

Supplementary Materials

Development of Synthetic mRNAs Encoding Split Cytotoxic Proteins for Selective Cell Elimination Based on Specific Protein Detection

Kendall Free, Hideyuki Nakanishi and Keiji Itaka

Table S1. List of primers used to prepare template DNAs for in vitro transcription.

Primer Name	Primer Sequence
HNC-542	CAGTGAATTGTAATACGACTCACTATAAGCGAATTAAGAGAGAAAAGAAGAGTAAGAA-GAAATATAAGACACCGGTGCCACCATG
HNT-7	CAGTGAATTGTAATACGACTCACTATAAGCGA
HNT-9	TTCTACTCAGGCTTATTCA

Table S2. Full sequences of template DNAs for in vitro transcription.

ID	hBarnase
Template pDNA	pUTR2-hBarnase
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, hBarnase gene: 88-417, Stop codon: 418-420
Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAGAGAGAAAAGAAGAGTAAGAA-GAA-GAAATATAAGACACCGGT GCCACCATG GCCCAGT GATCAACACCC TTGACGGCGTGG CCGACTACCTGCAGACATACCACAAAGCTGCCGACA ACTACAT CACCAA-GAGCGAGGCCAGGCTCTGGATGGGTTGCCTCTAAGGGAAACCTGGCGATGTGGCCC CTGGCAAGT CTATCGGCGCGACATCTTCAGCAACAGAGAGGGCAAGCTGCCTGGCAA-GAGCGGCAGAACTGGAGAGAGGCCGACATCAACTACACCAGCGGCTCCGGAACAGC GACCGGAT CTGTACAGCAGCGACTGGCTGATCTACAAGACCACCGACCACTAC-CAGAC- CTTCACCAAGATCAGATGA ATCTAGAC CTTCTGC GGGCTTG CCTCTGGCCATGCC CTT CTCTCCCTTG CAC CTGTACCTCTGG CTTGAATAAG CCTGAGTAG-GAAAAAA AAAAAA AAAAAAAAAA AAAAAA AAAAAAAAAA AAAAAA AAAAAAAAAA AAAAAA
ID	hBarnase-21N

Template pDNA	pUTR2-hBarnase-21N
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, N-terminal split Barnase gene: 88-147, Caged eNpu N-intein gene: 148-714, Anti-eDHFR nanobody Nb113 gene: 721-1122, Stop codon: 1123-1125
Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAAGAGAGAAAAGAAGAGTAA-GAA- GAAATATAAGACACCGGTC GCCACCATGGCCAAGT GATCAACACCTTCGACGGCGTGG CCGACTACCTGCAGACATACCCACAAGCTGTGCCTGAGCTACGAGACAGAGATCCTGAC- CGTG- GAATACGGCCTGCTGCCTATCGGAAGATCGTGGAAAAGCGGATCGAGTGCACCGTGA CAGCGTGGACAACAACGGCACACATCTACACCCCAGCCTGTGGCTCAGTGGCAC- GACAGAGGCAAGCAGAAGGTGTTGAGTACTGCCTGGAAGATGGCAGCCTGATCAGAG CCACCAAGGACCACAAGTTCATGACAGTGGACGCCAGATGCTGCCCATCAAAGA- GATCTCCGGCGGAAGCTGGACCTGATGAGAGTGGACAAACCTGCCTAATGGCTCTGGCG GCGAGAACCTGTACTTCAAGGGAAAATCTACTTCAAGGCGGCAGCGGCGG- CATCGA- GATGCCACAGAGAAGTATCTGGCGAGCAGAACGTGTACGACATCGCGTGGAACGG