

Application consideration

APS0008 : iPS-L1

Please keep in mind the following points:

For thawing cells;

- Please make sure to count the number of cells after thawing, and then start cultivating the cells by diluting them to the recommended cell density ($0.5\text{-}3.0 \times 10^5$ cells/100mm dish or $1.0\text{-}5.0 \times 10^4$ cells/60mm dish).
- Since the cell proliferation declines right after thawing, 4 to 5 days are required for subculturing the cells.
- Please subculture the cells after you see a colony as one shown in the morphological photo below (**Fig.1-C**).

For subculturing cells;

- Please make sure to count the number of cells, and then start cultivating the cells by diluting them to the recommended cell density for subculture ($1.0\text{-}3.0 \times 10^4$ cells/100mm dish or $0.5\text{-}1.0 \times 10^4$ cells/60mm dish).
- For washing the cells during subculturing, please use DMEM/F12 medium that does not contain KnockOut Serum Replacement (KSR).
- For detaching the cells during subculturing, please use 0.1% trypsin diluted with DMEM/F12 medium that does not contain KSR.
- For subculturing cells, they should be dispersed into single cells.

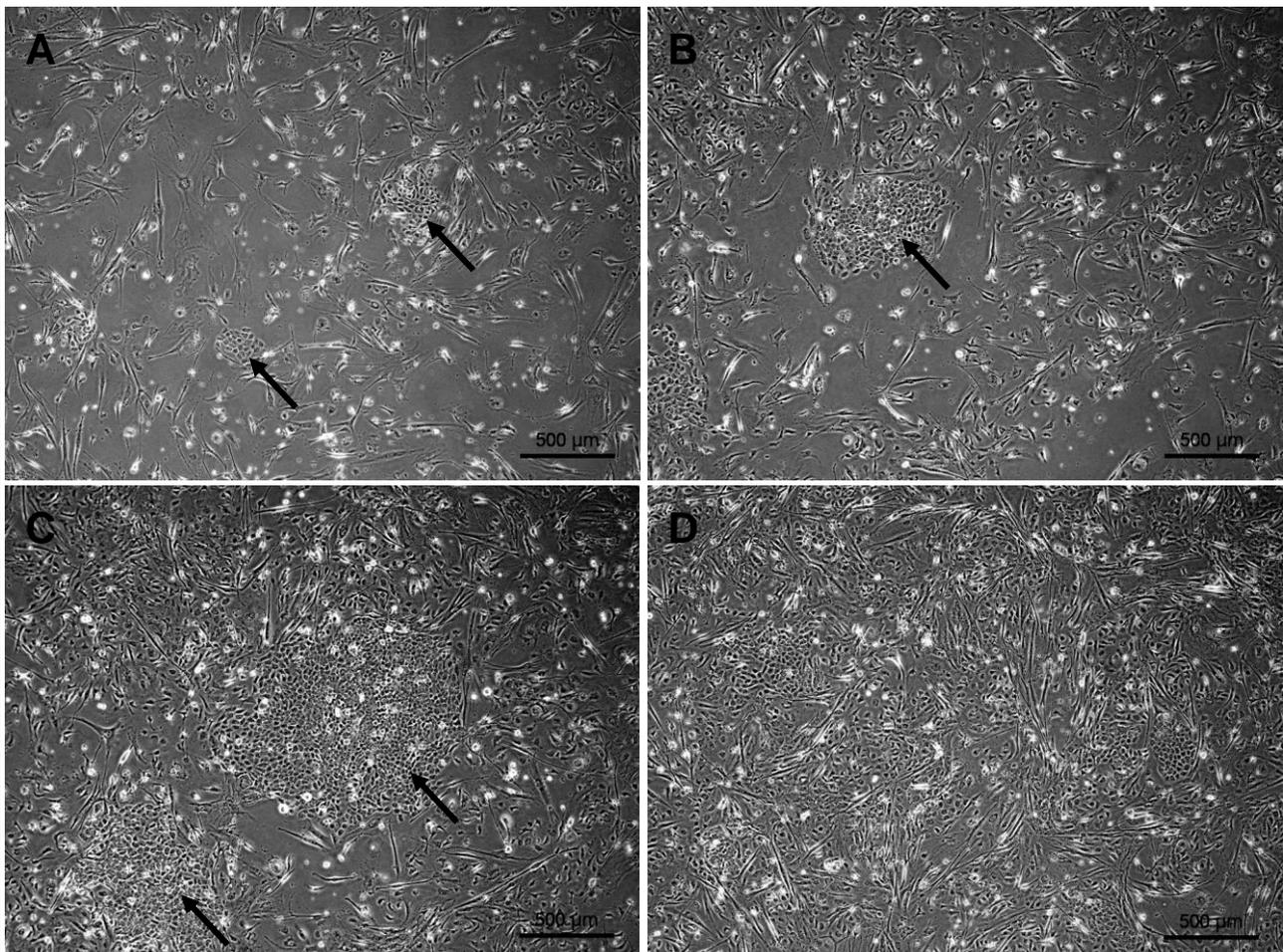


Fig.1 The proliferation of rabbit iPS cells are shown above (3-5 days after thawing, 3.0×10^5 cells/100mm dish). (A-C) The colonies of rabbit iPS cells with indistinct boundaries (as shown in arrows A: Day 3, B: Day 4, C: Day 5). (D) If the cells are plated at too high cell density (5.0×10^5 cells/100mm dish), it reduces cell proliferation since the cells become overcrowded before forming their colonies (5 days after thawing).