How to use GFP-LC3-RFP(-LC3ΔG)

The GFP-LC3-RFP(-LC3ΔG) fragments are inserted into the pMRX-IP vector for retrovirus generation

We strongly recommend making stable clones expressing the probe. It is difficult to detect changes in the GFP-RFP ratio in cells overexpressing this probe by transient transfection. For the same reason, we do not recommend using highly-expressing stable transformants.

For flow cytometry analysis, fixation is not required. Collect cells after trypsin treatment and place them on ice. See detail procedure in the following website.
http://proteolysis.jp/autophagy/protocol/protocol%20files/FACS%20analysis%20of%20GFP-LC3%20degradation.pdf

<<IMPORTANT>>
Isolation of single cell clones is essential for the GFP-LC3-RFP-LC3ΔG probe because this sequence frequently undergo homologous recombination between the two LC3 fragments during retrovirus infection (see below data).
PCR amplification of genomic DNA using the following primers yields a 1,533-bp fragment if the GFP-LC3-RFP-LC3ΔG probe correctly integrated. If homologous recombination occurs, a 480-bp fragment (GFP-LC3ΔG) is amplified.
Forward: 5′- CGCCGCCGGGATCACTCTCG -3′
Reverse: 5′- CCACCACACTGGGATCCTTA -3′
This type of homologous recombination does not occur in the GFP-LC3-RFP probe (without the second LC3). Therefore, this probe can be used without single cell cloning (see below data). Nevertheless, we think that the GFP-LC3-RFP-LC3ΔG probe is theoretically better because it produces an internal control containing the LC3 fragment.