SUPPORTING INFORMATION FOR


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**SEER peptide design.**

LacA portion of β-lactamase was constructed by PCR using 5’-GAGGAGGAGGGATC CCACCCAGAAACGCTGGTG-3’ as the forward primer and 5’-CTCCTCCTGCAGG CCAGTTAATAGTTTGCGCAACGTTTGCCATTTGCTACAGGAGTCG-3’ as the reverse primer, using pQE-30 (Qiagen) as the template. The reverse primer carried a mutation that gave an M182T conversion to further stabilize the fold of the peptide. The PCR product was purified over QIAquick PCR purification column (Qiagen). The purified product and a pMAL-c2x plasmid carrying ZnFn Zif268 with N-terminal 15aa linker were digested with PstI and BamHI for 2 hours at 37°C using NEB Buffer 2 (New England Biolabs; 10 mM Tris- HCl/ pH 7.9, 10 mM MgCl₂, 50 mM NaCl, 1 mM DTT). The digested products were visualized on a 1% agarose gel in TAE (40 mM Tris, pH 8.0, 20 mM Acetic Acid, 1 mM EDTA) at 100 V for 45 min (described in Sambrook and Russell, 2001, Chapter 5). The appropriate bands were cut from the gel and DNA was extracted using Montage columns (Millipore). The digested and purified vector and insert were ligated overnight at room temperature with T4 ligase (Promega) in 10 µl reaction volume and 2 µl of the ligation product was transformed into Top 10 cells (Invitrogen).

LacB portion of β-lactamase was generated by PCR using 5’-GAGGAGGAGGACCGGTGGGGTGAGGCGGTGGGGGTTCTGGTGGGGGTGGTACCCTACTTCTAGCTTCCCGGC -3’ as the forward primer and 5’-CTCCTCCTCAAGCTTCCAATGCTTAATCAGTGAGGC-3’ as the reverse primer. The forward primer carried a sequence coding for the15aa (GGGGS)₃ linker N-terminal of the LacB. The remaining procedures were similar with the construction of LacA-Zif268 except that LacB was cloned into C-terminal of pMAL-c3x vectors carrying either PBSII or PE1A ZnFn using AgeI and HindIII sites. The configuration and orientation of the SEER system is shown in Supplementary Figure 1. The full, annotated sequences of the proteins is offered in Supplementary Figure 2.

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Supplementary Figure 1: Configuration and orientation of LacA-Zif268 and PBSII–LacB constructs.
Ooi et al., SEER-LAC Supporting Information

CAG AAG CCC TTC CAG TGC CGC ATC TGC ATG CTC AAC TTC AGC AGC AGC GAC CAC CTC ACC
GTG TTC GGG AAG GTC AGC GCG ATC AGC TAG CTC AGG TGG GTC TGG CTC GAG TGG
Q K P F Q C R I C M R N F S R S D H L T>

Finger 2 - TGG

ACC CAC ATC CGC ACC AAC AAG CCC TCC GAG GTC TGG GGT TGC ATG CTC ACC
TGG GTG TAG GCG TGG TGG TAG CTT GCG CCG ATG AAG CCG AGG CAG CTT AGC
TH I R T H G E K P F A C D I C G R K>

AgeI

790 800 810 820 830 840
TTT GCC AGG AGC GAT GAA CGC AAG AGG CAT ACC AAC ATC CAC ACC GGT GAG CAG CAG AAG CTT
AAA CGG TCC TCG CTA CTT GCG TCG TCC GGT TGG TCC TAG TGG TGG CCA CTC GTC TTC GAA
F A R S D E R K R K I H T G E Q R L

Finger 3 - GCG

HindIII

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TGG GTG TAG GCG TGG TGG TAG CTT GCG CCG ATG AAG CCG AGG CAG CTT AGC
TH I R T H G E K P F A C D I C G R K>

AgeI

790 800 810 820 830 840
TTT GCC AGG AGC GAT GAA CGC AAG AGG CAT ACC AAC ATC CAC ACC GGT GAG CAG CAG AAG CTT
AAA CGG TCC TCG CTA CTT GCG TCG TCC GGT TGG TCC TAG TGG TGG CCA CTC GTC TTC GAA
F A R S D E R K R K I H T G E Q R L

Finger 3 - GCG

HindIII

PBSII-LacB: 1 to 588

BamHI XmaI

10 20 30 40 50 60
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Finger 1 - AAA

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C G K S F S R S D H L T T H Q R T H T G>

Finger 2 - TGG

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CTC TCC GGT ATG TTT ACA GGT CTT ACA ACG TCC AGA AAG AGG GCG TCG CTA CAC GAC CAC
E K P Y K C P E C G K S F S R S D H L T T H Q R T H T G>

Finger 3 - GTC

AgeI

250 260 270 280 290 300
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GGG GGG TGG TGG TGG TGG CCA TGG TGG CCA CCA CCC CCA CCC CCA CCC CCA CCC CCA CCC CCA CCC
G R H Q R T H T G C G S G G G G G S G G G G G>

KpnI 15aa Linker

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CCG CCA TGG GAT GAA GTA GAT GAG GCG GCC GTC TTT ATG TAT CTT GAC TAC GCC CTA
G T L L L T L A S R Q Q L I D W M E A D>

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PE1A-LacB: 1 to 588

BamHI XmaI AgeI KpnI

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K   V   A   G   P   L   R   S   A   L   P   A   G   W   F   I   A   D   K>
Supplementary Figure 2: Full, annotated sequences of the SEER fragments used in this study.

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Hairpin oligonucleotide DNA target design.

Hairpin oligonucleotide DNA targets used in this study had the general sequence shown in Supplementary Figure 3, where $X_1aX_1aX_1a$ is a three nucleotide subsite for zinc finger 1, and $X_1a'X_1a'X_1a'$ is its complement. A 4 nt hairpin was formed by four thymidines. Between the 9 bp binding sites for the two zinc finger proteins was spacer of 0, 6 or 10 bp, indicated as $(N)_{\text{spacerg}}$. The full sequence of all target site DNAs used in this study are shown in Supplementary Table 1. For simplicity, only the top strand (3’ end of the hairpin oligonucleotide) is shown.

Supplementary Figure 3: Hairpin oligonucleotide DNA target design

Supplementary Table 1: Full sequence of hairpin oligonucleotide DNA targets

1. For simplicity, only the top strand (3’ end of the hairpin oligonucleotide) is shown.
2. Zif268 target site shown in blue, G->T mutations shown in black
3. PBSII target site shown in red, PE1A target site shown in green.
No background due to pMAL-expressed β-lactamase.
All proteins were expressed from the vector pMAL-c2X (New England Biolabs). This plasmid also expresses a full-length β-lactamase as an antibiotic resistance marker. The full-length β-lactamase does not have an MBP-tag, and would therefore not be retained after protein purification. To ensure that trace contamination of the pMAL-C2X-expressed full-length β-lactamase was not contributing to background hydrolysis, samples containing individual LacB-PBSII and target DNA were incubated with the nitrocefin substrate under the standard assay conditions. No detectable hydrolysis was observed over the assay interval (Supplementary Fig. 4).

Supplementary Figure 4: Raw data from a nitrocefin assay showing SEER-Lac protein fragments with various concentrations of oligonucleotide targets. Top row contains both LacA-Zif268 and PBSII-LacB with indicated amount of Zif-n-PBSII DNA. Bottom row contains only PBSII-LacB with the same DNA. This image was captured after 30 min of incubation at room temperature.

References: