

Primer List for N-terminus tagged template

Primer type	Primer form	Primer name	Vector/tag type	Primer sequence (5'-3')
<1st PCR>				
Forward primer	Gene specific 1st Fw primer	1st-gene-specific-NF		5' side 18 mer, starts from CCAGCAGGGAGGTACT+ATG
Reverse primer	Gene specific 1st Rv primer	1st-gene-specific-NR		CCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTTA+3'side 18 mer excluding Stop codon.
<2nd PCR>				
Two primers are used as Forward Primer, 2nd-NF2 and one of 2nd protein tag(xxx)-NF1, where xxx is FLAG,His,HA,MYC or BAP. 2nd-STE-NCR2 and 2nd-STE-NR1 are used as Reverse Primer				
Forward primer	Universal 2nd Fw primer-tag	Universal 2nd Fw primer	2nd-T7-NF2	CCCGCGAAATAATACGACTCACTATAG
		2nd-NF1	No-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACCTTCAACTTCCTATTCTCCAGCAGGGAGGTACTATG
		2nd-protein tag(FLAG)-NF1	FLAG-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACCTTCAACTTCCTATTATGGACTACAAGGATGACGATGACAAGCTCCAGCAGGGAGGTACTATG
		2nd-protein tag(His)-NF1	His-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACCTTCAACTTCCTATTATGCATCATCATCATCATCTCCAGCAGGGAGGTACTATG
		2nd-protein tag(HA)-NF1	HA-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACCTTCAACTTCCTATTATGTATCCTTATGACGTGCCTGACTATGCCAGCCTGGGAGGACCTCTCCAGCAGGGAGGTACTATG
		2nd-protein tag(MYC)-NF1	MYC-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACCTTCAACTTCCTATTATGGAACAAAGCTAATCTCCGAGGAAGACTTGCTCCAGCAGGGAGGTACTATG
		2nd-protein tag(BAP)-NF1	Biotin-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACCTTCAACTTCCTATTATGGGCTGAACGACATCTTCGAGGCCAGAAGATCGAGTGGCACGAACTCCAGCAGGGAGGTACTATG
Reverse primer	Universal 2nd Rv primer	2nd-STE-NCR2		GGCCCCCCTCGAAGG
		2nd-STE-NR1		CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAA

■T7 Promoter, ■Tag, ■Linker, ■Start Codon

Primer List for C-terminus tagged template

Primer type	Primer form	Primer name	Vector/tag type	Primer sequence (5'-3')
<1st PCR>				
Forward primer	Gene specific 1st Fw primer	1st-gene-specific-CF		5' side 18 mer, starts from, CACAAAACATTTCCCTACATACAACCTTCAACTTCCTATT+ATG
Reverse primer	Gene specific 1st Rv primer	1st-gene-specific-CR		AGTACCTCCCTGCTGGAGACC+3'side 18 mer excluding Stop codon.
<2nd PCR>				
Two reverse primers are used, 2nd-STE-NCR2 and one of 2nd-protein tag(xxx)-CR1, where xxx is FLAG, His, MYC, HA or BAP				
Forward primer	Universal 2nd Fw primer	2nd-T7-CF		CCCGCGAAATTAATACGACTCACTATAGGGCTCACCTATCTCTCTACACAAAACATTTCC
Reverse primer	2nd Rv primer	2nd-STE-NCR2		GGCCCCCCTCGAAGG
	2nd Rv primer-tag	2nd-protein tag(FLAG)-CR1	FLAG-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTACTGTGCATCGTCATCCTTGTAGT AGTACCTCCCTGCTGG
		2nd-protein tag(His)-CR1	His-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTAATGATGATGATGATGATGAGTAC CTCCCTGCTGG
		2nd-protein tag(MYC)-CR1	MYC-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTACAAGTCTTCCTCGGAGATTAGC TTTTGTTAGTACCTCCCTGCTGG
		2nd-protein tag(HA)-CR1	HA-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTAAGGTCCTCCAGGCTGGCATA GTCAGGCACGTCATAAGGATAAGTACCTCCCTGCTGG
		2nd-protein tag(BAP)-CR1	Biotin-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTATTCGTGCCACTCGATCTTCTGG GCCTCGAAGATGTCGTTGAGGCCAGTACCTCCCTGCTGG

■ T7 Promoter, ■ Tag, ■ Linker, ■ Start Codon