

KASP thermal cycling conditions

Recycling protocol

If clear genotyping clusters have not been obtained after completion of the standard KASP thermal cycle, the reaction plate should be thermally cycled further. One KASP recycling step comprises of three additional PCR cycles, as outlined in the table below.

Protocol stage	Temperature	Duration	Number of cycles for each stage
Stage 1 Amplification	94 °C	20 seconds	x 3 cycles
	57 °C	60 seconds	
Optional stage 2 (read stage for qPCR instruments only)	30 °C (any temperature below 40 °C is suitable for the read stage)	60 seconds	x 1 cycle

* Please note that stage 2 of the above program is only required if running and reading KASP™ genotyping reactions on a qPCR machine. If running the KASP thermal cycle program on a Peltier block or a Hydrocycler, only Stage 1 is needed although you must ensure that the reaction plates are cooled to <40 °C before performing the template read.

For a detailed explanation of recycling and an illustration of the effect that this can have on your genotyping results, please view our <u>guide to further cycling of genotyping reactions</u>.

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For any queries about running KASP reactions in your laboratory please contact the technical support team at techsupport@lgcgroup.com.

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