KASP thermal cycling conditions

65-57°C touchdown protocol

Protocol Stage	Temperature	Duration	Number of cycles for each stage
Stage 1 Hot-start *Taq* activation	94°C	15 minutes	x 1 cycle
Stage 2 Touchdown	94°C	20 seconds	x 10 cycles
	65°C (65°C decreasing 0.8°C per cycle to achieve a final annealing / extension temperature of 57°C)	60 seconds	
Stage 3 Amplification	94°C	20 seconds	x 26 cycles
	57°C	60 seconds	
Optional Stage 4 (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 4 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 Stages 1, 2 and 3 are needed although you must ensure that the reaction plates are cooled to <40°C before performing the plate read.

Explanation of touchdown PCR

KASP™ chemistry utilises a two-step touchdown PCR method, with the elongation and annealing steps incorporated into a single step. The temperature used for the annealing stage determines the specificity of the reaction and hence the ability of the primers to anneal to the DNA template. A touchdown PCR involves starting with a high annealing temperature and incrementally decreasing the annealing temperature each PCR cycle. The higher annealing temperatures in the early cycles of a touchdown ensure that only very specific base pairing will occur between the DNA and the primer, hence the first sequence to be amplified is most likely to be the sequence of interest. The annealing temperature is gradually decreased to increase the efficiency of the reaction. The regions that were originally amplified during the highly specific early touchdown cycles will be further amplified and outcompete any non-specific amplification that may occur at the lower temperatures.

For any queries about running KASP reactions in your laboratory please contact the technical support team:
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