



stemgenomics
cell integrity

iCS-digital™ PSC 20q-only kit

Ready-to-use digital PCR Mix for the detection of the sub-karyotypic 20q11.21 recurrent genomic abnormality in human pluripotent stem cell lines

20 tests – reference: K101003-20

40 tests – reference: K101003-40

Store at -20°C
For Research Use Only



Table of contents

Version History	3
Description	3
Kit Content	4
Reagent Storage	4
Precautions for use	4
Required Reagents and Equipment.....	4
Instructions for Use	5
Sample preparation.....	5
DNA purity and quantification	5
Digital PCR reagent preparation.....	5
PCR program.....	6
Reader Setup	6
With the QuantaSoft™ software	6
With the QX Manager software	7
Analysis of results.....	8
QuantaSoft™ Analysis Pro (v1.0) cluster analysis	8
QX Manager (v1.2) cluster analysis	9



Version History

Version	Date	Description of changes
v9_2305	May, 2023	Addition of the product references Addition of the analysis protocol with QX Manager software Addition of the data analysis with the iCS-digital™ software
v8_2303	March, 2023	Adding 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™ PSC 20q-only kit allows the reliable quantification of the sub-karyotypic 20q11.21 amplification. Gain of 20q11.21 copy-number variant (CNV) is detected in more than 20% of worldwide cultured human Pluripotent Stem Cells (hPSCs)¹²³ and represents 22.9% of the recurrent structural variants identified in hPSCs 4, making it the most common genomic abnormality in hPSCs⁴. The mix assay allows the precise detection of the recurrent 20q11.21 CNVs in hPSCs, and testing can be performed in 8 samples simultaneously. The kit also includes a validated normal genomic DNA control.

¹ Halliwell J, Barbaric I, Andrews PW. Acquired genetic changes in human pluripotent stem cells: origins and consequences. Nat Rev Mol Cell Biol. 2020 Dec;21(12):715-728

² Baker D, Hirst AJ, Gokhale PJ, Juarez MA, Williams S, Wheeler M, Bean K, Allison TF, Moore HD, Andrews PW, Barbaric I. Detecting Genetic Mosaicism in Cultures of Human Pluripotent Stem Cells. Stem Cell Reports. 2016 Nov 8;7(5):998-1012

³ Avery S, Hirst AJ, Baker D, Lim CY, Alagaratnam S, Skotheim RI, Lothe RA, Pera MF, Colman A, Robson P, Andrews PW, Knowles BB. BCL-XL mediates the strong selective advantage of a 20q11.21 amplification commonly found in human embryonic stem cell cultures. Stem Cell Reports. 2013 Oct 31;1(5):379-86

⁴ Assou S, Girault N, Plinet M, et al. Recurrent Genetic Abnormalities in Human Pluripotent Stem Cells: Definition and Routine Detection in Culture Supernatant by Targeted Droplet Digital PCR. Stem Cell Reports. 2020;14(1):1-8

Kit Content

Product	Quantity		Content
	20 tests Ref: K101003-20	40 tests Ref: K101003-40	
Mix 20q	1 tube (60 µL)	2 tubes (2 x 60 µL)	- Reference assay (HEX) - Chr20q assay (FAM)
Normal Control DNA	1 tube (240 µL)	1 tube (240 µL)	Normal control DNA (male) with CNV = 1 at the ChrXp region and CNV = 2 in the other 23 regions

Reagent Storage

Upon receipt, the kit must be stored at -20°C and protected from light.
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
<ul style="list-style-type: none"> - 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
<ul style="list-style-type: none"> - 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
<ul style="list-style-type: none"> - ddPCR™ Supermix for Probes (No dUTP) from Bio-Rad (ref: 186033) - HindIII-HF enzyme (e.g. New England Biolabs ref: R3104L) - Nuclease-free water

Instructions for Use

Sample preparation

- 500,000 dissociated cells are sufficient for one test using the iCS-digital™ PSC 20q-only kit.
- Genomic DNA should be extracted using an appropriate DNA extraction method. It is recommended to use the QIAamp DNA Blood Mini Kit (Qiagen, ref: 51104) or the GenElute Mammalian Genomic DNA Miniprep Kits (Sigma-Aldrich, ref: G1N70-1KT) or other column extraction kits.

Washing and drying steps are very critical for the quality of the digital PCR. Follow the protocol carefully.

DNA purity and quantification

- Quantify the double-stranded DNA (dsDNA) in each sample using a Qubit™ fluorometer. At least 150 ng of dsDNA at a concentration of 5 ng/μL are necessary for one test.

Note: Use of the Qubit™ dsDNA HS Assay kit for DNA quantification is strongly encouraged because it generates highly accurate and precise results. Spectrophotometers tend to overestimate DNA concentrations, which can potentially increase the risk of errors in the subsequent data analysis.

