RCA of Gotcha V.1

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This protocol is to test that GotCha is functional and can works as designed.

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Preparation

1 Add **5 µL** of GotCha(functional beads) into eppendorf

2 Centrifuge for **15000 rpm, 00:05:00** and remove supernatant. Make sure that eppendorf should put on DynaMag when removing supernatant. 5m

Protocol

3 Add **3 µL** of 10X phi29 polymerase reaction buffer into eppendorf with GotCha

4 Add **20.4 µL** RNase-free water

5 Add **3 µL** of 100nM miRNA

6 Add **3 µL** of 2mM dNTPs

7 Add **0.6 µL** of 10U/ul phi29 polymerase

8 Pipetting to mix well

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

10 Add **1.5 µL** of 20X evagreen dye

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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