Activity-Guided Engineering of Natural Product Carrier Proteins

Andrew S. Worthington, Gene H. Hur and Michael D. Burkart*

Electronic Supplementary Information

Cloning of parent CP genes into pET22b

The vector ΔNEntB/pET22b was supplied by the Walsh laboratory at Harvard University.1 AcpP and FrnN were generated using 0.5 µM each primer for and reverse – NdeI and XhoI restriction sites (underlined) with 1 ng of alternate vector containing the gene of interest. PCR reaction mixture contains primers at 0.1 µM each, 50 mM KCl, 10 mM Tris pH 8.3, 2 mM MgCl2, 200 µM each dNTP, and 1 unit Pfu.

Thermocycling conditions were as follows:
95°C for 2 minutes
35 cycles of: 95°C for 30 seconds, 59°C for 30 seconds, 72°C for 30 seconds
72°C for 10 minutes

Forward and Reverse Primers

**AcpPforward**: ATTATATCATATGAGCACTATCGAAGAACGCG
**AcpPreverse**: TGATGTCCTCGAGCGCCTGGTGGCCGTTG
**FrnNforward**: ATTATATCATATGACCGACTGACCGTCGACG
**FrnNreverse**: TGATGTCCTCGAGGGCGGTGGCCGGGGTG
**EntBforward**: ATTATATCATATGCTCCTGAAATATGTGGCCG
**EntBreverse**: TGATGTCCTCGAGTTCACCTCGGC

PCR Ligation

Stocks of each primer set with a concentration of 2.5 µM per primer were made. One µL of each primer set (e.g. A3, F2F3) and 5 total primer sets were used per 25 µL reaction (0.1 µM for each primer in the final reaction) – see Supplementary Table 1.

The PCR reaction mixture contained primers at 0.1 µM each, 50 mM KCl, 10 mM Tris pH 8.3, 2 mM MgCl2, 200 µM each dNTP, and 1 unit Pfu.

Thermocycling conditions were as follows:
95°C for 2 minutes
30 cycles of: 95°C for 30 seconds, 64°C for 30 seconds, 72°C for 30 seconds
72°C for 10 minutes
Holding at 4°C
A second round of PCR reactions was performed, with 1 µL of this reaction with 0.5 µM (final concentration) of each CP specific primer (e.g. AcpPforward and FrnNreverse) with same reaction conditions (25 µL), except a different program was used (we had to vary the annealing temperature for each construct).

Thermocycling conditions were as follows:
95°C for 2 minutes
40 cycles of: 95°C for 30 seconds, 58-72°C for 30 seconds, 72°C for 30 seconds
72°C for 10 minutes
Holding at 4°C

The second PCR reaction was run on 2% agarose gel stained with 1 % ethidium bromide. Each properly sized band was excised and purified with the Qiaquick Gel Extraction Kit (Qiagen). Inserts were then digested with 20 units each of XhoI and NdeI (NEB) at 37°C overnight, followed by purification with the AccuPrep PCR Purification Kit (Bioneer, Inc.). Inserts were ligated to pET22b vector (Novagen) that had been digested with XhoI and NdeI and reacted with Calf Intestinal Alkaline Phosphatase (NEB). The resulting constructs were transformed into Subcloning Efficiency DH5a competent cells (Invitrogen) and plated on LB/AMP plates. Colonies were screened through sequencing of purified plasmid (Qiagen kit), which was performed by Eton Biosciences (San Diego, CA).

**Chimeric CP Panel Primers**

**A1 Primers**
AcpPforward
ACPA1rev:
CATTGGTAGAATTCATGCAACCGAGCTGCTTCGCGGATAAATTCTTAACCGGTTTCTTCGAT

**A2 Primer**
ACPA2rev:
CTCTTCTTCCAGAGCCATTACCAGCTCAACGGTGTCAAGAGAATCCGCCCAGGTCTTC

**A3 Primers**
ACPA3revA: GCCTGAACGGTGATTTTCTCACGCTTCGCGGATAATC
ACPA3forB: CACCACCGTTCCAGGCTGCATTGATTACATCAACCGGCCCCAGG

**F1 Primers**
FrnNforward
FrnNF1revA: CGGCGAGCAGTTTTCTTCAAGGAGGCTGACGCTCAGG
FrnNF1forB: GAAACTGCTCGCCGAGACCCGGGGAGGACG
FrnNF1revC: CGTGTGAGTTCTCCGGGAGGTGACGCTGCTGTCCTCCCG
F2 Primer
FrnNF2rev:
CCGCTGCTGGAGCACGGCGGCCGTCTCCAGCAGCGCGAGGGAGTCGTAGCCGAGGTCC