- Dilute the DNA samples to a concentration of 5 ng/μL in molecular grade H₂O.
- Vortex the diluted DNA samples for 5 seconds and centrifuge briefly.

Digital PCR reagent preparation

- If frozen, thaw the mix assay and the ddPCR™ Supermix for Probes (No dUTP) at room temperature. **Mix thoroughly by vortexing**, and briefly centrifuge.

Good homogenization of the kit reagents is critical to guarantee the quality of the final results. Therefore, we recommend that users **vigorously vortex** each mix assay tube twice for 5-10 seconds, and briefly centrifuge.

- Calculate the number of samples to be tested, including the control DNA.
- Dilute the HindIII-HF restriction enzyme using the recommended dilution buffer to a concentration of 2 U/μL.
- Prepare enough reaction mix for all samples. It is recommended to prepare at least 10% more master mix than required for the total number of reactions to be performed.

Table 1. Reaction mix preparation for one sample

Order	Reagent	Volume
1	H ₂ O	5 μL
2	Mix 20q	2 μL
3	ddPCR™ Supermix for Probes (No dUTP) 2X	11 μL
4	HindIII-HF (2 U/μL)	1 μL
5	Genomic DNA (5 ng/μL)	3 μL

- Mix thoroughly by vortexing and briefly centrifuging.
- Load the master reaction mix in tubes or 96 well plates and add 3 μL of DNA sample to each tube or well.
- Load 20 μL of the reaction mixture in a sample well of a DG8™ Cartridge.
- Centrifuge the tubes or plate briefly.

- Add 70 µL of Droplet Generation Oil for Probes to the bottom wells of the cartridge (oil wells).
- Attach a gasket across the top of the DG8™ cartridge and place it in the Droplet Generator.
- After droplet generation, remove the gasket and transfer the droplets (40 µL) from the upper wells of the DG8™ cartridge into a single column of a 96-well PCR plate by pipetting gently.
- Seal the PCR plate using heat seal pierceable foil and a thermal plate sealer.

PCR program

- Perform thermal cycling as detailed in Table 2.

Table 2. Thermal cycling program

Stage	Number of cycles	Duration	Temperature	Ramp rate
Enzyme activation	1	10 min	95°C	
Denaturation	45	30 sec	95°C	2.5°C/sec
Annealing	45	1 min	60°C	
Enzyme deactivation	1	10 min	98°C	
Hold	1	Infinite	12°C	

- If required, set the reaction volume to 40 µL.
- The recommended lid temperature is 105°C.

Note: Leave the PCR plate in the cycler for at least 2 hours; it is possible to leave the plate overnight in the cycler. This step significantly increases the number of droplets.

Reader Setup

With the QuantaSoft™ software

For more details, consult the latest version of the Bio-Rad user guide available online at <https://www.bio-rad.com/>.

- Place the PCR plate in the plate holder of the QX200 Droplet Reader.
- Open the QuantaSoft™ software from the computer connected to the droplet reader and configure a new plate template in the plate editor, as follows:
 - For all wells
 - Experiment type - CNV2
 - Supermix type - ddPCR Supermix for Probes (No dUTP)
 - Target 1 Type: Ch1 Unknown
 - Target 1 Label: 20q
 - Target 2 Type: Ch2 Reference
 - Target 2 Label: ref
 - For each well
 - Sample name - to be specified by the user


Notes: The assigned name should be exactly the same for the 8 cells of the column. The sample name should not exceed 17 characters and special characters should be avoided (e.g. ~ ! @ # \$ ^ % & * ? { }).




- Click Run and select the FAM/HEX dye set.

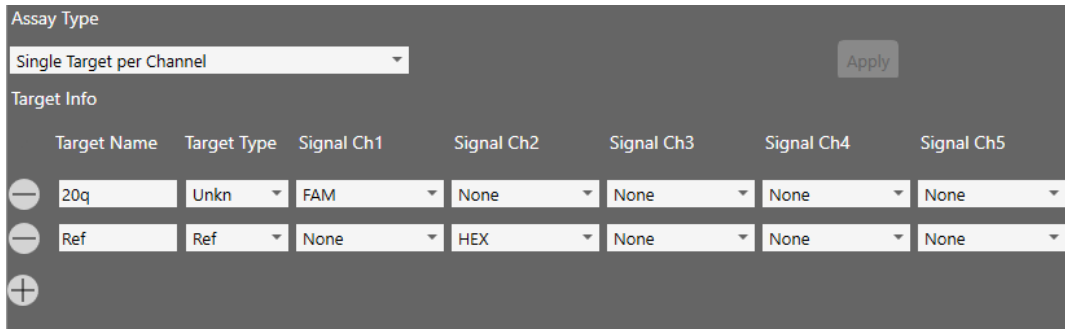
With the QX Manager software

For more details, consult the latest version of the Bio-Rad user guide available online at <https://www.bio-rad.com/>.

