Natural Antisense Transcript of MYOG Regulates Development and Injury Recovery in Chicken Skeletal Muscle by Shielding the Binding Sites of MicroRNAs in MYOG 3’UTR  
Yunqian Yin 1,2, Genghua Chen 1,2, Zetong Lin 1,2, Danlu Zhang 1,2,  
Wujian Lin 1,2, Wen Luo 1,2\*

**For cloning the following primers were used foR:PCR:**

MYOG full-length sequence cloning foR:pcDNA3.1:

MYOG-NAT-F: TTGCTCTTACGGAAATAAATTTTTTTTTTC

MYOG-NAT-R: GGACCCCCTGGGTCCCGGTGGCGGTCGGTG   
P1~P5 region cloning foR:pGL3-basic:   
P1-KpnI-F:CGGGGTACCCCGCTACATACAGCTTTCTTGCA

P2-KpnI-F:GGGTACCCTCACCAGCCTGGTGCCACTG

P3-KpnI-F:GGGTACCCCTCATGGAGCCTTTCCCACC

P4-KpnI-F:GGGTACCCGGGGAGATGGCCAAGGAGCT

P5-KpnI-F:GGGTACCCAGGCTATACAGTCACAATGT

Promoter-MluI-R:CAGTTGCCATTTGTTTGTGTCGACGCGTCG

**For mutagenesis the following primers were used foR:PCR**

MYOD1 binding sites on P4 region:  
P4-MUT1-F:TGCTTGTGCACCCCATTGTCACCCTCAGCTCC

P4-MUT1-R:GGAGCTGAGGGTGACAATGGGGTGCACAAGCA

P4-MUT2-F:CCCCGTGCCTCTCATCCGTGGCTGTTCTGCC

P4-MUT2-R:GGCAGAACAGCCACGGATGAGAGGCACGGGG

miRNAs binding sites on MYOG 3’UTR:

MUT1-F-MYOGUTR3: GGTGGCTGGCGGGTTAAAGGGGTCCCCCAGG

MUT1-R-MYOGUTR3: CCTGGGGGACCCCTTTAACCCGCCAGCCACC

MUT2-F-MYOGUTR3: GGCTGCCTTCGCATTAGGGCCTACTGACCGC

MUT2-R-MYOGUTR3: GCGGTCAGTAGGCCCTAATGCGAAGGCAGCC

MUT3-F-MYOGUTR3: GCTGGCTACCCTACCCGCGGCTCTC

MUT3-R-MYOGUTR3: GAGAGCCGCGGGTAGGGTAGCCAGC  
  
**For quantitative real time RT-PCR in chicken, the following primers were used:**

MYOG-NAT  
F:ACAGCGGTCAGTAGGAAAGC qPCR

R:CTTCCTTGGGGGCCAACAC  
MyoD1-qPCR (reference sequence: NM$\\_$204214.2):

F: 5’- GCTACTACACGGAATCACCAAAT

R: 5’- CTGGGCTCCACTGTCACTCA

MYOG-qPCR (reference sequence: NM$\\_$204184.1):

F: 5’- CGGAGGCTGAAGAAGGTGAA

R: 5’- CGGTCCTCTGCCTGGTCAT

MyHC-qPCR (reference sequence: NM$\\_$001319015.1):

F: 5’- CTCCTCACGCTTTGGTAA

R: 5’- TGATAGTCGTATGGGTTGGT

β-actin-qPCR (reference sequence: NM$\\_$205518.1):

F: 5’- TTGTTGACAATGGCTCCGGT

R: 5’- AACCATCACACCCTGATGTCT

ITGB1-qPCR (reference sequence: NM$\\_$001039254.2):

F: 5’- CCAAGTGGGATACGGGTGAA

R: 5’- TACGCTGCATACAGTGTCGT

TMEM182-qPCR (reference sequence: XM$\\_$416920.6):

F: 5’- GGTACACCAACCAGTCACCT

R: 5’- ACAAGACACCAGCAAGGATGT

Myomaker-qPCR (reference sequence: KP230536):

F: 5’- TGGGTGTCCCTGATGGC

R: 5’- CCCGATGGGTCCTGAGTAG

adult MyHC-qPCR (reference sequence: NM$\\_$001113709.1):

F: 5’- AGCATGAGCTGGAGGAA

R: 5’- CGCTTGAGGCATCTAC

eMyHC-qPCR (reference sequence: NM$\\_$001319304.1):

F: 5’- ACTCCCAATAAACAGCG

R: 5’- GATGACTTGGCATCAAAA

Desmin-qPCR (reference sequence: XM$\\_$015290092.2):

F: 5’- AACAACTTGGCTGCTTTCAGA

R: 5’- GCTCACGGATTTCCTCTTCG