iCS-digital™ PSC 24-probe kit

Ready-to-use digital PCR mix for the detection of recurrent genomic abnormalities reported in human pluripotent stem cell lines

20 tests – reference: K101001-20
40 tests – reference: K101001-40

Store at -20°C
For Research Use Only

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

Table of contents

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

Version History

Version	Date	Description of changes
v13_2312	December 2023	Browser settings for online analysis added. Weblink to access the iCS-digital online software added. Weblink to access the tutorial video added.
v12_2307	July 2023	Addition of the QX One platform in compatible instruments Washing protocol for cell pellet or cryopreserved cells Addition of the exported data format from QX Manager software in the appendix 3

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 24-probe kit detects more than 90% of the most frequent genomic abnormalities in hPSCs. The test relies on multiplex digital PCR with double-quenched probes. The eight mix assays make it possible to target 24 genomic regions with efficient coverage of the most recurrent genomic defects described in hPSCs (i.e. copy number variations)[1]. The kit also includes a validated normal genomic DNA sample (XY) to be used as a control for the targeted genomic regions. Data processing, statistical analysis, and graphical representation of the results can be easily performed using the online iCS-digital™ analysis software provided by Stem Genomics.

[1] 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.

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

Kit Content

Product	Quantity		Content
	20 tests Ref: K101001-20	40 tests Ref: K101001-40	
Mix 1	1 tube (60 µL)	2 tubes (2 x 60 µL)	- ChrXp assay (HEX high) - **Reference assay (HEX low)** - Chr20q assay (FAM high) - Chr12p assay (FAM low)
Mix 2	1 tube (60 µL)	2 tubes (2 x 60 µL)	- Chr9q assay (HEX high) - **Reference assay (HEX low)** - Chr18q assay (FAM high) - Chr17q assay (FAM low)
Mix 3	1 tube (60 µL)	2 tubes (2 x 60 µL)	- Chr17p assay (HEX high) - **Reference assay (HEX low)** - Chr1q assay (FAM high) - Chr5q assay (FAM low)
Mix 4	1 tube (60 µL)	2 tubes (2 x 60 µL)	- Chr13q assay (HEX high) - **Reference assay (HEX low)** - Chr11p assay (FAM high) - Chr7q assay (FAM low)
Mix 5	1 tube (60 µL)	2 tubes (2 x 60 µL)	- Chr4q assay (HEX high) - **Reference assay (HEX low)** - Chr1p assay (FAM high) - Chr3p assay (FAM low)
Mix 6	1 tube (60 µL)	2 tubes (2 x 60 µL)	- Chr14q assay (HEX high) - **Reference assay (HEX low)** - Chr19p assay (FAM high) - Chr8q assay (FAM low)
Mix 7	1 tube (60 µL)	2 tubes (2 x 60 µL)	- Chr6q assay (HEX high) - **Reference assay (HEX low)** - Chr15q assay (FAM high) - Chr7p assay (FAM low)
Mix 8	1 tube (60 µL)	2 tubes (2 x 60 µL)	- Chr16q assay (HEX high) - **Reference assay (HEX low)** - Chr22q assay (FAM high) - Chr2q assay (FAM low)
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

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

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
- Droplet Generator from Bio-Rad (recommended: QX200™, ref: 1864003) - Droplet Reader from Bio-Rad (recommended: QX200™, ref: 1864003 or QX600™, ref: 12013328) Note: the QX100™ Droplet Digital™ PCR System and the QX ONE ddPCR™ Droplet Digital PCR System are compatible with the iCS-digital™ PSC 24-probe kit - 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

Instructions for Use

Sample preparation

500,000 dissociated cells are sufficient for one test using the iCS-digital™ PSC 24-probe kit. Washing steps are required depending on the sample:

- Dry cell pellet: wash the cell pellet once.
    - Resuspend the cell pellet in 1 mL of PBS 1X.
    - Centrifuge 2 min at 20,000g.
    - Discard the supernatant.
    - Resuspend the cell pellet in 200 µL of PBS 1X for extraction.
- Cryopreserved cells or cells in medium: wash the cell pellet three times.
    - Resuspend the cell pellet in 1 mL of PBS 1X.
    - Centrifuge 2 min at 20,000g.

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

- Discard the supernatant.
- Repeat the previous steps twice.
- Resuspend the cell pellet in 200 µL of PBS 1X for extraction.

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 H_20.
- Vortex the diluted DNA samples for 5 seconds and centrifuge briefly.

Digital PCR reagent preparation

- If frozen, thaw the mix assays 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 (often included in the same box as the restriction enzyme) to a concentration of 2 U/µL.
- Prepare 8 Master Reaction Mixes according to table 1 and as detailed below. 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.
    - Prepare a working solution with ddPCR™ Supermix for Probes (No dUTP) + HindIII-HF + H_2O for the required number of reactions + 10% considering the 8 Assay Mixes
    - Prepare 8 new tubes (one per mix) and distribute the required volume of the working solution ddPCR™ Supermix for Probes (No dUTP) + HindIII-HF + H_2O to each of them
    - Add the required volume of each 8 Assay Mix provided in the kit, to each of the corresponding tubes

The 8 Assay Mixes **must be used every time** to guarantee the proper analysis and data interpretation through the online iCS-digital™ analysis software.

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

Table 1. Reaction mix preparation for one sample

Working Solution	H_2O	ddPCR™ Supermix for Probes (No dUTP) 2X	HindIII-HF (2 U/µL)
For 1 sample / MIX 1 to 8	32 µL	88 µL	8 µL

Distribution in the 8 tubes

Master reaction mix	Working Solution	Assay MIX
For 1 sample / **MIX 1**	16 µL	**3 µL**
For 1 sample / **MIX 2**	16 µL	**3 µL**
For 1 sample / **MIX 3**	16 µL	**3 µL**
For 1 sample / **MIX 4**	16 µL	**3 µL**
For 1 sample / **MIX 5**	16 µL	**3 µL**
For 1 sample / **MIX 6**	16 µL	**3 µL**
For 1 sample / **MIX 7**	16 µL	**3 µL**
For 1 sample / **MIX 8**	16 µL	**3 µL**

- Mix thoroughly by vortexing and briefly centrifuging.
- Load each 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 each reaction mixture in a sample well of a DG8™ Cartridge (refer to APPENDIX 1 - iCS-digital™ PSC 24-probe mix preparation for a schematic representation of the cartridge).
- Centrifuge the tubes or plate briefly.

Note: One full cartridge is necessary to test one sample (n=8 reaction mixtures).

- 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.

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

- 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 2 Label and type - Refer to Table 3

    - *For each column*
      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. ~ ! @ # $ ^ % & * ? { }).

    - *For each row*
      Target 1 Label and type - Refer to Table 3

Table 3. Plate editor configuration for the QX200 droplets reader

Mix	Label Target 1	Label Target 2
	Type: Ch1 Unknown	Type: Ch2 Reference
Mix 1	20q	Reference
Mix 2	18q	
Mix 3	1q	
Mix 4	11p	
Mix 5	1p	
Mix 6	19p	
Mix 7	15q	
Mix 8	22q	

- 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/.

