic albumin and nuclear HNF4A - the master transcription factor that defines the mature hepatocyte state - were both clearly expressed and comparable in distribution and intensity across conditions, with intact nuclei shown by DAPI. Cryopreservation did not erode pixHep™ identity at the protein level.

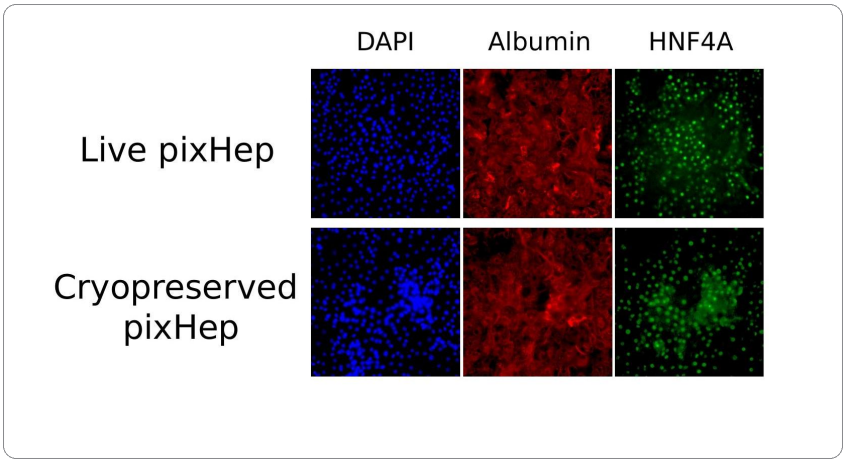


Figure 1. Immunofluorescence for DAPI (nuclei), albumin and HNF4A in live vs. cryopreserved pixHep™. Marker expression is comparable between conditions.

Basal and inducible CYP3A4 activity is preserved

CYP3A4 is the most abundant drug-metabolising enzyme in human liver and a demanding indicator of hepatocyte quality. Basal CYP3A4 activity in cryopreserved pixHep™ was comparable to live cultures of pixHep™, roughly 97% of live activity. Crucially, cryopreserved cells also remained **inducible**: vitamin D3 (100 nM) drove an ~2.8-fold rise in activity, closely matching the ~3.0-fold response of live cells. Preserved inducibility showed that not only the enzyme but the upstream nuclear-receptor signalling that regulates it survive cryopreservation - metabolism, not just viability, is retained.

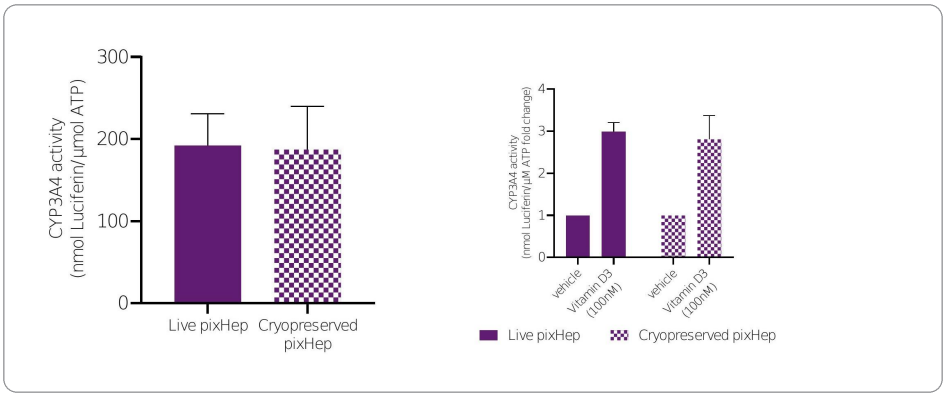


Figure 2. CYP3A4 activity in live vs. cryopreserved pixHep™. Left: basal activity (nmol luciferin/µmol ATP). Right: fold-induction by vitamin D3 (100 nM) over vehicle.

Table 1. Identity and metabolic endpoints. CYP3A4 activity in nmol luciferin/µmol ATP; induction as fold change over vehicle.