F3 Primers
FrnNF3revA:
CAGTTTCGCCGCGGGGTGCCCAGCCGCCGCCGCCGCCACGCTCGTCAGTCAG
FrnNF3forB:
CCCGCAGCGAAGCTGTCCGACGAGGTCAACACCACCCCGCCGCCACC
FrnNreverse

E1 Primers
EntBforward
EntBE1revA: CTTCAGTCATCACCACCCCGCAAGCTCGGCCACCGTCTCGTCAGTCAG
EntBE1forB: GTGATGACTGAAAGAATTACTGCGCAGCACCATTCCCCCGCCACC
EntBE1revC:
CATCGGACTGCTCCAGCAACGGCAGGATCACCTACGCACGCGCCGCTTTGCTGCCGGGGATAG

E2 Primer
EntBE2rev:
GCACTTTTCGCCGCGCCGCACCTCGACCTCGCCACCATACCGCACCAGCTCCAGACC
GTAGCTGATCAAGGGATGTC

E3 Primers
EntBE3revA: CCACCAGGCCGTCGATGTTGCTCGGGGTCTTTTCGCCACGATGCAAAG
EntBE3forB : CGACGCCCTTGGTGAAGCTACTCTCCGCCGAGGTGAAA
EntBreverse

Junction Primers
A1A2FOR : GTTACCAACAATGCTTCTTTCTGTTGAGAAGACCTGGGCACCGG
A1F2FOR : GTTACCAACAATGCTTCTTTCTGTTGAGAAGACCTGGGCACCG
A1E2FOR : GTTACCAACAATGCTTCTTTCTGTTGAGAAGACCTGGGCACCG
F1F2FOR : GGAGAATCACTCGACGCGCCCTTCTCGAGTGACATGCTCCAGAC
F1E2FOR : GGAGAATCACTCGACGCGCCCTTCTCGAGTGACATGCTCCAGAC
F1A2FOR : GGAGAATCACTCGACGCGCCCTTCTCGAGTGACATGCTCCAGAC
E1E2FOR : GACGAGCTCCGATAGAACCTCCGACCAACCTCCGACCAACCTCCGAC
E1A2FOR : GACGAGCTCCGATAGAACCTCCGACCAACCTCCGACCAACCTCCGAC
E1F2FOR : GACGAGCTCCGATAGAACCTCCGACCAACCTCCGACCAACCTCCGAC
A2A3FOR : GCTCTGGAAAGAAGAGTTTGATACGTGAGATTCCGCGACGAGAA
A2F3FOR : GCTCTGGAAAGAAGAGTTTGATACGTGAGATTCCGCGACGAGAA
A2E3FOR : GCTCTGGAAAGAAGAGTTTGATACGTGAGATTCCGCGACGAGAA
F2F3FOR : GCTCTGGAAAGAAGAGTTTGATACGTGAGATTCCGCGACGAGAA
F2E3FOR : GCTCTGGAAAGAAGAGTTTGATACGTGAGATTCCGCGACGAGAA
F2E3FOR : GCTCTGGAAAGAAGAGTTTGATACGTGAGATTCCGCGACGAGAA
F2A3FOR : GTGCTCCAGCAGCGTACGGCAGTCCGGAGATCGGACGAAAG
E2E3FOR : GGCAGGAAATGGGATGTGACTTCGACTTTCGACTTGCAGGC
E2A3FOR : GGCAGGAAATGGGATGTGACTTCGACTTTCGAGCAAG
E2F3FOR : GGCAGGAAATGGGATGTGACTTCGACTTTCGACTTGACGAGACG

**Supplementary Table 1.** Primer combinations used in the construction of the chimeric carrier proteins

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**Site-directed Mutagenesis (Quickchange)**

Site-directed mutagenesis was performed on constructs as necessary (many clones contained mutations from the PCR ligation, and some required two rounds of mutagenesis). PCR conditions were as follows: 100 ng template, 1 µM each primer, 1x Pfu Ultra Buffer, 200 µM each dNTP, and 2.5 units Pfu Ultra (Stratagene).