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

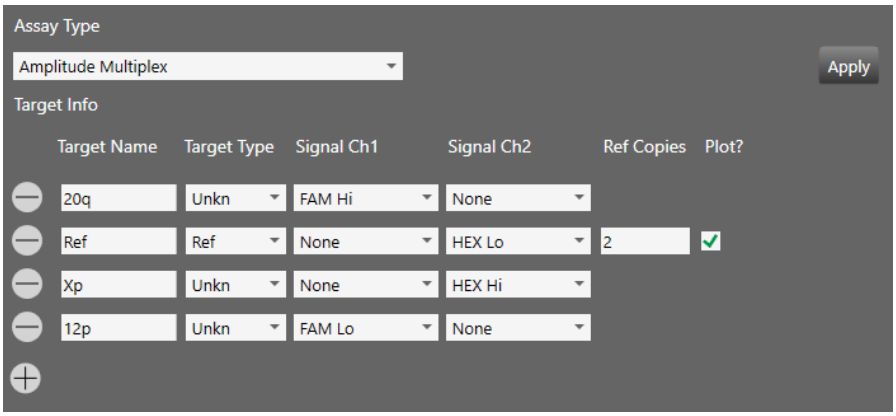
- Place the PCR plate in the plate holder of the QX200™ Droplet Reader or QX600™ Droplet Reader.
- Open the QX Manager software from the computer connected to the droplet reader.
- Go to the "Add Plate" tab  , click on the "Add Plate" button and then on "Configure Plate".
- Fill in the information in the "Plate Information (Required)" tab as follows:
    - 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:
    - *For all wells*
      Experiment type – CNV
      Assay Type: Amplitude Multiplex

    - *For each column*
      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., ~ ! @ # $ ^ % & * ? { }).

    - *For each row*
      Refer to the APPENDIX 2 - Plate Editor - Mix Assay Information to assign target and signal

Note: the **QX Manager v2.0** is suitable for 6-colour multiplex analysis. When "Amplitude Multiplex" is selected as the Assay type, 12 lines are displayed (low and high concentration for the 6 colors). Delete all the unused lines by clicking on the ⊖ symbol and keep only the four lines with the "FAM Hi", "HEX lo", "HEX Hi" and "FAM lo" labels.



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

Analysis of results

If you need help with this step, please watch the tutorial video here: https://www.youtube.com/watch?v=qqGDrJzrDLU. A troubleshooting guide is included at the end of these instructions for use in APPENDIX 5 - Troubleshooting for the iCS-digital™ PSC 24-probe kit.

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 wells in one row (i.e. all wells containing the same mix).
- In Assay Information, select "Amplitude multiplex" from the dropdown list.
- Refer to APPENDIX 2 - Plate Editor - Mix Assay Information to fill in the target name, type and signal.
- Press "Apply" to save changes.

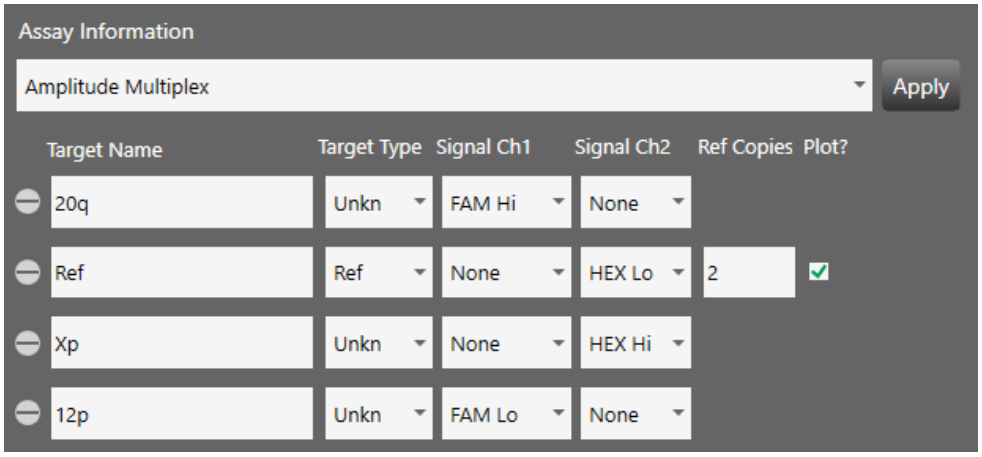


Figure 1. Example of QuantaSoft™ Analysis Pro Plate Editor tab parameters for Mix 1 assay

- 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.

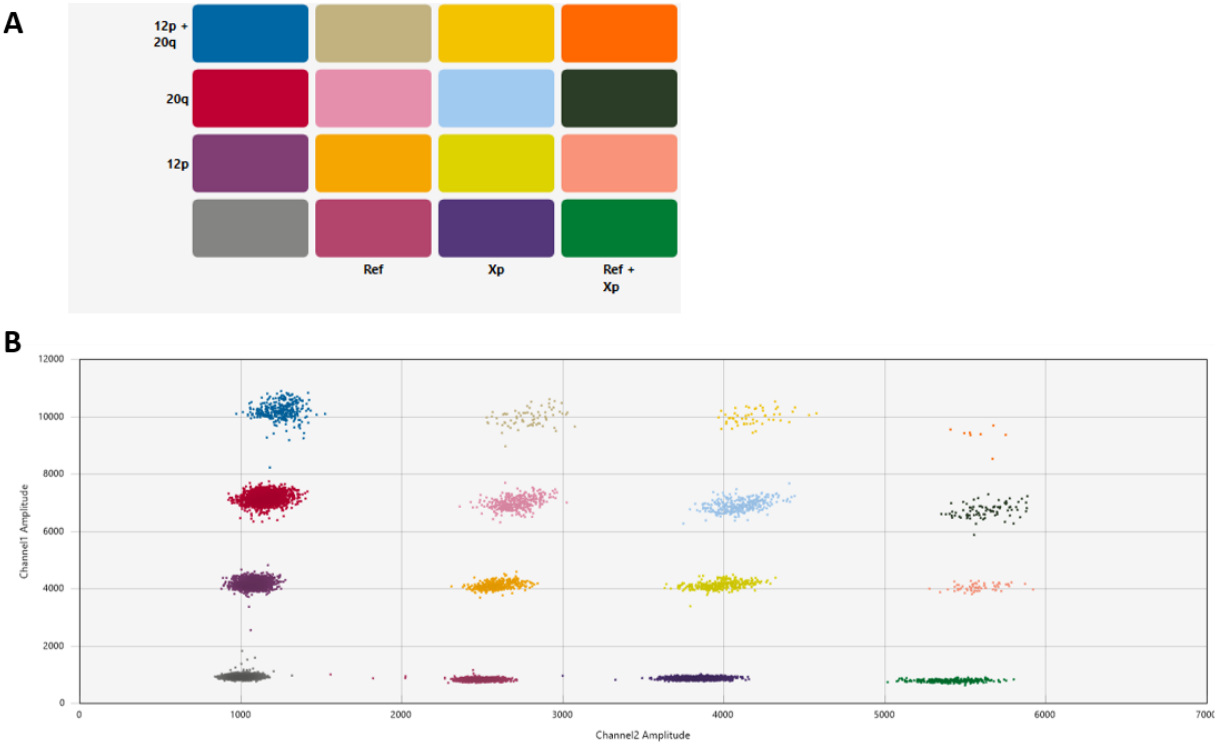


Figure 2. QuantaSoft™ Analysis Pro 2D Amplitude results. A. Example of Mix 1 assay target combination clusters. B. Example of a 2D plot after threshold assignment.

- In the 2D amplitude tab, select all wells for all samples in the Well Selector table and sort the lines by "**Sample**" name in the Well Data table (Figure 3; upward pointing arrow). Samples should then be listed in alphabetical

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

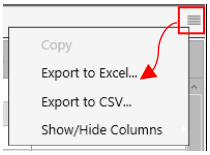
order and for each of them, the "**Target**" column must appear in the same order as in APPENDIX 2 - Plate Editor - Mix Assay Information and APPENDIX 3 - Example of an Excel file exported from Bio-Rad software (i.e. beginning with the Target named 20q and finishing with the Target named 2q).

