TaqMan® PreAmp Master Mix Kit for Real–Time Gene Expression Analysis with Sample Limited Specimens / P1219

Sarah Stanley, Cora Lee, Joyce Wilde, [1]Renata Coudry, [1]Cynthia Spittle, and Junko Stevens
Applied Biosystems, 850 Lincoln Center Drive, Foster City, CA, 94404, U.S.A. [1]Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA, 19111, U.S.A.

ABSTRACT

We have developed a robust solution for uniform amplification of cDNA prior to quantitative real-time PCR. TaqMan® PreAmp Master Mix Kit (P/N 4364130) allows up to 100 gene targets to be pre-amplified simultaneously using TaqMan® Gene Expression Assays as the source of pooled gene-specific primers. By incorporating a preamplification step into the sample preparation workflow, adverse sample splitting effects on biological samples are significantly reduced or eliminated.

INTRODUCTION

The main objective when performing preamplification is to retain the gene expression profile of the original cDNA sample. We have developed the analysis method to assess uniformity of preamplification (ΔΔCt method). ΔΔCt method compares the gene expression data generated from a cDNA sample to the same data generated from a preamplified amplicon as a starting template.

The TaqMan® PreAmp Master Mix Kit provides users with a simple workflow that results in over a thousand-fold increase of cDNA in less than 1.5 hours. First, RNA must be converted to single-stranded cDNA using AB High Capacity cDNA Archive Kit (P/N 4322171). Next, up to 100 TaqMan® Gene Expression Assays are pooled to create the assay pool. The assay pool is combined with the TaqMan® Preamp Master Mix and cDNA to create a reaction mixture that undergoes 10 or 14 cycles of PCR. The pre-amplified products are then diluted to eliminate any downstream assay inhibition and are used as the template for single-plex TaqMan® Gene Expression PCR.

Materials and Method

Degraded RNA Samples: Stratagene UHR Total RNA was degraded by adding 10 mM NaOH and heating at 37°C for 1 minute. The sample was immediately neutralized with 10 mM Tris, pH 7.0. The RNA integrity Number (RINA) was determined by Agilent 2100 Bioanalyzer. The degraded and untreated RNA samples were converted to cDNA using the AB High Capacity cDNA archiving kit. 25 ng of cDNA was used for 50 μL preamplification reactions.

Laser Capture Micro Dissection (LCM) Samples: Fox Chase Cancer Center obtained 20 to 1000 micro dissected cells from frozen human liver tissue by LCM. RNA was extracted using the Pico Pure RNA Isolation Kit (Acturus) and converted to cDNA using the AB High Capacity cDNA Archive Kit. RNA quality was evaluated using Agilent 2100 Bioanalyzer.

Pooling TaqMan® Gene Expression Assay Criteria: We recommend the following rules for pooling TaqMan® Gene Expression Assays. (1) Pool TaqMan® assays with Ct< 35 when using 0.3 ng/μL cDNA. (2) Do not include the 18S TaqMan® assay in the pool because it is highly expressed and introduces amplification bias. (3) Check the uniformity of preamplification using ΔΔCt method.

Δ Δ Ct Method for Checking Preamplification Uniformity: We recommend performing preamplification uniformity check using cDNA that is non-limiting prior to perform preamplification with limited biological samples. ΔΔCt method involves real time relative quantitation PCR with cDNA as a starting template and preamplified amplicon as a starting template. For Human target, CDK1NB (HS00153277_m1) is used as a uniformity reference gene because of its consistent gene expression profile.

(1) For each target, preamplified aver. Ct are normalized to aver. Ct of uniformity reference gene

ΔCt_{preamp} = aver. $Ct_{target\ X\ [preamp]}$ – aver. $Ct_{uniformity\ reference\ gene\ [preamp]}$

(2) Similarly, aver. Ct from cDNA template are normalized to aver. Ct of uniformity reference gene

ΔCt_{cDNA} = aver. $Ct_{target\ X\ [cDNA]}$ – aver. $Ct_{uniformity\ reference\ gene\ [cDNA]}$

(3) ΔΔCt is determined by the difference of the two ΔCt.

