cellenONE®: an innovative tool for high quality and sensitivity 3' scRNA-seq libraries in nanoliter volume

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Overview

Single-cell RNA sequencing (scRNA-seq) assays must combine both sensitivity and accuracy to capture, transcribe and amplify diverse transcripts in their relative proportions from a single cell. While widely implemented, there is no shortage of improvements to be made to scRNA-seq assays in order to fully realize cost efficient and highly sensitive transcriptomic analyses. Advancements in single cell sequencing methods are often developed first in open, accessible consumables such as microtiter plates (MTPs) with significant optimizations ultimately needed to reduce volume in order to improve costs per cell, sensitivities and increase throughput . Here we show;

Format	96-WP	384-WP	cellenCHIP, 384-unique barcodes
Reverse Transcription Reaction Volume	5 uL	2 uL	100 nL
Average Relative Cost / Cell	100%	62%	5%

Table 1. Volumes and relative reagent costs per cell for preparation of 3' scRNA-seq libraries: *Significant cost decreases are seen with reduction in reaction volumes through the adoption of substrate miniaturization and sub-nanoliter volume liquid handling.*

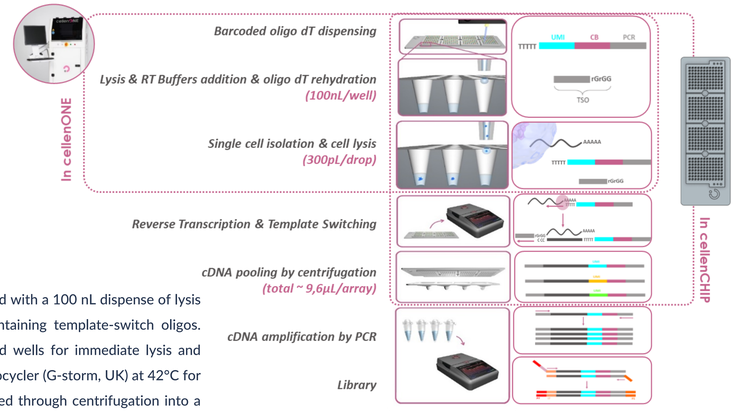
1) The typical workflow for miniaturization of an open-well 3' scRNA-seq assay from a 96 or 384-WP into the cellenCHIP™, a nanoliter volume 384-well chip.
2) Protocol optimizations to this baseline to optimize the performance in the cellenCHIP in order to obtain higher sensitivities at lower sequencing depths.
3) Implementation of this optimized protocol to interrogate the biological differences between liver cells (HepaRG™) cultured in 2D vs. 3D spheroid environments.

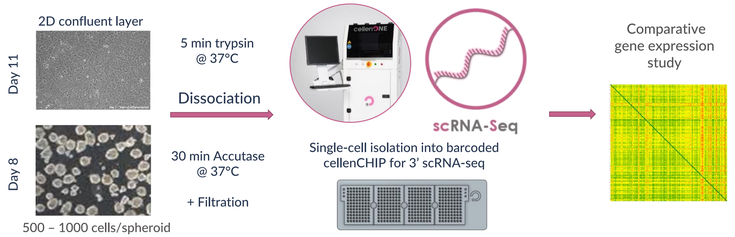
Methods

cellenCHIP Protocol Optimization:

The cellenCHIP is comprised of four 96-well arrays that are barcoded with either 96 (each array identical) or 384-unique oligo dT primers using Scienion sciFLEXARRAYERs (SCIENION AG, Germany). The oligo dT primers contain a preserved sequence for cDNA amplification, an individual well barcode (WB) and a unique molecular identifier (UMI) to respectively trace back sequencing reads to the corresponding wells and quantify the number of reads for each transcript. Barcoded chips are stored dry until use.

Using the cellenONE®, the oligo barcodes are rehydrated with a 100 nL dispense of lysis and three different reverse transcription (RT) buffers containing template-switch oligos. Single HEK293T cells were then isolated into the prefilled wells for immediate lysis and sealed for incubation on an *in situ* block inside a GS1 thermocycler (G-storm, UK) at 42°C for 90 min. The barcoded cDNA from the cells was then pooled through centrifugation into a recovery funnel and transferred to microcentrifuge tubes. The cDNA was amplified for a maximum of 18 cycles and used to generate Illumina sequencing libraries utilizing a 3' enrichment amplification strategy. The libraries were then QC'd for size distributions (Bioanalyzer, Agilent) and sequenced paired-end (NextSeq 500, Illumina).





HepaRG™ 2D & 3D Spheroids:

HepaRG™ cells (hepatic progenitor cell line; Biopredic, France) were cultivated in both 2D (monolayer) and 3D (spheroids) conditions in basal growth medium without any differentiation protocols. Cultures were dissociated (3D cultures on Day 8: Accutase for 15-30 min at 37°C) or trypsinized (2D cultures on Day 11: Trypsin for 5 min at 37°C) to form a single-cell dispersed suspension. Cells were isolated into pre-barcoded cellenCHIPs with the cellenONE and library preparation was performed using the first reverse transcriptase enzyme (RTase_1) from the optimization protocol.

