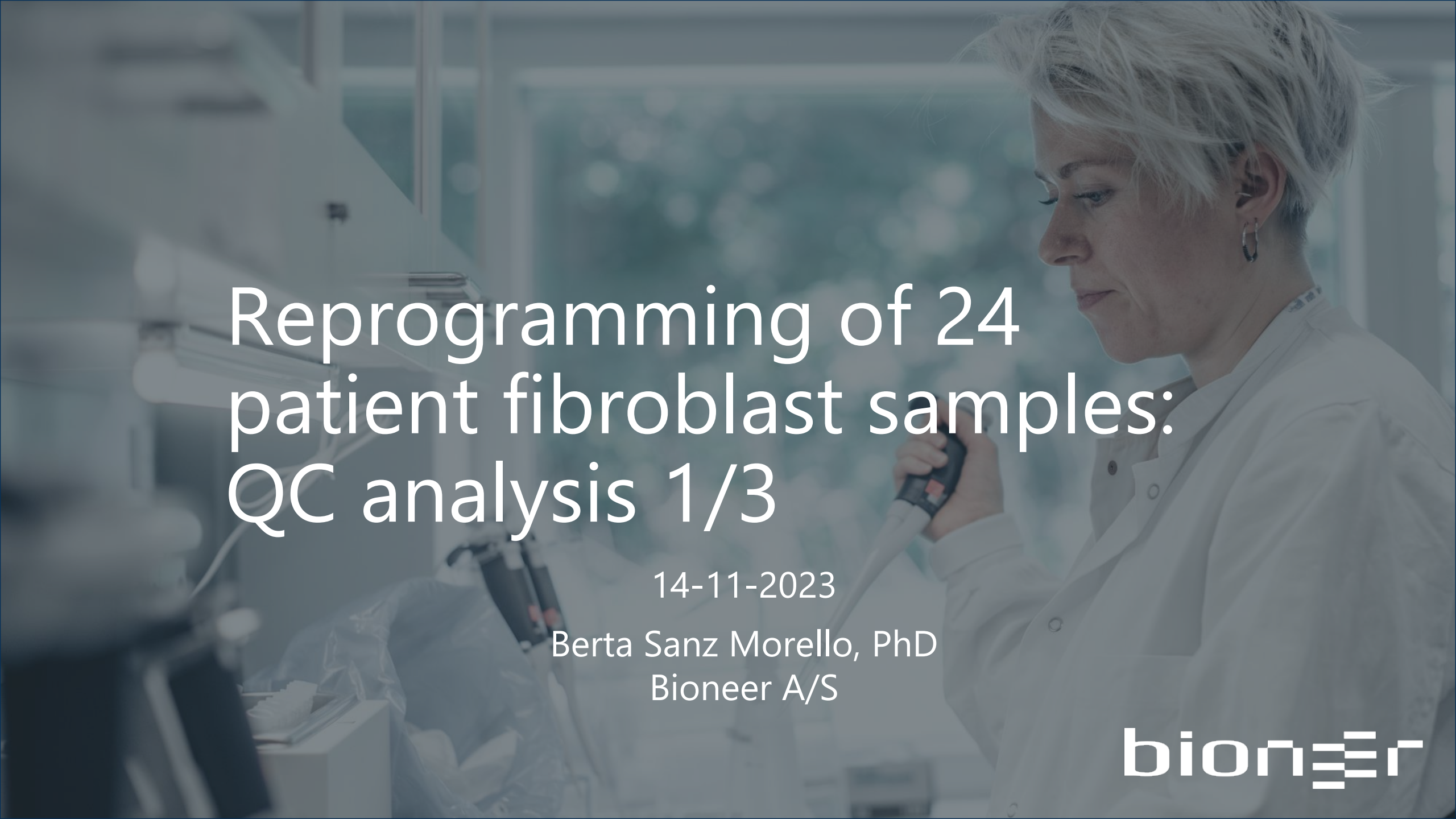


Reprogramming of 24 patient fibroblast samples: QC analysis 1/3

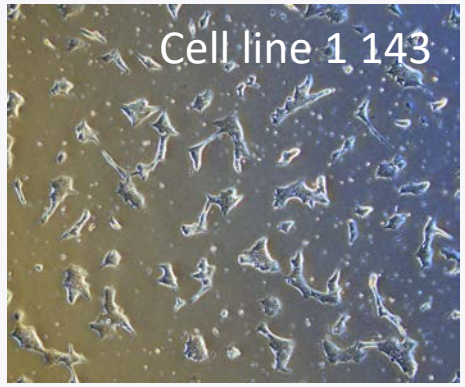
14-11-2023

Berta Sanz Morello, PhD
Bioneer A/S

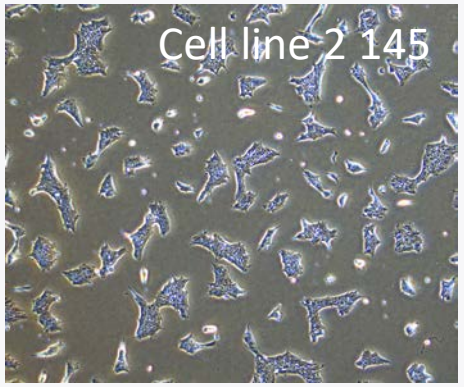
Morphology

Morphology of iPSCs 24 hours after thawing:

