

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

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

[illegible]

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	<p>CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAA-GAA-</p> <p>GAAATATAAGACACCGGTCGCCACCATGGCCCAAGTGATCAACACCTTCGACGGCGTGCCGACTACCTGCAGACATAACCACAAGCTGCCCCGACAACTACATCACCAA-GAGCGAGGCCCAGGCTCTCGGATGGGTTCCTCTAAGGGAAACCTGGCCGATGTGGCCCCTGGCAAGTCTATCGGCGGCGACATCTTCAGCAACAGAGAGGGCAAGCTGCCTGGCAA-GAGCGGCAGAACTTGGAGAGAGGCCGACATCAACTACACCAGCGGCTTCCGGAACAGCGACCGGATCCTGTACAGCAGCGACTGGCTGATCTACAAGACCACCGACCACTAC-CAGAC-</p> <p>CTTCACCAAGATCAGATGAATCTAGACCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAG-GAAA</p> <p>AAA</p> <p>AAAAAAAAAAAA</p>
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	<p>CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAA-GAA-</p> <p>GAAATATAAGACACCGGTCGCCACCATGGCCCAAGTGATCAACACCTTCGACGGCGTGGCCGACTACCTGCAGACATAACCACAAGCTGTGCCTGAGCTACGAGACAGAGATCCTGACCGTG-</p> <p>GAATACGGCCTGCTGCCTATCGGCAAGATCGTGGAAAAGCGGATCGAGTGCACCGTGTA CAGCGTGGACAACAACGGCAACATCTACACCCAGCCTGTGGCTCAGTGGCAC-</p> <p>GACAGAGGCAAGCAGAAGGTGTTTCGAGTACTGCCTGGAAGATGGCAGCCTGATCAGAG CCACCAAGGACCACAAGTTCATGACAGTGGACGGCCAGATGCTGCCCATCAAAGA-</p> <p>GATCTTCCGGCGGAAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCG GCGAGAACCTGTACTTCCAAGGGGAAAATCTCTACTTTCAAGGCGGCAGCGGCGG-</p> <p>CATCGA-</p> <p>GATCGCCACAGAGAAGTATCTGGGCGAGCAGAACGTGTACGACATCGGCGTGGAACGG GACCACAACCTTCGCCCTGAAGAACGGCGGCTACTTCCAGGGAATTGAGATTGCCAC-</p> <p>CGA-</p> <p>GAAATACCTCGGGGAACAGAATGTGTATGATATCGGAGTCGAGCGCGATCACAATTTTG CCCTGAAAAATGGCGGATCCAGGTCCAGCTGCAAGAGTCTGGCGGAGGACTT-</p> <p>GTTTCAGGCTGGCGGAAGCCTGAGACTGAGCTGTACTGCCAGCGGCAGAACCTTCAGCAG CTATGCCATGGGCTGGTTCAGACAGACCCCTGGCAAAGAACGCGAG-</p> <p>TTCGTGGCCGCCATTACATGGGGCGGAAGCACAACACTGTACGCCGACTCTGTGAAGGG CAGATTCACCATGAGCCGGGACAACGCCAAGAACACCGTGTACCTG-</p> <p>CAGATGAACAGCCTGAAGCCAGAGGACACCGCCGTGTACTATTGTGCCGCCGATGGCAG CCAGTACAGAAGCACCTACAGCTTCAGAGACAAGCCCGACTACGGCTCTT-</p> <p>GGGGCCAGGGAACACAAGTGACCGTGTCTCTCACCACCACCATCACCATTGAATCTAG ACCTTCTGCGGGGCTTGCCCTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTAC-</p> <p>CTCTT-</p> <p>GGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AA AA</p>
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	CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAAGAAGAGTAA-GAA- GAAATATAAGACACCGGTCGCCACCATGGCCCAGGTCCAGCTGCAAGAGTCTGGCGGAGGACTTGTTCAAGGCTGGCGGAAGCCTGAGACTGAGCTGTAAAGCCAGCGG-CATCATCTTCAGCGTGTACAAGATGACCTGGTACAGACAGGCCCTGGCAAAGAGAGAGAGCTGGTTGCCCTGATCACCACCAACAACAATACCATGACCGTGGACAGCGTGAAGGG-CAGATTCAACCATCAGCCGGGACAACGTGCAGAACACCGTGTACCTGGAAATGAACAATCTGAAGCCCCGAGGACACCGCCGTGTACTACTGCAACGCCAATAGAGGACTGGCCGGAC-CTGCCTATTGGGGCCAGGGAACACAAGTGACCGTGTCTCTCACCACCACCATCACCATGGATCCGGCGAACAAAGAAGTGTTTCGAGTACTGCCTGGAAGATGG-CAGCCTGATCAGAGCCAC-CAAGGACCACAAGTTCATGACCGTGGACGGCCAGATGCTGCCCATCGACGAGATCTTCGAGCGCGAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGGCGA-GAACCTG-TACTTCCAAGGGGAAAATCTCTACTTTCAAGGCGGCAGCGGCGGCATCAAGATCGCCACAAGAAAGTACCTGGGCAAGCAGAACGTGTACGACATCGGCGTGGAACGGGAC-CACAACTTCGCCCTGAAGAACGGCTTTATCGCCAGCAACTGCCCCGACAACTACATCACCAAGAGCGAGGCCAGGCTCTCGGATGGGTTGCCTCTAAGGGAAAC-CTGGCCGATGTGGCCCCTGGCAAGTCTATCGGCGGCGACATCTTCAGCAACAGAGAGGGCAAGCTGCCTGGCAAGAGCGGCAGAACTTGGAGAGAGGCCGACATCAACTACAC-CAGCGGCTTCCGGAACAGCGACCGGATCCTGTACAGCAGCGACTGGCTGATCTACAAGACCACCGACCACTACCAGACCTTCACCAAGATCAGATGAATCTAGAC-CTTCTGCGGGGCTT-GCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAG-GAAA AAA 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	CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAAGAAGAGTAA-GAA- GAAATATAAGACACCGGTCGCCACCATGGCCCAAGTGATCAACACCTTCGACGGCGTGGCCGACTACCTGCAGACATAACCACAAGCTGCCCCGACAACTACATCACCAA-GAGCGAGGCCAGGCTCTCGGATGGTGCCTGAGCTACGAGACAGAGATCCTGACCGTGG AATACGGCCTGCTGCCTATCGGCAAGATCGTGGAAGAGCGGATCGAGTGCACCGTG-TACAGCGTGGACAACAACGGCAACATCTACACCCAGCCTGTGGCTCAGTGGCACGACA

