Frequency	Problem	Possible Cause	Solution
Primer testing with qPCR			
Rare	Primer testing using qPCR provides inconsistent results (e.g. variable Ct between replicates with high standard deviation).	The ROX normalization is turned on in your instrument.	Make sure to turn the ROX normalization off before you start the qPCR run.
Medium	Primer testing using qPCR reveals poor efficiency over the different concentrations tested.	The PCR assay is not optimal in the dPCR mastermix.	Optimise the assay conditions or redesign the assay if needed.
dPCR droplets formation and reaction			
Medium	I observe foam and no droplets in one or more outlet well(s) of a dPCR cartridge.	This could be due to cartridge failure, instrument failure or incorrect loading of the cartridge (wrong order or volumes).	If the loading of the cartridge was done correctly, please contact support@samplix.com to evaluate other causes.
Rare	Double emulsion droplets are not forming in the dPCR cartridge after run.	This could be due to incorrect loading of the cartridge.	Remember to load the cartridge in the order A, D, C and B and to dilute the 2x dPCR buffer to 1x with molecular grade water.
Rare	During dPCR droplets break, the break solution is added, and the sample is centrifuged, however, in rare cases the aqueous phase turns solid after centrifugation.	Mixing strategy prior to centrifugation too vigorous.	Mix by gently flicking prior to centrifugation. If that does not solve the issue, let the tube sit for 15 minutes and then proceed to removing the aqueous phase.

dPCR droplets sorting			
Rare	I cannot identify any positive droplet	Slow ramping was not set on the thermal cycler.	Make sure that slow ramping is turned on in the PCR thermal cycler program, this is important as the oil layer act as insulator and temperature changes need more time to take effect.
		The dPCR reaction has not worked.	Make sure to test your primers in the Samplix dPCR mix before transferring into droplets.
		Wrong settings of the FACS.	Check you are applying the 488 nm (blue) laser and check that the optical filters fit with measurement settings applied.
Rare	I can't see a good separation between positive and negative droplets.	The intercalating dye can bind to impurities in the sample.	Perform a capture bead clean-up of the sample to remove potential impurities.
		The dPCR primers bind off-targets.	Remember to check for potential off-targets during dPCR primers design and testing by checking for only one melting point in the qPCR melting curve.
Rare	I can't find the droplets in SSC vs. FSC scatter plot.	Settings of the live plot and threshold might be wrong.	Make sure the live plot is showing at least 100,000 events, otherwise the positive droplet population might be difficult to identify. It might also be necessary to adjust detector and threshold settings.
Frequent	The droplet formation looks different between FACS runs.	It is expected that double emulsion droplets (dPCR) can look different on the FACS from production to production. Mostly it is the oil droplets in the production that either can make a S-shaped tail or C-shaped tail.	No need to take action. This is not affecting the downstream workflow. However, make sure to adjust the gates accordingly to each run.

dMDA droplets formation and reaction			
Rare	I cannot measure any DNA yield after the dMDA amplification reaction.	The dMDA enzyme has not been able to perform amplification due to remaining break solution.	Repeat dMDA droplet production and reaction. Make sure to accurately remove the leftovers of the breaking solution.
Rare	The volume recovered after the dMDA droplet production is less than 10 µl.	Collection of the dMDA droplets was not complete.	Remember to wash the outlet well with oil to collect all the dMDA droplets. Be very careful when separating the phases to reduce waste. Spin down the tube repeatedly if needed.
Enrichment calculation			
Frequent	Enrichment estimate measured by qPCR do not match the enrichment calculated on sequencing result.	qPCR enrichment estimate varies a lot depending on the positioning of the Validation Sequence used for the qPCR.	Use qPCR enrichment estimate only as indicative of positive enrichment whenever above 100x.
Instrument			
Rare	Instrument reports Error 8 (failure to maintain pressure)	This could be due to: cartridge failure, incorrect placement of the gasket, instrument failure or incorrect loading of the cartridge (wrong lanes selected).	Check that the cartridge and gasket are correctly placed. If this error appears repeatedly, contact support@samplix.com to evaluate the cause.