dragonfly® discovery
technical note

PCR reaction set-up by dragonfly® shows no cross contamination in sealed mixed samples

introduction

A vast number of advantages can be gained by semi-automating genomics workflows through use of low volume liquid handling instruments, such the mosquito® genomics and dragonfly® discovery offered by SPT Labtech. Benefits include the miniaturization of reaction volumes to reduce cost per sample and the elimination of most sources of human error to increase experimental reproducibility.

These semi-automated systems often require the use of a plate shaker or a vortexing instrument in order to ensure proper mixing of the different components of reactions. This can bring in the risk of cross-contamination due to micro-droplets and aerosol formation.

In this technical note we have tested the effect of mixing on cross-contamination between different wells during PCR set-up.

materials and methods

RT-PCR reactions were set up in 384-Well Skirted PCR Plates for Roche® Lightcycler® (STARLAB, E1042-9909) to a final volume of 5 µL per reaction using dragonfly® discovery. The reactions consisted of qPCR Control Kit (Jena Bioscience, PCR-354) and KAPA SYBR FAST qPCR Master Mix (2X) Universal (KAPA BIOSYSTEMS, KK4600), following the instructions of the manufacturers. qPCR-grade water was used as a negative control. The set-up of the plates was arranged as shown in figure 1.

Plates were sealed after reaction set-up using Clear Polypropylene Seals (STARLAB, E2796-0793/ E2796-9795/ E2796-9793). Mixing was performed in either way:

- MixMate® plate shaker (Eppendorf, 5353000510) for 5 minutes at 3000 rpm
- Personal Vortex Mixer (Grant Instruments™, PV-1) for 1 minute at maximum power

Reactions were performed in a LightCycler® 480 Instrument II (Roche Molecular Systems, 05015243001) following the cycle as described in table 1. Analysis of samples was performed using LightCycler® 480 Software.

results

The amplification analysis of plates mixed with both methods were analysed:

- Samples mixed with the MixMate® plate shaker showed no amplification in the negative controls (Fig 2. A). Two positive samples showed sporadic amplification due to lack of spin down after dispense (Fig 2. B). The Ct value for these samples was 25.39 (SD = 0.135; CV= 0.5)
- Samples mixed with the vortex mixer showed no detectable amplification in the negative controls (Fig 2. C). All positive samples show comparable amplification curves (Fig 2. D). The Ct value for these samples was 25.39 (SD = 0.124; CV= 0.5)

The threshold of CT >34 was used to consider negative samples.

conclusions

Mixing using either of the methods chosen should not be a cross contamination concern when using sealed plates.

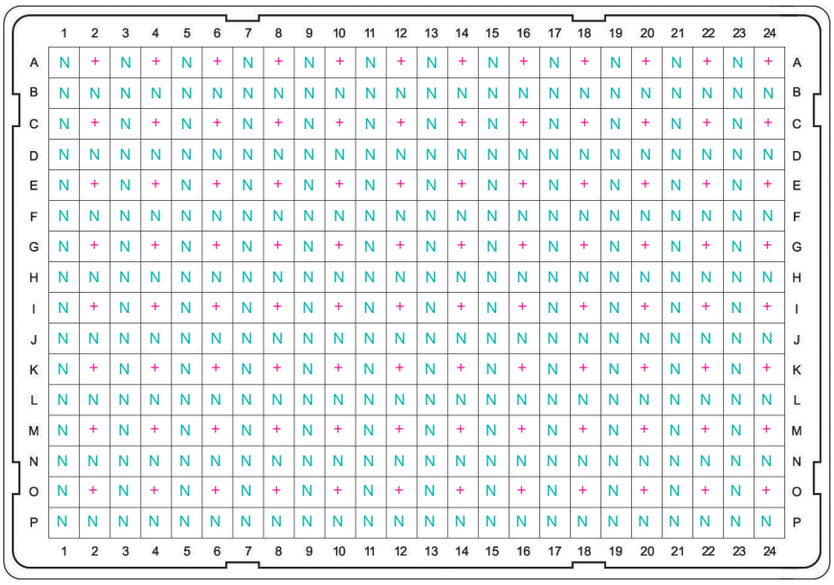

Fig 1: Plate layout for experiments. qPCR reactions (+) were arranged surrounded by negative controls (N).

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Table 1. PCR conditions that were used for testing.			
Stage	**Temperature**	**Duration**	**Number of cylces**
Initial denaturation	95°C	2 min	1x
Denaturation	95°C	10 sec	33x
Annealing	59°C	20 sec	
Elongation	72°C	30 sec	
Final elongation	72°C	2 min	1x

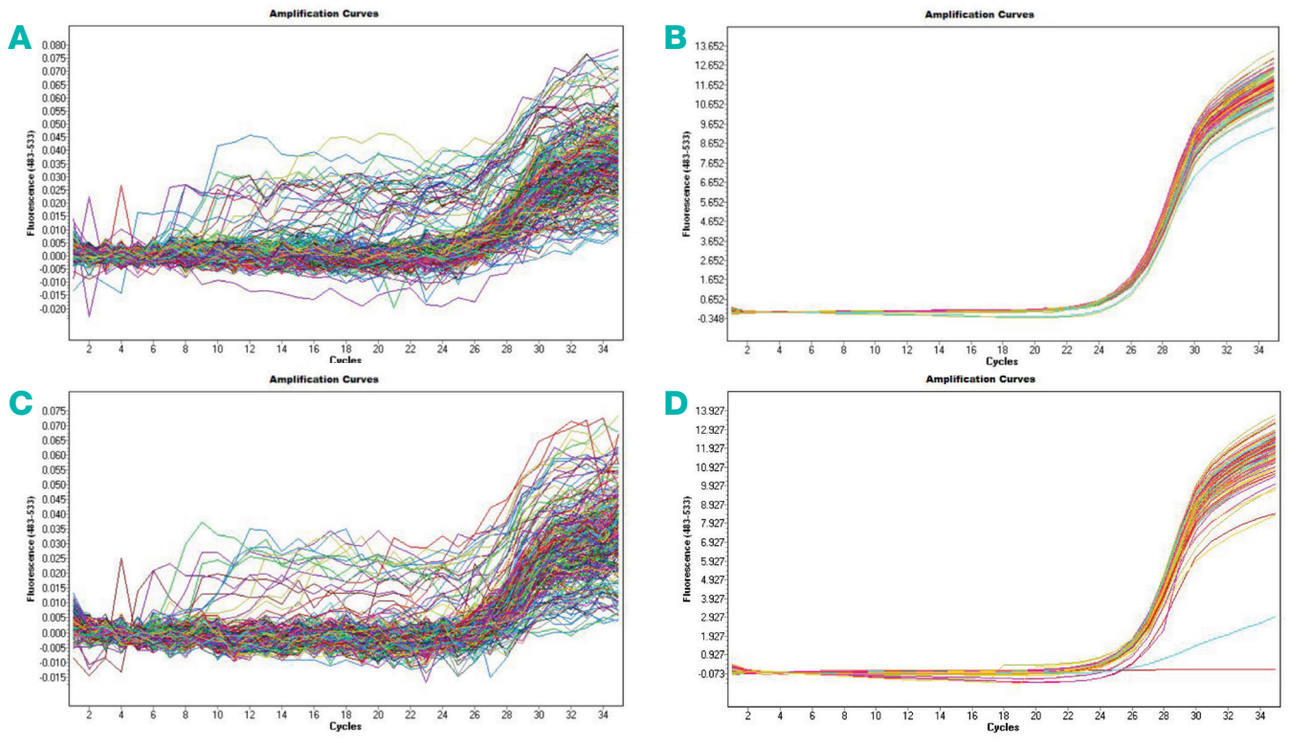

Fig 2. Negative controls for both types of mixing show no detectable amplification (A and C). Positive samples show comparable amplification curves in both cases (B and D). The Ct value for these samples is 25.39.

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For more information contact us at **discover@sptlabtech.com**

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