A woman with short blonde hair, wearing a white lab coat and a small hoop earring, is focused on her work in a laboratory. She is holding a pipette and appears to be transferring liquid into a multi-well plate. The background is slightly blurred, showing laboratory equipment and windows. The overall tone is professional and scientific.

Reprogramming of 24 patient fibroblast samples: QC analysis 2/3

01-12-2023

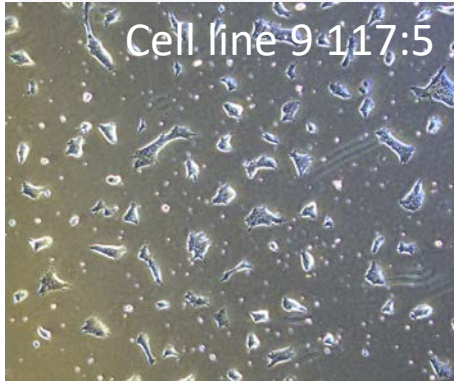
Berta Sanz Morello, PhD
Bioneer A/S

bioneer

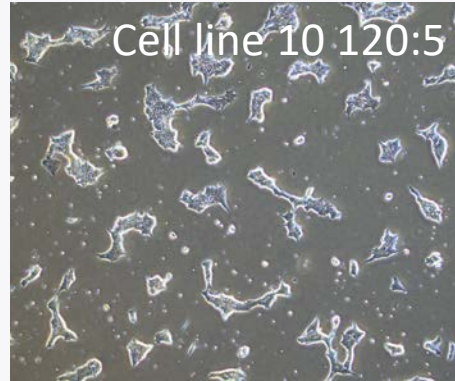
Morphology

Morphology of iPSCs 24 hours after thawing*:

