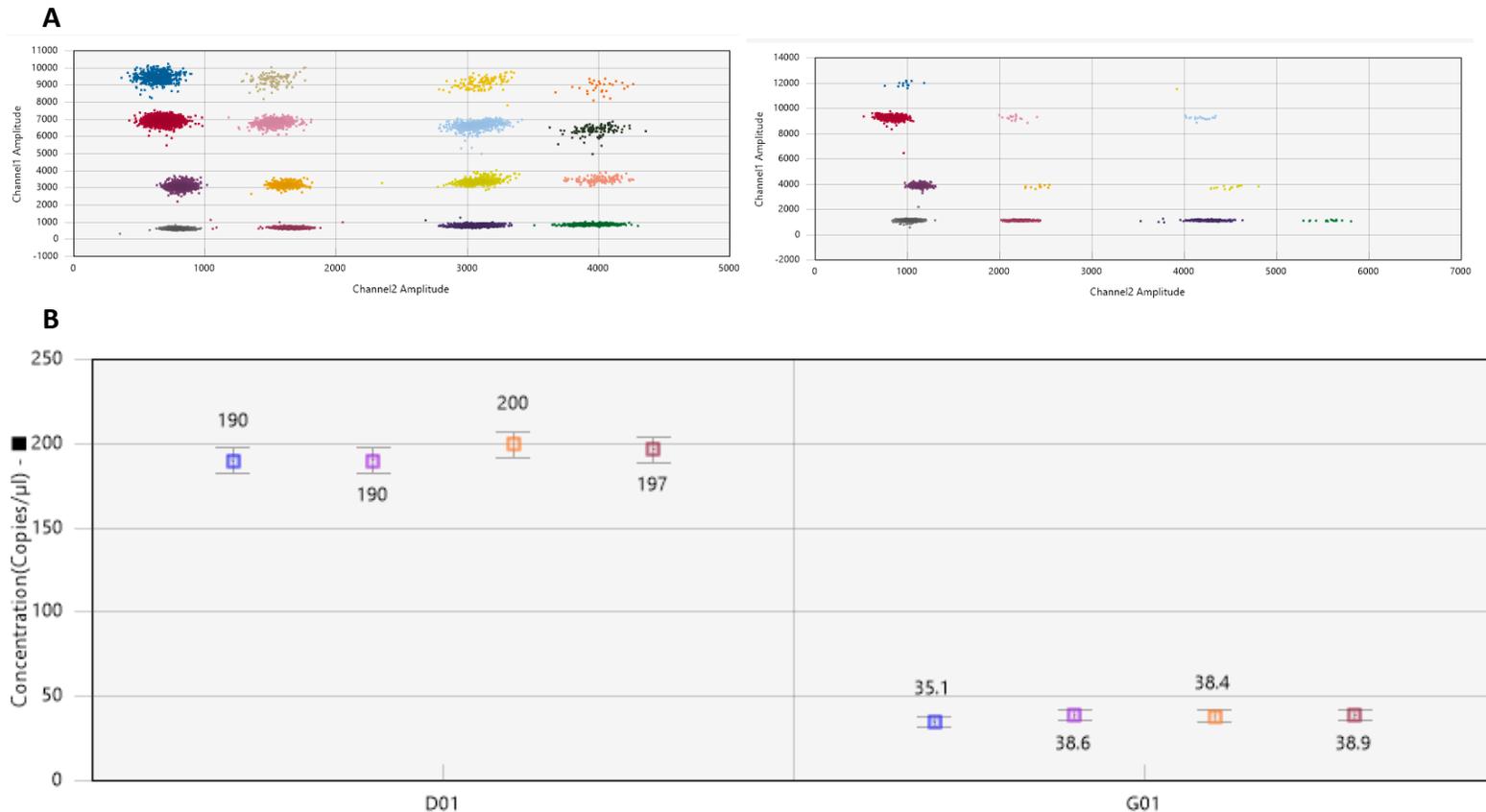# iCS-digital™ PSC kit Troubleshooting Guide

