Published in final edited form as:

Biochemistry. 2006 March 21; 45(11): 3620–3625. doi:10.1021/bi0517032.

SEquence-Enabled Reassembly of β -Lactamase (SEER-LAC):

a Sensitive Method for the Detection of Double-Stranded DNA†

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Abstract

This work describes the development of a new methodology for the detection of specific double-stranded DNA sequences. We previously showed that two inactive fragments of green fluorescent protein, each coupled to engineered zinc finger DNA-binding proteins, were able to reassemble an active reporter complex in the presence of a predefined DNA sequence. This system, designated SEquence-Enabled Reassembly (SEER), was demonstrated in vitro to produce a DNA-concentration-dependent signal. Here we endow the SEER system with catalytic capability using the reporter enzyme TEM1 β -lactamase. This system could distinguish target from non-target DNA in less than 5 minutes, representing a more than 1,000-fold improvement over our previous SEER design. A single base pair substitution in the DNA binding sequence reduced the signal to nearly background levels. Substitution of a different custom zinc finger DNA-binding domain produced a signal only on the new cognate target. Signal intensity was not affected by genomic DNA when present in equal mass to the target DNA. These results present SEER as a rapid and sensitive method for the detection of double-stranded DNA sequences.

Keywords

zinc finger; beta-lactamase; diagnostics; biosensors

Virtually all scientific methods for reading the sequence information of DNA rely on the hybridization properties of complementary nucleic acid molecules. Such methods, including PCR, Sanger sequencing, DNA microarray, Southern and Northern blotting, and in situ hybridization, all consequently require denaturation of the native DNA double helix into single strands and subsequent renaturation with specific primers or probes under carefully controlled conditions. In contrast, nature frequently relies on sequence-specific DNA-binding proteins to read the sequence information of DNA, such as occurs during the processes of transcription initiation, intron homing, and defense against invasive DNA by restriction endonucleases. In the human genome, DNA-binding transcription factors comprise one of the largest classes of

[†]D.S. was supported by American Cancer Society IRG7400125 and NIH P50CA95060. C.I.S. was supported by an NIH training grant.

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BRIEFS: A split protein system composed of the reporter enzyme β -lactamase and designed zinc-finger DNA-binding domains can detect specific double-stranded DNA sequences in less than five minutes.

SUPPORTING INFORMATION AVAILABLE: Details of the SEER-LAC protein fragment design, construction, and an annotated sequence, as well a full description of the oligonucleotide DNA targets used in this study and additional information on assay background are available free of charge via the Internet at <http://pubs.acs.org>.

known genes, with approximately 2,000 members (1). The most common type of DNA-binding domain is the Cys2-His2 class of zinc fingers.

Here we describe the development of a new methodology that may be readily applicable for the detection of specific double-stranded (ds) DNA sequences. This system, designated SEER (SEquence-Enabled Reassembly), consists of split-protein systems that are able to reassemble an active complex only in the presence of a cognate DNA sequence. This approach merges two rational protein design approaches, the ability to dissect proteins for complementation assays (PCA) and the ability to design custom zinc fingers (ZF) to most DNA targets in a rule-based manner.

PCA is a methodology initially described for detecting protein-protein interactions (2,3). A functional protein, typically a reporter molecule, is dissected into two non-functional fragments. Functionality is restored when the fragments are reassembled by attached protein-protein interaction domains, such as leucine zippers. Several such systems have been recently reported, including reassemblies of β -galactosidase (3), dihydrofolate reductase (DHFR) (4), green fluorescent protein (GFP) (5), TEM-1 β -lactamase (6), and firefly luciferase (7,8).

Custom DNA-binding proteins can be constructed from modified Cys2-His2 ZF DNA-binding domains. Each ZF domain contains 30 amino acids that form a $\beta\beta\alpha$ fold, stabilized by hydrophobic interactions and the chelation of a zinc ion between two histidines and two cysteines. Each domain typically recognizes 3-4 nucleotides of DNA. The domains can be found in covalent tandem arrays, facilitating recognition of extended DNA sequences. A protein containing six zinc fingers should have the capacity to recognize 18-base pairs of DNA, sufficiently large to specify a unique site in the human genome (9). A variety of combinatorial and rational design approaches have been used to modify the binding specificity of naturally occurring ZFs (10-13). In particular, Barbas and co-workers have produced a lexicon of interchangeable domains with the ability to recognize unique 3-4-base pair DNA sequences (14). Using these pre-defined recognition modules, DNA-binding proteins can be rapidly assembled to bind virtually any DNA-sequence or gene in the human genome (15). The three-finger proteins typically have affinities in the 1-50 nM range and are highly specific for their target site (9). These custom-made ZF proteins can be linked to functional domains to generate novel chimeric proteins that produce the desired activity at specific DNA sequences. This approach has been employed in designing targeted transcription factors (14,16), targeted endonucleases (17), and targeted integrases (18).

Previously, we demonstrated the chromophore regeneration of dissected ZF-linked fragments of GFP upon forming a ternary complex in the presence of a specific DNA sequence (SEER-GFP) (19). One advantage of the SEER-GFP approach was an extremely low background, due to the inability of the two unfolded GFP fragments to spontaneously reassemble. The signal produced by SEER-GFP was expected to be one GFP molecule for every copy of the target site. While this may be desirable in some instances, for example to have a signal that would be linearly proportional to target copy number, this feature may limit the use of SEER-GFP for the detection of low copy number sequences. In addition, poor protein solubility coupled with a slow rate of chromophore formation required assay times of 72-96 hours for maximal SEER-GFP signal intensity.

In the current study, we extend the SEER concept to the sequence-specific reassembly of an enzymatic activity, *E. coli* TEM-1 β -lactamase (SEER-LAC). β -lactamase is a promising candidate for SEER. This enzyme has previously been shown in a PCA approach (6). Its structure and function are well characterized. It is small (29kD), can be easily expressed, and is non-toxic to both prokaryotic and eukaryotic cells. Eukaryotic cells do not contain an endogenous β -lactamase activity. Its activity can be easily assayed by the hydrolysis of

nitrocefin, which changes from yellow to red (peak absorbance at 486nm) when the β -lactam ring is hydrolyzed by β -lactamase. We demonstrate that a SEER system can be generated by dissecting TEM-1 β -lactamase into two-halves with each fragment linked to a ZF domain. These protein fragments can be functionally reassembled when brought into proximity in the presence of target DNA sequences for the ZFs (Figure 1). The enzymatic signal amplification enables the sensitive detection of even a single base substitution within an 18-bp site in under 5 minutes, representing a greater than 1,000-fold rate improvement over our previous design. These results show the possibility of utilizing an enzymatic reporter protein to create a SEER system that has improved potential for further development into a useful detection tool.

EXPERIMENTAL PROCEDURES

Cloning, expression, and purification of the proteins

E. coli TEM-1 β -lactamase DNA was obtained by PCR using the bacterial expression vector pMAL-c2X (New England Biolabs) as the template. LacA (aa26-aa196) and LacB (aa198-aa290) were cloned into separate pMAL-c2X vectors using standard cloning procedures (see Supporting Information online). LacA contained an M182T mutation to enhance the stability of the protein (6). ZF proteins were constructed by PCR using overlapping primers. Zif268 was cloned C-terminal to LacA, whereas PBSII and PE1A were cloned N-terminal to LacB. The ZF and Lac domains were separated by a 15-aa linker, (GGGGS)₃. Protein was expressed in BL-21 Star cells (Invitrogen). 100 μ M of ZnCl₂ was added to 100 ml of LB growth media. At OD₆₀₀ = 0.6-0.8, protein expression was induced with 1 mM isopropyl β -D-thiogalactoside (IPTG) for 5 hours at 37°C. Cells were pelleted and resuspended in zinc buffer A (ZBA: 100 mM Tris base, 90 mM KCl, 1 mM MgCl₂, 100 μ M ZnCl₂, pH 7.5)/5 mM DTT. The MBP-tagged proteins were purified over amylose columns and eluted in ZBA/5 mM DTT/10 mM maltose, following the methodology of the Protein Fusion and Purification System (New England Biolabs).