- Place the PCR plate in the plate holder of the QX200™ Droplet Reader or QX600™ Droplet Reader.
- Open the QX Manager software from the computer connected to the droplet reader.
- Go to the “Add Plate” tab , click on the “Add Plate” button and then on “Configure Plate”.
- Fill in the information in the “Plate Information (Required)” tab as follows:
 - o Supermix - ddPCR Supermix for Probes (No dUTP)
- In the “Well Selection (Required)” tab, select the wells to be analyzed and validate by clicking on “Include Selected Wells”.
- In the “Well Information (Optional)” tab, configure the plate as follows:
 - o For all wells
 - Experiment type – CNV
 - Assay Type: Single Target per Channel
 - o For each well
 - Sample name – to be specified by the user

Note: The assigned name should be exactly the same for the 8 cells of the column. The sample name should not exceed 17 characters and special characters should be avoided (e.g., ~ ! @ # \$ ^ % & * ? { }).

Note: the **QX Manager v2.0** is suitable for 6-colour multiplex analysis. When “Single Target per Channel” is selected as the Assay type, 6 lines are displayed for the 6 colors. Delete all the unused lines by clicking on the  symbol and keep only the two lines with the “FAM” and “HEX” labels.



Assay Type		Target Info						
Single Target per Channel		Target Name	Target Type	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5
−	20q	Unkn	FAM	None	None	None	None	None
−	Ref	Ref	None	HEX	None	None	None	None
+								

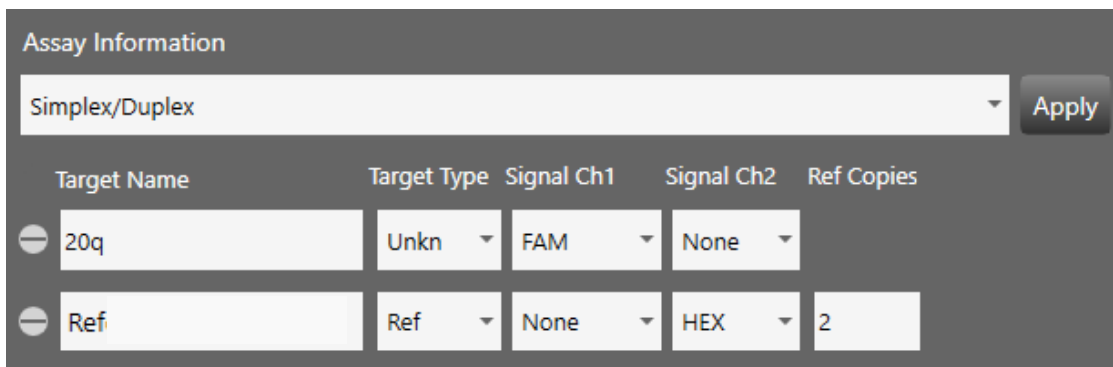
- Click Run.

Analysis of results

QuantaSoft™ Analysis Pro (v1.0) cluster analysis



For more details, consult the latest version of the Bio-Rad user guide available online at <https://www.bio-rad.com/>.

- In the “Plate Editor” tab (Figure 1), select all the wells to be analyzed.
- In Assay Information, select “Single Target per Channel” from the dropdown list.
- Refer to the Figure 1 below to fill in the target name, type and signal.
- Press “Apply” to save changes.



Assay Information		Target Name	Target Type	Signal Ch1	Signal Ch2	Ref Copies
Simplex/Duplex		20q	Unkn	FAM	None	
−	Ref	Ref	None	HEX		2

Figure 1. Example of QuantaSoft™ Analysis Pro Plate Editor tab parameters

- In the “2D Amplitude” tab (Figure 2), adjust the threshold using the Graph Tools, either manually (Threshold Cluster Mode ) or automatically (Threshold Line Mode ) , to assign each cluster to the appropriate target.