I. QuantaSoft™ Analysis Pro results troubleshooting table

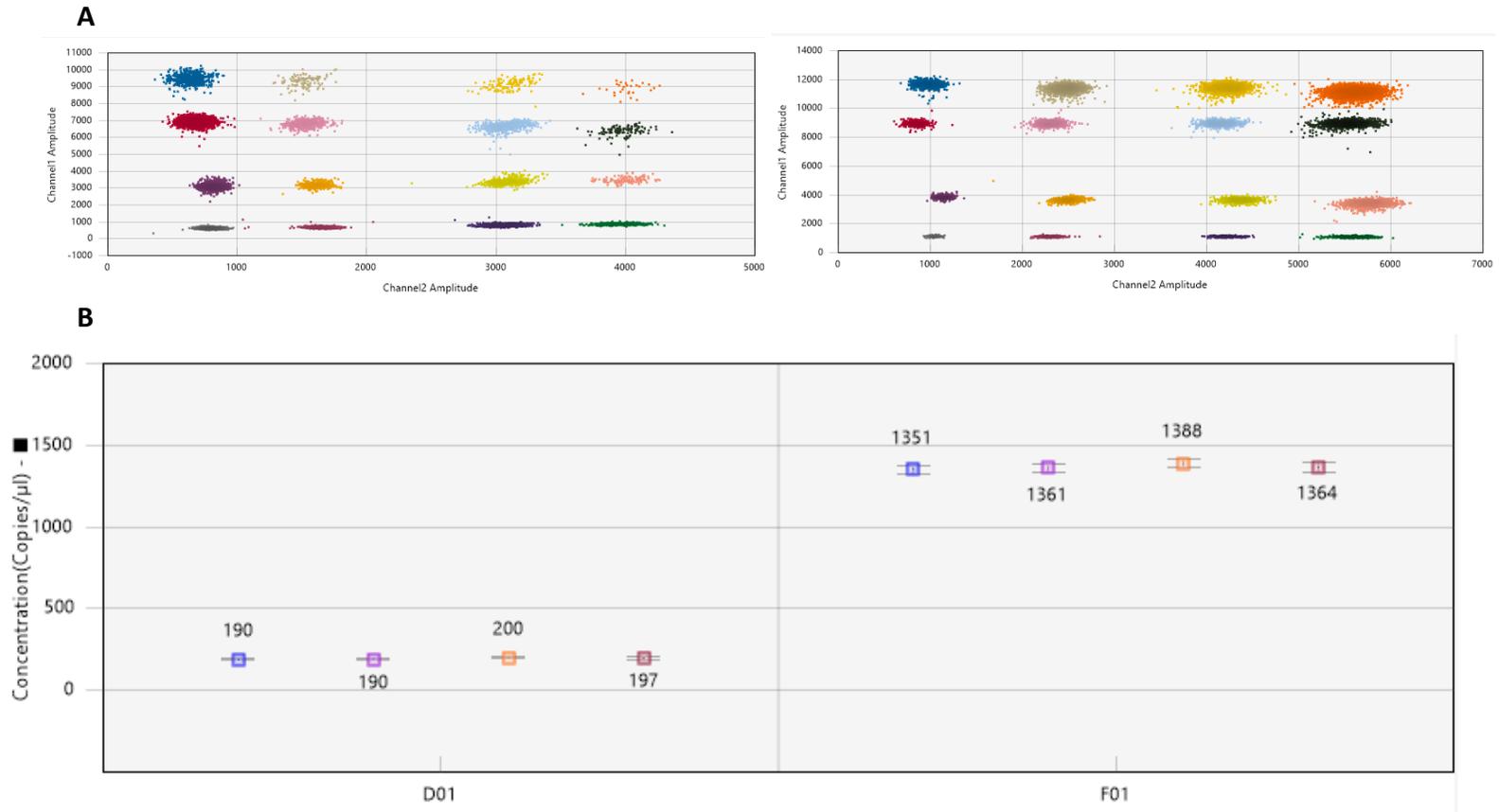| Problem observed			Possible reason	Solution
Small 2D amplitude clusters and low concentrations (<150 copies/µL) *See Fig.1. as an example*			- Low DNA concentrations were used	- Check the concentration of your DNA using a Qubit fluorometer and ensure you dilute the DNA samples to a concentration of 5 ng/µL
Large 2D amplitude clusters & high concentrations (>250 copies/µL) *See Fig.2. as an example*			- High DNA concentrations were used	
Intense Rain and/or duplicated clusters make the analysis difficult or impossible *See Fig.3. as an example*	All wells affected		- The Mix assays were not vortexed enough - PCR program is incorrect	- **Vortex vigorously** each Mix assay tube twice for 5-10 seconds, and briefly centrifuge the tubes between each vortexing steps - Check that the PCR program conforms to the protocol
	Only 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 vortexed enough	- **Vortex vigorously** each Mix assay tube twice for 5-10 seconds, and briefly centrifuge the tubes between each vortexing steps

Fig.1. Small 2D amplitude clusters and/or low concentrations (<200 copies/μL)

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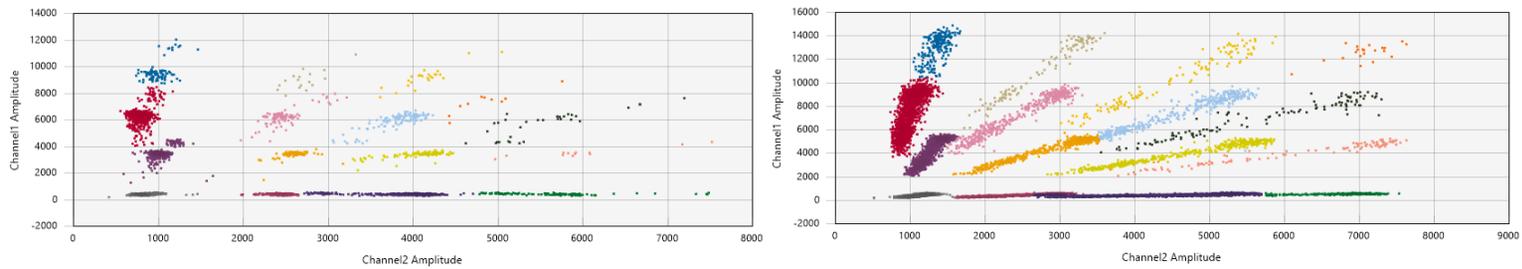
Fig.2. Large 2D amplitude clusters and/or high concentrations (>200 copies/µL)

A. 2D Amplitude tab. B. Concentration tab (normal values ~200 copies/µL).
Left panels: 2D amplitude & Concentration tab of a sample with correct DNA concentration. Right panels: 2D amplitude & Concentration tab of a sample with high DNA concentration.

Fig.3. Intense rain and/or duplicated clusters make the analysis difficult or impossible

Example of a low quality 2D-cluster well

II. 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 Page 6 & Pages 11-12 (APPENDIX 2) of the 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) **Refer to Page 6 of the User Manual**
	- Incorrect sorting of the data prior to their exportation 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 Page 9 (Figure 3) & Pages 13-14 (APPENDIX 3) of the User Manual**
	- A sample with concentration (copies/μL) and therefore CNV values equal to zero (e.g., used of a NCT)	- Do not include samples with 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 exportation 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 Page 9 (Figure 3) & Page 13-14 (APPENDIX 3) of the User Manual**