

Supplementary Material

1 Supplementary Data

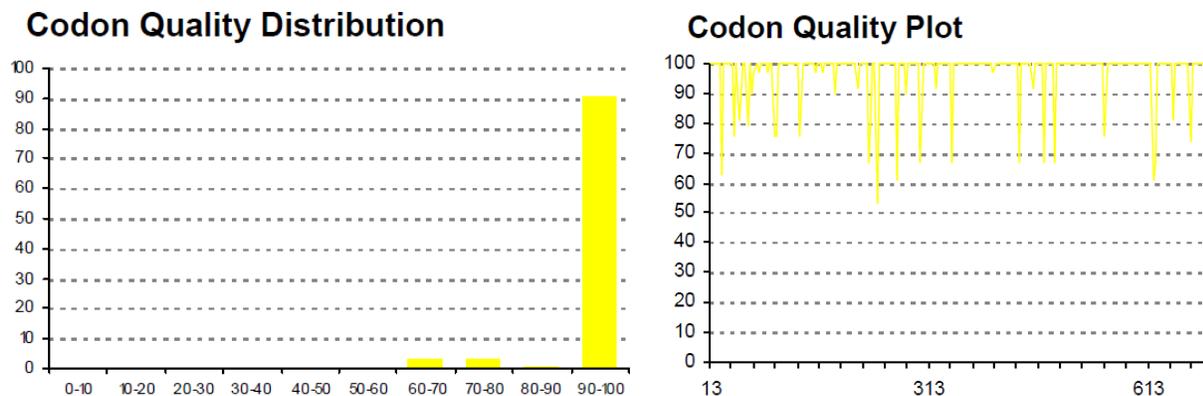
Sequence analysis for plasmid expressing FNDC5

1.1 Methods

The codon usage was adapted to the codon bias of *Mus musculus* genes. In addition, regions of very high (> 80 %) or very low (< 30 %) GC content have been avoided where possible.

During the optimization process the following cis-acting sequence motifs were avoided where applicable; internal TATA-boxes, chi-sites and ribosomal entry sites, AT-rich or GC-rich sequence stretches, RNA instability motifs, repeat sequences and RNA secondary structures, (cryptic) splice donor and acceptor sites in higher eucaryotes

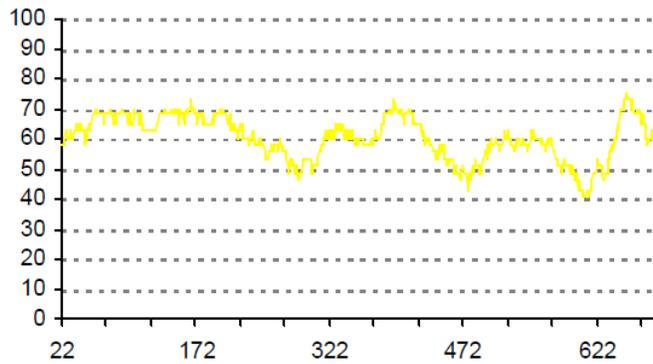
1.2 Results



Codon Adaptation Index : 0.96

The histograms show the percentage of sequence codons which fall into a certain quality class. The quality value of the most frequently used codon for a given amino acid in the desired expression system is set to 100, the remaining codons are scaled accordingly. The plots show the quality of the used codon at the indicated codon position.

GC Content



Average GC content: 60 %

The plot shows the GC content in a 40 bp window centered at the indicated nucleotide position.

1.3 Summary

The optimization was successful: Negative cis-acting sites (such as splice sites, TATA-boxes, etc.) which may negatively influence expression were eliminated wherever possible. GC content was adjusted to prolong mRNA half-life. Codon usage was adapted to the bias of *Mus musculus* resulting in a CAI* value of 0.96. The optimized gene should therefore allow high and stable expression rates in *Mus musculus*.

* CAI: The parameter CAI (codon adaptation index) describes how well the codons match the codon usage preference of the target organism. Thus, a CAI of 1.0 would be perfect. However, a CAI of > 0.9 is considered as very good (i.e. allowing high expression).

