Data collection for quality prediction
Evaluation target
Pipetting stresses
Stress 1
Stress 2
Stress 3
Different cell types
Normal cell
Sub-clone 1
Sub-clone 2
Image aquisition for morphological analysis
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Fluorescent staining
OCT3/4
rBC2LCN
Output:
Marker staining rate
Measurement of colony morphology using 16 morphological parameters
Input:
Morhological profile
Predicition model
Process evaluation using colony tracking analysis
Time-course analysis
Bulk evaluation
Colony number
Day2
Day3
Day4
Day5
Day6
Day7
Colony tracking analysis
Single colony evaluation
Colony size
Day 2
Day 3
Day 4
Day 5
Definition of standard colony growth
AVE
2SD
2SD
Average (AVE)
Stndard Deviation (SD)
95% confidence interval
Comparsion of "stresses"
Stress 1
Stress 2
Stress 3
Comparsion of "cell types"
Normal cell
Sub-clone1
Sub-clone2

Fig. 1

A
Shear stress level
Low
Medium
High
Day 3
Day 5
Day 7
B
Shear stress level
Low
Medium
High
Total colony area (×10^7) [μm^2]
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
Time [day]

Fig.2

A
Shear stress level (201B7)
Low
Medium
High
Day 2–3
Day 3–4
Day 4–5
Day 5–6
69(58.5%)
66(45.2%)
32(33.0%)
24(20.3%)
33(22.6%)
28(28.9%)
13(11.0%)
27(18.5%)
21(21.6%)
12(10.2%)
20(13.7%)
16(16.5%)
B
Shear stress level
201B7
201B7-1A
TIG108-4f3
0/69
2/69
1/66
5/66
0/32
1/32
13/60
3/60
7/63
1/63
1/44
1/44
27/79
3/79
5/40
1/40
4/32
2/32
Log10(colony area) [-]
Time [day]

Fig.3

Colonies in undifferentiated status
Colonies in deviated status
Phase contrast
OCT3/4
Phase contrast
OCT3/4
Phase contrast
rBC2LCN
Phase contrast
rBC2LCN

Fig. 4

A
Usage of morphological profies
OCT3/4
rBC2LCN
Prediction model number
Time [day]
RMSE value [-]
B
C
D
Predicted value [−]
Experimentally determined value [−]
Prediction model 9

Fig. 5

Supporting Information

Fig. S1. Scheme for the morphological measurements of iPSC colonies. The phase-contrast images of iPSC colonies were processed via 6 processing steps to segment the morphology of the colonies. The final goal of this processing scheme is to measure the morphological profiles of all recognizable colonies.

Fig. S2. Relationship between iPSC colony size and their expression rate of markers. X-axis: the marker expression rate in single colony, Y-axis: the size of single colony. 201B7 (blue), 201B7-1A (red), and TIG108-4f3 (green). The gray vertical dotted lines on the plots indicate two thresholds of colony diameters.

Fig. S3. The distribution profile at the end of tracks (day 7) plotted as bee-swarm plots. Three solid lines on the bee swarm plot indicate the first, second (median), and third quantiles from the top. The differences of colony number outside standard range at the end of tracks among culture conditions indicate their sensitivities to pipetting stress or cell lines. Statistical significance compared to the control cell line was determined by multiple comparison with Ryan's method: ***P-value<0.001. This indicate that by the differences of cell strains, there are optimized shear stress to fit the final colony size in the objective range.

Fig. S4. Predictive performance of OCT3/4 or rBC2LCN staining area (at day 7) only using single parameter (area at day 7). X-axis: the experimentally determined values, Y-axis: the predicted values from the morphological profiles. The gray regions on the plot indicate the possible deviation areas that can be affected by the experimental error (defined as the mean ± SD of experimental data). If the plots were included in this region, the prediction has a higher reliability.

Table S1. The number of colony tracks analyzed by their recognition start.

		Start of recognition [day]	Number of correctly tracked colonies [-]	Number of well-grown colonies [-] (diameter > 1 mm)
Shear stress level	Low	2-3	69	27
		3-4	24	0
		4-5	13	0
		5-6	12	2
	Medium	2-3	66	9
		3-4	33	0
		4-5	27	0
		5-6	20	0
	High	2-3	32	5
		3-4	28	0
		4-5	21	0
		5-6	16	0
Total		2-3	167	41
		3-4	85	0
		4-5	61	0
		5-6	48	2

Raw image
Processing step 1
Background adjustment
Processing step 2
Recognition of colony area
Processing step 3
Filling in the blank holes
Processing step 4
Removing noise objects
(< 225 μm diameter)
Processing step 5
Applying processing 1 to 4
to raw image
Processing step 6
Measurement of
colony morphology
(Morphological profile)
16 morphological parameters

Fig. S1

VIMENTIN
OCT3/4
201B7
201B7−1A
TIG108−4f3
Daimeter
= 1400 μm
Daimeter
= 800 μm
Colony area (×10⁶) [μm²]
Marker expression rate [%]
0
0.5
1.0
1.5
2.0
2.5
3.0
0
20
40
60
80
100

Fig. S2

***
***
Log10(colony area) [-]
6.5
6.0
5.5
5.0
4.5
Low
Medium
High
Low
Medium
High
Low
Medium
High
201B7
201B7-1A
TIG108-4f3

Fig. S3

OCT3/4
Predicted value [−]
Experimentally determined value [−]
40
50
60
70
80
90
100
rBC2LCN
Predicted value [−]
Experimentally determined value [−]
0
20
40
60
80
100

Fig. S4