ThermoFisher SCIENTIFIC

Sample prep

TaqMan Cells-to-C_T Express Kit: automation-friendly product for high-throughput gene expression analysis

Introduction

The Invitrogen™ TaqMan™ Cells-to-C_T™ Express Kit is a new addition to the Invitrogen™ TaqMan™ Cells-to-C_T™ portfolio, which enables gene expression analysis by reverse-transcription quantitative PCR (RT-qPCR) directly from cell lysates without the need to purify RNA. The TaqMan Cells-to-C_T Express Kit includes Express Lysis Solution and Express ezDNase™ enzyme to remove gDNA during cell lysis; SuperScript™ IV VILO™ Master Mix for RT; and TaqMan™ Fast Advanced Master Mix for qPCR. The major advantage of this kit version is a novel formulation of cell lysis solution, which does not require a stop solution to inactivate the lysis reaction prior to RT. Removing the stop solution step further simplifies the TaqMan Cells-to-C_T workflow and reduces the number of pipette tips required, which can amount to cost savings, especially for high-throughput users. The procedure does not require heating, centrifugation, or filtration steps, making it especially amenable to liquid handling systems. Here, we outline the use of a Tecan™ Fluent™ 780 automated liquid handling instrument configured with components to process an entire 96-well plate of cells and analyze gene expression for two different genes. To evaluate the performance of the automated method, we compared results of plates processed with either the automated or manual method.

Materials and methods

Cell culture

Sample plates were prepared by seeding 5,000 adherent human cervical adenocarcinoma cells into all wells of two flat-bottom 96-well cell culture plates—one to be used for the manual method and the other for the automated method. Cells were aliquoted into wells in 150 µL of Gibco™ Minimum Essential Medium α (MEMα) with 10% FBS. Plates were incubated overnight at 37^{0}C under 5% CO_2. After overnight incubation, the estimated cells doubled resulting in monolayers consisting of approximately 10,000 cells each.

Manual method

For manual TaqMan Cells-to-C_T Express sample preparation, the workflow outlined in Figure 1 was followed, and is detailed below. The manual 96-well cell culture plate was placed on ice while the Express Lysis Solution containing Express ezDNAse enzyme was prepared and 1X PBS for cell washing was cooled on ice. The cell culture medium was then removed from the wells using a multichannel pipette and discarded, and 150 µL of cold PBS was then added to the wells. The PBS wash was immediately drawn off and discarded, removing as much residual PBS as possible without disturbing the cell monolayer.

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The plate was taken off ice and placed on the benchtop for lysis. Express Lysis Solution mixed with Express ezDNAse enzyme was then added to each well in 50 µL aliquots using a multichannel pipette. The plate was then transferred to an orbital plate shaker and allowed to shake for 5 minutes to promote mixing and cell lysis. After 5 minutes, the plate was removed from the orbital shaker and placed on ice.

An RT plate was prepared by aliquoting 40 µL of SuperScript IV VILO Master Mix cocktail into wells of a 96-well PCR plate. Using a multichannel pipette, 10 µL of each cell lysate was added to corresponding wells of the RT plate and mixed by the pipette. The plate was then sealed with an adhesive seal and placed on a thermocycler for temperature cycling. After the RT program was completed, the plate was briefly centrifuged to collect reaction contents to the bottom of the tube.

For qPCR, two qPCR cocktails were prepared using the TaqMan Fast Advanced qPCR master mix, one containing a TaqMan gene expression assay targeting the *ACTB* gene and another targeting the *CDK4* gene. The qPCR cocktails were distributed into 192 wells of a 384-well real-time PCR plate such that half of the plate contained *ACTB* assays and the other half contained *CDK4* assays. Each qPCR well contained 8 µL of cocktail and 2 µL of cDNA was added to corresponding wells of the qPCR plate with duplicate reactions prepared for each cDNA sample. The plate was then sealed with an optical adhesive seal, vortexed briefly, and centrifuged to collect reaction contents at the bottom of the well. The plate was then transferred to an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, and the qPCR program was run.

