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**iCS-digital™ PSC 24-probes kit**

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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

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## Description

The iCS-digital™ PSC 24-probes 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 allow targeting 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 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)<br>- **Chr4 Reference assay (HEX low)**<br>- Chr20q assay (FAM high)<br>- Chr12p assay (FAM low) |
| **Mix 2** | 20 tests | - Chr9q assay (HEX high)<br>- **Chr4 Reference assay (HEX low)**<br>- Chr18q assay (FAM high)<br>- Chr17q assay (FAM low) |
| **Mix 3** | 20 tests | - Chr17p assay (HEX high)<br>- **Chr4 Reference assay (HEX low)**<br>- Chr1q assay (FAM high)<br>- Chr5q assay (FAM low) |
| **Mix 4** | 20 tests | - Chr13q assay (HEX high)<br>- **Chr4 Reference assay (HEX low)**<br>- Chr11p assay (FAM high)<br>- Chr7q assay (FAM low) |
| **Mix 5** | 20 tests | - Chr4q assay (HEX high)<br>- **Chr4 Reference assay (HEX low)**<br>- Chr1p assay (FAM high)<br>- Chr3p assay (FAM low) |
| **Mix 6** | 20 tests | - Chr14q assay (HEX high)<br>- **Chr4 Reference assay (HEX low)**<br>- Chr19p assay (FAM high)<br>- Chr8q assay (FAM low) |
| **Mix 7** | 20 tests | - Chr6q assay (HEX high)<br>- **Chr4 Reference assay (HEX low)**<br>- Chr15q assay (FAM high)<br>- Chr7p assay (FAM low) |
| **Mix 8** | 20 tests | - Chr16q assay (HEX high)<br>- **Chr4 Reference assay (HEX low)**<br>- Chr22q assay (FAM high)<br>- Chr2q assay (FAM low) |
| **Control DNA** | 10 tests<br>(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™, catalogue #186-4003)<br>- Droplet Reader from Bio-Rad (recommended: QX200™, catalogue #186-4003)<br>- 96-well Thermal Cycler<br>- Benchtop centrifuge<br>- Benchtop vortex<br>- Plate Sealer adapted for the Bio-Rad technology (recommended: PX1™ PCR Plate Sealer, catalogue #181-4000) |
| **Materials** |
| - Pipettes and pipette tips (delivering volumes from 1 µL to 1000 µL)<br>- 1.5 mL reaction tubes<br>- 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)<br>- HindIII-HF enzyme (e.g., New England Biolabs #R3104L)<br>- Nuclease-free water |

# Instructions for Use

### Sample preparation

*Using cell culture supernatants 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 (3.5 cm culture plate) may be added to the supernatant sample to obtain more DNA.
- DNA isolation should be preferentially performed using a magnetic bead system kit.

*Using cell pellets as starting material:*

- $1.10^6$ dissociated cells are sufficient for one test using the iCS-digital™ PSC 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. 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.
- Vortex the DNA samples and the control DNA for at least 5 seconds, and centrifuge briefly.
- Dilute the DNA samples and control DNA to a concentration of 5 ng/µL in 30 µL of molecular grade $H_20$.
- 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 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 corresponding to the 8 Assay Mixes provided by the kit. 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 8 Assay Mixes **must be used every time** to guaranty the proper analysis and data interpretation through the online iCS-digital™ analysis software.

- The reaction volumes for one sample are detailed in Table 1 and in Appendix 1.
- Mix thoroughly by vortexing the tubes and centrifuge briefly.

**Table 1. Reaction mix preparation for one sample**

| Example for Mix 1 | Volume for 1 sample |
|---|---|
| ddPCR™ Supermix for Probes (No dUTP) 2X | 11 µL |
| Mix 1 | 3 µL |
| HindIII-HF (2 U/µL) | 1 µL |
| Genomic DNA (≥ 5 ng/µL) | 3 µL |
| $H_2O$ | q.s. 22 µL |

- 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).
  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 | |

- 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, 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 | Chr4 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 the changes.

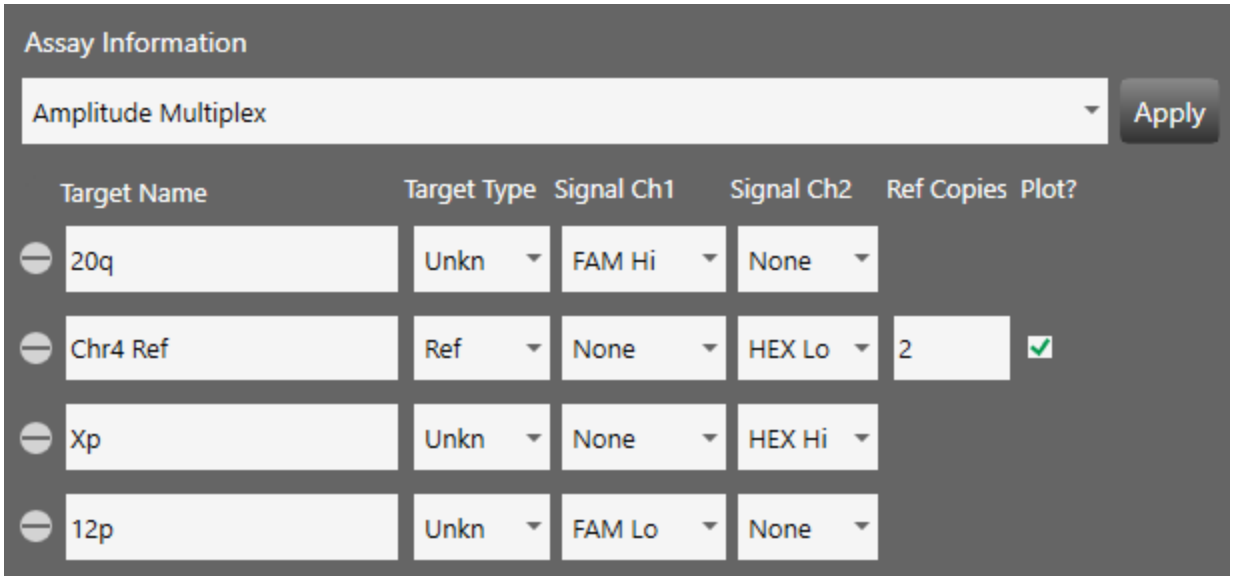



**Figure 1. Example of QuantaSoft™ Analysis Pro Plate Editor tab parameters for Mix 1 assay**

- 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.