GACCACAACCTCGCCCTGAAGAACGGCGCTACTTCCAGGGATTGAGATTGCCAC- CGA- GAAATACCTCGGGAACAGAACAGAACATGTATGATATCGGAGTCGAGCGCGATACAATTG CCCTGAAAAATGGCGGATCC CAGGTCCAGCTGCAAGAGTCTGGCGAGGACTT- GTTCAGGCTGGCGGAAGCCTGAGACTGAGCTGTACTGCCAGCGCAGAACCTTCAGCAG CTATGCCATGGCTGGTTCAGACAGACAGACCCCTGGCAAAGAACCGCAG- TCGTGGCGCCATTACATGGGGCGGAAGCACAAACACTGTACGCCGACTCTGTGAAGGG CAGATTACCATGAGCCGGACAACGCCAAGAACACCGTGTACCTG- CAGATGAACAGCCTGAAGCCAGAGGACACCGCCGTACTATTGTGCCGCCATGGCAG CCAGTACAGAACCTACAGCTCAGAGACAAGCCGACTACGGCTCTT- GGGGCCAGGGAACACAAGTGACCGTGTCTCACCACCACCATACCAT TGA ATCTAG ACCTTCTGGGGCTTGCCTCTGCCATGCCCTCTCTCCCTTGACACTGTAC- CTCTT- GGTCTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAA AAAAAAAA ID hBarnase-21C
Template pDNA	pUTR2-hBarnase-21C
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, Anti-eDHFR nanobody CA1698 gene: 91-459, Caged eNpu C-intein gene: 466-792, C-terminal split Barnase gene: 793-1065, Stop codon: 1066-1068

Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAGAGAGAAAAGAAGAGTAA-GAA- GAAATATAAGACACCGGTcGCCACCATGGCCCAGGTCCAGCTGCAAGAGTCTGGCGGA GGACTTGTTCAGGCTGGCGGAAGCCTGAGACTGAGCTGTAAAGCCAGCGG-C CATCATCTTCAGCGTGTACAAGATGACCTGGTACAGACAGGCCCCGGCAAAGAGAGAG AGCTGGTTGCCCTGATCACCACCAACAATACCATGACCGTGGACAGCGTGAAGGG- CAGATTCAACCATCAGCCGGACAACGTGCAGAACACCGTGTACCTGGAAATGAACAAT CTGAAGCCCCGAGGGACACCGCCGTGTACTACTGCAACGCCAATAGAGGACTGGCCGGAC- CTGCCTATTGGGCCAGGGAACACAAGTGACCGTGTCTCACCAACACCACATACCAT GGATCCGGCGAACAGAAGTGTGAGTACTGCCTGGAAGATGG- CAGCCTGATCAGAGCCAC- CAAGGACCACAAGTTCATGACCGTGGACGGCCAGATGCTGCCATCGACGAGATCTCG AGCGCGAGCTGGACCTGATGAGAGTGAGACAACCTGCCATAATGGCTCTGGCGGCA- GAACCTG- TACTTCCAAGGGAAAATCTCTACTTCAAGGCGGCAGCGCGGCATCAAGATGCCAC AAGAAAGTACCTGGCAAGCAGAACGTGTACGACATCGCGTGGAACCGGAC- CACAACCTGCCCTGAAGAACGGCTTATGCCAGCAACTGCCCGACAACATACATCAC CAAGAGCGAGGCCAGGCTCTGGATGGTGCCTCTAAGGGAAAC- CTGGCCGATGTGGCCCTGGCAAGTCTATCGCGGGACATCTCAGCAACAGAGAGGG CAAGCTGCCCTGGCAAGAGCGGCAGAACCTGGAGAGAGGCCGACATCAACTACAC- CAGCGGCTCCGGAACAGCGACCGGATCCTGTACAGCAGCGACTGGCTGATCTACAAGA CCACCGACCACCTACCAAGACCTCACCAAGATCAGATGAATCTAGAC- CTTCTGCGGGGCTT- GCCTTCTGGCCATGCCCTCTCTCTCCCTGCACCTGTACCTCTGGTCTTGAATAAAGC CTGAGTAG- GAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAA
ID	hBarnase-36N
Template pDNA	pUTR2-hBarnase-36N
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, N-terminal split Barnase gene: 88-192, Caged eNpu N-intein gene: 193-759, Anti-eDHFR nanobody Nb113 gene: 766-1167, Stop codon: 1168-1170
Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAGAGAGAAAAGAAGAGTAA-GAA- GAAATATAAGACACCGGTcGCCACCATGGCCCAGGTGATCAACACCTCGACGGCGTGG CCGACTACCTGCAGACATACCCACAAGCTGCCGACAACATCACCAA- GAGCGAGGCCAGGCTCTGGATGGTGCCTGAGCTACGAGACAGAGATCCTGACCGTGG AATACGGCCTGCTGCCTATCGGCAAGATCGTGGAAAAGCGGATCGAGTCACCGTG- TACAGCGTGGACAACACGGCAACATCTACACCCAGCCTGTGGTCAGTGGCACGACA

	GAGGCAAGCAGAAGGTGTTCGAGTACTGCCCTGGAAGATGGCAGCCTGATCAGAGCCAC- CAAGGACCACAAGTTCATGACAGTGGACGGCCAGATGCTGCCATCAAAGAGATCTTCC GGCGGAAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGGCGA- GAACCTG- TACTTCCAAGGGAAAATCTCTACTTCAGGCGGAGCAGAACGAGTACGACATCGCGTGGAACGGAC- CACAACTTCGCCCTGAAGAACGGGGCTACTTCAGGAATTGAGATTGCCACCGAGAA ATACCTCGGGGAAACAGAATGTGTATGATATCGGAGTCGAGCGCGATCACAATTT- GCCCTGAAAAATGGCGGATCC CAGGTCCAGCTGCAAGAGTCTGGCGAGGACTTGTCA GGCTGGCGGAAGCCTGAGACTGAGCTGTACTGCCAGCGCAGAACCTTCAGCAGC- TATGCCATGGCTGGTCAGACAGACAGCCCCTGGCAAAGAACCGAGTTCTGGCCGCCAT TACATGGGGCGGAAGCACAACACTGTACGCCGACTCTGTGAAGGGCAGATTCAC- CATGAGCCGGACAACCCAAGAACACCCGTGTACCTGCAGATGAACAGCCTGAAGCCA GAGGACACCGCCGTGTACTATTGTGCCGCCATGGCAGCCAGTACAGAACGCAC- CTACAGCTTCAGAGACAAGCCGACTACGGCTCTGGGCCAGGAACACAAGTGACC GTGTCTCTCACCAACCACCATCACCAT TGA ATCTAGACCTCTGCCGGGCTT- GCCTCTGCCATGCCCTCTCTCCCTGCACCTGTACCTCTGGTCTTGAATAAACG CTGAGTAG- GAA AAAAAA AAAAAA AAAAAA AAAAAA AAAAAA
ID	hBarnase-36C
Template pDNA	pUTR2-hBarnase-36C
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, Anti-eDHFR nanobody CA1698 gene: 91-459, Caged eNpu C-intein gene: 466-792, C-terminal split Barnase gene: 793-1020, Stop codon: 1021-1023
Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAAGAGAGAAAAGAACAGTAA- GAA- GAAATATAAGACACCGGTC GCCACCATGGCCCAGGTCCAGCTGCAAGAGTCTGGCGGA GGACTTGTTCAGGCTGGCGGAAGCTGAGACTGAGCTGTAAAGCCAGCGG- CATCATCTTCAGCGTGTACAAGATGACCTGGTACAGACAGGCCCCTGGCAAAGAGAGAG AGCTGGITGCCCTGATCACCACCAACAACAATACCATGACCGTGGACAGCGTGAAGGG- CAGATTCACCATCAGCCGGACAACGTGCAGAACACCCGTGTACCTGGAAATGAACAAT CTGAAGCCCGAGGGACACCGCCGTGTACTACTGCAACGCCAATAGAGGACTGCCGGAC- CTGCCTATTGGGCCAGGGAACACAAGTGACCGTGTCTCACCACCACCATCACCAC GGATCCGGCGAACAGAACAGTGTGAGTACTGCCCTGGAAGATGG- CAGCCTGATCAGAGCCAC- CAAGGACCACAAGTTCATGACCGTGGACGGCCAGATGCTGCCATCGACGAGATCTTCG

	<p>AGCGCGAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGGCGA-GAACCTG-</p> <p>TACTTCCAAGGGAAAATCTCTACTTCAAGGCCAGCGCGGCATCAAGATGCCAC</p> <p>AAGAAAGTACCTGGCAAGCAGAACGTGTACGACATCGCGTGGAACGGAC-</p> <p>CACAACCTCGCCCTGAAGAACGGCTTATGCCAGCAACTGCGTTGCCCTAAGGGAAA</p> <p>CCTGGCCGATGTGGCCCTGGCAAGTCTATGCCAGCAACTGCGTTGCCCTAAGGGAAA</p> <p>GAGG-</p> <p>CAAGCTGCCTGGCAAGAGCGGCAGAACCTGGAGAGAGGCCGACATCAACTACACCAGC</p> <p>GGCTTCCGGAACAGCGACCGGATCCTGTACAGCAGCGACTGGCTGATCTACAAGAC-</p> <p>CAC-</p> <p>CGACCACTACCAGACCTCACCAAGATCAGATGAATCTAGACCTCTGCCGGGCTGCC</p> <p>TCTGGCCATGCCCTCTTCTCTCCCTGCACCTGTACCTCTGGCTTTGAATAAA-</p> <p>GCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA</p> <p>AA</p> <p>AAAAAAAAAAAAAAAAAAAAAA</p>
ID	hBarnase-65N
Template pDNA	pUTR2-hBarnase-65N
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, N-terminal split Barnase gene: 88-279, Caged eNpu N-intein gene: 280-846, Anti-eDHFR nanobody Nb113 gene: 853-1254, Stop codon: 1255-1257
Sequence	<p>CACTGAATTGTAATACCACTACTATAAGGCGAATTAAAGAGAGAAAAGAAGACTAA-GAA-</p> <p>GAAATATAAGACACCGGTCCCACCATGCCCAAGTGTACACACCTTCGACGGCGTGG</p> <p>CCGACTACCTGCAGACATACCAACAGCTGCCGACAACACTACATCACCAA-</p> <p>GAGCGAGGCCAGGCTCTGGATGGTTGCCTAAGGGAAACCTGCCGATGTGGCCC</p> <p>CTGGCAAGTCTATCGCGGCGACATCTCAGCAACAGAGAGGGCAAGCTGCCCTT-GCCTGAGC-</p> <p>TACGAGACAGAGATCCTGACCGTGGAAATACGGCCTGCTGCCATCGGCAAGATCGTGGAAAGCGGATCGACTGCACCGTGTACAGCGTGGACAACAAACGG-</p> <p>CAACATCTACACCCAGCCTGTGGCTAGTGGCACGACAGAGGGCAAGCAGAGGTGTTCG</p> <p>AGTACTGCCCTGGAAGATGGCAGCCTGATCAGAGGCCACCAAGGAC-CACAAGTTCATGACAGTG-</p> <p>GACGGCCAGATGCTGCCATCAAAGAGATCTCCGGCGGAAGCTGGACCTGATGAGAGTGGACAACCTGCCATGGCTCTGGCGGCGAGAACCTGTACTTCAAGGGAAAATCTCTACTTCAAGGCCAGCGCGGATCGAGATGCCACAGAGAGAAGTATCTGGCGAGCAGAACCGTGTACGACATCGCGTGGAACGGGACCAACTCGCCCTGAAGAACGGCGC-</p> <p>TACTTCCAGGAAATTGAGATTGCCACCGAGAAATACCTCGGGAACAGAACAGAATGTGTATGA</p>

