

# Full Protocol of SA mini for Single-Cell Cloning

## Step 1: Cell Suspension Preparation

1. Harvest adherent cells and make them into cell suspension.
2. Transfer 2  $\mu$ L 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 15-30. If too much/little, dilute/enrich it to match the number.
5. Add 100  $\mu$ L of medium to the cell suspension and pipette cells to be fully suspended.
6. Aspirate the 100  $\mu$ L of cell suspension (250-500 cells/mL) into pipette tip.

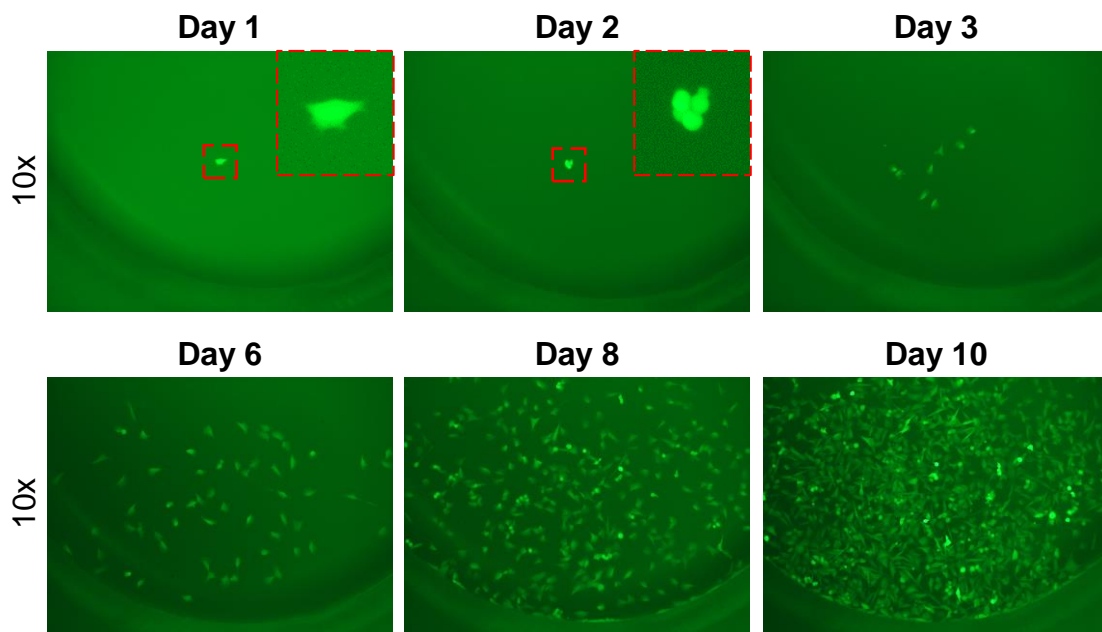
## Step 2: Single-Cell Isolation

1. Vertically insert pipette tip into the Inlet Adaptor.
2. Press pipette plunger down (100  $\mu$ L +P) to load cell suspension into the 32 Outlet Wells.
3. After loading, remove the Inlet Adaptor by tilting the pipette tip with holding the SA mini. DO NOT release the plunger button. The Inlet Adaptor will be easily detached from SA mini.

## Step 3: Single-Cell Identification

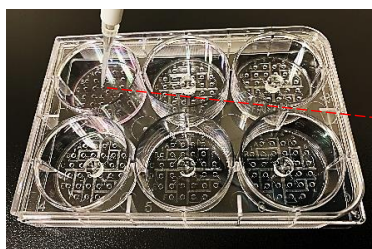
1. Put the SACE under microscope with 10x objective (bright field or fluorescence).
2. Identify and note all Outlet Wells containing only single cells.

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## Step 4: Single-Cell Cloning

1. Add 3 mL of cell culture medium into each SA mini.
2. Culture the isolated single cells for about 2 weeks to generate clonal cells.



A pipette tip is above a well.

## Step 5: Clonal Cells Harvesting

1. Remove cell culture medium from the SA mini.
2. Wash residual medium 2-3 times by PBS and then remove PBS from the SA mini.
3. Carefully remove PBS from the Outlet Wells containing desired clonal cells.
4. Add about 2  $\mu$ l of trypsin into each Outlet Well 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 SA mini.