The position of the inserts/promoter on this plasmid is listed below:

pCMV: 272 – 926 (655 bp)

FLEx: 990 – 1122 (133 bp) & 1978 – 2118 (141)

HA-Tags: 1135 – 1161 (27 bp), 1168 – 1194 (27 bp) & 1198 – 1224 (27 bp) -> in frame with TetOn3G (C-terminally fused)

TetOn3G: 1977 – 1234 (744 bp) -> The sequence is located inverse (start at 1977 bp) on the plasmid and is only expressed correctly after inversion from cre recombinase.

The backbone for this plasmid is the ‘AAV-cDNA6-V5His’ from Vector Bioloabs. We inserted the insert downstream of the CMV promoter using Gibson Cloning. The primer for inserting the FLEx-TetOn3G-3xHa-FLEx cassette behind the CMV promoter are listed below:

Insert\_f: cgtttaaacttaagcttggtaccgagc

Insert\_r: gaggctgatcagcgggtt

Vector\_f: aacccgctgatcagcctc

Vector\_r: gctcggtaccaagcttaagtttaaacg