Use siRNA to knowdown Cebpα in 3T3-L1

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ABSTRACT

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, 荧光是怎么与扩增数相关的。

The method of siRNA to knowndown the expression of gene.(Why we use dsRNA).

The points, which we should metation.

电转 (受用面广，但有毒性)

Two siRNA , one qPCR primer, one positive control (GADPH)

MATERIALS AND METHODS

Firstly, from the He Y, Li Y, Zhao T, Wang Y, Sun C (2013), we choose the CCAAT/enhancer-binding protein alpha from Mus musculus. Cebpa-α encodes a transcription factor that contains a basic leucine zipper (bZIP) domain and recognizeas the CCAAT motif in the promoters of target genes. The encoded protein functions in homodimers and also heterodimers with CCAAT/enhancer-binding protein beta and gamma. Activity of this protein can modulate the expression of genes involved in cell cycle regulation as well as in body weight homeostasis. The target sequence is following, and Accession ID: NM_007678.3 from https://www.ncbi.nlm.nih.gov/nuccore/NM_007678.3.

1 ttcgcgacc cgaagctgcg cgggcgcgag ccagttgggg cactgggtgg gcggcggcga

61 cagcggcgcc acgcgcaggc tggaggccgc cgaggctcgc catgccggga gaactctaac

121 tccccc.../atgg agtcggccga cttctacgag gtggagccgc ggcccccgat gagcagtcac

181 ctccagagcc ccccgcacgc gcccagcaac gccgcctttg gctttccccg gggcgcgggc

241 cccgcgccgc ccccagcccc acctgccgcc ccggagccgc tgggcggcat ctgcgagcac
301 gagacgtcta tagacatcag cgcctacatc gacccggccg ccttcaacga cgagttcctg
361 gccgacctct tccagcacag ccgacagcag gagaaggcca aggcggcggc gggccccgcg
421 ggtggcggcg gtgactttga ctacccggga gccccggcgg gccccggcgg cgcggtcatg
481 tccgcggggg cgcacgggcc ccctcccggc tacggctgtg cggcggccgg ctacctggac
541 ggcaggctgg agcccctgta cgagcgcgtc ggggcgcccg cgctacg gcc gctggtgatc
601 aaacaagagc cccgcgagga ggacgaggcg aagcagctgg cgctggccgg cctcttcccc
661 taccagccac cgccgccacc gccaccgccg cacccgcacg cgtctcccgc gcacctggcc
721 gccccccact tgcagttcca gatcgcgcac tgcggccaga ccaccatgca cctgcagcct
781 ggccacccca caccgccgcc cacgcccgtg cccagcccgc acgctgcgcc cgccttgggt
841 gctgcgggcc tgcctggccc cgggagcgcg ctcaagggct tggccggtgc gcaccccgac
901 ctccgcacgg gaggcggcgg cggtggcagc ggtgccggtg cgggcaaagc caagaagtcg
961 gtggacaaga acagcaacga gtaccgggta cggcgggaac gcaacaacat cgcggtgcgc
1021 aagagccgag ataaagccaa acaacgcaac gtggagacgc aacagaaggt gctggagttg
1081 accagtgaca atgaccgcct gcgcaagcgg gtggaacagc tgagccgtga actggacacg
1141 ctgcggggca tcttccgcca gctgcctgag agctccttgg tcaaggccat gggcaactgc
1201 gcgtga.../ggcg cgcggctgcg ggaccgcctt gggccggccc cctggctgga gacccagagg
1261 atggtttcgg gtcgctggat ctctaggctg cccgggccgc gcaagccagg actaggagat
1321 tccggtgtgg cctgaaagcc tggcctgctc cgcgtgtccc ctcccttcct ctgagccgga
1381 ctcggtgcgt ctaagatgag ggagtcaggc cgtggtggtt tctccttgag accgagagac
1441 tttccgcgga gctgagctgg gggcccggca gtactagtat taaggaagta accttgtgcc
1501 ttggatactc aaaactcgct ccttttccta ccgagtaggg ggagcaaaaa tgtgccttga
1561 tattttattt ggaggattcc tgcttcctct cgggcctcag ctggccccgt gagaaaaatg
1621 aagggtgcag gcccagggca ggaggaagat acaggaagct gagatcccgg cagtgccctg
1681 agctgcccct cagtccctgt ctttagaggg gagggactta ggtgttgggg atttgagtct
1741 gtgtcctcac ccccagctac agggaggtgg agggctccta atcccttgct ttttgcacct
1801 ccacctacat ccccccccccc ccactcagct tacaacaggc caggtttcct gggtgagttc
1861 atggagaatg ggggcaccac ccccagtcag accagaaagc tgagttgtga gttagccatg
1921 tggtaggaga cagagaccta ggtttctggg ctttgtgggg tgggggatag gaggacacgg
1981 ggaccattag ccttgtgtgt actgtatgtc gccagccgct gttgctgaag gaacttgaag
2041 cacaatcgat ccatcccaga gggactggag ttatgacaag cttcccaaat attttgcttt
2101 atcatccgat atcaacactt gtatctggtc tctgtgtccc agcggtgcct tgtgcaatgg
2161 cagtgtgcac gtctatgcta aaccaccatt ttatttggtc ttttgttttg ttttggtttt
2221 gctctgattc ttgccaaact gagactcttc actaacggct gggggaagga gctgagtgag
2281 gctctcattc tttttggttt agggatgttt gggttttttc gtctgcctcc cagaggacca
2341 atgaaatgaa gtgggcttcc ccctctcccc tagttgtcca agggtgtatg tagtagtggg
2401 tcttagcttc ctccggctaa gacttaggct tccccaccca cccaacccca tccccaacgg
2461 ccctggctct gggtctggaa agaaggccac ctccagccag ttcatacaca cacccctgtg
2521 gctgggagca gggctggacc gcttccttct cttctttttt ttgggggggg gggacacaaa
2581 gtttcatgct agatgtcgta tgtattatat ctataatata aacatatcaa actcaaaaaa
2641 aaaaaaaaaa a

