

**Table 1.** Basic and additional LIC vectors constructed.

Vector name	Protein sequence	Protein sequence after TEV protease cleavage	Expression host
pREnLIC	M-His <sub>10</sub> -G-TEV site-protein	G-protein	<i>L. lactis</i> NZ9000
pREcLIC	MGGGFA-protein-TEV site-His <sub>10</sub>	MGGGFA-protein-ENLYFQ	<i>L. lactis</i> NZ9000
pREcLIC-GFP	MGGGFA-protein-TEV site-GFP-His <sub>10</sub>	MGGGFA-protein-ENLYFQ	<i>L. lactis</i> NZ9000
pRE-USP45-nLIC	M-ssUSP45 <sup>1)</sup> -His <sub>10</sub> -G-TEV site-protein	G-protein	<i>L. lactis</i> NZ9000
pBADnLIC	M-His <sub>10</sub> -G-TEV site-protein	G-protein	<i>E. coli</i>
pBADcLIC	MGGGFA-protein-TEV site-His <sub>10</sub>	MGGGFA-protein-ENLYFQ	<i>E. coli</i>
pBADcLIC-GFP	MGGGFA-protein-TEV site-GFP-His <sub>10</sub>	MGGGFA-protein-ENLYFQ	<i>E. coli</i>
pBAD-OmpA-nLIC	M-ssOmpA <sup>2)</sup> -His <sub>10</sub> -G-TEV site-protein	G-protein	<i>E. coli</i>

<sup>1)</sup> ssUSP45 indicates the signal sequence of the *L. lactis* USP45 protein. The pRE-USP45-nLIC vector is used if the N-terminus of the membrane protein of interest is predicted to be on the outside of the cytoplasmic membrane, or to replace the signal sequence of the protein of interest with the ssUSP45.

<sup>2)</sup> ssOmpA indicates the signal sequence of the *E. coli* OmpA protein. The pBAD-OmpA-nLIC vector is used if the N-terminus of the membrane protein of interest is predicted to be on the outside of the cytoplasmic membrane, or to replace the signal sequence of the protein of interest with the ssOmpA.

**Table 2.** Primer extensions required for the ligation-independent cloning procedure.

Type of primer	Sequence (5'→3')
nLIC forward	AT GGT GAG AAT TTA TAT TTT CAA GGT + gene specific (no start)
nLIC reverse	T GGG AGG GTG GGA TTT TCA TTA + gene specific (no stop)
cLIC forward	ATG GGT GGT GGA TTT GCT + gene specific (no start)
cLIC reverse	TTG GAA GTA TAA ATT TTC + gene specific (no stop)