	TATCGGAGTCGAGCGCGATCACAATTGCCCTGAAAAATGGCG-GATCC CAGGTCCAGCTG- CAAGAGTCTGGCGGAGGACTTGTTCAGGCTGGCGAAGCCTGAGACTGAGCTGTACTGC CAGCGGCAGAACCTTCAGCAGCTATGCCATGGGCTGGTCAGACAGACCCCTGGCAAAGAAC- GCGAGTTCTGGCCGCCATTACATGGGGCGGAAGCACAACACTGTACGCCGACTCTGTG AAGGGCAGATTACCATGAGCCGGACAACGCCAAGAACACCCGTACCTG-C CAGATGAACAGCCTGAAGCCAGAGGACACCCTGACTATTGTGCCGCCATGGCAG CCAGTACAGAACGACCTACAGCTTCAGAGACAAGCCCCTACGGCTCTT- GGGCCAGGGAACACAAGTGACCGTGTCTCACCACCACCATACCAT TGA ATCTAG ACCTTCTGCAGGGCTTGCCTCTGCCATGCCCTCTCCCTGCACCTGTAC- CTCTT- GGTCTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAA AA AAAAAAAAAAAAAAAA ID hBarnase-65C Template pUTR2-hBarnase-65C Primers HNC-542, HNT-7 and HNT-9 Features T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, Anti-eDHFR nanobody CA1698 gene: 91-459, Caged eNpu C-intein gene: 466-792, C-terminal split Barnase gene: 793-933, Stop codon: 934-936 Sequence CACTGAATTG TAATAACGACTCACTATAAGG CGAATTAAGAGAGAAAAGAAGAGTAA- GAA- GAAATATAAGACACCGGTG GCCACCATGGCCCAGGTCCAGCTGCAAGAGTCTGGCGGA GGACTTGTTCAGGCTGGCGGAAGCCTGAGACTGAGCTGTAAAGCCAGCGG- CATCATCTTCAGCGTGTACAAGATGACCTGGTACAGACAGGCCCCGGCAAAGAGAGAG AGCTGGTTGCCCTGATCACCAACAAACAATACCATGACCGTGGACAGCGTGAAGGG- CAGATTCACCATCAGCCGGACAACGTGCAGAACACCGTGTACCTGGAAATGAACAAT CTGAAGCCCGAGGACACCGCCGTGTACTACTGCAACGCCAATAGAGGACTGCCGGAC- CTGCCTATTGGGCCAGGGAACACAAGTGACCGTGTCTCACCACCACCATACCAT GGATCCGGCGAACAAAGAAGTGTGAGTACTGCCCTGGAAGATGG- CAGCCTGATCAGAGCCAC- CAAGGACCACAAGTTCATGACCGTGGACGCCAGATGCTGCCATCGACGAGATCTCG AGCGCGAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGCGA- GAACCTG- TAATTCCAAGGGAAAATCTCTACTTCAAGGCCAGCGCGGCATCAAGATGCCAC AAGAAAGTACCTGGCAAGCAGAACGTGTACGACATCGCGTGGAACGGGAC- CACAACTCGCCCTGAAGAACGGCTTATGCCAGCAACTGCCAGAACAGCGGCAGAA CTTGGAGAGAGGCCGACATCAACTACACCAGCGGCTCCGGAACAGCGACCG- GATCCTG- TACAGCAGCGACTGGCTGATCTACAAGACCACCGACCACTACCAGACCTCACCAAGAT
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	CAGATGA ATCTAGACCTTCTGCGGGGCTTGCCTCTGCCATGCCCTCTCTCCCTT-GCACCTGTACCTCTGGCTTGAAATAAAGCCTGAGTAGGAAAAAAA AAA AAAAAAAAAAAAAAAAAAAAA
ID	hBarnase-81N
Template pDNA	pUTR2-hBarnase-81N
