In vitro Transcription

Aim of the experiment

We used in vitro transcription (IVT) to synthesize RNA from DNA templates. The first protocol describes IVT from complete double stranded templates. For generation of crRNAs we developed a protocol using incomplete templates to facilitate varyation of target sequences. crRNA templates were completed using DNA polymerase and transcribed in a one-batch reaction.

Materials

Single tube reaction system (20 μL) :

	Positive control group	Experimental groupA	Experimental groupB	Experimental groupC
T7 Control template	2μL			
DNA Template (100 ng/uL)		1μL	2μL	3μL
10X Transcription Buffer	2μL	2μL	2μL	2μL
rNTP (100 mM ench)	5μL	5μL	5μL	5μL
T7 RNA Polymerase	2μL	2μL	2μL	2μL
RNase Free H2O	9μL	10μL	9μL	8μL
Total	20μL	20μL	20μL	20μL

Procedure

1. According to the experimental materials prepare the transcription mix.

2. The incubation:

(1) Gently absorb and mix the reaction system with a pipette (do not brush strong

oscillation).Instantaneous centrifugation ensures that all the solution is collected at the bottom of the tube.

(2) The reaction tube was placed in the PCR instrument (or other thermostatic equipment) and incubated with 2-4h at 37°C (partially inactivated at 70 °C).

Take out the reaction products and place them on ice to measure the concentration.

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4. Next test or -80C store as soon as possible.