Specificity test of toehold

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1 Works for me This protocol is published without a DOI.

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Preparation

1 Sterilize the bench, and put on a labmat

2 Thaw the reagents including 1. DNase/ RNase free water (store in -20°C) 2. solution A (store in -80°C) 3. solution B (store in -80°C) 4. RNase inhibitor (store in -20°C) 5.toehold switches with invertase DNA (store in 4°C) 6.miRNA as the trigger (store in 4°C) **On ice**

Prtotocols 2h 30m 20s

3 Gently shake and spin down the tube before adding (especially solution A and DNA plasmid)

4 Add below reagents in to PCR tubes in order:
1. ddH2O(till totally **5 µl**)
2. solution A **2 µl**
3. solution B **1.5 µl**
4. inhibitor **0.2 µl**
5. toehold switches with invertase DNA **50 ng**
6. miRNA **0 µl** **50 µl** **100 µl** **150 µl** **200 µl**

5 Centrifuge **00:00:20** **4000 rpm** 20s

6 Place the PCR tubes in the dry bath incubator **37 °C** **02:00:00** 2h

7 Put the PCR tubes **On ice** after 2 hours

8 Measure the glucose concentrations of each PCR tubes by glucometer

9 Put the PCR tubes in the dry bath incubator **55 °C** **00:30:00** 30m

10 Measure the glucose concentrations of each PCR tubes by glucometer again