

*Appendix A*

## DNA SEQUENCES AND PLASMID MAPS

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**A.1 Bacterial Strains**

**DH5 $\alpha$ F'** Genotype: F'  $\phi$ 80*lacZ* $\Delta$ M15  $\Delta$ (*lacZYA-argF*) U169 *recA1 endA1*

*hsdR17*( $r_k^-$ ,  $m_k^+$ ) *phoA supE44*  $\lambda^-$  *thi-1 gyrA96relA1*

**BL21(DE3) pLysS** Genotype: *E. coli* B F' *dcm ompT hsdS*( $r_B^-$   $m_B^-$ ) *gal*  $\lambda$ (DE3)

[pLysS Cam<sup>r</sup>]

## A.2 pUC19-CBD

**Submitted by** Julie C. Liu **Date** 21 March 2006

**Strain name/  
(plasmid name)** DH5 $\alpha$ F'/ pUC19-6RGD  
 DH5 $\alpha$ F'/ pUC19-13RDG  
 DH5 $\alpha$ F'/ pUC19-16PHSRN  
 DH5 $\alpha$ F'/ pUC19-22NHRPS  
 DH5 $\alpha$ F'/ pUC19-3CS1'

**Vector (kb)** pUC19-6RGD  
 pUC19-13RDG  
 pUC19-16PHSRN  
 pUC19-22NHRPS  
 pUC19-3CS1'

### **Construction**

The cell-binding domain oligonucleotides were ordered from the Caltech DNA Synthesis Facility. The single-stranded strands were annealed and inserted into the pUC19 vector digested with *EcoRI/BamHI*.

### **Available Sources:**

1. 12% glycerol culture in Julie Liu's -80 °C freezer box  
 Culture conditions: 2 $\times$ YT, 200 mg/L ampicillin, 37 °C
2. Plasmid DNA in Julie Liu's -20 °C freezer box

**Gene Sequences:****RGD sequence**

Sall  
 aattogctagctaagtgcac tat gct gtc act ggc cgt gga gac agc ccc gca agc agc aag cca att gcg gtgccgctcgagactcgtg  
 gcgacgattcagctg ata cga cag tga ccg gca cct ctg tcg ggg cgt tcg tcg ttc ggt taa cgc caccggcgagctctgagcacctag  
 ▶ Y A V T G R G D S P A S S K P I A

BamI XhoI

**RDG sequence**

Sall  
 aattogctagctaagtgcac tat gct gtc act ggc cgt gac gga agc ccc gca agc agc aag cca att gcg gtgccgctcgagactcgtg  
 gcgacgattcagctg ata cga cag tga ccg gca ctg cct tcg ggg cgt tcg tcg ttc ggt taa cgc caccggcgagctctgagcacctag  
 ▶ Y A V T G R D G S P A S S K P I A

BamI XhoI

**PHSRN sequence**

Sall  
 aattogctagctaagtgcac ggg gaa gat cgg gtc ccc cac tct cgg aac tcc atc acc ctg acc aac gcg gtg ccg ctg gagactcgtg  
 gcgacgattcagctg ccc ctt cta gcc cag ggg gtg aga gcc ttg agg tag tgg gag tgg ttg cgc cac ggc gag ctctgagcacctag  
 ▶ G E D R V P H S R N S I T L T N A

BamI XhoI

**NHRPS sequence**

Sall  
 aattogctagctaagtgcac ggg gaa gat cgg gtc aac cac cgg ccc tct tcc atc acc ctg acc aac gcg gtg ccg ctg gagactcgtg  
 gcgacgattcagctg ccc ctt cta gcc cag ttg gtg gcc ggg aga agg tag tgg gag tgg ttg cgc cac ggc gag ctctgagcacctag  
 ▶ G E D R V N H R P S S I T L T N A

BamI XhoI

**CS1' sequence**

Sall  
 aattogctagctaagtgcac gac gaa ctg cct cag ctg gtt acc ttg cca cac ccg aac  
 gcgacgattcagctg ctg ctt gac gga gtc gac caa tgg aac ggt gtg ggc ttg  
 ▶ D E L P Q L V T L P H P N

BamI XhoI  
 ttg cat ggt cct gaa atc gtt gat ctg ccg agc acc ggg gtgccgctcgagactcgtg  
 aac gta cca gga ctt tag caa cta gag ggc tcg tgg ccc caccggcgagctctgagcacctag  
 ▶ L H G P E I V D L P S T G

### A.3 pEC2-CBD

**Submitted by** Julie C. Liu **Date** 21 March 2006

**Strain name/  
(plasmid name)** DH5 $\alpha$ F'/ pEC2-6RGDb  
 DH5 $\alpha$ F'/ pEC2-13RDGa  
 DH5 $\alpha$ F'/ pEC2-16PHSRNa  
 DH5 $\alpha$ F'/ pEC2-22NHRPSa  
 DH5 $\alpha$ F'/ pEC2-3CS1'b

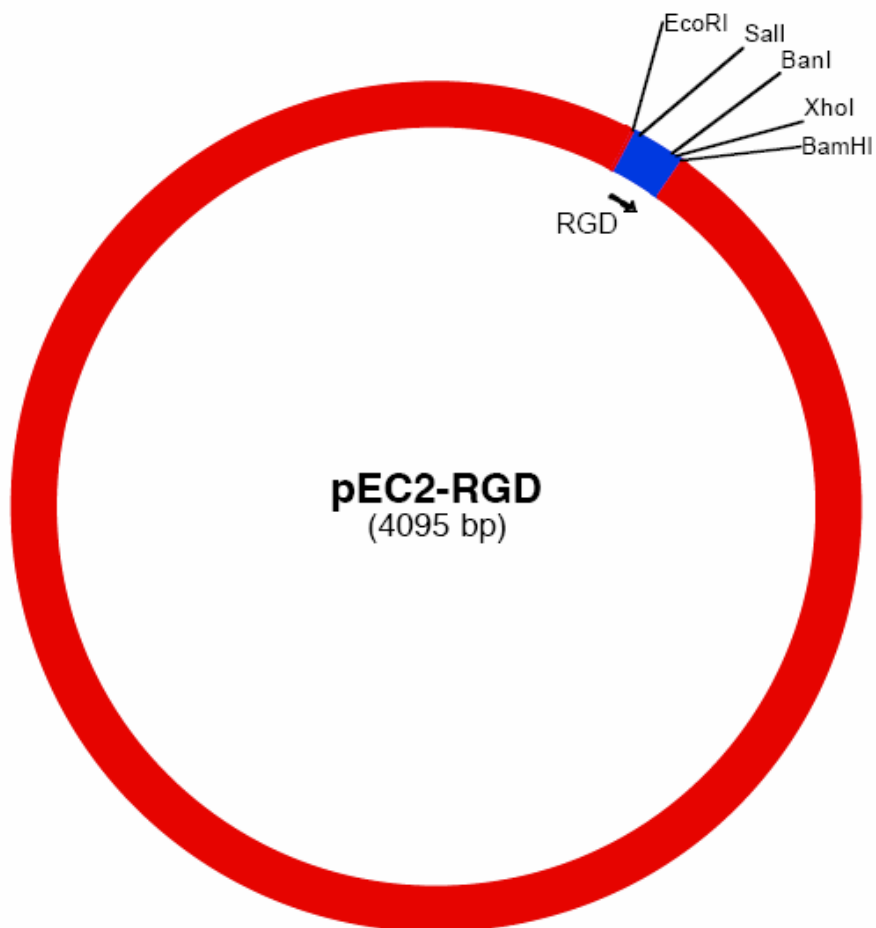
**Vector (kb)** pEC2-6RGDb  
 pEC2-13RDGa  
 pEC2-16PHSRNa  
 pEC2-22NHRPSa  
 pEC2-3CS1'b

#### **Construction**

The cell-binding domain fragments were cut out of pUC19-6RGD, pUC19-13RDG, pUC19-16PHSRN, pUC19-22NHRPS, and pUC19-3CS1' with *EcoRI/BamHI* digestion. The inserts were then ligated into the pEC2 vector (designed by Eric Cantor) digested with *EcoRI/BamHI*.

#### **Available Sources:**

1. 12% glycerol culture in Julie Liu's -80 °C freezer box  
 Culture conditions: 2 $\times$ YT, 25 mg/L kanamycin, 37 °C
2. Plasmid DNA in Julie Liu's -20 °C freezer box

**Plasmid map (pEC2-RGD)****Positions of elements**

Vector	Vector size (bp)	Insert region (bp)
pEC2-6RGDb	4095	315-404
pEC2-13RDGa	4095	315-404
pEC2-16PHSRNa	4095	315-404
pEC2-22NHRPSa	4095	315-404
pEC2-3CS1'b	4122	315-431

**Full sequence (pEC2-RGD)**

1 GTTGACGCCGGGCAAGAGCAACTCGGTCGCCGATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCA  
CAACTGCGGCCCGTTCTCGTTGAGCCAGCGGCGTATGTGATAAGAGTCTTACTGAACCACTCATGAGTGGTCAGT

77 CAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGC  
GTCTTTTCGTAGAATGCCTACCGTACTGTCACTTCTTAATACGTACGACGGTATTGGTACTCACTATTGTGACG

153 GGCCAACTTACTTCTGACAACGATCGGAGACCGAAGGAGCTAACCCTTTTTTGCACAACATGGGGGATCATGTA  
CCGGTTGAATGAAGACTGTTGCTAGCCTCCTGGCTTCTCGATTGGCGAAAAACGTGTGTACCCTTAGTACAT

229 ACTCGCCTTGATCGTTGGGAACGGAGCTGAATGAAGCATAACCAACGACGAGCGTGACACCACGATGCCTGCAG  
TGAGCGGAACTAGCAACCCTTGGCCTCGACTTACTTCGGTATGGTTTGTCTGCTCGCACTGTGGTGTACGGACGT

305 **EcoRI** **Sall**  
ATCTGATCAGaattcgctagctaagtcgac tat gct gtc act ggc cgt gga gac agc ccc gca  
TAGACTAGTCTTAAGcgcgattcagctg ata cga cag tga ccg gca cct ctg tcg ggg cgt  
1▶ Y A V T G R G D S P A

368 **BanI** **XhoI** **BamHI**  
agc agc aag cca att gcg gtgccgctcgagactcgtgGATCCATCTAGAGTCGACGTCGGCCGTTAACCT  
tcg tcg ttc ggt taa cgc cacggcgagctctgagcaacctagGTAGATCTCAGCTGCAGCCGGCAATTGGA  
12▶ S S K P I A

438 AGGAGATCTGCAGCAATGGCAACAACGTTGCGCAAATATTAAGTGGCGAACTACTTACTCTAGCTTCCCGGCAAC  
TCCTCTAGACGTCGTTACCGTTGTTGCAACGCGTTTGATAATTGACCGCTTGATGAATGAGATCGAAGGGCGGTTG

514 AATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCCTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTAT  
TTAATTATCTGACCTACCTCCGCCTATTTCAACGTCCTGGTGAAGACGCGAGCCGGGAAGGCCGACCGACCAATA

590 TGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCC  
ACGACTATTTAGACCTCGGCCACTCGCACCCAGAGCGCCATAGTAACGTCGTGACCCCGGTCTACCATTCCGGGAGG

666 CGTATCGTAGTTATCTACACGACGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTG  
GCATAGCATCAATAGATGTGCTGCCCTCAGTCCGTTGATACTACTTGTCTTATCTGTCTAGCGACTCTATCCAC

742 CGTGCCTCACTGATTAAGCATTGGTAACTGTGACACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTC  
GCACGGAGTGACTAATTCGTAACCATTGACAGTCTGGTTCAAATGAGTATATATGAAATCTAACTAAATTTTGAAG

818 ATTTTAAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAAAGTGAAGTTTC  
TAAAAATTAATTTTCTAGATCCACTTCTAGGAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAAG

894 GTTCCACTGAGCGTCAGACCCCTTAATAAGATGATCTTCTTGAGATCGTTTTGGTCTGCGCGTAATCTCTGTCTCT  
CAAGTGACTCGCAGTCTGGGGAATTATTCTACTAGAAGAACTCTAGCAAAACCAGACGCGCATTAGAGAACGAGA

970 GAAAACGAAAAAACCGCCTTGACGGGCGGTTTTTGAAGGTTCTCTGAGCTACCAACTCTTTGAACCGAGGTAAC  
CTTTTGCTTTTTTGGCGAACGTCGCCCAAAAAGCTTCCAAGAGACTCGATGGTTGAGAACTTGGCTCCATTGA

1046 GGCTTGGAGGAGCGCAGTCACCAAACTTGTCCTTTTCAAGTTTGCCTTAAACCGGCGCATGACTTCAAGACTAACTC  
CCGAACCTCCTCGCGTCAAGTGGTTTTGAAACAGGAAAGTCAAATCGGAATTGGCCGCGTACTGAAGTTCTGATTGAG

1122 CTCTAAATCAATTACAGTGGCTGCTGCCAGTGGTCTTTTTGCATGTCTTTCCGGGTTGGACTCAAGACGATAGTT  
GAGATTTAGTTAATGGTCACCGACGACGGTCAACCACGAAAACGTACAGAAAGGCCAACCTGAGTTCTGTATCAA

1198 ACCGGATAAGGCGCAGCGGTGCGACTGAACGGGGGTTTCGTGCATACAGTCCAGCTTGGAGCGAACTGCCTACCCG  
TGGCCTATTTCCGCGTCGCCAGCCTGACTTGCCCCCAAGCAGTATGTCAGGTGCAACCTCGCTTACCGGATGGCC

1274 GAACTGAGTGTGAGGCGTGAATGAGACAAACGCGCCATAACAGCGGAATGACACCGGTAACCGGAAAGGCAGGA  
CTTGACTCACAGTCCGCACCTTACTCTGTTTGGCCGGTATTGTGCGCTTACTGTGGCCATTTGGCTTTCCGCTCT

1350 ACAGGAGAGCGCACGAGGGAGCCGACGGGGAAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCCGCCACCACT  
TGCTCTCGCGTGCTCCCTCGGCGGTCCCTTTGCGGACCATAGAAATATCAGGACAGCCAAAGCGGTGGTGA

1426 GATTTGAGCGTCAGATTTCTGTGATGCTTGTGAGGGGGCGGAGCCTATGGAAAAACGGCTTTGCCGGCGCCCTCTC  
CTAAACTCGCAGTCTAAAGCACTACGAACAGTCCCCCGCCTCGGATACCTTTTTGCCGAAACGGCGCCGGGAGAG

1502 ACTTCCTGTTAAGTATCTTCCTGGCATCTTCAGGAAATCTCCGCCCCGTTGCGTAAGCCATTTCCGCTCGCCGCA  
TGAAGGGACAATTCATAGAAGGACCGTAGAAGGTCCTTTAGAGGCGGGGCAAGCATTTCGTAAGGCGAGCGGCGT

1578 GTCGAACGACCGAGCGTAGCGAGTCAGTGAGCGAGGAAGCGGAATATATCCTGTATCACATATTTGCTGACGCAC  
CAGCTTGCTGGCTCGCATCGCTCAGTCACTCGCTCCTTCGCCTTATATAGGACATAGTGATAAGACGACTGCGTG

1654 CGGTGCAGCCTTTTTCTCCTGCCACATGAAGCACTTCACTGACACCCTCATCAGTGCCAACATAGTAAGCCAGTA  
GCCACGTCCGAAAAAGAGGACGGTGTACTTCGTGAAGTGAAGTGTGGGAGTAGTCACGGTTGTATCATTCCGGTCA

1730 TACACTCCGCTAGCGCTGAGGTCTGCCTCGTGAAGAAGGTGTTGCTGACTCATAACCAGGCTGAATCGCCCCATCA  
ATGTGAGGCGATCGCGACTCCAGACGGAGCACTTCTCCACAACGACTGAGTATGGTCCGGACTTAGCGGGGTAGT

1806 TCCAGCCAGAAAGTGAGGGAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTT  
AGTTCGGTCTTTCACTCCCTCGGTGCCAACTACTCTCGAAACAACATCCACCTGGTCAACCACTAAAACCTGAAAA

