# Use siRNA to knowdown 3T3

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## 1 Introduction

We choose two target gene from paper.

Tranditinal PCR is an in vitro techniques which allows the amplification of a specific DNA region that lies between two regions of known DNA sequence. Here are three phases of PCR, including exponential phase, linear phase and plateau phase. Nowadays, we

Relative PCR

## 2 Method

### 2.1 Choose a target gene

### 2.2 Design siRNA and qPCR primer

### 2.3 Abstracte qPCR

### 2.4 Test Primers for Real-time PCR

To determine if an assay is optimal, we will use stardard curve to test primers for real-time PCR.Calculate the Ct and E

### 2.5 siRNA Transfection

### 2.6 Isolation RNA & reverse RNA

### 2.7 qPCR

Firstly, we prepare threePCR mixture as following table.(Table 1)

| Reagent | Volume (3.5 tubles) | 18srRNA premix (60 tubes) |
|---|---|---|
| SYBR Premix EX Taq II(Tli RNaseH plus)(2x) | 3.5 | 600 |
| PCR Forward Primer (10μl) | 2.8 | 48 |
| PCR Reverse Primer (10μl) | 1.4 | 48 |
| ROX Reference Dye | 2.8 | 48 |
| Template (<100 ng) | 7 | |
| $H_2O$(sterile distilled water) | 21 | 360 |
| Total | 70 | 1080 |

**Table 1.**

Then, we made a PCR set-up sheet in 96-well PCR plate as following table.

| | 18s rRNA | | | GAPDH | | | 18s rRNA | | | Target gene | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #1 | #2 | #3 | #1 | #2 | #3 | #1 | #2 | #3 | |
| NC-1 | | | | | | | | | | | | | Test 1-1 |
| NC-2 | | | | | | | | | | | | | Test 1-2 |
| NC-3 | | | | | | | | | | | | | Test 1-3 |
| PC-1 | | | | | | | | | | | | | Test 2-1 |
| PC-2 | | | | | | | | | | | | | Test 2-2 |
| PC-3 | | | | | | | | | | | | | Test 2-3 |
| | | | | | | | | | | | | | NTC |
| | | | | | | | | | | | | | Target Gene |
| | | | | NC-1 | | | NC-2 | | | NC-3 | | | |

**Table 2.**

For test sample: $2^{-[(\mathrm{Ct}_{\text{target gene}}-\mathrm{Ct}_{18\mathrm{srRNA}})-(\mathrm{Ct}_{18\mathrm{sRNA}})}$

For postive control $2^{-[(\mathrm{Ct}_{\mathrm{GAPDH}}-\mathrm{Ct}_{18s\,\mathrm{rRNA}})-(\mathrm{Ct}_{\mathrm{GAPDH\,gene}}-\mathrm{Ct}_{18s\,\mathrm{rRNA}})]}$

# 3 Results

# 4 Discussion

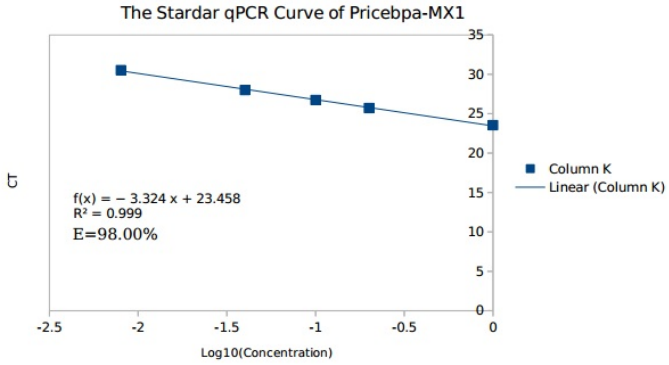
# 5 Reference

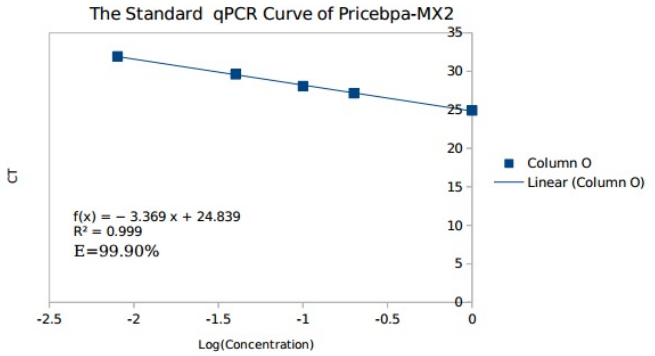
Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, *29*(9), e45.