** In some rare cases negatively cis acting motifs cannot be removed due to the particular amino acid sequence or restriction site

1.4 Optimized sequence

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                PflMI
BamHI      NcoI
GGATCCGCCACCAATGGACTGGACCTGGATTCTGTTCCCTGGTGGCCGCTGCCACACGGGTG
1 -----+-----+-----+-----+-----+-----+-----+-----+
CCTAGGCGGTGGTACCTGACCTGGACCTAAGACAAGGACCACCGGCGACGGTGTGCCAC
                M D W T W I L F L V A A A T R V
                1   3   5   7   9  11  13  15

CACTCTATGCCACCTGGCCCTTGGCCCTGCCCTCCCAGAGCTGCTCTGAGACTGTGGCTG
61 -----+-----+-----+-----+-----+-----+-----+
GTGAGATACGGTGGACCGGGAACCGGACCGGAGGGTCTCGACGAGACTCTGACACCGAC
H S M P P G P C A W P P R A A L R L W L
17  19  21  23  25  27  29  31  33  35

GGCTGCGTGTGCTTCGCTCTGGTGCAGGCCGACAGCCCTAGCGCCCTGTGAACGTGACC
121 -----+-----+-----+-----+-----+-----+
CCGACGCACCGAAGCGAGACCACGTCCGGCTGTCCGGATCGCGGGGACACTTGCACCTGG
G C V C F A L V Q A D S P S A P V N V T
37  39  41  43  45  47  49  51  53  55

GTGCGGCACCTGAAGGCCAACAGCGCCGTGGTGTCCITGGGACGTGCTGGAAGATGAGGTG
181 -----+-----+-----+-----+-----+-----+
CACGCCGTGGACTTCCGGTGTGCGCGGCACCACAGGACCCTGCACGACCTTCTACTCCAC
V R H L K A N S A V V S W D V L E D E V
57  59  61  63  65  67  69  71  73  75

GTCATCGGCTTCGCCATCAGCCAGCAGAAAAGGACGTGCGGATGCTGAGATTCAATCCAG
241 -----+-----+-----+-----+-----+-----+
CAGTAGCCGAAGCGGTAGTCCGGTCTTTTTCTGCACGCCTACGACTCTAAGTAGGTG
V I G F A I S Q Q K K D V R M L R F I Q
77  79  81  83  85  87  89  91  93  95

GAAGTGAACACCACCACAGATCCTGCGCCCTGTGGGACCTGGAAGAGGACCCGAGTAC
301 -----+-----+-----+-----+-----+-----+
CTTCACTTGTGGTGGTCTAGGACCGCGGACCCCTGGACCTTCTCCTGTGGCTCATG
E V N T T T R S C A L W D L E E D T E Y
97  99  101 103 105 107 109 111 113 115

ATCGTGCACGTGCAGGCCATCAGCATCCAGGGCCAGAGCCCTGCCAGCGAGCCCGTGTG
361 -----+-----+-----+-----+-----+-----+
TAGCACGTGCACGTCCGGTATGTCGTAGGTCCCGGTCTCGGGACGGTTCGCTCGGGCACGAC
I V H V Q A I S I Q G Q S P A S E P V L
117 119 121 123 125 127 129 131 133 135

TTCAAGACCCCCAGAGAAGCCGAGAAGATGGCCTCCAAGAACAAGGACGAAGTGACCATG
421 -----+-----+-----+-----+-----+-----+
AAGTTCGGGGTCTCTTCGGCTCTTCTACCGGAGGTTCTTGTTCCTGCTTCACTGGTAC
F K T P R E A E K M A S K N K D E V T M
137 139 141 143 145 147 149 151 153 155

                PvuII                BclI
AAGGAAATGGGCAGAAACCAGCAGCTGAGAACCGGCGAGGTGCTGATCATCGTGGTGGTG
481 -----+-----+-----+-----+-----+-----+
TTCCTTTACCCGTCITTTGGTTCGTCGACTCTTGGCCGCTCCACGACTAGTAGCACCACCAC
K E M G R N Q Q L R T G E V L I I V V V
157 159 161 163 165 167 169 171 173 175

