KASP genotyping - guide to further cycling of genotyping reactions

The purpose of this document is to explain the process of recycling (further cycling of reactions) and to illustrate the positive effect that this can have on your genotyping results.

Introduction

The efficiency of KASP™ genotyping reactions is dependent on a number of factors including concentration of sample DNA and composition of the DNA sequence surrounding the SNP site. DNA sequence composition will impact the efficiency of primer binding and hence affect the rate of the PCR reaction. For this reason, different KASP assays will reach completion at different rates and can require additional PCR cycles to produce clear genotyping clusters.

Following completion of the standard KASP thermal cycle (10 cycles of touchdown PCR and 26 cycles of standard PCR), it is possible that your data points will not have separated into distinct clusters. Rather than indicating that the KASP genotyping assay is not working, it is more likely that the PCR has not undergone a sufficient number of cycles to reach completion. At this stage, LGC, Biosearch Technologies™ recommend further cycling of the reaction plate – termed 'recycling'.

Effects of recycling

Recycling allows for each data point to reach reaction completion, helping to tighten clusters and aid with genotype scoring. Figure 1 illustrates the effect that recycling can have on the genotyping cluster plot. After the initial KASP thermal cycle, the data points are beginning to form three clusters, but the data points within each cluster are fairly spread out (Figure 1A). This indicates that there are considerable differences in the amount of signal that has been generated for samples of the same genotype. At this stage, the signal generation is too low which is causing the no template control (NTC) samples to appear as if they are amplifying.

The reaction plate was further thermally cycled for one recycle step (3 PCR cycles), and the plate re-read (Figure 1B). With further cycling, more signal is generated and the groups tighten. More differentiation is seen between the DNA sample clusters and the NTCs that are not amplifying. A second recycle step (3 PCR cycles) was run, and the plate re-read for the final time (Figure 1C). At this stage, tight genotyping clusters are evident indicating that the PCR has reached completion.

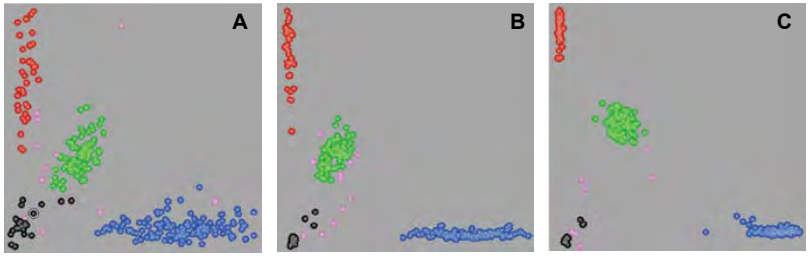

Figure 1. Genotype clustering improves after each recycle step. Initial clusters (A) are not tight, one recycling step improves the clustering (B) and a second recycling step brings the reactions to completion resulting in tight, easy to score clusters (C).

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KASP recycling program

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 Table 1.

Step	Description	Temperature	Time	Number of cycles per step
1	Denaturation	94 °C	20 sec	3 cycles
2	Annealing/ elongation	57 °C	60 sec	

Table 1. Conditions for further cycling (recycling) of KASP chemistry.

Hints and tips for recycling of KASP genotyping reactions

- Each KASP Assay will differ in the optimal number of cycles required to reach reaction completion.
- Biosearch Technologies recommends optimising your assay by performing several individual recycle steps with subsequent plate reads, rather than combining all of the additional cycles together and reading once at the end.
- Following assay optimisation you can increase the number of cycles in the initial KASP thermal cycle by the number of additional cycles that are required to take the reaction to completion. The assay can then be run on the remainder of your DNA sample plates without the need for additional recycling. For example: if SNP assay 1 required two recycling steps (+ 6 PCR cycles) to produce tight clustering, the standard KASP thermal cycle can be adjusted to comprise of 10 touchdown PCR cycles, and 32 standard PCR cycles (26 + 6).
- The maximum number of recycle steps (3 PCR cycles each) that Biosearch Technologies recommend is four. This equates to an additional 12 PCR cycles to the standard KASP thermal cycle (+12). If tight clusters are not attained after four recycle steps, the assay will require further troubleshooting e.g. redesign, DNA concentration optimisation.

Technical support

For any queries about this guide please contact:
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