Nitrocefin Assay

Hairpin oligonucleotide DNA target *Zif268-0-PBSII* had the sequence, 5'-GGC TTT CCA CAC CGC CCA CGC GGG TTTT CCC GCG TGG GCG GTG TGG AAA GCC-3', and *Zif268-0-PE1A* had the sequence, 5'-GGC GTT ATT TAT CGC CCA CGC GGG TTTT CCC GCG TGG GCG ATA AAT AAC GCC-3', where 5'-CGC TGG GCG-3', 5'-GTT TGG AAA-3' and 5'-ATA AAT AAC-3' are the target sites for ZFs Zif268, PBSII and PE1A, respectively. All oligonucleotides were heated to 95°C for 10 minutes at 10 μ M in ZBA, then slowly cooled to room temperature to form hairpins with a four-thymidine loop. A more complete description of the DNA targets use in this study is offered in the Supporting Information online.

In a 96-well plate, 120 μ l of ZBA was added to the wells followed by 20 μ l of 10 μ M, 200 nM, 2 nM, or no hairpin oligonucleotide DNA targets (1 μ M, 20 nM, 200 pM, 0 final concentration, Operon). For the experiment in Figure 5, the volume of ZBA was reduced 20 μ l, and 20 μ l of 160 μ g/ml sheared, double-stranded Herring Sperm DNA (HS-DNA, Invitrogen) was added. 20 μ l of 5 μ M protein fragments LacA-Zif268 and PBSII-LacB/PE1A-LacB (0.5 μ M final concentration each fragment) was added to the wells before adding 20 μ l of 1 mM nitrocefin (0.1 mM final concentration, Calbiochem). All steps were carried out at room temperature. Absorbance at 486 nm was monitored over 20 minutes with a SpectraMax M2 (Molecular Devices). The *relative signal* was determined by subtracting the background (samples containing no DNA) from triplicate measurements, then normalizing to the highest signal value. Error bars indicate the standard deviation of triplicate samples. To ensure that trace contamination of the pMAL-C2X-expressed full-length β -lactamase was not contributing to background hydrolysis, samples containing individual SEER fragments were incubated with

substrate. No detectable hydrolysis was observed over the assay interval (see Supporting Information online).

RESULTS

Protein design

SEER-LAC proteins consisted of two inactive fragments of β -lactamase fused to zinc finger proteins with the ability to recognize specific DNA sequences. The two fragments were designed to bind near each other at adjacent sites in the presence of a user-defined DNA target site to generate a signal. Two 3-finger ZF proteins binding in this way would have the collective capacity to recognize 18 bp of DNA, a target site sufficiently large to be unique in the human genome (9). However, since biologically relevant target sites could not be chosen until the optimal spacer and orientation parameters were established, initial experiments employed designed target sites that were recognized by existing, well-characterized ZF. Zif268 is a naturally occurring 3-finger ZF that has been extensively studied structurally and biochemically (20,21). It binds the 9 bp sequence 5'-GCG TGG GCG-3'. PBSII and PE1A are designed 3-finger ZFs assembled from predefined modified ZF domains (14,15), and recognize the sequences 5'-GTG TGG AAA-3' and 5'-ATA AAT AAC-3', respectively.

Two inactive fragments of the 290-amino acid TEM1 β -lactamase protein can be generated by splitting the protein between residues 196 and 198 (6). To maintain the correct polarity of the protein fragments, Zif268 was appended to the C-terminus of β -lactamase residues 26-196 (LacA-Zif268; lacking the N-terminal secretory signal sequence), and PBSII or PE1A was appended to the N-terminus of residues 198-290 (PBSII-LacB or PE1A-LacB). The ZF and β -lactamase domains were separated by a 15-aa linker, (GGGGS)₃ (Figure 1). All proteins were expressed from the vector pMAL-c2X, which additionally appended the 392-amino acid Maltose Binding Protein (MBP) to the N-terminus of all three protein fragments. All experiments were performed with the MBP domain attached.

DNA-dependent enzyme activity

β -lactamase activity assays were conducted using the colorimetric substrate nitrocefin, which changes from yellow to red (486 nm) upon hydrolysis. Based on similar studies with chimeric ZF-endonucleases (see Discussion), we expected the spacing between the two ZF sites ("spacer") on the target DNA to be crucial for efficient enzyme reassembly. To examine this relationship, nitrocefin assays were performed in triplicate with 0.5 μ M of each LacA-Zif268 and PBSII-LacB proteins in the presence of hairpin oligonucleotides containing the two target sites at spacer lengths of 0, 6, and 10 bp (labeled *Zif-0-PBSII*, *Zif-6-PBSII*, and *Zif-10-PBSII* in Figure 2), at concentrations of 1 μ M, 20 nM, 200 pM, and no DNA as a control. DNA-assisted enzyme reassembly was shown with all three spacer lengths (Figure 2A). The relative signal intensity after 20 minutes of incubation was highest for the 0 bp spacer, followed by 10 and 6 bp (Figure 2B). The signal intensity was generally proportional to the DNA concentration for all spacings, although a slightly higher correlation was observed for the 0 bp spacer, followed by 10 and 6 bp (R^2 correlation coefficients of 0.986, 0.971 and 0.887, respectively).

