A MICROFLUIDIC DESKTOP SYSTEM FOR AUTOMATED GENOME EDITING OF SINGLE CELLS

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INTRODUCTION

Genome editing is rapidly becoming an essential tool across all areas of life sciences R&D such as gene therapy and regenerative medicine, synthetic biology or bio-manufacturing.

Current approaches, dependent upon manual labour and extensive screening, are highly inefficient and time-consuming, thus new methods that reduce costs and handling time for the generation and recovery of edited cells would be highly welcome.

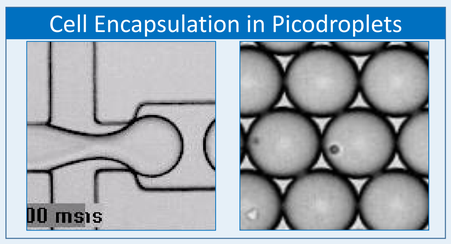
Using picodroplet microfluidic technology, Sphere Fluidics is developing a novel system that will automate and miniaturise many of the steps of the genome editing pipeline, yielding high-quality, precision engineered cells in a high-throughput manner.

WORKFLOW:

Sample loading

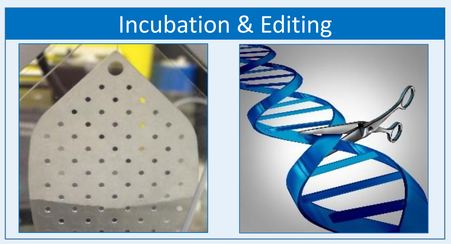
1. User's cells and reagents are loaded into the biochip.

Cell Encapsulation in Picodroplets



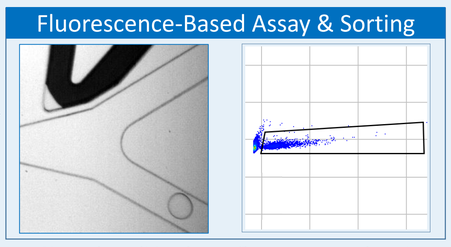
2. Cells and reagents are encapsulated in picolitre volume picodroplets, together with gene-delivery and editing reagents. The distribution of cells in picodroplets follows a Poisson distribution. Hundreds of thousands to several million cells can be encapsulated and processed in a single run.

Incubation & Editing



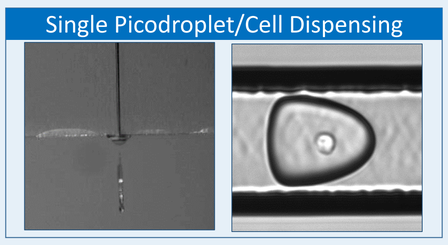
3. Picodroplets containing cells are incubated at 37ºC for several days. During incubation, cell transfection and editing will take place.

Fluorescence-Based Assay & Sorting



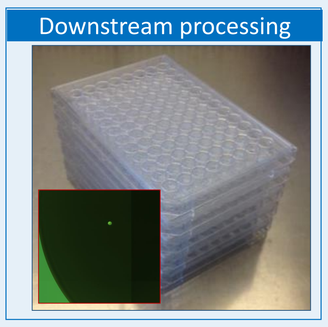
4. Given the editing has a fluorescent readout (e.g. fluorescent tag, promoter driven expression of fluorescent protein, correction of stop codon), picodroplets containing edited single cells can be detected based on the fluorescent signal. Positive cells in picodroplets can then be sorted based on the gate settings defined by the user.

Single Picodroplet/Cell Dispensing



5. Sorted positive picodroplets containing single cells are dispensed one by one into individual wells of 96- or 384-well plates. Monoclonality is assured by recording multiple images of each picodroplet before dispensing.

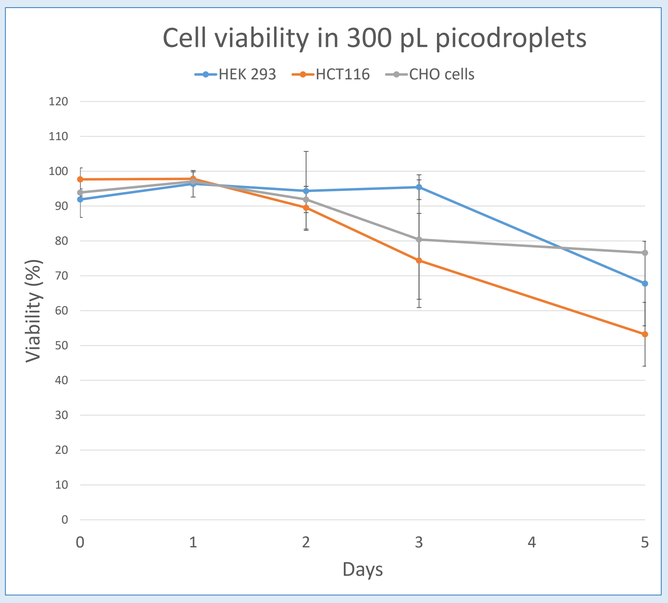
Downstream processing



6. Individual cells dispensed into the wells can be directly processed for downstream analysis (sequencing) or further cultured to grow into single-cell derived clones.

Cyto-Mine®

CELL VIABILITY IN 300 PL PICODROPLETS



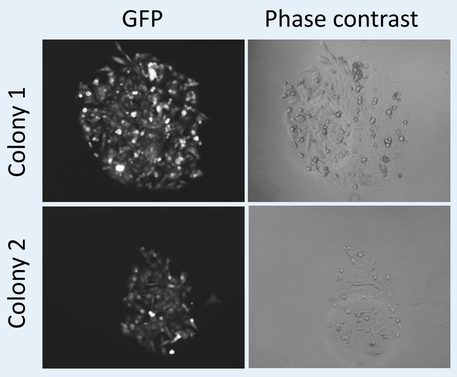
- Cells were encapsulated in 300pL picodroplets using a PDMS biochip and pump driven flow.
- Cell concentration: $1x10^6$ cells/ml.
- Incubation in a 1.5 mL microtube in static incubator, 37°C, 6% CO_2.
- Around 50 µL of sample was tested per time point. To break the emulsion, 1H,1H,2H,2H-Perfluoro-1-octanol was added and the aqueous phase was collected after a brief spin.
- Viability was tested immediately after breaking emulsion using staining with Acridine Orange (all cells) and DAPI (dead cells).

GENE EDITING IN PICODROPLETS: TRANSFECTION

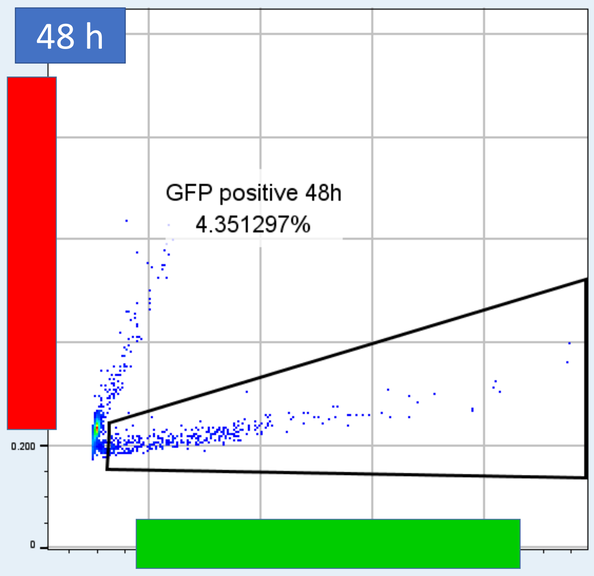
Expected picodroplet occupancy

λ	0.30
% of empty picodroplets	74.08%
% of picodroplets contain 1 cell	22.22%
% of picodroplets contain 2 cells	3.33%
% of picodroplets contain 2+ cells	0.36%