Then, we use BLOCK_iT RNAi Designer ad GeneBank to design siRNA and qPCR perimers as following

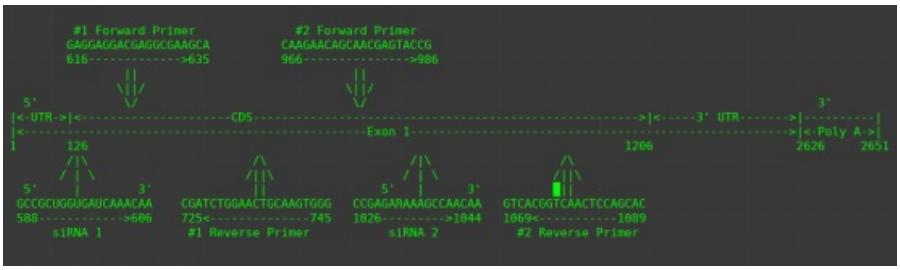

Figure 1. The siRNA and qPCR primers for Cebp-α

We use absolute qPCR to compare the effectity of two qPCR primers. Prepare the PCR mixture showm below. Diveide Primer-1&2 premixers into seven new tubes, respectively.(63μl each). cDNA wre added into tybes(7μl for 1:1, 1:5, 1:10, 1:25, 1:125, 1:3125, and control group. Then divide qPCR mixture into each well. After qPCR, draw standard curve and calculate the E from $10^{-1/\text{slope}} - 1$ and observe the melt curves.

Reagent	Volume(μL)	Total volume	Final concentration
SYBR Premix ExIaqII(The RNase Plus)(2x	10	390	1x
PCR Forward Primer(10μM)	0.8	28	0.4μM
PCR Reverse Primer(10μM)	0.8	28	0.4μM
ROX reference Dye(50x)	0.8	14	1x
Templete(<100g)	2	~	
dWater	6	210	
Total	20	630	

To transfecte siRNA to 3T3-L cell, we use electroporation.

Then we isoalte RNA from cell and resverse RNA to cDNA.

Finally we use qPCR to measure the expression of Cebp-α.

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

RESULTS

The primer Pricebpa-MX2 is better than Pricebpa-MX1 because the E is closer 100% than Pricebpa-MX1.

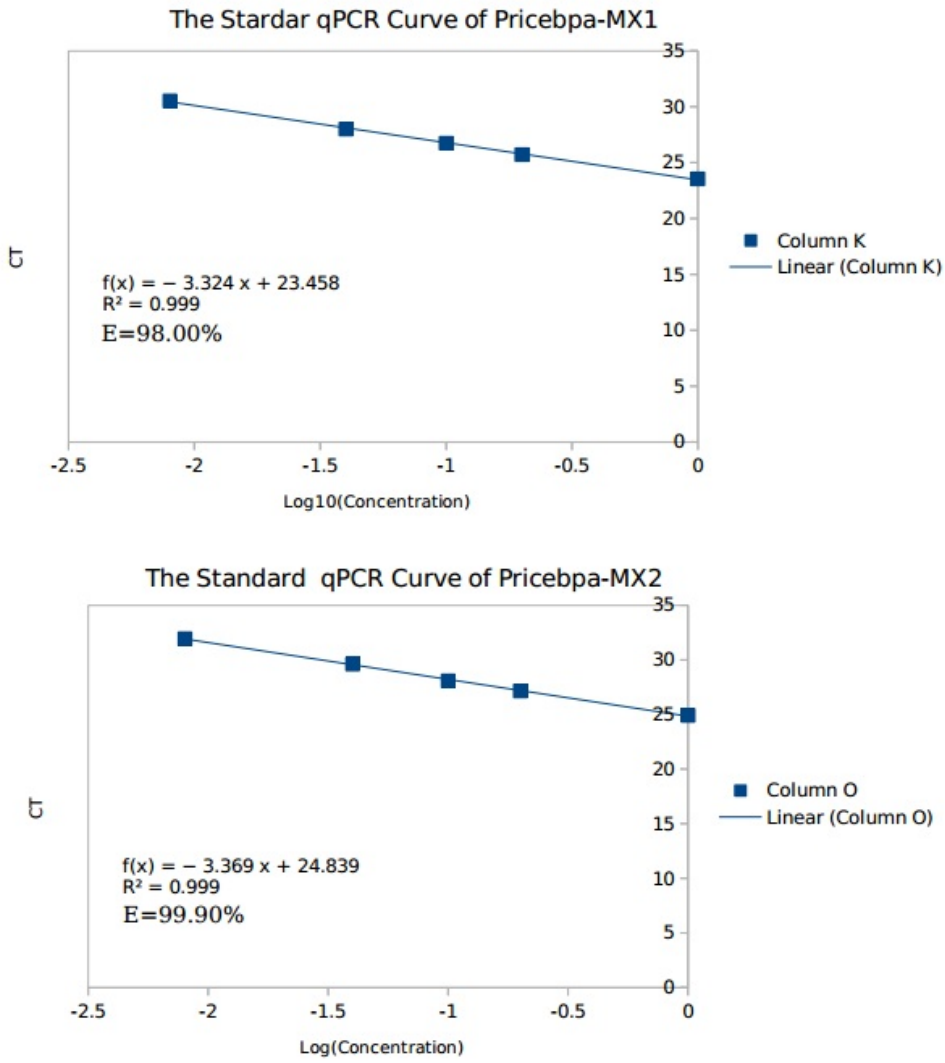

Figure 2. The stardard qPCR curve of Pricebpa-MX1 and Pricebpa-MX2.

Figure 3. **Figure 4.**

The

Gel analysis of total RNA.