1882 GCTTTGCCACGGAACGGTCTGCGTTGTGCGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGTTGCGATTTAT  
CGAAACGGTGCCTTGCCAGACGCAACAGCCCTTCTACGCACTAGACTAGGAAGTTGAGTCGTTTTCAAGCTAATA

1958 TCAACAAAGCCACGTTGTGTCTCAAATCTCTGATGTTACATTGCACAAGATAAAAAATATATCATCATGAACAATA  
AGTTGTTTCGGTGCACACAGAGTTTTAGAGACTACAATGTAACGTGTTCTATTTTTATATAGTAGTACTTGTAT

2034 AAACGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCGAGGC  
TTTGACAGACGAATGTATTTGTCATTATGTTCCCAACAATACTCGGTATAAGTTGCCCTTTGCAGAACGAGCTCCG

2110 CGCGATTAAATTCACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGC  
CGCTAAATTTAAGGTTGTACCTACGACTAAATATACCCATATTTACCCGAGCGCTATTACAGCCCGTTAGTCCACG

2186 GACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAAT  
CTGTTAGATAGCTAACATACCCTTCGGGCTACGCGGTCTCAACAAAGACTTTGTACCGTTTCCATCGCAACGGTTA

2262 GATGTTACAGATGAGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTATCC  
CTACAATGTCTACTCTACCAGTCTGATTTGACCGACTGCCTTAAATACGGAGAAGGCTGGTAGTTCGTAATAATAG

2338 GTACTCCTGATGATGCATGGTTACTCACCCTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCC  
CATGAGGACTACTACGTACCAATGAGTGGTGACGCTAGGGGCCCTTTGTGCGTAAGGTCCATAATCTTCTTATAG

2414 TGATTCAGGTGAAAATATTGTTGATGCGTGGCAGTGTTCCTGCGCCGGTTGCATTGATTCCTGTTTGTAAATTGT  
ACTAAGTCCACTTTTATAACAACACTACGCGACCGTACAAGGACGCGGCCAACGTAAGCTAAGGACAAACATTAACA

2490 CCTTTAACAGCGATCGCGTATTTGCTCTGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTG  
GGAAAATTGTCGCTAGCGCATAAAGCAGAGCGAGTCCGCGTTAGTGCTTACTTATTGCCAAACCACTACGCTCAC

2566 ATTTTGTGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGAAAGAAATGCATAAGCTTTTGCATTCTCACC  
TAAACTACTGCTCGCATTACCGACCGGCAACTGTTTCAGACCTTCTTTACGTATTGAAAACGGTAAGAGTGG

2642 GGATTAGTCGTCACCTCATGGTATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGTTGTATT  
CCTAAGTCAGCAGTGAGTACCCTAAAGAGTGAACATTGGAATAAAAACCTGCTCCCCTTAATTATCCAACATAA

2718 GATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACGCTCGGTGAGTTTTCTC  
CTACAACCTGCTCAGCCTTAGCGTCTGGCTATGGTCTAGAACGGTAGGATACCTTGACGGAGCCACTCAAAAGAG

2794 CTTCAATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATGCAGTTTCATTGAT  
GAAGTAATGTCTTTGCCGAAAAAGTTTTTATACCATAACTATTAGGACTATACTTATTTAACGTCAAAGTAAACTA

2870 GCTCGATGAGTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGAC  
CGAGTACTCAAAAAGATTAGTCTTAACCAATTAACCAACATTGTGACCGTCTCGTAATGCGACTGAACTGCCCTG

2946 GGCGGCTTTGTTGAATAAATCGAACTTTTGTGAGTTGAAGGATCAGATCACGCATCTTCCGACAACGCAGACCG  
CCGCCGAAACAACCTATTTAGCTTGAACGACTCACTTCTAGTCTAGTGCGTAGAAGGGCTGTTGCGTCTGGC



3022 TTCCGTGGCAAAGCAAAGTTCAAATCACCAACTGGTCCACCTACAACAAAGCTCTCATCAACCGTGGCTCCCTC  
AAGGCACCGTTTCGTTTTCAAGTTTTAGTGGTTGACCAGGTGGATGTTGTTTTCGAGAGTAGTTGGCACCGAGGGAG

3098 ACTTTCTGGCTGGATGATGGGGCGATTACAGCCTGGTATGAGTCAGCAACACCTTCTTACAGAGGCAGACCTCAGC  
TGAAAGACCGACCTACTACCCCGCTAAGTCCGGACCATACTCAGTCGTTGTGGAAGAAGTGCTCCGTCTGGAGTCG

3174 GCTCAAAGATGCAGGGGTAAGCTAACCGCATCTTACCACAAAGGCATCCGGCAGTTCAACAGATCGGGAAGGG  
CGAGTTTCTACGTCCCATTTTTGATTGGCGTAGAAATGGCTGTTCCGTAGGCCGTCAAGTTGTCTAGCCCTTCCC

3250 CTGGATTTGCTGAGGATGAAGGTGGAGGAAGGTGATGTCTTCTGGTGAAGAAGCTCGACCGTCTTGGCCGCGACA  
GACCTAAACGACTCCTACTTCCACCTCCTTCCACTACAGTAAGACCACTTCTTCGAGCTGGCAGAACCGGCGCTGT

3326 CCGCCGACATGATCCAAGTATAAAGAGTTTATGCTCAGGGTGTAGCGGTTTCGTTTTATTGACGACGGGATCAG  
GGCGGCTGTACTAGGTTGACTATTTTCTCAAACCTACGAGTCCACATCGCCAAGCCAAATAACTGCTGCCCTAGTC

3402 TACCGACGGTATATGGGGCAAATGGTGGTCACCATCCTGTGCGCTGTGGCACAGGCTGAACGCCGGAGGATCGAT  
ATGGCTGCCACTATACCCGTTTACCACCAAGTGGTAGGACAGCCGACACCGTGTCCGACTTGGCGCTCCTAGCTA

3478 CCTAGAGCGCACGAATGAGGGCCGACAGGAAGCAAGCTGAAAGGAATCAAATTTGGCCGAGGCGTACCGTGGAC  
GGATCTCGCGTCTTACTCCCGCTGTCTTCTGTTTCGACTTTCCTTAGTTTTAAACCGGCGTCCGCATGGCACCTG

3554 AGGAACGTCGTGCTGACGCTTCATCAGAAGGGCACTGGTGCACGGAAATGCTCATCAGCTCAGTATTGCCCGCT  
TCCTTGACAGCACGACTGCGAAGTAGTCTTCCCGTGACCAGTTCGCTTTAACGAGTAGTCGAGTCATAACGGGCGA

3630 CCACGGTTTTATAAAATTTCTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATA  
GGTGCCAAATATTTAAGAACTTCTGCTTCCCGGAGCACTATGCGGATAAAAAATATCCAATTACAGTACTATTAT

3706 ATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATAC  
TACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAAGATTTATG

3782 ATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAGGAAGAGTATGAG  
TAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCTTCTCATACTC

3858 TATTCAACATTTCCGTGTGCGCCCTTATCCCTTTTTTGCGCATTTTGCCTTCCTGTTTTGCTCACCCAGAAACG  
ATAAGTTGTAAAGGCACAGCGGGAATAAGGGAAAAACGCCGTAAAACGGAAGGACAAAAACGAGTGGGTCTTTGC

3934 CTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTA  
GACCACTTTCATTTTCTACGACTTCTAGTCAACCCAGTGTCAACCAATGTAGCTTGACCTAGAGTTGTGCGCAT

4010 AGATCCTTGAGAGTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGT  
TCTAGGAACCTCATAAAGCGGGGCTTCTTGCAAAAGGTTACTACTCGTGAATAATTTCAAGACGATACACCGGCCA

4086 ATTATCCCGT  
TAATAGGGCA

**A.4 pEC2-CBD-EL<sub>4</sub>**

**Submitted by** Julie C. Liu **Date** 21 March 2006

**Strain name/  
(plasmid name)** DH5 $\alpha$ F'/ pEC2-6RGDb.41  
 DH5 $\alpha$ F'/ pEC2-13RDGa.13  
 DH5 $\alpha$ F'/ pEC2-16PHSRNa.64  
 DH5 $\alpha$ F'/ pEC2-22NHRPSa.25  
 DH5 $\alpha$ F'/ pEC2-3CS1'b.12