4.35% GFP positive picodroplets = **20% edited cells**

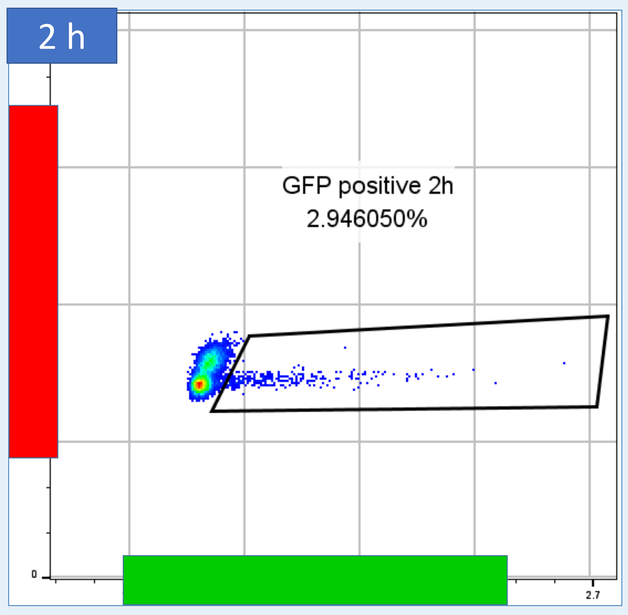


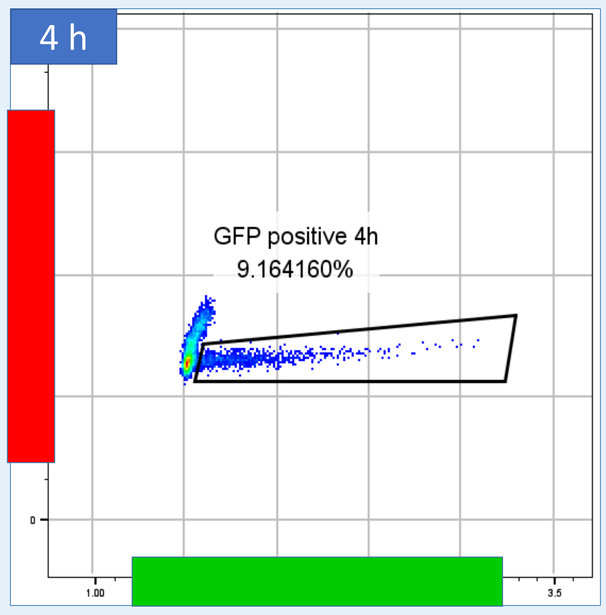
plate#	recovery (%)
1	20.48
2	13.54
3	15.22
4	11.46
5	18.75
6	14.58
average	15.67
stdev	3.36



- HTC116 FIRE-line (kindly provided by Horizon Discovery): inactive GFP under control of CMV promoter integrated into HPRT locus.
- HTC116 cells, RNPs, DNA repair template and DharmaFect transfection reagent were co-encapsulated in 400 pL picodroplets using a Y-junction biochip and pump driven flow.
- Cells were incubated in picodroplets in 1.5 mL microtubes at 37°C, 6% CO_2 for 48 hours and analysed using Cyto-Mine®.
- Picodroplets, containing GFP-expressing cells were dispensed into 96-well plates and cell outgrowth was analysed 3 weeks later.

MRNA TRANSFECTION IN PICODROPLETS





- HCT116 cells ($1x10^6$ cells/ml) were co-encapsulated in 300pL picodroplets with Cas9/GFP mRNA (100nM) and DharmaFect transfection reagent using Cyto-Mine®.
- Picodroplets were incubated for 2h and 4h in Cyto-Mine®, at 37°C.
- Picodroplets containing GFP-expressing cells were analysed at different time points.
- Following a 4h incubation, picodroplets were sorted based on fluorescent signal using Cyto-Mine®.
- Positive picodroplets were then dispensed into five 96-well plates and successful cells outgrowth was monitored for 2 weeks post-dispensing.