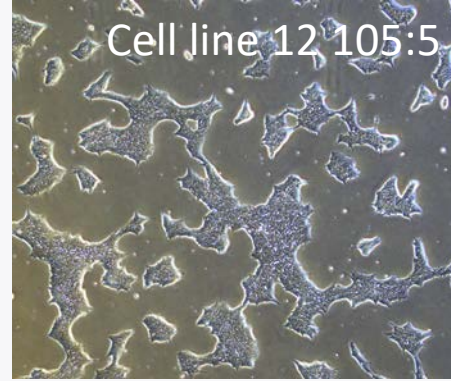
hPSCreg ID
SUHi010-A



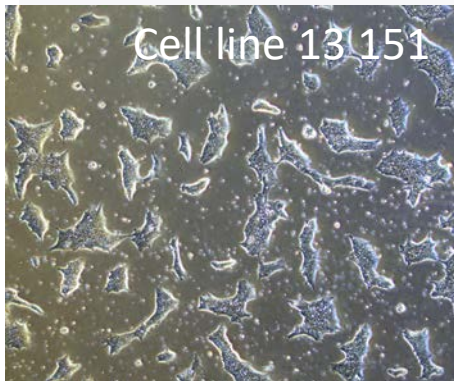
hPSCreg ID
SUHi014-A



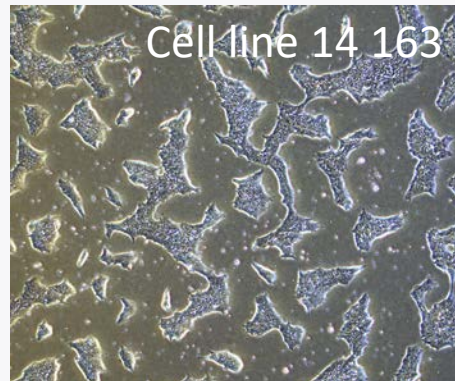
hPSCreg ID
SUHi009-A



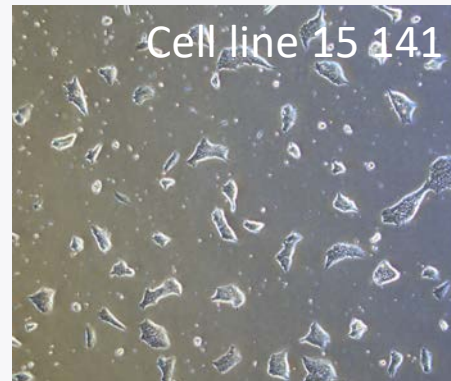
hPSCreg ID
SUHi011-A



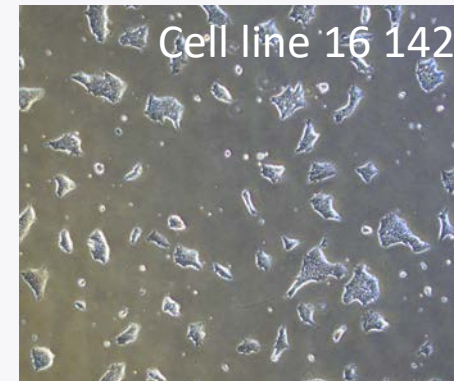
hPSCreg ID
SUHi012-A



hPSCreg ID
SUHi015-A



hPSCreg ID
SUHi016-A

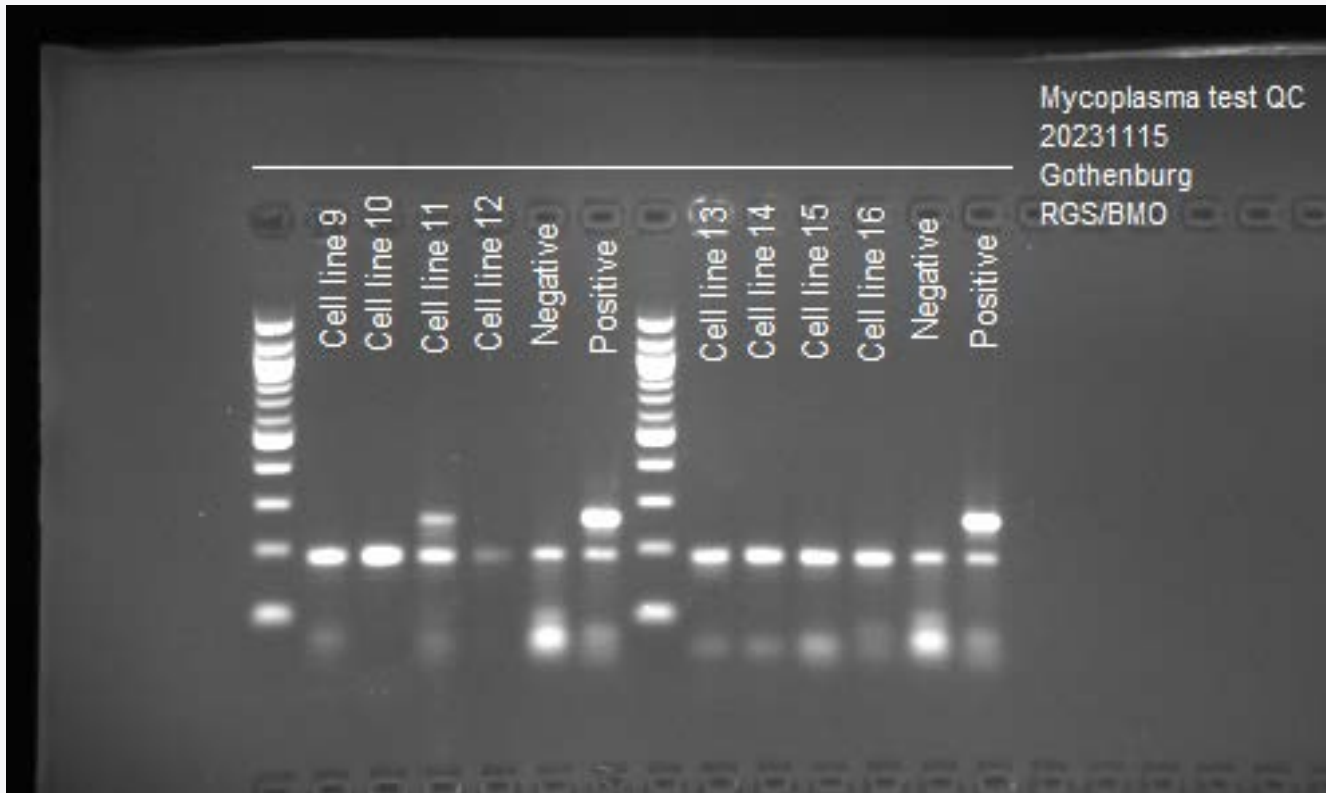


*Photos of the morphology of the iPSC lines before banking will be sent in a separate format (photos are too heavy to be included here).

QC Overview

hPSCreg ID	Line ID	Clone	Mycoplasma	Sterility	Trilineage diff.
SUHi010-A	Cell line 9 117:5	3	Passed	Passed	Passed
SUHi014-A	Cell line 10 120:5	3	Passed	Passed	Passed
SUHi009-A	Cell line 12 105:5	3	Passed	Passed	Passed
SUHi011-A	Cell line 13 151	1	Passed	Passed	Passed
SUHi012-A	Cell line 14 163	2	Passed	Passed	Passed
SUHi015-A	Cell line 15 141	3	Passed	Passed	Passed
SUHi016-A	Cell line 16 142:3	1	Passed	Passed	Passed

Mycoplasma test



hPSCreg ID	Line ID	Clone
SUHi010-A	Cell line 9 117:5	3
SUHi014-A	Cell line 10 120:5	3
SUHi009-A	Cell line 12 105:5	3
SUHi011-A	Cell line 13 151	1
SUHi012-A	Cell line 14 163	2
SUHi015-A	Cell line 15 141	3
SUHi016-A	Cell line 16 142:3	1

*Cell line 11 110:5 cl 1 gave a false positive in the mycoplasma test and, therefore, was excluded from the trilineage differentiation. However, in a second and third mycoplasma test, it was shown to be negative. QC results for cell line 11 will be included in the next report.

Sterility test

No contamination was detected in any of the lines 48 hours after thawing (data not shown).

Trilineage differentiation

hPSCreg ID	SAMPLES	Fold over reference sample			