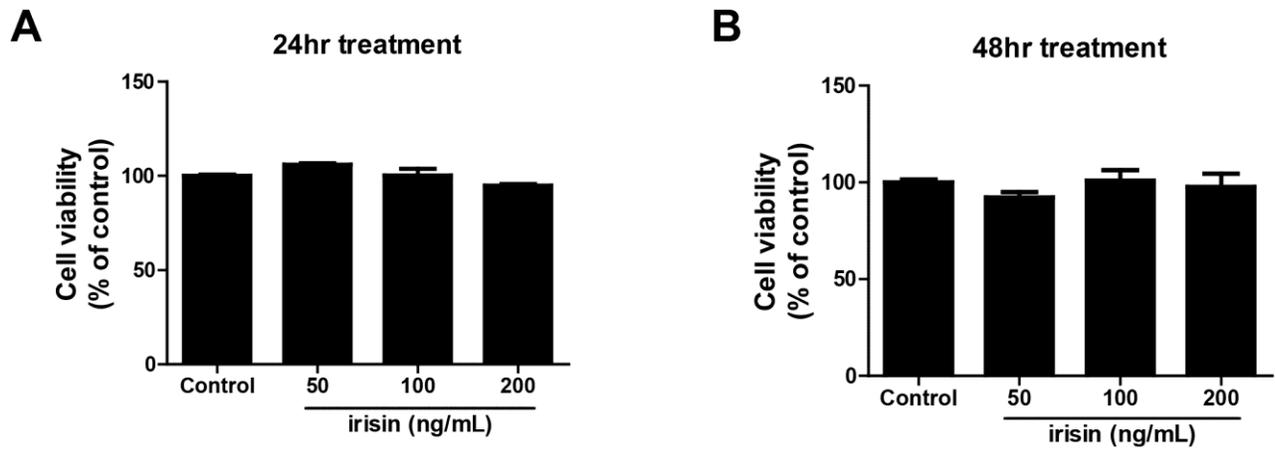
                PstI
CTGTTCAITGTTGGCTGGCGTGTATCGCCCTGTTCTGCAGACAGTACGACATCATCAAGGAC
541 -----+-----+-----+-----+-----+-----+
GACAAGTACACCCGACCGCACTAGCGGACAAGAGCTCTGTCATGCTGTAGTAGTCTCTG
L F M W A G V I A L F C R Q Y D I I K D
177 179 181 183 185 187 189 191 193 195

AACGAGCCCAACAACAACAAGAAAAGACCAAGAGCGCCAGCGAGACAAGCACCCCCGAG
601 -----+-----+-----+-----+-----+-----+
TTGCTCGGGTGTGTTGTTGTTCTTTCTGGTCTCGCGGTCGCTCTGTTCTGGGGGCTC
N E P N N N K E K T K S A S E T S T P E
197 199 201 203 205 207 209 211 213 215

