iCS-digital™ Control

Ready-to-use positive and normal controls for recurrent genomic abnormalities reported in human pluripotent stem cell lines

20 reactions
40 reactions

Store at -20°C
For Research Use Only

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Version History

Version	Date	Description changes
v4_2406	June, 2024	Updated address and phone number for Stem Genomics
v3_2305	May, 2023	Addition of the product references
v2_230330	March, 2023	Addition of the version history table **Modification of protocol to use normal control DNA: dilution is no longer required; the normal control DNA is ready-to-use and concentrated to 5ng/µL**

Description

During their time in culture, human Pluripotent Stem Cells (hPSCs, i.e. embryonic stem cells and induced pluripotent stem cells) are prompt to acquire genomic alterations. These variants are often recurrent and non-random and affect the same genomic regions. These alterations confer a selective growth advantage or reduce the cells' differentiation capacities. Abnormal cells can completely take over the culture in a maximum of 5 passages.

The most common abnormalities are Copy Number Variations (CNV), DNA segments of one kilobase (kb) or larger that are present at an abnormal copy number compared to a reference genome. Normal copy number should be equal or close to the value of 2 for the human genome.

The iCS-digital™ Control products can be used with our iCS-digital™ PSC kit range. It includes abnormalities found on all the targeted positions in our 24-probe or 20q kits, ie. the most frequent abnormalities found in human pluripotent stem cells. A normal DNA control for the 24 targets is also available.

Kit Content

Product	Quantity (volume)		Content
	20 reactions	**40 reactions**	
Control 1	1 tube (60 µL – 5 ng/µL) *Ref: K101005-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101005-40*	Positive control DNA (male) with Xp, 12p and 20q anomalies
Control 2	1 tube (60 µL – 5 ng/µL) *Ref: K101006-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101006-40*	Positive control DNA (male) with 18q, 9q and 17q anomalies
Control 3	1 tube (60 µL – 5 ng/µL) *Ref: K101007-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101007-40*	Positive control DNA (male) with 1q, 17p and 5q anomalies
Control 4	1 tube (60 µL – 5 ng/µL) *Ref: K101008-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101008-40*	Positive control DNA (male) with 11p, 13q and 7q anomalies
Control 5	1 tube (60 µL – 5 ng/µL) *Ref: K101009-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101009-40*	Positive control DNA (male) with 1p, 4q and 3p anomalies
Control 6	1 tube (60 µL – 5 ng/µL) *Ref: K101010-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101010-40*	Positive control DNA (male) with 19p, 14q and 8q anomalies
Control 7	1 tube (60 µL – 5 ng/µL) *Ref: K101011-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101011-40*	Positive control DNA (male) with 15q, 6q and 7p anomalies
Control 8	1 tube (60 µL – 5 ng/µL) *Ref: K101012-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101012-40*	Positive control DNA (male) with 22q, 16q and 2q anomalies
Control 20q-only	1 tube (60 µL – 5 ng/µL) *Ref: K101013-20*	2 tubes (2 x 60 µL – 5ng/µL) *Ref: K101013-40*	Positive control DNA (male) with 20q anomaly
Normal Control DNA (male)	1 tube (240 µL – 5 ng/µL) *Ref: K101014-20*	2 tubes (2 x 240 µL – 5ng/µL) *Ref: K101014-40*	**CNV = 1 at the ChrXp** region and **CNV = 2** in the other 23 regions
Full panel of Positive Control 1 to 8	8 tubes (60 µL – 5 ng/µL) *Ref: K101004-20*	2 x 8 tubes (60 µL – 5ng/µL) *Ref: K101004-40*	See above

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Reagent Storage

Upon receipt, the positive and normal controls must be stored at -20°C.

Repeated freezing and thawing must be avoided.

Precautions for use

Laboratory coats and gloves must be worn for all handling

Required Reagents and Equipment

Instruments
- Droplet Generator from Bio-Rad (recommended: QX200™, ref: 1864003) - Droplet Reader from Bio-Rad (recommended: QX200™, ref: 1864003 or QX600™, ref: 12013328) - 96-well Thermal Cycler - Benchtop centrifuge - Benchtop vortex - Plate Sealer adapted for the Bio-Rad technology (recommended: PX1™ PCR Plate Sealer, ref: 1814000)
Materials
- Pipettes and pipette tips (delivering volumes from 1 µL to 1000 µL) - 1.5 mL reaction tubes - QX200/QX600 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 (ref: 186033) - HindIII-HF enzyme (e.g. New England Biolabs ref: R3104L) - Nuclease-free water - iCS-digital™ PSC 24-probe 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 and normal control are ready-to-use with a concentration of 5ng/µL. **No dilution is required**.
- 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 **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) or the QX Manager software (version 1.2) 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 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
Normal control DNA (male)	MIX 1 to 8	CNV = 1 at the ChrXp region and CNV = 2 in the other 23 regions

Example of a normal control DNA and a positive control:
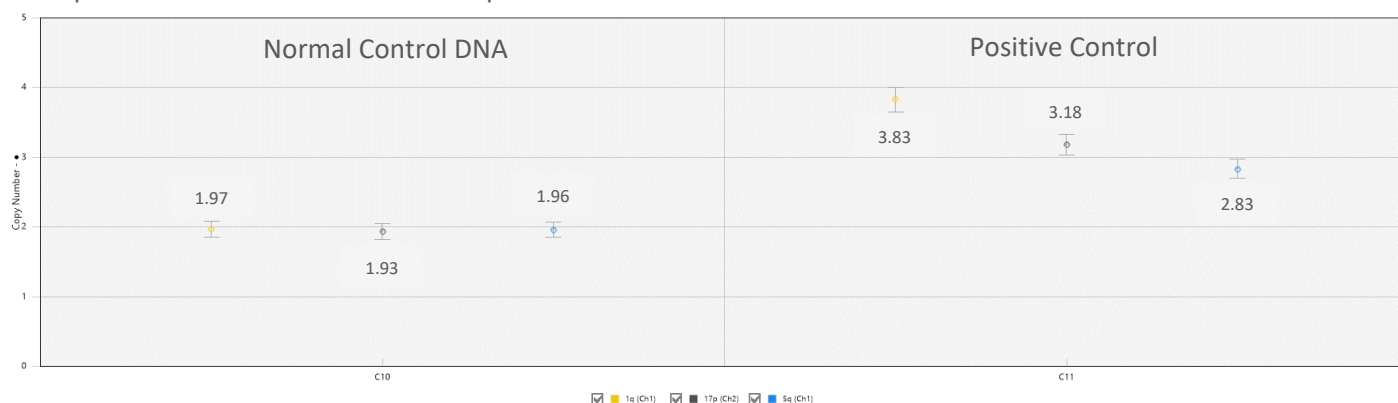
If you desire, you can obtain a profile of the positive control thanks to the iCS-digital™ software provided by Stem Genomics (https://kit.stemgenomics.com). The 8 mixes (MIX 1 to 8) are mandatory for an analysis with the iCS-digital™ software.

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

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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)
- Then rename all the lines "Control 1"
- 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.