

Cloning Strategy for expression of a gene of interest under a constitutively active promoter with an epitope tag

I. N-terminal FLAG tag

Vector: pTCdc6N-Myc-FLAG-N#7: -----CMV promoter-Intron-ATG-FLAG-ggatcc (BamH1)-Cdc6N-Myc-Stop codon-----ggtacc (Asp718)---SV40 polyA-----

Insert: -----ggatcc (BamH1) gene of interest in frame-Stop codon-ggtacc (Asp718)

II. C-terminal FLAG tag

Vector: pTCdc6N-Myc-FLAG-C#1: -----CMV promoter-Intron-ggatcc (BamH1)-Cdc6N-Myc-STOP---ggtacc (Asp718)-FLAG-StopCodon GAATTC (R1)--- SV40 polyA---

Insert:-----ggatcc (BamH1) acc ATG gene of interest in frame-ggtacc (Asp718)

III. N-terminal HA tag

Vector: pTCdc6N-Myc-HA-N#16: -----CMV promoter-Intron-ATG-HA-ggatcc (BamH1)-Cdc6N-Myc-Stop codon---ggtacc (Asp718)---SV40 polyA-----

Insert: -----ggatcc (BamH1) gene of interest in frame-Stop codon-ggtacc (Asp718)

IV. C-terminal HA tag

Vector: pTCdc6N-Myc-HA-C#9: -----CMV promoter-Intron-ggatcc (BamH1)-Cdc6N-Myc-ggtacc (Asp718)-HA-StopCodon-GAATTC (R1)--- SV40 polyA---

Insert:-----ggatcc (BamH1) acc ATG gene of interest in frame-ggtacc (Asp718)

V. N-terminal Myc tag

Vector: pTCdc6N-Myc-Myc-N#19: -----CMV promoter-Intron-ATG-Myc-ggatcc (BamH1)-Cdc6N-Myc-Stop codon----ggatcc (Asp718)---SV40 polyA-----

Insert: -----ggatcc (BamH1) gene of interest in frame-Stop codon-ggtacc (Asp718)

VI. C-terminal Myc tag

Vector: pTCdc6N-Myc-Myc-C#20: -----CMV promoter-Intron-ggatcc (BamH1)-Cdc6N-Myc---ggtacc (Asp718)-Myc-StopCodon GAATTC (R1)--- SV40 polyA---

Insert:-----ggatcc (BamH1) acc ATG gene of interest in frame-ggtacc (Asp718)

Oligo design for subcloning of cDNA

N-terminal tag:

Forward primer: X GGA TCC (BamH1) XXX XXX of the gene of interest (18-21 nt)

Reverse Primer: X GGT ACC (Asp718) TCA (STOP) XXX XXX of the gene of interest (18-21 nt)

C-terminal tag:

Forward primer: X GGA TCC (BamH1) ACC ATG (START) XXX XXX of the gene of interest (18-21 nt)

Reverse Primer: X GGT ACC (Asp718) XXX XXX of the gene of interest (18-21 nt)