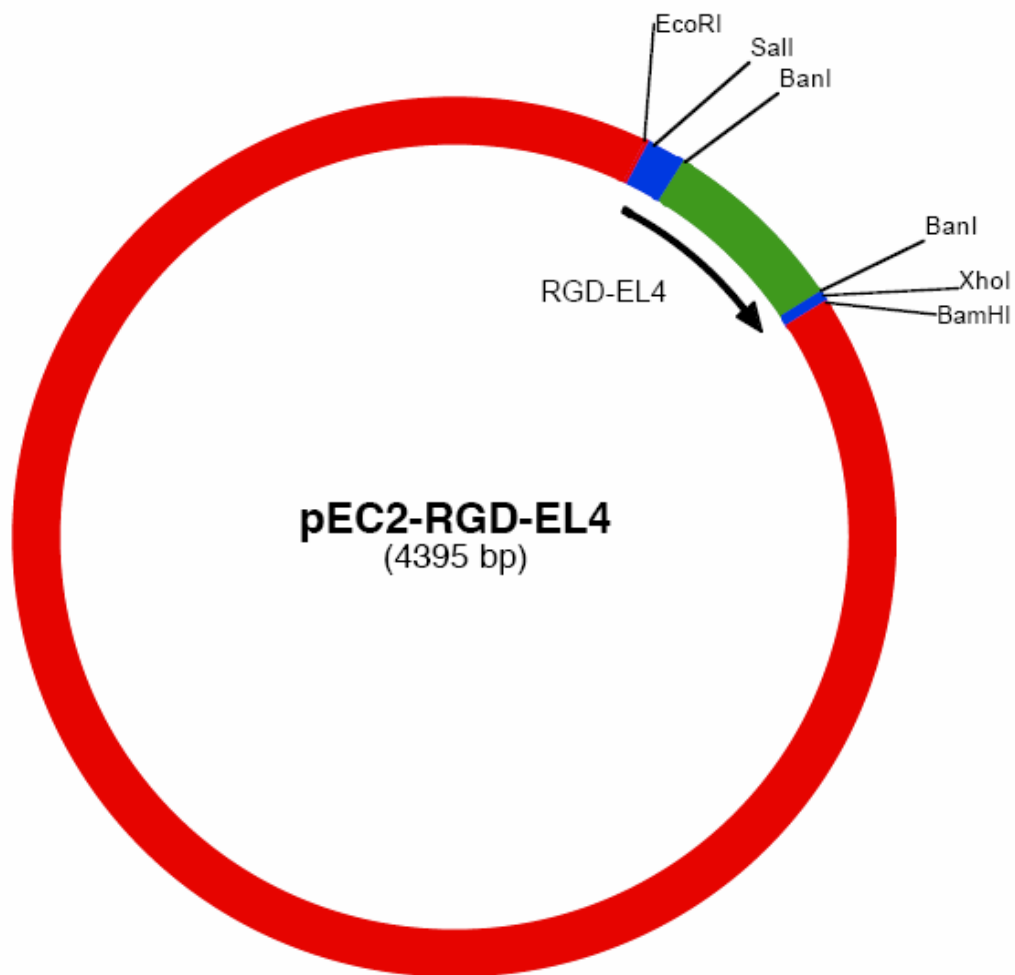
**Vector (kb)** pEC2-6RGDb.41  
 pEC2-13RDGa.13  
 pEC2-16PHSRNa.64  
 pEC2-22NHRPSa.25  
 pEC2-3CS1'b.12

**Construction**

Cells containing elastin (EL) monomers with internal lysine residues in a pUC19 vector (pUC19-EL) were obtained from Kathleen Di Zio. The EL monomers were isolated from pUC19-EL digested with *Ban*I and multimerized (T4 ligase, 16 °C, 35 min) to form tetramers. The elastin tetramers were ligated into *Ban*I-digested pEC2-6RGDb, pEC2-13RDGa, pEC2-16PHSRNa, pEC2-22NHRPSa, and pEC2-3CS1'.

**Available Sources:**

1. 12% glycerol culture in Julie Liu's -80 °C freezer box  
 Culture conditions: 2 $\times$ YT, 25 mg/L kanamycin, 37 °C
2. Plasmid DNA in Julie Liu's -20 °C freezer box

**Plasmid map (pEC2-RGD-EL4)****Positions of elements**

Vector	Vector size (bp)	Insert region (bp)
pEC2-6RGDb.41	4395	315-704
pEC2-13RDGa.13	4395	315-704
pEC2-16PHSRNa.64	4395	315-704
pEC2-22NHRPSa.25	4395	315-704
pEC2-3CS1'b.12	4422	315-731

**Full sequence (pEC2-RGD-EL<sub>4</sub>)**

1 GTTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCA  
CAACTGCGGCCCGTTCTCGTTGAGCCAGCGGCGTATGTGATAAGAGTCTTACTGAACCAACTCATGAGTGGTTCAGT

77 CAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGC  
GTCTTTTCGTAGAATGCCTACCGTACTGTCACTTCTTAATACGTACGACGGTATTGGTACTCACTATTGTGACC

153 GGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCCTTTTTTGCACAACATGGGGGATCATGTA  
CCGGTTGAATGAAGACTGTTGCTAGCCTCCTGGCTTCTCGATTGGCGAAAAACGTGTTGTACCCCTAGTACAT

229 ACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCATAACCAACGACGAGCGTGACACCACGATGCCTGCAG  
TGAGCGGAACTAGCAACCCTTGGCCTCGACTTACTTCGGTATGGTTTGTCTGCTCGCACTGTGGTGTACGGACGTC

305 EcoRI Sall  
ATCTGATCAGaattcgctagctaagtcgac tat gct gtc act ggc cgt gga gac agc ccc gca  
TAGACTAGTCTTAAGcgcgattcagctg ata cga cag tga ccg gca cct ctg tcg ggg cgt  
1▶ Y A V T G R G D S P A

368 BclI  
agc agc aag cca att gcg gtg ccg ggt atc ggc gtt ccg ggc atc ggt gta ccg ggc  
tcg tcg ttc ggt taa cgc cac ggc cca tag ccg caa ggc ccg tag cca cat ggc ccg  
12▶ S S K P I A V P G I G V P G I G V P G

425 aaa ggt gtt ccg ggc atc ggt gtt ccg ggt atc ggg gtg ccg ggt atc ggc gtt ccg  
ttt cca caa ggc ccg tag cca caa ggc cca tag ccc cac ggc cca tag ccg caa ggc  
31▶ K G V P G I G V P G I G V P G I G V P

482 ggc atc ggt gta ccg ggc aaa ggt gtt ccg ggc atc ggt gtt ccg ggt atc ggg gtg  
ccg tag cca cat ggc ccg ttt cca caa ggc ccg tag cca caa ggc cca tag ccc cac  
50▶ G I G V P G K G V P G I G V P G I G V

539 ccg ggt atc ggc gtt ccg ggc atc ggt gta ccg ggc aaa ggt gtt ccg ggc atc ggt  
ggc cca tag ccg caa ggc ccg tag cca cat ggc ccg ttt cca caa ggc ccg tag cca  
69▶ P G I G V P G I G V P G K G V P G I G

596 gtt ccg ggt atc ggg gtg ccg ggt atc ggc gtt ccg ggc atc ggt gta ccg ggc aaa  
caa ggc cca tag ccc cac ggc cca tag ccg caa ggc ccg tag cca cat ggc ccg ttt  
88▶ V P G I G V P G I G V P G I G V P G I G V P G K

653 BclI XhoI BamHI  
ggg gtt ccg ggc atc ggt gtt ccg ggt atc ggg gtg ccg gct cgagactcgtgGATCCATCTAGAG  
cca caa ggc ccg tag cca caa ggc cca tag ccc cacggcgagctctgagcacctagGTAGATCTC  
107▶ G V P G I G V P G I G

718 TCGACGTGCGCCGTTAACCTAGGAGATCTGCAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGGCGAACTACT  
AGCTGCAGCCGCAATTGGATCCTCTAGACGTCGTTACCGTGTGCAACGCGTTTGATAATTGACCGCTTGATGA

794 TACTCTAGTTCCTCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC  
ATGAGATCGAAGGGCCGTTGTTAATTATCTGACCTACCTCCGCTATTTCAACGTCCTGGTGAAGACGCGAGCCGG

870 CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGG  
GAAGGCCGACCGACCAATAACGACTATTTAGACCTCGGCCACTCGCACCCAGAGCGCCATAGTAACGTCGTGACC

946 GGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAG  
CCGGTCTACCATTCCGGAGGGCATAGCATCAATAGATGTGTGCCCTCAGTCCGTTGATACCTACTTGTCTTATC

1022 ACAGATCGCTGAGATAGGTGCGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACT  
TGTCTAGCGACTCTATCCACGCACGGAGTGACTAATTCGTAACCATTGACAGTCTGGTTCAAATGAGTATATATGA

1098 TTAGATTGATTTAAACTTCATTTTTAATTTAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACAAA  
AATCTAACTAAATTTTGAAGTAAAAATTAATTTTCTAGATCCACTTCTAGGAAAACTATTAGAGTACTGGTTT

1174 ATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCTTAATAAGATGATCTTCTTGAGATCGTTTTGGTC  
TAGGGAATTGCACTCAAAGCAAGGTGACTCGCAGTCTGGGAATTATTCTACTAGAAGAACTCTAGCAAAACCAG

1250 TGC GCGTAATCTCTTGCTCTGAAAACGAAAAACCGCCTTG CAGGGCGGTTTTTCGAAGTTCTCTGAGCTACCAA  
ACGCGCATTAGAGAACGAGACTTTTGCTTTTTTGCGGAACGTCCCGCCAAAAAGCTTCCAAGAGACTCGATGGTT

1326 CTCTTTGAACCGAGGTAAGTGGCTTGAGGAGCGCAGTCACCAAACTTGTCTTT CAGTTTAGCCTTAACCGGCG  
GAGAACTTGGCTCCATTGACCGAACCTCCTCGCGTCAGTGGTTTTGAAACAGGAAAGTCAAATCGGAATTGGCCCG

1402 CATGACTTCAAGACTAACTCCTCTAAATCAATTACCAGTGGCTGCTGCCAGTGGTGTCTTTGCATGTCTTTCCGGG  
GTACTGAAGTTCTGATTGAGGAGATTTAGTTAATGGTCACCGACGACGGTCACCACGAAAACGTACAGAAAGGCC

1478 TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGACTGAACGGGGGGTTCGTGCATACAGTCCAGCT  
AACCTGAGTTCTGCTATCAATGGCCTATTCGCGTCGCCAGCCTGACTTGCCCCCAAGCACGTATGTCAGGTCGA

1554 TGGAGCGAACTGCCTACCCGGAAGTGTGAGTGTGAGCGTGGAAATGAGACAAACGCGGCCATAACAGCGGAATGACAC  
ACCTCGCTTGACGGATGGCCCTTGACTCACAGTCCGCACCTTACTCTGTTTGCGCCGGTATTGTCGCCTTACTGTG

1630 CGGTAAACCGAAAGGCAGGAACAGGAGAGCGCACGAGGGAGCCCGCAGGGGAAACGCCTGGTATCTTTATAGTCC  
GCCATTTGGCTTTCCGTCTTGTCTCTCGCGTGTCCCTCGGCGGTCCCCCTTGCGGACCATAGAAATATCAGG

1706 TGTGGGTTTTCGCCACCCTGATTTGAGCGTCAGATTTCTGTGATGCTTGTGAGGGGGCGGAGCCTATGGAAAAAC  
ACAGCCCAAAGCGGTGGTGACTAACTCGCAGTCTAAAGCACTACGAACAGTCCCCCGCCTCGGATACCTTTTTG

1782 GGCTTTGCCGGGCCCTCTCACTTCCCTGTTAAGTATCTTCTGCGCATCTTCCAGGAAATCTCCGCCCGTTGTA  
CCGAAACGGCGCCGGGAGAGTGAAGGGACAATTCATAGAAGGACCGTAGAAGGTCTTTAGAGGCGGGGCAAGCAT

1858 AGCCATTTCCGCTCGCCGAGTCGAACGACCGAGCGTAGCGAGTCAGTGAGCGAGGAAGCGGAATATATCTGTAT  
TCGGTAAAGGCGAGCGCGTCAGCTTGTGGCTCGCATCGCTCAGTCACTCGCTCCTTCGCCTTATATAGGACATA

1934 CACATATTCTGCTGACGCACCGGTGCAGCCTTTTTTCTCCTGCCACATGAAGCACTTCACTGACACCCTCATCAGT  
GTGTATAAGACGACTGCGTGGCCACGTTCGAAAAAAGAGGACGGTGTACTTCTGTAAGTACTGTGGGAGTAGTCA

2010 GCCAACATAGTAAGCCAGTATACACTCCGCTAGCGCTGAGGTCTGCCTCGTGAAGAAGGTGTTGCTGACTCATAAC  
CGGTTGTATCATTGGTCATATGTGAGGCGATCGCGACTCCAGACGGAGCACTTCTTCCACAACGACTGAGTATGG

2086 AGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAAGGAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCA  
TCCGACTTAGCGGGTAGTAGGTGCGTCTTCACTCCCTCGGTGCCAACTACTCTCGAAACAACATCCACCTGGT

2162 GTTGGTGATTTTGAACTTTTGCTTTGCCACGGAACGGTCTGCGTTGTCGGGAAGATGCGTGATCTGATCCTTCAAC  
CAACCACTAAACTTGAAAACGAAACGGTGCCTTGCCAGACGCAACAGCCCTTCTACGCACTAGACTAGGAAGTTG

2238 TCAGCAAAGTTCGATTTATTCAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAA  
AGTCGTTTTCAAGCTAAATAAGTTGTTTCCGGTGCACACAGAGTTTTAGAGACTACAATGTAACGTGTTCTATTTT

2314 ATATATCATCATGAACAATAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTTCAACG  
TATATAGTAGTACTTGTATTTTGGACAGACGAATGTATTTGTCATTATGTTCCCCACAATACTCGGTATAAGTTGC

2390 GGAAACGTCTTGCTCGAGGCCGCGATTAATTTCAAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGAT  
CCTTTGCAGAACGAGCTCCGGCGCTAATTTAAGGTTGTACCTACGACTAAATATACCCATATTTACCCGAGCGCTA

2466 AATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATG  
TTACAGCCCGTTAGTCCACGCTGTAGATAGCTAACATACCCTTCGGGCTACGCGGTCTCAACAAAGACTTTGTAC

2542 GCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTTCC  
CGTTTCCATCGCAACGGTTACTACAATGTCTACTCTACCAGTCTGATTTGACCGACTGCCTTAAATACGGAGAAGG

2618 GACCATCAAGCATTTTTATCCGTACTCCTGATGATGCATGGTTACTCACCCTGCGATCCCGGGAAAAACAGCATT  
CTGGTAGTTCGTAATAAGGCATGAGGACTACTACGTACCAATGAGTGGTGACGCTAGGGGCCCTTTTGTGCGTAA

2694 CAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATT  
GTCCATAATCTTCTTATAGGACTAAGTCCACTTTTATAACAACCTACGCGACCGTCAACAAGGACGCGGCCAACGTAA

2770 CGATTCCTGTTTGAATTTGCTTTTTAACAGCGATCGCGTATTTGCTCTCGCTCAGGCGCAATCACGAATGAATAA  
GCTAAGGACAACATTACAGGAAAATTGTCGCTAGCGCATAAAGCAGAGCGAGTCCGCGTTAGTCTTACTTATT

2846 CGGTTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCAT  
GCCAAACCAACTACGCTCACTAAAACACTGCTCGCATTACCGACCGACAACCTTGTTTCAGACCTTTCTTTACGTA

2922 AAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTGACGAGG  
TTCGAAAACGGTAAGAGTGGCCTAAGTCAGCAGTGAGTACCACTAAAGAGTGAACCTATTGGAATAAAAACCTGCTCC

2998 GGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAA  
CCTTTAATTATCCAACATAACTACAACCTGCTCAGCCTTAGCGTCTGGCTATGGTCTAGAACGGTAGGATACCTT