		GATA4 (Endo and Meso marker)	CRCR4 (Meso and Endo marker)	Pax 6 (Ecto marker)	GAPDH
SUHi010-A	Cell line 9 117:5 Cl. 3 iPSC	1	1	1	1
SUHi014-A	Cell line 10 120:5 Cl. 3 iPSC	1	1	1	1
SUHi009-A	Cell line 12 105:5 Cl. 3 iPSC	1	1	1	1
SUHi011-A	Cell line 13 151 cl 1 iPSC	1	1	1	1
SUHi012-A	Cell line 14 163 cl 2 iPSC	1	1	1	1
SUHi015-A	Cell line 15 141 cl 3 iPSC	1	1	1	1
SUHi016-A	Cell line 16 142:3 cl 1 iPSC	1	1	1	1
SUHi010-A	Cell line 9 117:5 Cl. 3 Endo	6266,02	66,87	24,59	1
SUHi014-A	Cell line 10 120:5 Cl. 3 Endo	37208,21	271,22	639,15	1
SUHi009-A	Cell line 12 105:5 Cl. 3 E Endo	17722,98	236,11	58,22	1
SUHi011-A	Cell line 13 151 cl 1 Endo	4379,85	91,35	36,93	1
SUHi012-A	Cell line 14 163 cl 2 Endo	797,86	17,63	1,58	1
SUHi015-A	Cell line 15 141 cl 3 Endo	3484,33	78,07	6,74	1
SUHi016-A	Cell line 16 142:3 cl 1 Endo	5196,53	78,07	9,08	1
SUHi010-A	Cell line 9 117:5 Cl. 3 Meso	1499,22	137,82	0,04	1
SUHi014-A	Cell line 10 120:5 Cl. 3 Meso	1382,76	826,00	0,07	1
SUHi009-A	Cell line 12 105:5 Cl. 3 Meso	1040,70	481,04	0,02	1
SUHi011-A	Cell line 13 151 cl 1 Meso	1136,20	346,49	0,06	1
SUHi012-A	Cell line 14 163 cl 2 Meso	758,32	216,77	0,01	1
SUHi015-A	Cell line 15 141 cl 3 Meso	712,46	424,61	0,02	1
SUHi016-A	Cell line 16 142:3 cl 1 Meso	812,75	387,13	0,02	1
SUHi010-A	Cell line 9 117:5 Cl. 3 Ecto	0,19	9,11	564,18	1
SUHi014-A	Cell line 10 120:5 Cl. 3 Ecto	2,26	49,64	12795,36	1
SUHi009-A	Cell line 12 105:5 Cl. 3 Ecto	0,42	16,37	3404,75	1
SUHi011-A	Cell line 13 151 cl 1 Ecto	0,33	23,00	7315,12	1
SUHi012-A	Cell line 14 163 cl 2 Ecto	0,70	9,92	1888,90	1
SUHi015-A	Cell line 15 141 cl 3 Ecto	0,16	12,52	2057,49	1
SUHi016-A	Cell line 16 142:3 cl 1 Ecto	0,39	6,68	526,39	1

