Application Note

CL-Quant Add-on Module

Tracking of human iPS cell colonies using "hPSC Colony Tracking"

＜BioStation CT usage example＞

Human pluripotent stem cells (hPS cells) including embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells) have the unique characteristics of self-renewal and pluripotency. Due to these characteristics, these cells are expected to be useful as a tool for regenerative medicine and pharmaceutical research. On the other hand, it has been reported that hPS cells show genomic instability during prolonged periods in culture, and exhibit abnormal cell growth as transformed clones are generated. It is known that such kind of clones have reduced differentiation potential. When culturing conditions for hPS cells are changed, it is necessary to evaluate the phenotype stability over several passages. Evaluation of cell growth of hPS cells is an important criterion to assess cell quality and phenotype.
CL-Quant image analysis software, in combination with the Add-on Module "hPSC Colony Tracking", allows the user to track hPS cell colonies and automatically digitize hPS cell colony regions. As a result, changes in the growth of individual colonies can be detected.
To evaluate the quality and phenotype of human iPS cells, human iPS cells were analyzed from phase-contrast images acquired using BioStation CT, and individually tracked colony areas were digitized over time.

Observation device

- BioStation CT (Nikon, MLA10000)

Image analysis software

- CL- Quant ver. 5.02 (Nikon, MLS21000)

CL-Quant Add-on Module

- Tracking Module (Nikon, MLS21020)
- PC-QE01 hPSC Colony Tracking (Nikon, MLS30501)

Cells

- Human iPS cell line 253G1 (for use in private companies: Kyoto University iPS Cell Laboratory/iPS Academia Japan; for academic institutions: HPS0002, RIKEN BRC through National BioResource Project of MEXT/AMED, Japan)

Reagents and materials

- StemFit® AK02N (Ajinomoto, AK02N)
- iMatrix-511 solution (0.5mg/ml) (MATRIXOME, 892011)
- Y-27632 (FUJIFILM Wako Pure Chemical, 257-00511)
- TrypLE™ Select(1x), no Phenol Red (Thermo Fisher Scientific, 12563011)
- PBS, pH 7.4 (Thermo Fisher Scientific, 10010023)
- Costar® 6-well clear TC-treated multiple well plates(Corning, 3516)

Methods

Human iPS (253G1) cells were dissociated into single cells using TrypLE™ Select, and seeded at a density of 6.5 x 10^4 cells per well in AK02N medium supplemented with Y-27632 in a 6-well plate coated with iMatrix-511. The cells were then cultured in a Biostation CT at 37°C, in a humidified atmosphere of 5% CO_2. Phase-contrast images of the 8 x 8 fields of view (approximately 15.5 mm x 15.5 mm) at the center of the wells were captured with a 4x objective lens every 6 hours, starting two hours after seeding and confirming the cell attachment. The obtained image data was analyzed using CL-Quant and the Add-on Module "hPSC Colony Tracking", and each individually tracked colony area "Ph-cell region-area (μm²)" was automatically digitized. The cell area to be tracked was adjusted by setting the "Remove size" parameter to "500". The tracked iPS cell colony mask and its digitized values were confirmed on the operation screen and output in Microsoft Excel® format. The changes over time of each individual colony area was graphed and displayed as "Colony area (μm²)".

Results

Images were acquired with the BioStation CT every six hours, from two hours after seeding up to 140 hours. The images were imported into CL-Quant and analyzed using the Add-on Module "hPSC Colony Tracking".

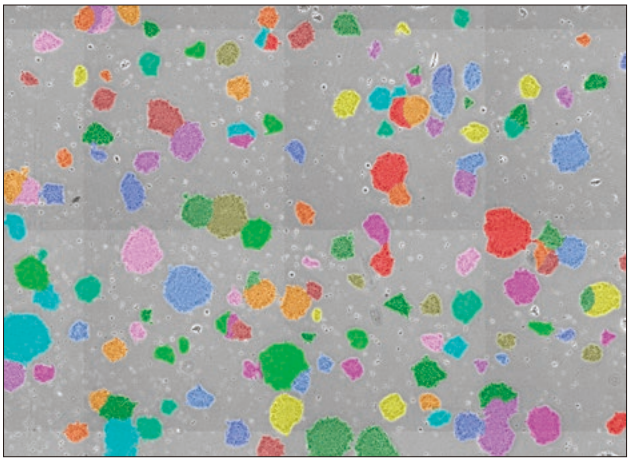

Fig. 1 Phase contrast image overlaid with mask for identifying colony areas

A mask identifying the tracked cell area is overlaid on a phase contrast image 110 hours after seeding. The colors in the mask show each tracked object.