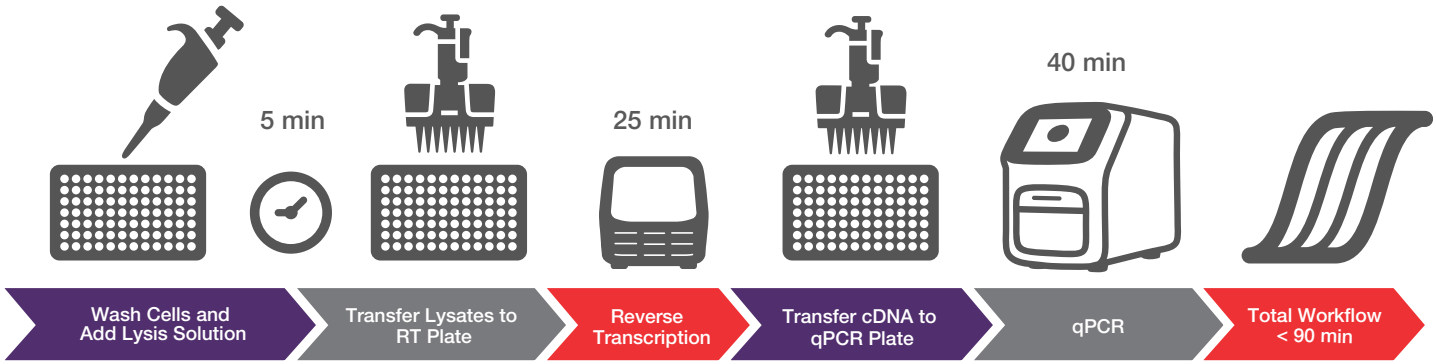

Figure 1. Overview of the TaqMan Cells-to-C_T Express workflow. The culture medium is removed and cells are washed with cold PBS. Next, Express Lysis Solution with Express ezDNase enzyme is added to the culture wells and pipette-mixed 5 times or placed on an orbital shaker. The lysate is then incubated for 5 minutes. SuperScript IV VILO RT cocktail is prepared and distributed into a PCR plate; an aliquot of cell lysate is then added directly to the RT reaction; and the reactions are run on a thermocycler. TaqMan Fast Advanced qPCR cocktail, including an Applied Biosystems™ TaqMan™ gene expression assay is aliquoted into a real-time PCR plate, cDNA is added, and the plate is run on a real time PCR instrument.

Automated method

For the automated TaqMan Cells-to-C_T Express workflow preparation, a Tecan Fluent 780 automated liquid handling instrument configured with components outlined in Figure 2 was utilized. The 96-well cell culture plate for the automated method was placed on ice while preparing the system.

To prepare for the experiment, Express Lysis Solution containing Express ezDNase enzyme was prepared off-system and aliquoted into wells of a 96 deep-well plate and loaded onto the instrument deck at position 8 in Figure 2. A reagent trough was filled with cold PBS for washing cells and an empty reagent trough for liquid waste was additionally added to the instrument deck at positions 7 and 9, respectively, in Figure 2. RT and qPCR cocktails were prepared off-system, aliquoted into 2 mL screw- cap tubes, and placed into the tube holder at position 13 in Figure 2. Once all consumables and reagents were loaded onto the instrument, the culture plate was placed on the plate shaker at position 3 in Figure 2.

The automation script consisting of three segments was created for this experiment. In the first segment, the 96-channel arm is used to aspirate the cell culture media from wells of the culture plate and transfer it to the waste trough. The 96-channel arm is then used to wash cells by dispensing 100 µL of PBS to the culture plate and then subsequently aspirating the PBS and disposing it to the waste trough. Following aspiration of PBS, we observed ~15 µL of residual PBS dead volume remaining in the culture plate. The 96-channel arm is then used to transfer 50 µL of Express Lysis Solution to the culture plate, the plate is then shaken at 900 rpm for 5 minutes on the BioShake™ 3000-T elm plate shaker (QInstruments). During the plate shaking step, the RT plate is prepared using the 8-channel arm to distribute 40 µL of RT cocktail to wells of the 96-well PCR plate on the instrument deck. Once plate shaking is complete, the 96-channel arm is used to transfer 10 µL of each lysate to corresponding wells of the prepared RT plate. The RT reaction is then pipette-mixed 4 times by the 96-channel arm using the same tips. The RT plate is then removed from the instrument, sealed with an adhesive seal, and placed on a thermocycler to run the RT program.