Fold over reference sample is based on the formula:

$$2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct \text{ target tissue} - \Delta Ct \text{ reference tissue})}$$

Where:


ΔCt = Ct gene of interest – Ct house keeping gene

Target tissue = endo, ecto or mesoderm
 Reference tissue = undifferentiated iPSCs
 Gene of interest = GATA4, CXCR4, or Pax6
 Housekeeping gene = GAPDH

Trilineage differentiation

hPSCreg ID	SAMPLES	Fold over reference sample			
		GATA4 (Endo and Meso marker)	CRCR4 (Meso and Endo marker)	Pax 6 (Ecto marker)	GAPDH
SUHi010-A	Cell line 9 117:5 Cl. 3 iPSC	1	1	1	1
SUHi014-A	Cell line 10 120:5 Cl. 3 iPSC	1	1	1	1
SUHi009-A	Cell line 12 105:5 Cl. 3 iPSC	1	1	1	1
SUHi011-A	Cell line 13 151 cl 1 iPSC	1	1	1	1
SUHi012-A	Cell line 14 163 cl 2 iPSC	1	1	1	1
SUHi015-A	Cell line 15 141 cl 3 iPSC	1	1	1	1
SUHi016-A	Cell line 16 142:3 cl 1 iPSC	1	1	1	1
SUHi010-A	Cell line 9 117:5 Cl. 3 Endo	6266,02	66,87	24,59	1
SUHi014-A	Cell line 10 120:5 Cl. 3 Endo	37208,21	271,22	639,15	1
SUHi009-A	Cell line 12 105:5 Cl. 3 E Endo	17722,98	236,11	58,22	1
SUHi011-A	Cell line 13 151 cl 1 Endo	4379,85	91,35	36,93	1
SUHi012-A	Cell line 14 163 cl 2 Endo	797,86	17,63	1,58	1
SUHi015-A	Cell line 15 141 cl 3 Endo	3484,33	78,07	6,74	1
SUHi016-A	Cell line 16 142:3 cl 1 Endo	5196,53	78,07	9,08	1
SUHi010-A	Cell line 9 117:5 Cl. 3 Meso	1499,22	137,82	0,04	1
SUHi014-A	Cell line 10 120:5 Cl. 3 Meso	1382,76	826,00	0,07	1
SUHi009-A	Cell line 12 105:5 Cl. 3 Meso	1040,70	481,04	0,02	1
SUHi011-A	Cell line 13 151 cl 1 Meso	1136,20	346,49	0,06	1
SUHi012-A	Cell line 14 163 cl 2 Meso	758,32	216,77	0,01	1
SUHi015-A	Cell line 15 141 cl 3 Meso	712,46	424,61	0,02	1
SUHi016-A	Cell line 16 142:3 cl 1 Meso	812,75	387,13	0,02	1
SUHi010-A	Cell line 9 117:5 Cl. 3 Ecto	0,19	9,11	564,18	1
SUHi014-A	Cell line 10 120:5 Cl. 3 Ecto	2,26	49,64	12795,36	1
SUHi009-A	Cell line 12 105:5 Cl. 3 Ecto	0,42	16,37	3404,75	1
SUHi011-A	Cell line 13 151 cl 1 Ecto	0,33	23,00	7315,12	1
SUHi012-A	Cell line 14 163 cl 2 Ecto	0,70	9,92	1888,90	1
SUHi015-A	Cell line 15 141 cl 3 Ecto	0,16	12,52	2057,49	1
SUHi016-A	Cell line 16 142:3 cl 1 Ecto	0,39	6,68	526,39	1

