Electronic Supplementary Material (ESI) for Analyst

Efficient Division and Sampling of Cell Colonies Using Microcup Arrays

Supplemental Information

Jeng-Hao Pai, Kimberly Kluckman, Dale O. Cowley, Donna M. Bortner, Christopher E. Sims, Nancy L. Allbritton

Jeng-Hao Pai • Christopher E. Sims • Nancy L. Allbritton

Department of Chemistry,

University of North Carolina,

Chapel Hill, NC 27599

E-mail: nlallbri@unc.edu

Fax: +1 (919) 962-2388

Tel: +1 (919) 966-2291

Kimberly Kluckman • Dale O. Cowley • Donna M. Bortner

TransViragen, Inc.

PO Box 110301

Research Triangle Park, NC 27709

Nancy L. Allbritton

Department of Biomedical Engineering,

University of North Carolina,

Chapel Hill, NC 27599

North Carolina State University,

Raleigh, NC 27695

Supplemental Methods

Murine ES Cell Culture

Murine 129 E14Tg2a strain ES cells were obtained from the University of North Carolina Animal Models Core Facility (UNC-AMC, Chapel Hill, NC). C57BL/6N (JM8) murine ES cells were obtained from the Knockout Mouse Project (KOMP) Repository at the University of California (Davis, CA). FBS was heat inactivated in 56 °C water bath for 30 min before use. Cell culture medium was prepared from MEM, L-glutamine, sodium pyruvate, penicillin/streptomycin, 2-Mercaptoethanol, 1% GMEM, 15% FBS, and 533 U/mL LIF (pH 7.4).

Transfection Protocol

The plasmid used for generation of stably transfected cells contained the neomycin-resistance gene under the control of the PGK promoter, and the gene for the fluorescent protein mCherry with an upstream splice acceptor element, but with no promoter. The plasmid should confer resistance to the antibiotic G418 through expression of the neomycin-resistance gene which encodes an aminoglycoside 3´-phosphotransferase, APH 3´ II. The expression of mCherry occurs in cells where the plasmid integrates into an actively-expressed gene in a manner that allows correct splicing to the mCherry gene. Feederless E14Tg2a ES cells were grown in GMEM supplemented with 15% FBS, 2mM Glutamax, 1mM Sodium Pyruvate, 0.1mM nonessential amino acids, 1000 units/ml LIF, and 55 µM 2-mercaptoethanol. 7×10^6 E14Tg2a ES cells were electroporated at 280 volts and 250 microFarads with 20 µg of the mCherry plasmid. Following electroporation, cells were resuspended in ES media and plated onto two 10 cm, 0.1% gelatin coated tissue culture plates. At 48 hours after transfection, cells were trypsinized and transferred

to microarrays. Cells were then subjected to 200 µg/ml G418 selection.

ES Cell Lysate Preparation

Cell lysates from 96 well clones were prepared by adding 30 µl of trypsin to each well for 5 minutes. 8 µl of the cell/trypsin mix was transferred into a PCR wells containing 20 µl of freshly prepared lysis buffer (1.0 ml TE pH 8.0, 20 µl 10% Triton, and 20 µl Proteinase K (10mg/ml). The plate was heat inactivated in a PCR machine at 55° C for 30 min, 94° C for 10 min and 20° C for 1 min. Cell lysates were stored at -20° C.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) analysis of DNA lysates prepared from ES cell colonies was performed using the forward primer 5'-TGATGAACTTCGAGGACGGCGGCGTGGTGA-3' and reverse primer 5'–CCTTCAGGGCGCCGTCCTCGGGGTACATCC-3' that produce a 189 bp product corresponding to a region of the mCherry gene. PCR was performed in a 25 µl reaction containing 1× Ex Taq Polymerase Buffer, 800 µM dNTPs, 0.4 µM each primer, 0.5 µl Taq Polymerase and 3 µl DNA lysate. The following conditions were utilized for PCR amplification: 35 cycles of denaturation at 95° C for 30s, annealing at 58° C for 30s and extension at 72° C for 30s.