	<p>GAGGCAAGCAGAAGGTGTTTCGAGTACTGCCTGGAAGATGGCAGCCTGATCAGAGCCAC- CAAGGACCACAAGTTCATGACAGTGGACGGCCAGATGCTGCCCATCAAAGAGATCTTCC GGCGGAAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGGCGA- GAACCTG- TACTTCCAAGGGGAAAATCTCTACTTTCAAGGCGGCAGCGGCGGCATCGAGATCGCCAC AGAGAAGTATCTGGGCGAGCAGAACGTGTACGACATCGGCGTGGAACGGGAC- CACAACCTCGCCCTGAAGAACGGCGGCTACTTCCAGGGAATTGAGATTGCCACCGAGAA ATACCTCGGGGAACAGAATGTGTATGATATCGGAGTCGAGCGCGATCACAATTTT- GCCCTGAAAAATGGCGGATCCCAGGTCCAGCTGCAAGAGTCTGGCGGAGGACTTGTTC GGCTGGCGGAAGCCTGAGACTGAGCTGTACTGCCAGCGGCAGAACCTTCAGCAGC- TATGCCATGGGCTGGTTCAGACAGACCCCTGGCAAAGAACGCGAGTTCGTGGCCGCCAT TACATGGGGCGGAAGCACAACACTGTACGCCGACTCTGTGAAGGGCAGATTAC- CATGAGCCGGGACAACGCCAAGAACACCGTGTACCTGCAGATGAACAGCCTGAAGCCA GAGGACACCGCCGTGTACTATTGTGCCGCCGATGGCAGCCAGTACAGAAGCAC- CTACAGCTTCAGAGACAAGCCCGACTACGGCTCTTGGGGCCAGGGAACACAAGTGACC GTGTCCTCTCACCACCACCATCACCATTGAATCTAGACCTTCTGCGGGGCTT- GCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGC CTGAGTAG- GAA AAA AAAAAAAAAAAA</p>
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	<p>CAGTGAATTGTAATACGACTCACTATAAGCGCAATTAAGAGAGAAAAGAAGAGTAA- GAA- GAAATATAAGACACCGGTCGCCACCATGGCCCAGGTCCAGCTGCAAGAGTCTGGCGGA GGACTTGTTCAAGGCTGGCGGAAGCCTGAGACTGAGCTGTAAAGCCAGCGG- CATCATCTTCAGCGTGTACAAGATGACCTGGTACAGACAGGCCCTGGCAAAGAGAGAG AGCTGGTTGCCCTGATCACCACCAACAACAATACCATGACCGTGGACAGCGTGAAGGG- CAGATTCACCATCAGCCGGGACAACGTGCAGAACACCGTGTACCTGGAAATGAACAAT CTGAAGCCCGAGGACACCGCCGTGTACTACTGCAACGCCAATAGAGGACTGGCCGGAC- CTGCCTATTGGGGCCAGGGAACACAAGTGACCGTGTCTCTCACCACCACCATCACCAT GGATCCGGCGAACAAGAAGTGTTCGAGTACTGCCTGGAAGATGG- CAGCCTGATCAGAGCCAC- CAAGGACCACAAGTTCATGACCGTGGACGGCCAGATGCTGCCCATCGACGAGATCTTCG</p>

