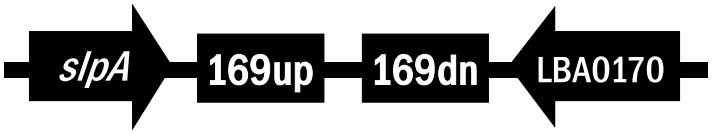
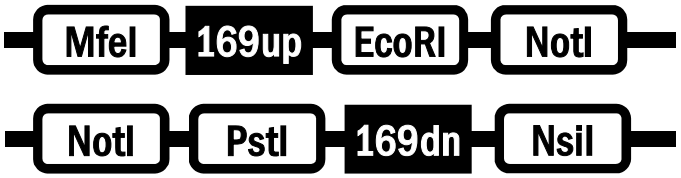
Flow chart of gene cloning of a homologous recombination vector for Lactobacillus acidophilus

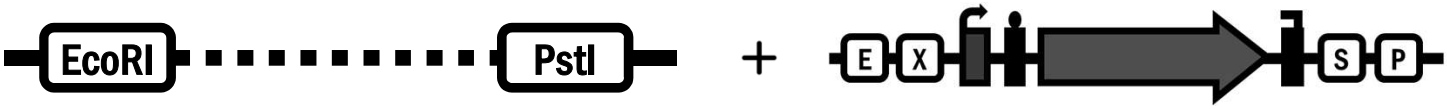
STEP 1. Extract genomic DNAs of Lactobacillus acidophilus



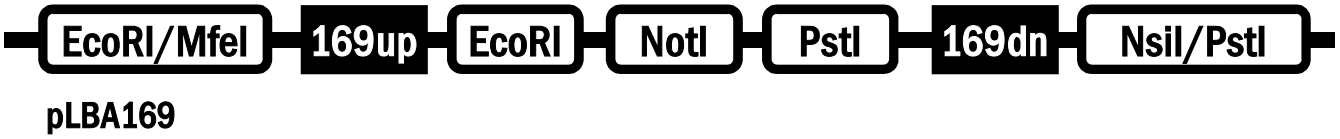
STEP 2. Amplify DNA fragments of 169up and 169dn by PCR and cut by MfeI & NotI or NotI & NsiI



STEP 3. Cut Part: BBa_K1033280 with EcoRI and PstI, and keep the fragments of "CP29-RBS-aeBlue"



STEP 4. Ligate through EcoRI plus MfeI, NotI with NotI, and PstI plus NsiI, which formed scars in the flanking regions and generate EcoRI and PstI sites within.



pLBA169

STEP 5. Assemble with "CP29-RBS-aeBlue" using EcoRI & PstI, and build the standard BioBrick assembly restriction enzyme recognition sites. Unfortunately, an extra SpeI site was found on 169dn region.

CP29-RBS-aeBlue/pLBA169 (SpeI)

STEP 6. Delete the extra SpeI sequence by site-directed mutagenesis

CP29-RBS-aeBlue/pLBA169