3074 CTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAAT  
GACGGAGCCACTCAAAAGAGGAAGTAATGTCTTTGCCGAAAAAGTTTTTATACCATAACTATTAGGACTATACTTA

3150 AAATTGCAGTTTCATTTGATGCTCGATGAGTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCA  
TTTAACTGCAAAGTAACTACGAGCTACTCAAAAAGATTAGTCTTAACCAATTAACCAACATTGTGACCGTCTCGT

3226 TTACGCTGACTTGACGGGACGGCGGCTTTGTTGAATAAATCGAACTTTTGCTGAGTTGAAGGATCAGATCACGCAT  
AATGGACTGAACTGCCCTGCCCGCAACAACCTATTTAGCTTGAAAACGACTCAACTTCCTAGTCTAGTGCCTA

3302 CTTCCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAGTTCAAAATCACCAACTGGTCCACCTACAACAAAGCTC  
GAAGGCTGTTGCGTCTGGCAAGGCACCGTTTCGTTTTCAAGTTTTAGTGGTTGACCAGGTGGATGTTGTTTCGAG

3378 TCATCAACCGTGGCTCCCTCACTTTCTGGCTGGATGATGGGGCGATTGAGGCTGGTATGAGTCAGCAACACCTTC  
AGTAGTTGGCACCGAGGAGTGAAAGACCGACCTACTACCCCGCTAAGTCCGGACCATACTCAGTCGTTGTGGAAG

3454 TTCACGAGGCAGACCTCAGCGCTCAAAGATGCAGGGTAAAAGCTAACCGCATCTTTACCGACAAGGCATCCGGCA  
AAGTGTCCGTCTGGAGTCGCGAGTTTCTACGTCCCATTTTTCGATTGGCGTAGAAATGGCTGTTCCGTAGGCCGT

3530 GTTCAACAGATCGGGAAGGGCTGGATTTGCTGAGGATGAAGGTGGAGGAAGGTGATGTCATTCTGGTGAAGAAGCT  
CAAGTTGTCTAGCCCTTCCCGACTAAACGACTCTACTTCCACCTCCTTCCACTACAGTAAGACCACCTTCTTCGA

3606 CGACCGTCTTGCCCGACACCCCGACATGATCCAACCTGATAAAAAGAGTTTGATGCTCAGGGTGTAGCGGTTCCG  
GCTGGCAGAACCAGCGCTGTGGCGGCTGTACTAGGTTGACTATTTTCTCAAACCTACGAGTCCACATCGCCAAGCC

3682 TTTATTGACGACGGGATCAGTACCGACGGTGATATGGGGCAAATGGTGGTCACCATCCTGTGGTGTGGCACAGG  
AAATAACTGCTGCCCTAGTCATGGCTGCCACTATAACCCGTTTACCACCAGTGGTAGGACAGCCGACACCGTGTCC

3758 CTGAACGCCGGAGGATCGATCCTAGAGCGCACGAATGAGGGCCGACAGGAAGCAAAGCTGAAAGGAATCAAATTTG  
GACTTGGCGCCTCCTAGCTAGGATCTCGCGTGTACTCCGGCTGTCTTCCGTTTCGACTTTCCCTTAGTTTAAAC

3834 GCCGACGGCGTACCGTGGACAGGAACGTGCTGCTGACGCTTCATCAGAAGGGCACTGGTGCAACGGAAATGCTCA  
CGGCTCCGCATGGCACCTGTCTTGCAGCACGACTGCGAAGTAGTCTTCCGTGACCAGGTTGCCTTTAACGAGT

3910 TCAGCTCAGTATTGCCCGCTCCACGGTTTATAAAATCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTAT  
AGTCGAGTCATAACGGCGAGGTGCCAAATATTTAAGAACCTTCTGCTTTCCCGGAGCACTATGCGGATAAAAATA

3986 AGGTTAATGTCATGATAATAATGGTTTCTTAGACGTGAGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTAT  
TCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCTTTACACGCGCCTTGGGGATA

4062 TTGTTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATAT  
AACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATA

4138 TGAAAAGGAAGAGTATGAGTATCAACATTTCCGTGTGCGCCTTATCCCTTTTTTGCGGCATTTTGCCTTCTCG  
ACTTTTTCTTCTCATACTCATAAGTTGTAAAGGCACAGCGGGAATAAGGGAAAAACGCCGTAAAACGGAAGGAC

4214 TTTTTGCTCACCCAGAAACGCTGGTGAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGA  
AAAAACGAGTGGGTCTTTGCGACCACTTTCAATTTCTACGACTTCTAGTCAACCCACGTGCTCACCCAATGTAGCT

4290 ACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAA  
TGACCTAGAGTTGTGCGCATTCTAGGAACCTCAAAAAGCGGGGCTTCTTGCAAAGGTTACTACTCGTGAATAATTT

4366 GTTCTGCTATGTGGCGCGGTATTATCCCGT  
CAAGACGATACACCGCGCCATAATAGGGCA

**A.5 pET28aRW-(CBD-EL<sub>4</sub>)<sub>3</sub>**

**Submitted by** Julie C. Liu **Date** 21 March 2006

**Strain name/  
(plasmid name)** DH5αF' or BL21(DE3) pLysS / pET28aRW-(RGD-EL<sub>4</sub>)<sub>3.10</sub>  
 DH5αF' or BL21(DE3) pLysS / pET28aRW-(RDG-EL<sub>4</sub>)<sub>3.1</sub>  
 DH5αF' or BL21(DE3) pLysS / pET28aRW-(PHSRN-EL<sub>4</sub>)<sub>3.1</sub>  
 DH5αF' or BL21(DE3) pLysS / pET28aRW-(NHRPS-EL<sub>4</sub>)<sub>3.4</sub>  
 DH5αF' or BL21(DE3) pLysS / pET28aRW-(CS1'-EL<sub>4</sub>)<sub>3.4</sub>

**Vector (kb)** pET28aRW-RGD or pET28aRW-(RGD-EL<sub>4</sub>)<sub>3.10</sub>  
 pET28aRW-RDG or pET28aRW-(RDG-EL<sub>4</sub>)<sub>3.1</sub>  
 pET28aRW-PHSRN or pET28aRW-(PHSRN-EL<sub>4</sub>)<sub>3.1</sub>  
 pET28aRW-NHRPS or pET28aRW-(NHRPS-EL<sub>4</sub>)<sub>3.4</sub>  
 pET28aRW-CS1' or pET28aRW-(CS1'-EL<sub>4</sub>)<sub>3.4</sub>

**Construction**

The pET28a vector with a unique polylinker inserted between the *Nco*I and *Xho*I sites (pET28aRW) was obtained from Ralf Weberskirch. The polylinker contains a heptahistidine tag, a T7 tag for antibody identification, and an enterokinase cleavage site. The cell-binding domain attached to an elastin tetramer (CBD-EL<sub>4</sub>) was isolated from pEC2-6RGDb.41, pEC2-13RDGa.13, pEC2-16PHSRNa.64, pEC2-22NHRPSa.25, and pEC2-3CS1'b.12 digested with *Xho*I and *Sal*I. These fragments were sequentially inserted into the pET28aRW vector digested with *Xho*I. The intermediate cloning vectors (available in the DH5αF' strain) are:

***pET28aRW-(CBD-EL<sub>4</sub>)<sub>1</sub> (one cassette)***

pET28aRW-(RGD-EL<sub>4</sub>)<sub>1.3</sub>  
 pET28aRW-(RDG-EL<sub>4</sub>)<sub>1.1</sub>  
 pET28aRW-(PHSRN-EL<sub>4</sub>)<sub>1.4</sub>  
 pET28aRW-(NHRPS-EL<sub>4</sub>)<sub>1.3</sub>