Thermocycling conditions were as follows:
95°C for 2 minutes
16 cycles of: 95°C for 1 minute, 52°C for 1 minute, 68°C for 12 minutes
Holding at 4°C
PCR reactions were incubated with 1 µL DpnI (NEB) overnight at 37ºC. 5 µL each reaction was transformed into Subcloning Efficiency DH5a competent cells (Invitrogen) and plated on LB/AMP plates.

**Mutagenesis Primers**

EntBqc1: GCGCATGATGGAGCTGGCGGCGCGCTGGCGG
EntBqc2: GCCGCCAGCTCCATCATGCGCACCAGATCC

For constructs: A2, A6
A2QC1: GACACCGTTGAGCTGGTAATGGCTCTGGAAG 3’
A2QC2: CCATTACCAGCTCAACGGTGTCAAGAGAATC 3’
For construct: A3
QCA3A: CCAACAATGCTTTCTTGTTGAGACCTTG
QCA3B: CAACGAAAGAAGCATTTGTGTAACTTCTTC
For construct: A4
QC16A: GTGCGACCTCGCCGAGAACTCGACAGAGCCTTCTGGAAG
QC16B: GCGCTGTCGAGTTCTCAGGAGGTCCACGCTGTGC
For constructs: A4, E2, E9, F3
F1QC1 : CATATGAGCGCACTGACCGTCGACGACCTC 3’
F1QC2 : CGACGGTGTCAGTGCGCTCATATGTATATCTC 3’
For construct: E2
F1QC3: CGACCTCAAGAAACTGCTCGCCGAGACCAGC
F1QC4: GAGCAGTTTCTTGAGGTCGTCGACGGTCAG
For construct: E3
F3QC3 : CGGCCACCGCCTCGACACACACCACCACCACC
F3QC4: GGTGCTCGAGGGCGGTGGCGGAGGTCGC
For construct: E4
QCE4A: CAATGCTTTCTTGATGACGCAGCAACCTGATC
QCE4B: GTGCTCATCGAAAGAAGCATTTGTGTAAC
For constructs: E4, E7
E2QC1 : CGCATGTGGCGCTGGCGGCGCGCTGGCGG
E2QC2 : CCATTACCAGCTCAACGGTGTCAAGAGAATC 3’
For construct: E5
E3QC1: GCGAAAAACCCGACCATCGACGCCTGGTGGAAG
E3QC2: GGCGTCGATGGTCGGGTTTTTCGCCAGCATGAC
For construct: E8
QCE8A: GGTGATATCGCGCTGACTGACGAGACGGTC
QCE8B: CAGTCAGCGCAGATATCACCAGCAGACTTTC
For construct: E8
F3QC1 : CGCGAACTGCTCGAGCGAGGTCAACACCACC
F3QC2 : CCTCGCTGAGCAGTTGCTGGCGGCGG
For constructs: F5, F8
F2QC1 : CCTCGCGCTGAGCAGCGGCGGCGGCGG
F2QC2 : GGCGCTTCAGCGCGAGGAGGTGAG

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For construct: F6
F2QC3B: CTACGACTCCCTCGCGCTGCTGGAGACGGCCGCCGTGCTCCAG
F2QC4B: GGC GGCCGTCTCCAGCAGCGCGAGGGAGTCGTAGCCGAGGTCC

Supplementary Figure 1. SDS-Page gel of purified AcpP-helix II mutants
Supplementary Figure 2. SDS-Page gel of purified FrnN-helix II mutants
Supplementary Figure 3. SDS-Page gel of purified ΔEntB-helix II mutants

References