A kinetic analysis of the reaction using the 0 bp spacer (Figure 2C) revealed hydrolysis rates of 25 mU/min at 1 μ M DNA (red), 20 mU/min at 20 nM (orange), 7 mU/min at 200 pM (yellow), and a background rate of 6 mU/min with no DNA (black). A hydrolysis rate of 3 mU/min was observed for the negative control of LacA-Zif268 paired with PE1A-LacB on the above target DNA (green). A difference in the relative signal intensity between the 1 μ M DNA sample and background was distinguishable by the earliest time point in our assay (3 minutes), and became more pronounced over time. All hydrolysis rates were linear over the 23 minute assay interval, with correlation coefficients generally greater than 0.99.

Effects of target site mutations on the SEER signal intensity

In order to determine the sensitivity of SEER to mutations, nitrocefin assays were performed using oligonucleotide targets carrying different mutations on either one or both of the ZF binding sites (Figure 3). At 1 μ M DNA concentration and 0.5 μ M each protein, a single mutation in the Zif268 target site reduced the relative signal 90% to nearly background levels. A single base pair mutation in the PBSII target site resulted in a 36% reduction in relative signal intensity. Target sites carrying two or more mutations also lowered the relative signal levels by at least 90%.

SEER binding domains are interchangeable

In order to demonstrate the generality of SEER-LAC to target significantly different binding sites, a nitrocefin assay was performed with two different DNA target sequences (Figure 4). One target contained Zif268 and PBSII binding sites with no spacer (*Zif-0-PBSII*, black bars) and the other contained Zif268 and PE1A sites with no spacer (*Zif-0-PE1A*, white bars). Both SEER combinations reassembled in the presence of their cognate DNA sequences. Inappropriate target DNA produced a signal similar to the background signal of no target DNA.

SEER binding in the presence of genomic DNA

The previous experiments were conducted with purified DNA targets. However, some applications of this technology might require it to recognize its target in the presence of complex DNA, such as a genome, which might contain multiple alternative sites for the individual SEER proteins. To investigate if the presence of complex double-stranded DNA would interfere with this assay, a nitrocefin assay was performed in the presence or absence of herring sperm DNA (HS-DNA). The concentration of HS-DNA used was equimolar in base pairs (ie: equal in mass) to 1 μ M of the oligonucleotide target DNA. Under these conditions, there was no difference in relative signal intensity when 0.5 μ M each of LacA-Zif268 and PBSII-LacB proteins were incubated with 1 μ M of *Zif-0-PBSII* target DNA in the presence or absence of HS-DNA (Figure 5, black bars). As a negative control, the proteins were also incubated with the *Zif-0-PE1A* target DNA (white bars). Although the relative signal generated using this target was somewhat higher than in previous assays, there was essentially no change in the signal intensity in the presence of HS-DNA.

DISCUSSION

In this study, the SEER-LAC system produced a DNA-concentration-dependent signal. Signal amplification remained linear over the assay time, and target DNA could be distinguished from non-target DNA in less than 5 minutes. The proteins were expressed from the plasmid pMAL-C2X, which appends an MBP domain to the N-terminus. Based on previous experience (9, 22,23), the presence of the MBP domain was not expected to interfere with SEER binding or activity. In fact, the MBP domain may have contributed to the solubility of these protein fragments, facilitating their use for in vitro assays. In our previous studies with a SEER-GFP system (19), GFP-containing fragments were insoluble, requiring an extensive (48 hours) renaturation process, followed by a lengthy (24-48 hours) incubation for chromophore formation. The use of soluble protein fragments, coupled with the enzymatic signal amplification provided by β -lactamase, enabled the detection of DNA in less than 5 minutes. This enhancement represents a greater than 1,000-fold time improvement over our previous design.

