Attachment to Rapid Communication in Physiological Research No. 6/2008:

Real Time RT-PCR with a Newly Designed Set of Primers Confirmed the Presence of 2b and 2x/d Myosin Heavy Chain mRNAs in the Rat Slow Soleus Muscle

J. ŽURMANOVÁ, F. PÙTA, R. STOPKOVÁ, T. SOUKUP

Appendix 1. Sequencing analysis.

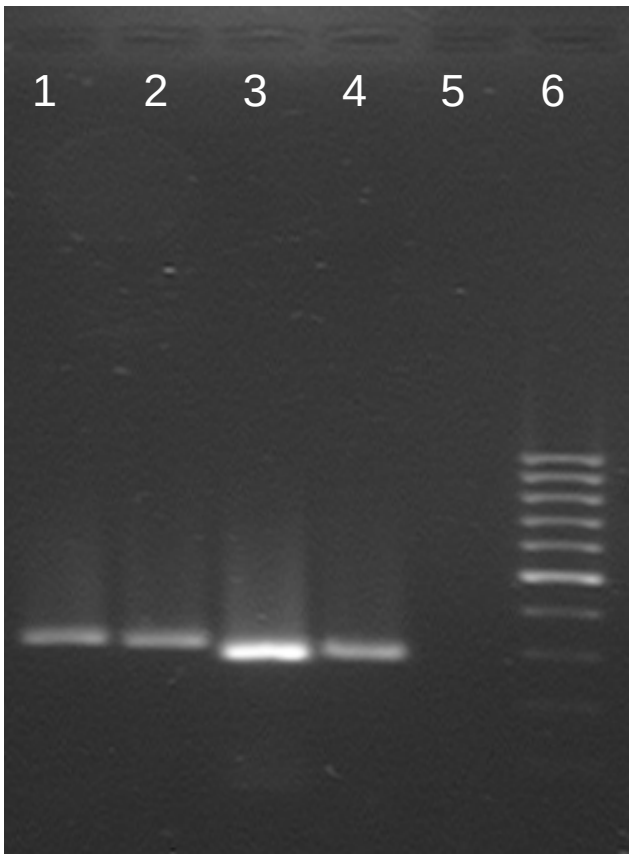
MYHC 2b (284 bps product)
GAGGACCGCAAGAACGTGCTGAGGCTGCAGGACCTAGTGGATAAATTACAGACTAAAGTGAAAGC
CTACAAGAGACAAGCAGAGGAGGCTGAGGAACAATCCAACGTCAACCTGGCCAAGTTCCGCAAGA
TCCAGCACGAGCTGGAGGAAGCCGAGGAGCGGGCCGACATCGCCGAGTCCCAGGTCAACAAGCTG
CGGGTGAAGAGCCGAGAGGTTCACACCAAAGTCATAAGCGAAGAATAGCTCAATTCCTTCTGTTGA
AAGGTGACAGAAGAAATCACA
Translation:
DRKNVLRLQDLVDKLQTKVKAYKRQAEEAEEQSNVNLAKFRKIQHELEEAEERADIAESQVNKLRVKS
REVHTKVISEE

MyHC 2x/d (318 bps product)
AAGACCGCAAGAACGTTCTCAGGCTCCAGGACCTTGTGGACAAACTGCAATCAAAGGTCAAAGCA
TACAAGAGACAAGCCGAGGAAGCGGAGGAACAATCCAACGTCAACCTGGCCAAGTTCCGCAAGAT
CCAGCACGAGCTGGAGGAAGCCGAGGAGCGGGCTGACATCGCCGAGTCCCAGGTCAACAAGCTGC
GGGTGAAGAGCCGCGAGGTTCACACCAAAATCATAAGCGAAGAGTGATCGATCCAAAGCAGGAAA
GTGACCAAAGAGATGAGCAAAATGTGAAGATCTTTGTCACTCCATTTTGTACTTACGA
Translation:
DRKNVLRLQDLVDKLQSKVKAYKRQAEEAEEQSNVNLAKFRKIQHELEEAEERADIAESQVNKLRVKS
REVHTKIISEE

MyHC 2a (309 bps product)
TCCTCAGGCTTCAAGATTTGGTGGATAAACTCCAAGCAAAAGTAAAATCTTACAAGAGACAAGCCG
AGGAGGCTGAGGAACAATCCAACACAAATCTATCCAAGTTCCGCAAGCTGCAGCATGAGCTGGAG
GAAGCCGAGGAGCGGGCTGACATCGCCGAGTCCCAGGTCAACAAGCTGCGGGTGAAGAGCCGCGA
GGTTCACACTAAAGTCATAAGTGAAGAGTAAGGCAGCTCTGATGCTGTAGAATGACCGAAGAAAG
GCACAAAATGTGAAGCCTTTGGTCATGTCCCCATGTGATTCTATTTAA
Translation:
LRLQDLVDKLQAKVKSYKRQAEEAEEQSNTNLSKFRKLQHELEEAEERADIAESQVNKLRVKSREVHT
KVISEE