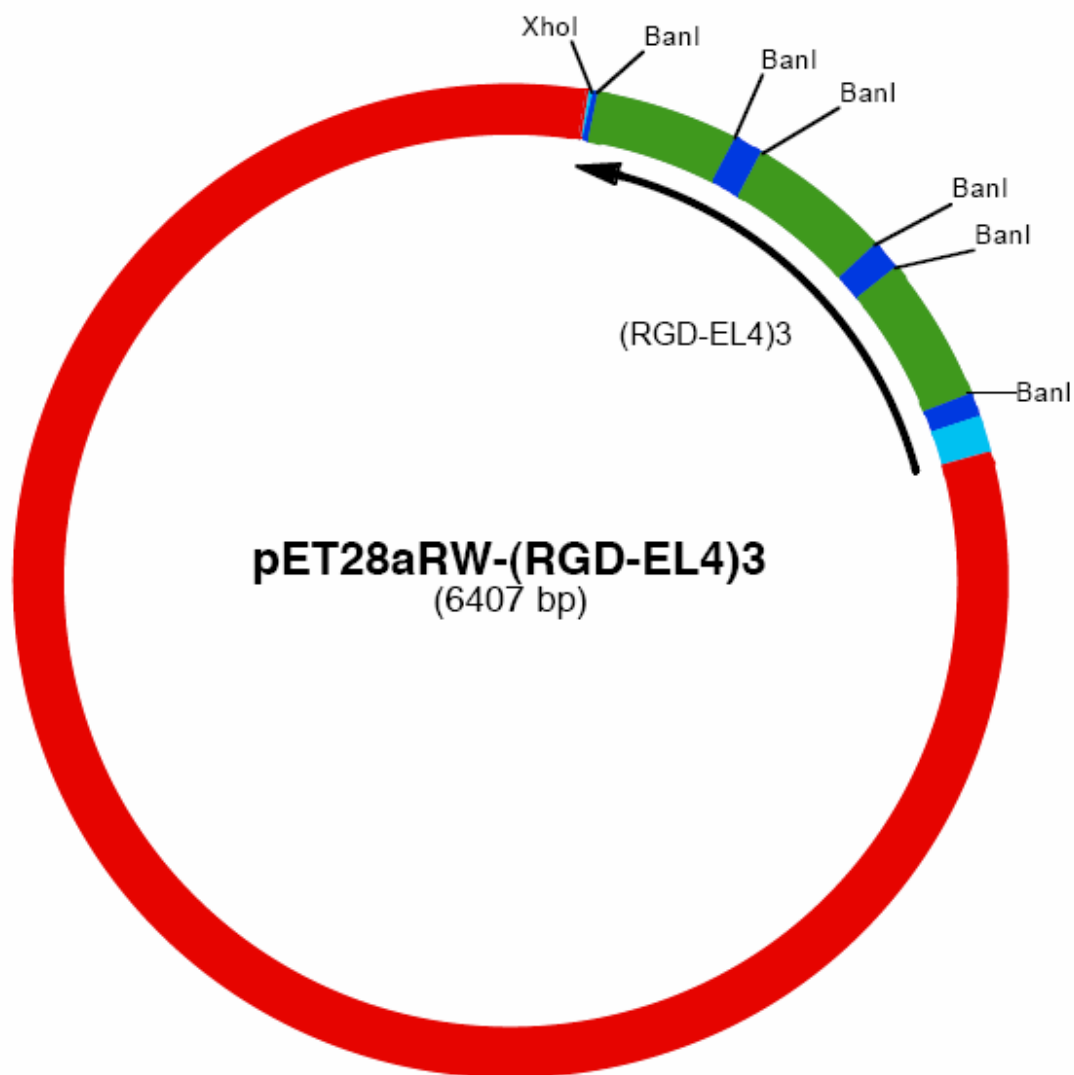
***pET28aRW-(CBD-EL<sub>4</sub>)<sub>2</sub> (two cassettes)***

pET28aRW-(RGD-EL<sub>4</sub>)<sub>2.1</sub>  
 pET28aRW-(NHRPS-EL<sub>4</sub>)<sub>2.1</sub>  
 pET28aRW-(CS1'-EL<sub>4</sub>)<sub>2.9</sub>

**Available Sources:**

1. 12% glycerol culture in Julie Liu's -80 °C freezer box  
 Culture conditions: 2×YT, 25 mg/L kanamycin,  
 35 mg/L chloramphenicol for BL21(DE3) pLysS, 37 °C
2. Plasmid DNA in Julie Liu's -20 °C freezer box

**Plasmid map (pET28aRW-(RGD-EL<sub>4</sub>)<sub>3</sub>)**



**Positions of elements**

Vector	Vector size (bp)	Insert region (bp)
pET28aRW-(RGD-EL <sub>4</sub> ) <sub>3</sub> .10	6407	165-1337
pET28aRW-(RDG-EL <sub>4</sub> ) <sub>3</sub> .1	6407	165-1337
pET28aRW-(PHSRN-EL <sub>4</sub> ) <sub>3</sub> .1	6407	165-1337
pET28aRW-(NHRPS-EL <sub>4</sub> ) <sub>3</sub> .4	6407	165-1337
pET28aRW-(CS1'-EL <sub>4</sub> ) <sub>3</sub> .4	6488	165-1418



**Full sequence (pET28aRW-(RGD-EL<sub>4</sub>)<sub>3</sub>)**

1 ATCCGGATATAGTTCCCTCTTTTCAGCAAAAAACCCCTCAAGACCCGTTTAGAGGCCCAAGGGTTATGCTAGTT  
 TAGGCCTATATCAAGGAGGAAAGTCGTTTTTTGGGGAGTTCTGGGCAATCTCCGGGGTTCCCAATACGATCAA  
 76 ATTGCTCAGCGGTGGCAGCAGCCAACCTCAGCTTCTTTTCGGGCTTGTGTAGCAGCCGATCTCAGTGGTGGTGGT  
 TAACGAGTCGCCACCGTCGTCGGTTGAGTCGAAGGAAAGCCCGAAACAATCGTCGGCTAGAGTCACCACCACCA

XhoI                      BlnI

151 GGTGGTGC tgc act tta tta ctc gag cgg cac ccc gat acc cgg aac acc gat gcc  
 CCACCACG agc tga aat aat gag ctc gcc gtg ggg cta tgg gcc ttg tgg cta cgg  
 391 ◀ • • E L P V G I G P V G I G

207 cgg aac acc ttt gcc cgg tac acc gat gcc cgg aac gcc gat acc cgg cac ccc gat  
 gcc ttg tgg aaa cgg gcc atg tgg cta cgg gcc ttg cgg cta tgg gcc gtg ggg cta  
 377 ◀ P V G K G P V G I G P V G I G P V G I G P V G I  
 264 acc cgg aac acc gat gcc cgg aac acc ttt gcc cgg tac acc gat gcc cgg aac gcc  
 tgg gcc ttg tgg cta cgg gcc ttg tgg aaa cgg gcc atg tgg cta cgg gcc ttg cgg  
 358 ◀ G P V G I G P V G K G P V G I G P V G  
 321 gat acc cgg cac ccc gat acc cgg aac acc gat gcc cgg aac acc ttt gcc cgg tac  
 cta tgg gcc gtg ggg cta tgg gcc cta cgg gcc ttg tgg aaa cgg gcc atg tgg  
 339 ◀ I G P V G I G P V G I G P V G K G P V  
 378 acc gat gcc cgg aac gcc gat acc cgg cac ccc gat acc cgg aac acc gat gcc cgg  
 tgg cta cgg gcc ttg cgg cta tgg gcc gtg ggg cta tgg gcc ttg tgg cta cgg gcc  
 320 ◀ G I G P V G I G P V G I G P V G I G P

BlnI

435 aac acc ttt gcc cgg tac acc gat gcc cgg aac gcc gat acc cgg cac cgc aat tgg  
 ttg tgg aaa cgg gcc atg tgg cta cgg gcc ttg cgg cta tgg gcc gtg cgc tta acc  
 301 ◀ V G K G P V G I G P V G I G P V A I P

