Experiment time: 2025-06-21, 13:00 - 2025-06-21, 13:30

Reverse Transcription

I. Objective of the experiment

The RNA of human bladder transitional cell carcinoma cell lines (T24) extracted in the early stage, namely the wild-type (control), low-, medium-, and high-drug-resistant T24-RC48 cell lines, were reverse transcribed to obtain cDNA, which laid the foundation for qPCR in the later stage.

II. Experimental content

2.1 Experimental design

In this experiment, the total RNA of human bladder transitional cell carcinoma cell line (T24) and its drug-resistant subtype (T24-RC48) were reverse transcribed into cDNA to prepare for subsequent qPCR experiments.

First, based on RNA quantification results, calculate the required volume of each reverse transcription reagent for every sample according to the manufacturer's instructions to prepare a 20 μL reaction system. Next, retrieve the RNA samples and reverse transcription reagents from the -80°C freezer and thaw them on ice. Distribute the reagents into corresponding RNA extraction tubes (EP tubes) by group and gently mix. Place the EP tubes in a metal bath: first incubate at 50°C for 15 minutes, then cool to 85°C for 5 seconds. Finally, after allowing the temperature to slightly decrease, seal the EP tubes with sealing film and store them in the -80°C freezer. The entire experiment strictly followed RNase-free protocols conducted under ice bath conditions to ensure RNA quality and obtain high-quality cDNA.

2.2 Sample types

T24 wild-type cell line, low/medium/high drug resistance T24-RC48 cell line.

2.3 Measurement principle

Reverse transcriptase synthesizes complementary cDNA strands using RNA as a template and guided by primers. This critical step converts unstable RNA into stable double-stranded cDNA, providing a DNA template for subsequent qPCR amplification and quantification. As the essential pretreatment process in RT-qPCR analysis of RNA (e.g., gene expression studies), it ensures reliable data acquisition through precise DNA preparation.

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III. Materials and reagents

3.1 Materials

Micropipettes: All purchased from Eppendorf

RNase-free pipette tips: Purchased from Axygen

EP tubes: Purchased from Axygen

Parafilm

Lens cleaning paper

3.2 Reagents

RNA previously extracted from drug-resistant cell lines

Reverse transcription reagent: purchased from Vazyme

IV. Experimental instruments

Constant temperature metal bath heater: purchased from Biosafer

Ultra-micro spectrophotometer: purchased from Thermo Fisher Scientific

-80 degree refrigerator

V. Experimental steps

This experiment strictly followed the RNase-free operation protocol, and was carried out under ice bath to prevent RNA degradation and nonspecific amplification. The experimental steps were as follows:

5.1 Based on the results of the last RNA extraction and the ratio shown in the instruction manual, calculate the amount of each reverse transcription reagent added.

#	样品名称	ng/μL	A260/A280
1	样品 1	719.7	1.91
2	样品 2	1302.8	1.93
3	样品 3	1015.1	1.91
4	样品 4	1115.4	1.92
5	样品 5	967.1	1.93

Figure 1 RNA quantification results

Table 1. The amount of each reagent in the reverse transcription system of each group

	RNA amount (ng/μl)	**Enzyme Mix (μl)**	**5×All-in-one qRT SuperMix (μl)**	**RNase-free ddH_2O (μl)**
Wild type	1302.8	23.4504	117.252	333.756
Low levels of resistance	1015.1	18.2718	91.359	256.077
Intermediate drug resistance	1115.4	20.0772	100.386	283.158
High levels of resistance	967.1	17.4078	87.039	243.117

5.2 Sample pretreatment: Retrieve the cryopreserved RNA samples (four tubes total) from the -80°C freezer and thaw them gradually on ice. Meanwhile, the reverse transcription reagent is slowly thawed on ice.

5.3 Carefully aspirate the calculated volumes of reagents and add them to the respective RNA-containing tubes for the wild-type, low-, medium-, and high-resistance groups. Take care to avoid introducing air bubbles during pipetting.

5.4 After adding all components, mix the reaction gently by pipetting up and down, and briefly centrifuge to collect the contents at the bottom of the tube. Wipe any condensation from the outside of the tube. Place the tubes in a pre-heated metal bath set at 50°C for 15 minutes for the reverse transcription reaction, followed by incubation at 85°C for 5 seconds to inactivate the reverse

transcriptase. This process converts the RNA template into complementary DNA (cDNA).

5.5 Once the tubes have cooled slightly, seal them with Parafilm and store at -80°C for subsequent qPCR experiments.

VI. Experimental results

The RNA samples used for reverse transcription had A260/A280 ratios between 1.8 and 2.0, indicating high purity. High-quality cDNA was successfully synthesized, as inferred from the quality of the input RNA.

VII. Results analysis

The experimental procedure was rigorous and well-executed. The A260/A280 ratios of 1.8-2.0 indicate high RNA purity with minimal protein contamination, ensuring a high-quality reverse transcription reaction suitable for subsequent qPCR experiments.