

Expression of Human ACE2 in *Lactobacillus* and Beneficial Effects in Diabetic Retinopathy in Mice

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Following publication, the authors realized that the design of CTB fusion constructs used in the study had not been sufficiently explained in the Materials and Methods. This information is now provided below and has been incorporated into the original online file.

Expression Vector Construction

The concept of using CTB fusion for systemic protein delivery by the oral route was originally reported by Limaye et al.⁸⁹ and has been used for oral delivery of a number of therapeutic proteins, including ACE2 and Ang-(1-7).^{35,90} A mutant form of CTB, which retains the binding to GM1-ganglioside for cellular uptake but lacks immunomodulatory and toxic activity,^{91,92} was used in this study. The codons of the CTB sequence were also extensively changed for optimal expression in *Lactobacillus*. The FC site was based on our previously published sequence,³⁶ which is an artificial FC site designed after careful comparison of available natural protein substrates of furin protease, with enhanced affinity for FC. The codons were also optimized for expression in *Lactobacillus* species. The DNA sequence for codon-optimized CTB for expression in *Lactobacillus* species is as follows: 5'-ATGATTAAGTTAAAGTTTGGTGTTTTTTTTACTGTTTTATTATCATCAGCTTACGCTCACGG TACTCCACAAAACATTACTGATTTATGTGCTGAATACCACAACACTCAAATTCACACTTTAAACGATAAGATTTTTTCATACACT GAATCATTAGCTGGTAAGCGTGAAATGGCTATTACTTTTTAAGAACGGTGCTACTTTTCAAGTTGAAGTTCAGGTTCCAGGTTCCACAA GCTATTGATTCACAAAAGAAGGCTATTGAACGTATGAAGGATACTTTACGTATTGCTTACTTAACTGAAGCTAAGGTTGAAAAG TTATGTGTTTGGAAACAACAAGACTCCACACGCTATTGCTGCTATTTCAATGGCTAAC-3'.

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