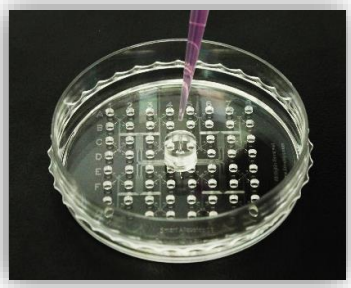
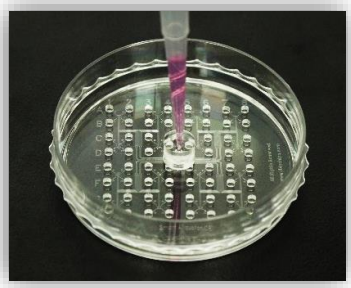
Full Protocol of SACE for Single-Cell Cloning



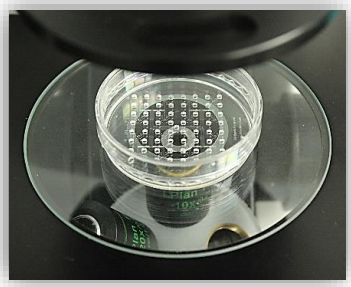
Step 1: Cell Suspension Preparation

1. Harvest adherent cells and make them into cell suspension.
2. Transfer 2 µl of cell suspension to a Petri Dish.
3. Identify the cell suspension and roughly estimate cell numbers under the microscope.
4. The ideal number of cells is 30-50. If too much/little, dilute/enrich it to match the number.
5. Add 200 µL of medium to the cell suspension and pipette cells to be fully suspended.
6. Aspirate the 200 µl of cell suspension into pipette tip.



Step 2: Single-Cell Isolation

1. Vertically insert pipette tip into the Inlet Adaptor.
2. Press pipette plunger down (200 µL +P) to aliquot cell suspension into the 64 Outlet Wells.
3. After aliquoting, hold the device and tilt the pipette tip to remove the Inlet Adaptor, without releasing plunger button.



Step 3: Single-Cell Identification

1. Put the SACE under microscope with 10x objective (bright field or fluorescence).
2. Identify and note all Outlet Wells containing only single cells.

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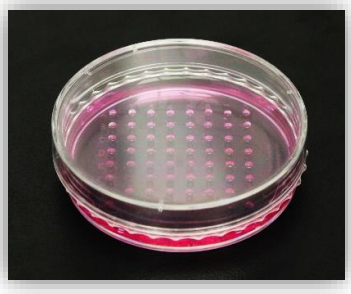
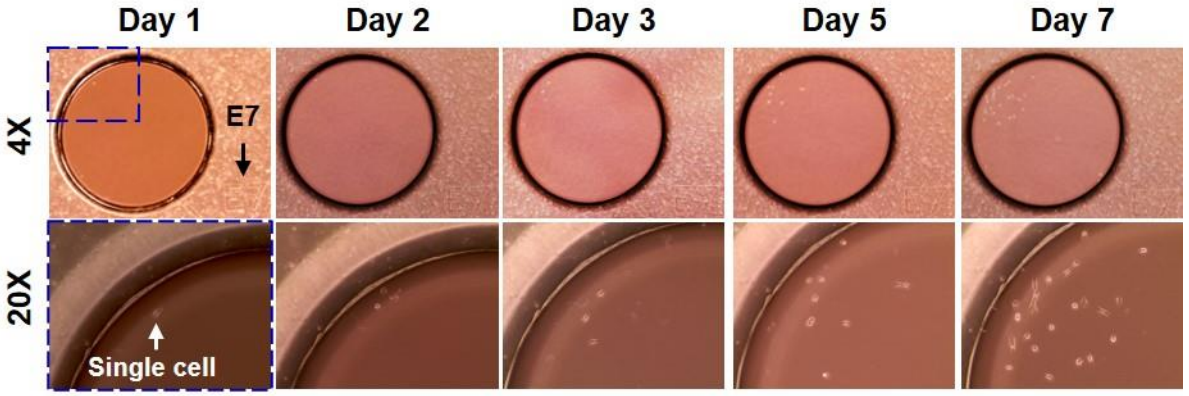
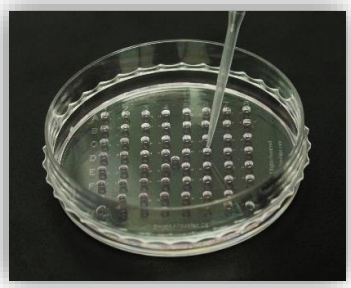


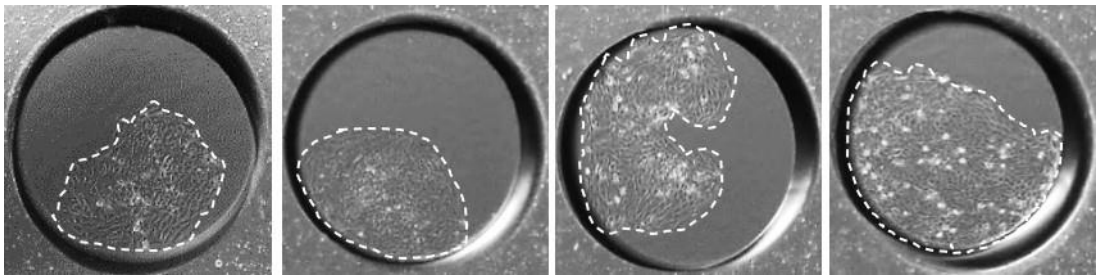


Step 4: Single-Cell Cloning

1. Add 5 mL of cell culture medium into the SACE.
2. Culture the isolated single cells for ~2 weeks to generate clonal cells.



Four clonal cells indicated by white dotted line



Step 5: Clonal Cells Harvesting

1. Remove cell culture medium from the SACE.
2. Wash residual medium 2-3 times by PBS and then remove PBS from the SACE.
3. Carefully remove PBS from the Outlet Wells containing desired clonal cells.
4. Add about 2 µl of trypsin into the Outlet Wells containing desired clonal cells.
5. Put the SACE into the cell culture hood for 3-5 minutes to dissociate adherent cells from the bottom.
6. Use pipette to harvest the clonal cells from the Outlet Wells of SACE.

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