Proper data sorting by "**Sample**" name is mandatory to ensure correct subsequent data processing and report generation using the iCS-digital™ software provided by Stem Genomics.

Well Data

Well	Sample	Target	Conc(copies/µL)	Status	Experiment	SampleType	TargetType	Supermix	Dye
A01	Control	20q	3.77	Manual	CNV	Unknown	Unknown	ddPCR Su...	FAM
A01	Control	Ref	3.21	Manual	CNV	Unknown	Reference	ddPCR Su...	HEX
A01	Control	Xp	1.96	Manual	CNV	Unknown	Unknown	ddPCR Su...	HEX
A01	Control	12p	2.93	Manual	CNV	Unknown	Unknown	ddPCR Su...	FAM
B01	Control	18q	4.19	Manual	CNV	Unknown	Unknown	ddPCR Su...	FAM
B01	Control	Ref	4.75	Manual	CNV	Unknown	Reference	ddPCR Su...	HEX
B01	Control	9q	2.65	Manual	CNV	Unknown	Unknown	ddPCR Su...	HEX
B01	Control	17q	2.86	Manual	CNV	Unknown	Unknown	ddPCR Su...	FAM
C01	Control	1q	3.18	Manual	CNV	Unknown	Unknown	ddPCR Su...	FAM
C01	Control	Ref	3.53	Manual	CNV	Unknown	Reference	ddPCR Su...	HEX
C01	Control	17p	3.46	Manual	CNV	Unknown	Unknown	ddPCR Su...	HEX
C01	Control	5q	3.46	Manual	CNV	Unknown	Unknown	ddPCR Su...	FAM
D01	Control	11p	4.25	Manual	CNV	Unknown	Unknown	ddPCR Su...	FAM

Figure 3. Example of QuantaSoft™ Analysis Pro Well Data table with results ordered by "Sample" name.



- Select all wells from the Well Data table and export the data in an Excel format by clicking on for subsequent analysis.

Do not include the NTC sample (Non-Template Control) in the exported data. Our software cannot analyze targets for which CNV = 0. An error occurs in this case and does not allow you to continue your analysis.

Note: For proper subsequent data processing, the exported file should contain the totality of the Well Data table columns (refer to APPENDIX 3 - Example of an Excel file exported from Bio-Rad software for an illustration of an exported file. In this example only the first 7 columns are displayed).

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 (Figure 4), select all the wells in one row (i.e. all wells containing the same mix).
- In Assay Information, select "Amplitude multiplex" from the dropdown list.
- Refer to APPENDIX 2 - Plate Editor - Mix Assay Information to fill in the target name, type and signal.
- Press "Apply" to save changes.

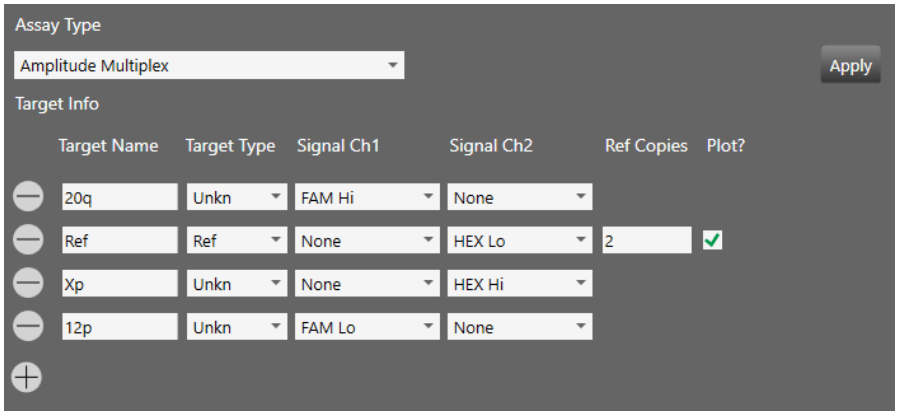


Figure 4. Example of QX Manager Plate Editor tab parameters for Mix 1 assay

Note: the **QX Manager v2.0** is suitable for 6-colour multiplex analysis. When "Amplitude Multiplex" is selected as Assay type, 12 lines are displayed (low and high concentration for the 6 colors). Delete all the unused lines by clicking on the ⊖ symbol and keep only the four lines with the "FAM Hi", "HEX lo", "HEX Hi" and "FAM lo" 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.

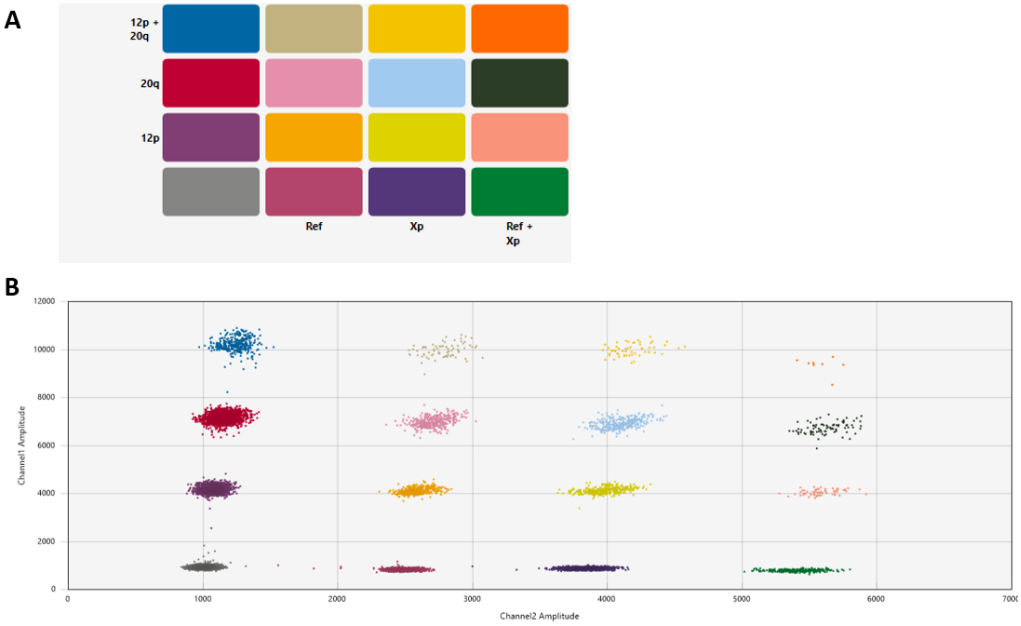


Figure 5. QX Manager 2D Amplitude results. A. Example of Mix 1 assay target combination clusters. B. Example of a 2D plot after threshold assignment

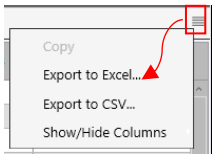
- In the "Data table" tab, select all wells for all samples in the Well Selector table and sort the lines by "**Sample**" name in the Well Data table (Figure 6; upward pointing arrow). Samples should then be listed in alphabetical order and for each of them, the "**Target**" column must appear in the same order as in APPENDIX 2 - Plate Editor - Mix Assay Information and APPENDIX 3 - Example of an Excel file exported from Bio-Rad software (i.e. beginning with the Target named 20q and finishing with the Target named 2q).

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

Well Data

Well	Sample description 1	Sample description 2	Sample description 3	Sample description 4	Target	Conc(
A01	#1				20q	
A01	#1				Ref	
A01	#1				Xp	
A01	#1				12p	
B01	#1				17q	
B01	#1				18q	
B01	#1				Ref	
B01	#1				9q	
C01	#1				5q	
C01	#1				1q	
C01	#1				Ref	
C01	#1				17p	
D01	#1				7q	

Figure 6. Example of QX Manager Well Data table with results ordered by "Sample" name.

