SUPPORTING INFORMATION

A zinc finger protein array for the visual detection of specific DNA sequences for diagnostic applications

Moon-Soo Kim[1], Gulnaz Stybayeva[2], Ji Youn Lee[2], Alexander Revzin[2], David J. Segal[1,†]

[1]*Department of Pharmacology and Genome Center,* [2]*Department of Biomedical Engineering,*

451 Health Sciences Drive, University of California, Davis, CA 95616

Sequences of hairpin DNA target oligonucleotides

All sequences written 5' to 3' with ZFP binding site shown in bold.

Target site1 for rrsA27 and rrsA62:
GAC **GAT TGA ACG CTG GCG GCA GGC CTA ACA CAT GCA AGT** GGG TTTT CCC **ACT TGC ATG TGT TAG GCC TGC CGC CAG CGT TCA ATC** GTC

Target site 2 for rrsA125 and rrsA160:
GAC **TGG GAA ACT GCC TGA TGG AGG GGG ATA ACT ACT GGA** GGG TTTT CCC **TCC AGT AGT TAT CCC CCT CCA TCA GGC AGT TTC CCA** GTC

Target site 3 for rrsA1175 and rrsA1192:
GAC **GAG GAA GGT GGG GAT GAC** GGG TTTT CCC **GTC ATC CCC ACC TTC CTC** GTC

Hairpin oligonucleotide of Zif268/PBSII site, used as an irrelevant control:
GGC TTT CCA CAC CGC CCA CGC GGG TTTT CCC **GCG TGG GCG GTG TGG AAA** GCC

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   1 AAATTGAAGA GTTTGATCAT GGCTCAGATT GAACGCTGGC GGCAGGCCTA
  51 ACACATGCAA GTCGAACGGT AACAGGAAGA AGCTTGCTTC TTTGCTGACG
 101 AGTGGCGGAC GGGTGAGTAA TGTCTGGGAA ACTGCCTGAT GGAGGGGGAT
 151 AACTACTGGA AACGGTAGCT AATACCGCAT AACGTCGCAA GACCAAAGAG
 201 GGGTACCTTC GGGCCTCTTG CCATCGGATG TGCCCAGATG GGATTAGCTA
 251 GTAGGTGGGG TAACGGCTCA CCTAGGCGAC GATCCCTAGC TGGTCTGAGA
 301 GGATGACCAG CCACACTGGA ACTGAGACAC GGTCCAGACT CCTACGGGAG
 351 GCAGCAGTGG GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCCATGC
 401 CGCGTGTATG AAGAAGGCCT TCGGGTTGTA AAGTACTTTC AGCGGGGAGG
 451 AAGGGAGTAA AGTTAATACC TTTGCTCATT GACGTTACCC GCAGAAGAAG
 501 CACCGGCTAA CTCCGTGCCA GCAGCCGCGG TAATACGGAG GGTGCAAGCG
 551 TTAATCGGAA TTACTGGGCG TAAAGCGCAC GCAGGCGGTT TGTTAAGTCA
 601 GATGTGAAAT CCCCGGGCTC AACCTGGGAA CTGCATCTGA TACTGGCAAG
 651 CTTGAGTCTC GTAGAGGGGG GTAGAATTCC AGGTGTAGCG GTGAAATGCG
 701 TAGAGATCTG GAGGAATACC GGTGGCGAAG GCGGCCCCCT GGACGAAGAC
 751 TGACGCTCAG GTGCGAAAGC GTGGGGAGCA AACAGGATTA GATACCCTGG
 801 TAGTCCACGC CGTAAACGAT GTCGACTTGG AGGTTGTGCC CTTGAGGCGT
 851 GGCTTCCGGA GCTAACGCGT TAAGTCGACC GCCTGGGGAG TACGGCCGCA
 901 AGGTTAAAAC TCAAATGAAT TGACGGGGGC CCGCACAAGC GGTGGAGCAT
 951 GTGGTTTAAT TCGATGCAAC GCGAAGAACC TTACCTGGTC TTGACATCCA
1001 CGGAAGTTTT CAGAGATGAG AATGTGCCTT CGGGAACCGT GAGACAGGTG
1051 CTGCATGGCT GTCGTCAGCT CGTGTTGTGA AATGTTGGGT TAAGTCCCGC
1101 AACGAGCGCA ACCCTTATCC TTTGTTGCCA GCGGTCCGGC CGGGAACTCA
1151 AAGGAGACTG CCAGTGATAA ACTGGAGGAA GGTGGGGATG ACGTCAAGTC
1201 ATCATGGCCC TTACGACCAG GGCTACACAC GTGCTACAAT GGCGCATACA
1251 AAGAGAAGCG ACCTCGCGAG AGCAAGCGGA CCTCATAAAG TGCGTCGTAG
1301 TCCGGATTGG AGTCTGCAAC TCGACTCCAT GAAGTCGGAA TCGCTAGTAA
1351 TCGTGGATCA GAATGCCACG GTGAATACGT TCCCGGGCCT TGTACACACC
1401 GCCCGTCACA CCATGGGAGT GGGTTGCAAA AGAAGTAGGT AGCTTAACCT
1451 TCGGGAGGGC GCTTACCACT TTGTGATTCA TGACTGGGGT GAAGTCGTAA
1501 CAAGGTAACC GTAGGGGAAC CTGCGGTTGG ATCACCTCCT TA
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Figure S-1. Location of three target regions (27-62 bp, 125-160 bp, and 1175-1192 bp) in *rrsA* gene (1542 bp). The target regions are high-lighted in yellow, light green, and cyan for ZFP rrsA27 and rrsA62, ZFP rrsA125 and rrsA160, and ZFP rrsA1175 and rrsA1192, respectively.