Table 1. Colony area of tracked human iPS cells during 140 hrs culturing

Time (hours)	Colony area (μm^2)				
	Tracked object 2	Tracked object 5	Tracked object 18	Tracked object 107	Tracked object 122
2					
8					
14	9056	15520			
20	10096	18736	8672		
26	8352	15456	16480		
32	10272	16928	13056		
38	10928	18464	16464		
44	11808	17504	17792		
50	14592	22240	20304		
56	15360	27808	27808		
62	19328	32448	33472	10416	
68	22688	40608	38960	11264	9088
74	30288	52272	46656	11504	9328
80	37520	61728	58976	12832	13344
86	46480	77824	74976	16144	15200
92	62928	96528	96704	18560	18528
98	84768	125696	125664	23968	23312
104	112112	170392	162288	30464	30288
110	142944	212812	199456	39456	38944
116	183440	255580	248032	50016	53792
122	233200	301356	293808	65712	67168
128	287872	346512	337448	76608	80496
134	340784	372188	381860	99056	99104
140	392412	389436	411528	116620	123700

The graph shows the time course of the area of individual colonies (Fig.2A), and the tracked cell areas of several representative colonies are displayed with a mask in Fig.2B. Figures 2A and 2B show that the area of each colony was observed to vary from 32 hours after seeding and gradually increased.

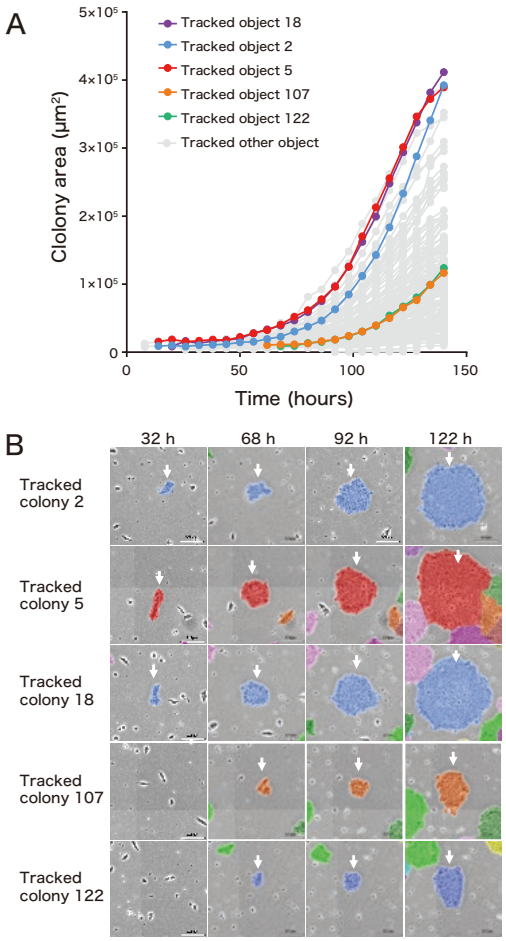

Fig.2 Change in colony area of tracked human iPS cells over time

A. The time course of individual colony areas is shown. Several selected colonies are displayed as color symbols.

B. The individual tracked colonies at each indicated time after seeding are displayed with different color masks. The scale bar indicates 200 μm. The arrow indicates the colony whose area was digitized.

Summary

- Tracking analysis of phase contrast images of iPS cell colonies using CL-Quant and Add-on Modules enables automatic calculation of changes over time in each colony area.
- By referring to the mask image, one can easily check whether the colony area is correctly recognized.
- Even during culturing, increase over time in a colony area can be monitored, so the quality of hPS cells can be evaluated by measuring colony growth.

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