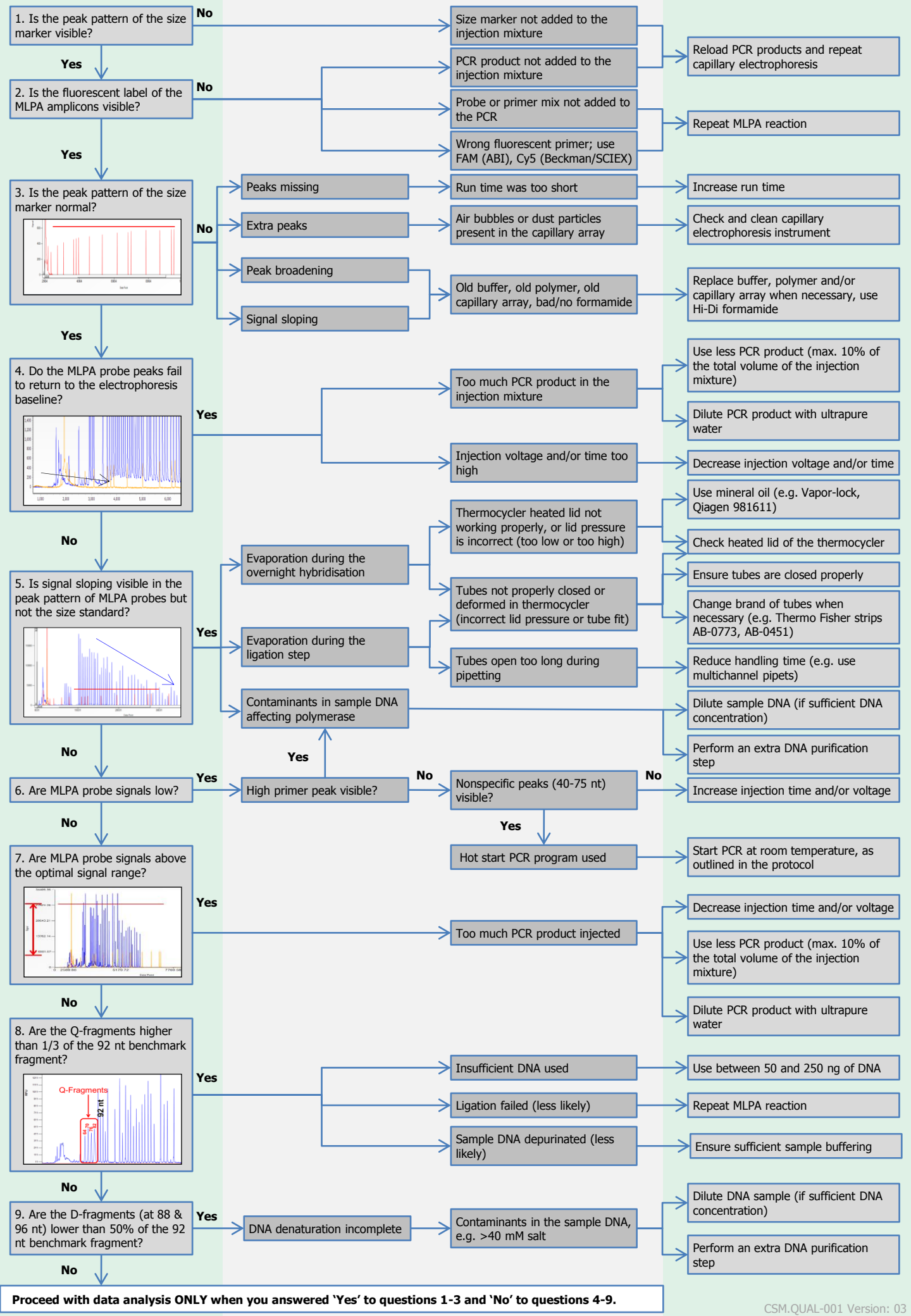
1. Is the peak pattern of the size marker visible?
No
Size marker not added to the injection mixture
Yes
2. Is the fluorescent label of the MLPA amplicons visible?
No
PCR product not added to the injection mixture
Probe or primer mix not added to the PCR
Wrong fluorescent primer; use FAM (ABI), Cy5 (Beckman/SCIEX)
Reload PCR products and repeat capillary electrophoresis
Repeat MLPA reaction
Yes
3. Is the peak pattern of the size marker normal?
No
Peaks missing
Extra peaks
Peak broadening
Signal sloping
Run time was too short
Air bubbles or dust particles present in the capillary array
Old buffer, old polymer, old capillary array, bad/no formamide
Increase run time
Check and clean capillary electrophoresis instrument
Replace buffer, polymer and/or capillary array when necessary, use Hi-Di formamide
Yes
4. Do the MLPA probe peaks fail to return to the electrophoresis baseline?
Yes
Too much PCR product in the injection mixture
Injection voltage and/or time too high
Use less PCR product (max. 10% of the total volume of the injection mixture)
Dilute PCR product with ultrapure water
Decrease injection voltage and/or time
No
5. Is signal sloping visible in the peak pattern of MLPA probes but not the size standard?
Yes
Evaporation during the overnight hybridisation
Evaporation during the ligation step
Contaminants in sample DNA affecting polymerase
Thermocycler heated lid not working properly, or lid pressure is incorrect (too low or too high)
Tubes not properly closed or deformed in thermocycler (incorrect lid pressure or tube fit)
Tubes open too long during pipetting
Use mineral oil (e.g. Vapor-lock, Qiagen 981611)
Check heated lid of the thermocycler
Ensure tubes are closed properly
Change brand of tubes when necessary (e.g. Thermo Fisher strips AB-0773, AB-0451)
Reduce handling time (e.g. use multichannel pipets)
Dilute sample DNA (if sufficient DNA concentration)
Perform an extra DNA purification step
No
6. Are MLPA probe signals low?
Yes
High primer peak visible?
Yes
No
Nonspecific peaks (40-75 nt) visible?
No
Increase injection time and/or voltage
Yes
Hot start PCR program used
Start PCR at room temperature, as outlined in the protocol
No
7. Are MLPA probe signals above the optimal signal range?
Yes
Too much PCR product injected
Decrease injection time and/or voltage
Use less PCR product (max. 10% of the total volume of the injection mixture)
Dilute PCR product with ultrapure water
No
8. Are the Q-fragments higher than 1/3 of the 92 nt benchmark fragment?
Q-Fragments
92 nt
Yes
Insufficient DNA used
Ligation failed (less likely)
Sample DNA depurinated (less likely)
Use between 50 and 250 ng of DNA
Repeat MLPA reaction
Ensure sufficient sample buffering
No
9. Are the D-fragments (at 88 & 96 nt) lower than 50% of the 92 nt benchmark fragment?
Yes
DNA denaturation incomplete
Contaminants in the sample DNA, e.g. >40 mM salt
Dilute DNA sample (if sufficient DNA concentration)
Perform an extra DNA purification step
No
Proceed with data analysis ONLY when you answered 'Yes' to questions 1-3 and 'No' to questions 4-9.