Single-cell cloning in optically clear chambers and small volumes

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Background

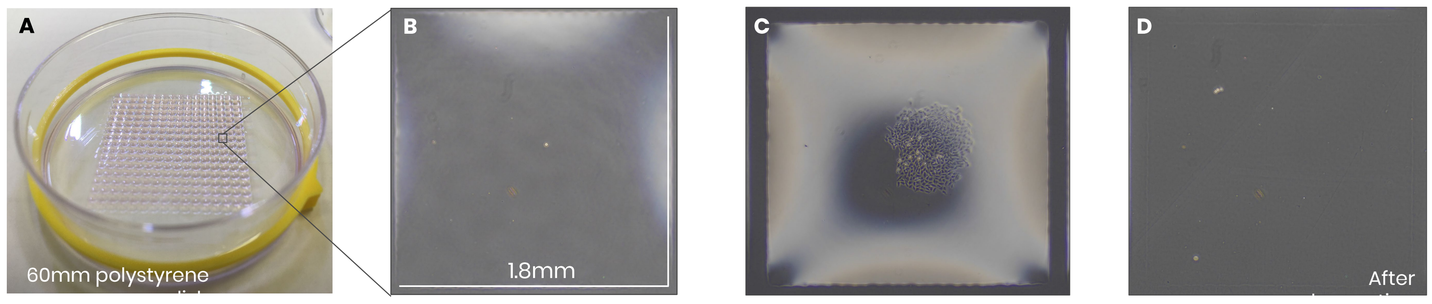
Single-cell cloning poses a critical bottleneck in the development of homogenous cell models. Traditional single-cell cloning methods suffer from a high uncertainty of single-cell clonality as well as lengthy and wasteful culture phases, which can render the generation of adequate cell systems laborious, expensive and inefficient.

We present a novel microfluidic cell culture method that exploits interfacial tension to create 'GRIDs' - individual cell culture chambers that are isolated and sealed from each other via the immiscible fluid FC40[STAR]. GRID chambers allow easy verification of single-cell clonality on standard brightfield microscopes and utilize volumes of < 1µl.

We demonstrate that diverse cell types can readily be cloned using this novel approach, including both suspension and adhesion cells, as well as human induced pluripotent stem cells (hiPSCs) growing on substrates.

1 Single-cell cloning workflow in GRIDs

GRID fabrication → Single-cell verification → Culture phase → Harvest clonal colonies



A: GRIDs - 256 isolated culture chambers separated and overlaid with the immiscible liquid FC40[STAR]

B: 'Whole chamber' image of a GRID chamber containing a single cell (brightfield microscopy with 4x objective)

C: Single-cell derived colony – single cells grow in GRID chambers into colonies in volumes < 1µl

D: Image of GRID chamber after harvesting the clonal colony in (c)

2 Efficient single-cell cloning of adherent and suspension cells in GRIDs

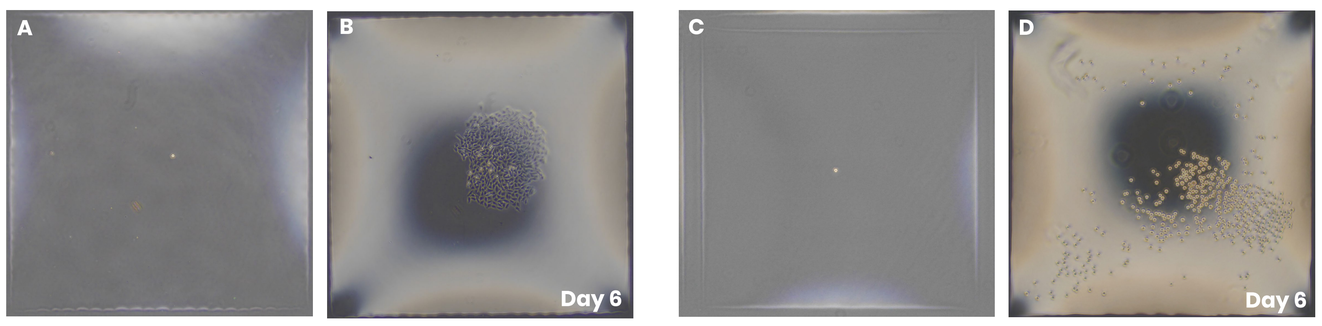


Figure 2A: Brightfield microscopy images of adherent and suspension cells in GRID chambers
(A) A single CHO-K1 cell after plating, (B) Clonal population derived from the cell shown in A after 6 days of culturing. (C) A single K562 cell after plating, (D) Clonal population derived from the cell shown in C after 6 days of culturing. A-D, 4x objective, culture volume in A, C = 200 nl; B, D = 800 nl)