	<p>AGCGCGAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGGCGCA-GAACCTG-</p> <p>TACTTCCAAGGGGAAAATCTCTACTTTCAAGGCGGCAGCGGCGGCATCAAGATCGCCAC</p> <p>AAGAAAGTACCTGGGCAAGCAGAACGTGTACGACATCGGCGTGGAACGGGAC-</p> <p>CACAACTTCGCCCTGAAGAACGGCTTTATCGCCAGCAACTGCGTTCCTCTAAGGGAAA</p> <p>CCTGGCCGATGTGGCCCCTGGCAAGTCTATCGGCGGCGACATCTTCAGCAACAGA-GAGGG-</p> <p>CAAGCTGCCTGGCAAGAGCGGCAGAACTTGGAGAGAGGCCGACATCAACTACACCAGC</p> <p>GGCTTCCGGAACAGCGACCGGATCCTGTACAGCAGCGACTGGCTGATCTACAAGAC-CAC-</p> <p>CGACCACTACCAGACCTTCACCAAGATCAGATGAATCTAGACCTTCTGCGGGGCTTGCCT</p> <p>TCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAA-</p> <p>GCCTGAGTAGGAAA</p> <p>AAA</p> <p>AAAAAAAAAAAAAAAAAAAAAAAAA</p>
ID	hBarnase-65N
Template pDNA	pUTR2-hBarnase-65N
Primers	HNC-542, HNT-7 and HNT-9
Features	<p>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</p>
Sequence	<p>CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAA-GAA-</p> <p>GAAATATAAGACACCGGTCGCCACCATGGCCCAAGTGATCAACACCTTCGACGGCGTGG</p> <p>CCGACTACCTGCAGACATAACCACAAGCTGCCCCGACAACCTACATCACCAA-</p> <p>GAGCGAGGCCCAGGCTCTCGGATGGGTTGCCTCTAAGGGAAACCTGGCCGATGTGGCCC</p> <p>CTGGCAAGTCTATCGGCGGCGACATCTTCAGCAACAGAGAGGGCAAGCTGCCTT-GCCTGAGC-</p> <p>TACGAGACAGAGATCCTGACCGTGGAATACGGCCTGCTGCCTATCGGCAAGATCGTGGA</p> <p>AAAGCGGATCGAGTGCACCGTGTACAGCGTGGACAACAACGG-</p> <p>CAACATCTACACCCAGCCTGTGGCTCAGTGGCACGACAGAGGCAAGCAGAAGGTGTTCC</p> <p>AGTACTGCCTGGAAGATGGCAGCCTGATCAGAGCCACCAAGGAC-</p> <p>CACAAGTTCATGACAGTG-</p> <p>GACGGCCAGATGCTGCCCATCAAAGAGATCTTCCGGCGGAAGCTGGACCTGATGAGAGT</p> <p>GGACAACCTGCCTAATGGCTCTGGCGGCGAGAACCTGTACTTCCAAGGGGAAAATCTC-</p> <p>TACTTTCAAGGCGGCAGCGGCGGCATCGAGATCGCCACAGAGAAGTATCTGGGCGAGC</p> <p>AGAACGTGTACGACATCGGCGTGGAACGGGACCACAACCTTCGCCCTGAAGAAC-GGCGGC-</p> <p>TACTTCCAGGGAATTGAGATTGCCACCGAGAAATACCTCGGGGAACAGAATGTGTATGA</p>

