In fusion cloning

pMSCV-loxp-dsRed-loxp-eGFP-Puro-WPRE and pMSCV-loxp-dsRed-loxp-3xHA-Puro-WPRE have the same 30bp sequence around the HpaI site. Therefore, after an HpaI digest, the same 15bp sequence is present upstream and downstream in both plasmids, which can be used for in fusion cloning (Clontech).

AGATCTCTCGAGGTTAACGAATTCGACTAC

After HpaI cutting

AGATCTCTCGAGGTT AACGAATTCGACTAC

After Cloning

AGATCTCTCGAGGTTgccaccATGXXX…XXX(without STOP codon)AACGAATTCGACTAC-FLAG, 3x HA or eGFP (in frame)

To design the primer for cDNA amplification:

- generate primers to amplify your cDNA
    - forward primer: add an optimal Kozak sequence to it (gccacc)
    - reverse primer: don't forget to remove the STOP codon, otherwise the Tag will not be expressed

To design the in fusion primer:

- You can use the following webside: http://bioinfo.clontech.com/infusion/convertPcrPrimersInit.do
- Paste some sequence around the HpaI side of the MSCV vector in the webside

GCTGAAGGGCGAGATCCACAAGGCGCTGAAGCTGAAGGGCGGCGGCCACTACCTGGTGGAGTTCAAGTCAATCT
ACATGGCCAAGAAGCCCGTGAAGCTGCCCGGCTACTACTACGTGGACTCCAAGCTGGACATCACCTCCCACAACG
AGGACTACACCGTGGTGGAGCAGTACGAGCGCGCCGAGGCCCGCCACCACCTGTTCCAGTAGAGCTAGCTGAAT
AAGGCCGCTCGAATAACTTCGTATAGCATACATTATACGAAGTTATTCGAGTCTAGAGGGCCCGTTAGATCTCTCG
AGGTTAACGAATTCGACTACTACCCCTACGACGTGCCCGACTACGCCgctggagcaTACCCCTACGACGTGCCCGACT
ACGCCgctggagcaTACCCCTACGACGTGCCCGACTACGCCTGAGTCGACGCGGCCGCCAATTCTACCGGGTAGGGG
AGGCGCTTTTCCCAAGGCAGTCTGGAGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTGGCC
TCTGGCCTCGCACACATTCCACATCCACCGGTAGGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCT

- Paste your forward and reverse primers in the webside
- Tell the program the restriction side to use (HpaI)
- You can choose to preserve or delete the HpaI side (normally we delete it)

Alternatively, you can just add the following sequences by hand in front of your primers:

- forward: AGATCTCTCGAGGTTgccaccXXXXXXXXX
- reverse GTAGTCGAATTCGTTXXXXXXX