During RT cycling, the second script segment is initiated to distribute 8 µL of each qPCR cocktail to wells of the 384-well real-time PCR plate using the 8-channel arm. Once the RT thermocycling program has been completed, the RT plate is centrifuged briefly to collect reaction contents to the bottom of the well and returned to the instrument deck.

In the third script segment, the 96-channel arm is used to transfer 2 µL of cDNA from the RT plate to wells of the 384-well real-time PCR plate. Each cDNA sample was transferred to duplicate qPCR reactions. After each sample is dispensed into the qPCR plate, the reaction is pipette-mixed 4 times by the 96-channel arm using the same tips. Once complete, the qPCR plate was removed from the instrument, sealed with an optical adhesive seal, and transferred to a QuantStudio 5 system. The qPCR program was then run.

Figure 2. Configuration deck of the Fluent 780 system as outlined and described in Table 1 below.

Table 1. Tecan Fluent 780 system deck configuration.

Position	Component	Utilization
1	8-channel flexible channel arm	Automated pipetting
2	96-channel multichannel arm	Automated pipetting
3	BioShake 3000-T elm plate shaker	Shaker for lysis plate
4	200 µL conductive tips, filtered	Tips for 8-channel arm
5	Boxes of 50 µL tips, filtered	Tips for 96-channel arm
6	Boxes of 150 µL tips, filtered	Tips for 96-channel arm
7	300 mL reagent trough	Container for PBS wash
8	96-well deep-well plate	Container for Express Lysis Solution
9	300 mL reagent trough	Container for reagent waste
10	96-well semi-skirted PCR plate	Reverse transcription plate
11	384-well real-time PCR plate	qPCR plate
12	96-well flat-bottom cell culture plate	Culture/lysis plate
13	2 mL flip-cap tubes	Containers for RT/qPCR cocktail

Results

Data from qPCR were analyzed using QuantStudio 5 software with automatic C_t settings for both the manual and automation plates. C_t values were averaged and standard deviations were calculated across all 192 qPCR replicates for each plate and gene target. We observed little difference in average C_t values for both genes tested between the manual and automated methods (Figure 3). Comparison of the standard deviation values between the two methods as well as amplification plot data (Figure 4) show that the automated method produced a slightly greater spread of C_t values across replicates. This is most likely due to the variable amount of residual PBS observed in wells of the automation plate after the cell washing portion of the script. We suspect that the automation process could be optimized to remove more of the residual PBS to minimize this difference.

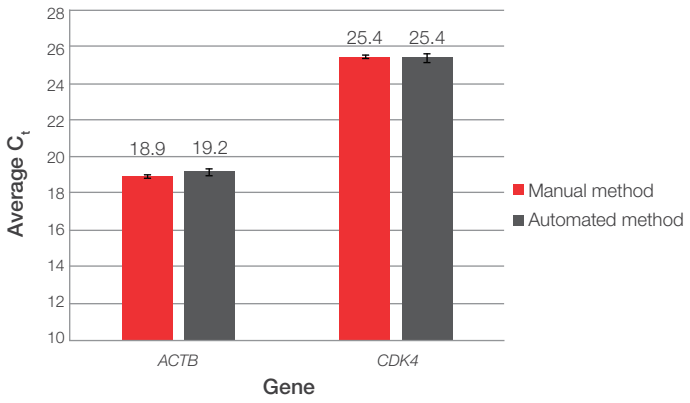

Figure 3. Comparison of average C_t values obtained from manual and automated methods. Bars represent the mean ± standard deviation of 192 replicates.