	<p>TATCGGAGTCGAGCGCGATCACAATTTTGCCTGAAAAATGGCG- GATCCCAGGTCCAGCTG- CAAGAGTCTGGCGGAGGACTTGTTCAAGGCTGGCGGAAGCCTGAGACTGAGCTGTACTGC CAGCGGCAGAACCTTCAGCAGCTATGCCATGGGCTGGTTCAGACAGACCCCTGGCAAA- GAAC- GCGAGTTTCGTGGCCGCCATTACATGGGGCGGAAGCACAACACTGTACGCCGACTCTGTG AAGGGCAGATTACCATGAGCCGGGACAACGCCAAGAACACCGTGTACCTG- CAGATGAACAGCCTGAAGCCAGAGGACACCGCCGTGTACTATTGTGCCGCCGATGGCAG CCAGTACAGAAGCACCTACAGCTTCAGAGACAAGCCCGACTACGGCTCTT- GGGGCCAGGGAACACAAGTGACCGTGTCTCTCACCACCACCATCACCATTGAATCTAG ACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTAC- CTCTT- GGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AA AA</p>
ID	hBarnase-65C
Template pDNA	pUTR2-hBarnase-65C
Primers	HNC-542, HNT-7 and HNT-9
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Sequence	<p>CAGTGAATTGTAATACGACTCACTATAAGCGCAATTAAGAGAGAAAAGAAGAGTAA- GAA- GAAATATAAGACACCGGTCGCCACCATGGCCCAGGTCCAGCTGCAAGAGTCTGGCGGA GGACTTGTTCAAGGCTGGCGGAAGCCTGAGACTGAGCTGTAAAGCCAGCGG- CATCATCTTCAGCGTGTACAAGATGACCTGGTACAGACAGGCCCTGGCAAAGAGAGAG AGCTGGTTGCCCTGATCACCAACAACAATACCATGACCGTGGACAGCGTGAAGGG- CAGATTCACCATCAGCCGGGACAACGTGCAGAACACCGTGTACCTGGAAATGAACAAT CTGAAGCCCGAGGACACCGCCGTGTACTACTGCAACGCCAATAGAGGACTGGCCGGAC- CTGCCTATTGGGGCCAGGGAACACAAGTGACCGTGTCTCTCACCACCACCATCACCAT GGATCCGGCGAACAAGAAGTGTTTCGAGTACTGCCTGGAAGATGG- CAGCCTGATCAGAGCCAC- CAAGGACCACAAGTTCATGACCGTGGACGGCCAGATGCTGCCCATCGACGAGATCTTCG AGCGCGAGCTGGACCTGATGAGAGTGGACAACCTGCCTAATGGCTCTGGCGGCGA- GAACCTG- TACTTCCAAGGGGAAAATCTCTACTTTCAAGGCGGCAGCGGCGGCATCAAGATCGCCAC AAGAAAGTACCTGGGCAAGCAGAACGTGTACGACATCGGCGTGGAACGGGAC- CACAACCTTCGCCCTGAAGAACGGCTTTATCGCCAGCAACTGCGGCAAGAGCGGCAGAA CTTGAGAGAGGCCGACATCAACTACACCAGCGGCTTCCGGAACAGCGACCG- GATCCTG- TACAGCAGCGACTGGCTGATCTACAAGACCACCGACCACTACCAGACCTTCACCAAGAT</p>

