

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

Store at -20°C

For Research Use Only

Description

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)¹⁻³ and represents 22.9% of the recurrent structural variants identified in hPSCs⁴, making it the most common genomic abnormality in hPSCs. 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 (volume)	Content
Mix 20q	20 tests	Chr4 Reference assay (HEX) Chr20q Target assay (FAM)
Control DNA	10 tests (30 μ L - 50 ng/ μ L)	Normal control DNA (male) with 2 copies of the 20q11.21 region

Reagent Storage

Upon reception, the kit must be stored at -20°C and protected from light.
Repeated freezing and thawing must be avoided.

Use Precautions

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

Required Reagents and Equipment

Instruments
<ul style="list-style-type: none"> - Droplet Generator from Bio-Rad (recommended: QX200™, catalogue #186-4003) - Droplet Reader from Bio-Rad (recommended: QX200™, catalogue #186-4003) - 96-well Thermal cycler - Benchtop centrifuge - Benchtop vortex - Plate sealer suitable for the Bio-Rad technology (recommended: PX1™ PCR Plate Sealer, catalogue #181-4000)
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 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 (#186033) - HindIII-HF enzyme (e.g., New England Biolabs #R3104L) - Nuclease-free water

Instructions for Use

Sample preparation

If cell culture supernatants are used as starting material:

Please, refer to our online video for instructions on how to collect supernatants:
<https://www.stemgenomics.com/resources>.

- Cells must be at least 70% confluent.
- Culture medium must have been in contact with the cells for at least 24 hours.
- Collect 1.5 mL of cell culture supernatant before cell passaging.
- At passaging, one fifth of dissociated cells (3.5cm culture plate) may be added to the supernatant sample to obtain more DNA.
- DNA isolation should be preferentially performed using a magnetic bead-based kit.

If cell pellets are used as starting material:

- 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.

DNA purity and quantification

- Quantify the double-stranded DNA (dsDNA) in each sample using a Qubit fluorometer. You will need at least 3 µL of dsDNA at a concentration of 5 ng/µL for one test.
Note: Using 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 and this can potentially increase the risk of errors in the subsequent data analysis.
- Vortex the DNA samples and the control DNA for at least 5 seconds, and centrifuge briefly.
- Dilute the DNA samples and control DNA to 5 ng/µL in 3 µL of molecular grade H₂O.
- Vortex the diluted DNA samples for 5 seconds, and centrifuge briefly.

Note: The A260/230 ratio of DNA samples should be between 1.8 and 2.2.

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 users to **vortex vigorously** the Mix assay tube twice for 5-10 seconds, and to briefly centrifuge the tube between each vortexing steps.

- 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 what required for the total number of reactions to be performed.
- The reaction volumes for one sample are detailed in Table 1.

- Mix thoroughly by vortexing the tubes and centrifuge briefly.

Table 1. Reaction mix preparation for one sample

Example for 1 sample	Volume
ddPCR™ Supermix for Probes (No dUTP) 2X	11 µL
Mix 20q	2 µL
HindIII-HF (2 U/µL)	1 µL
Genomic DNA (5 ng/µL)	3 µL
H ₂ O	q.s. 22 µL

- Load 20 µL of each reaction mixture in a sample well of a DG8™ Cartridge.
Note: 8 samples (including the control DNA) can be run in the same cartridge.
- Add 70 µL of Droplet Generation Oil for Probes in the bottom wells of the cartridge (oil wells).
- Attach a gasket across the top of the DG8™ cartridge and place it in the QX200 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	

- 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 4 hours, and if possible, overnight. This step increases significantly the number of droplets.