Primer amplification efficiency: 杂峰

siRNA efficent: knowdown efficiency

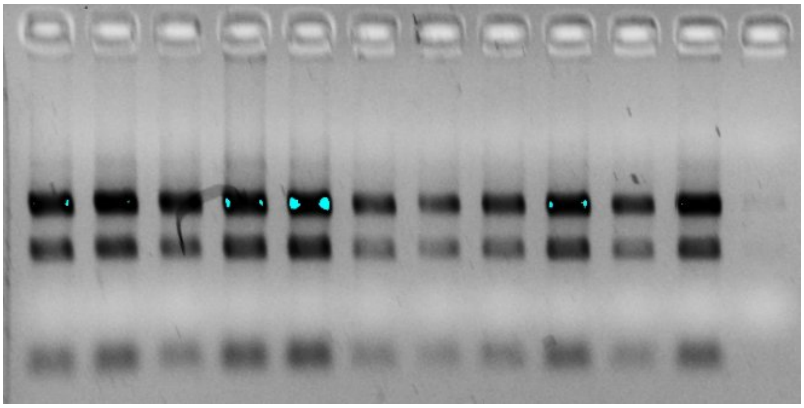

Figure 5. The Gel Graph of 4 isolativte RNAs. (From left to right: Postive Control, Negative control, siRNA 1, siRNA 2