$\Delta\Delta Ct = \Delta Ct_{preamp} - \Delta Ct_{cDNA}$

A ΔΔCt close to zero indicates preamplification uniformity. Typically, 90% of TaqMan® Gene Expression Assays produce Δ Δ Ct within ± 1.5.

Preamplification Reaction Conditions

Table 1 shows the reaction components of preamplification.

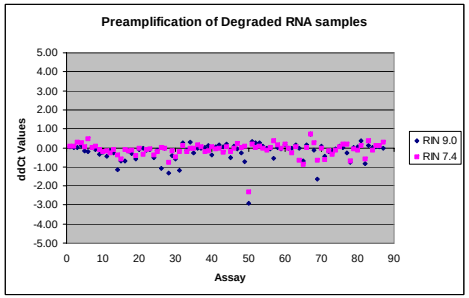
Component	Volume* (μL/Reaction)	Final Concentration
TaqMan PreAmp Master Mix (2X)	25.0	1X
Pooled assay mix (0.2X)	12.5	0.05X (each assay)
1–250 ng cDNA sample + nuclease-free water	12.5	0.02–50 ng/μL
Total	50.0	—

Table 2 shows the preamplification thermal cycling conditions.

	Enzyme Activation	Preamplification PCR	
	HOLD	CYCLE (10 or 14 cycles)	
		Denature	Anneal/ Extend
Temp	95 °C	95 °C	60 °C
Time	10 min	15 sec	4 min

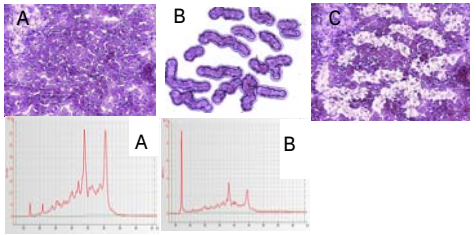
Results

Figure 1. Preamplificaton of Degraded RNA and Untreated RNA



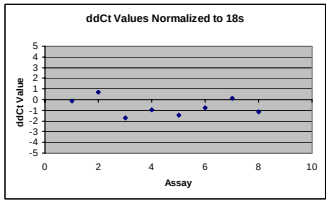
The ΔΔCt from the degraded (RIN 7.4) and untreated (RIN 9.0) preamplified templates are plotted together on this graph. The ΔΔCt are along the Y-axis and the assays are along the X-axis.

Figure 2. A. Human Liver Tissue before LCM, B. Hepatocytes in the cap after being harvested. C. Liver tissue after harvest.



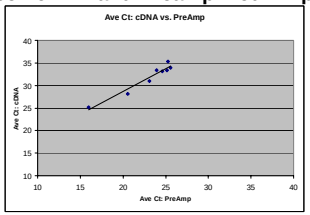
Bioanalyzer profile from 1,000 (A) and 20 (B) hepatocytes collected using LCM. Both electropherograms show 18 and 28s peaks, demonstrating very good quality of the RNA.

Figure 3.LCM Liver Sample ΔΔCt Analysis



The ave. Ct for CDKN1B in cDNA was > 35, the data were normalized to 18S (not included in the PreAmp Kit reactions).

Figure 4. LCM Study- Comparison of Ct Between cDNA and Preamplified Amplicons



The aver. Ct for the PreAmp reaction are plotted on the X-axis and the aver. Ct values for the cDNA sample (0.3ng/uL) are plotted on the Y-axis.

Conclusions

TaqMan® PreAmp Master Mix and optimized cycling parameters enable nearly 100% efficient amplification of target sequences. Data obtained from the Applied Biosystems 7900HT Sequence Detection System demonstrate the wide utility of this process in many gene expression arenas including the profiling of cells obtained by laser capture micro dissection. Results using the TaqMan® PreAmp Master Mix Kit is amenable to partially degraded RNA samples.

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