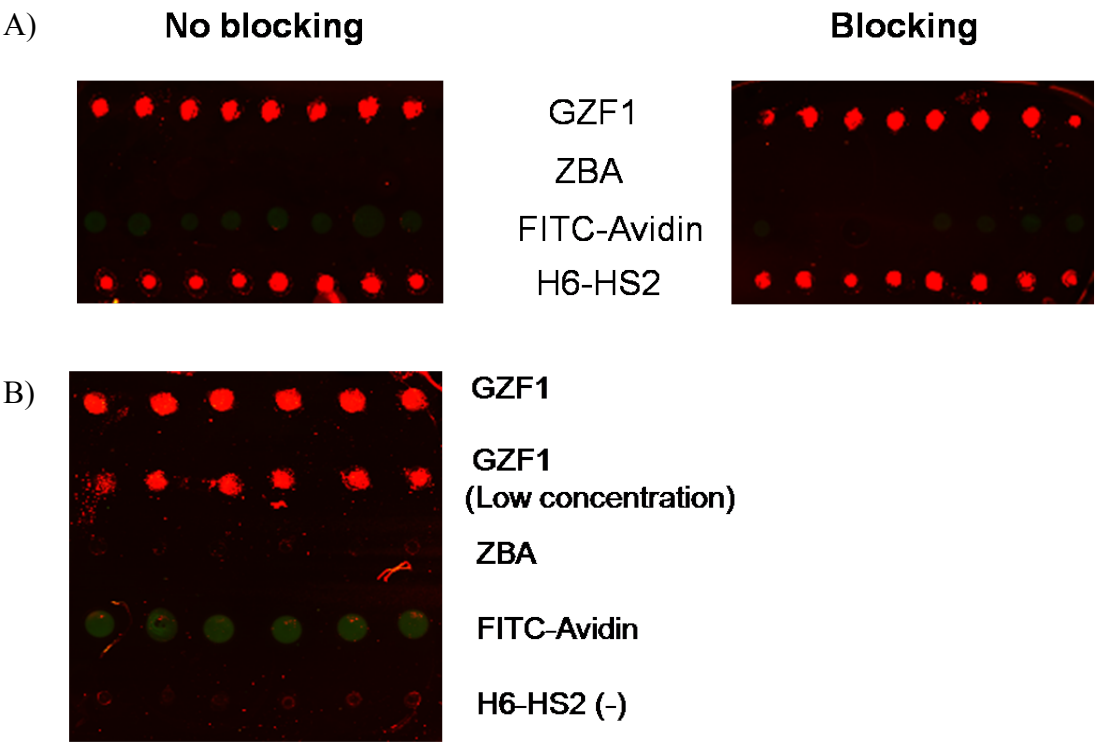

Figure S-2. (A) ZFP microarray to detect the immobilized GZF1 on the slide by Cy5-labeled secondary antibody. ZFP GZF1 fused with MBP (Maltose binding protein) was printed on the PEG gel slide. The slide was blocked with ZBA/1% BSA (Right panel) and washed with ZBA and air-dried. The slide was incubated with primary antibody anti MBP-HRP and washed with ZBA and ZBA/0.05% TWEEN. Incubation with Cy5-labeled mouse IgG secondary antibody and washing were performed as in the case of the primary antibody. The slide was scanned with an Agilent Microarray Fluorescent Scanner. H6-HS2 is also ZFP fused with MBP. (B) ZFP microarray for detection of GZF1 binding to target DNA labeled with Cy5. ZFP GZF1 was printed on the PEG gel slide which was then incubated with Cy5-labeled with target DNA. After washing with ZBA and air-drying, the slide was scanned an Agilent Microarray Fluorescent Scanner. Another ZFP, H6-HS2, designed to bind a different target site was used as a negative control. GZF1 concentration was 1.9 μM and its lower concentration was 1.3 μM.