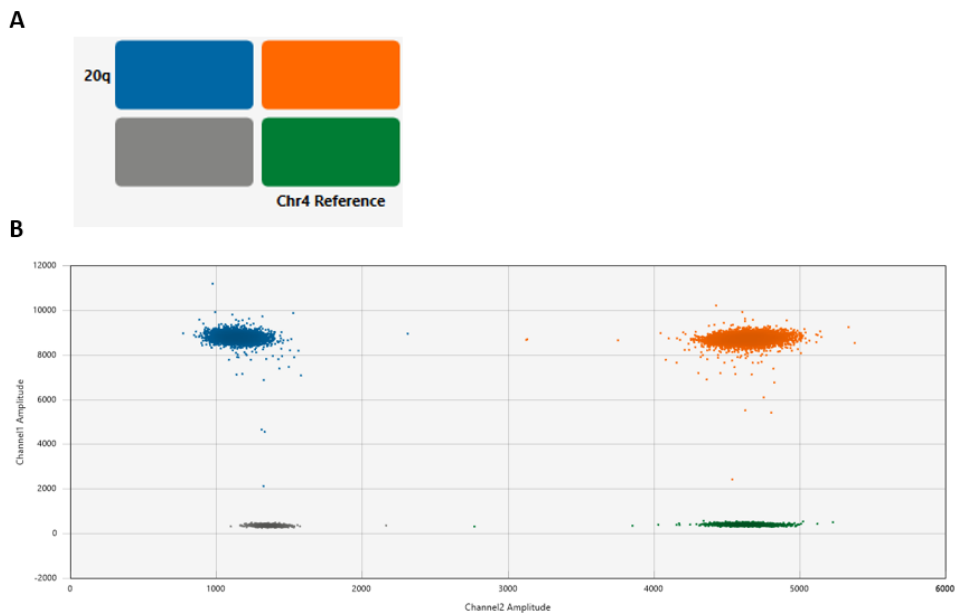
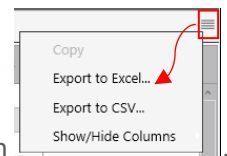


Figure 2. QuantaSoft™ Analysis Pro 2D Amplitude results. A. Example of 20q genetic assay target combination clusters. B. Example of a 2D plot after threshold assignment.

- Refer to the “Copy Number” and the “Data table” tabs for results analysis.



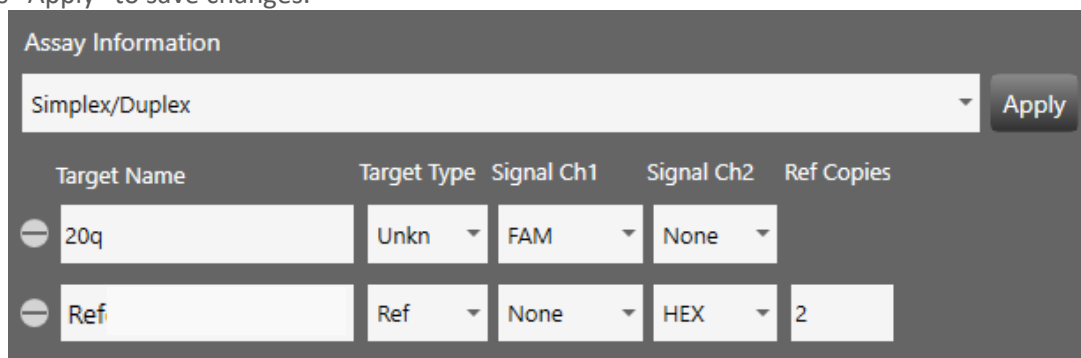
- Data can be exported for subsequent analysis through the “Data table” by clicking on

QX Manager (v1.2) cluster analysis

For more details, consult the latest version of the Bio-Rad user guide available online at <https://www.bio-rad.com/>.

Note: Plate configuration can be performed during the reader setup step (see the paragraph “Reader Setup – With the QX Manager software”).

- In the “Plate Editor” tab, select all the wells.
- In Assay Information, select “Single Target per Channel” from the dropdown list.
- Refer to the Figure 3 below to fill in the target name, type and signal.
- Press “Apply” to save changes.




Assay Information



Simplex/Duplex Apply

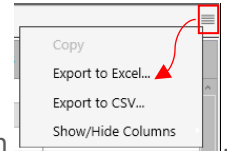
Target Name	Target Type	Signal Ch1	Signal Ch2	Ref Copies
20q	Unkn	FAM	None	
Ref	Ref	None	HEX	2

Figure 3. Example of QX Manager Plate Editor tab parameters



Note: the **QX Manager v2.0** is suitable for 6-colour multiplex analysis. When “Single Target per Channel” is selected as the Assay type, 6 lines are displayed for the 6 colors. Delete all the unused lines by clicking on the  symbol and keep only the two lines with the “FAM” and “HEX” labels.

- In the “2D Amplitude” tab (Figure 5), adjust the threshold using the Graph Tools, either manually (Threshold Cluster Mode ) or automatically (Threshold Line Mode ), to assign each cluster to the appropriate target.
- Refer to the “Copy Number” and the “Data table” tabs for results analysis.



- Data can be exported for subsequent analysis through the “Data table” by clicking on