iCS-digital™ Controls

Ready-to-use positive and normal controls for recurrent genomic abnormalities reported in human pluripotent stem cell lines. These controls can be used with the iCS-digital™ PSC 24-probe and iCS-digital™ 20q-only kits.
20 reactions for the positive controls / 10 tests for the normal control
Store at -20°C

For Research Use Only

Content

Product	Quantity (volume)	Content
Control 1	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with Xp, 12p and 20q anomalies
Control 2	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with 18q, 9q and 17q anomalies
Control 3	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with 1q, 17p and 5q anomalies
Control 4	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with 11p, 13q and 7q anomalies
Control 5	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with 1p, 4q and 3p anomalies
Control 6	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with 19p, 14q and 8q anomalies
Control 7	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with 15q, 6q and 7p anomalies
Control 8	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with 22q, 16q and 2q anomalies
Control 20q-only	20 reactions (30 µL – 5 ng/µL)	Positive control DNA (male) with 20q anomaly
Control DNA (male)	10 tests (30 µL – 50 ng/µL)	**CNV = 1 at the ChrXp** region and **CNV = 2** in the other 23 regions

Reagent Storage

Upon reception, the positive and normal controls must be stored at -20°C.
Repeated freezing and thawing must be avoided.

Use Precautions

For all handling, laboratory coats and gloves must be worn.

Required Reagents and Equipment

Instruments
- Droplet Generator from Bio-Rad (recommended: QX200™ #186-4003) - Droplet Reader from Bio-Rad (recommended: QX200™ #186-4003) - 96-well Thermal Cycler - Benchtop centrifuge - Benchtop vortex - Plate Sealer adapted for the Bio-Rad technology (recommended: PX1™ PCR Plate Sealer #181-4000)
Materials
- Pipettes and pipette tips (delivering volumes from 1 µL to 1000 µL) - 1.5 mL reaction tubes - QX200 Bio-Rad ddPCR™ consumables (Droplet Generation Oil for Probes, DG8™ Cartridges, DG8 Cartridge Holder, DG8 Gaskets, ddPCR™ 96-Well PCR Plates and Heat Seal Pierceable Foil)
Reagents
- ddPCR™ Supermix for Probes (No dUTP) from Bio-Rad (#186033) - HindIII-HF enzyme (e.g., New England Biolabs #R3104L) - Nuclease-free water - iCS-digital™ PSC 24 probes or iCS-digital™ 20q-only kit

Instructions for Use

Each positive control must be used with the mix including primers and probes to detect the corresponding anomalies (see table below). Positive controls are used to verify the digital PCR workflow and reagents.

Product	MIX 1	MIX 2	MIX 3	MIX 4	MIX 5	MIX 6	MIX 7	MIX 8
Control 1	+	-	-	-	-	-	-	-
Control 2	-	+	-	-	-	-	-	-
Control 3	-	-	+	-	-	-	-	-
Control 4	-	-	-	+	-	-	-	-
Control 5	-	-	-	-	+	-	-	-
Control 6	-	-	-	-	-	+	-	-
Control 7	-	-	-	-	-	-	+	-
Control 8	-	-	-	-	-	-	-	+
Control 20q-only	+	-	-	-	-	-	-	-
Normal Control	-	-	-	-	-	-	-	-

Sample preparation

Positive controls are ready-to-use with a concentration of 5ng/µL. No dilution is required.
The normal control must be diluted to 5 ng/µL before use.

- If frozen, thaw the controls to room temperature. **Mix thoroughly by vortexing**, and briefly centrifuge. Good homogenization of the controls is critical to guarantee the quality of the final results. Therefore, we recommend that users **vigorously vortex** each control tube for 5-10 seconds, and then briefly centrifuge the tubes.

- Add 3 µL of control instead of sample in each corresponding mix.

Example of an experimental design with controls:

		1	2	3	4	5	6	7	8	9	10	11	12
MIX 1	A	Control 1	Control DNA	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
MIX 2	B	Control 2	Control DNA	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
MIX 3	C	Control 3	Control DNA	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
MIX 4	D	Control 4	Control DNA	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
MIX 5	E	Control 5	Control DNA	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
MIX 6	F	Control 6	Control DNA	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
MIX 7	G	Control 7	Control DNA	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
MIX 8	H	Control 8	Control DNA	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10

For more details on how to perform iCS-digital™ tests, please refer to the following user manual:

iCS-digital™ PSC 24 probe

iCS-digital™ 20q-only

Analysis of results

To analyze the PCR data, use the QuantaSoft™ Analysis Pro software (version 1.0.596) and refer to the user manual of the iCS-digital™ test to define the clusters.

A control can be validated according to the raw data values of CNV analyzed on the "Copy Number" tab of the QuantaSoft™ Analysis Pro software.

Product	Mix analyzed	CNV value
Control 1	MIX 1	Chr20q: CNV > 2.5 Chr12p: CNV > 2.5 Chr Xp: CNV > 2.0 (male sample)
Control 2	MIX 2	Chr18q: CNV > 2.5 Chr9q: CNV > 2.5 Chr17q: CNV > 2.5
Control 3	MIX 3	Chr1q: CNV > 2.5 Chr17p: CNV > 2.5 Chr5q: CNV > 2.5
Control 4	MIX 4	Chr11p: CNV > 2.5 Chr13q: CNV > 2.5 Chr7q: CNV > 2.5
Control 5	MIX 5	Chr1p: CNV > 2.5 Chr4q: CNV > 2.5 Chr3p: CNV > 2.5
Control 6	MIX 6	Chr19p: CNV > 2.5 Chr14q: CNV > 2.5 Chr8q: CNV > 2.5
Control 7	MIX 7	Chr15q: CNV > 2.5 Chr6q: CNV > 2.5 Chr7p: CNV > 2.5
Control 8	MIX 8	Chr22q: CNV > 2.5 Chr16q: CNV > 2.5 Chr2q: CNV > 2.5
Control 20q-only	MIX 1	Chr20q: CNV > 2.5
Control DNA (male)	MIX 1 to 8	**CNV = 1 at the ChrXp** region and **CNV = 2** in the other 23 regions

	Normal DNA Control		Positive Control
	1.97	1.96	3.83 / 3.18
	1.93		2.83

Legend: ☑ 1q (Ch1)   ☑ 17p (Ch2)   ☑ 5q (Ch1)
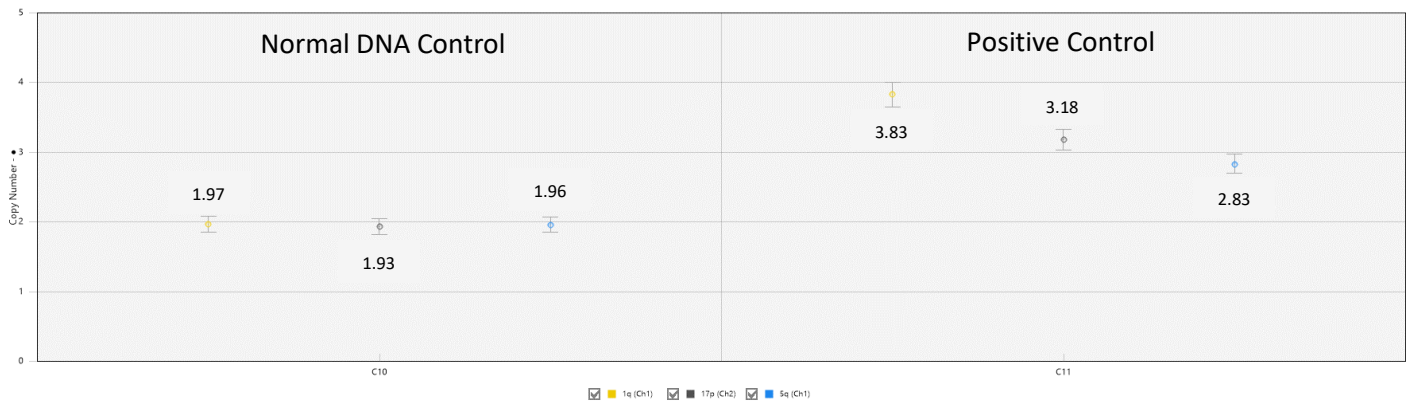
If you desire, you can obtain a profile of the positive control thanks to the iCS-digital™ software provided by Stem Genomics (https://kit.stemgenomics.com). The 8 mixes (MIX 1 to 8) are mandatory for an analysis with the iCS-digital™ software.

- Export the data of the column with positive controls and the column with the normal control in an Excel format

Note: For proper subsequent data processing, the exported file should contain the totality of the Well Data table columns.

- Copy/Paste all the lines referring to the normal DNA control including the table header in a new Excel file
- Copy the 4 lines corresponding to the MIX 1 for the CONTROL 1 (Chr20q, Reference, Chr12p and Chr Xp position) and paste the values over the values of the MIX 1 of the normal DNA control (see example below)

Left table:

	Well	Sample	Target	Conc(copies/µL)	Status	Experiment	SampleType	TargetType	Supermix	DyeName(s)
2	A10	Normal DNA control	20q	168.88	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
3	A10	Normal DNA control	RPP30	167.58	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
4	A10	Normal DNA control	Xp	80.95	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
5	A10	Normal DNA control	12p	166.12	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
6	B10	Normal DNA control	18q	155.02	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
7	B10	Normal DNA control	RPP30	169.32	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
8	B10	Normal DNA control	9q	172.48	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
9	B10	Normal DNA control	17q	160.33	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
10	C10	Normal DNA control	1q	199.89	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
11	C10	Normal DNA control	RPP30	202.79	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
12	C10	Normal DNA control	17p	195.96	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
13	C10	Normal DNA control	5q	199.04	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
14	D10	Normal DNA control	11p	172.98	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
15	D10	Normal DNA control	RPP30	170.69	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
16	D10	Normal DNA control	13q	166.11	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
17	D10	Normal DNA control	7q	160.79	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
18	E10	Normal DNA control	1p	152.96	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
19	E10	Normal DNA control	RPP30	155.01	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
20	E10	Normal DNA control	4q	157.06	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
21	E10	Normal DNA control	3p	160.78	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
22	F10	Normal DNA control	19p	154.56	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
23	F10	Normal DNA control	RPP30	166.14	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
24	F10	Normal DNA control	14q	158.28	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
25	F10	Normal DNA control	8q	168.87	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
26	G10	Normal DNA control	15q	171.76	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
27	G10	Normal DNA control	RPP30	187.21	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
28	G10	Normal DNA control	6q	179.50	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
29	G10	Normal DNA control	7p	177.32	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
30	H10	Normal DNA control	22q	175.77	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
31	H10	Normal DNA control	RPP30	188.86	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
32	H10	Normal DNA control	16q	185.69	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
33	H10	Normal DNA control	2q	178.76	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo

Right table:

	Well	Sample	Target	Conc(copies/µL)	Status	Experiment	SampleType	TargetType	Supermix	DyeName(s)
66	A11	Control 1	20q	392.69	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
67	A11	Control 1	RPP30	195.39	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
68	A11	Control 1	Xp	213.69	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
69	A11	Control 1	12p	316.68	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
70	B10	Normal DNA control	18q	155.02	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
71	B10	Normal DNA control	RPP30	169.32	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
72	B10	Normal DNA control	9q	172.48	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
73	B10	Normal DNA control	17q	160.33	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
74	C10	Normal DNA control	1q	199.89	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
75	C10	Normal DNA control	RPP30	202.79	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
76	C10	Normal DNA control	17p	195.96	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
77	C10	Normal DNA control	5q	199.04	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
78	D10	Normal DNA control	11p	172.98	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
79	D10	Normal DNA control	RPP30	170.69	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
80	D10	Normal DNA control	13q	166.11	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
81	D10	Normal DNA control	7q	160.79	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
82	E10	Normal DNA control	1p	152.96	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
83	E10	Normal DNA control	RPP30	155.01	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
84	E10	Normal DNA control	4q	157.06	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
85	E10	Normal DNA control	3p	160.78	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
86	F10	Normal DNA control	19p	154.56	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
87	F10	Normal DNA control	RPP30	166.14	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
88	F10	Normal DNA control	14q	158.28	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
89	F10	Normal DNA control	8q	168.87	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
90	G10	Normal DNA control	15q	171.76	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
91	G10	Normal DNA control	RPP30	187.21	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
92	G10	Normal DNA control	6q	179.50	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
93	G10	Normal DNA control	7p	177.32	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
94	H10	Normal DNA control	22q	175.77	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
95	H10	Normal DNA control	RPP30	188.86	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
96	H10	Normal DNA control	16q	185.69	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
97	H10	Normal DNA control	2q	178.76	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo

- Then rename all the lines "Control 1"

	A	B	C	D	E	F	G	H	I	J
1	Well	Sample	Target	Conc(copies/µL)	Status	Experiment	SampleType	TargetType	Supermix	DyeName(s)
66	A11	Control 1	20q	392.69	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
67	A11	Control 1	RPP30	195.39	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
68	A11	Control 1	Xp	213.69	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
69	A11	Control 1	12p	316.68	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
70	B10	Control 1	18q	155.02	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
71	B10	Control 1	RPP30	169.32	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
72	B10	Control 1	9q	172.48	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
73	B10	Control 1	17q	160.33	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
74	C10	Control 1	1q	199.89	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
75	C10	Control 1	RPP30	202.79	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
76	C10	Control 1	17p	195.96	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
77	C10	Control 1	5q	199.04	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
78	D10	Control 1	11p	172.98	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
79	D10	Control 1	RPP30	170.69	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
80	D10	Control 1	13q	166.11	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
81	D10	Control 1	7q	160.79	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
82	E10	Control 1	1p	152.96	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
83	E10	Control 1	RPP30	155.01	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
84	E10	Control 1	4q	157.06	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
85	E10	Control 1	3p	160.78	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
86	F10	Control 1	19p	154.56	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
87	F10	Control 1	RPP30	166.14	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
88	F10	Control 1	14q	158.28	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
89	F10	Control 1	8q	168.87	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
90	G10	Control 1	15q	171.76	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
91	G10	Control 1	RPP30	187.21	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
92	G10	Control 1	6q	179.50	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
93	G10	Control 1	7p	177.32	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo
94	H10	Control 1	22q	175.77	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Hi
95	H10	Control 1	RPP30	188.86	Manual	CNV	Unknown	Reference	ddPCR Super	HEX Lo
96	H10	Control 1	16q	185.69	Manual	CNV	Unknown	Unknown	ddPCR Super	HEX Hi
97	H10	Control 1	2q	178.76	Manual	CNV	Unknown	Unknown	ddPCR Super	FAM Lo

- Repeat the 3 previous steps to analyze all the positive controls on the same Excel file
- Submit the Excel data file to the iCS-digital™ software

For any inquiries regarding the use of the iCS-digital™ software, please contact our technical support team at services@stemgenomics.com.