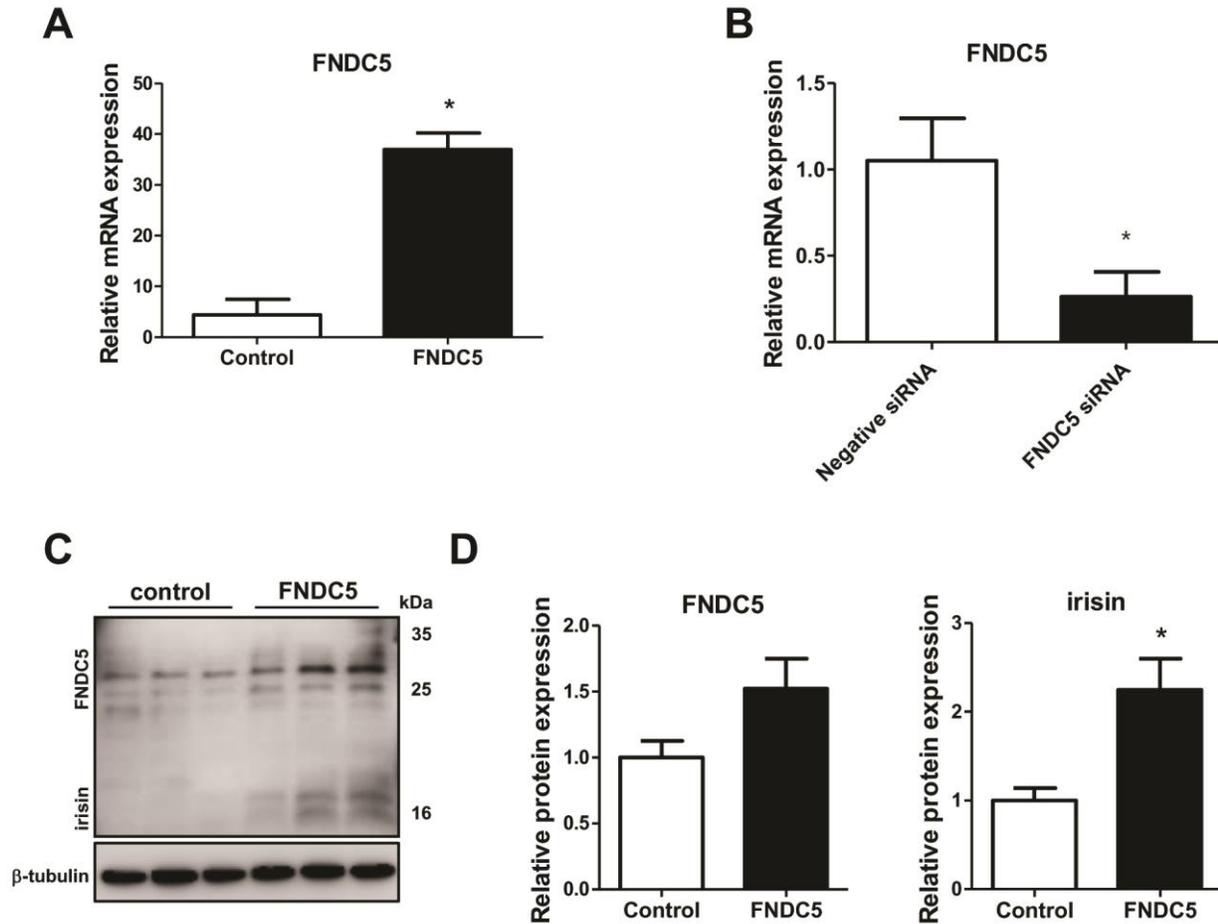
                NotI
                StuI                BglII                EagI
CACCAGGGCGGAGGCCTGCTGAGAAGCAAGATCTGATGAGCGGCCGC
661 -----+-----+-----+-----+-----+-----+
GTGTCGCCGCTCCGGACGACTCTTCTGTTCTAGACTACTCGCCGCG
H Q G G G L L R S K I
217 219 221 223 225 227

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2 Supplementary Figures



Supplementary Figure S1. The effect of recombinant irisin on cell viability (A-B) 3T3-L1 cells were treated with various concentrations of irisin for 24 and 48 hours. Cell viability was measured using MTT assay reagent.



Supplementary Figure S2. The efficiency of cell transfection. (A) FNDC5 was overexpressed in 3T3-L1 preadipocytes using plasmid encoding FNDC5 gene. (B) FNDC5 was knocked down in 3T3-L1 preadipocytes using 25 nmol/L siRNA. Gene expression of FNDC5 was examined by real-time PCR and normalized to 18s. (C, D) Western blot analysis of FNDC5 and irisin protein expression in 3T3-L1 preadipocytes overexpressed with FNDC5. Each target has been normalized with β -tubulin as an internal control. *P < 0.05 compared with control.