QuantaSoft™ Experiment Setup

- 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 (Figure 1), as follows:
 - For all wells:
 - Experiment type - CNV2
 - Supermix type - ddPCR Supermix for Probes (No dUTP)
 - Target 1
 - Type: Ch1 Unknown
 - Label: 20q
 - Target 2
 - Type: Ch2 Reference
 - Label: Chr4 Reference
 - For each well:
 - Sample name - to be specified by the user

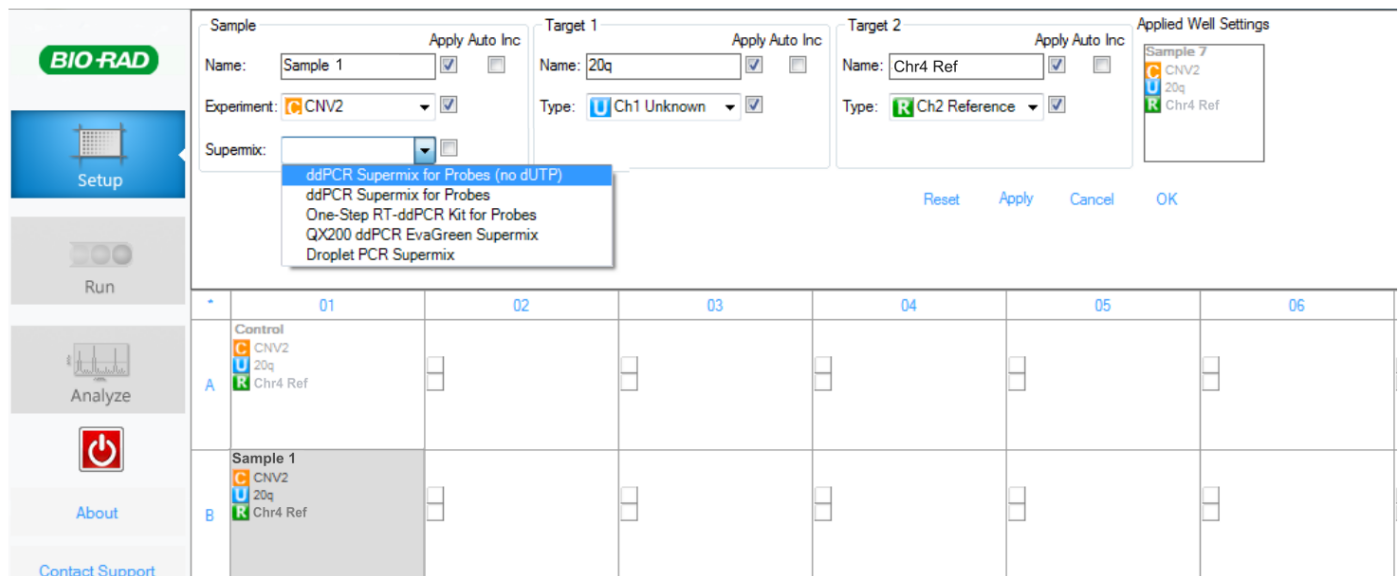


Figure 1. Example of plate configuration

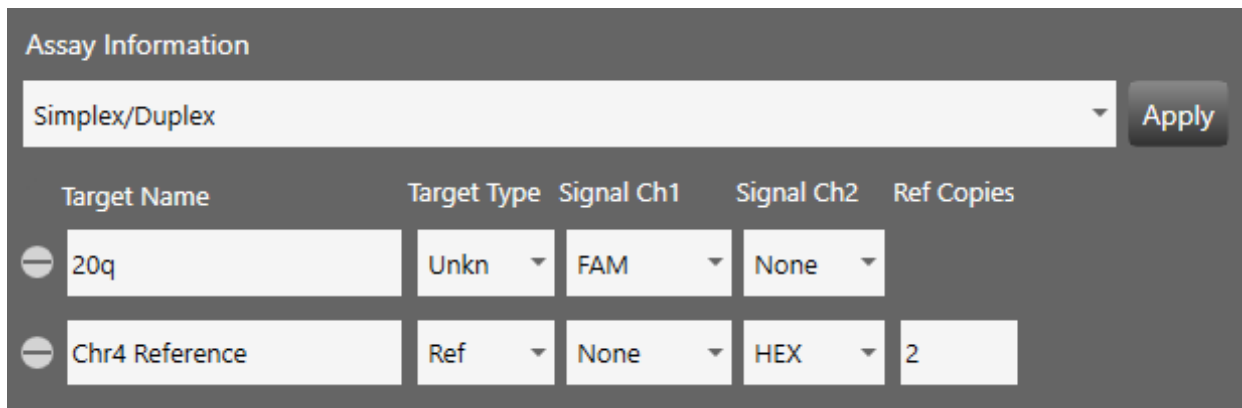
- Click Run and select the FAM/HEX dye set.