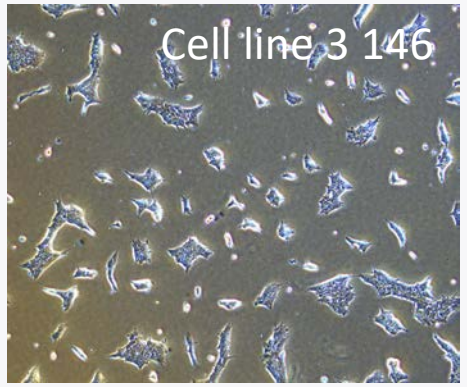
hPSCreg ID
SUHi001-A



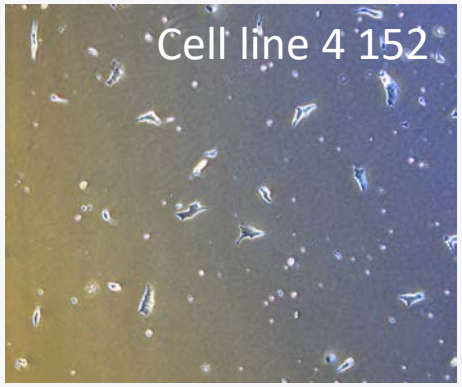
hPSCreg ID
SUHi002-A



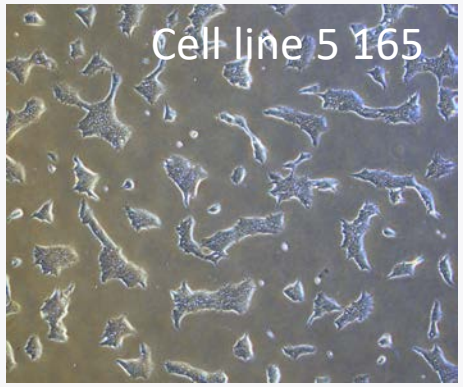
hPSCreg ID
SUHi003-A



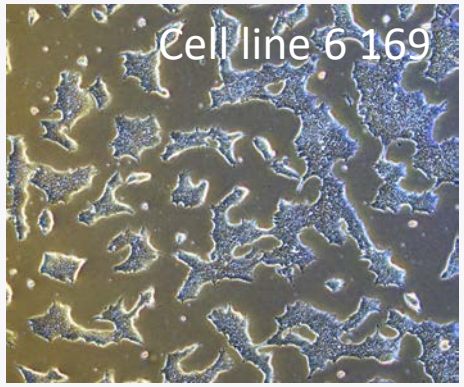
hPSCreg ID
SUHi004-A



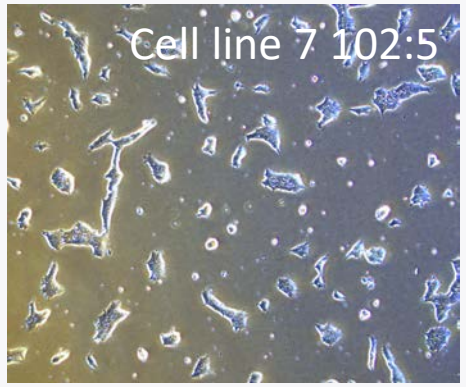
hPSCreg ID
SUHi005-A



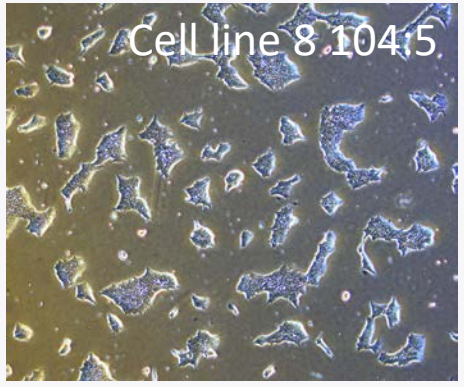
hPSCreg ID
SUHi006-A



hPSCreg ID
SUHi007-A



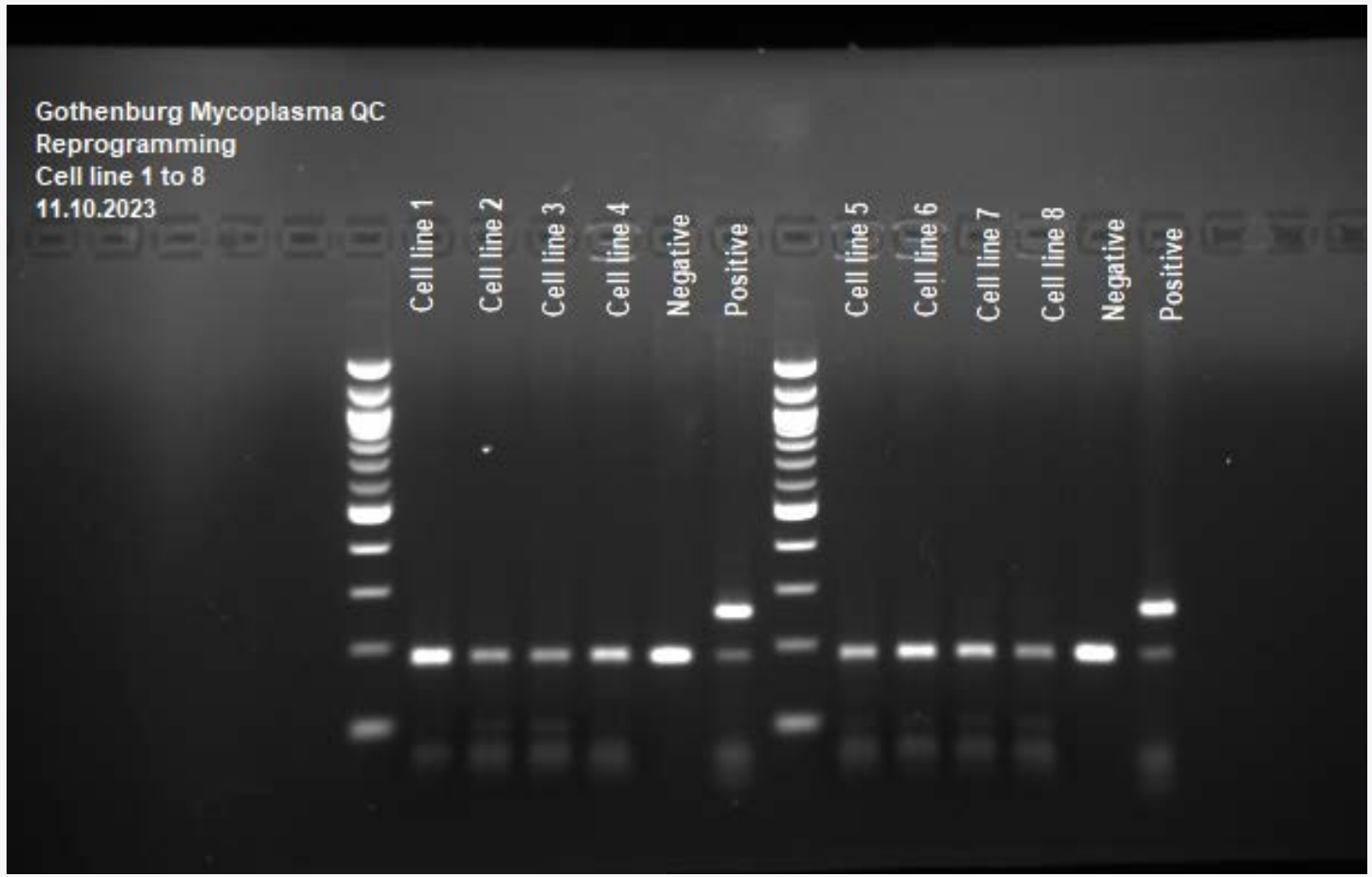
hPSCreg ID
SUHi008-A



QC Overview

hPSCreg ID	Line ID	Clone	Mycoplasma	Sterility	Trilineage diff.
SUHi001-A	Cell line 1 143	1	Passed	Passed	Passed
SUHi002-A	Cell line 2 145	2	Passed	Passed	Passed
SUHi003-A	Cell line 3 146	1	Passed	Passed	Passed
SUHi004-A	Cell line 4 152	3	Passed	Passed	Passed
SUHi005-A	Cell line 5 165	2	Passed	Passed	Passed
SUHi006-A	Cell line 6 169	3	Passed	Passed	Passed
SUHi007-A	Cell line 7 102:5	1	Passed	Passed	Passed
SUHi008-A	Cell line 8 104:5	2	Passed	Passed	Passed

