

### **pLenti-EGFP-hChibby1**

(vector)

EcoRI/MfeI

GAATTG-ACC-**EGFP** (no stop codon)-GAA/TTC-GGG/GCC/ATT/ACG/GCC-  
EcoRI **SfiI**  
(Unique)

hCby(0.4kb)-GGCCGCCTCGGCC-GCGGCCGC

**SfiI** NotI(vector)

The lentiviral vector has a pEF1 $\alpha$ -IRES-EGFP backbone but the original IRES-EGFP cassette was removed.

The vector can be used to subclone a gene of your interest for an N-terminal EGFP fusion.

For cloning a gene of your interest, first order a pair of primers with SfiI overhangs:

5' primer: GAATTCGGCCATTACGGCC-(20 bases from a gene of your interest)

3' primer: GAATTCGGCCGAGGCGGCC-(20 bases from a gene of your interest; make sure to include the stop codon!!)

We found that SfiI sites work well for subcloning, and the SfiI sites in this vector have different overhangs, enabling a directional cloning.

5' sequencing primer: ACCACATGGTCCTTCTTCAG (GFP primer)

3' sequencing primer: ACGCCACGTTGCCTGACAAC