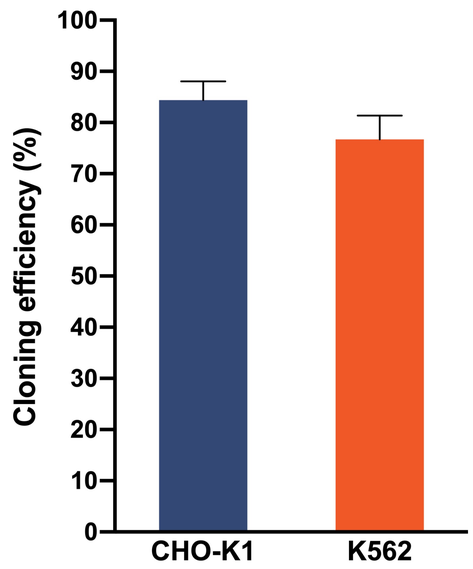


Figure 2B: Cloning efficiency (%) of CHO-K1 and K562 cells at day 6

Cloning efficiency was determined as:

$$cloning\ efficiency\ (\%) = \frac{c}{n} * 100$$

c = number of single cell-derived colonies at day 6
n = number of chambers containing a single cell at day 0

3 Compatibility of GRIDs for cloning gene-edited hiPSCs

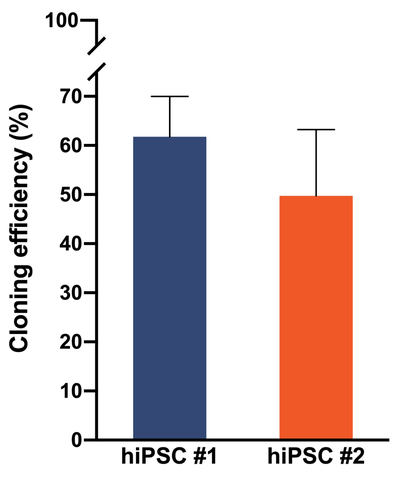


Figure 3A: Cloning efficiency (%) of genetically independent hiPSCs in GRIDs

Cloning efficiency was determined as

$$cloning\ efficiency\ (\%) = \frac{c}{n} * 100$$

c = number of single cell-derived colonies at day 6
n = number of chambers containing a single cell at day 0

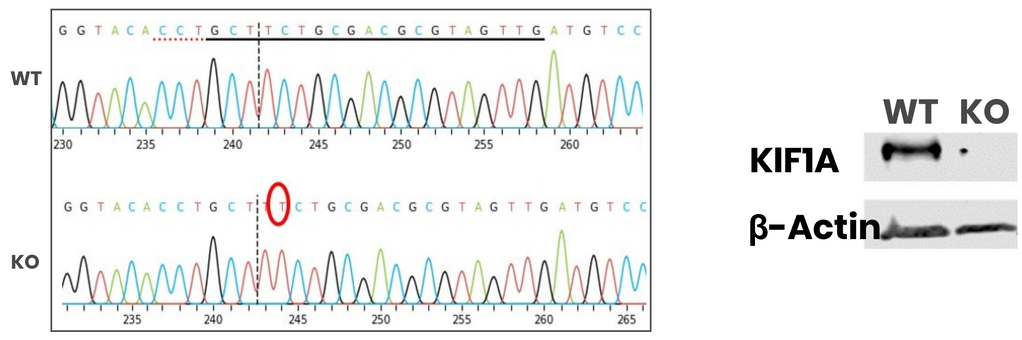


Figure 3B: Sanger sequencing and Western blot of wild-type (WT) and KIF1A knock-out (KO) hiPSC clones
Human iPSCs were gene-edited using CRISPR/Cas9, followed by single-cell cloning in GRIDs

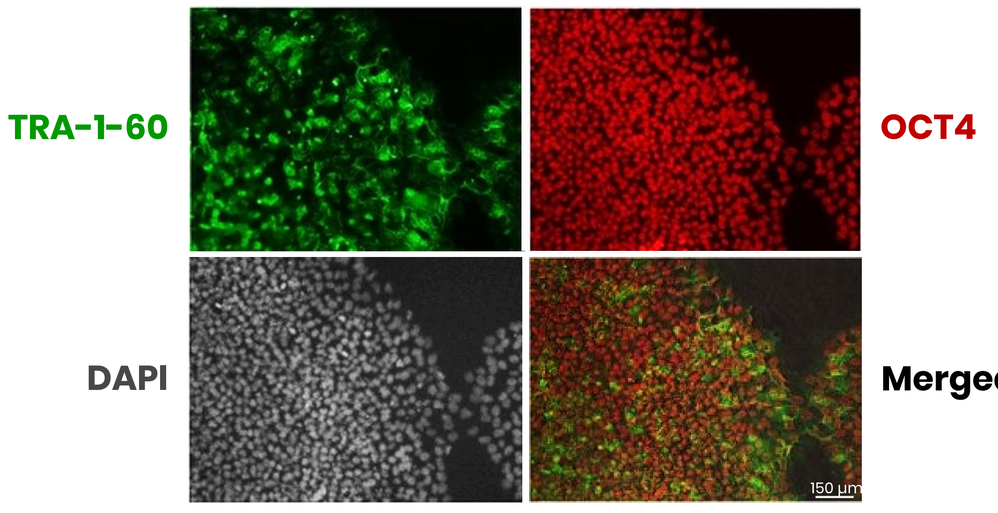


Figure 3C: Characterisation of GRID-derived clonal hiPSC cultures
Clonal hiPSCs cultures derived from GRIDs maintain the expression of pluripotency markers TRA-1-60 and OCT4

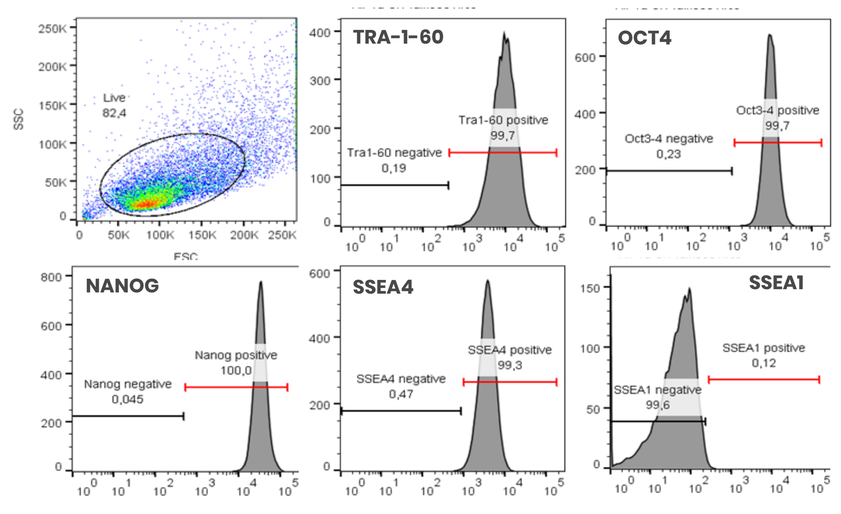


Figure 3D: Flow cytometry analysis of pluripotency (TRA-1-60, OCT4, NANOG, SSEA4) and differentiation (SSEA1) markers
GRID-derived clonal hiPSC cultures express pluripotency and differentiation markers as expected

Conclusions

- GRIDs are suitable for single-cell cloning of adherent and suspension cells, as well as cells dependent on substrate coatings (e.g., hiPSCs)
- Efficient single-cell cloning in small volumes (< 1µL)
- Optical clarity of chambers allows easy verification of monoclonality on standard brightfield microscopes

isoCell

- ✓ Fabricates GRIDs
- ✓ Automates cell plating, feeding, harvesting
- ✓ Compact and user-friendly system

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