Endpoint	Live pixHep™	Cryopreserved pixHep™
Albumin (IF)	Positive	Positive
HNF4A (IF)	Positive	Positive
Basal CYP3A4 activity	≈ 193 units	≈ 187 units (~97%)
CYP3A4 induction (Vit D3)	≈ 3.0-fold	≈ 2.8-fold
Verdict	**Reference**	**Equivalent** ✓

The hepatic transcriptional programme is maintained genome-wide

RNA-seq across a curated liver function panel showed that cryopreserved pixHep™ reproduce the expression signature of live pixHep™ in every functional category - plasma/secreted proteins, phase I (cytochrome P450) and phase II conjugation enzymes, transporters, urea-cycle and energy metabolism, coagulation factors, and the hepatocyte transcription factors and surface markers that define cell identity. The most highly expressed hepatic genes (ALB, SERPINA1, the fibrinogen cluster) remained dominant in both conditions. Where differences appeared, several metabolic and conjugation genes were modestly **higher** in cryopreserved cells rather than lost - arguing against selective loss of the most fragile, metabolically active hepatocytes during freeze-thaw.

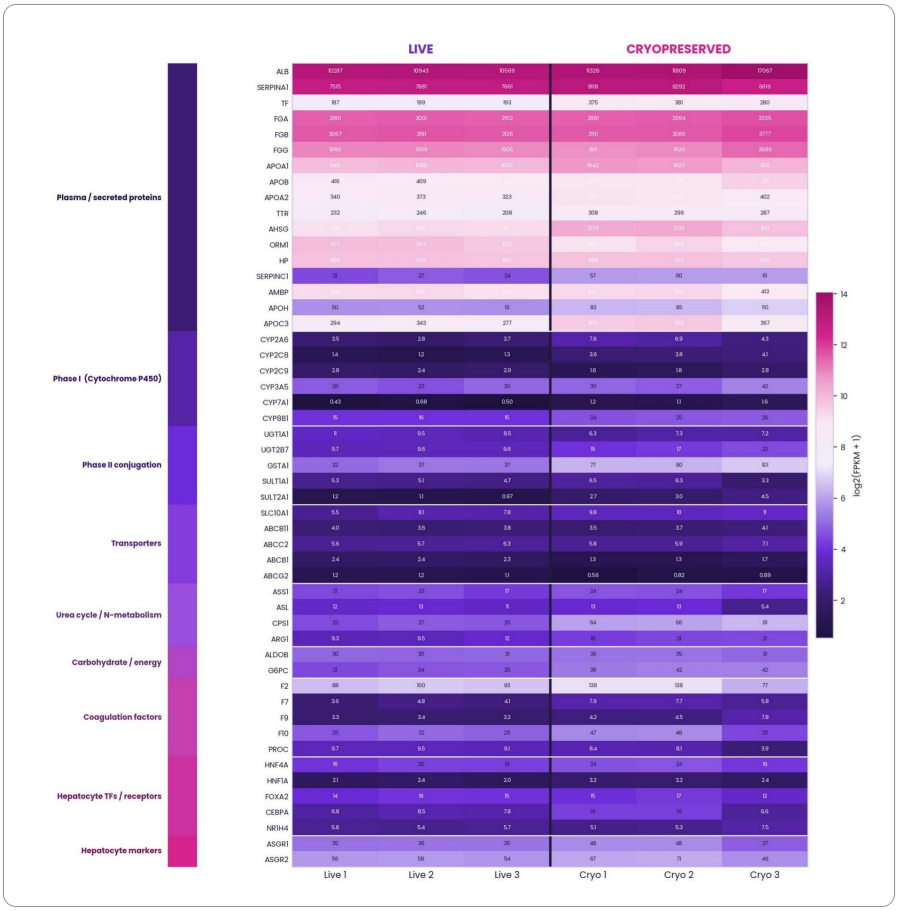


Figure 3. Liver-panel gene expression heatmap (log2 FPKM+1) across live (Live 1–3) and cryopreserved (Cryo 1–3) replicates, grouped by hepatic function. Patterns are conserved across conditions.

