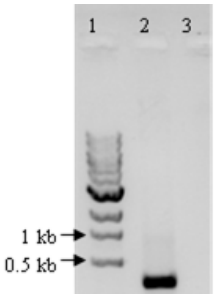
Cloning and generation of *myl7 t*ransgenic line

The 278 bp promoter region of myl7 was PCR amplified using zebrafish genomic DNA (Fig 1) using primer SSB_P1210 and SSB_P951 containing BglII cloning RE site (Huang et al., 2003). Subsequently, the PCR-amplified product was initially cloned into the TOPO-TA vector (TOPO™ TA Cloning™ Kit), ThermoFisher Scientific. The sequences of the cloned product were confirmed through Sanger sequencing. Finally, the verified myl7 TOPO-TA vector was used to clone the myl7 promoter sequence using BglII RE sites upstream of the RFP gene into a mini-Tol2 vector. To generate a stable myl7 transgenic line, the *myl7*:RFP Tol2 vector and Tol2 transposase mRNA, as described earlier, were injected into the one-cell stage of zebrafish. The embryos were raised to adulthood and crossed to wild type to identify the germline transgenesis.

Forward Primer (SSB_P1210): AAAAGATCTGCGAATTCCGGCCGCTAAAT
Reverse Primer (SSB_P951): AAAAGATCTGCAGGTTTAAACGAATTCGC
(BglII RE sites are underlined in the primers)



Lane	Sample	Expected	Observed
1	1 kb ladder		
2	Cmlc2	278	Below 0.5 kb

Fig1.: Gel picture showing 278 bp PCR amplification in lane 2 of cmlc2 minimal promoter.

Sequencing results for Cmlc2/pSS536

Following sequencing results showed that the 278 bp cmlc-2 promoter region was cloned in the TOPO-TA vector. The sequencing was done using universal T7 and T3 primers.

> LM304_T7-1.ab1

GAGCAATAGCGAGTGTAGTACTTGAGTAATTTTACTTGATTACTGTACTTAAGTATTATTTTTGGGGATTTTTACTTT
ACTTGAGTACAATTAAAAATCAATACTTTTACTTTTACTTAATTACATTTTTTTAGAAAAAAAAGTACTTTTTACTCC
TTACAATTTTATTTACAGTCAAAAAGTACTTATTTTTTGGAGATCACTTCATTCTATTTTCCCTTGCTATTACCAAAC
CAATTGAATTGCGCTGATGCCCAGTTTAATTTAAAT**AGATCT**GCAGGTTTAAACGAATTCGCCCTTCATCCCTCAAAT

CTCTCATTCACGTCCCCCTCCCCATCTGCACACTTTATCTCATTTTCCACCCTGCTGGAATCTGAGCACTTGTGCAGT
TATCAGGGCTCCTGTATTTAGGAGGCTCTGGGTGTCCATGTAGGGGACGAACAGAAACACTGCAGACCTTTATAGAAG
AACAAATGATAAGAGTCCTCATACATAAAGACTCCATTAGAAACGTCAGTGACCCAGGAGCCCAGACCAACAGCAAAG
CAGACAGTGAACATGGTGAGTAGACAAAGCAAGGGCGAATTCCGGCCGCTAAAT**AGATCT**GGCCA**TCTAGA**GCGGCCG
CGCGCACTAGTGAATTCCATGGCCAGCTCCGAGGATGTCATCAAAGAGTTTATGAGATTTAAGGTCAAGATGGAGGGA
AGCGTCAACGGACACGAGTTCGAGATTGAGGGAGAAGGAGAAGGCCGGCCTTACGAGGGCACACAAACCGCTAAGCTC
AAGGTCACAAAAGGAGGACCCCTCCCCTTCTCCTGGGATATTCTGAGCCCTCAGTTCCAGTACGGAAGCAAAGCCTAT
GTTAAACACCCTGCCGACATCCCTGACTATCTGAAGCTCTCCTTCCCTGAAGGCTTCAAGTGGGAGAGATTCATGAAC
TTCGAGGACGGAGGCGTGGTGACAGTCACACAAGATAGCACCCTCCAGGACGGAGAGTTTATTTATAAGTGAAACTCA
GAGGACCAACTTCCCCTCCGATGGCCCTGTCATGCAAAAAAACATGGGATGGCAGCTCCACCGAAAGATGTATCCTGA
AGATGCGCTCTGAAGGCGAATTAAATGAGACTGAAACTCAAGACGGAGGACTACCGATGCGAGTCAAACA

- LM304_p1195.ab1

GGGATTGTCGTTGAGCTTCCTCCATCTTGACCTTAAATCTCATAAACTCTTTGATGACATCCTCGGAGCTGGCCATGG
AATTCACTAGTGCGCGCGGCCGC**TCTAGA**TGGCC**AGATCT**ATTTAGCGGCCGGAATTCGCCCTTGCTTTGTCTACTCA
CCATGTTCACTGTCTGCTTTGCTGTTGGTCTGGGCTCCTGGGTCACTGACGTTTCTAATGGAGTCTTTATGTATGAGG
ACTCTTATCATTTGTTCTTCTATAAAGGTCTGCAGTGTTTCTGTTCGTCCCCTACATGGACACCCAGAGCCTCCTAAA
TACAGGAGCCCTGATAACTGCACAAGTGCTCAGATTCCAGCAGGGTGGAAAATGAGATAAAGTGTGCAGATGGGGAGG
GGGACGTGAATGAGAGATTTGAGGGATGAAGGGCGAATTCGTTTAAACCTGC**AGATCT**ATTTAAATTAAACTGGGCAT
CAGCGCAATTCAATTGGTTTGGTAATAGCAAGGGAAAATAGAATGAAGTGATCTCCAAAAAATAAGTACTTTTTGACT
GTAAATAAAATTGTAAGGAGTAAAAAGTACTTTTTTTTCTAAAAAAATGTAATTAAGTAAAAGTAAAAGTATTGATTT
TTAATTGTACTCAAGTAAAGTAAAAATCCCCAAAAATAATACTTAAGTACAGTAATCAAGTAAAATTACTCAAGTACT
TTACACCTCTGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTG
GGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAAGG
CCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTTGAATGGCGAATGGACGCGCCCTGTAGCGGGCGCATTAAGC
GCGCGGGTGTGGTGGTTTACGCGCAGCGTGAACGCTACACTTTGCAGCGCCCTAGCGCCCGCTCATTCGCTTTCCTTC
CCTCCTTTCTCGCCACGTCGCAGGCTTCCCGGTCAGCTCTAATCGGGGCTCCCTTAGTCGATTATGCTACCGACCTTG
ACAAAAACCTGAATAAGGTTAATGGTCAGCAGGTGAGATG

Huang, C.J., Tu, C.T., Hsiao, C.D., Hsieh, F.J., and Tsai, H.J. (2003). Germ-line transmission of a myocardium-specific GFP transgene reveals critical regulatory elements in the cardiac myosin light chain 2 promoter of zebrafish. *Dev Dyn* 228(1)**,** 30-40. doi: 10.1002/dvdy.10356.