[illegible]

[illegible]

	<p>CCGACTACCTGCAGACATACCACAAGCTGCCCCGACAACTACATCACCAA- GAGCGAGGCCAGGCTCTCGGATGGGTTGCCTCTAAGGGAAACCTGGCCGATGTGGCCC CTGGCAAGTCTATCGGCGGCGACATCTTCAGCAACAGAGAGGGCAAGCTGCCTGGCAA- GAGCGGCAGAACTTGGAGAGAGGGCCGACATCAACTACACCAGCTGCCTGAGCTACGAG ACAGAGATCCTGACCGTGGAATACGGCCTGCTGCCTATCGGCAAGATCGTGGA AAA- GCG- GATCGAGTGCACCGTGTACAGCGTGGACAACAACGGCAACATCTACACCCAGCCTGTGG CTCAGTGGCACGACAGAGGCAAGCAGAAGGTGTTTCGAGTACTGCCTGGAAGATGG- CAGCCTGATCAGAGCCACCAAGGACCACAAGTTCATGACAGTGGACGGCCAGATGCTG CCCATCAAAGAGATCTTCCGGCGGAAGCTGGACCTGATGAGAGTGGACAAC- CTGCCTAATGGCTCTGGCGGCGAGAACCTGTACTTCCAAGGGGAAAATCTCTACTTTCAA GGCGGCAGCGGCGGCATCGAGATCGCCACAGAGAAGTATCTGGGCGAGCAGAACGTG- TAC- GACATCGGCGTGGAACGGGACCACAACCTTCGCCCTGAAGAACGGCGGCTACTTCCAGG GAATTGAGATTGCCACCGAGAAATACCTCGGGGAACAGAATGTGTATGATATCGGAG- TCGAGCGCGATCACAATTTGCCCTGAAAAATGGCGGATCCGCTCAGGTGCAGCTGGTG GAATCTGGTGGTAGACTGGTGCAGGCCGGCGATAGCCTGAGACTGTCTT- GTGCCGCCAGCGG- CAGAACCTTTAGCACATCTGCCATGGCCTGGTTCAGACAGGCCCTGGCAGAGAAAGGG AATTCGTGGCCGCCATCACATGGACCGTGGGCAATAC- CATCCTGGGCGACTCTGTGAAGGG- CAGATTCACCATCAGCCGGGACAGAGCCAAGAACACCGTGGACCTGCAGATGGACAAC CTGGAACCTGAGGACACCGCCGTGTACTACTGCAGCGCCAGATCTAGAGGCTAC- GTGCTGTCCGTGCTGAGAAGCGTGGACAGCTACGATTATTGGGGCCAGGGCACCCAAGT GACCGTGTCTTGAATCTAGACCTTCTGCGGGGCTT- GCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGC CTGAGTAG- GAA AAA AAAAAAAAAAAAA</p>
ID	GFPenhNb-hBarnase-81C
Template pDNA	pUTR2-GFPenhNb-NpuCcage-hBarnase-81C
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Sequence	<p>CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAA- GAA- GAAATATAAGACACCGGTCGCCACCATGGCTCAGGTGCAGCTTGTGTAATCTGGCGGAG</p>

