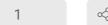




# Background control of Gotcha RCA V.2

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Oct 17, 2021

protocol.

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This protocol aims to verify that GotCha design works as expected.

Chia-Hsien Shih 2021. Background control of Gotcha RCA. **protocols.io** https://protocols.io/view/background-control-of-gotcha-rca-by5jpy4n Chia-Hsien Shih

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## Preparation

- 1 Add **□5 μL** of functional beads(Gotcha) into eppendorf
- 2 Centrifuge for **315000 rpm**, **00:05:00** and remove supernatant. Make sure that eppendorf should put on DynaMag when removing supernatant.

Protocol of Control group without miRNA

3 Add  $\blacksquare 3 \mu L$  of 10X phi29 polymerase reaction buffer into eppendorf with functional beads

- 4 Add **□23.4** µL RNase-free water
- 5 Add **3 μL** of 2mM dNTPs
- 6 Add  $\mathbf{\Box 0.6} \, \mu \mathbf{L}$  of 10U/ul phi29 polymerase
- 7 Pipetting to mix well
- 8 Incubate for © 02:00:00 at & Room temperature
- 9 Add  $\blacksquare$ 1.5  $\mu$ L of 20X evagreen dye
- 10 Add ■1.5 µL of 0.5M EDTA buffer to suspend the reaction

Protocol of Control group without phi29 polymerase

11 Add  $\blacksquare 3 \mu L$  of 10X phi29 polymerase reaction buffer into eppendorf with functional beads

2h

- 12 Add **□21 µL** RNase-free water
- 13 Add  $\blacksquare 3 \mu L$  of 2mM dNTPs

- 14 Add □3 µL of 100nM miRNA
- 15 Pipetting to mix well
- 16 Add  $\blacksquare$ 1.5  $\mu$ L of 20X evagreen dye
- 17 Add  $\blacksquare$ 1.5  $\mu$ L of 0.5M EDTA buffer to suspend the reaction

# Measuring

- 18 Load  $\mathbf{20} \, \mu \mathbf{L}$  of reaction solution into 384-well plate
- 19 Measure the fluorescence excitation and emission intensity