- Export the data in an Excel format by clicking on 

for subsequent analysis.

Do not include the NTC sample (Non-Template Control) in the exported data. Our software cannot analyze targets for which CNV = 0. An error occurs in this case and does not allow you to continue your analysis.

Note: For proper subsequent data processing, the exported file should contain the totality of the Well Data table columns (refer to APPENDIX 3 - Example of an Excel file exported from Bio-Rad software for an illustration of an exported file. In this example only the first 7 columns are displayed).

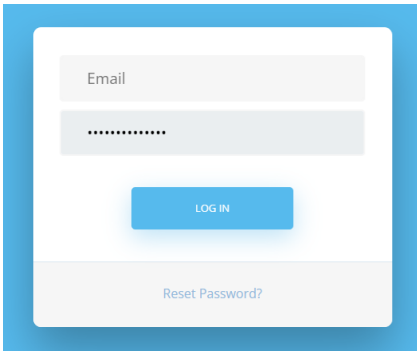
Data processing and graphical representation of the results

If you need help with this step, please watch the tutorial video here: https://www.youtube.com/watch?v=qqGDrJzrDLU. A troubleshooting guide is included at the end of these instructions for use in APPENDIX 5 - Troubleshooting for the iCS-digital™ PSC 24-probe kit.

Important note to **read before data processing**: if you use Google Chrome as browser, please refer to the APPENDIX 4 – Google Chrome settings.

Results obtained using the Bio-Rad software must be analyzed using the iCS-digital™ software provided by Stem Genomics (https://kit.stemgenomics.com).

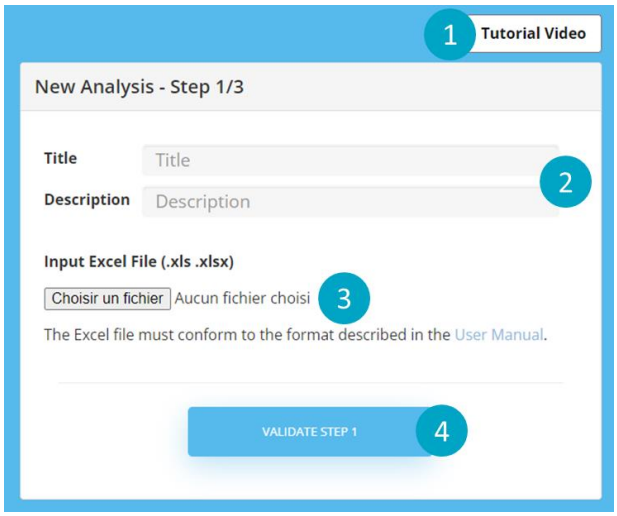
- After creating your account, sign in to the iCS-digital™ software with your email and password.



- At the top of the home page, a menu is available:
  1) "NEW ANALYSIS" allows you to launch a new analysis
  2) "ANALYSIS LIST" allows you to access the list of all the analyses performed by the user
  3) "PROFILE" allows you to set up your profile by clicking on "Edit Profile" at the bottom
  4) "LOG OUT" allows you to log out and change the user
  5) "CONTACT" allows you to find the postal address, phone number and contact form on the Stem Genomics website

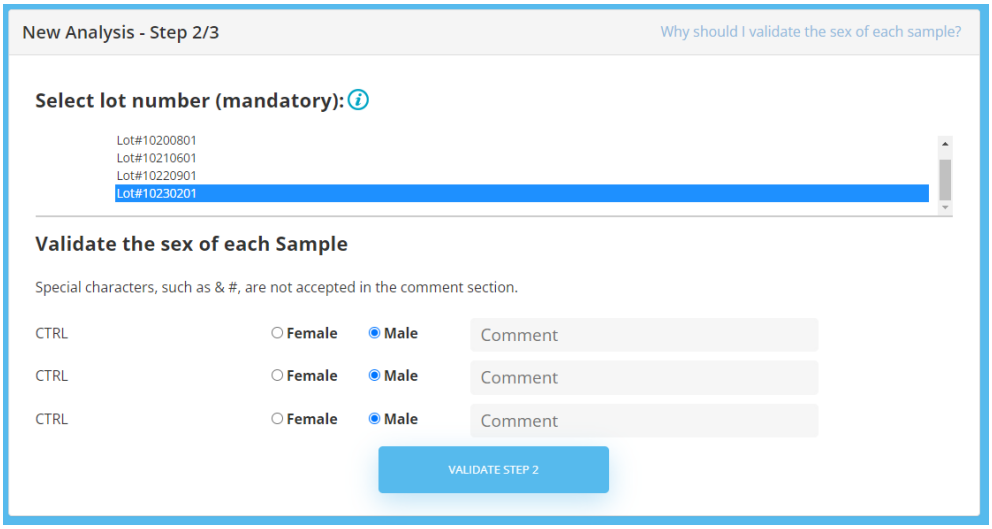
- To perform your first analysis, click on "NEW ANALYSIS"
  1) A tutorial video is available that explains how to analyze the data
  2) Fill in the "Title" and "Description" fields
  3) Import the raw data file in .xls or .xlsx format
  4) Click on "VALIDATE STEP 1"

Do not remove any columns or lines from the exported Excel file prior to its importation in the iCS-digital™ software.

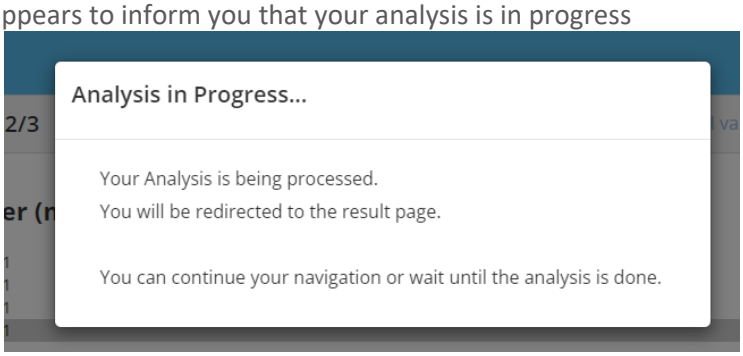


- Select the lot number to ensure the correct analysis of your data
- Specify the sex of each sample and add a comment (optional)

- Click on "VALIDATE STEP 2"



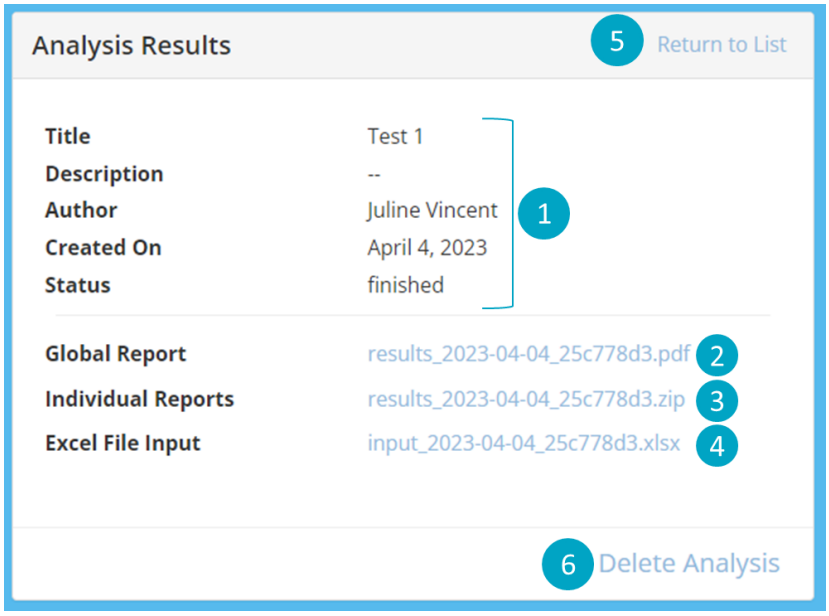
- A pop-up window appears to inform you that your analysis is in progress



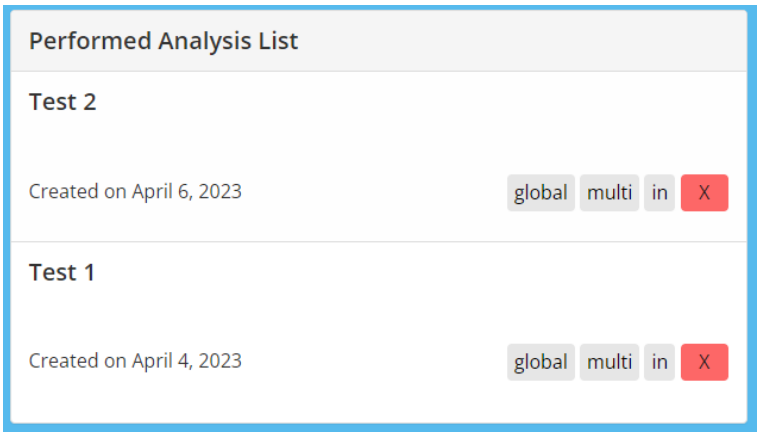
- The analysis results are available with different information and options:
  1) General information about the test: test name, operator, date and status
  2) The global report is available for your results. It gathers all your samples in one pdf file
  3) The individual reports allow you to obtain a one-page pdf report with the results for one sample

Note: If a sample is not interpretable, its detailed results can only be found in an individual report.

  4) The Excel file with raw data from the Bio-Rad software can be exported from this page
  5) The list of the analyses performed by the user is available by clicking on this link
  6) The analyses and all the associated results are **permanently deleted** by clicking on this link



- In the "Performed Analysis List", all the analyses performed by the user can be found sorted by date. There are several buttons:
  - The "global" button allows you to download the global report of the analysis
  - The "multi" button allows you to download a zip file with the individual reports
  - "In" allows you to download the Excel input file
  - "X" (red cross) **permanently deletes** the analysis



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

Note: Stem Genomics software access is restricted to iCS-digital™ PSC kit customers.

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

APPENDIX 1 - iCS-digital™ PSC 24-probe mix preparation

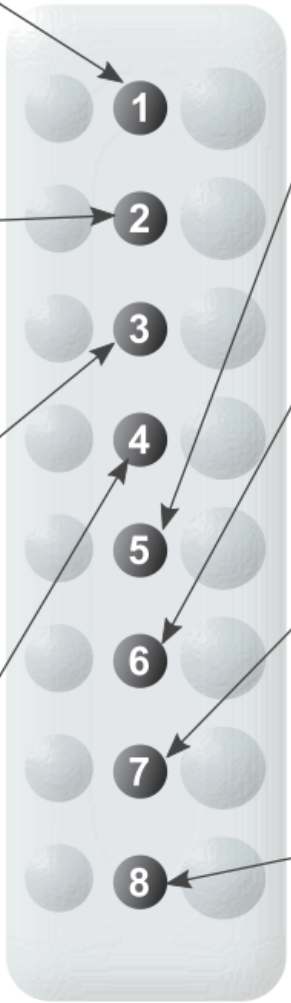
One cartridge per genomic DNA sample

Reaction 1	Volume (µL)
H_20	4
Mix1	3
Supermix 2x	11
HindIII	1
gDNA	3

Reaction 2	Volume (µL)
H_20	4
Mix2	3
Supermix 2x	11
HindIII	1
gDNA	3

Reaction 3	Volume (µL)
H_20	4
Mix3	3
Supermix 2x	11
HindIII	1
gDNA	3

Reaction 4	Volume (µL)
H_20	4
Mix4	3
Supermix 2x	11
HindIII	1
gDNA	3



Reaction 5	Volume (µL)
H_20	4
Mix5	3
Supermix 2x	11
HindIII	1
gDNA	3

Reaction 6	Volume (µL)
H_20	4
Mix6	3
Supermix 2x	11
HindIII	1
gDNA	3

Reaction 7	Volume (µL)
H_20	4
Mix7	3
Supermix 2x	11
HindIII	1
gDNA	3

Reaction 8	Volume (µL)
H_20	4
Mix8	3
Supermix 2x	11
HindIII	1
gDNA	3

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

APPENDIX 2 - Plate Editor - Mix Assay Information

Mix	Target Name	Target Type	Signal Ch1	Signal Ch2	Reference Copies
Mix 1	20q	Unknown	FAM High	None	
	Ref	Reference	None	HEX Low	2
	Xp	Unknown	None	HEX High	
	12p	Unknown	FAM Low	None	
Mix 2	18q	Unknown	FAM High	None	
	Ref	Reference	None	HEX Low	2
	9q	Unknown	None	HEX High	
	17q	Unknown	FAM Low	None	
Mix 3	1q	Unknown	FAM High	None	
	Ref	Reference	None	HEX Low	2
	17p	Unknown	None	HEX High	
	5q	Unknown	FAM Low	None	
Mix 4	11p	Unknown	FAM High	None	
	Ref	Reference	None	HEX Low	2
	13q	Unknown	None	HEX High	
	7q	Unknown	FAM Low	None	

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

Mix	Target Name	Target Type	Signal Ch1	Signal Ch2	Reference Copies
Mix 5	1p	Unknown	FAM High	None	
	Ref	Reference	None	HEX Low	2
	4q	Unknown	None	HEX High	
	3p	Unknown	FAM Low	None	
Mix 6	19p	Unknown	FAM High	None	
	Ref	Reference	None	HEX Low	2
	14q	Unknown	None	HEX High	
	8q	Unknown	FAM Low	None	
Mix 7	15q	Unknown	FAM High	None	
	Ref	Reference	None	HEX Low	2
	6q	Unknown	None	HEX High	
	7p	Unknown	FAM Low	None	
Mix 8	22q	Unknown	FAM High	None	
	Ref	Reference	None	HEX Low	2
	16q	Unknown	None	HEX High	
	2q	Unknown	FAM Low	None	

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

APPENDIX 3 - Example of an Excel file exported from Bio-Rad softwares

In these examples, **only the first seven columns (A to G) are displayed**.

Quantasoft™ Analysis Pro software

The complete Excel file should contain at least 65 columns for. The two samples (Sample 1 and Sample 2) shown in this example are in different text colors.

A	B	C	D	E	F	G	...
Well	Sample	Target	Conc (copies/µL)	Status	Experiment	SampleType	...
A01	Sample 1	20q	150.00	Manual	CNV	Unknown	...
A01	Sample 1	Ref	156.09	Manual	CNV	Unknown	...
A01	Sample 1	Xp	77.65	Manual	CNV	Unknown	...
A01	Sample 1	12p	152.48	Manual	CNV	Unknown	...
B01	Sample 1	18q	155.50	Manual	CNV	Unknown	...
B01	Sample 1	Ref	160.98	Manual	CNV	Unknown	...
B01	Sample 1	9q	160.91	Manual	CNV	Unknown	...
B01	Sample 1	17q	156.51	Manual	CNV	Unknown	...
C01	Sample 1	1q	158.00	Manual	CNV	Unknown	...
C01	Sample 1	Ref	161.97	Manual	CNV	Unknown	...
C01	Sample 1	17p	154.05	Manual	CNV	Unknown	...
C01	Sample 1	5q	163.63	Manual	CNV	Unknown	...
D01	Sample 1	11p	155.17	Manual	CNV	Unknown	...
D01	Sample 1	Ref	160.18	Manual	CNV	Unknown	...
D01	Sample 1	13q	157.03	Manual	CNV	Unknown	...
D01	Sample 1	7q	156.94	Manual	CNV	Unknown	...
E01	Sample 1	1p	160.59	Manual	CNV	Unknown	...
E01	Sample 1	Ref	164.17	Manual	CNV	Unknown	...
E01	Sample 1	4q	167.48	Manual	CNV	Unknown	...
E01	Sample 1	3p	163.89	Manual	CNV	Unknown	...
F01	Sample 1	19p	159.46	Manual	CNV	Unknown	...
F01	Sample 1	Ref	156.05	Manual	CNV	Unknown	...
F01	Sample 1	14q	153.92	Manual	CNV	Unknown	...
F01	Sample 1	8q	156.90	Manual	CNV	Unknown	...
G01	Sample 1	15q	164.37	Manual	CNV	Unknown	...
G01	Sample 1	Ref	162.83	Manual	CNV	Unknown	...
G01	Sample 1	6q	171.37	Manual	CNV	Unknown	...
G01	Sample 1	7p	169.23	Manual	CNV	Unknown	...
H01	Sample 1	22q	163.55	Manual	CNV	Unknown	...
H01	Sample 1	Ref	169.84	Manual	CNV	Unknown	...
H01	Sample 1	16q	168.19	Manual	CNV	Unknown	...
H01	Sample 1	2q	163.32	Manual	CNV	Unknown	...

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

A02	Sample 2	20q	160.92	Manual	CNV	Unknown	...
A02	Sample 2	Ref	153.08	Manual	CNV	Unknown	...
A02	Sample 2	Xp	79.30	Manual	CNV	Unknown	...
A02	Sample 2	12p	157.17	Manual	CNV	Unknown	...
B02	Sample 2	18q	155.17	Manual	CNV	Unknown	...
B02	Sample 2	Ref	159.07	Manual	CNV	Unknown	...
B02	Sample 2	9q	154.69	Manual	CNV	Unknown	...
B02	Sample 2	17q	159.83	Manual	CNV	Unknown	...
C02	Sample 2	1q	147.77	Manual	CNV	Unknown	...
C02	Sample 2	Ref	149.82	Manual	CNV	Unknown	...
C02	Sample 2	17p	149.11	Manual	CNV	Unknown	...
C02	Sample 2	5q	146.28	Manual	CNV	Unknown	...
D02	Sample 2	11p	156.60	Manual	CNV	Unknown	...
D02	Sample 2	Ref	156.37	Manual	CNV	Unknown	...
D02	Sample 2	13q	149.92	Manual	CNV	Unknown	...
D02	Sample 2	7q	157.30	Manual	CNV	Unknown	...
E02	Sample 2	1p	158.66	Manual	CNV	Unknown	...
E02	Sample 2	Ref	160.42	Manual	CNV	Unknown	...
E02	Sample 2	4q	159.62	Manual	CNV	Unknown	...
E02	Sample 2	3p	154.50	Manual	CNV	Unknown	...
F02	Sample 2	19p	161.18	Manual	CNV	Unknown	...
F02	Sample 2	Ref	158.99	Manual	CNV	Unknown	...
F02	Sample 2	14q	153.53	Manual	CNV	Unknown	...
F02	Sample 2	8q	161.11	Manual	CNV	Unknown	...
G02	Sample 2	15q	163.64	Manual	CNV	Unknown	...
G02	Sample 2	Ref	164.01	Manual	CNV	Unknown	...
G02	Sample 2	6q	165.47	Manual	CNV	Unknown	...
G02	Sample 2	7p	158.25	Manual	CNV	Unknown	...
H02	Sample 2	22q	128.09	Manual	CNV	Unknown	...
H02	Sample 2	Ref	136.01	Manual	CNV	Unknown	...
H02	Sample 2	16q	129.18	Manual	CNV	Unknown	...
H02	Sample 2	2q	129.46	Manual	CNV	Unknown	...

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

QX Manager software

The complete Excel file should contain at least 81 columns. The two samples (Sample 1 and Sample 2) shown in this example are in different text colors.

A	B	C	D	E	F	G	…
Well	Sample description 1	Sample description 2	Sample description 3	Sample description 4	Target	Conc(copies/µL)	…
A01	Sample 1				20q	488.44	…
A01	Sample 1				RPP30	494.34	…
A01	Sample 1				Xp	478.77	…
A01	Sample 1				12p	491.90	…
B01	Sample 1				18q	381.44	…
B01	Sample 1				RPP30	379.49	…
B01	Sample 1				9q	379.95	…
B01	Sample 1				17q	368.68	…
C01	Sample 1				1q	371.73	…
C01	Sample 1				RPP30	374.22	…
C01	Sample 1				17p	357.96	…
C01	Sample 1				5q	377.28	…
D01	Sample 1				11p	373.80	…
D01	Sample 1				RPP30	381.03	…
D01	Sample 1				13q	377.64	…
D01	Sample 1				7q	355.57	…
E01	Sample 1				1p	366.54	…
E01	Sample 1				RPP30	371.57	…
E01	Sample 1				4q	359.36	…
E01	Sample 1				3p	362.01	…
F01	Sample 1				8q	392.45	…
F01	Sample 1				RPP30	387.89	…
F01	Sample 1				14q	378.71	…
F01	Sample 1				19p	362.10	…
G01	Sample 1				15q	378.39	…
G01	Sample 1				RPP30	392.18	…
G01	Sample 1				6q	389.81	…
G01	Sample 1				7p	387.70	…
H01	Sample 1				22q	419.17	…
H01	Sample 1				RPP30	408.42	…
H01	Sample 1				16q	408.71	…
H01	Sample 1				2q	414.89	…

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

A02	Sample 2				20q	340.97	...
A02	Sample 2				RPP30	232.50	...
A02	Sample 2				Xp	105.59	...
A02	Sample 2				12p	215.99	...
B02	Sample 2				18q	171.63	...
B02	Sample 2				RPP30	190.65	...
B02	Sample 2				9q	178.96	...
B02	Sample 2				17q	152.20	...
C02	Sample 2				1q	200.45	...
C02	Sample 2				RPP30	213.50	...
C02	Sample 2				17p	245.52	...
C02	Sample 2				5q	207.98	...
D02	Sample 2				11p	237.49	...
D02	Sample 2				RPP30	211.97	...
D02	Sample 2				13q	211.01	...
D02	Sample 2				7q	207.03	...
E02	Sample 2				1p	252.51	...
E02	Sample 2				RPP30	210.77	...
E02	Sample 2				4q	191.03	...
E02	Sample 2				3p	216.35	...
F02	Sample 2				8q	220.87	...
F02	Sample 2				RPP30	214.72	...
F02	Sample 2				14q	219.21	...
F02	Sample 2				19p	254.81	...
G02	Sample 2				15q	229.94	...
G02	Sample 2				RPP30	211.42	...
G02	Sample 2				6q	211.83	...
G02	Sample 2				7p	228.13	...
H02	Sample 2				22q	230.48	...
H02	Sample 2				RPP30	212.96	...
H02	Sample 2				16q	172.60	...
H02	Sample 2				2q	198.71	...

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

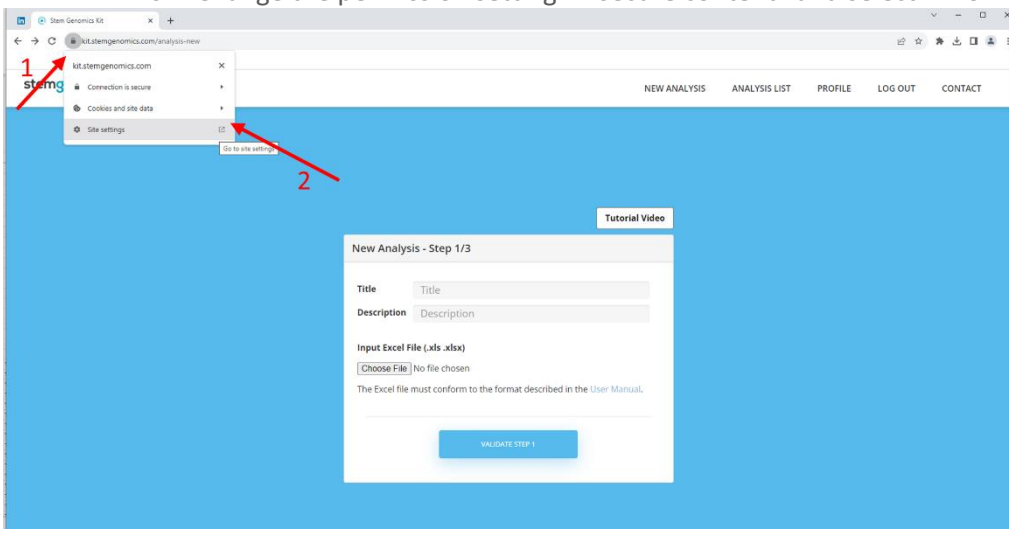
APPENDIX 4 – Google Chrome settings

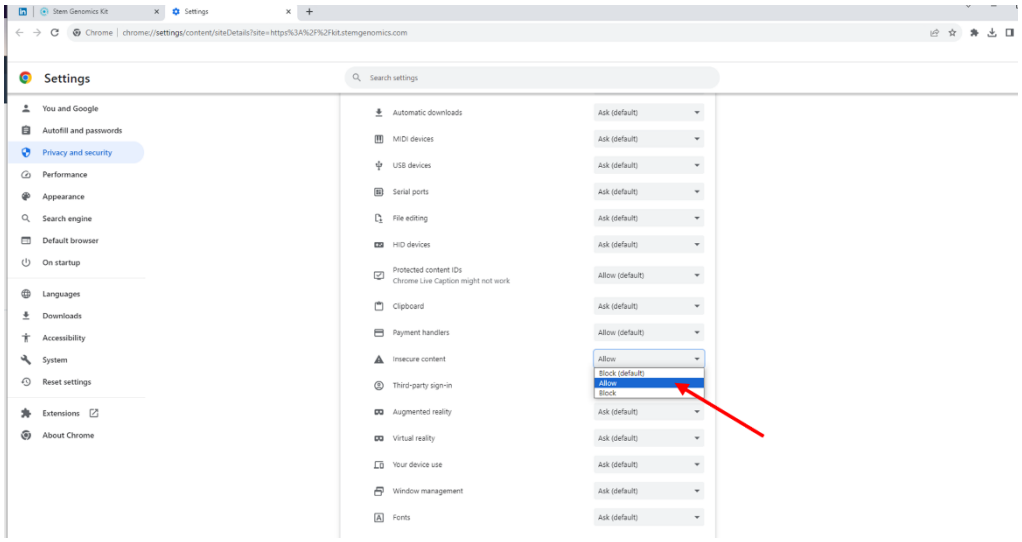
Since the last Google Chrome's update, security settings have changed and downloads from external sites are blocked by default.

If you use Google Chrome, this may impact the download of your iCS-digital PSC reports (global report, individual report, and input file) when generated via the iCS-digital software (kit.stemgenomics.com). If you are impacted, you may receive an error code "Step 2" when trying to generate your report.

To resolve this issue, there are two options:

1) You can use another browser to perform the analysis (recommended).
2) You can change the permissions of the iCS-digital software settings by following these instructions:
   - On your computer, open Chrome.
   - Go to the site kit.stemgenomics.com.
   - To the left of the web address, click on the padlock icon and "Site settings".
   - Change the permission setting "Insecure content" and select "Allow".





Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

APPENDIX 5 - Troubleshooting for the iCS-digital™ PSC 24-probe kit

QuantaSoft™ Analysis Pro results troubleshooting table

Problem observed			Possible reason	Solution
Small 2D amplitude clusters and low concentrations (<150 copies/µL) *See Fig. 7. as an example*			Low DNA concentrations were used	Check the concentration of your DNA using a Qubit™ fluorometer and make sure that you dilute the DNA samples to a concentration of 5 ng/µL
Large 2D amplitude clusters & high concentrations (>250 copies/µL) *See Fig. 8. as an example*			High DNA concentrations were used	
Intense Rain and/or duplicated clusters make the analysis difficult or impossible *See Fig. 9. as an example.*	All wells affected		The Mix assays were not sufficiently vortexed. The PCR program is incorrect	Vigorously vortex each mix assay tube twice for 5-10 seconds and briefly centrifuge the tubes between each vortexing step Check that the PCR program complies with the protocol
	Only a few wells affected	Droplet number < 10 000	Probable issue with the QX instrument (dust, bubbles, etc.)	Perform the test in duplicate
		Droplet number ≥ 10 000	Some mix assays were not sufficiently vortexed	Vigorously vortex each mix assay tube twice for 5-10 seconds and briefly centrifuge the tubes between each vortexing step

