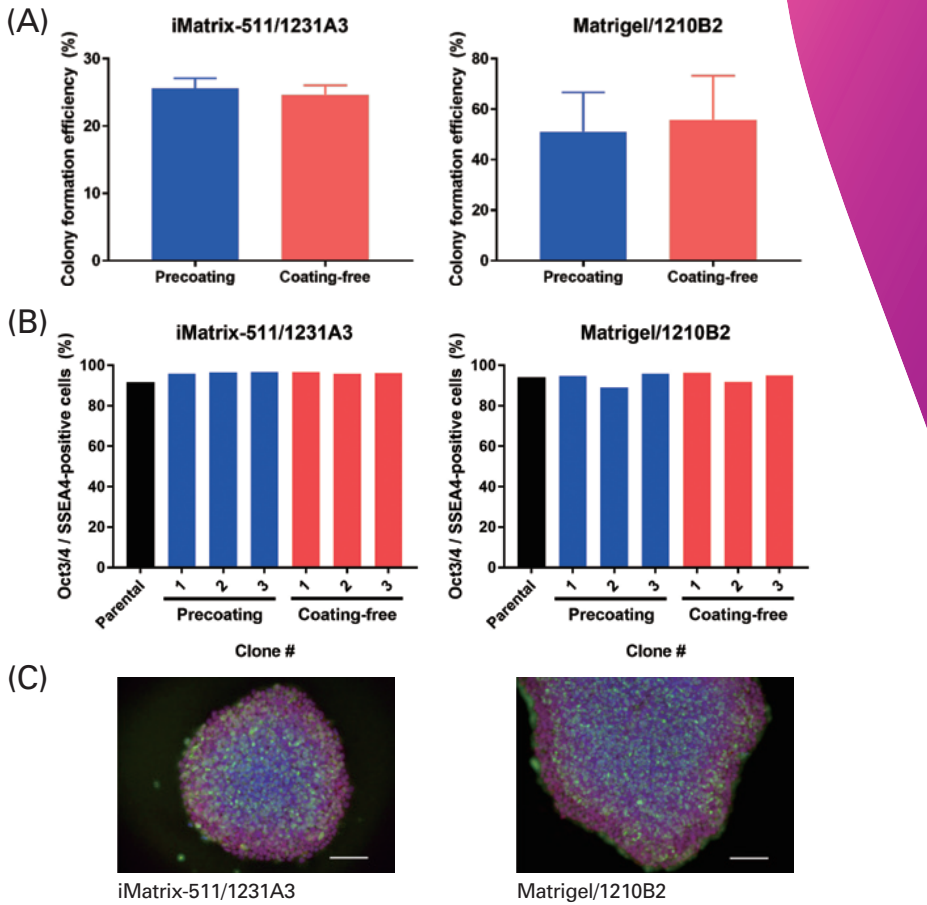
Cloning efficiency with coating-free protocol and pluripotency analysis of isolated hiPSC clones



1231A3 and 1210B2 (Human episomal iPSC lines established by CiRA) were cloned on iMatrix-511 or Matrigel® by the precoating or coating-free method.
(A) Comparison of the cloning efficiency of hiPSCs by two different methods. Bars represent the means ± S.D. (n=3). (B,C) Analysis of pluripotent markers in isolated hiPSC clones. Expression levels of pluripotent markers were evaluated by (B) FACS and (C) ICC (Blue: DNA, Green: Tra1-60, Red: Oct3/4). Scale bars: 100 µm.

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Feeder-free medium for ES/iPS cells

StemFit Technical tips

Key Points for ***single-cell cloning*** with ***coating-free method***

Benefit 1

Superior colony-forming efficiency

Enables efficient single-cell cloning

Benefit 2

Coating-free protocol

No coating process, No incubation

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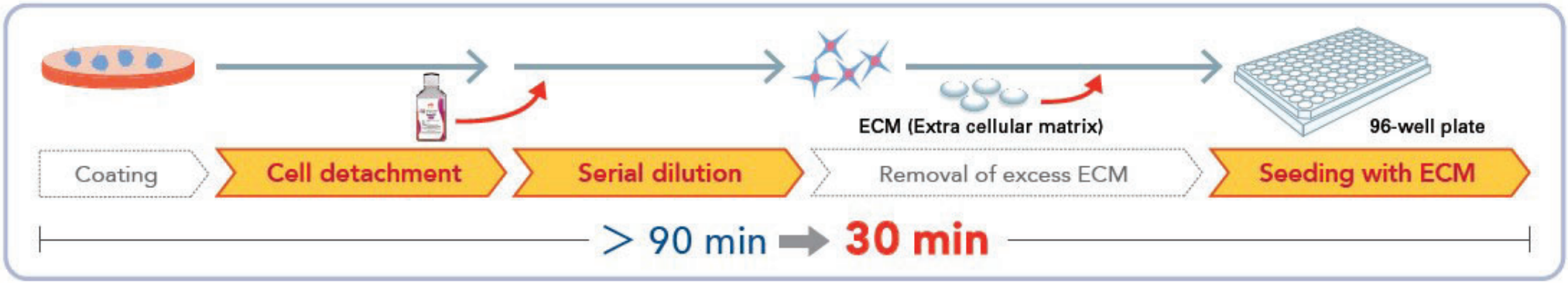
● Brief protocol for single-cell cloning by the coating-free method

StemFit Technical tips



1 Detach cells and resuspend in culture medium supplemented with 10 μM Y-27632.

* Also see our Technical tips: Key points for successful single-cell passage

2 Prepare 10 mL of 10 cell/mL cell suspension by serial dilution with culture medium with 10 μM Y-27632.

3 Add iMatrix-511 or Matrigel® to the prepared cell suspension and mix thoroughly.

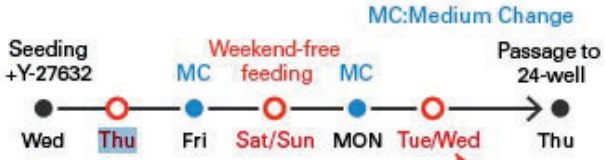
Point-1
Concentration of ECMs !

ECM	Amount	Final conc.
iMatrix-511 (0.5 mg/ml)	35 μl	1.75 μg/ml
Matrigel®	100 μl (x1/100)	10 μl/ml

4 Plate 100 μL (= 1 cell) in each well of the 96-well plate immediately.

5 Replace medium with fresh culture medium without Y-27632 at least every three days. Around day 8, select single colonies to be passaged to a 24-well plate.

<Medium Change Schedule Example>



Point-2
Extend or shorten culture period for colonies to be appropriate sizes

6 After washing the colonies with 100 μl of PBS, detach the cells with 50 μl of cell detaching solution and incubate at 37 ℃ for 10 min.

* Accutase or TrypLE™ can be used
* Incubation times may vary

7 Carefully remove cell detaching solution.

Point-3
Remove very carefully because the colonies easily detach

8 Dissociate colonies by pipetting with 100 μl of culture medium with 10 μM Y-27632. Transfer resuspended cells to ECM-coated 24-well plate with 400 μl of culture medium containing 10 μM Y-27632 immediately.

Point-4
Detach the colonies one by one as reattachment can occur soon after adding the culture medium

9 Change the medium to fresh culture medium without Y-27632 at least every three days.

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