BlnI

492 ctt gct gct tgc ggg gct gtc tcc acg gcc agt gac agc ata gtc gag cgg cac ccc  
 gaa cga cga acg ccc cga cag agg tgc cgg tca ctg tgc tat cag ctc gcc gtg ggg  
 282 ◀ K S S A P S D G R G T V A Y D L P V G  
 549 gat acc cgg aac acc gat gcc cgg aac acc ttt gcc cgg tac acc gat gcc cgg aac  
 cta tgg gcc ttg tgg cta cgg gcc ttg tgg aaa cgg gcc atg tgg cta cgg gcc ttg  
 263 ◀ I G P V G I G P V G K G P V G I G P V  
 606 gcc gat acc cgg cac ccc gat acc cgg aac acc gat gcc cgg aac acc ttt gcc cgg  
 cgg cta tgg gcc gtg ggg cta tgg gcc ttg tgg cta cgg gcc ttg tgg aaa cgg gcc  
 244 ◀ G I G P V G I G P V G I G P V G K G P  
 663 tac acc gat gcc cgg aac gcc gat acc cgg cac ccc gat acc cgg aac acc gat gcc  
 atg tgg cta cgg gcc ttg cgg cta tgg gcc gtg ggg cta tgg gcc ttg tgg cta cgg  
 225 ◀ V G I G P V G I G P V G I G P V G I G  
 720 cgg aac acc ttt gcc cgg tac acc gat gcc cgg aac gcc gat acc cgg cac ccc gat  
 gcc ttg tgg aaa cgg gcc atg tgg cta cgg gcc ttg cgg cta tgg gcc gtg ggg cta  
 206 ◀ P V G K G P V G I G P V G I G P V G I  
 777 acc cgg aac acc gat gcc cgg aac acc ttt gcc cgg tac acc gat gcc cgg aac gcc  
 tgg gcc ttg tgg cta cgg gcc ttg tgg aaa cgg gcc atg tgg cta cgg gcc ttg cgg  
 187 ◀ G P V G I G P V G K G P V G I G P V G

BlnI

834 gat acc cgg cac cgc aat tgg ctt gct gct tgc ggg gct gtc tcc acg gcc agt gac  
 cta tgg gcc gtg cgc tta acc gaa cga cga acg ccc cga cag agg tgc cgg tca ctg  
 168 ◀ I G P V A I P K S S A P S D G R G T V

BlnI

891 agc ata gtc gag cgg cac ccc gat acc cgg aac acc gat gcc cgg aac acc ttt gcc  
 tgc tat cag ctc gcc gtg ggg cta tgg gcc ttg tgg cta cgg gcc ttg tgg aaa cgg  
 149 ◀ A Y D L P V G I G P V G I G P V G I G P V G K G  
 948 cgg tac acc gat gcc cgg aac gcc gat acc cgg cac ccc gat acc cgg aac acc gat  
 gcc atg tgg cta cgg gcc ttg cgg cta tgg gcc gtg ggg cta tgg gcc ttg tgg cta  
 130 ◀ P V G I G P V G I G P V G I G P V G I  
 1005 gcc cgg aac acc ttt gcc cgg tac acc gat gcc cgg aac gcc gat acc cgg cac ccc  
 cgg gcc ttg tgg aaa cgg gcc atg tgg cta cgg gcc ttg cgg cta tgg gcc gtg ggg  
 111 ◀ G P V G K G P V G I G P V G I G P V G  
 1062 gat acc cgg aac acc gat gcc cgg aac acc ttt gcc cgg tac acc gat gcc cgg aac  
 cta tgg gcc ttg tgg cta cgg gcc ttg tgg aaa cgg gcc atg tgg cta cgg gcc ttg  
 92 ◀ I G P V G I G P V G K G P V G I G P V

1119 gcc gat acc cgg cac ccc gat acc cgg aac acc gat gcc cgg aac acc ttt gcc cgg  
 cgg cta tgg gcc gtg ggg cta tgg gcc ttg tgg cta cgg gcc ttg tgg aaa cgg gcc  
 73 ◀ G I G P V G I G P V G I G P V G K G P  
 BanI

1176 tac acc gat gcc cgg aac gcc gat acc cgg cac cgc aat tgg ctt gct gct tgc ggg  
 atg tgg cta cgg gcc ttg cgg cta tgg gcc gtg cgc tta acc gaa cga cga acg ccc  
 54 ◀ V G I G P V G I G P V A I P K S S A P

1233 gct gtc tcc acg gcc agt gac agc ata gtc gag ttt atc atc atc atc atc atc atc atc  
 cga cag agg tgc cgg tca ctg tcg tat cag ctc aaa tag tag tag tag tac cac cac  
 35 ◀ S D G R G T V A Y D L K D D D D H H H

1290 gtg gtg gtg gtg acc cat ttg ctg tcc acc agt cat gct agc cat cat GGTATATCTCC  
 cac cac cac cac tgg gta aac gac agg tgg tca gta cga tcg gta gta CCATATAGAGG

16 ◀ H H H H G M Q G G T M S A M M

1349 TTCTTAAAGTTAAACAAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACAAATCCCTATAGTGAGTGTATT  
 AAGAATTTCAATTTGTTTTAATAAAGATCTCCCCTTAAACAATAGGCGAGTGTAAAGGGGATATCACTCAGCATAA  
 1424 AATTTTCGGGGATCGAGATCTCGATCCTCTACGCCGACGCATCGTGGCCGGCATCACCGCGCCACAGGTGCGG  
 TTAAAGCGCCCTAGCTCTAGAGCTAGGAGATCGGCCCTGCGTAGCACCGCCGTAGTGGCCGCGGTGTCCACGCC  
 1499 TTGCTGGCGCCTATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGT  
 AACGACCGGGATATAGCGGCTGTAGTGGCTACCCCTTCTAGCCGAGCGGTGAAGCCCGAGTACTCGGAACAA  
 1574 TCGGCGTGGGTATGGTGGCAGGCCCGTGGCCGGGGACTGTTGGGCGCCATCTCCTTGCATGCACATTCCTTG  
 AGCCGCACCCATAACCACCGTCCGGGGCACCGGCCCTTGACAACCCGCGGTAGAGGAACGTACGTGGTAAGGAAC  
 1649 CGGCGCGGGTGTCAACGGCCTCAACCTACTACTGGGCTGCTTCCCTAATGACGAGGTGCGATAAGGGAGAGCGTC  
 GCCGCCGCCACGAGTTGCCGGAGTTGGATGATGACCCGACGAAGGATTACGTCTCAGCGTATTCCTCTCGCAG  
 1724 GAGATCCCGACACCATCGAATGGCGAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTC  
 CTCTAGGGCCTGTGGTAGCTTACCGCGTTTTGGAAAGGCCATACCGTACTATCGCGGCCCTTCTCAGTTAAG  
 1799 AGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGAGATATGCCGGTGTCTCTTATCAGACCGTTTCC  
 TCCCACCACTTACTTTGGTCAATGCAATATGCTACAGCGTCTCATACGGCCACAGAGAATAGTCTGGCAAAGG  
 1874 CGCGTGTGAACCAGGCCAGCCACGTTTCTGCGAAAACCGGGGAAAAAGTGAAGCGCGCATGGCGGAGCTGAAT  
 GCGCACCACTTGGTCCGGTCCGGTGCAAAGACGCTTTTTCGCGCCCTTTTTCACCTTCGCGCGTACCGCCTCGACTTA  
 1949 TACATCCCAACCGCGTGGCACAACAACCTGGCGGGCAACAGTCTGTTGCTGATTGGCGTGGCCACCTCCAGTCTG  
 ATGTAAGGGTTGGCGCACCGTGTGTTGACCGCCGTTTGTGACGCAACGACTAACCGCAACGGTGGAGGTCAGAC  
 2024 GCCCTGCACGCGCCGTGCGCAAATTTGTCGGCGGATTAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTG  
 CGGGACGTGCGCGGCAGCGTTTAAACAGCGCCCTAATTTAGAGCGCGGCTAGTTGACCCACGGTGCACACCAC  
 2099 TCGATGGTAGAACAAGCGCGCTCGAAGCCTGTAAGCGGGCGGTGCACAATCTTCTCGCGCAACCGCTCAGTGGG  
 AGCTACCATTGCTTTCGCGCAGCTTCGCGCATTTCCGCCACGTGTTAGAAGAGCGCGTGGCGCATCCACC  
 2174 CTGATCATTAACCTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCATAATGTTCCGGCGTTA  
 GACTAGTAATTGATAGCGACCTACTGGTCTACGGTAACGACACCTTCGACGGACGTGATTACAAGGCCGCAAT  
 2249 TTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTTATTTCTCCCATGAAGACGGTACGGGACTGGCGGTG  
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6374 TAATGCGCCGCTACAGGGCGCGTCCCATTCGCCA  
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