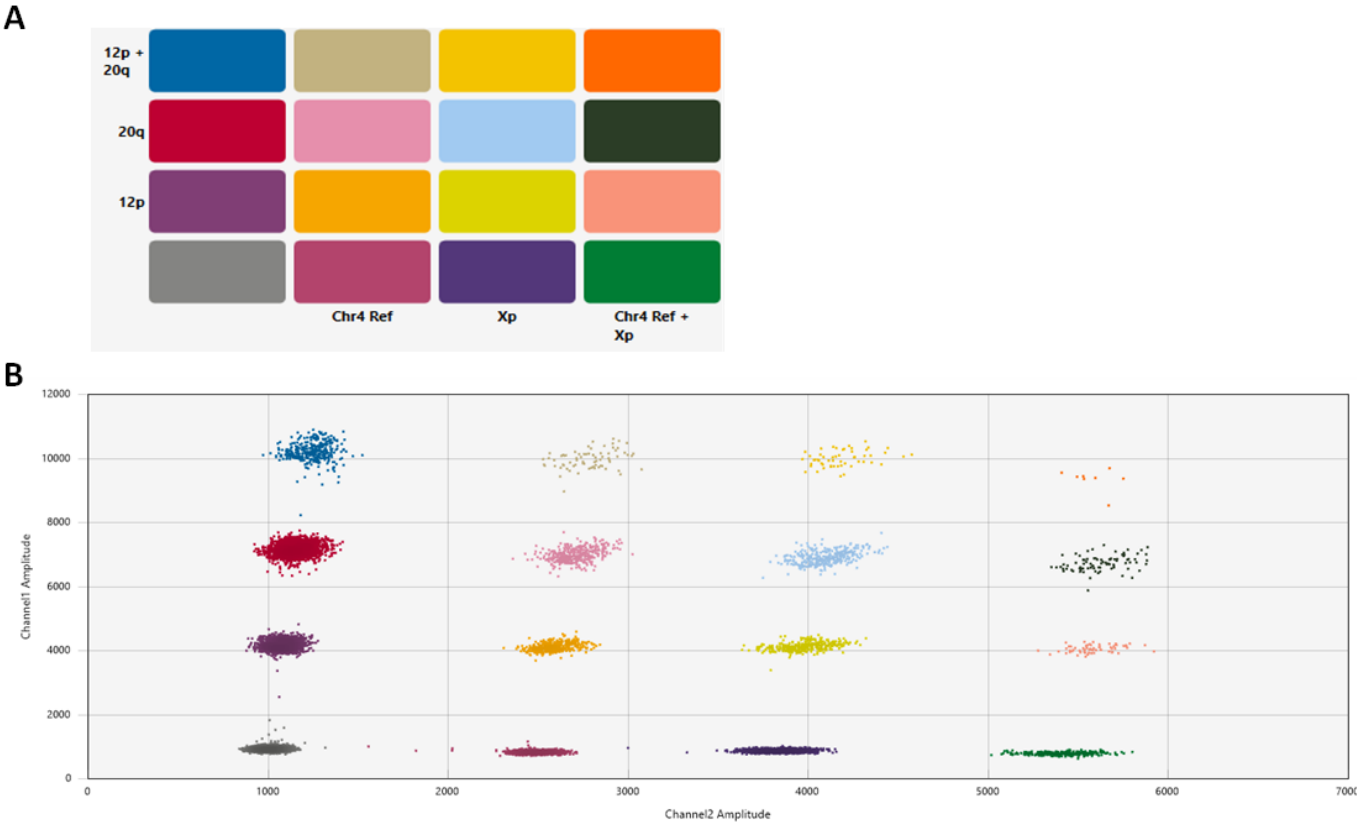



**Figure 2. QuantaSoft™ Analysis 2D Amplitude results. A. Example of Mix 1 assay target combination clusters. B. Example of a 2D plot after threshold assignment.**

- 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 | DyeName(s) | Copies/20µLWell |
|---|---|---|---|---|---|---|---|---|---|---|
| A01 | Sample 1 | 20q | 240 | Manual | CNV | Unknown | Unknown | ddPCR Su... | FAM Hi | 4802 |
| A01 | Sample 1 | Chr4 Ref | 237 | Manual | CNV | Unknown | Reference | ddPCR Su... | HEX Lo | 4738 |
| A01 | Sample 1 | Xp | 203 | Manual | CNV | Unknown | Unknown | ddPCR Su... | HEX Hi | 4055 |
| A01 | Sample 1 | 12p | 227 | Manual | CNV | Unknown | Unknown | ddPCR Su... | FAM Lo | 4547 |
| B01 | Sample 1 | 18q | 134 | Manual | CNV | Unknown | Unknown | ddPCR Su... | FAM Hi | 2675 |
| B01 | Sample 1 | Chr4 Ref | 242 | Manual | CNV | Unknown | Reference | ddPCR Su... | HEX Lo | 4835 |
| B01 | Sample 1 | 9q | 228 | Manual | CNV | Unknown | Unknown | ddPCR Su... | HEX Hi | 4563 |
| B01 | Sample 1 | 17q | 214 | Manual | CNV | Unknown | Unknown | ddPCR Su... | FAM Lo | 4286 |
| C01 | Sample 1 | 1q | 249 | Manual | CNV | Unknown | Unknown | ddPCR Su... | FAM Hi | 4985 |
| C01 | Sample 1 | Chr4 Ref | 245 | Manual | CNV | Unknown | Reference | ddPCR Su... | HEX Lo | 4902 |
| C01 | Sample 1 | 17p | 252 | Manual | CNV | Unknown | Unknown | ddPCR Su... | HEX Hi | 5032 |
| C01 | Sample 1 | 5q | 240 | Manual | CNV | Unknown | Unknown | ddPCR Su... | FAM Lo | 4791 |
| D01 | Sample 1 | 11p | 236 | Manual | CNV | Unknown | Unknown | ddPCR Su... | FAM Hi | 4719 |
| D01 | Sample 1 | Chr4 Ref | 239 | Manual | CNV | Unknown | Reference | ddPCR Su... | HEX Lo | 4787 |
| D01 | Sample 1 | 13q | 217 | Manual | CNV | Unknown | Unknown | ddPCR Su... | HEX Hi | 4343 |

**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 Excel format for subsequent analyses. The exported file should contain 65 columns and 32 lines per sample (refer to Appendix 3 for an example of exported file).

*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).

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.

