Oct 18, 2021

# RCA of Gotcha

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dx.doi.org/10.17504/protocols.io.by53py8n

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

DOI

dx.doi.org/10.17504/protocols.io.by53py8n

Chia-Hsien Shih 2021. RCA of Gotcha. **protocols.io** https://dx.doi.org/10.17504/protocols.io.by53py8n

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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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