A 15-aa linker between the zinc fingers and the β -lactamase domains was originally chosen to avoid possible steric clashes. A linker of this length may be useful for applications in which the target sites are separated by 1 to 10 bp. However, future SEER variations may benefit from the use of shorter linkers. Insights can be gained from studies of chimeric ZF endonucleases

(24). In this analogous system, two monomers of the dimeric endonuclease FokI are brought together by DNA-binding ZF domains to create an active cleavage enzyme. The optimal DNA spacing between the target sites was found to be related to the length of the protein linker. Long linkers (15 aa) allowed greater flexibility in the spacer size between the ZF binding sites. However, reducing the linker length to 0 aa improved the specificity of interaction (allowing reassembly only at 6 bp) and improved cleavage efficiency. In the current study, hydrolysis rates were highest on the targets with 0 and 10 bp spacers, with 0 bp providing the maximal signal. Thus, maximum efficiency was achieved with the current SEER design when the β -lactamase fragments were positioned on the same face of the DNA helix and close together. As in the endonuclease studies, shorter linkers might provide a more rigid positioning of the β -lactamase fragments, resulting in more efficient reassembly and less tolerance to variations in target site spacing.

A single base pair substitution in the Zif268 DNA-binding sequence reduced the relative signal 10-fold to background levels, demonstrating the sensitivity of SEER to detect point mutations. However, a similar substitution in the PBSII site reduced the relative signal by only 36%. This discrepancy likely results from differences in the binding properties of the two ZF proteins. Interestingly, the mutant site was recognized by the same ZF domain in both fragments. However, other differences, such as inter-domain amino acid interactions and sequence-dependent DNA deformation, may influence specificity. These results identify the quality of the ZF-DNA interaction as an important determinant of SEER function. Generally, the specificity of designed zinc finger proteins has been shown to be excellent (9,11,25).

Substitution of a different custom zinc finger DNA-binding domain generated a new SEER that produced a signal only on the new cognate target. These results clearly demonstrate that the observed effects were not due to special properties of ZFs Zif268 and PBSII, but can be achieved using other custom ZFs, such as PE1A. This feature suggests that SEERs can be rapidly designed to detect virtually any desired DNA sequence using custom ZF DNA-binding technology (14,15).

In summary, we have demonstrated the use of TEM-1 β -lactamase to improve the sequence-enabled enzyme reactivation method for the detection of double-stranded DNA. The assay can detect its target sequence rapidly and with high specificity. The technology could be advantageous for certain applications, particularly those for which purified DNA is not limiting. For example, SEER-LAC might be useful for the rapid detection of specific sequences in amplified DNA. The linear relationship between signal and DNA target concentration suggests SEER could be developed for the quantitative determination of target copy number, or for measuring the length of repeated sequences such as telomeres. In principle, the ability to directly detect double-stranded DNA could provide some novel capabilities. However, further studies on the range and utility of the SEER-LAC system will be necessary before its general applicability can be assessed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

ZF PBSII was kindly supplied by Carlos F. Barbas III. We thank Carlos F. Barbas III and members of the Ghosh and Segal lab for helpful discussions.

ABBREVIATIONS

ZF, zinc finger; GFP, green fluorescent protein; MBP, maltose binding protein; HS-DNA, herring sperm DNA; ZBA, zinc buffer A.