Expected picodroplet occupancy (Poisson distribution)

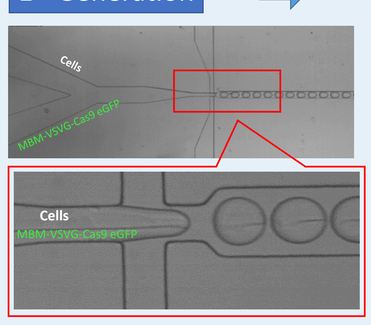
λ	0.12
% of empty picodroplets	88.69%
% of picodroplets contain 1 cell	10.64%
% of picodroplets contain 2 cells	0.64%
% of picodroplets contain 2+ cells	0.03%

2h incubation = **~30% of cells transfected**

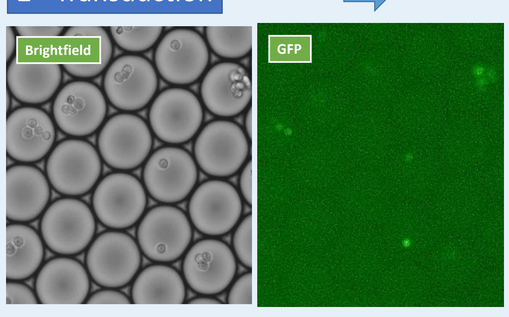
4h incubation = **~90% of cells transfected**

GENE EDITING IN PICODROPLETS: VIRAL TRANSDUCTION

1 - Generation

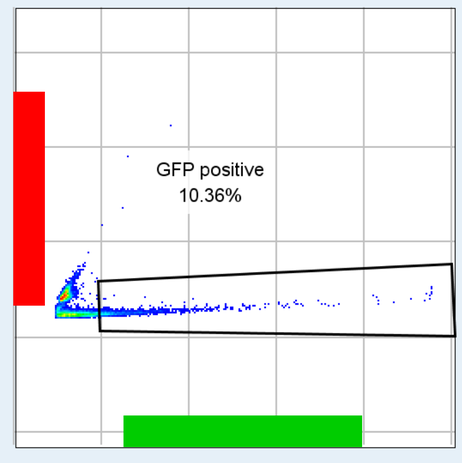


2 - Transduction

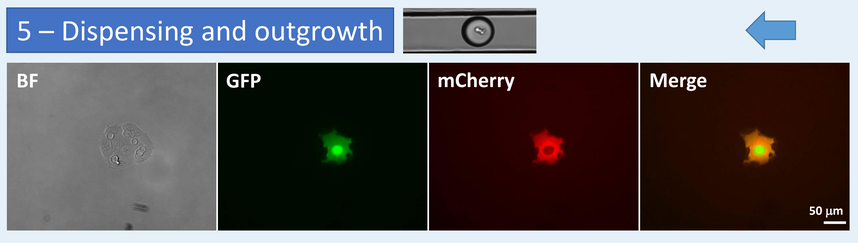


3 - Re-injection

4 - Sorting



5 – Dispensing and outgrowth



- HCT116 cells were co-encapsulated in 400 pL picodroplets with MBM-VSVG-Cas9 eGFP virus (MOI=0.25) via a Y-junction PDMS biochip and pump driven flow.
- After 24h incubation at 37ºC, picodroplets were re-injected into Cyto-Mine®.
- GFP-positive picodroplets were sorted and dispensed using Cyto-Mine® into 96-well plates.
- Cell outgrowth was inspected 4 days post-dispensing.
- RFP (mCherry) signal as readout of genome-editing (insertion of RFP into ACTB gene safe harbour) was detected using fluorescence microscope

CONCLUSIONS:

We successfully performed CRISPR-mediated gene-editing of adherent mammalian cells in water-in-oil picodroplets, either by lipofection or through viral gene delivery. These editing events were detected via a fluorescent readout; single, positive cells were selectively sorted, dispensed into MTP plates and subsequently grown into single-cell derived clones. The system enables monoclonality assurance, providing a comprehensive solution for accelerated cell line development. Further development work is ongoing, aiming to develop a multiplexing workflow that will allow simultaneous editing of libraries of genes and/or cell lines.

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