Results

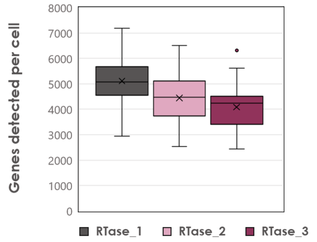


Figure 1. Total number of genes detected per HEK293T cell *utilizing the same protocol with three different commercially available reverse transciptases (RTase).*

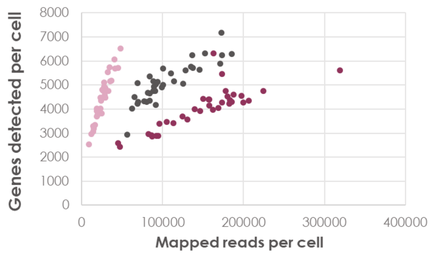
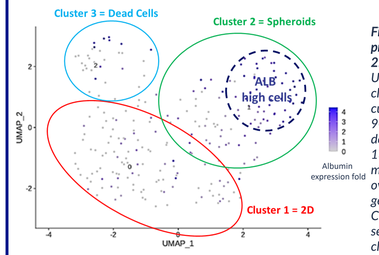


Figure 2. Genes detected per cell *for each RTase tested as a function of mapped reads, indicating that an average of 5,000 genes may be detected with as few as 100,000 reads.*



Condition	Cluster 1	Cluster 2	Cluster 3	Total
Sc-2D	**100**	10	7	117
Sc-Spheroid	8	**93**	25	126

Figure 3. Global transcriptomic profile between HepaRG cells in 2D and 3D culture conditions: *Unsupervised analysis reveals 3 clusters: Cluster 1 = 92.6% 2D culture derived cells, Cluster 2 = 90.3% 3D Spheroid culture derived cells and Cluster 3 = 13.2% of total cells consisting mainly of spheroid-derived cells overexpressing mitochondrial genes (typical of apoptotic cells). Cells strongly expressing albumin segregate into the same sub-cluster into cluster 3 (ALB high cells).*

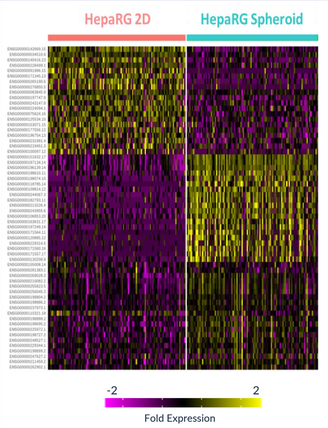
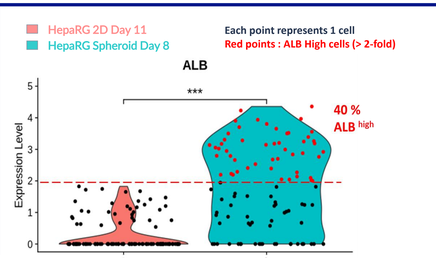
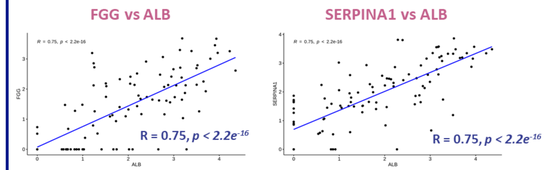


Figure 4. Differential transcriptional profile: *Average 50 genes present a differential expression between HepaRG cells grown in 2D and spheroid-derived cells.*

	Alcohol & Lipid Metabolism	Biosynthesis & Detoxification	Phase II Enzymes	Extracellular Matrix	Others
Over expressed in 3D	ALDH1A1 APOC1 APOE PLIN2	AKR1B10 AKRR1C1 AKR1C2 AKR1C3 AKR1C4	GSTA1 GSTA2 GSTA3 GSTA5	UGDH FN1	FGG ORM1 PTGR1 SERPINA1 SPP1
Over expressed in 2D					CCDC80 NUTF2 SERPINE1

Table 2. Differential transcriptional profile: *Top 20 most differentially expressed genes for each HepaRG culture condition. Most genes identified are involved in liver metabolic function and are overexpressed in spheroid-derived-cells.*





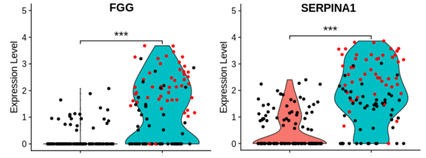


Figure 5. Main expression correlations. *Albumin, one of the main hepatic markers, is overexpressed in 3D (80% of total positive cells and 40% designated high positive cells with >2-fold increased expression: red dots) compared to 2D culture condition (30 % of positive cells). Notably, albumin expression strongly correlates with FGG and SERPINA1 genes (plasmatic proteins expressed by hepatocytes, upregulated in acute phase response). Each point represents one cell.*

Conclusions

- Combining cellenCHIP's high-density nanowell arrays with cellenONE's nanoliter volume dispensing and cell isolation capabilities enables miniaturization of many microtiter plate-based protocols in sub-microliter volumes. In this study, a miniaturized 3' scRNA-seq protocol was developed to generate quality libraries with high sensitivity and significantly reduced costs.
- Using the 3' scRNA-seq cellenCHIP kit developed, with as little as 100 single cells per condition, it was successfully demonstrated that 2D and 3D spheroid derived HepaRG cells had highly differentiated transcriptional profiles after only 1 week of culture (without any chemical induction).
- As described in the literature, HepaRG cells cultivated in 3D showed overexpression of key hepatic function related-genes (Albumin, ALK or GST) which emphasize their more liver-like phenotype as compared to cells grown as traditional 2D monolayers. Moreover, it was possible to confirm that in these models that Albumin gene expression was strongly correlated with both FGG and SERPINA 1 transcriptional level.
- Future work using the 3' scRNA-seq cellenCHIP kit developed here will explore the effect of small molecules on spheroids under different culture conditions in order to assess their liver toxicity and further decipher their underlying mechanisms of action.

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