REFERENCES

1. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, et al. The sequence of the human genome. *Science* 2001;291:1304–51. [PubMed: 11181995]
2. Johnsson N, Varshavsky A. Split ubiquitin as a sensor of protein interactions in vivo. *Proc Natl Acad Sci U S A* 1994;91:10340–4. [PubMed: 7937952]
3. Rossi F, Charlton CA, Blau HM. Monitoring protein-protein interactions in intact eukaryotic cells by beta-galactosidase complementation. *Proc Natl Acad Sci U S A* 1997;94:8405–10. [PubMed: 9237989]
4. Pelletier JN, Campbell-Valois FX, Michnick SW. Oligomerization domain-directed reassembly of active dihydrofolate reductase from rationally designed fragments. *Proc Natl Acad Sci U S A* 1998;95:12141–6. [PubMed: 9770453]
5. Ghosh I, Hamilton AD, Regan L. Antiparallel leucine zipper-directed protein reassembly: application to the green fluorescent protein. *J. Am. Chem. Soc* 2000;122:5658–5659.
6. Galarneau A, Primeau M, Trudeau LE, Michnick SW. beta-Lactamase protein fragment complementation assays as in vivo and in vitro sensors of protein protein interactions. *Nat Biotechnol* 2002;20:619–22. [PubMed: 12042868]
7. Ray P, Pimenta H, Paulmurugan R, Berger F, Phelps ME, Iyer M, Gambhir SS. Noninvasive quantitative imaging of protein-protein interactions in living subjects. *Proc Natl Acad Sci U S A* 2002;99:3105–10. [PubMed: 11854471]
8. Paulmurugan R, Umezawa Y, Gambhir SS. Noninvasive imaging of protein-protein interactions in living subjects by using reporter protein complementation and reconstitution strategies. *Proc Natl Acad Sci U S A* 2002;99:15608–13. [PubMed: 12438689]
9. Segal DJ, Beerli RR, Blancafort P, Dreier B, Effertz K, Huber A, Koksche B, Lund CV, Magnenat L, Valente D, Barbas CF III. Evaluation of a modular strategy for the construction of novel polydactyl zinc finger DNA-binding proteins. *Biochemistry* 2003;42:2137–2148. [PubMed: 12590603]
10. Segal D, Barbas CF III. Custom DNA-binding proteins come of age: polydactyl zinc-finger proteins. *Curr. Opin. Biotech* 2001;12:632–637. [PubMed: 11849947]
11. Liu Q, Xia Z, Zhong X, Case CC. Validated zinc finger protein designs for all 16 GNN DNA triplet targets. *J Biol Chem* 2002;277:3850–6. [PubMed: 11726671]
12. Lee DK, Seol W, Kim JS. Custom DNA-binding proteins and artificial transcription factors. *Curr Top Med Chem* 2003;3:645–57. [PubMed: 12570857]
13. Klug A. Towards therapeutic applications of engineered zinc finger proteins. *FEBS Lett* 2005;579:892–4. [PubMed: 15680969]
14. Blancafort P, Segal DJ, Barbas CF 3rd. Designing transcription factor architectures for drug discovery. *Mol Pharmacol* 2004;66:1361–71. [PubMed: 15340042]
15. Segal DJ. The use of zinc finger peptides to study the role of specific factor binding sites in the chromatin environment. *Methods* 2002;26:76–83. [PubMed: 12054906]
16. Jamieson AC, Miller JC, Pabo CO. Drug discovery with engineered zinc-finger proteins. *Nat Rev Drug Discov* 2003;2:361–8. [PubMed: 12750739]
17. Carroll D. Using nucleases to stimulate homologous recombination. *Methods Mol Biol* 2004;262:195–207. [PubMed: 14769963]
18. Tan W, Zhu K, Segal DJ, Barbas CF 3rd, Chow SA. Fusion proteins consisting of human immunodeficiency virus type 1 integrase and the designed polydactyl zinc finger protein E2C direct integration of viral DNA into specific sites. *J Virol* 2004;78:1301–13. [PubMed: 14722285]
19. Stains CI, Porter JR, Ooi AT, Segal DJ, Ghosh I. DNA sequence-enabled reassembly of the green fluorescent protein. *J Am Chem Soc* 2005;127:10782–3. [PubMed: 16076155]

20. Elrod-Erickson M, Rould MA, Nekludova L, Pabo CO. Zif268 protein-DNA complex refined at 1.6 Å: a model system for understanding zinc finger-DNA interactions. *Structure* 1996;4:1171–1180. [PubMed: 8939742]
21. Wolfe SA, Nekludova L, Pabo CO. DNA recognition by Cys2His2 zinc finger proteins. *Annu Rev Biophys Biomol Struct* 2000;29:183–212. [PubMed: 10940247]
22. Segal DJ, Dreier B, Beerli RR, Barbas CF III. Toward controlling gene expression at will: selection and design of zinc finger domains recognizing each of the 5'-GNN-3' DNA target sequences. *Proc Natl Acad Sci U S A* 1999;96:2758–2763. [PubMed: 10077584]
23. Beerli RR, Segal DJ, Dreier B, Barbas CF III. Toward controlling gene expression at will: specific regulation of the erbB-2/HER-2 promoter by using polydactyl zinc finger proteins constructed from modular building blocks. *Proc Natl Acad Sci U S A* 1998;95:14628–14633. [PubMed: 9843940]
24. Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, Chandrasegaran S. Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. *Mol Cell Biol* 2001;21:289–97. [PubMed: 11113203]
25. Tan S, Guschin D, Davalos A, Lee YL, Snowden AW, Jouvenot Y, Zhang HS, Howes K, McNamara AR, Lai A, Ullman C, Reynolds L, Moore M, Isalan M, Berg LP, Campos B, Qi H, Spratt SK, Case CC, Pabo CO, Campisi J, Gregory PD. Zinc-finger protein-targeted gene regulation: genomewide single-gene specificity. *Proc Natl Acad Sci U S A* 2003;100:11997–2002. [PubMed: 14514889]
26. Choo Y, Klug A. Selection of DNA binding sites for zinc fingers using rationally randomized DNA reveals coded interactions. *Proc. Natl. Acad. Sci. U. S. A* 1994;91:11168–72. [PubMed: 7972028]

Figure 1.

The SEER-LAC strategy. LacA-Zif268 comprises residues 26-196 of β -lactamase (green) fused by a 15-aa linker (orange) to the DNA binding ZF Zif268 (blue). PBSII-LacB comprises the ZF PBSII (red) fused by a 15-aa linker (magenta) to residues 198-290 of β -lactamase (yellow). In the presence of target DNA containing binding sites for Zif268 (cyan) and PBSII (pink) with an appropriate spacer (0-bp spacer is shown), SEER fragments reassemble to form an active reporter enzyme.

Figure 2.

The DNA concentration-dependent SEER signal. A) Digital image of triplicate nitrocefin assays after 30 minutes incubation. DNA target oligonucleotides (with target site spacings of 0, 6 and 10 bp) and their concentrations are indicated above the image; SEER fragments (0.5 μ M each) are indicated to the left. B) Relative Signal vs. DNA concentration plot for the assay shown in A. Relative Signal (normalized and background subtracted) after 20 minutes of incubation was calculated on *Zif-0-PBSII* DNA (blue diamonds), *Zif-6-PBSII* DNA (gray squares), and *Zif-10-PBSII* DNA (purple circles) as described in the Experimental Procedures. C) Kinetic data for the reactions with *Zif-0-PBSII* DNA. Absorbance at 486 nm was measured at 3 minutes and every 2 minutes after. Raw data were plotted for LacA-Zif268 & PBSII-LacB with 1 μ M DNA (red diamonds), 20 nM (orange diamonds), 200 pM (yellow diamonds), and no DNA (black triangles), as well as the non-cognate LacA-Zif268 & PE1A-LacB (green diamonds) with 1 μ M *Zif-0-PBSII* DNA. Hydrolysis rates were calculated as the slope of the linear fit of the kinetic data (solid lines).

Figure 3.

Sensitivity of SEER to mutations in the target DNA. A) Digital image of triplicate nitrocefin assays after 30 minutes incubation. A series of modified *Zif-0-PBSII* target oligonucleotides were used at 1 μ M, containing 1, 2, 3 or 5 G to T substitutions (boxed) in the Zif268 (blue) or PBSII (red) binding sites, as indicated. SEER fragments LacA-Zif268 & PBSII-LacB were used at 0.5 μ M each. B) Graphical representation of the relative signal intensities after 20 minutes of incubation for the assay shown in A.

Figure 4.

SEER activity using various combinations of ZF binding domains and DNA targets. The relative signal intensities after 20 minutes of incubation for triplicate nitrocefin assays are shown. Target oligonucleotides at 1 μ M are indicated above the graph (black and white bars); SEER fragments at 0.5 μ M each are indicated below.

Figure 5. SEER binding in the presence of genomic DNA. LacA-Zif268 & PBSII-LacB at 0.5 μ M each were incubated with 1 μ M *Zif-0-PBSII* (black bars) or 1 μ M *Zif-0-PE1A* (white bars) for 20 minutes in the presence or absence (as indicated) of 3.2 μ g of sheared, double-stranded Herring Sperm DNA. This concentration is equal in moles of base pairs (5.2 nmoles bp) to 1 μ M of the target oligonucleotide.