Application consideration

APS0010 : iPS-S1

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 (1.0-3.0×10^5 cells/100mm dish or 3.0-10.0×10^4 cells/60mm dish).
- Since the cell proliferation declines right after thawing, 4 to 5 days are required for subculturing 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 subculturing (1.0-3.0×10^4 cells/100mm dish or 0.5-1.0×10^4 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.

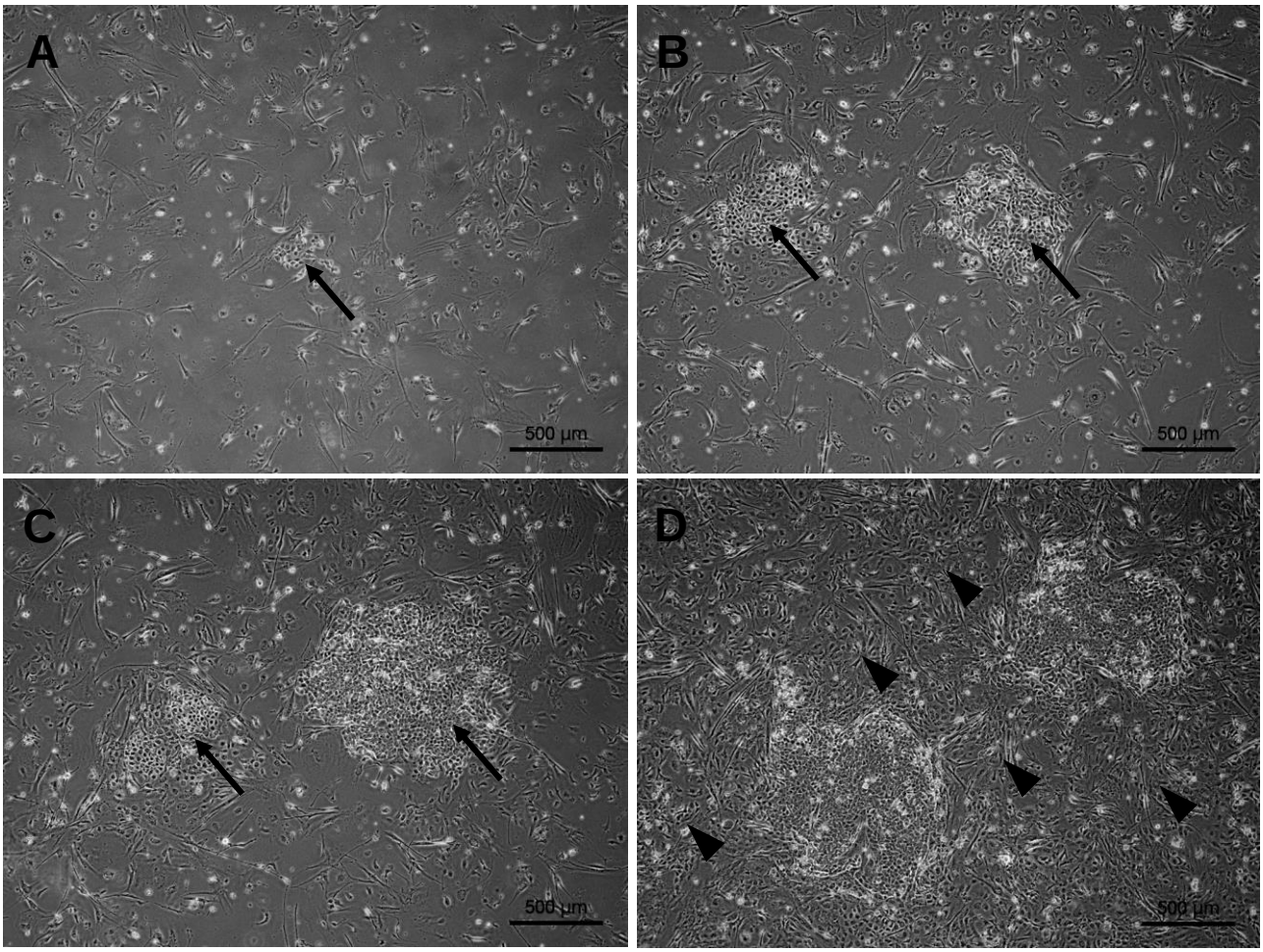

Fig.1 The proliferation of rabbit iPS cells are shown above (3-5 days after thawing, 3.0×10^5 cells/100mm dish). (A-C) The colonies of rabbit iPS cells with indistinct boundaries (as shown in arrows A: Day 3, B: Day 4, C: Day 5). **(D) If the cells are plated at too high cell density (5.0×10^5 cells/100mm dish), it reduces cell proliferation since the cells which do not form a colony (arrow-head) become overcrowded around the normal colonies (5 days after thawing).**

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