

## Primer List for N-terminus tagged template

Primer type	Primer form	Primer name	Vector/tag type	Primer sequence (5'-3')
<b>&lt;1st PCR&gt;</b>				
Forward primer	Gene specific 1st Fw primer	1st-gene-specific-NF		5' side 18 mer, starts from CCAGCAGGGAGGACT+ATG
Reverse primer	Gene specific 1st Rv primer	1st-gene-specific-NR		CCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTTA+3'side 18 mer excluding Stop codon.

<b>&lt;2nd PCR&gt;</b>				
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-SU-NCR2 and 2nd-SU-NR1 are used as Reverse Primer				
Forward primer	Universal 2nd Fw primer	2nd-T7-NF2		CCCGCGAAATTAATACGACTCACTATAG
	Universal 2nd Fw primer-tag	2nd-NF1	No-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACITTCCTATTCTCCAGCAGGGAGGACTATG
		2nd-protein tag(FLAG)-NF1	FLAG-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACITTCCTATTATGGACTCAAGGATGACGATGACAAGCTCCAGCAGGGAGGACTATG
		2nd-protein tag(His)-NF1	His-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACITTCCTATTATGCATCATCATCATCATCTCCAGCAGGGAGGACTATG
		2nd-protein tag(HA)-NF1	HA-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACITTCCTATTATGTATCCTATGACGTGCCTGACTATGCCAGCCTGGGAGGACCTCTCCAGCAGGGAGGACTATG
		2nd-protein tag(MYC)-NF1	MYC-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACITTCCTATTATGGAACAAGGTAATCTCCGAGGAAGACTTGCTCCAGCAGGGAGGACTATG
		2nd-protein tag(BAP)-NF1	Biotin-tag	CGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTTCCCTACATACAACITTCCTATTATGGGCCTGAACGACATCTTCGAGGCCAGAAGATCGAGTGGCACGAACTCCAGCAGGGAGGACTATG
Reverse primer	Universal 2nd Rv primer	2nd-SU-NCR2		GGCCCCCCTCGAAGG
		2nd-SU-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, CACAAAACATTCCCTACATACAACITTC AACITTCCTAT + 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-SU-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		CCCGCGAAATAATACGACTCACTATAGGGCTCACCTATCTCTACACAAAACATTCC
Reverse primer	2nd Rv primer	2nd-SU-NCR2		GGCCCCCCTCGAAGG
	2nd Rv primer-tag	2nd-protein tag(FLAG)-CR1	FLAG-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTACTGTGCATCGTCATCCTTGTAGTAGTACCTCCCTGCTGG
		2nd-protein tag(His)-CR1	His-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTAATGATGATGATGATGATGAGTACCTCCCTGCTGG
		2nd-protein tag(MYC)-CR1	MYC-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTACAAGTCTCCTCGGAGATTAGCTTTGTTAGTACCTCCCTGCTGG
		2nd-protein tag(HA)-CR1	HA-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTAAAGTCTCCTCCAGGCTGGCATAGTCAGGCACGTCATAAGGATAAGTACCTCCCTGCTGG
		2nd-protein tag(BAP)-CR1	Biotin-tag	CCCTCGAAGGATCAGGCCCTTATGGCCGGATCCAAGAGCTCTTTTTTTTTTATTCGTGCCACTCGATCTCTGGGCTCGAAGATGTCGTTAGGCCAGTACCTCCCTGCTGG

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