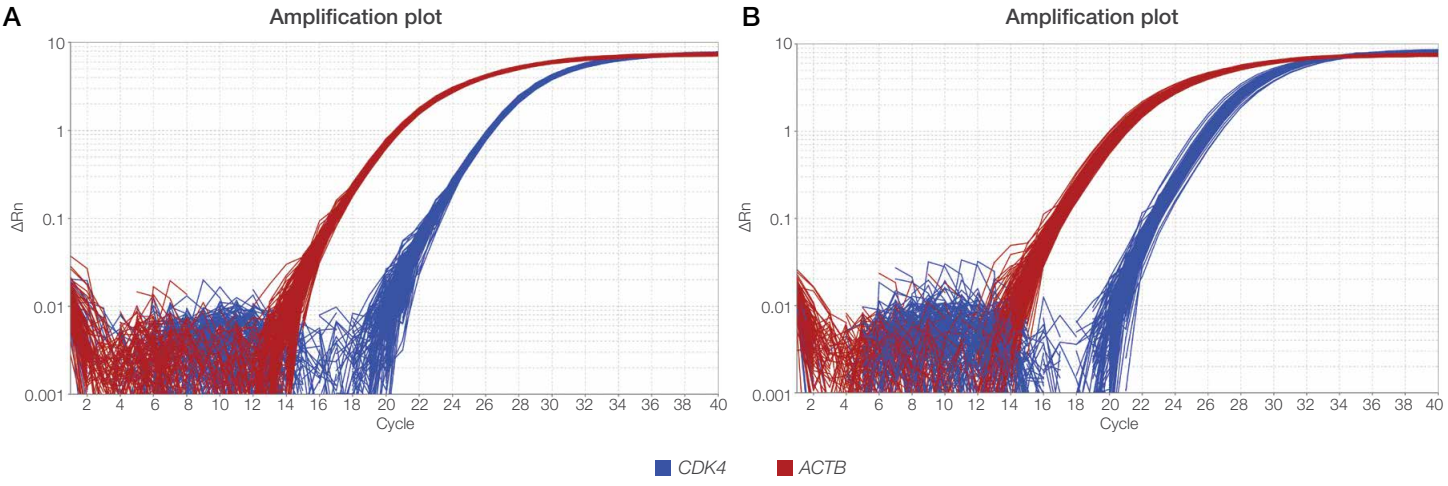

Figure 4. Amplification plots for manual and automated workflows. The amplification plot from the QuantStudio 5 software for **(A)** manual and **(B)** automated workflows for *ACTB* and *CDK4* genes.

Here we demonstrate that automation of the TaqMan Cells-to-C_T Express workflow can be readily achieved on a Tecan Fluent 780 liquid handler and that the results generated with this automated implementation are equivalent to those obtained using the manual method. Due to the simplicity of the procedure with no heating, centrifugation, or filtration steps, we anticipate broad compatibility with most liquid handling systems. The method and system components outlined here can be used as a guide to adopt the workflow on other automated systems.

Conclusions

Automating the TaqMan Cells-to-C_T Express workflow procedure enables efficient usage of time, labor, and plastics, yet maintains the high-quality gene expression data with accuracy comparable to that of the conventional methods. The automated method described here requires very little off-instrument setup, which could be minimized further by incorporating the preparation of RT and qPCR cocktails into the liquid handling program, resulting in a fully automated workflow. The TaqMan Cells-to-C_T Express workflow provides flexibility and ease of use and is well-suited for a high-throughput screening.

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Ordering information

Description	Quantity	Cat. No.
TaqMan Cells-to-C_T Express Kit	40 reactions	A57985
	100 reactions	A57986
	400 reactions	A57987
	2,500 reactions	A57988
TaqMan Cells-to-C_T Express Lysis Reagents	2,500 reactions; Express Lysis Solution and Express ezDNase enzyme only	A57989

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