Genome-wide sample-to-sample correlation confirmed the concordance quantitatively. Replicates clustered tightly within each condition, and cross-condition correlations were high (Pearson r ≈ 0.91–0.97). Restricting the analysis to liver function genes associated with hepatocyte performance yielded an even stronger correlation of **r = 0.989, with 86% of genes within 2-fold** of their live-cell expression.

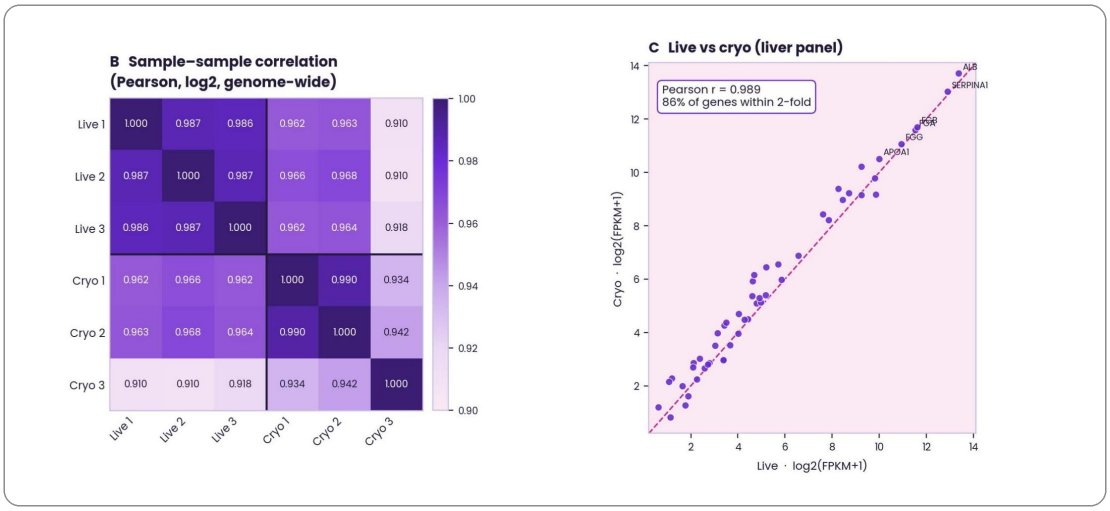


Figure 4. Left: genome-wide sample–sample Pearson correlation matrix (log2). Right: live vs. cryopreserved expres sion across the liver panel (log2 FPKM+1); dashed line is identity.

Table 2. Sample–sample correlations, genome-wide and across the liver function panel.

Correlation comparison	Pearson r	Interpretation
Live vs. Live (within group)	0.986 – 0.987	High reproducibility
Cryo vs. Cryo (within group)	0.934 – 0.990	High reproducibility
Live vs. Cryo (genome-wide)	0.910 – 0.968	Strong concordance
Live vs. Cryo (liver panel)	0.989	Near-identical

Why cryopreservation performance matters

A hepatocyte product is only useful at scale if freezing does not quietly degrade it. The three readouts here con-verge on a single result: cryopreserved pixHep™ are functionally interchangeable with freshly prepared cells. Iden-tity was retained at protein level, metabolic function is retained at ~97% of live activity while remaining fully indu-cible, and the underlying transcriptional programme is retained genome-wide (liver-panel r = 0.989). The absence of systematic gene loss - and the modest upregulation of several metabolic genes rather than their depletion - in dicated that the cryopreservation process does not preferentially sacrifice the most valuable, metabolically active cells. Cryopreserved pixHep™ can therefore be deployed as a ready-to-use, drop-in alternative to fresh hepato-cytes.

Conclusions

Cryopreserved pixHep™ retain the hepatocyte identity, metabolic competence and genome-wide hepatic transcriptional programme of matched live cells. With pre-served albumin and HNF4A expression, ~97% retention of basal CYP3A4 activity, intact CYP3A4 inducibility, and near-identical liver-panel gene expression (r = 0.989), cryop-reserved pixHep™ perform as functional equivalents of freshly prepared cells - giving drug-metabolism, toxicology and liver-modelling teams a bankable, ready-to-use human hepatocyte with no measurable loss of function.

www.pixl.bio · Babraham Research Campus, Cambridge, UK