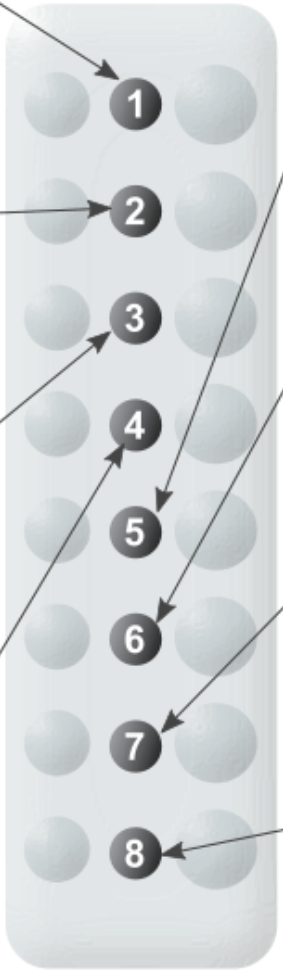
APPENDIX 1: iCS-digital™ PSC Mix preparation (one cartridge per genomic DNA sample)

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

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

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

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




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

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

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

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

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 | |
| | Ch4 Ref | Reference | None | HEX Low | 2 |
| | Xp | Unknown | None | HEX High | |
| | 12p | Unknown | FAM Low | None | |
| | | | | | |
| **Mix 2** | 18q | Unknown | FAM High | None | |
| | Ch4 Ref | Reference | None | HEX Low | 2 |
| | 9q | Unknown | None | HEX High | |
| | 17q | Unknown | FAM Low | None | |
| | | | | | |
| **Mix 3** | 1q | Unknown | FAM High | None | |
| | Ch4 Ref | Reference | None | HEX Low | 2 |
| | 17p | Unknown | None | HEX High | |
| | 5q | Unknown | FAM Low | None | |
| | | | | | |
| **Mix 4** | 11p | Unknown | FAM High | None | |
| | Ch4 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 | |
| | Ch4 Ref | Reference | None | HEX Low | 2 |
| | 4q | Unknown | None | HEX High | |
| | 3p | Unknown | FAM Low | None | |
| | | | | | |
| **Mix 6** | 19p | Unknown | FAM High | None | |
| | Ch4 Ref | Reference | None | HEX Low | 2 |
| | 14q | Unknown | None | HEX High | |
| | 8q | Unknown | FAM Low | None | |
| | | | | | |
| **Mix 7** | 15q | Unknown | FAM High | None | |
| | Ch4 Ref | Reference | None | HEX Low | 2 |
| | 6q | Unknown | None | HEX High | |
| | 7p | Unknown | FAM Low | None | |
| | | | | | |
| **Mix 8** | 22q | Unknown | FAM High | None | |
| | Ch4 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, eight columns (A to H) from a total of 65 are displayed. The two samples (Sample 1 and Sample 2) shown in this example are in different text colours.

