Background control of Gotcha RCA V.2

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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
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Preparation

1 Add **5 µL** of functional beads(Gotcha) 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 of Control group without miRNA

3 Add **3 µL** 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 **0.6 µL** of 10U/ul phi29 polymerase

7 Pipetting to mix well

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

9 Add **1.5 µL** 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 **3 µL** of 10X phi29 polymerase reaction buffer into eppendorf with functional beads

12 Add **21 µL** RNase-free water

13 Add **3 µL** of 2mM dNTPs

14 Add **3 µL** of 100nM miRNA

15 Pipetting to mix well

16 Add **1.5 µL** of 20X evagreen dye

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

Measuring

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

19 Measure the fluorescence excitation and emission intensity