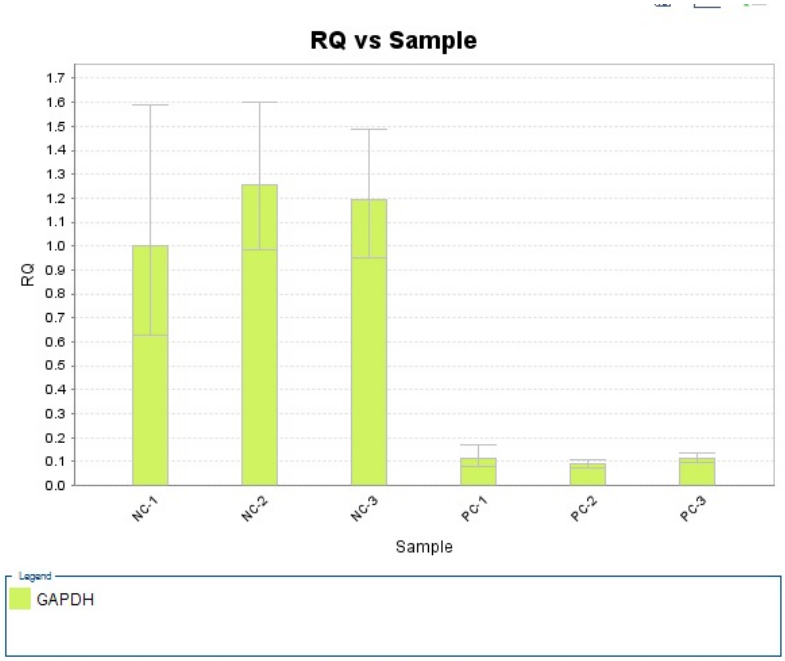

Figure 6. The experssion of GADPH

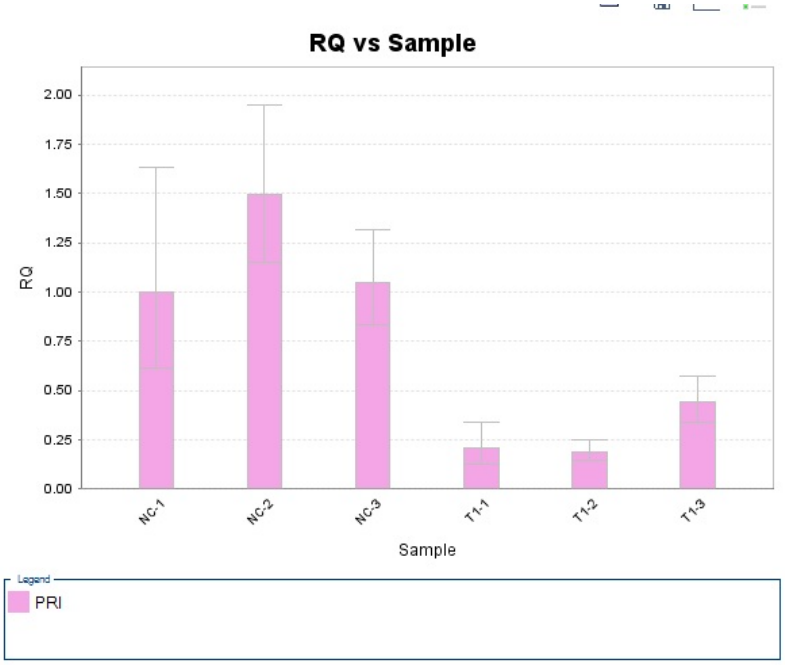

Figure 7. The experssion of Cebp α with siRNA

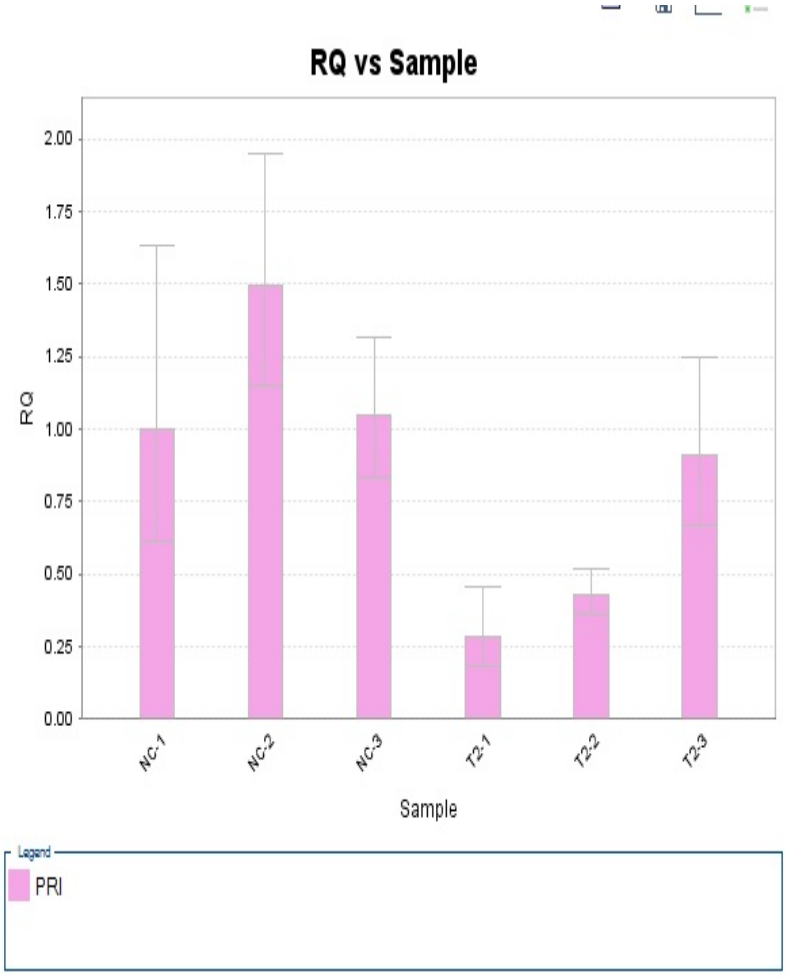

Figure 8. The expressino of Cebp α with siRNA 2

DISCUSSION

E%>100% 有非特异性扩增, E%<100% 效率

CONCLUSION

ACKNOWLEDGEMENTS

Xu wenxin and I finish this work together. *Conlict of interest statement.* None declared.

REFERENCES

- He Y, Li Y, Zhao T, Wang Y, Sun C (2013) Ursolic Acid Inhibits Adipogenesis in 3T3-L1 Adipocytes through LKB1/AMPK Pathway. PLoS ONE 8(7): e70135. https://doi.org/10.1371/journal.pone.0070135
- Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, *29*(9), e45.

图要有图注，p value