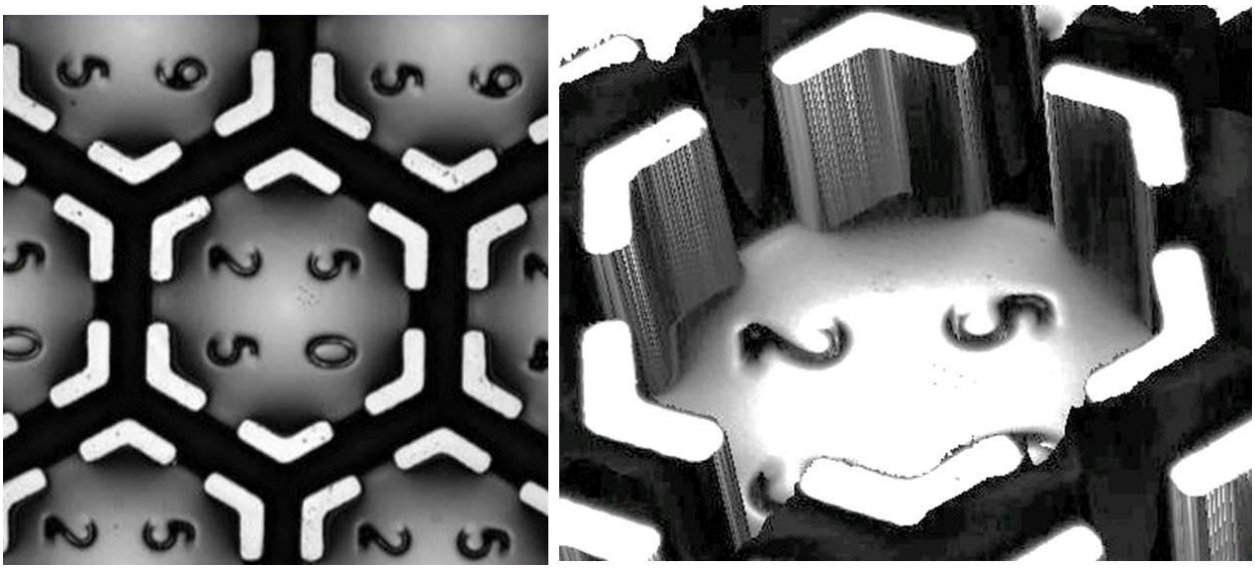

Fig. S1. 2D image and 3D rendition of the split wall microcup. The 2D image was acquired using an Olympus LEXT 3D Measuring Laser Microscope OLS-4000 and converted to a 3D rendition (50° altitude from the horizontal) with accompanying software.

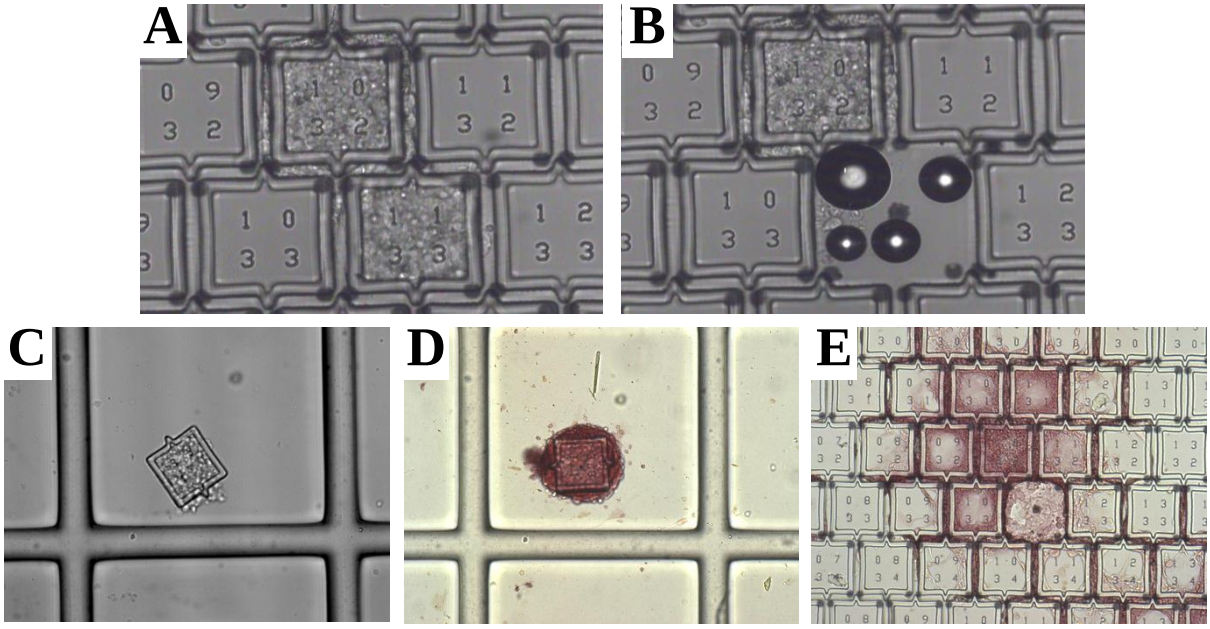

Fig. S2. Growth and differentiation state of murine ES cell colonies after sampling. (A) Colony cultured for 5 days on microcups with titanium-patterned "dots" at their corners. The cells have expanded from microcup "1032" to microcup "1133". The dimensions of the microcups were 100 × 100 μm, 10 μm wall width, and 15 μm gaps. (B) Microcup "1133" was released to obtain a sample of cells from the colony while leaving microcup "1032" on the array. (C) Microcup "1133" with its cargo of cells after release and collection into a microwell plate. (D) The cell sample in microcup "1133" maintained in culture for 48 h after release followed by alkaline phosphatase staining. The cells continued to grow out of the cup and remained undifferentiated as indicated by the purple coloration. (E) The colony contained in microcup "1032" after 48 h of continued culture followed by alkaline phosphatase staining. The cells have continued to expand into additional microcups and have begun to fill in the empty area left by removal of microcup "1133".