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RCA of Circular Probe

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This protocol is to verify that circular probe can conduct RCA reaction.

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Preparation

1 Dilute the 1μM Circular probe into 100nM

1.1 Add **5 μL** of 1μM circular probe

1.2 Add **45 μL** of RNase-free water

1.3 Spin down after vortex

Protocol

2 Add **17.4 µL** RNase-free water into a eppendorf

3 Add **3 µL** of 10X phi29 polymerase reaction buffer

4 Add **3 µL** of 100nM circular probe

5 Add **3 µL** of 100nM miRNA

6 Add **3 µL** of 2mM dNTPs

7 Add **0.6 µL** of 10U/µl Phi29 polymerase

8 Pipetting the reaction solution

9 Incubate for **02:00:00** at **Room temperature** 2h

10 Add **1.5 µL** of 20X evagreen dye

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11 Add **1.5 µL** of 0.5M EDTA buffer to suspend the reaction

Measuring

12 Load **20 µL** of reaction solution into 384-well plate

13 Measure the fluorescence excitation and emission intensity

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