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Hybridization and Immobilization

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This protocol is to assemble GotCha with circular probe, immobilization probe and magnetic beads.

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Preparation

1 Dilute immobilization probe into 10μM

Protocol of Hybridization

2 Add **4.5 μL** of 1μM circular probe

3 Add **9 µL** of 10X **pH 7.4** PBS buffer

4 Add **0.9 µL** of 10µM immobilization probe

5 Add **75.6 µL** RNase-free water

6 Vortex slightly and spin down

7 Heated to **90 °C** for **00:00:30** then cool down to **Room temperature** and keep for **00:30:00** 30m 30s

Protocol of Immobilization 36m

8 Take **22.5 µL** of 10mg/ml magnetic beads into a eppendorf

9 Vortex then spin down in Centrifuge **15000 rpm, 00:02:00** 2m

10 Carefully take the eppendorf onto the DynaMag and wait for **00:01:00** to collect the beads 1m

11 Wash beads with 1X PBS for three times. Make sure that eppendorf should put on DynaMag when removing supernatant.

12 Add **90 µL** of hybridization solution into the eppendorf with dry beads

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13 Incubate at **Room temperature** for **00:30:00** , then spindown in Centrifuge **15000 rpm, 00:02:00** 32m

14 Carefully take the eppendorf onto the DynaMag and wait for **00:01:00** to collect the beads 1m

15 Wash beads with 1X PBS for three times. Make sure that eppendorf should put on DynaMag when removing supernatant.

16 Preserve the functional beads in **90 µL** 1X PBS

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