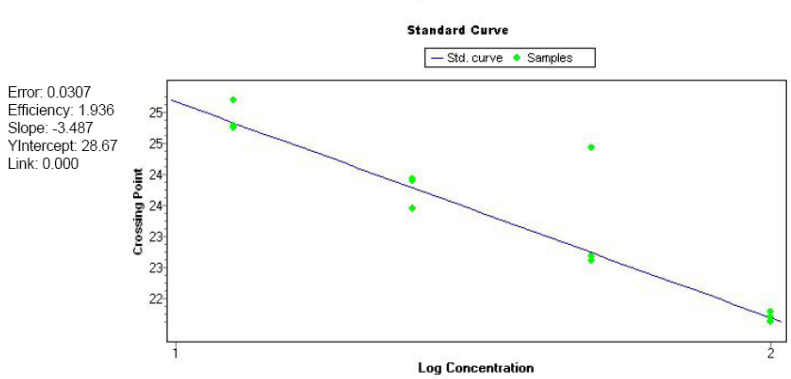
MyHC 1 (285 bps product)
AGAGGAAGACAGGAAGAACCTACTGCGACTGCAGGACCTGGTGGACAAGCTGCAGTTAAAGGTGA
AGGCCTACAAGCGCCAGGCTGAGGAGGCGGAGGAACAGGCCAACACCAACCTGTCCAAGTTCCGC
AAGGTGCAGCACGAGCTGGATGAGGCAGAGGAGAGGGCGGACATTGCCGAGTCCCAGGTCAACA
AGCTGCGGGCCAAGAGCCGTGACNTTGGCGCCAAGGGCCTGAATGAAGAGTAGATCTTGTGCTAC
CCAACCNTAANGATGCCTGTGAAGCC
Translation:
EDRKNLLRLQDLVDKLQLKVKAYKRQAEEAEEQANTNLSKFRKVQHELDEAEERADIAESQVNKLRA
KSRDxGAKGLNEE

Appendix 2. Demonstration of the MyHC isoform PCR product size. The mRNA was converted into cDNA and amplified by 50 cycles in real time PCR configuration using isoform specific primers, run on agarose gel, stained by ethidium bromide, and photographed under the UV illumination. Lanes: 1 - 2x/d; 2 - 2a; 3 - 1; 4 - 2b and 6 - 100 bp GeneRulerTM Ladder (SM0243, Fermentas).

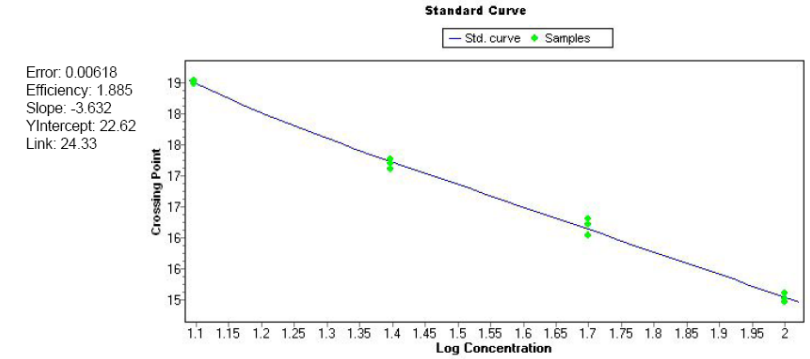


Appendix 3. The calibration curves documenting consistent efficiency of the experimental procedure for each pair of primers used in PCR reaction.

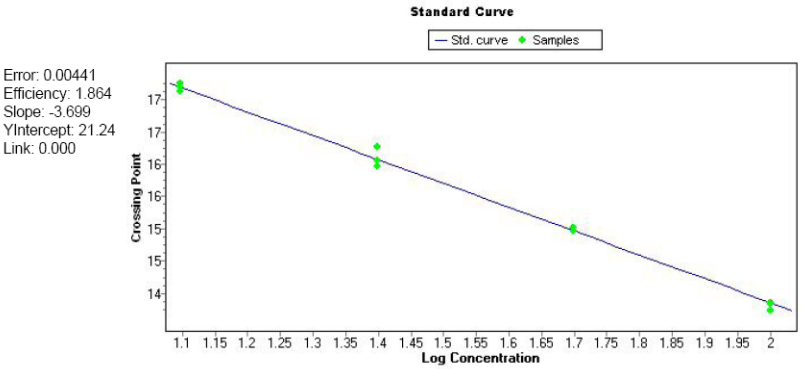
GAPDH (Efficiency: 1.936)



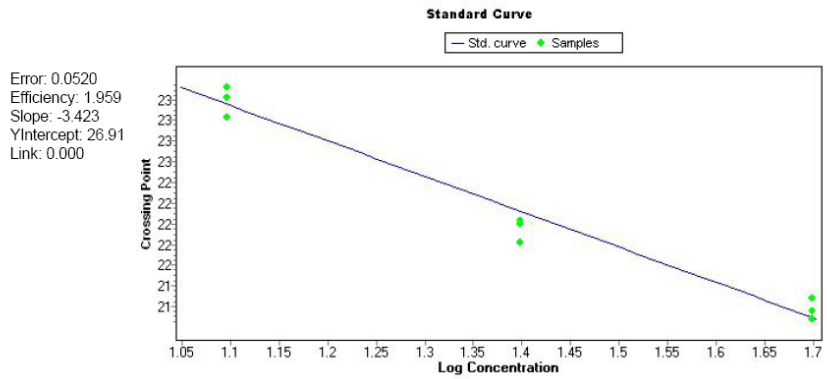
MyHC 1 (Efficiency: 1.885)



MyHC 2a (Efficiency: 1.864)



MyHC 2x/d (Efficiency: 1.959)



MyHC 2b (Efficiency: 1.883)