Design AarI primers
Adapted from Sergio Peisajovich

MCS for iGEM pRS315 acceptor vectors:

GGGCCC_CTCGAGggagcaag**gcaggtg**gacaagaggagtcccgggagctggaactccc**acctgc**aacatgcgGGATCCTGAGTAAATAAGCGGCCGCGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTATAAAAAAAATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTTAAAACGAAAATTCTTATTCTTGAGTAACTCTTTCCTGTAGGTCAGGTTGCTTTCTCAGGTATAGCATGAGGTCGCTCTTATTGACCACACCGAGCTC

Restriction sites (yellow) are in the following order (AarI recognition is bolded): PspOMI, XhoI, XmaI, BamHI, NotI and SacI (the AarI sites "A" and "D" are marked in red).

In Green: 3 STOPS in different frames.

In Purple: Adh-Terminator

Promoters (including ATG) are between PspOMI and XhoI.

A four part ligation would look like this:
(colors different, matching that in the text preceding the sequence)

1-ATG-XhoI-"A" and 2 adaptor bases: ATGCTCGAGGGAGCT

2-7 Adaptor bases, followed by "B" and another adaptor base: GGTAGTTCCCTA

3- 4 Adaptor bases, followed by "C" followed by another adaptor base: GGTAGCGAT

7-STOP, followed by "D" (TGCG), followed by BamHI, 3 STOPS in three different frames, then NotI, the Adh terminator and SacI.

Example of primer design for a three-part ligation
(acceptor and two donors specifying protein domains for
fusion)

Add these sequences to your primers:
AB part (amino terminal part of protein fusion)

FOR 5':CACCTGCAACAGGAGCT—YOUR PRIMER-3'
REV 5':CACCTGCCTTGAGGGAACTACC—-3'

BD part (carboxy terminal part of protein fusion)

FOR 5':CACCTGCAACACCCTA—YOUR PRIMER-3'
REV 5':CACCTGCCTTGCGCATTA-YOUR PRIMER-3'