Analysis of results:

QuantaSoft™ cluster analysis

To analyse the PCR data, use the QuantaSoft™ Analysis Pro software (version 1.0.596):

- In the “Plate Editor” tab (Figure 2), select all the wells to be analysed.
- In Assay Information, select from the dropdown list “Simplex/Duplex”.
- Refer to Figure 1 below for filling in target name, type and signal.
- Press “Apply” to save the changes.

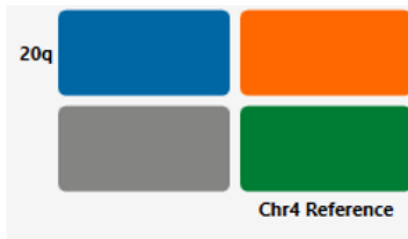


Target Name	Target Type	Signal Ch1	Signal Ch2	Ref Copies
20q	Unkn	FAM	None	
Chr4 Reference	Ref	None	HEX	2

Figure 2. Example of QuantaSoft™ Analysis Pro Plate Editor tab parameters

- In the “2D Amplitude” tab (Figure 3), 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.

A



B

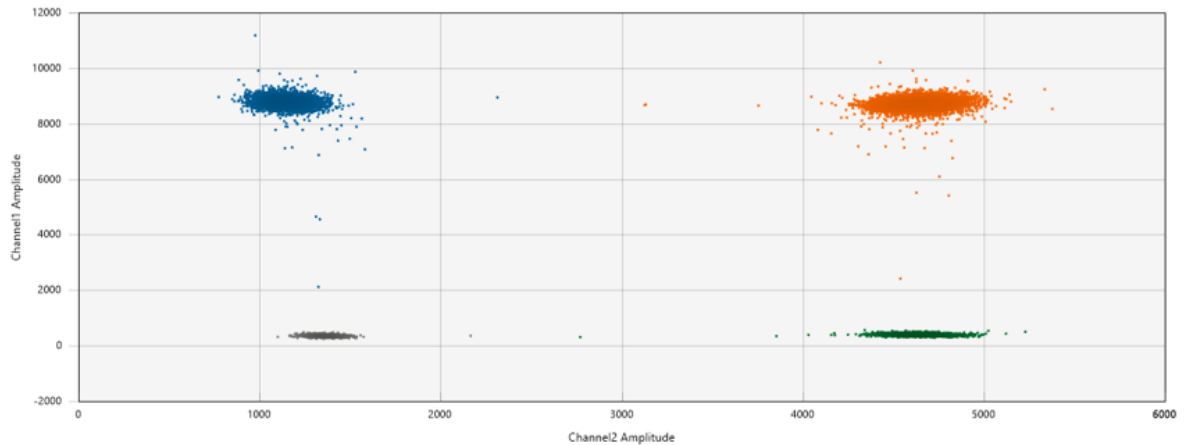


Figure 3. QuantaSoft™ Analysis 2D Amplitude results. A. Example of 20q genetic assay target combination clusters. B. Example of 2D plot after threshold assignment.

- Refer to the “Concentration” and “Copy Number” tabs for results analysis.
- Data can be exported for subsequent analyses through the Well Data table.