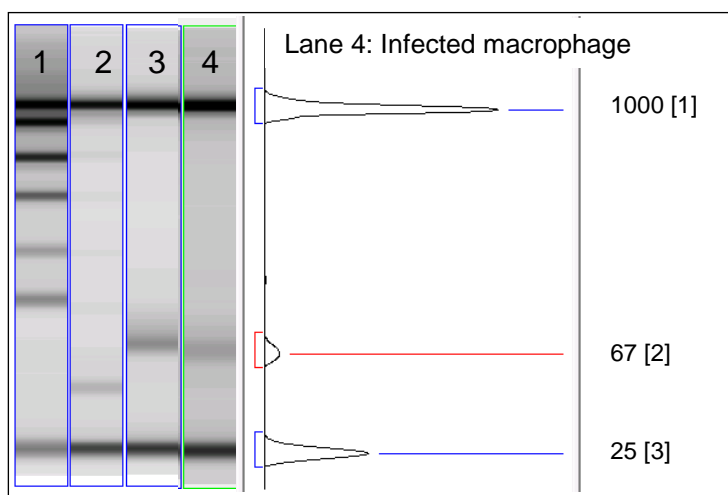


ScreenTape® enables fast Quality Control of miRNA qPCR reactions

Quantitative PCR (qPCR) is routinely used to assess miRNA expression levels. Quality control of the reaction by gel electrophoresis confirms the presence of a single product of the correct size. This control can be achieved using ScreenTape, within 10 minutes, providing automated, hands-free analysis without reagent or gel preparation.



Tape Used

ScreenTape D800

Total time to result

for 7 samples

10 minutes

Sample volume per test – 1µl

Analysis Results

Precise and automated sizing of
65-70bp qPCR products

Figure 1: Results from qPCR products run on ScreenTape showing amplified MCMV miRNA from infected 3T3 cells in lane 3 and from infected macrophages in lane 4. Lane 2 shows a result from un-infected 3T3 cells.

Introduction

Following qPCR analysis, it is important to check that the reaction only contains amplified products that are specific to the chosen primers. This is routinely achieved by melting curve analysis on the qPCR instrument. However, this method is not always precise as it often distinguishes poorly contaminating bands with small size or sequence differences. Many scientists therefore prefer to use traditional gel electrophoresis. ScreenTape provides a novel and more efficient method of analysing and precisely sizing qPCR products.

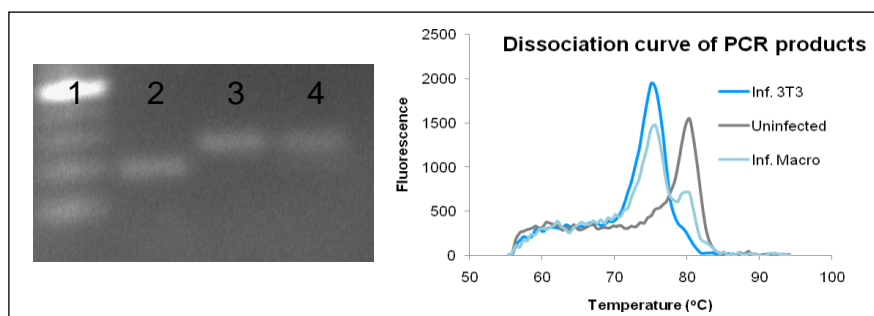


Figure 2: Results from miRNA qPCR reactions run on an agarose gel showing an uninfected sample in lane 2 and MCMV infected 3T3 cells and macrophages in lanes 3 and 4 respectively. Dissociation curves of these PCR products can be seen in the associated graph.

Materials and Methods used in qPCR

Purified RNA from MCMV infected macrophages and 3T3 cells were poly-A tailed and reverse transcribed using the NCode™ miRNA First strand cDNA synthesis kit (Invitrogen), following the manufacturer's instructions. SYBR green PCR was performed using qPCR supermix-UDG™ (Invitrogen) and conditions were optimized to generate only one product of around 65-70bp for each primer in infected cells, whereas a product of 44bp was observed for uninfected cells, showing the presence or absence of a specific regulating viral miRNA.

ScreenTape DNA Analysis Procedure

Samples were mixed 1:1 with loading buffer and placed in the TapeStation™ with pre-packaged ScreenTape. After clicking "START" on the software driven menu, full analysis and archiving of the results was achieved with no user intervention within 10 minutes.

Results on ScreenTape

Analysed results shown in Figure 1 immediately provide band sizes for each of the qPCR reactions. Results agree with the gel and dissociation curve analysis seen in Figure 2. Sample 2 shows a product of 44bp diagnostic of an uninfected sample, whereas samples 3 in macrophages and 4 in 3T3 cells have a product of 66bp, indicating that they are from MCMV infected cells. Lanes 4, 5 and 7 are negative controls with no PCR product present.

Benefits of using ScreenTape for qPCR QC.

Like the dissociation curve analysis, ScreenTape provides the convenience of an automated solution to qPCR QC with the precision and certainty of slab gel analysis. Band sizes are accurately and automatically determined, with minor contaminants being easily identified. ScreenTape offers significant time saving for gel analysis of qPCR reactions, by providing a fully automated, walk-away solution. ScreenTape electrophoresis is convenient and integrates seamlessly with qPCR analysis. As no reagent preparation is needed and Ethidium Bromide and UV light are eliminated; user-safety is enhanced. The TapeStation instrument is no bigger than a desk-top printer which saves space and keeps the lab bench free of spilt buffers.