All iPSC lines tested were able to differentiate to the 3 germ layers.

A woman with short blonde hair, wearing a white lab coat and a small hoop earring, is shown in profile from the chest up. She is holding a pipette and looking down at it. The background is a blurred laboratory setting with various pieces of equipment and glassware. The entire image has a semi-transparent dark blue overlay.

Reprogramming of 24 patient fibroblast samples: QC analysis. Pluripotency test

22-03-2024

Berta Sanz Morello, PhD
Bioneer A/S

bioneer

Pluripotency analysis

hPSCreg ID	SAMPLES	OCT4	NANOG	GAPDH
SUHi001-A	Cell line 1 143 cl 1	1.19	0.95	1
SUHi002-A	Cell line 2 145 cl 2	0.99	0.92	1
SUHi003-A	Cell line 3 146 cl 1	1.23	0.88	1
SUHi004-A	Cell line 4 152 cl 3	1.00	0.56	1
SUHi005-A	Cell line 5 165 cl 2	0.98	0.75	1
SUHi006-A	Cell line 6 169 cl 3	1.16	0.65	1
SUHi007-A	Cell line 7 102:5 cl 1	1.41	0.75	1
SUHi008-A	Cell line 8 104:5 cl 2	1.21	0.94	1
SUHi010-A	Cell line 9 117:5 cl 3	1.16	1.11	1
SUHi014-A	Cell line 10 120:5 cl 3	0.92	0.62	1
SUHi013-A	Cell line 11 110:5 cl 1	0.81	0.82	1
SUHi009-A	Cell line 12 105:5 cl 3	1.20	0.77	1
SUHi011-A	Cell line 13 151 cl 1	1.11	0.79	1
SUHi012-A	Cell line 14 163 cl 2	1.01	0.87	1
SUHi015-A	Cell line 15 141 cl 3	0.65	0.84	1
SUHi016-A	Cell line 16 142:3 cl 1	0.80	1.15	1
SUHi018-A	Cell line 17 RES168 cl 1	0.91	0.89	1
SUHi020-A	Cell line 19 RES114:5 cl 3	1.13	0.66	1
SUHi021-A	Cell line 20 RES116:5 cl 2	0.74	0.73	1
SUHi022-A	Cell line 21 RES118:5 cl 1	0.80	0.60	1
SUHi023-A	Cell line 22 RES121:5 cl 5	0.81	0.38	1
SUHi024-A	Cell line 23 RES174 cl 4	0.78	0.47	1
SUHi017-A	Cell line 24 RES158 cl 4	0.93	0.55	1
Control	Control iPSC line: BIONi010-C	1	1	1

Fold over reference sample is based on the formula:

$$2^{-\left(\Delta\text{Ct target tissue} - \Delta\text{Ct reference tissue}\right)}$$

Where:

$\Delta\text{Ct} = \text{Ct gene of interest} - \text{Ct house keeping gene}$

Target tissue = reprogrammed cell lines

Reference tissue = iPSC line BIONi010-C

Genes of interest = OCT4, NANOG

Housekeeping gene = GAPDH

All reprogrammed lines tested showed pluripotency ability.