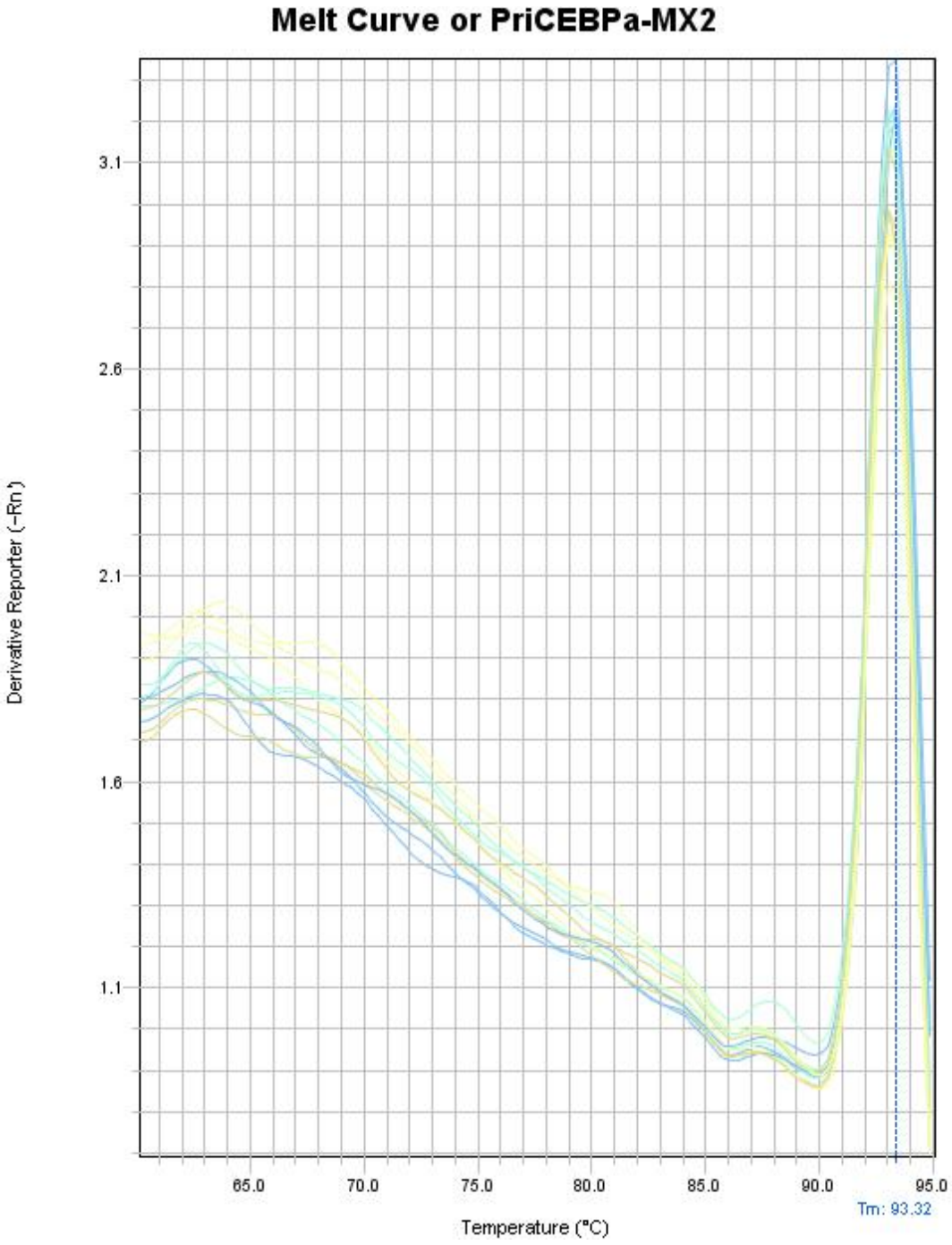
# 6 Contribution

Xu wenxin and I finish this work together.

# 7 Figures


The Stardar qPCR Curve of Pricebpa-MX1
CT
f(x) = − 3.324 x + 23.458
R² = 0.999
E=98.00%
Log10(Concentration)
Column K
Linear (Column K)


The Standard qPCR Curve of Pricebpa-MX2
CT
f(x) = − 3.369 x + 24.839
R² = 0.999
E=99.90%
Log(Concentration)
Column O
Linear (Column O)


Melt Curve or PriCEBPa-MX2
Derivative Reporter (-Rn')
3.1
2.6
2.1
1.6
1.1
65.0
70.0
75.0
80.0
85.0
90.0
95.0
Tm: 93.32
Temperature (°C)

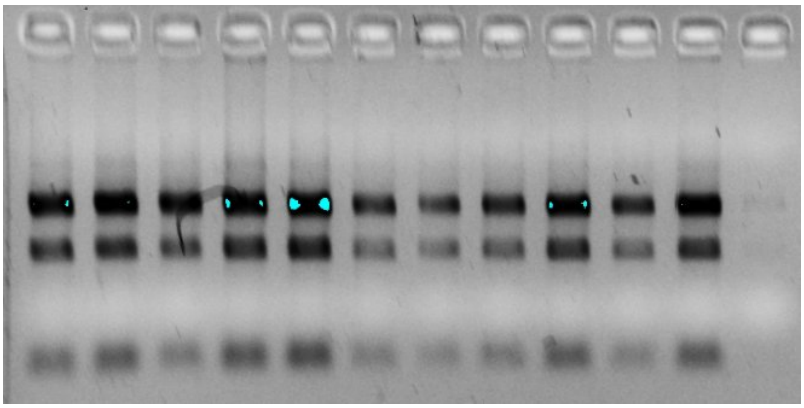


**Figure 1.** The Gel Graph of 4 isolativte RNAs. (From left to right: Postive Control, Negative control, siRNA 1, siRNA 2