| A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|
| Well | Sample | Target | Conc(copies/µL) | Status | Experiment | SampleType | TargetType |
| A01 | Sample 1 | 20q | 150.00 | Manual | CNV | Unknown | Unknown |
| A01 | Sample 1 | Ch4 Ref | 156.09 | Manual | CNV | Unknown | Reference |
| A01 | Sample 1 | Xp | 77.65 | Manual | CNV | Unknown | Unknown |
| A01 | Sample 1 | 12p | 152.48 | Manual | CNV | Unknown | Unknown |
| B01 | Sample 1 | 18q | 155.50 | Manual | CNV | Unknown | Unknown |
| B01 | Sample 1 | Ch4 Ref | 160.98 | Manual | CNV | Unknown | Reference |
| B01 | Sample 1 | 9q | 160.91 | Manual | CNV | Unknown | Unknown |
| B01 | Sample 1 | 17q | 156.51 | Manual | CNV | Unknown | Unknown |
| C01 | Sample 1 | 1q | 158.00 | Manual | CNV | Unknown | Unknown |
| C01 | Sample 1 | Ch4 Ref | 161.97 | Manual | CNV | Unknown | Reference |
| C01 | Sample 1 | 17p | 154.05 | Manual | CNV | Unknown | Unknown |
| C01 | Sample 1 | 5q | 163.63 | Manual | CNV | Unknown | Unknown |
| D01 | Sample 1 | 11p | 155.17 | Manual | CNV | Unknown | Unknown |
| D01 | Sample 1 | Ch4 Ref | 160.18 | Manual | CNV | Unknown | Reference |
| D01 | Sample 1 | 13q | 157.03 | Manual | CNV | Unknown | Unknown |
| D01 | Sample 1 | 7q | 156.94 | Manual | CNV | Unknown | Unknown |
| E01 | Sample 1 | 1p | 160.59 | Manual | CNV | Unknown | Unknown |
| E01 | Sample 1 | Ch4 Ref | 164.17 | Manual | CNV | Unknown | Reference |
| E01 | Sample 1 | 4q | 167.48 | Manual | CNV | Unknown | Unknown |
| E01 | Sample 1 | 3p | 163.89 | Manual | CNV | Unknown | Unknown |
| F01 | Sample 1 | 19p | 159.46 | Manual | CNV | Unknown | Unknown |
| F01 | Sample 1 | Ch4 Ref | 156.05 | Manual | CNV | Unknown | Reference |
| F01 | Sample 1 | 14q | 153.92 | Manual | CNV | Unknown | Unknown |
| F01 | Sample 1 | 8q | 156.90 | Manual | CNV | Unknown | Unknown |
| G01 | Sample 1 | 15q | 164.37 | Manual | CNV | Unknown | Unknown |
| G01 | Sample 1 | Ch4 Ref | 162.83 | Manual | CNV | Unknown | Reference |
| G01 | Sample 1 | 6q | 171.37 | Manual | CNV | Unknown | Unknown |
| G01 | Sample 1 | 7p | 169.23 | Manual | CNV | Unknown | Unknown |
| H01 | Sample 1 | 22q | 163.55 | Manual | CNV | Unknown | Unknown |
| H01 | Sample 1 | Ch4 Ref | 169.84 | Manual | CNV | Unknown | Reference |
| H01 | Sample 1 | 16q | 168.19 | Manual | CNV | Unknown | Unknown |
| H01 | Sample 1 | 2q | 163.32 | Manual | CNV | Unknown | Unknown |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A02 | Sample 2 | 20q | 160.92 | Manual | CNV | Unknown | Unknown |
| A02 | Sample 2 | Ch4 Ref | 153.08 | Manual | CNV | Unknown | Reference |
| A02 | Sample 2 | Xp | 79.30 | Manual | CNV | Unknown | Unknown |
| A02 | Sample 2 | 12p | 157.17 | Manual | CNV | Unknown | Unknown |
| B02 | Sample 2 | 18q | 155.17 | Manual | CNV | Unknown | Unknown |
| B02 | Sample 2 | Ch4 Ref | 159.07 | Manual | CNV | Unknown | Reference |
| B02 | Sample 2 | 9q | 154.69 | Manual | CNV | Unknown | Unknown |
| B02 | Sample 2 | 17q | 159.83 | Manual | CNV | Unknown | Unknown |
| C02 | Sample 2 | 1q | 147.77 | Manual | CNV | Unknown | Unknown |
| C02 | Sample 2 | Ch4 Ref | 149.82 | Manual | CNV | Unknown | Reference |
| C02 | Sample 2 | 17p | 149.11 | Manual | CNV | Unknown | Unknown |
| C02 | Sample 2 | 5q | 146.28 | Manual | CNV | Unknown | Unknown |
| D02 | Sample 2 | 11p | 156.60 | Manual | CNV | Unknown | Unknown |
| D02 | Sample 2 | Ch4 Ref | 156.37 | Manual | CNV | Unknown | Reference |
| D02 | Sample 2 | 13q | 149.92 | Manual | CNV | Unknown | Unknown |
| D02 | Sample 2 | 7q | 157.30 | Manual | CNV | Unknown | Unknown |
| E02 | Sample 2 | 1p | 158.66 | Manual | CNV | Unknown | Unknown |
| E02 | Sample 2 | Ch4 Ref | 160.42 | Manual | CNV | Unknown | Reference |
| E02 | Sample 2 | 4q | 159.62 | Manual | CNV | Unknown | Unknown |
| E02 | Sample 2 | 3p | 154.50 | Manual | CNV | Unknown | Unknown |
| F02 | Sample 2 | 19p | 161.18 | Manual | CNV | Unknown | Unknown |
| F02 | Sample 2 | Ch4 Ref | 158.99 | Manual | CNV | Unknown | Reference |
| F02 | Sample 2 | 14q | 153.53 | Manual | CNV | Unknown | Unknown |
| F02 | Sample 2 | 8q | 161.11 | Manual | CNV | Unknown | Unknown |
| G02 | Sample 2 | 15q | 163.64 | Manual | CNV | Unknown | Unknown |
| G02 | Sample 2 | Ch4 Ref | 164.01 | Manual | CNV | Unknown | Reference |
| G02 | Sample 2 | 6q | 165.47 | Manual | CNV | Unknown | Unknown |
| G02 | Sample 2 | 7p | 158.25 | Manual | CNV | Unknown | Unknown |
| H02 | Sample 2 | 22q | 128.09 | Manual | CNV | Unknown | Unknown |
| H02 | Sample 2 | Ch4 Ref | 136.01 | Manual | CNV | Unknown | Reference |
| H02 | Sample 2 | 16q | 129.18 | Manual | CNV | Unknown | Unknown |
| H02 | Sample 2 | 2q | 129.46 | Manual | CNV | Unknown | Unknown |