Design of primers for tagging at the C-term of the GOI (knock-in) using one of the pLinker vector as template

Plasmids that can be used as template:

- pTUB1:YFP-AID-3HA, DHFR-TS:HXGPRT

- pTUB1:YFP-mAID-3HA, DHFR-TS:HXGPRT

- pLinker-2xTy-DHFR-LoxP

- pLinker-2xTy-HXGPRT-LoxP

- pLinker-6xHA-HXGPRT-LoxP

Forward primer:

30-32 bp of homology with the 3’ end of the gene without the stop + GCTAGCAAGGGCTCGGG

Reverse primer:

30-32 bp of homology within the 3’ UTR of the gene (rev compl) + ATACGACTCACTATAGGG

PCR done with Q5 and 57°C as annealing temperature

Design of primers for inserting the Tet system at the N-term of the GOI

Template:

V1-5'COR-pT8TATi1-HX-tetO7S1myc

Fwd primer for PCR

GOI homology + CATGTTTGCGGATCCGGGG

Rev primer with Myc for PCR

GOI homology + CAGGTCCTCCTCGGAGATGA

Rev primer without Myc for PCR

GOI homology + CCATTTTGATATCCCTAGGAATTCAC