Mycoplasma test



hPSCreg ID	Line ID	Clone
SUHi001-A	Cell line 1 143	1
SUHi002-A	Cell line 2 145	2
SUHi003-A	Cell line 3 146	1
SUHi004-A	Cell line 4 152	3
SUHi005-A	Cell line 5 165	2
SUHi006-A	Cell line 6 169	3
SUHi007-A	Cell line 7 102:5	1
SUHi008-A	Cell line 8 104:5	2

Sterility test

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

Trilineage differentiation

		Fold over reference sample			
hPSCreg ID	SAMPLES	GATA4 (Endo and Meso marker)	CXCR4 (Meso and Endo marker)	Pax6 (Ecto marker)	GAPDH
SUHi001-A	Cell line1 143 CL1 iPSC	1	1	1	1
SUHi002-A	Cell line2 145 CL2 iPSC	1	1	1	1
SUHi003-A	Cell line3 146 CL1 iPSC	1	1	1	1
SUHi004-A	Cell line4 152 CL3 iPSC	1	1	1	1
SUHi005-A	Cell line5 165 CL2 iPSC	1	1	1	1
SUHi006-A	Cell line6 169 CL3 iPSC	1	1	1	1
SUHi007-A	Cell line7 102:5 CL1 iPSC	1	1	1	1
SUHi008-A	Cell line8 104:5 CL2 iPSC	1	1	1	1
SUHi001-A	Cell line1 143 CL1 Endo	13873,08	328,56	152,57	1
SUHi002-A	Cell line2 145 CL2 Endo	843,36	61,11	8,00	1
SUHi003-A	Cell line3 146 CL1 Endo	388,92	36,34	1,04	1
SUHi004-A	Cell line4 152 CL3 Endo	9464,65	454,04	435,54	1
SUHi005-A	Cell line5 165 CL2 Endo	543,70	381,80	163,90	1
SUHi006-A	Cell line6 169 CL3 Endo	768,91	98,70	9,23	1
SUHi007-A	Cell line7 102:5 CL1 Endo	6762,46	249,58	303,03	1
SUHi008-A	Cell line8 104:5 CL2 Endo	1376,38	293,39	32,07	1
SUHi001-A	Cell line1 143 CL1 Meso	672,47	692,98	0,07	1
SUHi002-A	Cell line2 145 CL2 Meso	115,36	399,86	0,03	1
SUHi003-A	Cell line3 146 CL1 Meso	499,15	550,02	0,02	1
SUHi004-A	Cell line4 152 CL3 Meso	350,92	463,58	0,12	1
SUHi005-A	Cell line5 165 CL2 Meso	50,80	542,45	0,05	1
SUHi006-A	Cell line6 169 CL3 Meso	300,25	574,70	0,08	1
SUHi007-A	Cell line7 102:5 CL1 Meso	243,31	558,99	0,12	1
SUHi008-A	Cell line8 104:5 CL2 Meso	222,86	705,91	0,10	1
SUHi001-A	Cell line1 143 CL1 Ecto	0,65	52,83	4781,78	1
SUHi002-A	Cell line2 145 CL2 Ecto	0,08	23,00	2057,49	1
SUHi003-A	Cell line3 146 CL1 Ecto	0,18	33,51	1266,53	1
SUHi004-A	Cell line4 152 CL3 Ecto	0,17	53,69	5832,91	1
SUHi005-A	Cell line5 165 CL2 Ecto	0,02	27,22	3026,29	1
SUHi006-A	Cell line6 169 CL3 Ecto	0,08	21,01	2929,96	1
SUHi007-A	Cell line7 102:5 CL1 Ecto	0,35	20,73	4544,80	1
SUHi008-A	Cell line8 104:5 CL2 Ecto	0,09	62,25	8441,79	1

Fold over reference sample is based on the formula:

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

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

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

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

22-03-2024

Berta Sanz Morello, PhD
Bioneer A/S

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:

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

Where:
ΔCt = Ct gene of interest – 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.