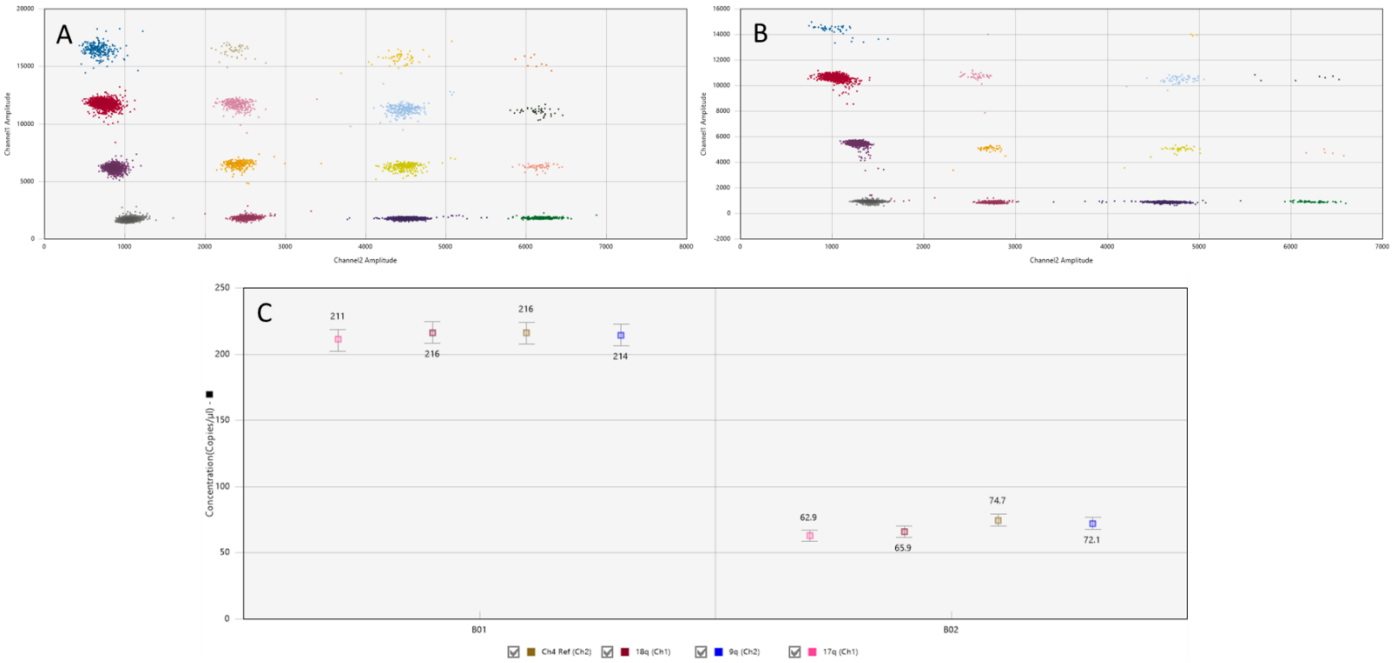


Figure 7. Small 2D amplitude clusters and/or low concentrations (<200 copies/µL) A-B. *2D Amplitude tab.* ***C.*** *Concentration tab (normal values ~200 copies/µL). Left panels: 2D amplitude & Concentration tab of a sample with a correct DNA concentration. Right panels: 2D amplitude & Concentration tab of a sample with a low DNA concentration.*

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

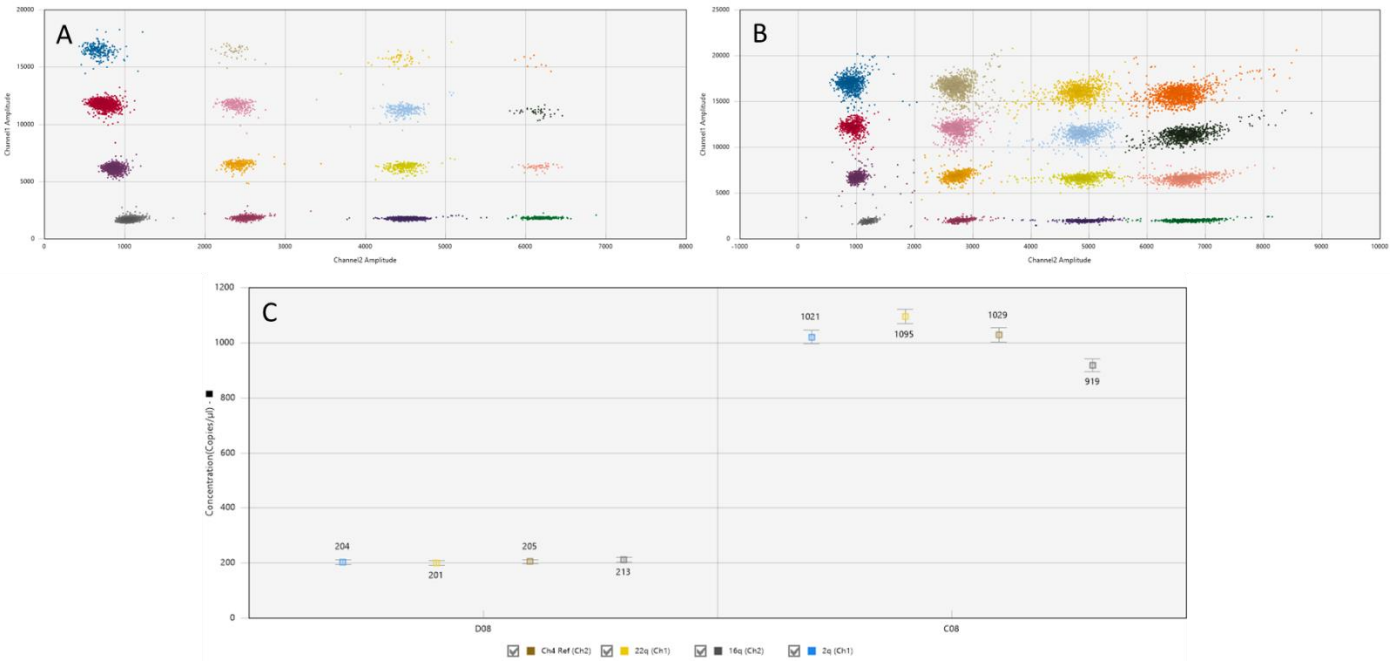


Figure 8. Large 2D amplitude clusters and/or high concentrations (>200 copies/µL). A-B. *2D Amplitude tab.* ***C.*** *Concentration tab (normal values ~200 copies/µL). Left panels: 2D amplitude & Concentration tab of a sample with a correct DNA concentration. Right panels: 2D amplitude & Concentration tab of a sample with a high DNA concentration.*

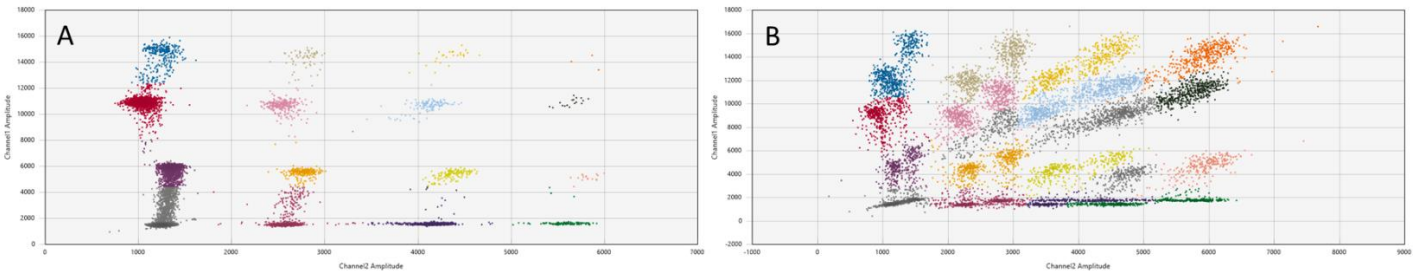


Figure 9. Intense rain and/or duplicated clusters make the analysis difficult or impossible. *Example of a low quality 2D-cluster well*

Stem Genomics SAS
IRMB - Hopital St Eloi ▪ 80 avenue Augustin Fliche
34295 Montpellier - Cedex 5 ▪ France
Tel: +33 (0)9 85 03 61 60
Email: services@stemgenomics.com
www.stemgenomics.com

Online iCS-digital™ analysis tool troubleshooting table

Problem observed	Possible reason	Problem resolution
"502 bad gateway nginx/1.19.6" Error message	Incorrect naming of the Target Example: "3q" instead of "3p"	Validate the correct naming of the targets (column C of the Input Excel file) Refer to the APPENDIX 2 of this User Manual
	Use of a special character for the Sample name (e.g. ~ ! @ # $ ^ % & * ? { })	Remove any special characters present in the Sample names (column B of the Input Excel file)
	Incorrect sorting of the data prior to their export from the QuantaSoft™ Analysis Pro software	In the "2D Amplitude" tab of the QuantaSoft™ Analysis Pro software, sort the lines by "Sample" name in the Well Data table prior to exporting the data in Excel format Refer to the APPENDIX 3 of this User Manual
	A sample with a concentration (copies/µL) and therefore CNV values equal to zero (e.g. use of an NCT)	Do not include samples with a concentration (column "Conc" of the Well Data table) equal or close to zero when exporting the data from the QuantaSoft™ Analysis Pro software
Incoherent sample name in the final report	Incorrect sorting of the data prior to their export from the QuantaSoft™ Analysis Pro software	In the "2D Amplitude" tab of the QuantaSoft™ Analysis Pro software, sort the lines by "Sample" name in the Well Data table prior to exporting the data in Excel format Refer to the APPENDIX 3 of this User Manual