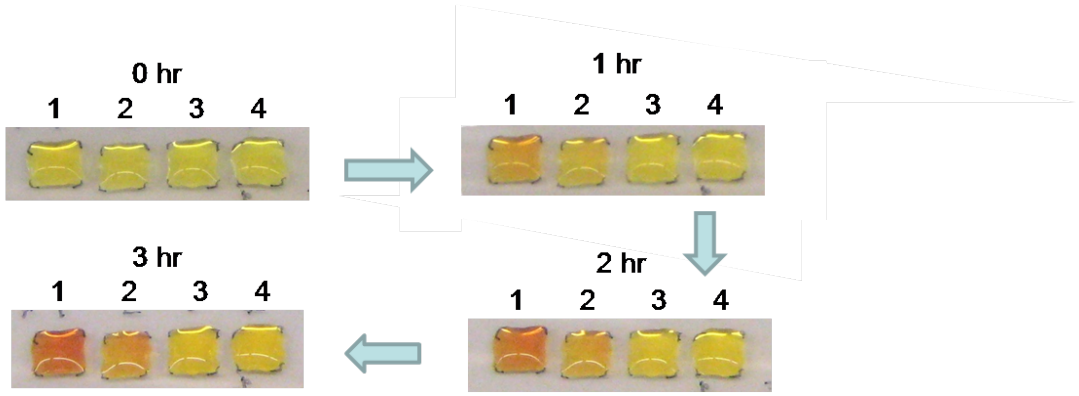

Figure S-3. Spotted ZFP microarrays using the SEER-LAC system detect *E. coli* DNA sequences in a concentration-dependant manner. MBP-LacA-ZFP rrsA125 was printed on the PEG gel slide using a manual arraying tool. Each array (square) contains 20 spots (5 x 4 rows). The arrays were then incubated for 20 min with MBP-ZFP rrsA160-LacB and target DNA oligonucleotides (1: DNA 2.5 µM, 2: DNA 0.25 µM, 3: DNA 5 nM, and 4: No DNA). The slide was washed with ZBA and air-dried, followed by incubation with nitrocefin.

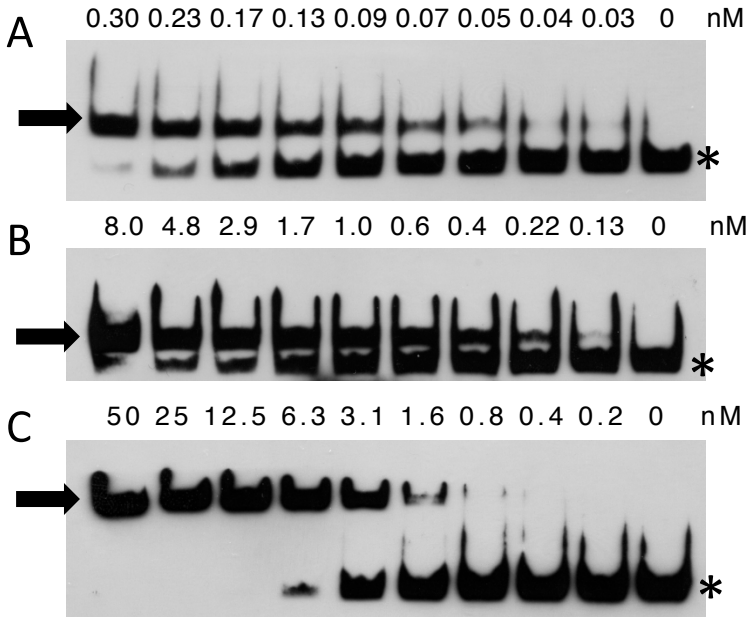

Figure S-4. Electromobility shift assay (EMSA) of engineered zinc finger proteins. Exemplary data are shown for (A) LacA-rrsA27, (B) LacA-rrsA125, and (C) rrsA160-LacB. The concentrations of the protein are indicated. Arrows, bound probe; asterisks, free probe.