Real-time PCR using Platinum Direct PCR Universal Master Mix

Introduction

Invitrogen™ Platinum™ Direct PCR Universal Master Mix is designed to amplify DNA sequences directly from a variety of sample types, without requiring DNA purification. The master mix is formulated with Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase, which is engineered for high inhibitor tolerance, fast DNA synthesis, and high sensitivity. Real-time PCR allows for faster target detection than endpoint PCR because it does not require gel analysis of completed reactions. Here we describe how Platinum Direct PCR Universal Master Mix can be used with SYBR™ Green dye in real-time PCR assays for rapid target detection.

Important notes

- Cell samples can be added directly to the real-time PCR reaction mix without the lysis step. However, this is not recommended for samples containing more than 1,000 cells, as excess cell debris in the reaction may hinder the detection of fluorescent signal.
- When adding cell samples directly to the real-time PCR reaction mix, do not exceed 5% of the final reaction volume since excess sample buffer (e.g., PBS) may inhibit the reaction and reduce real-time PCR sensitivity.
- The green dye in the master mix enables better visual control of the solutions in the reaction plate. The dye is inert and has no effect on the efficiency of PCR.
- ROX™ dye serves as an internal reference for normalization of fluorescent signal. Supplement the real-time PCR reaction mix with ROX dye to the concentration recommended for your real-time PCR instrument.
- To verify the specificity of primers and identity of PCR products, check the melting curve of your real-time PCR reactions. Primer-dimers may form during PCR (if the primer design is not optimal) and have a lower melting temperature than the desired PCR products.
- For analysis on Invitrogen™ E-Gel™ agarose gels, 2- to 20-fold dilution of the PCR samples is recommended for optimal separation.

Materials and methods

Materials

- Platinum Direct PCR Universal Master Mix (**Cat. No. A44647100**)
- Invitrogen™ SYBR™ Green I Nucleic Acid Gel Stain (**Cat. No. S7585**)
- Thermo Scientific™ ROX™ Solution (**Cat. No. R1371**)
- Forward and reverse PCR primers for targets of interest
- Purified mouse genomic DNA or human genomic DNA
- Invitrogen™ E-Gel™ EX Agarose Gels, 1% (**Cat. No. G401001**), or equivalent
- Invitrogen™ E-Gel™ 1 Kb Plus Express DNA Ladder (**Cat. No. 10488091**)

Cell line samples

Direct PCR was performed using lysates of BALB/c mouse primary embryonic fibroblasts, HeLa S3 human cervical cancer cells, and Daudi human B lymphoblast tumor cells.

Sample lysis

A lysis solution was prepared by adding 0.6 µL of proteinase K to 20 µL of lysis buffer (both included with the master mix) and briefly mixing. Varying numbers of cells, from 10 to 10,000 cells, were added to tubes with lysis solution and incubated at room temperature for at least 1 minute. Thereafter, the samples were heated at 98°C for 1 minute and centrifuged, and the supernatants were kept at room temperature for later use in PCR.

Real-time PCR

Real-time PCR assays were set up as 20 µL reactions (Table 1). Reaction mixes were supplemented with SYBR Green I dye for detection of amplification, and ROX dye for signal normalization. For the BALB/c lysates, primers designed to generate a 114 bp amplicon from the *IRG-47* (interferon gamma-inducible protein 47) gene were used. For the HeLa S3 and Daudi lysates, primers designed to generate a 139 bp amplicon from the *ACTB* (β-actin) gene were used. As templates, 2 µL of each cell lysate supernatant was used, or 10-fold dilution series of purified mouse or human genomic DNA were prepared as standards for target quantification.

Real-time PCR was performed on an Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System using a 2-step cycling protocol with data acquisition during the annealing and extension step (Table 2).

Table 1. Reaction setup.

Component	Per 20 µL reaction	Final concentration
Platinum Direct PCR Universal Master Mix, 2X	10 µL	1X
50 µM ROX dye	0.012 µL	30 nM
100X SYBR Green dye	0.08 µL	0.4X
10 µM forward primer	0.4 µL	200 nM
10 µM reverse primer	0.4 µL	200 nM
Water, nuclease-free	To 18 µL	–
Template (lysate supernatant, or purified DNA standard)	2 µL	–

Table 2. Cycling protocol.

PCR cycles	Step	Temp.	Time
1	Activation	94°C	2 min
40	Denaturation	94°C	15 sec
	Annealing and extension*	60°C	45 sec
Hold		4°C	Indefinitely

* Data acquisition should be performed during the annealing and extension step.

Results

To assess whether Platinum Direct PCR Universal Master Mix can be used in SYBR Green dye–based real-time PCR, we tested several gene-specific assays with mouse and human cell lines. Cell samples serially diluted to contain 10 to 10,000 cells were lysed, and for each lysate supernatant, 2 µL (representing 10% of the initial cell sample lysed) was added directly to the real-time PCR reaction mix.

The analysis demonstrates that Platinum Direct PCR Universal Master Mix can be used to reliably detect target sequences in cell lysates (Figure 1). Real-time PCR signals from lysates from varying cell number inputs follow linear regression, similar to the controls containing purified genomic DNA, enabling relative estimation of target abundance in the samples.

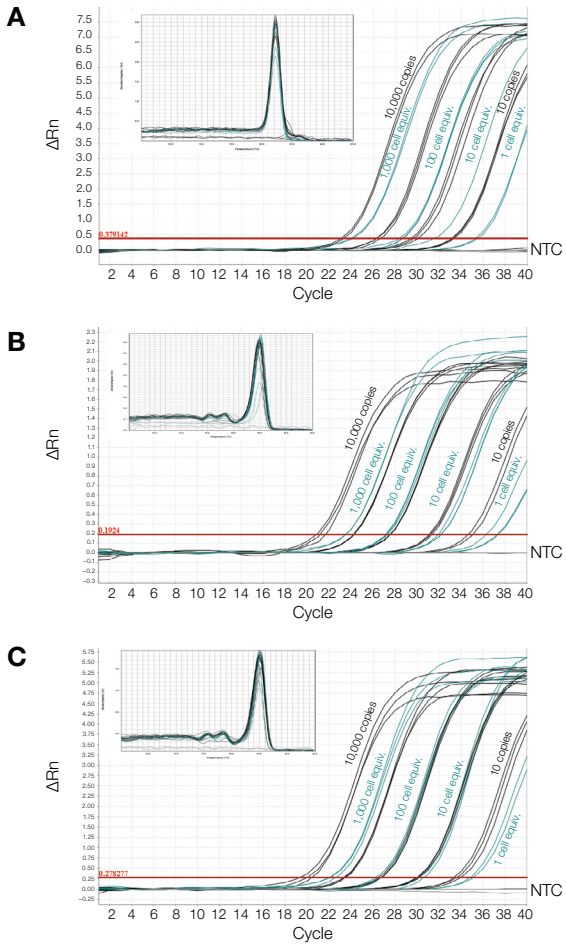

Figure 1. Real-time PCR assays using Platinum Direct PCR Universal Master Mix and SYBR Green dye. Lysates of **(A)** BALB/c, **(B)** HeLa S3, and **(C)** Daudi cells were used directly in real-time PCR for target detection. The numbers indicated in green reflect the cell equivalents that were added to each reaction and correspond to 10% of the initial numbers of cells lysed. qPCR amplified target genes from standards prepared with purified **(A)** mouse or **(B, C)** human genomic DNA, as described in the "Real-time PCR" section. The 10-fold serially diluted standards (black) are labeled for estimated numbers of target copies. The melting curve analyses are shown in the insets.

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To verify PCR specificity, a melting curve analysis was performed (Figure 1), and completed reactions were further analyzed by gel electrophoresis (Figure 2). For the latter, PCR products were separated in 1% E-Gel EX Agarose Gels containing Invitrogen™ SYBR™ Gold II DNA stain. Results showed that Platinum Direct PCR Universal Master Mix specifically amplified fragments of the expected sizes—114 bp for mouse *IRG-47* and 139 bp for human *ACTB*—in all experiments.

Summary

Platinum Direct PCR Universal Master Mix reliably amplifies targets from a variety of cell types. When supplemented with SYBR Green I dye, the master mix helps enable detection and quantification of targets by real-time PCR.

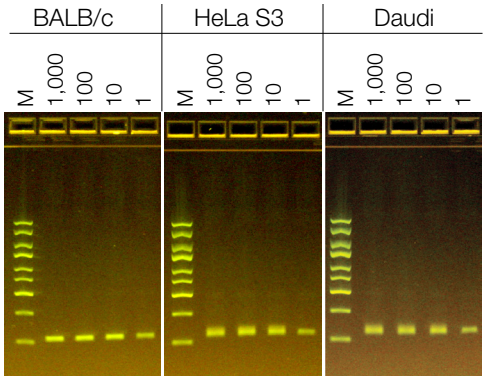

Figure 2. Analysis of PCR by gel electrophoresis. Completed PCR reactions of cell lysates with Platinum Direct PCR Universal Master Mix were loaded on 1% E-Gel EX Agarose Gels containing SYBR Gold II DNA stain. Numbers above the lanes are cell equivalents present in each reaction. The E-Gel 1 Kb Plus Express DNA Ladder was used as a size standard (M).

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