Protocol: Cloning of hiPSCs using StemFit®Basic02 and iMatrix-511
By "Coating-free method"

1. Materials required

- StemFit® Medium (amsbio #SFB-500)
- iMatrix-511 (amsbio #AMS.892)
- Y-27632 (amsbio #1596-1)
- PBS (Ca-/Mg-)
- TrypLE Select (Thermo Fisher Scientific)
- 96-well cell culture plate
- 24-well cell culture plate
- bFGF (amsbio #AMS-480)

2. Media preparation

1. Before use, thaw frozen "Liquid A" and "Liquid B" with occasional mixing at room temperature (15-25 ˚C). *(CAUTION: Do not thaw "Liquid B" at 37 ˚C, as it accelerates the degradation of the medium ingredients.)*

2. Aseptically mix medium components by adding the full volume of "Liquid B" to "Liquid A". Mix thoroughly.

3. Upon thawing, StemFit®Basic02 medium can be aseptically aliquoted and stored at below -20˚C. Before use, thaw an aliquot in the refrigerator overnight.

4. Add bFGF at a concentration of 40 ng/ml. (Basic02+F)

(We recommend adjusting the concentration of bFGF according to suit your cell line.)

5. Store the thawed medium in the refrigerator.

(CAUTION: Thawed StemFit®Basic02 medium may be stored at 2 - 8˚C for up to two weeks.)

(CAUTION: We recommend storing the medium in the dark.)

6. Before use, warm aliquots to room temperature and use immediately.

(Do not heat the thawed medium to 37 ˚C.)

3. Cloning on uncoated 96-well plates

3-1. Serial dilution of hiPSCs in StemFit® and seeding

1. Detach hiPSCs by TrypLE Select and resuspend in the Basic02+F supplemented with 10 µM Y-27632.

2. Count the cells using a cell counter which is optimized for hiPSCs.

3. Prepare 10 mL of 10 cell/mL cell suspension by serially diluting hiPSCs by Basic02+F with 10 µM Y-27632.

4. Add 35 µL of 0.5 mg/mL iMatrix-511 to 10 mL of 10 cell/ mL cell suspension prepared above and mix thoroughly.
5. Add 100 µL (= 1 cell) to each well of the 96-well plate immediately.
6. Culture in 5% CO_2 incubator at 37 °C.

3-2. Medium change

1. After two days, carefully aspirate media from the plate and add 100 µL of Basic02+F (without Y-27632) to each well.
2. Change media as above at least every three days.

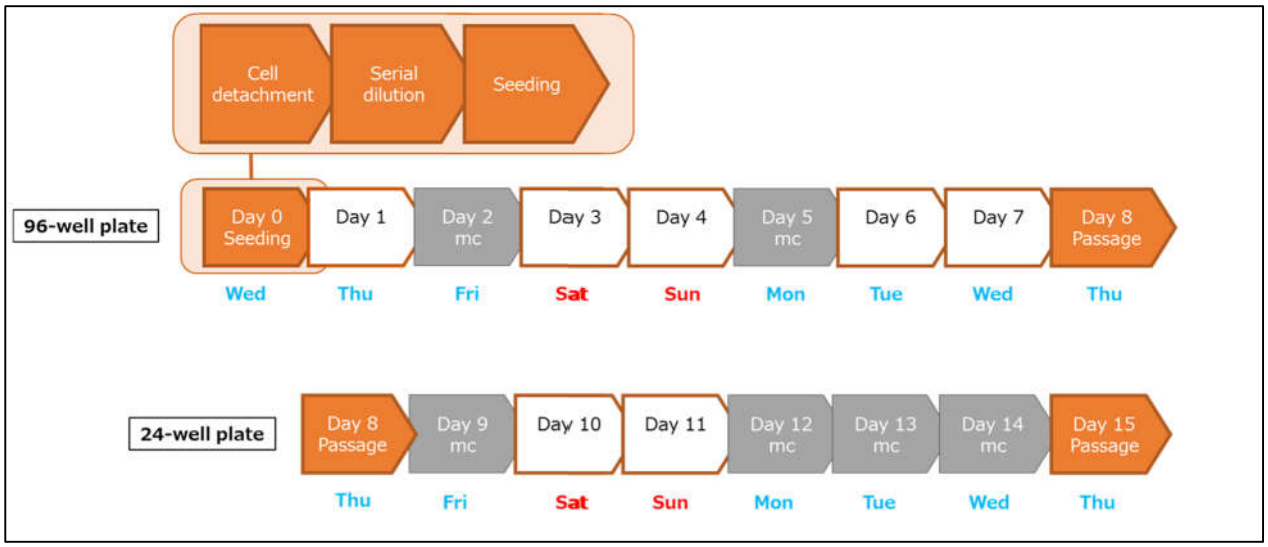
4. Passaging the colonies

4-1. Preparation of 24-well plate (for 10 well)

1. Add 20 µL of 0.5 mg/mL iMatrix-511 in 4 mL of Basic02+F supplemented with 10 µM Y-27632.
2. Add 400 µL of diluted iMatrix-511 solution to each well of the 24-well plate.
3. Pre-warm the plate in the 5% CO_2 incubator at 37 °C. (Prepare in the day of passage)

4-2. Picking up the colonies and seeding

1. Around day 8, select single colonies on the 96-well plate to be passaged to 24-well plate.
(Extend or shorten culture period for colonies to be appropriate sizes. Usually, colonies can be grown enough for passaging in 7-8 days.)
2. Mark positions of the selected colonies.
3. Aspirate media from the selected wells of the 96-well plate and wash wells by 100 µL of PBS.
4. After removing PBS, add 50 µL of cell detaching solution (50% TrypLE Select/PBS, 0.75 mM EDTA).
5. Incubate at 37 °C for 10 min.
6. Carefully remove cell detaching solution by micropipette and detach colonies by pipetting with 100 µL of Basic02+F supplemented with 10 µM Y-27632.
(Remove cell detaching solution very carefully since hiPSC colonies could be easily detached after Trypsin treatment).
(If several colonies are simultaneously Trypsin-treated, please firstly remove cell detaching solution from all the wells, and then detach colonies one by one.)
7. Dissociate colonies into single hiPSC by pipetting 10 times.
(Pipetting should be performed immediately after addition of Basic02+F medium. Otherwise, reattachment of colonies can occur.)
8. Add dissociated hiPSC suspension to pre-warmed 24-well plate.
9. Culture in 5% CO_2 incubator at 37 °C.
10. After 24 h, remove media and add 500 µL of Basic02+F (without Y-27632).
11. Change media according to the weekend-free culture protocol.



Appendix. An example of the schedule of weekend-free cloning.

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85