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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
Store at -20°C

For Research Use Only

Description

The iCS-digital™ PSC 24-probe kit detects more than 90% of the most frequent genomic abnormalities in human Pluripotent Stem Cells (hPSCs, i.e. embryonic stem cells and induced pluripotent stem cells). The test relies on multiplex digital PCR with double-quenched probes. The eight mix assays enable to target 24 genomic regions with an 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.

Kit Content

Product	Quantity (volume)	Content
Mix 1	20 tests	- ChrXp assay (HEX high) - **Reference assay (HEX low)** - Chr20q assay (FAM high) - Chr12p assay (FAM low)
Mix 2	20 tests	- Chr9q assay (HEX high) - **Reference assay (HEX low)** - Chr18q assay (FAM high) - Chr17q assay (FAM low)
Mix 3	20 tests	- Chr17p assay (HEX high) - **Reference assay (HEX low)** - Chr1q assay (FAM high) - Chr5q assay (FAM low)
Mix 4	20 tests	- Chr13q assay (HEX high) - **Reference assay (HEX low)** - Chr11p assay (FAM high) - Chr7q assay (FAM low)
Mix 5	20 tests	- Chr4q assay (HEX high) - **Reference assay (HEX low)** - Chr1p assay (FAM high) - Chr3p assay (FAM low)
Mix 6	20 tests	- Chr14q assay (HEX high) - **Reference assay (HEX low)** - Chr19p assay (FAM high) - Chr8q assay (FAM low)
Mix 7	20 tests	- Chr6q assay (HEX high) - **Reference assay (HEX low)** - Chr15q assay (FAM high) - Chr7p assay (FAM low)
Mix 8	20 tests	- Chr16q assay (HEX high) - **Reference assay (HEX low)** - Chr22q assay (FAM high) - Chr2q assay (FAM low)
Control DNA	10 tests (30 µL – 50 ng/µL)	Normal control DNA (male) with 1 CNV at the ChrXp region and 2 CNVs in the other 23 regions

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 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

Instructions for Use

Sample preparation

- 500,000 dissociated cells are sufficient for one test using the iCS-digital™ PSC 24-probe kit.
- Genomic DNA should be extracted using an appropriate DNA extraction method. It is recommended to use the QIAamp DNA Blood Mini Kit (Qiagen, #51104) or the GenElute Mammalian Genomic DNA Miniprep Kits (Sigma-Aldrich, #G1N70-1KT).

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 will be necessary for one test.

Note: The 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 and control DNA 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) to 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** each Mix assay tube twice for 5-10 seconds, and to briefly centrifuge the tubes 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 8 Master Reaction Mixes according to the 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 what is required for the total number of reactions to be performed.
  - Prepare a bulk of enzyme 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 necessary volume of the bulk H_2O + ddPCR™ Supermix for Probes (No dUTP) + HindIII-HF in each of them
  - Add the required volume of each 8 Assay Mix provided by the kit, in 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.

Table 1. Reaction mix preparation for one sample

Enzyme bulk	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	Enzyme Bulk	Assay MIX n
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 in each tube or well
- Load 20 µL of each reaction mixture in a sample well of a DG8™ Cartridge (refer to Appendix 1 for a schematic representation of the cartridge).
- Centrifuge briefly the tubes or plate

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

- 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	

- 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.

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, 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.

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

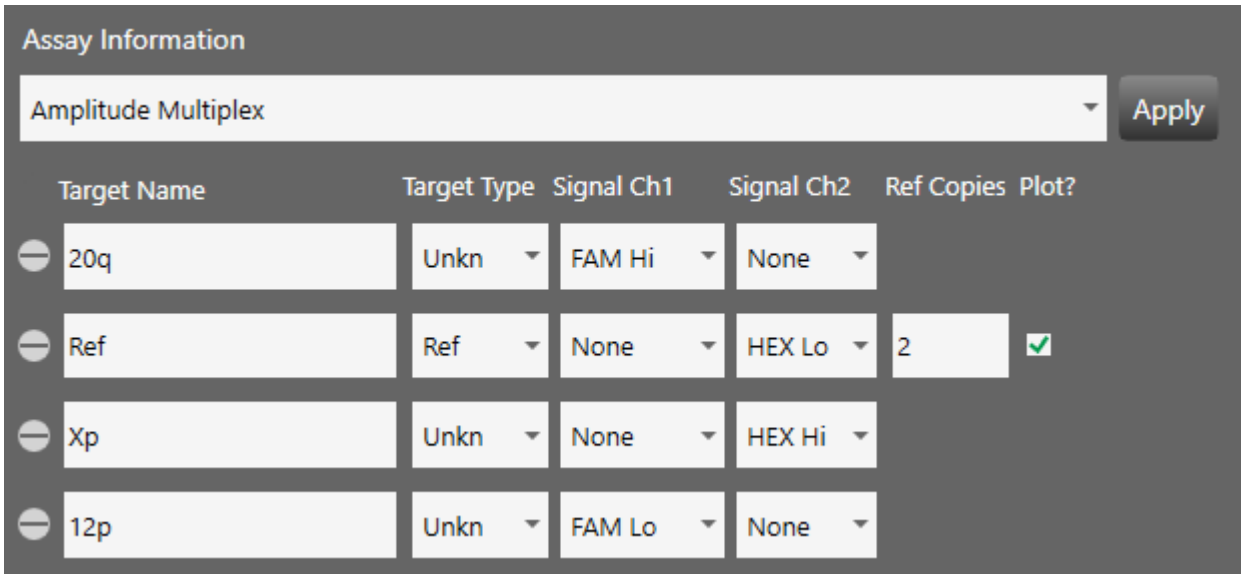


Figure 1. Example of QuantaSoft™ Analysis Pro Plate Editor tab parameters for Mix 1 assay

- 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.

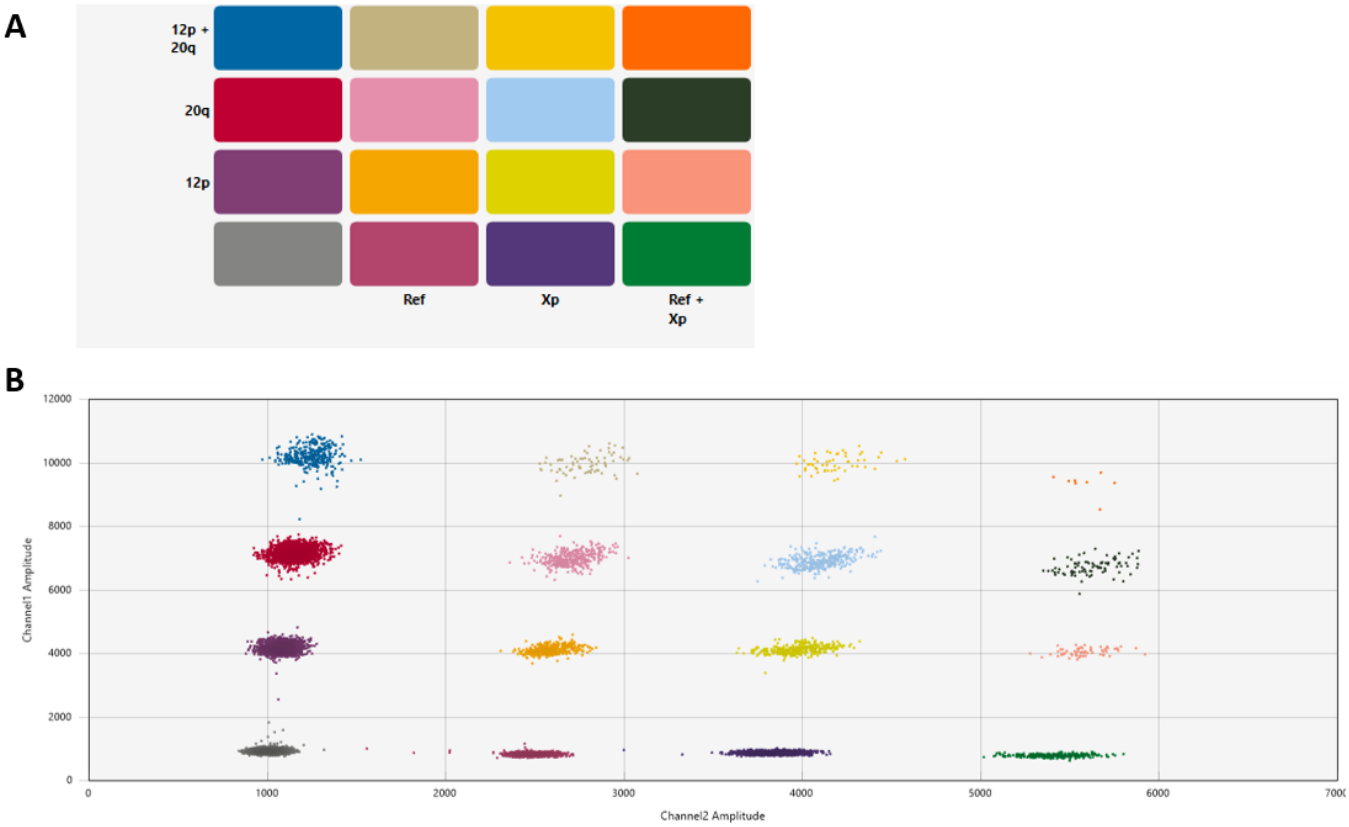


Figure 2. QuantaSoft™ Analysis 2D Amplitude results. A. Example of Mix 1 assay target combination clusters. B. Example of a 2D plot after threshold assignment.

- In the 1D amplitude or 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 order and for each of them, the "**Target**" column must appear in the same order as in Appendix 2 and Appendix 3 (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™ 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 for subsequent analysis.

Do not include the NTC sample (Non-Template Control) in the exported data. Our software cannot analyse 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 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

Results obtained using the QuantaSoft™ Analysis Pro software must be analysed using the iCS-digital™ software provided by Stem Genomics (https://kit.stemgenomics.com).

Do not remove any columns or lines from the exported Excel file prior to its importation in 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.

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

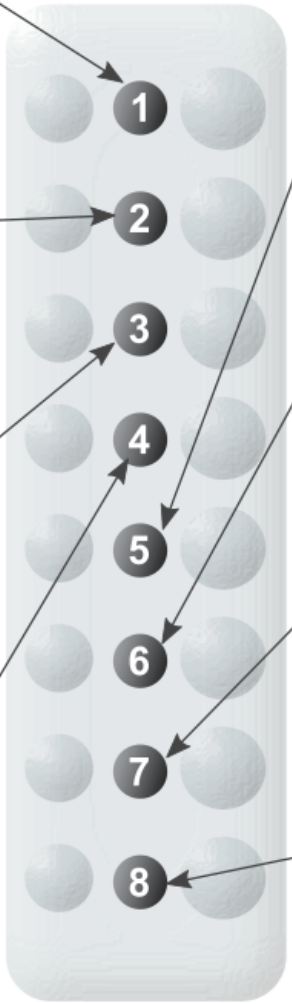
APPENDIX 1: iCS-digital™ PSC Mix preparation (one cartridge per genomic DNA sample)

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

APPENDIX 2: QuantaSoft™ 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	

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	

APPENDIX 3: Example of an Excel file exported from the QuantaSoft™ Analysis Pro software. In this example, **only the first seven columns (A to G) are displayed**. The complete Excel file should contain at least 65 columns. The two samples (Sample 1 and Sample 2) shown in this example are in different text colours.

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	…

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	…