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, N-terminal split Barnase gene: 88-327, Caged eNpu N-intein gene: 328-894, Anti-eDHFR nanobody Nb113 gene: 901-1302, Stop codon: 1303-1305
Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAAGAGAGAAAAGAAGAGTAA-GAA- GAAATATAAGACACCGGTC GCCACCATG GCCCAAGT GATCAACACCTTCGACGGCGTGG CCGACTACCTGCAGACATAC CCACAAGCTGCCGACAACACTACATCACCA-GAGCGAGGCCAGGCTCTGGATGGGTTGCCTCTAAGGGAAACCTGCCGATGTGCC CTGGCAAGTCTATCGGCGCGACATCTTCAGCAACAGAGAGGGCAAGCTGCCGGCAA-GAGCGGCAGAA CTTGAGAGAGGCCGACATCAACTACACCAGCTGCCTGAGCTACGAG ACAGAGATCCTGACCGTGAATACGGCCTGCTGCCTATCGCAAGATCGTGGAAAAA-GCG- GATCGAGTGCACCGTGTACAGCGTGGACAACAACGGCAACATCTACACCCAGCCTGTGG CTCAGTGGCACGACAGAGGCAAGCAGAAGGTGTTGAGTACTGCCCTGGAAGATGG-CAGCCTGATCAGAGCCACCAAGGACCACAAGTTCATGACACTGGACGCCAGATGCTG CCCCTCAAAGAGATCTCCGGCGAACCTGTACTTCAAGGGAAAATCTACTTTCAA GGCGGCAGCGCCGCATCGAGATGCCACAGAGAAGTATCTGGCGAGCAGAACGTG-TAC- GACATCGGCGTGAACGGGACCACAACCTCGCCCTGAAGAACGGCGCTACTTCCAGG GAATTGAGATTGCCACCGAGAAATACCTGGGGAAACAGAAATGTGTATGATATCGGAG-TCGAGCGCGATCACAATTGCCCTGAAAAATGGCGGATCC CAGGTCCAGCTGCAAGAG TCTGGCGAGGACTTGTTCAGGCTGGCGGAAGCCTGAGACTGAGCTGTACTGCCAGCGG -CAGAACCTTCAGCAGCTATGCCATGGGCTGGTTAGACAGAGACCCCTGGCAAAGAACCG AGTTCGTGGCCGCCATTACATGGGGCGGAAGCACAACACTGTAC-GCCGACTCTGTGAAGGG- CAGATTCAACCATGAGCCGGACAACGCCAAGAACACCGTGTACCTGCAGATGAACAGC CTGAAGCCAGAGGAACCCCGTGTACTATTGTGCCGCCATGGCAGCCAGTACAGAA- GCAC- CTACAGCTTCAGAGACAAGCCGACTACGGCTCTGGGCCAGGGAACACAAGTGACC GTGTCCTCTCACCACCACTACCCAT TGA ATCTAGACCTCTGCACCTTGGTCTTGAATAAAGC CTGAGTAG- GAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAA
ID	hBarnase-81C
Template pDNA	pUTR2-hBarnase-81C
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, Anti-eDHFR nanobody CA1698 gene: 91-459, Caged eNpu C-intein gene: 466-792, C-terminal split Barnase gene: 793-885, Stop codon: 886-888
Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAAGAGAGAAAAGAAGAGTAA- GAA- GAAATATAAGACACCGGTC GCCACCATG GCCAGGTCCAGCTGCAAGAGTCTGGCGGA GGACTTGTTCAGGCTGGCGGAAGCCTGAGACTGAGCTGTAAAGCCAGCGG- CATCATCTTCAGCGTGTACAAGATGACCTGGTACAGACAGGCCCCTGGCAAAGAGAGAG AGCTGGTTGCCCTGATCACCAACAAACAATACCATGACCGTGGACAGCGTGAAGGG- CAGATTCAACCATCAGCCGGACAACGTGCAGAACACCGTGTACCTGGAAATGAACAAT CTGAAGCCCGAGGACACCGCCGTGTACTACTGCAACGCCAATAGAGGACTGCCGGAC- CTGCCTATTGGGCCAGGGAACACAAGTGACCGTGTCCCTCTCACCACCACCATCACC GGATCCGGCGAACAGAAGTGTGAGTACTGCCTGGAAGATGG- CAGCCTGATCAGAGCCAC- CAAGGACCACAAGTTCATGACCGTGGACGGCCAGATGCTGCCATCGACGAGATCTCG AGCGCGAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGCGA- GAACCTG- TACTTCCAAGGGAAAATCTCTACTTCAAGGCGGCAGCGCGGCATCAAGATGCCAC AAGAAAGTACCTGGCAAGCAGAACGTGTACGACATCGCGTGGAACAGGGAC- CACAACCTGCCCTGAAGAACGGCTTATGCCAGCAACT TGCGGCTTCCGGAACAGCGA CCGGATCCTGTACAGCAGCGACTGGCTGATCTACAAGACCAACCGACCACTACCAGAC- CTTCACCAAGATCAGAT GA ATCTAGACCTCTGCAGGGCTTGCTTGAATAAGCCTGAGTAG- GAA AAAAAA AAAAAA
ID	hBarnase-81N-Lag16
Template pDNA	pUTR2-hBarnase-81N-eNpuNcage-Lag16
Primers	HNC-542, HNT-7 and HNT-9
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Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAAGAGAGAAAAGAAGAGTAA- GAA- GAAATATAAGACACCGGTC GCCACCATG GCCCAAGTGATCAACACCTCGACGGCGTGG

	<p>CCGACTACCTGCAGACATACCAACAAGCTGCCGACAACATACATCACCAA-</p> <p>GAGCGAGGCCAGGCTCTGGATGGGTGCCTCTAAGGGAAACCTGCCGATGTGGCCC</p> <p>CTGGCAAGTCTATCGGCCGACATCTTCAGCAACAGAGAGGGCAAGCTGCCCTGGCAA-</p> <p>GAGCGGCAGAACTGGAGAGAGGCCGACATCAACTACACCAGCTGCCCTGAGCTACGAG</p> <p>ACAGAGATCCTGACCGTGAATACGGCCTGCTGCCCTATCGCAAGATCGTGGAAAAA-</p> <p>CGC-</p> <p>GATCGAGTGCACCGTGTACAGCGTGGACAACAACGGCAACATCTACACCCAGCCTGTGG</p> <p>CTCAGTGGCACGACAGAGGCAGCAGAAGGTGTTGAGTACTGCCCTGGAAAGATGG-</p> <p>CAGCCTGATCAGAGCCACCAAGGACCACAAGTTCATGACAGTGGACGGCCAGATGCTG</p> <p>CCCATCAAAGAGATCTCCGGCGAAGAACCTGTACTTCCAAGGGAAAATCTCTACTTCAA</p> <p>GGCGGCAGCGCCGCATCGAGATGCCACAGAGAAGTATCTGGCGAGCAGAACGTG-</p> <p>TAC-</p> <p>GACATCGCGTGGAACGGGACCACAACCTCGCCCTGAAGAACGGCGCTACTTCCAGG</p> <p>GAATTGAGATTGCCACCGAGAAATACCTCGGGAACAGAAATGTGTATGATATCGGAG-</p> <p>TCGAGCGCGATCACAATTTCGCCCTGAAAAATGCCGGATCCGCTCAGGTGCAGCTGGTG</p> <p>GAATCTGGTGGTAGACTGGTGCAGGCCGGCATGCCCTGAGACTGTCTT-</p> <p>GTGCCGCCAGCGG-</p> <p>CAGAACCTTACGACATGCCATGGCCTGGTTGAGACAGGGCCCTGGCAGAGAAAGGG</p> <p>AATTCTGGCCGCCATCACATGGACCGTGGCAATAC-</p> <p>CATCCTGGCGACTCTGTGAAGGG-</p> <p>CAGATTCAACATCAGCCGGACAGAGCCAAGAACACCGTGGACCTGCAGATGGACAAC</p> <p>CTGGAACCTGAGGACACCGCCGTGTACTACTGCAGCGCCAGATCTAGAGGCTAC-</p> <p>GTGCTGTCCGTGCTGAGAACGCGTGGACAGCTACGATTATTGGGCCAGGGCACCCAAAGT</p> <p>GACCGTGTCTGAATCTAGACCTCTGCCGGGCTT-</p> <p>GCCTCTGGCCATGCCCTCTCTCCCTGCACCTGTACCTCTGGTCTTGAATAAGC</p> <p>CTGAGTAG-</p> <p>GAA</p> <p>AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA</p> <p>AAAAAA</p>
ID	GFPenhNb-hBarnase-81C
Template pDNA	pUTR2-GFPenhNb-NpuCcage-hBarnase-81C
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Sequence	CAGTGAATTG TAATACGACTCACTATAAGG CGAATTAAGAGAGAAAAGAAGAGTAA- GAA- GAAATATAAGACACCGGTC GCCACCATGGCTCAGGTGCAGCTTGTGAATCTGGCGGAG

	CACTGGTTCAGCCTGGCGGATCTCTGAGACTGTCTT-GTGCCGCCTCTGGCTCCCCGTGAAC-CGGTACAGCATCGGGTGGTATAGACAGGCCCCTGGCAAAGAACGAGAGTGGGTGGCCCGAATGTCTAGCGCTGGCGATAGAACAGCTACGAGGACAGCGTAAGGGCAGATTACACATCAGCAGGGACGACGCCAGAAACACCGTGTACCTGCAGATGAACAGCCTGAAGCCTGAGGACACCGCCGTGTACTACTGCAACGTGAACGTGGGCTTCGAG-TACTGGGCCAGGGAACCCAAGTGACCCTTCTGGATCCGGCGAACACAAGTTCATGAC-CGTG-GACGGCCAGATGCTGCCATCGACGAGATCTCGAGCGCGAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGGCAGAACCTGTACTTCCAAGGGAAAATCTCTACTTCAAGGCGGCAGCGGCCATCAAGATGCCACAAGAAAGTACCTGGGCAAGCAGAACCGTGTACGACATCGGCGTGAACGGGACCACAAACTTCGCCCTGAAGAAC-GGCTTATGCCAGCAACTGCGGCTCCGGAACAGCGACCGGATCCTGTACAGCAGCGACTGGCTGATCTACAAGACCAACGACCACTACCAGACCTCACCAA-GATCAGATGAATCTA-GACCTTCTGCGGGGCTTGCCTCTGGCATGCCCTCTCTCCCTGCACCTGTACCTCTTGGCTTGAAATAAGCCTGAGTAG-GAA
ID	hBarstar
Template pDNA	pUTR2-hBarstar
Primers	HNC-542, HNT-7 and HNT-9
Features	T7 promoter (for CleanCap AG Reagent): 11-30, Kozak sequence (including start codon): 79-87, hBarstar Gene: 88-357, Stop Codon: 358-360
Sequence	CAGTGAATTGTAATACGACTCACTATAAGCGAATTAAGAGAGAAAAGAACAGTAA-GAA-GAAATATAAGACACCGGTCGCCACCATGCCAAGAAAGCCGTGATCACGGCGAGCAGATCCGGTCCATCAGCGACCTGCACCAAGACACTGAAGAAAGAGCTGGCCCTGCCTGAG-TACTACGGCGAGAATCTGGATGCCCTGTGGATTGCTGACCGGCTGGTCGAATACCCCTGGTTCTGGAATGGCGGCAGTTCGAGCAGAGCAAGCAGCTGACAGA-GAATGGCGCCGA-GAGCGTGCCTCAGGTGTTCAGAGAACCCAAGGCCAGGGCTGCGACATCACCATCATCTGAGCTGAATCTAGACCTCTGGGGCTTGCCTCTGCCATGCCCTCTCTCCCTTGCACCTGTACCTCTGGTCTTGAATAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAA